DE GRUYTER

Karsten Schrör

ACETYLSALICYLIC ACID

3RD EDITION

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Acetylsalicylic Acid

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düsseldorf university press

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Preface

Acetylsalicylic acid, best known by its first trade name "Aspirin," belongs to the small number of drugs that are well known to health professionals and laymen and enjoy great popularity among both of them. Aspirin is not only one of the most intensively studied but also one of the most frequently used drugs worldwide, today with an impressive annual production rate of 8 billion tablets alone in the German Bayer plant. Actually, about 2,500–3,000 entries for the term "aspirin" (acetylsalicylic acid) can be found in the PubMed database – every year. All this happened more than 100 years after the first pharmacologist studying the compound supposed "the substance is of no value" and the first clinician who used the drug for treatment of inflammatory pain did so with "not little distress." We all know now that the reality soon became another one.

What were the reasons for these exciting developments? Around 1900, there was an urgent need for effective and well-tolerated antipyretic, antiinflammtory analgesics that were on-hand to everybody and this for a reasonable prize. In this context, aspirin became soon very popular as a household remedy for almost any condition associated with flu-like symptoms, headache or other kinds of "malaise" – "take an aspirin." The pharmacological breakthrough was the discovery of a mode of action – inhibition of prostaglandin synthesis. This offered for the first time a plausible mechanistic explanation for the multitude of pharmacological actions of the compound. Later, the antiplatelet/antithrombotic properties of aspirin came into focus and opened the door to an entirely new and still growing clinical field of aspirin usage in prevention and treatment of thrombotic vessel occlusions. Aspirin is the drug of first choice in many of these indications, most notably secondary prevention of myocardial infarction. More recently, prevention of certain forms of venous thromboembolism and preeclampsia became new clinical indications for aspirin. There are also multiple actual research topics. These include the effects of aspirin on gene regulation and transcription as well as posttranslational effects, for example its application as an adjunct in severe systemic inflammatory reactions, including acute respiratory distress syndrome, sepsis and, most recently, viral infections. Malignancies are another actual area of clinical research, in particular prevention of colorectal cancer.

This book provides an overview on all aspects of clinically relevant aspirin actions and the underlying modes of action. The pharmacological focus is on the unique structural properties of the compound, consisting of two bioactive groups, the reactive acetyl group of the intact aspirin molecule with multiple acetylation targets and the salicylate moiety with its unique physicochemical properties.

Subsequent to an introductory section on the fascinating history of the detection of aspirin and important early findings, the pharmacology, toxicology and clinical application of aspirin are discussed in three main sections, each divided into several subsections. More than 100 clinical aspirin trials are presented and critically discussed in

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more detail. A list of references is found after each subsection including a selection of papers that have been published by the end of 2021.

Subsequent to three German, two English and one Chinese edition, this is the third completely revised English edition. Many friends and colleagues worldwide have again extended their help and support to cover the issue of “aspirin” as complete as possible. I am most grateful to all of them. The continued help of Petra Rompel (Düsseldorf) in generating the illustrations and helping me with many other technical issues is particularly gratefully acknowledged.

Dresden, May 3, 2022 Karsten Schrör

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Contents

Preface —— V

1 General aspects —— 1
1.1 History —— **1**
1.1.1 From willow bark to salicylic acid —— **9**
1.1.1.1 Antiinflammatory and analgesic effects of willow bark —— **9**
1.1.1.2 Salicylates as the active ingredients of willow bark and other natural sources —— **10**
1.1.1.3 Chemical synthesis of salicylic acid —— **10**
1.1.2 Synthesis of acetylated salicylic acid (Aspirin) and first medical use —— **13**
1.1.2.1 The invention of acetylated salicylic acid —— **13**
1.1.2.2 Introduction of acetylsalicylic acid in the clinic —— **20**
1.1.3 Search for pharmacological modes of aspirin action —— **23**
1.1.3.1 Salicylates and energy metabolism of the cell —— **24**
1.1.3.2 Inhibition of cyclooxygenases by salicylates —— **25**
1.1.3.3 Further actions of salicylates on cell signaling —— **30**
1.1.4 Clinical applications – a piece of history —— **34**
1.1.4.1 Antiinflammatory/analgesic and antipyretic actions —— **34**
1.1.4.2 Antiplatelet/antithrombotic actions and bleeding —— **35**
1.1.4.3 Aspirin and the history of prevention of myocardial infarction and stroke —— **37**
1.1.4.4 Aspirin and tumor prevention —— **41**
1.1.5 Current research topics —— **44**
1.1.5.1 Clinical research —— **44**
1.1.5.2 Basic research —— **45**
1.2 Chemistry —— **49**
1.2.1 Structures and chemical properties of salicylates —— **50**
1.2.1.1 Salicylates in clinical use – chemical properties —— **51**
1.2.1.2 Aspirin formulations —— **53**
1.2.2 Determination of salicylates —— **56**
1.2.2.1 General aspects —— **56**
1.2.2.2 Methods of determination —— **57**

2 Pharmacology —— 61
2.1 Pharmacokinetics —— **62**
2.1.1 Absorption and distribution —— **63**
2.1.1.1 Absorption and bioavailability —— **63**
2.1.1.2 Aspirin formulations and application modes —— **66**
2.1.1.3 Distribution and plasma levels —— **71**

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2.1.1.4 Modifying factors — 73
2.1.2 Biotransformation and excretion — 77
2.1.2.1 Biotransformations of aspirin — 77
2.1.2.2 Biotransformations of salicylic acid — 81
2.1.2.3 Excretion of salicylates — 83
2.2 Cellular modes of action — 87
2.2.1 Inhibition of cyclooxygenases — 89
2.2.1.1 General aspects — 89
2.2.1.2 Inhibition/modulation of cyclooxygenases by aspirin — 91
2.2.1.3 The different pharmacology of aspirin and NSAIDs — 98
2.2.1.4 Further actions of salicylates on lipid mediators — 100
2.2.2 COX-independent actions of aspirin on cell function — 104
2.2.2.1 General aspects — 104
2.2.2.2 Salicylates in the plant kingdom — 105
2.2.2.3 Non-COX acetylation actions of aspirin — 106
2.2.2.4 Aspirin and nitric oxide formation — 106
2.2.2.5 Salicylates and kinases — 109
2.2.2.6 Salicylates and transcription factors — 113
2.2.3 Energy metabolism — 119
2.2.3.1 General aspects — 119
2.2.3.2 Salicylates and fatty acid β-oxidation — 121
2.2.3.3 Salicylates and uncoupling of oxidative phosphorylation — 125
2.2.3.4 Metabolic actions of salicylates and Reye´s syndrome — 128
2.3 Actions on organs and tissues — 133
2.3.1 Hemostasis and thrombosis — 134
2.3.1.1 General aspects — 134
2.3.1.2 Platelets — 137
2.3.1.3 Endothelial cells — 145
2.3.1.4 Plasmatic coagulation — 150
2.3.1.5 Fibrinolysis — 150
2.3.2 Inflammation, microbial infections, pain and fever — 160
2.3.2.1 General aspects — 160
2.3.2.2 Modes of antiinflammatory actions of aspirin — 162
2.3.2.3 Modes of antimicrobial actions of aspirin — 170
2.3.2.4 Modes of analgesic actions of aspirin — 172
2.3.2.5 Modes of antipyretic actions of aspirin — 177
2.3.3 Aspirin and malignancies — 185
2.3.3.1 General aspects — 185
2.3.3.2 Pathophysiology of intestinal neoplasias — 187
2.3.3.3 Modes of aspirin action — 191
2.3.3.4 COX-related actions of aspirin — 192
2.3.3.5 Non-COX-related antitumor actions of aspirin — 196

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3 Toxicity and drug safety — 207
3.1 Systemic side effects — **208**
3.1.1 Acute and chronic toxicity — **209**
3.1.1.1 General aspects — **209**
3.1.1.2 Pathophysiology and clinical symptoms of acute toxicity — **211**
3.1.1.3 Pathophysiology and clinical symptoms of chronic toxicity — **214**
3.1.1.4 Treatment — **215**
3.1.1.5 Habituation — **216**
3.1.2 Bleeding time and bleeding risk — **221**
3.1.2.1 General aspects — **221**
3.1.2.2 Bleeding time — **221**
3.1.2.3 Modes of aspirin action — **225**
3.1.2.4 Aspirin-related bleeding in long-term prevention — **227**
3.1.2.5 Aspirin-related bleeding in surgery and withdrawal — **228**
3.1.2.6 Prevention and treatment of bleeding — **230**
3.1.3 Safety pharmacology in particular life situations — **237**
3.1.3.1 General aspects — **237**
3.1.3.2 Pregnancy and fetal development — **237**
3.1.3.3 The elderly patient — **241**
3.2 Organ toxicity — **248**
3.2.1 Gastrointestinal tract — **248**
3.2.1.1 General aspects — **248**
3.2.1.2 Pathophysiology of gastrointestinal (stomach) injury — **249**
3.2.1.3 Modes of aspirin action — **252**
3.2.1.4 Clinical studies — **258**
3.2.1.5 Aspirin and other drugs — **262**
3.2.2 Liver — **269**
3.2.2.1 General aspects — **269**
3.2.2.2 Pathophysiology and mode of aspirin action — **270**
3.2.2.3 Clinical studies — **270**
3.2.3 Kidney and heart failure — **273**
3.2.3.1 General aspects — **273**
3.2.3.2 Mode of aspirin action — **274**
3.2.3.3 Clinical studies – individuals without kidney diseases — **275**
3.2.3.4 Clinical studies – individuals with preexisting kidney diseases — **276**
3.2.3.5 Heart failure — **279**
3.2.4 Audiovestibular system — **284**
3.2.4.1 General aspects — **284**
3.2.4.2 Pathophysiology of aspirin-induced hearing loss — **285**
3.2.4.3 Clinical studies — **287**
3.3 Hypersensitivity to aspirin and Reye's syndrome — **291**

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3.3.1 Aspirin-exacerbated respiratory disease (“aspirin-induced asthma,” Widal syndrome) —— **292**
3.3.1.1 History and epidemiology —— **292**
3.3.1.2 Pathophysiology of AERD —— **293**
3.3.1.3 Mode of aspirin action —— **297**
3.3.1.4 Clinical studies —— **299**
3.3.1.5 Aspirin and other drugs —— **301**
3.3.2 Urticaria/angioedema, Stevens–Johnson syndrome and Lyell syndrome —— **306**
3.3.2.1 Urticaria/angioedema —— **306**
3.3.2.2 Stevens–Johnson syndrome and Lyell syndrome —— **307**
3.3.3 Reye’s syndrome —— **309**
3.3.3.1 History and epidemiology —— **309**
3.3.3.2 Clinics, laboratory and morphological findings —— **311**
3.3.3.3 Etiology and pathogenesis —— **313**
3.3.3.4 Clinical studies —— **316**
3.3.3.5 Actual situation —— **323**

4 Clinical applications of aspirin —— 331
4.1 Thromboembolic diseases —— **337**
4.1.1 Coronary vascular disease —— **338**
4.1.1.1 General aspects —— **338**
4.1.1.2 Thrombotic risk and mode of aspirin action —— **340**
4.1.1.3 Clinical trials – primary prevention in apparently healthy individuals —— **344**
4.1.1.4 Clinical trials – primary prevention in individuals with vascular risk factors —— **353**
4.1.1.5 Clinical trials – primary prevention in patients with diabetes —— **357**
4.1.1.6 Clinical trials – patients with chronic coronary vascular disease —— **362**
4.1.1.7 Clinical trials – patients with acute coronary syndrome (ACS) —— **363**
4.1.1.8 Clinical trials – long-term secondary prevention —— **367**
4.1.1.9 Clinical trials – coronary artery bypass graft surgery and other surgical interventions —— **373**
4.1.1.10 Aspirin and other drugs —— **376**
4.1.1.11 Actual situation —— **390**
4.1.2 Cerebrovascular diseases —— **409**
4.1.2.1 General aspects —— **409**
4.1.2.2 Thrombotic risk and mode of aspirin action —— **411**
4.1.2.3 Clinical trials – primary prevention —— **415**
4.1.2.4 Clinical trials – secondary prevention —— **416**
4.1.2.5 Aspirin and other drugs —— **424**
4.1.2.6 Actual situation —— **435**

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4.1.3 Peripheral arterial occlusive disease — 445
4.1.3.1 General aspects — 445
4.1.3.2 Thrombotic risk and mode of aspirin action — 447
4.1.3.3 Clinical trials: primary prevention — 449
4.1.3.4 Clinical trials: secondary prevention — 451
4.1.3.5 Clinical trials: peripheral transluminal angioplasty — 452
4.1.3.6 Aspirin and other drugs — 454
4.1.3.7 Actual situation — 457
4.1.4 Venous thromboembolism — 461
4.1.4.1 General aspects — 461
4.1.4.2 Thrombotic risk and modes of aspirin action — 464
4.1.4.3 Clinical trials – primary prevention — 464
4.1.4.4 Clinical trials – secondary prevention — 471
4.1.4.5 Aspirin and other drugs – NOACs — 473
4.1.4.6 Actual situation — 474
4.1.5 Pregnancy-induced hypertension (preeclampsia) and preterm delivery — 479
4.1.5.1 General aspects — 479
4.1.5.2 Vascular risk and modes of aspirin action — 481
4.1.5.3 Clinical trials — 486
4.1.5.4 Clinical trials – reasons for data variability — 488
4.1.5.5 Actual situation — 492
4.1.6 Aspirin “high on treatment platelet reactivity” (HTPR, aspirin resistance) — 500
4.1.6.1 General aspects — 500
4.1.6.2 Definition and types of aspirin – HTPR — 501
4.1.6.3 Detection of aspirin HTPR — 504
4.1.6.4 Mechanisms of aspirin HTPR — 507
4.1.6.5 Clinical trials — 515
4.1.6.6 Actual situation — 519
4.2 Pain, fever and inflammatory diseases — 528
4.2.1 Analgesia and antipyresis — 529
4.2.1.1 General aspects — 529
4.2.1.2 Pain, fever and mode of analgesic/antipyretic aspirin action — 530
4.2.1.3 Clinical trials — 534
4.2.1.4 Aspirin and other drugs — 538
4.2.1.5 Actual situation — 539
4.2.2 Inflammatory diseases and viral infections — 542
4.2.2.1 General aspects — 542
4.2.2.2 Rheumatoid arthritis – pathophysiology, mode of aspirin action and clinical trials — 544

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4.2.2.3 Osteoarthritis – pathophysiology, mode of aspirin action and clinical trials —— **547**
4.2.2.4 Systemic inflammatory response syndrome (SIRS). Pathophysiology, mode of aspirin action and clinical trials. Sepsis and acute respiratory distress syndrome (ARDS) —— **548**
4.2.2.5 HIV – pathophysiology, mode of aspirin action and clinical trials —— **552**
4.2.2.6 COVID-19 and flu-like conditions: pathophysiology, modes of aspirin action and clinical trials —— **555**
4.2.2.7 Aspirin and other drugs —— **559**
4.2.2.8 Actual situation —— **560**
4.2.3 Kawasaki disease —— **567**
4.2.3.1 General aspects —— **567**
4.2.3.2 Pathophysiology and mode of aspirin action —— **568**
4.2.3.3 Clinical trials —— **569**
4.2.3.4 Aspirin and other drugs —— **571**
4.2.3.5 Actual situation —— **571**
4.3 Further potential clinical indications —— **574**
4.3.1 Colorectal tumors (adenomas, carcinomas) —— **575**
4.3.1.1 General aspects —— **575**
4.3.1.2 Epidemiology, etiology and pathogenesis —— **576**
4.3.1.3 Modes of aspirin action —— **578**
4.3.1.4 Clinical trials – primary prevention —— **581**
4.3.1.5 Clinical trials – secondary prevention —— **587**
4.3.1.6 Aspirin and other drugs —— **593**
4.3.1.7 Actual situation —— **594**
4.3.2 Alzheimer's disease and other neurological disorders —— **602**
4.3.2.1 General aspects —— **602**
4.3.2.2 Epidemiology, etiology and pathophysiology —— **602**
4.3.2.3 Modes of aspirin action —— **603**
4.3.2.4 Clinical trials —— **605**
4.3.2.5 Aspirin and other drugs —— **609**
4.3.2.6 Actual situation —— **609**

Abbreviations —— 613

Acronyms of clinical trials —— 617

Index —— 623

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1 General aspects

1.1 History

The medical history of salicylates is long and stretches back more than 2000 years, when bark from the willow tree was introduced as an antiinflammatory agent and antipyretic analgesic [1]. This view did not change over many centuries until the redetection of the usefulness of extracts of willow bark for treatment of "aigues, fever and intermitting disorders" in 1763 by the English Edward Stone. A major step forwards was the identification of salicin and salicylic acid as the active ingredients, allowing for chemical synthesis in the nineteenth century, thanks to rapid advances in pharmaceutical chemistry. The availability of synthetic salicylate, easy to be produced in unlimited amounts, then led to the search for appropriate chemical modifications to improve the efficacy and to reduce side effects of the compound – a common and frequently used procedure for optimization of pharmacological properties of natural products. In the case of salicylate, the sought-after chemical modification was acetylation, eventually resulting in the purely synthetic compound acetylsalicylic acid, which soon entered the market under its first brand name "Aspirin" in 1899. Acetylation of salicylate resulted in the generation of a more potent and better tolerable product. However, it was only in 1971 when *John Vane* from Beckenham (UK) detected inhibition of prostaglandin biosynthesis by aspirin as its principal mode of action.

The discovery of inhibition of platelet-derived thromboxane formation by aspirin then opened the door for new and widespread use of aspirin as an agent to prevent and to treat thromboinflammatory disorders. Best studied are the multiple actions of aspirin on platelet function and thrombosis in a plethora of arterial and venous thrombotic diseases, such as myocardial infarction and ischemic stroke, peripheral arterial occlusions and venous thrombosis. Other thrombotic diseases of interest and subject of actual research are preeclampsia, sepsis and immunothromboses, for example in Kawasaki's disease and other forms of vascular inflammation. Currently, systemic inflammatory response syndrome (SIRS), acute respiratory distress syndrome (ARDS) and sepsis are studied intensively, and it has even been speculated that aspirin might be valuable for treatment of COVID-19 because of its unique combination of antiplatelet and antiinflammatory actions in one molecule. Of considerable interest is also the possible beneficial effect of aspirin in tumor prevention, specifically prevention of colorectal carcinomas. This clinical research is accompanied by intense basic research on the mode(s) of action which appear to be many more than only inhibition of COXs and subsequent prostaglandin production and are associated with the detection of new aspirin-sensitive pathways of inflammation and tumorigenesis.

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Table 1.1 summarizes some important discoveries during the more than 2000 years of history of salicylates and more than 100 years of its most important synthetic derivative – acetylsalicylic acid, best known under its first trade name "Aspirin."

Table 1.1: The history of salicylates and acetylsalicylic acid.

Date	Discovery
400 BC–AD 100	*Hippokrates* recommends bark and leaves of the willow tree (*Salix alba*) for medical use. This recommendation is later compiled by *Plinius* and *Dioscurides* as popular medical knowledge of the time.
1763 Rev.	*Edward Stone* recommends the use of willow bark extracts for treatment of "Aigues and intermitting disorders."
1826–1830	*Brugnatelli* and *Fontana* as well as Johann *Buchner* identify salicin as the active antipyretic ingredient of the willow bark. Pierre-Joseph *Leroux* in 1829 is the first to isolate salicin in crystalline form. The compound, prepared from willow bark, is later sold by Ernst *Merck* (Darmstadt) as an antipyretic drug for half the prize of quinine.
1839	Raffaele *Piria* prepares salicylic acid from salicin and correctly determines the brutto formula $C_7H_6O_3$.
1835–1843	New, rich sources of natural salicylates are detected, most notably wintergreen oil from the American Wintergreen (*Gaultheria procumbens*), containing > 96 % methylsalicylate. This finding markedly increases the availability of salicylates for practical use.
1859–1874	Hermann *Kolbe* synthesizes for the first time pure salicylic acid from the already known decomposition products phenol and carbonic acid. His assistant Rudolf Wilhelm *Schmitt* improves the synthesis and elucidates the reaction kinetics ("Kolbe–Schmitt" synthesis). Schmitt's student, Friedrich *von Heyden*, further improves the procedure in order to produce the compound in industrial amounts and, together with Kolbe, receives a patent for his new technology. Von Heyden founds the first salicylic acid-producing factory in Radebeul (near Dresden) in 1874. The plant soon produces tons of salicylic acid every year. This provides unlimited amounts of the compound for practical purposes and makes its availability independent of natural sources.
1875	*Ebstein* and *Müller* detect the blood sugar-lowering action of salicylates.
1876	Franz *Stricker* publishes the first report on the clinical usefulness of salicylic acid as an analgesic/antirheumatic drug and introduces it for this indication at the Charité in Berlin. Shortly thereafter, Thomas *MacLagan*, a Scottish physician, and Germain *Sée*, a Frenchman from Strasbourg (Alsace), also describe an antipyretic/analgesic activity of the compound.
1897	Felix *Hoffmann*, working in the pharmaceutical department at Bayer laboratories in Elberfeld under the direction of Arthur *Eichengrün*, synthesizes for the first time acetylsalicylic acid as a chemically pure and stable compound. This work is substantially supported by Carl *Duisberg*, the then head of Bayer research.

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Table 1.1: (continued)

Date	Discovery
1899	Heinrich *Dreser*, head of the pharmacological research laboratories at Bayer, becomes informed about these findings and publishes the first report on the pharmacology of acetylsalicylic acid. He considers the compound as a prodrug of the active metabolite salicylic acid with the advantage of a better taste and less toxicity to the stomach. The first clinical studies by Kurt *Witthauer* and Julius *Wohlgemuth* are published the same year.
1899	Introduction of acetylsalicylic acid to the market under the trade name "Aspirin®." Since then, Aspirin® is used worldwide as a standard medication and most popular household remedy for treatment of fever, pain and inflammation ("take an aspirin!").
1902	First description of a hypersensitivity reaction (dyspnea, urticaria, angioedema) to aspirin after oral intake of 1 g by G. *Hirschberg* from Posen (Poland). These hypersensitivity reactions are later named "aspirin-exacerbated respiratory disease" (AERD) or "aspirin-exacerbated cutaneous disease" (AECD).
1945–1952	Rudolf *Singer*, an ETN physician from the United States, describes a bleeding tendency after tonsillectomies if aspirin was used as analgesic. No such effect was seen with metamizole (dipyrone). This observation was confirmed in further case reports. Singer explains this action of aspirin by reduced prothrombin levels in blood.
1949–1950	Paul C. *Gibson* from London (UK) reports on positive results with aspirin for treatment of angina pectoris. He ascribes this effect to a combination of analgesic and antithrombotic activities of aspirin, the latter being similar to those of coumarins from which salicylate was already known to be generated as a metabolic intermediate in the liver by Karl Paul Link from Madison (Wisconsin) (1943).
1950–1956	Lawrence L. *Craven*, a general practitioner from Glendale (California), publishes a series of studies using aspirin as "coumarin-light" for prevention of myocardial infarction and stroke. According to data from his first study, daily administration of 650–1,950 mg aspirin completely prevented myocardial infarctions in 400 medium-aged male patients during an observation period of 2 years. In the following years, he increased the number of patients to about 8,000 – reportedly without having seen any myocardial infarction – and finally reduces the daily aspirin dose to one tablet (1 tablet = 5 gran = 325 mg aspirin) per day. Craven himself saw these data critically, in particular because of the absence of untreated controls and strongly recommended controlled studies for validation of these findings. However, his data and conclusions were not appreciated by the scientific community at the time, possibly influenced by the way they were published (mainly "letters to the editor") and the low impact factors of the journals.
1960	Armand J. *Quick* publishes the first mechanistic approach to explain the prolonged bleeding time after oral aspirin intake (6 g). He suggests an action on thrombin and the plasmatic coagulation system, possibly by inhibition of prothrombin biosynthesis. Later, he suggested additional, aspirin-sensitive factors in vivo, because he did not see any prolongation of bleeding time by aspirin or salicylic acid in vitro.

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Table 1.1: (continued)

Date	Discovery
1967–1968	Several authors, among them H. Klaus *Breddin*, John R. *O'Brien*, Majorie *Zucker*, Harvey J. *Weiss*, James F. *Mustard* and their coworkers, publish the first mechanistic studies on an antiplatelet activity of aspirin as the explanation of the increased bleeding tendency after in vivo application. Daily doses of 150 mg were found sufficient to inhibit platelet function over several days. According to his data, O'Brien recommends a clinical trial with aspirin for thrombosis prevention in patients at elevated vascular thrombotic risk.
1969	The successful Apollo 11 mission to the moon has aspirin in the board pharmacy.
1971	*Sir John Vane* detects the inhibition of prostaglandin synthesis by aspirin (and salicylate) and considers this as the mechanism of its antiinflammatory and antipyretic actions. This work and the later detection of prostacyclin are acknowledged with the Nobel Prize for Medicine in 1982.
1971	J. Brian *Smith* and Al *Willis*, both working in John Vane's institution, describe the inhibition of prostaglandin biosynthesis by aspirin in human blood platelets. The (thrombin-induced) platelet secretion reaction (serotonin release) remains unaffected. This was also the first demonstration – although less appreciated by the scientific community – that inhibition of prostaglandin (thromboxane) formation by platelets and inhibition of platelet function(s) by aspirin are two separate phenomena.
1972	Gabriel L. *Gasic* and his group describe for the first time an antimetastatic action of aspirin in mice and explain this by its antiplatelet effects. This finding was in line with earlier discoveries (1968) of this group on the inhibition of tumor metastasis by experimental platelet depletion.
1974	Peter C. *Elwood*, Archibald L. *Cochrane* and coworkers from Cardiff (Wales) publish the first prospective randomized, placebo-controlled study with aspirin (300 mg/day) in 1,239 male survivors of an acute myocardial infarction. Compared to placebo, aspirin intake reduced 1-year mortality by 25 %. This finding was not statistically significant but further studies were strongly suggested.
1975	Andrzej *Szczeklik* and his group from Kraków (Poland) detect that precipitation of asthma attacks in sensitive persons by aspirin and related drugs (nonsteroidal antiinflammatory drugs [NSAIDs]) involves inhibition of prostaglandin biosynthesis. After the detection of leukotrienes, this hypothesis was extended to an imbalance in eicosanoid formation and action in these patients.
1975	Philip W. *Majerus* and his postdoc Gerald *Roth* detect the irreversible acetylation of platelet COX by aspirin and explain by this mechanism the inhibition of thromboxane formation and thromboxane-dependent platelet functions. Later work of this laboratory and others identifies a serine residue inside the substrate channel of COX-1 as the molecular target of acetylation.
1976	Martin *Hemler*, William E. M. *Lands* and William L. *Smith* from the University of Michigan purify the COX from sheep seminal glands and identify a specific acetylation site ($serine_{530}$) which is associated with inhibition of enzyme activity by aspirin.

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Table 1.1: (continued)

Date	Discovery
1979	Philip W. *Majerus* and his group publish the first double-blind, randomized, placebo-controlled clinical trial on the antithrombotic effects of an "antiplatelet dose" (160 mg/day) of aspirin. Aspirin treatment reduces the incidence of arteriovenous (AV) shunt thromboses in hemodialysis patients within an observation period of 1 month highly significantly by 65 %.
1979	A. J. *Crandon* and D. M. *Isherwood* report that regular intake of aspirin in pregnancy reduces the risk of preeclampsia. H. C. *Wallenburg* and colleagues confirm the prevention of preeclampsia in women at high risk by low-dose aspirin (60 mg/day) in a placebo-controlled trial in 1986. They report no adverse effects of aspirin in mothers or infants.
1983	H. D. *Lewis* Jr. and colleagues publish the first placebo-controlled, randomized, double-blind trial (Veterans Administration Study) on the prophylactic use of aspirin (324 mg/day) in men with acute coronary syndromes. The study finds a 50 % reduction of the incidence of (recurrent) myocardial infarctions and death within a follow-up period of 3 months.
1988–1989	Publication of the first large placebo-controlled prospective long-term trial on primary prevention in apparently healthy male physicians in the United States (US-PHS). Ingestion of 325 mg aspirin every other day resulted in a 44 % reduction of the incidence of a first myocardial infarction within 5 years. There was a nonsignificant increase in the incidence of gastrointestinal ulcers and hemorrhagic strokes but an increased general bleeding tendency. Total mortality remained unchanged.
1988	The ISIS-2 trial, a prospective, placebo-controlled, randomized trial in patients with acute myocardial infarction, demonstrates a 23 % reduction in mortality by aspirin alone (162 mg/day) and a 38 % reduction in combination with fibrinolysis (streptokinase) during an observation period of 5 weeks. This study resulted in guideline recommendation of aspirin for secondary prevention of acute coronary syndromes.
1988	Gabriel *Kune* and colleagues from Melbourne (Australia) publish the first study on aspirin intake and prevention of colon cancer. In a retrospective, exploratory case-control study, regular (daily) use of aspirin reduced the risk of (incident) colon cancer by 40 %. These findings were principally confirmed and extended by many others in large observational and some randomized clinical trials.
1988–1990	William L. *Smith*, *David*, L. *De Witt* and colleagues demonstrate that the molecular mode of action of aspirin is steric hindrance of access of substrate (arachidonic acid) to binding sites inside the substrate channel of the enzyme (COX) and does not involve acetylation of the active center at tyrosine$_{385}$.
1991	A prospective, randomized, placebo-controlled study (SALT-Trial) on low-dose (75 mg/day) aspirin in 1,360 patients with previous cerebral ischemic events (TIA, minor stroke) is published. Aspirin reduced the incidence of recurrent strokes (TIA) myocardial infarctions and mortality, while the number of fatal hemorrhagic cerebral infarctions increased. All these changes were significant. The benefit/risk ratio was considered to be in favor of prevention. However, the authors also concluded that an overall risk reduction by only 17–25 % in these high-risk patients suggests a low efficacy of aspirin, since the majority of recurrent thrombotic events was not prevented.

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Table 1.1: (continued)

Date	Discovery
1991	Kenneth K. *Wu*, Xiao-Ming *Xu* and colleagues show for the first time inhibition of cytokine-induced expression of COX-2 in human endothelial cells in vitro by aspirin and salicylate in nanomolar concentrations. No inhibition is seen with indomethacin. This suggests salicylate-mediated inhibition of COX gene transcription. Later work of this group identifies the C/EBP-β and NF-κB transcription factors as possible molecular targets of this salicylate action.
1994	Daniel *Picot*, Patrick J. *Loll* and R. Michael *Garavito* describe the crystal structure of COX-1 and its molecular mode of inactivation by aspirin.
1994	The Oxford Group around Sir Richard *Peto* has developed the technology of metaanalysis for evaluation of drug efficacy/safety profiles in clinical trials. The method allows the evaluation of combined data from different trials after appropriate standardization of the raw data. This results in large numbers of patients and a more reliable data background than with small single studies. The analysis data can also be updated as long as necessary. The first of a series of studies with antiplatelet agents, mainly aspirin, is published in 1994. The results, elaborated by the "Antiplatelet (Antithrombotic) Trialists' Collaboration" (ATTC), show an overall reduction of about 20 % of the risk of new severe thrombotic vascular events upon secondary cardiovascular prevention by regular aspirin. Overall, a positive benefit/risk ratio is found, despite increased bleeding events. In an updated version, published in 2009, efficacy of aspirin is also documented in primary prevention, although much lower in absolute terms because of the lower thrombotic risk. According to these data and numerous subsequent clinical trials, aspirin is currently (2022) considered as a drug of first choice in secondary prevention of cardiovascular events (1A level of recommendation), whereas aspirin use for primary prevention should be decided on an individual basis and is limited to adults aged 40 to 59 years with a 10 % or greater 10-year cardiovascular disease (CVD) risk who are not at increased risk for bleeding.
1995	Joan *Claria* and Charles N. *Serhan* detect the generation of "aspirin-triggered lipoxin" (ATL) by the interaction of acetylated COX-2 with white cell lipoxygenases.
2001	The "Clopidogrel in Unstable Angina to prevent Recurrent Events" (CURE) trial is conducted to study the efficacy of aspirin combined with the ADP-P2Y12 antagonist clopidogrel in patients with acute coronary syndromes without ST elevation. Aspirin plus clopidogrel reduced the incidence of recurrent vascular events or death during a 12-month follow-up period by 18 % compared with aspirin alone ($P < 0.001$). In the combined treatment group, there were significantly more severe bleeding events, 3.7 % vs. 2.7 % ($P < 0.001$), but no change in mortality and no increased incidence of hemorrhagic strokes were observed. This study eventually resulted in the introduction of dual antiplatelet therapy (aspirin plus ADP antagonist) in treatment of acute coronary syndromes.
2005	The first prospective, randomized, placebo-controlled primary prevention trial in women – the Women's Health Study (WHS) – is published. The study demonstrates a modest, nonsignificant reduction of cardiovascular events by 9 % ($P = 0.13$) by aspirin (100 mg each other day) in apparently healthy women (≥45 years) during a 10-year observation period. There was no change in the rate of myocardial infarctions but a reduction of ischemic strokes ($P = 0.04$) and a significant increase of severe gastrointestinal bleeding events ($P = 0.02$) were observed.

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Table 1.1: (continued)

Date	Discovery
2006	The CHARISMA study compares low-dose aspirin (75–162 mg/day) alone and in combination with clopidogrel (75 mg/day) for primary prevention of vascular events in persons at high risk of atherothrombosis. While the combination was useful in secondary prevention, the comedication of clopidogrel with aspirin did not reduce the vascular risk but significantly increased bleeding in subjects with risk factors but without a preexisting event.
2007	Igor *Mazur* and colleagues from Münster (Germany) describe for the first time an antiviral effect of high-dose aspirin in vitro and in vivo (mice) by inhibition of IKK/NF-κB signaling in host cells. This pathway is "misused" by the virus for replication, subsequent caspase activation and nuclear export of new viruses. This new host cell-directed antiviral strategy will not induce resistant virus variants and might be useful for antiinfluenza virus interventions with aerosolized salicylate for treatment of viral affections of the respiratory tract.
2011/2012	Peter W. *Rothwell* from Oxford (UK) and colleagues publish a series of articles (meta-analyses) on the chemopreventive effect of aspirin in primary and secondary prevention of colorectal cancer. The three main findings were as follows. (i) A significant reduction of cancer mortality in both primary and secondary prevention after regular aspirin intake for ca. 8–10 years. This reduced cancer mortality accounts for the long-term survival benefit in these persons rather than protection from vascular events. (ii) The incidence of severe and/or life-threatening bleeding is markedly (about 50 %) reduced with time, starting at about 5 years of aspirin use. (iii) The beneficial effects of aspirin on cancer prevention are seen at antiplatelet doses of around 100 mg/day and do not become stronger with increasing doses, suggesting no clear dose dependency.
2014	Leslie A. *Bateman* and colleagues identify 112 new proteins that are long-term (lysine-)acetylated by aspirin. Threshold aspirin concentrations are in the range of 50–100 µM. At least some of these proteins (enzymes) might be relevant to cell proliferation and energy metabolism, considering the (long) survival time of (many) acetylated proteins and the possible accumulation of acetylated sites at the proteins with repeated drug application.
2014	A new fast disintegrating oral aspirin formulation with a threefold higher peak plasma level of unmetabolized aspirin and a more than twice faster onset of action is introduced to the German market by Bayer Company.
2016	Michael L. *Lucido* and colleagues working in the group of Michael G. *Malkowski* identify the crystal structure of acetylated COX-2 and propose a reaction scheme for the transformation of the acetylated enzyme into a 15-lipoxygenase.
2017	Daniel L. *Rolnik* and colleagues publish a large randomized, prospective, placebo-controlled double-blind study on aspirin as a preventive of preterm preeclampsia (pregnancy-induced hypertension [PIH]) in high-risk women (ASPRE-trial). The risk of these women was determined by a complex score based on clinical and laboratory parameters. The women received enteric-coated aspirin (150 mg/day) or placebo, starting early (week 11–14) in gestation. Aspirin treatment reduced the incidence of preterm PIH (36th week of gestation) significantly from 4.3 % to 1.6 % (a reduction of more than 60 %). Term preeclampsia was not significantly affected. There were no differences in the incidence of neonatal adverse outcomes or other adverse events of mother and child.

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Table 1.1: (continued)

Date	Discovery
2018	The randomized, placebo-controlled "Aspirin in Reducing Events in the Elderly" (ASPREE) trial is published. Aspirin (100 mg/day) was given to an elderly population (average age 74.9 years) without known cardiovascular disease and physical or mental disabilities. The study was stopped prematurely at 4.7 years. At this time, aspirin caused a significantly higher risk of major hemorrhages and all-cause mortality. It did not reduce the risk of cardiovascular diseases or cancer incidence but rather increased cancer malignancy. The data caused an adaptation of the United States Preventive Services Task Force (USPSTF) recommendations for aspirin in primary prevention, removing the rationale for considering low-dose aspirin for prevention of colorectal cancer.
2021	Salim *Yusuf* and colleagues publish the Polycap study-3 (TIPS-3). The study investigates not only the effects of a polypill containing statins and multiple antihypertensives versus aspirin alone, but also aspirin in combination with the polypill versus placebo. The study was a population-based, randomized primary prevention trial in men and women at elevated cardiovascular risk but without known cardiovascular disease. The incidence of major cardiovascular events during a 4.6-year follow-up period was reduced from 5.8 % to 4.1 % (a reduction of 29 %) in the polypill plus aspirin group as compared to placebo. There were no differences in major bleeding events. Aspirin alone reduced the event rate by 15 %, the polypill alone by 20 %. The conclusion was that combined treatment with a polypill plus aspirin reduces significantly the incidence of cardiovascular events among persons without cardiovascular disease who are at an intermediate cardiovascular risk.
2020/2021	Along with the COVID-19 pandemic, the repeated occurrence of new SARS-CoV-2 mutants and the absence of specific medical treatment options, the antiviral/antiinflammatory effects of aspirin are rediscovered and come increasingly into research focus. Observational trials in COVID-19 patients using antiplatelet doses (75–162 mg/day) of aspirin on top of standard medical care provided mixed results by the end of 2021. The first large randomized, prospective trial using 150 mg aspirin/day (RECOVERY) was negative with respect to mortality rates at 28 days. One explanation for a variable outcome might be a too low aspirin dose. This may not cover the full spectrum of the unique combination of antiinflammatory, antithrombotic and antiviral properties of aspirin in one molecule. Paul A. *Gurbel* and coworkers hypothesize about the clinical usefulness of high-dose soluble aspirin (LASAG), admitted directly to the lung as aerosol by a nebulizer for treatment of COVID-19. Inhalation will immediately allow high local salicylate levels in the lung for inhibition of both aspirin-sensitive platelet- and NF-κB-dependent signaling pathways that account for the pathology of the disease. Appropriately sized prospective clinical trials are strongly suggested.
2032	Planned end of the RECOVERY-II trial on medical treatment of COVID-19, including 40,000 participants. Study is underway since November 2021 and will compare 15 (!) different therapeutic approaches, including low-dose oral aspirin (150 mg/day), for their usefulness in COVID-19 treatment.

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1.1.1 From willow bark to salicylic acid

1.1.1.1 Antiinflammatory and analgesic effects of willow bark

Medical effects of willow bark. Treatment of maladies by plants or extracts thereof is as old as the history of mankind. This is also true for fever and pain, two particularly frequent and inconvenient symptoms of acute illnesses but also typical for osteoarthritis and rheumatism, two examples for chronic painful diseases. Rheumatism was already known in old Egypt, as seen from cartilage alterations in Egyptian mummies. The Egyptians were also aware of the pain-relieving effects of potions made from myrtle and willow leaves. Clay tablets from the Sumerian period also contained information about the use of willow leaves as medicines. *Hippokrates* recommended leaves of the willow tree for medical purposes about 400 BC. *Pliny* (compilations) and *Dioscurides* (Materia Medica) also recommended decocts of willow leaves or ash from willow bark for treatment of sciatica (lumbago) and gout at about AD 100. Outside Europe, it were the Nama (Hottentots) in Southern Africa who had a "for a long time" used tea made from bark of willow trees for treatment of rheumatic diseases [2] (cited after Gross & Greenberg, 1948). This comment was made by a certain Dr. Ensor from Capetown (South Africa) in reply to a publication of Dr. MacLagan in 1876 [3], describing for the first time positive experiences with salicylates at 2 g/day for treatment of rheumatism.

The first published clinical trial. The first known public communication on the medical use of willow bark extracts in modern times came from Reverend *Edward Stone* [4] from Chipping Norton (Oxfordshire, England). He treated some 50 cases of "aigues, fever and intermitting disorders" with a redissolved powdered dry bark preparation of willow tree. The doses were about "20 gr(ains) [≈ 1.3 g] to a dram of water every 4 hours." On June 2, 1763, he wrote a letter to the Earl of Macclesfield, then the President of the Royal Society in London, entitled "An account of the success of the bark of the willow in the cure of aigues." In this letter he summarized his opinion about this treatment as follows:

> ... As this tree delights in moist or wet soil where agues chiefly abound, the general maxim, that many natural maladies carry their cure along with them or that their remedies lie not far from their causes, was so very apposite to this particular case, that I could not help applying it; and this might be the intention of providence here, I must own had some little weight with me ....

After claiming to have obtained good results he concluded:

> ... I have no other motives for publishing this valuable specific than that it may have a fair and full trial in all its variety of circumstances and situations, and that the world may reap the benefits accruing from it.

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1.1.1.2 Salicylates as the active ingredients of willow bark and other natural sources

Detection and preparation of salicin from willow bark. In 1828, the German pharmacist *Johann Andreas Buchner* was the first to prepare a yellowish mash with bitter taste from boiled willow bark which he named Salicin, according to the Latin word for willow (*salix*). He considered salicin as the active antipyretic ingredient of willow bark and recommended its use for treatment of fever. A similar conclusion had earlier been reached by the Italians *Brugnatelli* and *Fontana* in 1826 using a less purified preparation of willow bark. They also considered salicin as the active principle of willow bark (cit. after Sharp [5]). In 1830, the Frenchman Henry *Leroux* was the first to obtain salicin in crystalline form. Only 3 years later, in 1833, the pharmacist *Merck* in Darmstadt (Germany) announced highly purified salicin from willow bark for use as an antipyretic for half of the prize of quinine (cit. after [6]) – a really attractive offer at that time.

Salicin from natural sources as starting material to make salicylic acid. Salicin is not only the active antipyretic ingredient of willow bark but also causes its strong bitter taste. This and the irritation of stomach mucosa limited its practical use. Salicin hydrolyzes in aqueous media to glucose and salicylic alcohol (saligenin). Saligenin has no bitter taste and can easily be oxidized to salicylic acid. The Italian *Raffaele Piria* was the first who successfully synthesized salicylic acid (acide salicique ou salicylique) from salicin in 1839 and also correctly determined the empirical formula $C_7H_6O_3$. As a result, it was now possible to replace the poorly palatable salicin by salicylic acid, for example in form of its well-water-soluble sodium salt. This became practically relevant after new and abundant natural sources for salicylates were detected. These included wintergreen oil obtained from the American Wintergreen *Gaultheria procumbens* and spireic acid (Acidum salicylicum) from the American teaberry (*Spirea ulmaria*). Gaultheria oil (Wintergreen oil) consists of > 96 % methyl salicylate, from which free salicylic acid can easily be obtained. However, production of salicylates by plants is also an important defense mechanism for themselves.

Efficient communication between pest-colonized and noncolonized plants is vital for timely manifestation of defenses that restrict systemic spread of pests. Airborne signals are involved in these processes. Methyl salicylate is a volatile compound that is made by a number of plants and is suggested to act as such a mobile airborne signal in plant defense by activation of systemic acquired resistance. This confers enhanced resistance against a broad spectrum of pathogens (Section 2.2.2) [7].

1.1.1.3 Chemical synthesis of salicylic acid

Kolbe–Schmitt synthesis. The modern pharmaceutical history of salicylates and its derivatives starts with the chemical synthesis of the compound. In 1859, the German

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Hermann Kolbe, Professor of Chemistry in Marburg, produced the first fully synthetic salicylic acid from the already known decomposition products phenol and carbonic acid, that is, sodium phenolate and carbon dioxide. Kolbe then stimulated his assistant *Rudolf Wilhelm Schmitt* to further improve the technology, eventually resulting in doubling of the salicylic acid yield. Schmitt also elucidated the reaction kinetics. This base-promoted carboxylation of phenols under high pressure allowing the synthesis of salicylic acid derivatives is known since then as the "Kolbe–Schmitt reaction." *Friedrich von Heyden*, a student of Schmitt, was introduced to Kolbe and encouraged by him to develop a procedure to make the compound in industrial amounts. Von Heyden was the first to receive a patent for this new technology. This allowed the synthesis of large amounts of salicylate and made investigators independent of the limited availability of natural sources with varying content and seasonal variations of salicin. It also opened the door for its broad practical use and caused a massive drop in price: The prize of 100 g of salicylic acid prepared from salicin from natural sources (gaultheria oil) dropped from 10 to 1 taler (dollar = American for taler) for the chemical product of Kolbe's synthesis (cit. after [8]).

Von Heyden started the large-scale production of salicylic acid in the kitchen of his mansion, the "Villa Adolpha" in Dresden (Germany). In 1874, the site was moved to Radebeul, a provincial town west to Dresden, where he founded the "Salizylsäurefabrik Dr. von Heyden." This plant was extremely effective: After making 4 tons of salicylic acid in the first year, the annual production was increased to 25 tons only four years later and continued to grow steadily. Kolbe and von Heyden obtained patents for the synthesis of salicylate in many European countries and the US [9]. Interestingly, after solving some legal issues, von Heyden's plant also produced the salicylic acid which was later used by Bayer to make aspirin [10].

Practical use of salicylate. After salicylate became available as a cheap chemical in unlimited amounts, the compound was tested for new practical applications. For example, salicylic acid was soon found to have antiseptic properties which could be used to preserve milk and meat. The compound was also recommended as an alternative to phenol (carbolic acid), which, at the time, was the antiseptic of choice in surgery. The antipyretic action of salicylate was for a time also attributed to its antiseptic activity, until it was shown that the sodium salt with little antiseptic properties was an equally effective antipyretic (cit. after [2]). Importantly, salicylic acid was also studied as a potential drug to treat a variety of diseases and thus became the first synthetic drug ever developed. In 1875, *Ebstein* and *Müller* [11] detected the blood sugar-lowering action of the compound. Shortly thereafter, the uricosuric action of salicylate was described. Thus, salicylates appeared to be useful for treatment of diabetes and gout.

Salicylic acid as an antiinflammatory antirheumatic agent. Among these discoveries regarding the medical applications of salicylates, the most far-reaching finding was

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the detection that synthetic salicylates were potent antiinflammatory analgesics and proved to be extremely useful for treatment of rheumatic diseases. *Franz Stricker* from Berlin was the first to publish that sodium salicylate was not only an antipyretic but also an effective antiinflammatory drug, useful for treatment of rheumatic bone and joint diseases [12]. He was the first to clinically introduce salicylate in 1876 for this indication at the Charité in Berlin [13]. Two months later, the Scottish physician *Thomas J. MacLagan* [14] published the first of a series of articles showing that administration of salicylate to patients with rheumatic fever resulted in the rapid disappearance of fever and pain. Similar results were reported by the Frenchman *Germain Sée* one year later [15]. The papers of these three authors mark the beginning of the systematic medical use of salicylates as analgesic antiinflammatory drugs.

Summary

Extracts or other preparations from willow bark or leaves were used since ancient times as household remedies for treatment of fever, inflammation and pain. These medical uses have been rediscovered only in the eighteenth century: In 1763, the first communication on successful use of an aqueous extract of powdered willow bark in the treatment of "aigue and feverish diseases" was published in the UK by Rev. Edward Stone.

The search for the active ingredient of willow bark initially resulted in the detection of salicin, from which salicylate as the active fraction could be prepared. Further rich natural sources of salicylates were found, including the American Wintergreen *G. procumbens* and spireic acid (Acidum salicylicum) from the American teaberry (*S. ulmaria*).

The German Kolbe was the first who, in 1859, succeeded to make fully synthetic salicylate from sodium phenolate and carbon dioxide, a procedure later improved by Schmitt. Some further improvements by von Heyden eventually resulted in the foundation of the "Salizylsäurefabrik Dr. von Heyden" in 1874 and large-scale industrial production of salicylic acid. This now allowed for broad practical use of the new compound which – among other applications – also became the first entirely synthetic drug worldwide and was first introduced in the clinics as an analgesic antirheumatic by Franz Stricker in Berlin 1876.

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1.1.2 Synthesis of acetylated salicylic acid (Aspirin) and first medical use

1.1.2.1 The invention of acetylated salicylic acid

Despite the undisputed benefits of sodium salicylate in the treatment of pain, fever and inflammatory disorders, there were several problems with the practical handling of the compound. These included an unpleasant sweetish taste and, in particular, irritations of the stomach, often associated with nausea and vomiting. Another disturbing side effect was a hearing disorder called tinnitus. These side effects occurred quite frequently at the high doses of several grams of salicylate per day which had to be taken regularly at the time by patients suffering from chronic (rheumatic) pain. Thus, after an effective technology to generate large amounts of entirely synthetic and cheap salicylate became available, efforts were now made to improve the pharmacological properties of the compound by appropriate chemical modifications, eventually resulting in increased efficacy as well as improved gastric tolerability. Acetylation was a favored chemical method at the time to reach this goal. Several researchers addressed this issue by acetylating salicylic acid with different results [1–3] until chemists of the firm of "Farbenfabriken Bayer" in Elberfeld, today part of Wuppertal (Germany), succeeded in synthesizing acetylated salicylic acid in a chemically pure and stable form.

The history of Bayer Aspirin. Three persons at Bayer were intimately involved in this development. All three were chemists, and all of them were of the same age group – born in the 1860s, when the knowledge in organic chemistry just started to explode. The first to be named was *Carl Duisberg* (Fig. 1.1.2-1). After completing his dissertation in Jena on acetoacetate esters (!) he decided to pursue a career in the chemical industry. In 1883 he joined Bayer Company, and he became head of research only 5 years

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Arthur Eichengrün (1867–1949) | Felix Hoffmann (1868–1946) | Carl Duisberg (1861–1935)

Figure 1.1.2-1: Arthur Eichengrün (1867–1949), Felix Hoffmann (1868–1946) and Carl Duisberg (1861–1935) – with kind permission of Bayer AG.

later [4]. *Arthur Eichengrün* (Fig. 1.1.2-1) joined Bayer Company in 1895 and became head of the Pharmaceutical Research Department that was newly founded by Duisberg [5]. According to a report, written by Eichengrün 50 years later [6], it was his idea to acetylate salicylate in order to made it more palatable, that is, to avoid the unpleasant irritation of the stomach and, possibly, to obtain a stronger action. As mentioned above, the concept of acetylation of drugs to improve their efficacy was not new at the time. It had already been successfully used to make phenacetin (acetophenetidin), a powerful analgesic. Phenacetin was synthesized via acetaminophen (paracetamol or dipyrome) from *p*-nitrophenol, a waste product of Bayer's dye fabrication – according to a suggestion of Duisberg [4]. This positive experience probably stimulated the company to extend the acetylation procedures to other chemicals and drugs. These included guaiacol, cinchonine, morphin – and salicylic acid. *Felix Hoffmann* (Fig. 1.1.2-1) was the chemist working on this issue "*on my* [*Eichengrün's*] *advice*" [6]. He was the first person to develop a technology to produce chemically pure and stable acetylsalicylic acid from salicylic acid and acetic anhydride. According to a handwritten note in his laboratory diary, this success was achieved on August 10, 1897 (Fig. 1.1.2-2). Later he wrote:

> . . . When salicylic acid (100.0 parts) is heated with acetic anhydride (150.0 parts) for 3 hours under reflux, the salicylic acid is quantitatively acetylated . . . By its physical properties, e. g. its sour taste without being corrosive, the acetylsalicylic acid differs favorably from salicylic acid, and is now being tested in this respect for its usefulness . . . .

Another person at Bayer has also to be mentioned in this context: *Heinrich Dreser*, the then head of the Department of Pharmacology. Dreser was not interested in this kind of research, initially did not believe in any clinically useful properties of the new com-

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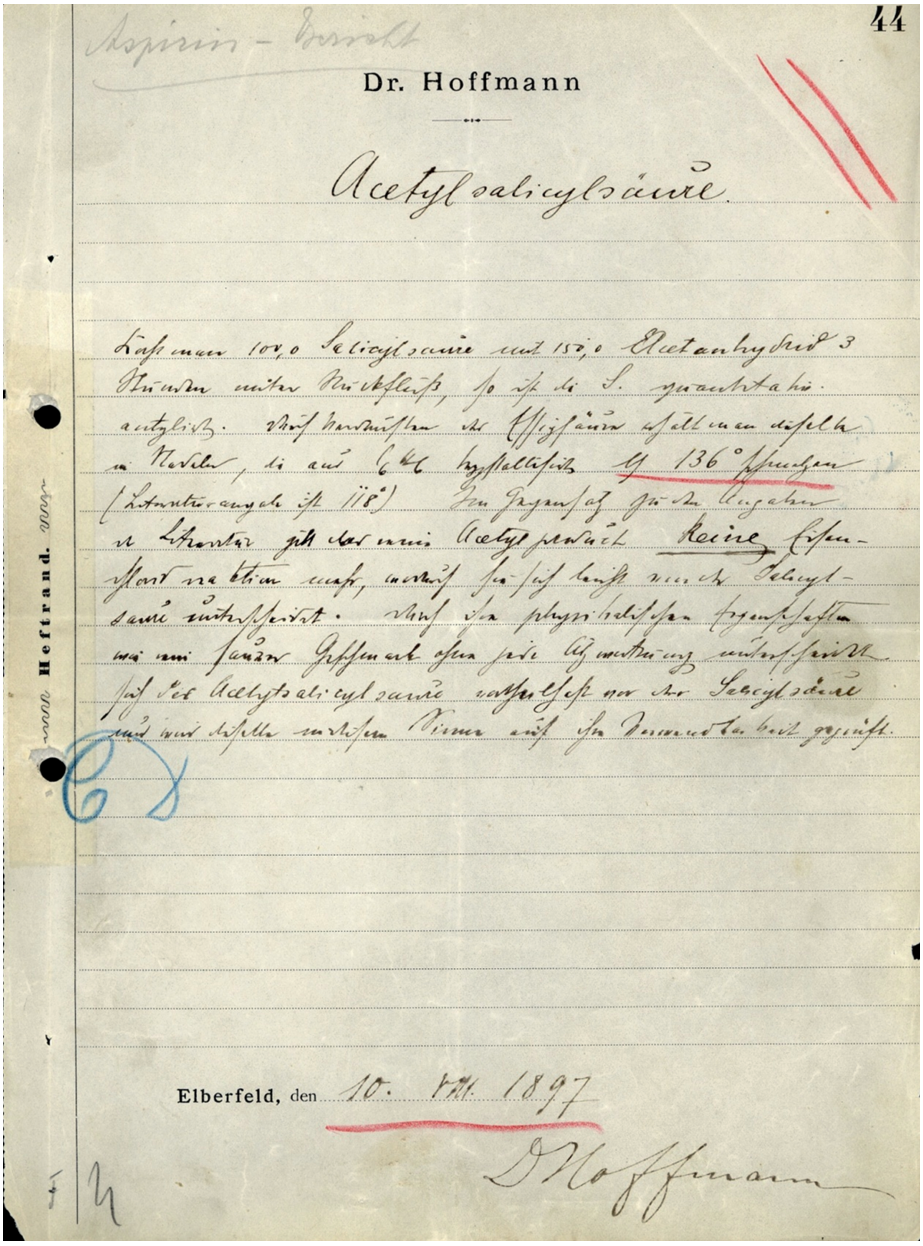

Figure 1.1.2-2: Laboratory record of Dr. Felix Hoffmann from August 10, 1897 containing the first description of successful synthesis of acetylsalicylic acid – with kind permission of Bayer AG.

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pound ("*the compound is of no value*") and, consequently, was also not involved in any research activities. However, in his later description of the pharmacology of aspirin, he acknowledged the better taste and less gastric irritation [7]. Initially, he was also not informed by the pharmacists about its successful clinical testing, although according to his contract with the company, the pharmacists should have reported this finding to him before undertaking further actions [1]. Thus, he was probably not amused to learn that without his knowledge and against his declared intention, the new compound was – even successfully – tested in patients. According to Eichengrün and other sources, he did everything to block the further development of aspirin while Duisberg emphatically supported the activities of Eichengrün and Hoffmann and, as expected, finally succeeded. The further development and clinical introduction of acetylated salicylate as an antipyretic analgesic, eventually resulting in the worldwide spread of the compound, is his merit.

The new drug received the trade name "aspirin" which is composed from "*a*cetic" and "*spir*eic acid", a former name of o-hydroxybenzoic acid (salicylic acid), originally prepared from Spirea ulmaria, one of the richest natural sources of salicylates.

The first description of the pharmacology of aspirin was published in 1899 by Dreser (Dreser 1899). The names of Hoffmann and Eichengrün were not mentioned in this paper. Dreser considered aspirin as a better tolerable prodrug of the active metabolite salicylic acid with the positive pharmacodynamic property not to be cardiotoxic [7]. According to Eichengrün [6], Dreser had nothing to do with the invention. However, it was Dreser who took the financial benefits from it, not Eichengrün or Hoffmann. According to a contract with Bayer, the products invented under the directorate of Eichengrün had to be patented in Germany to get a royalty for the inventor from the company [6]. Acetylsalicylic acid was registered on February 1, 1899 under the trade name "Aspirin®" by the Imperial Patent Bureau ("Kaiserliches Patentamt") in Berlin, and shortly later it was introduced as the first drug in tablet form (1 tablet = 5 gran = 325 mg). This was also the first time that a drug was dispensed as a product made by chemists according to quality standards of their company and not dispensed as a product manufactured (as a powder) by a pharmacist. This caused long-lasting and intense discussions about the role of pharmacists as the primary controller of drug production [8].

"Aspirin" did not receive recognition as a drug to be patented in Germany or any other European country, except the United Kingdom, where Bayer held a patent until 1905. This patent was declared futile by a British Court in 1905 after a legal action of von Heyden company, the provider of salicylic acid for Bayer. Von Heyden also produced and sold acetylated salicylic acid [9], but under its chemical name "acetylsalicylic acid" [8].

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Bayer marketed the new compound under the Bayer-owned trade name “Aspirin.” In the labeling, the product was identified as “monoacetic acid ester of salicylic acid” and advertised as a better tasting replacement for salicylic acid. The aspirin packages did not indicate that aspirin was pure acetylsalicylic acid. Bayer took every effort to keep this trade name as sole property of Bayer. The (numerous) copycats had to use other labels; mostly they preferred the chemical term “acetylsalicylic acid,” while Bayer advertised aspirin as “best replacement for salicylic acid.” Doctors (probably) never learned from Bayer’s advertising that aspirin was solely a trade name and found it easier to prescribe “Aspirin(um)” [sic!] than “acetylsalicylic acid” [8].

Aspirin was patented in 1900 exclusively in the United States (Fig. 1.1.2-3).

UNITED STATES PATENT OFFICE.

FELIX HOFFMANN OF ELBERFELD GERMANY ASSIGNOR TO THE FARBENFABRIKEN OF ELBERFELD COMPANY OF NEW YORK.

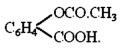
ACETYL SALICYLIC ACID.

SPECIFICATION forming part of Letters Patent No. 644,077, dated February 27, 1900.
Application filed August 1, 1898 Serial No. 087,385 (Specimens.)

To all whom it may concern:

Be it known that I, FELIX HOFFMANN, doctor of philosophy, chemist, (assignor to the FARBENFABRIKEN OF ELBERFELD COMPANY, of New York) residing at Elberfeld, Germany, have invented a new and useful Improvement in the Manufacture or Production of Acetyl Salicylic Acid; and I hereby declare the following to be a clear and exact description of my invention.

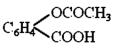
In the *Annalen der Chemie und Pharmacie*, Vol. 150, pages 11 and 12, Kraut has described that he obtained by the action of acetyl chlorid on salicylic acid a body which he thought to be acetyl salicylic acid. I have now found that on heating salicylic acid with acetic anhydride a body is obtained the properties of which are perfectly different from those of the body described by Kraut. According to my researches the body obtained by means of my new process is undoubtedly the real acetyl salicylic acid



Therefore the compound described by Kraut cannot be the real acetyl salicylic acid but is another compound. In the following I point out specifically the principal differences between my new compound and the body described by Kraut.

If the Kraut product is boiled even for a long while with water, (according to Kraut's statement,) acetic acid is not produced, while my new body when boiled with water is readily split up, acetic and salicylic acid being produced. The watery solution of the Kraut body shows the same behavior on the addition of a small quantity of ferric chlorid as a watery solution of salicylic acid when mixed with a small quantity of ferric chlorid—that is to say, it assumes a violet color. On the contrary, a watery solution of my new body when mixed with ferric chlorid does not assume a violet color. If a melted test portion of the Kraut body is allowed to cool it begins to solidify (according to Kraut's statement) at from 118° to 118.5° centigrade while a melted test portion of my product solidifies at about 70° centigrade. The melting-points of the two compounds cannot be compared because Kraut does not give the melting-point of his compound. It follows from those details that the two compounds are absolutely different.

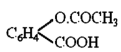
In producing my new compound I can proceed as follows, (without limiting myself to the particulars given:) A mixture prepared from fifty parts of salicylic acid and seventy-five parts of acetic anhydride is heated for about two hours at about 150° centigrade in a vessel provided with a reflex condenser. Thus a clear liquid is obtained, from which on cooling a crystalline mass is separated, which is the acetyl salicylic acid. It is freed from the acetic anhydride by pressing and then recrystallized from dry chloroform. The acid is thus obtained in the shape of glittering white needles melting at about 135°centigrade, which are easily soluble in benzene, alcohol, glacial acetic acid, and chloroform, but difficultly soluble in cold water. It has the formula



and exhibits therapeutical properties.

Having now described my invention and in what manner the same is to be performed, what I claim as new, and desire to secure by Letters Patent, is—

As a new article of manufacture the acetyl salicylic acid having the formula:



being when crystallized from dry chloroform in the shape of white glittering needles, easily soluble in benzene, alcohol and glacial acetic acid, difficultly soluble in cold water being split by hot water into acetic acid and salicylic acid, melting at about 135° centigrade substantially as herein before described.

In testimony whereof I have signed my name in the presence of two subscribing witnesses.

FELIX HOFFMANN.

Witnesses:
R. E. JAHN,
OTTO KONIG.

Figure 1.1.2-3: The US acetylsalicylic acid patent from February 27, 1900 – with kind permission from Bayer AG.

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As a consequence of World War I, in 1917, all patents and trade names of German firms were held enemy property in the United States and, thus, were confiscated [10]. German companies were also no longer allowed to sell their products in the United States [8]. The Bayer assets were auctioned by the US Alien Property Custodian and sold the same year for 5.3 million USD to Sterling Drugs, Inc. of New York [8, 9, 11]. This company then produced "genuine Bayer Aspirin" for the US market [12]: It was only in 1994 that the German Bayer AG could buy back the rights of the trademark and the Bayer Cross in the United States (for details see [8]).

Hoffmann or Eichengrün as the inventor of aspirin? According to a publication by Sneader and some followers, not Hoffmann but rather Eichengrün should be considered as the true inventor of aspirin [13]. As Eichengrün was Jewish, he could not enjoy the fruits of his remarkable scientific research, including also the invention of several other products in addition to aspirin, such as acetate silk, because of political reasons during the Nazi regime. Eichengrün was interned in 1944 in a concentration camp and remained there until the end of World War II. Eichengrün in the year of his death (1949) stated in an article, published in the German scientific journal *Die Pharmazie*, that it was him and Felix Hoffmann who should be considered as the inventors of aspirin [6].

According to Eichengrün [6], it was Hoffmann who had worked out the acetylation technology (... "*welcher* [*Hoffmann*] *die Acetylierung ausgearbeitet hatte*" ...), eventually resulting in the first synthesis of pure and chemically stable acetylated salicylic acid [14], although, again according to Eichengrün, he did so following "*my chemical advices*" ("*er* [*führte*] *meine chemischen Anordnungen aus*") [6]. However, these "advices" were not specified by Eichengrün. Obviously, it was Hoffmann who did the experiments and also worked out the study protocol himself. The sole, unopposed mention of Hoffmann's name on the US patent application form of 1900 (Fig. 1.1.2-3) clearly would not have been possible without the knowledge or even against the will of his two supervisor chemists, Eichengrün and Duisberg. This clearly suggests that both agreed to consider Hoffmann's activities in this research as very fundamental, justifying his name as the sole inventor of aspirin. Because of the complexity of the issue, as discussed above, one should, however, also pay tribute to the significant contributions of Eichengrün and Duisberg in the research and development of aspirin. This will not reduce the outstanding contribution of Hoffmann in this discovery.

Further attempts to make acetylsalicylic acid. At this point it should be noted that Hoffmann was not the first person who tried to chemically synthesize acetylated salicylic acid. In 1853, *Charles Frédéric Gerhardt*, a Frenchman from Strasbourg (Alsace), described the synthesis of a new compound from acetyl chloride and sodium salicylate which he named "salicylate acétique" [15].

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This publication of Gerhardt was taken by several authors as evidence to ascribe the invention of acetylsalicylic acid to him (e. g., [3, 9, 16]). This is not correct for several reasons. The "acetylsalicylic acid" of Gerhardt, if it was formed at all, solely might have existed as a labile, intermediate raw product of the reaction between acetyl chloride (prepared by him by a suboptimal procedure) and sodium salicylate [1]. The chemical structure of „salicylate acétique" was not determined. The physicochemical properties were not those of acetylsalicylic acid but rather those of salicylic acid [17, 18]. The technical procedure was suboptimal and resulted in simultaneous formation of large amounts of acetic acid anhydride together with acetosalicylic acid anhydride because of an inappropriate processing of the raw product. As a stable end product Gerhardt only obtained salicylic acid. From his experiments, he concluded that acetylated salicylic acid is unstable and in water immediately breaks down to salicylic acid and acetate [15]. Both statements are wrong and do not qualify Gerhardt for the claim to have invented the synthesis of acetylsalicylic acid [18].

In 1859, *Hugo von Gilm*, a pharmacist from Innsbruck (Austria), reported on the synthesis of acetylsalicylic acid [19], as did *Karl Kraut* and his group from Hannover (Prussia) 10 years later [17]. Kraut and his coworkers *Schröder* and *Prinzhorn* were also the first to assign the correct structure with the acetyl moiety connected to the phenolic oxygen to the compound. However, these preparations still were impure and contained significant amounts of salicylic acid, as seen from the positive red "Gerhardt reaction" of salicylate with ferric chloride. In addition, it exhibited physicochemical properties different from acetylsalicylic acid (see also comments of Hoffmann in his patent application (Fig. 1.1.2-3). Nevertheless, it was the publication of Kraut which was the reason for the decline of patent protection of Hoffmann synthesis by the German Patent Authorities [20].

Acetylsalicylic acid – Organic Chemistry vs. Pharmacology. In contrast to the natural product salicylic acid, *acetyl*salicylic acid could be made only by synthetic organic chemistry – although Karl Kraut started his experiments for its synthesis with gaultheria oil as a natural salicylate (salicin) source. During the following 30 years, there were no further attempts to improve the synthesis procedure, although significant progress was made in organic and pharmaceutical chemistry at the time. According to an organic chemist, acetylsalicylic acid was of no particular interest, but solely made to confirm the feasibility of its synthesis. There also were no ideas or concepts about any possible practical application, including its use as a medicine. Thus, acetylated salicylic acid probably would have suffered the fate of several hundreds of chemicals before and many thousands thereafter – a product of chemical synthesis, principally easy to make but more difficult in pure and chemically stable form and without any practical value.

Hoffmann and Eichengrün, in contrast, combined the available medical knowledge about curative properties of a product from nature with the contemporary organic chemistry with the clear intention to make a new and better therapeutic out of it. These studies would not have been possible without the substantial and continuous support

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by *Carl Duisberg*, then the head of Bayer research. Duisberg later became Chief Executive and Director General at Bayer. His numerous and outstanding efforts inside and outside Bayer Company gained considerable and consistent recognition in Germany and abroad [4]. In an obituary in 1935, the London "Times" noted: "*his country loses a man who, all things considered, … may be regarded as the greatest industrialist the world has yet had …*" Therefore, the company had good reason to duly celebrate the 100th anniversary of "his" compound, which in the meantime has become the most popular drug in the world [13].

In the context of priorities in science, an interesting comparison between the discovery of aspirin and the discovery of prostacyclin can be made – both also tightly connected with the name of John Vane. Its chemical structure as well as a suggested (later confirmed) enzymatic synthetic pathway was originally described in 1971 by Pace-Asciak & Wolfe. These authors considered the (labile) product as just another prostaglandin – in addition to the dozen of already known compounds. The authors assumed that prostacyclin was possibly overlooked by earlier investigators because of its low biological activity, tested at the time in bioassay experiments using the rat stomach strip. It also remained uncertain whether the compound was synthesized at all in the intact stomach wall and, if so, if it was released in biologically active amounts [21].

A completely different approach was followed by the group of John Vane. Their work on prostacyclin started with the discovery of a biological effect – inhibition of platelet aggregation – by an enzymatic product made from prostaglandin endoperoxides by artery walls [22]. This prostaglandin, originally named as PGX, differed in its biological properties from all other known prostaglandins. PGX was later identified as the already known enzymatic product of prostaglandin endoperoxides, described by Pace-Asciak & Wolfe and was renamed prostacyclin (PGI_2).

Despite the originality and merits of Pace-Asciak & Wolfe regarding the detection and original description of biosynthetic pathways of natural prostacyclin and its suggested chemical structure, the medical history of prostacyclin starts with the work of Vane's group who were the first to discover the biological significance of prostacyclin, here in control of hemostasis and thrombosis.

1.1.2.2 Introduction of acetylsalicylic acid in the clinic

The first clinical trials. *Kurt Witthauer* [23], the then senior physician in the (still existent!) city hospital (Diakonie-Krankenhaus) in Halle/Saale (Germany), and *Julius Wohlgemuth* [24] from Berlin published the first clinical investigations on aspirin in 1899. In his publication, Witthauer first outlined the pharmacological advantages of aspirin over salicylate, that is, its chemical stability in the acidic stomach juice, while release of active salicylate only occurs in the alkaline intestinal fluid. Because of this, he would expect a better gastric tolerance of the new compound. Then, he reported on treatment results in about 50 patients suffering from a variety of inflammatory, mostly rheumatic, diseases. They received 4–5 g of aspirin daily, obviously without any complaints. Witthauer started his account as follows:

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> ... Nowadays, certain courage is necessary to recommend a new drug. Almost every day those are thrown on the market and one has to have an excellent memory to keep all the new names and brands in mind. Many drugs appear, are praised and recommended by the companies and certain authors but after a short time have disappeared without any further comment ....

The author also did not forget to instruct his readership that he did this study with "*no little distrust*." Regarding the results, he comments:

> ... the treatment result was at least as good as that of natron [salicylate], sometimes [aspirin] was even effective when natron [salicylate] failed ...

and added that:

> ... the patients were unsatisfied, if it became necessary to interrupt the aspirin treatment because of an insufficient supply ...

Witthauer concluded:

> ... According to my positive results, the company is now prepared – after waiting for quite a time – to introduce the new compound on the market. I sincerely hope that the difficult technology to make it will not cause a too high price, to allow the broad general use of this – as far as I believe – valuable new drug [23].

Aspirin as a household remedy against fever, inflammation and pain. Soon after the introduction of acetylsalicylic acid into medical use under the brand name "aspirin," the new drug became a most popular remedy against fever, inflammation and pain. A local German newspaper in the Leverkusen area ("Kölner Stadtanzeiger") published the following advice for treatment of flu on March 6, 1924:

> ... As soon as you feel yourself ill, you should go to bed and have a hot-water bottle at your feet. You should drink hot chamomile tea or grog in order to sweat and should take 3 tablets of aspirin a day. If you follow these instructions you will recover within a few days, in most cases ....

This citation is remarkable for several reasons: During the past 25 years of practical use, aspirin had become a drug whose name was not only well known to health professionals but also to the general public – and accepted without reservation by both. Certainly, the limited availability of antipyretic and antiinflammatory analgesics other than aspirin will have significantly contributed to this. The compound was recommended – and accepted – both by the lay press and doctors – as a general "household remedy" for treatment of pain, fever, inflammation and other kinds of "feeling bad," although very little if anything at all was known about the pharmacological mechanism of action behind these multiple activities (Section 1.1.3). Thus, in public opinion, a reliable medical effect for the user was considered much more important than

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an occasional dyspepsia or some bleeding tendency. It took more than half a century of intense practical use before the first reports on clinically relevant (postoperative) bleeding were published (Section 1.1.4). However, at the time, enhanced (gastro)intestinal blood loss and minor bleeding events were already known as typical side effects of salicylates. At daily doses of 1–3 grams, about half of the aspirin-treated patients were reported to have an estimated daily loss of 2–6 ml occult blood with the feces. Over 1 month, this amount was comparable to the blood loss during menstruation (50–100 ml) [25]. In addition, bloodletting was a frequently used therapeutic measure. Thus, bleeding was not considered a serious clinical problem by the vast majority of patients, particularly those taking aspirin only once or twice daily or occasionally a few days to treat headache, flu or other feverish discomfort. It took about half a century before the first reports on clinically relevant bleeding under the influence of aspirin were published [26].

Summary

The unlimited availability of synthetic salicylic acid as a result of marked progress in organic chemistry of the late nineteenth century and the positive results with the compound in daily practice, including its medical use as an antiinflammatory analgesic, eventually stimulated the interest in chemical modifications. For medical use, the major aim was to improve the taste and (gastric) tolerance.

The first successful synthesis of a pure and stable acetylated salicylic acid was performed by Felix Hoffmann in the group of Arthur Eichengrün at Bayer in Elberfeld (Germany) in 1897. This research and further drug development was substantially supported by Carl Duisberg, the then head of research at Bayer, and resulted in a commercial preparation of acetylsalicylic acid (Aspirin®) that was essentially free of unreacted salicylate and the first industrial drug sold in tablet form worldwide.

After rather enthusiastic first reports about the clinical efficacy and tolerability – as opposed to salicylic acid – "Aspirin" was launched in 1899 and rapidly became a well-known and well-accepted household remedy for treatment of fever, pain, inflammation, flu-like symptoms and other manifestations of "feeling bad" ("take an aspirin!"). Side effects, except occasional gastric dyspepsia (nausea, vomiting), were rare at the time in the conventional short-term use, despite the rather high doses of several grams taken. Even the enhanced, about doubled, occult blood loss with the feces appeared not to be a serious problem except the very rare but severe bleeding events.

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1.1.3 Search for pharmacological modes of aspirin action

"The successful use of a drug in medicine is not precluded by a lack of knowledge about its mode of action … salicylates could be used as one of the better illustrations of [this] dictum …" wrote M. J. H. Smith in his monography "The Salicylates" published in 1966 [1]. This statement is further underlined by the fact that most drugs act at more than one site in a living organism. Thus, the molecular targets for salicylates

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might be similar or even the same at the cellular level but the consequences of an interaction with them may be quite different at the tissue and organ levels. In addition, aspirin is a unique compound, bearing two biologically active structures in one and the same molecule: the reactive acetyl group of the nonmetabolized compound and salicylic acid, the primary metabolite. As outlined in detail in Section 2, both components differ with respect to their molecular targets as well as their pharmacodynamic and pharmacokinetic behavior.

The present chapter is focused on the three most relevant aspects regarding the mode of action of aspirin: (i) its effects on cellular energy metabolism as the historically first and undisputable pharmacological mode of direct actions of salicylates; (ii) inhibition of COXs as the probably best studied pharmacodynamic action of aspirin and salicylate at the transcriptional, translational and posttranslational levels (this also includes the generation of new antiinflammatory and inflammation resolving products, such as "aspirin-triggered lipoxin" [ATL]); and (iii) the numerous actions of aspirin (mainly acetylations) on other macromolecules and transcription factors.

1.1.3.1 Salicylates and energy metabolism of the cell

Uncoupling of oxidative phosphorylation. The first reports on basic pharmacology of aspirin and salicylates were published in the 1950s. One key finding was the detection of an interaction of salicylates with the energy metabolism of cells, in particular an increase in oxygen uptake subsequent to uncoupling of oxidative phosphorylation [2], associated with marked depletion of cellular ATP levels and subsequent inhibition of β-oxidation of fatty acids was found (Section 2.2.3). This effect required analgesic/antiinflammatory concentrations of 2–4 mM of salicylates. These concentrations could be obtained by the intake of about 2–4 g aspirin per day, which was the conventional antipyretic/antiinflammatory dose at the time. The uncoupling of energy-producing from energy-consuming processes clinically resulted in sweating (removal of excess heat) and hyperventilation, followed by metabolic acidosis in salicylate poisoning (Section 3.1.1). All of these actions were caused primarily by salicylate and, for the most part, could be sufficiently explained by the unique physicochemical properties of the compound (Section 2.2.3) [3–5].

ATP depletion and kinase inhibition. Exhaustion of ATP levels, that is, depletion of cellular energy stores, is a very fundamental event with considerable consequences for all energy-dependent functions of the cell. These include maintenance of the structure of the cytoskeleton, cell proliferation and, in the end, cell survival. ATP depletion will inhibit or even completely prevent every kinase activation, that is, the phosphorylation of target substrates, for example, enzymes and transcription factors, intimately involved in cell signaling and protein synthesis (Section 2.2.3) [6].

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For these reasons, the explanation of the multiple pharmacological actions of aspirin by one ubiquitous mechanism – kinase inhibition subsequent to inhibition of oxidative phosphorylation – was an attractive and convincing concept at the time. It also was in agreement with the general idea that all actions of aspirin are salicylate-mediated. Today these metabolic actions of salicylates are considered to be primarily of toxicological interest because of the high concentrations required and the possibility to dissociate those from acetylation reactions, for example in blood platelets. These and some other pharmacological effects of aspirin on pain and inflammation were seen at substantially lower concentrations and were not associated with ATP depletion. In addition, the "energy depletion" hypothesis" did not answer the question why aspirin – and other salicylates – preferably acted on inflamed or otherwise injured tissue but had no clear-cut effects on healthy tissues. Also, inhibition of oxidative phosphorylation by another well-known uncoupling agent, 2,4-dinitrophenol (DNP), had no antiinflammatory effect [7]. Thus, there should be other, more specific molecular targets to understand the pharmacological effects of aspirin in inflammation and pain.

1.1.3.2 Inhibition of cyclooxygenases by salicylates

Algesic actions of arachidonic acid and bradykinin are inhibited by aspirin. A new and finally quite successful search for a more specific mode of action of aspirin began with studies about the pathomechanism, mediators and symptoms of inflammation. Here inflammatory pain was one of the most disturbing symptoms which could be treated with aspirin. In 1959, it was shown by R. *Jaques* from the CIBA company in Basel (Switzerland) that pricking of diluted emulsions of low-dose arachidonic acid into the volar face of the human fore-arm caused pain. This pain started after a latency period of 15–20 s, lasted for several minutes and was followed by a long-lasting (15–30 min) erythema without itching. In vitro experiments using smooth muscle contractions as a pain surrogate parameter additionally showed that arachidonic acid at low concentration (0.1 µg/ml) contracted the guinea pig ileum smooth muscle in vitro after a latency period of 10–15 s and reached a peak after 45–90 s. These contractions could be blocked by pretreatment with several agents, including analgesics such as aspirin (25 µg/ml), while salicylic acid, atropine (anticholinergic) and mepyramine (antihistaminergic) were inactive. This suggested that these effects were not mediated by acetylcholine or histamine but, probably, by a hitherto unknown lipid mediator, generated from arachidonic acid within less than a minute.

From these and other data Jaques concluded that:

> Arachidonic acid . . . which is a constituent of body lipids or a substance with similar pharmacological characteristics. . . present in a preactive form might be set free by some enzyme system . . . and among other things cause pain [8].

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In 1969, another remarkable finding was published by *Priscilla Piper* and *John R. Vane* [9]. These authors showed that stimulation of isolated guinea pig lung by bradykinin but not histamine caused release of "rabbit aorta contracting substance" (RCS). Release of RCS – later identified as a mixture of prostaglandin endoperoxides and thromboxane A_2 – was blocked by aspirin. A similar observation had been reported previously by *Collier* and *Shorley*, who found that aspirin and – to a lower extent – salicylate antagonized the bronchoconstrictor response to bradykinin but not responses to other spasmogens in guinea pigs in vivo [10]. Others showed that intraarterial injection of bradykinin in man caused transient pain for about 40 s after an initial latency period of ca. 20 s. This effect could again be blocked by aspirin but not by placebo [11]. Taken together, these findings suggested that aspirin prevented the generation rather than the action of hitherto unknown mediator(s) of pain and smooth muscle contraction subsequent to stimulation by bradykinin. However, the mode of algesic action of arachidonate and bradykinin as well as of the analgesic action of aspirin remained unknown.

Inhibition of prostaglandin synthesis by aspirin. In 1971, the journal "*Nature*" published three papers of the group of *John R. Vane* (Fig. 1.1.3-1), the then Professor of Pharmacology at the Royal College of Surgeons of England. In his article, Vane demonstrated for the first time a new mode of action of aspirin that was able to explain its antiinflammatory and antipyretic actions by *one* single pharmacological mechanism:

Figure 1.1.3-1: Sir John R. Vane – with kind permission from Science Photo Library/US National Library of Medicine.

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inhibition of (enhanced) biosynthesis of prostaglandins, a group of proinflammatory lipid mediators after tissue injury [12].

In his pioneering paper on aspirin and prostaglandins, the later Sir John Vane showed by as simple as elegant bioassay experiments that aspirin like indomethacin – and the somewhat less potent salicylate – inhibited prostaglandin formation in cell-free tissue homogenates of the guinea pig lung after addition of the natural precursor arachidonic acid – imitating endogenous arachidonic acid release after tissue injury (Fig. 1.1.3-2). Vane suggested that the inhibition could be brought about by competition of these (acidic) drugs with arachidonic acid for binding in the region of the active site of the prostaglandin-generating enzyme(s). He postulated that this mechanism accounts for the antipyretic and antiinflammatory actions of salicylates but also their gastrointestinal and ulcer-promoting actions by inhibition of gastroprotective protective prostaglandin synthesis in the stomach mucosa. He did, however, not postulate that this was the only explanation for all effects of antiinflammatory drugs of the salicylate or indomethacin type. Specifically, he saw no link between a peripheral analgesic action of these compounds and inhibition of prostaglandin synthesis. These findings of John Vane, including "discoveries concerning prostaglandins and related biologically active substances," specifically prostacyclin, were acknowledged with the Nobel Prize for Medicine in 1982.

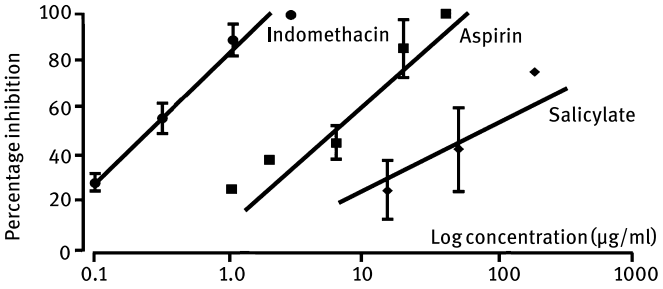

Figure 1.1.3-2: First description of inhibition of prostaglandin biosynthesis by aspirin and salicylate and the reference compound indomethacin. Note the dose dependency of the reaction with about 50 % inhibition of prostaglandin production by aspirin at <10 µg/ml (<60 µM) (mod. after Ref. [12]).

A separate paper, published by *J. Bryan Smith* and *Al Willis* from the same institution on the following pages of the same issue of Nature, showed that a similar mechanism was also likely to work in human platelets. In this study, aspirin treatment of platelets in vitro or ex vivo largely abolished thrombin-induced prostaglandin formation. However, it did not affect the thrombin-induced "release reaction" as seen from an unchanged thrombin-induced secretion of platelet-stored serotonin [13]. This suggested that prostaglandin formation and platelet function (secretion) could be separated from each other – a very important observation that, unfortunately, was not paid the necessary attention in later years.

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Prostaglandins and other eicosanoids – a short summary. Prostaglandins, thromboxanes, leukotrienes and lipoxins are members of a group of natural lipid mediators that are all peroxidation products of arachidonic acid (5,8,11,14-all-*cis*-eicosatetraenoic acid). Consequently, they all bear a 20-carbon backbone and are summarized as "eicosanoids" (Greek: *eikos* = twenty). Today more than 150 eicosanoids are known and have been structurally identified. Arachidonic acid, the precursor fatty acid, is an essential constituent of the cell membrane phospholipids and is released from there by phospholipases. Eicosanoid synthesis starts with the availability of free arachidonic acid in close proximity to the metabolizing enzymes without requiring entry into the cytosol or the extracellular space.

The first oxidation step of arachidonic acid to generate prostaglandins is catalyzed by cyclooxygenases (COXs) and results in the formation of the prostaglandin endoperoxide PGG_2. PGG_2 is than reduced to the endoperoxide PGH_2 by a peroxidase. COX and peroxidase together form the prostaglandin H synthase (PGHS) complex (Fig. 2.2.1-2). There are two isoforms of the COX- enzyme – the constitutively expressed isoform COX-1, which is present in about every cell and tissue of the organism, and the inducible isoform COX-2, an "immediate early gene" that becomes rapidly upregulated in response to all kinds of stimuli, most notably humoral, immunological and inflammatory factors, and then accounts for the majority of prostaglandin formation. The prostaglandin endoperoxides are then converted to the terminal products of this pathway, that is, prostaglandins (PGs) and thromboxane (TX) A_2, by different isomerases and synthases in a cell-specific manner. The local eicosanoid level is solely controlled by biosynthesis. The active products are not stored but released upon cell stimulation, act on their cellular target via specific receptors and are rapidly degraded afterwards to inactive metabolites by specific enzymes.

Prostaglandins exert their multiple actions via specific G-protein-coupled receptors, located at the cell membrane. The about ten currently known prostaglandin receptors determine direction and intensity of the biological response that is highly organ- and tissue-specific. Prostaglandins act as local autocrine or paracrine mediators that dispatch signals between cells [14]. Although prostaglandins can be formed in and act on probably all cells of the body, dependent on the existing receptor population(s), they are not essential for vital cell functions, such as energy metabolism or maintenance of the cell cytoskeleton, but rather act as modulators of cell function.

In a living organism, the cellular prostaglandin synthesis is usually at a low basal level. However, it can be markedly increased in response to disturbed homeostasis (injury) or humoral activation (sexual steroids, angiotensin and others) in order to adapt cell function to changes in the environmental conditions. This increased prostaglandin synthesis "on demand" reflects a cell-specific response. This frequently also includes amplification of the alarming signal "pain." Examples of prostaglandin-associated physiological situations are hemostasis and pregnancy, whereas the increased prostaglandin production in inflammation, immune reactions, atherosclerosis and tumorigenesis reflects the response to pathological stimuli.

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Thus, any change in generation of prostaglandins or the related thromboxanes *per se* is neither good nor bad but rather reflects a functioning cell-based adaptation or defense mechanisms. Consequently, in functional terms, pharmacological interaction (inhibition) of prostaglandin formation may be either positive or negative. Functional disorders may arise when prostaglandins become critical factors for control of cell and organ function. However, in most cases their removal is not associated with any measurable alterations at the organ level as long as other mediator systems can sufficiently compensate for it.

Aspirin and cyclooxygenases. Today, two genes are known that encode COXs: COX-1 and COX-2. Both COX isoforms are molecular targets of aspirin. In addition, a considerable number of splice variants of these genes has been detected. Some of them are also transcriptionally regulated and can generate gene products, such as "COX-3," a splice variant of COX-1 [16].

Aspirin blocks the biosynthesis of prostaglandins and thromboxane A_2 at the level of COX in the prostaglandin endoperoxide synthase (PGHS) complex by covalent binding (acetylation) to a critical serine near the active site of the enzyme (Section 2.2.1). This prevents the access of substrate (arachidonic acid) to the catalytic region ("active site") within the substrate channel of the enzyme [17] and explains the antiplatelet effect of the agent. This was first described by the group of *Philip Majerus* [18]. The group of *Garret FitzGerald* [19] confirmed this finding for the cloned enzyme from human platelets, while *William Smith* and coworkers described the molecular reaction kinetics of COXs with aspirin. Importantly, this acetylation process is enhanced at least 100-fold by binding heme to the apoprotein of COX. This probably explains why the PGH synthase complex (COX plus peroxidase) is the principal target of aspirin acetylation in intact cells [20].

The molecular interaction of aspirin with COX-1 could be further analyzed after the crystal structure of the enzyme became elucidated by *Michael Garavito* and coworkers [21, 22].

The crystal structure of the acetylated COX-2 has been elucidated by *Michael J. Lucido*, working in the group of *Michael G. Malkowski*. These authors showed that serine acetylation of COX-2 by aspirin prevents the access of substrate to the hydrophobic side pocket of the enzyme and also provided for the first time a model of the reaction kinetics of the 15-lipoxygenase [23]. New details about the acetylation kinetics of COX-2 by aspirin, including the generation of 15(R)- prostaglandins, have just been described [24].

The detection of inhibition of prostaglandin synthesis was the first plausible explanation for the multiple clinical actions of aspirin in inflammation, pain, fever and hemostasis via one ubiquitous class of endogenous mediators: prostaglandins and thromboxanes. With the increasing knowledge of the complex nature of these reactions, specifically after detection of further eicosanoids and the multiple interactions

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of prostaglandins with other mediator systems, more details of the functional consequences of these findings became evident and are now interpreted in a more complex manner.

COX-2 acetylation and lipoxin formation as an explanation for the antiinflammatory/analgesic action of aspirin. The main effect of aspirin-induced acetylation of COX-2 in addition to inhibition of prostaglandin synthesis is conversion of the enzyme activity to that of a 15-lipoxygenase, resulting in generation of 15(R)-hydroxyeicosatetraenoic acid (15-(R)-HETE). This fatty acid serves as a substrate for the subsequent generation of "Aspirin-triggered lipoxin" (ATL) or (15-epi-LTA_4) by interaction with the 5-lipoxygenase from white cells (Sections 2.2.1 and 2.3.2). Lipoxins and the related resolvins represent new classes of antiinflammatory and inflammation-resolving mediators that are formed by interaction of aspirin with COX-2 but not by any other NSAID or coxib.

ATL was first described by *Charles Serhan* and his group [25]. More recent work by *Derek Gilroy* and his group demonstrated that aspirin, even at low, antiplatelet doses of 75 mg/day, is able to stimulate the generation of antiinflammatory ATL and subsequently inhibits white cell accumulation in inflammatory skin exudates of men at only minor reductions of PGE_2 levels (Section 2.3.2) [26]. This finding was the first to show an antiinflammatory effect of low-dose aspirin via modulation rather than inhibition of COX-2 and enhanced lipoxin production.

1.1.3.3 Further actions of salicylates on cell signaling

Aspirin and salicylate are unique compounds. In contrast to most other drugs, they do not act via specific receptors or binding sites but rather by more or less specific acetylation of all kinds of target macromolecules (Section 1.1.5). Interestingly, acetylation of the $serine_{529}$ hydroxyl group by aspirin is enhanced at least 100-fold by binding heme to the apoprotein of COX. This is one explanation why the PGH synthase complex (COX plus peroxidase) is the principal target of aspirin acetylation in intact cells [20]. However, there are numerous other effects of both salicylates and aspirin, which are caused by secondary modifications of cell signaling molecules at both the transcriptional and translational levels [27].

Kenneth K. Wu (Fig. 1.1.3-3) and his group were the first to show that both aspirin and salicylate can interact independently with the binding of transcription factors to the promoter region of the COX-(2) gene.

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Figure 1.1.3-3: Kenneth K. Wu – with kind permission.

In tissue injury, transcription factors regulate gene expression levels after stimulation by inflammatory mediators in many cell types, including white cells and endothelial cells [28, 29], and are known targets of salicylates (Fig. 1.1.3-4). Later work of this group eventually identified salicylate-induced phosphorylation of CCAAT/enhancer-binding protein-β (C/EBP-β or NF/interleukin-6 [IL-6]) as one critical transcription factor [27, 30]. Thus, antiinflammatory actions of aspirin and salicylate also involve inhibition of transcription of the COX gene(s), followed by reduced generation of enzyme protein. This effect is not shared with other "aspirin-like" drugs and independent of the inhibition of enzyme activity by acetylation. Only naproxen, an NSAID with a very long half-life of 13 h, was also found to transcriptionally downregulate COX-2 (and COX-1) protein expression [31].

Interestingly, as also shown by the group of Kenneth Wu, control of gene transcription by aspirin and salicylate is not restricted to COX-(2) but also occurs with other immediate early genes that are regulated by the same transcription factors, for example the inducible NO synthase (iNOS), which generates large amounts of NO, another most important inflammatory mediator [30, 32, 33]. A number of signaling pathways that have been detected are discussed in Section 2.2.2.

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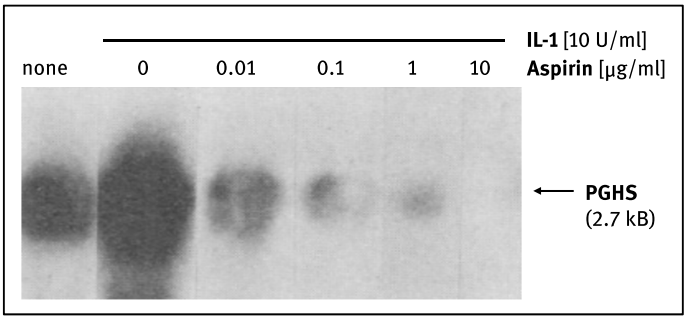
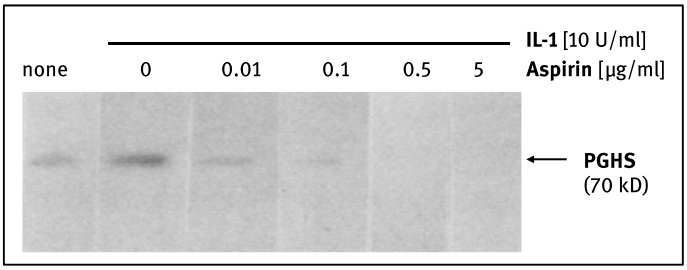


(a)



(b)

Figure 1.1.3-4: Inhibition by aspirin of PGH synthase (PGHS) mRNA (a) and protein (b) levels in quiescent and IL-1-treated human umbilical endothelial cells. Note the marked increase of PGHS mRNA and protein expression after stimulation by IL-1 in comparison to quiescent cells (none) and the concentration-dependent inhibition by aspirin, suggesting an action at the level of transcription. This was accompanied by a marked concentration-dependent reduction of product (PGI_2) formation (not shown). Similar inhibition was seen by salicylate but not by indomethacin (not shown).(modified after [28, 29]. (With kind permission of Kenneth K. Wu).

Summary

In the 1950s, uncoupling of oxidative phosphorylation was described as a first pharmacological mode of action of aspirin and salicylates. These metabolic effects were salicylate-mediated and required high local concentrations of the compounds, frequently in the low millimolar range. They explained the metabolic effects of high-dose aspirin (hyperventilation, sweating and metabolic acidosis) as well as most symptoms of salicylate intoxication. However, they lacked a direct relationship to the inflammatory processes of local tissue injury.

In 1971, Sir John Vane suggested for the first time inhibition of prostaglandin synthesis by aspirin as a uniform mode of the antipyretic and antiinflammatory actions of aspirin. This finding explained the clinical efficacy of aspirin in treatment of fever and inflammatory pain. In molecular terms this was later explained by specific and irreversible acetylation of a serine in the substrate channel of COX, upstream to the active site of the enzyme.

After the detection of two genetically defined COX isoforms, COX-1 and COX-2, it became apparent that the molecular mode of action of aspirin is the same on both enzymes – irreversible acetylation of a target serine – but the consequences were different. In COX-1 this resulted in (almost complete) inhibition of prostaglandin and thromboxane formation, which was most effective in platelets. These are unable to replace the acetylated COX-1 by fresh enzyme because of their very

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low protein-synthesizing capacity. In COX-2, an enzyme with a much broader substrate specificity and significant turnover rate, acetylation by aspirin caused only partial inhibition of prostaglandin production but also modulation of enzyme activity towards that of a 15-lipoxygenase. This eventually resulted in the generation of 15-(R)-HETE, a precursor of ATL and other antiinflammatory mediators. Additionally, both aspirin and salicylate inhibit the cytokine-induced protein expression of COX-2 and other immediate early genes, such as iNOS, at the transcriptional level. These actions can be detected at submillimolar concentrations of aspirin and salicylate in vitro and are not shared by NSAIDs.

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1.1.4 Clinical applications – a piece of history

1.1.4.1 Antiinflammatory/analgesic and antipyretic actions

Aspirin as an antiinflammatory drug. The disclosure of a causal relationship between inhibition of prostaglandin synthesis and the antipyretic and antiinflammatory actions of aspirin provided for the first time a plausible explanation for its therapeutic efficacy in the treatment of inflammatory and feverish diseases. It also stimulated mechanism-based research for new antiinflammatory compounds. These compounds were designed to block prostaglandin biosynthesis but should be more potent and

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better tolerable than high-dose aspirin. Indomethacin was the first of these so-called "aspirin-like" drugs [1] and was already used as a reference compound in the pioneering experiments of Sir John Vane (Fig. 1.1.3-2). Many others followed. In 2021, there were more than 20 different chemicals alone on the German market, which were developed as reversible-type inhibitors of prostaglandin biosynthesis and were approved for clinical use as antipyretic/antiinflammatory analgesics. In Germany, this included more than 40 (!) brands containing ibuprofen, 19 containing diclofenac and 4 containing indomethacin, most of them available in different galenic formulations and doses. On the other hand, there were 10 brands containing only acetylsalicylic acid as the active ingredient. Only one single agent has been developed but not yet introduced clinically: 2-(acetoxy-phenyl)hept-2-ynyl sulfide (APHS), which mimics the irreversible mode of COX serine acetylation of aspirin by covalent binding and exhibits significant COX-2 selectivity (Section 2.2.1) [2, 3]. Thus, the invention of aspirin has significantly stimulated basic research for new antiinflammatory analgesics – and still does. However, aspirin has lost its outstanding position as a medicine for treatment of chronic inflammatory diseases, such as rheumatoid arthritis – the indication it was originally introduced for into medicine – because of the availability of improved drug alternatives (Section 4.2.2).

Aspirin as an antipyretic analgesic. Through the first 50 years of its practical use, aspirin became a well-accepted and increasingly used antiinflammatory/antipyretic over-the-counter (OTC) analgesic worldwide. Today, the compound in its numerous commercial formulations still belongs to the most frequently used antipyretic analgesics for self-medication of headache, flu and other acute inflammatory/painful conditions. Actually, aspirin has to compete in these indications with ibuprofen and the nonantiinflammatory paracetamol (acetaminophen), coxibs, such as celecoxib, but still does well [4]. Regarding the clinical use, there are distinct advantages and disadvantages with either of these compounds in OTC use which are discussed in more detail in the clinical Section 4.2.2–4.3.

1.1.4.2 Antiplatelet/antithrombotic actions and bleeding

Aspirin and bleeding. As mentioned in Section 1.1.2, bleeding events were not considered a serious clinical problem for the vast majority of individuals who took aspirin shortly to treat headache, flu-like symptoms or other feverish discomfort. First reports about a bleeding tendency with aspirin as a possible clinical problem were only published in 1945, half a century after its introduction. *Rudolf Singer*, a US ETN physician, reported late bleeding events ≥3 days (!) after tonsillectomies. He attributed this to prescription of aspirin for analgesic purposes. No bleeding events of this type were seen when aspirin was withdrawn or replaced by the nonantiinflammatory analgesic metamizol (dipyrone) [5]. Prolonged bleeding time was also reported in aspirin-treated

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cardiac patients [6] with subsequent reports also on aspirin intake and bleeding in minor surgeries, such as tooth extractions.

A few years later, the first mechanistic concepts were developed by relating the bleeding tendency of aspirin to thrombin and the coagulation system [7, 8]. It required, however, another 10 years before *Armand J. Quick* and coworkers [9] suggested for the first time a causal relationship between aspirin and blood coagulation. In 1960 they hypothesized that the prolonged bleeding time after high-dose (6 g) aspirin to healthy individuals might be due to diminution of a stable procoagulatory factor in plasma (probably prothrombin) [9]. In 1966, Quick and others additionally demonstrated that a prolonged bleeding time ex vivo was specific for aspirin and was not seen with comparable doses of salicylate (Fig. 3.1.2-1) [10, 11].

Aspirin and platelet function. In 1967/1968, several groups showed independently of each other that within the clotting system, platelets were a target for aspirin and that aspirin inhibited various aspects of platelet function. *H. Klaus Breddin* showed that aspirin treatment of patients with peripheral arterial occlusive disease resulted in inhibition of platelet aggregation and a prolongation of bleeding time by nearly 2 min after intake of 500 mg [12]. *Harvey J. Weiss* found that only aspirin but not salicylate inhibited ADP release and "secondary platelet aggregation" and that this reaction was irreversible during the platelet life span [10]. Later this group also showed that inhibition of platelet function resulted in disturbed platelet thrombus formation and thrombus adhesion to the subendothelium [13]. The group of *James F. Mustard* [14] confirmed the data of Quick for several animal species and further demonstrated that inhibition of platelet aggregation by aspirin was dependent on the kind of platelet stimulus. Specifically, aspirin did not inhibit ADP-induced primary aggregation, that is, the contraction of the platelet cytoskeleton. Interestingly, even a high dose of 1 mg/ml (!) aspirin did not block high-dose thrombin-induced platelet aggregation but did so when low-dose thrombin was used [14]. This agrees with the missing inhibition of platelet secretion by aspirin, later reported by Smith and Willis [15], and also demonstrates that inhibition of cellular prostaglandin biosynthesis is not necessarily paralleled by changes in cellular function. In 1968, *John R. O'Brien* reported a "permanent" inhibition of platelet function by aspirin already at a "subclinical" single dose of 150 mg and strongly recommended a clinical trial of the compound in patients at elevated thrombotic risk to determine its therapeutic potential. He also noted that this aspirin-induced platelet "abnormality" could be corrected by addition of 10 % untreated platelets [16].

Detection of an antithrombotic mode of action and first clinical trials. *Philip W. Majerus* and his group were the first to show that aspirin irreversibly binds to a particular protein fraction (COX) in platelets and acetylates a specific serine ($serine_{529}$) inside the COX channel [17, 18]. This was also their explanation for antiplatelet and – perhaps – some antiinflammatory actions of the compound. This group also initiated the

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first double-blind, placebo-controlled clinical trial to study the antithrombotic effects of an antiplatelet dose (160 mg/day) of aspirin in hemodialysis patients who were at high risk for thrombotic shunt occlusion [19]. Some details of the study design and results were written down 25 years later by Dr. Majerus and are still interesting to read:

> ... This study was done in 1978 when clinical trials were much easier to carry out than it is today. Late one afternoon, I looked into the St. Louis phone directory for aspirin and found a company in town, Rexall, that made aspirin tablets. I called after hours, and a man answered the phone. I explained what I wanted: 100 bottles of 100 tablets containing 160 mg aspirin and the same number of bottles of a matched placebo. The man said he could make them without any problem and he delivered them to my lab next morning at no charge. We continued the study for 6 months by which time 18 of 25 patients in the placebo group had a thrombosis compared to 6 of 19 of those given aspirin for a relative reduction of 3-fold, a highly significant result .... [20].

This first clinical trial was followed by others, done independently by several groups at about the same time. These studies provided the rationale for inhibition of platelet function by aspirin as a treatment option to prevent atherothrombotic vessel occlusions, specifically myocardial infarction and ischemic stroke (Sections 4.1.1 and 4.1.2).

1.1.4.3 Aspirin and the history of prevention of myocardial infarction and stroke

First observations. In 1948, *Paul C. Gibson* (London, UK) suggested that salicylic acid, which was a known metabolic intermediate from coumarin metabolism, could act similarly to dicoumarol as an anticoagulant and, therefore, might be potentially useful for treatment of coronary thrombosis [21]. One year later, he published a first report on successful use of aspirin for prevention of anginal pain and coronary thrombosis in patients suffering from various thrombotic diseases. In a questionnaire he asked 20 doctors who treated cardiac patients about their experience with aspirin. The majority of them considered aspirin as being of "undoubted value" in relieving and preventing anginal pain while none thought that it was useless. Gibson explained these beneficial effects by a combination of coumarin-like and analgesic effects of the compound. He recommended doses of 1,300 mg (20 grains) every 4 hours for 10 days, followed by 650 mg (10 grains) aspirin "as long as desirable" for treatment of patients with myocardial infarction [22].

The Craven Studies. The first systematic investigations on the significance of antithrombotic effects of aspirin for prevention of myocardial infarction and stroke were published by *Lawrence L. Craven* (Fig. 1.1.4-1), a suburban general practitioner from Glendale (California) [23]. His studies were initiated by the fact that coumarin-type anticoagulants (dicoumarol) had already been very successfully used at the time for prevention of coronary thrombosis, that is, myocardial infarctions [24]. Though the clinical efficacy of dicoumarol was impressive – reduction of reinfarctions by 30–50 %

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Figure 1.1.4-1: Photograph of Dr. Lawrence L. Craven in 1914, at the age of 31, when he graduated from the University of Minnesota College of Medicine and Surgery. (Courtesy of University of Minnesota Archives, University of Minnesota, Twin Cities – with kind permission).

– there were problems with its practical handling. The percentage of time when patients were at the desired therapeutic drug level was suboptimal, i. e., too short (low) (ineffective) or too long (high) (bleeding). This was assumed to be due to insufficient treatment control by physicians and laboratories and/or the patient's drug adherence rather than due to a failure of the drug to act [25]. Salicylate had been previously identified as an intermediate in the metabolism of coumarins in men [26]. This was then the explanation of the anticoagulant action of aspirin and the absent effects of coumarins (dicumarol) on bleeding time in vitro. Moreover, studies of the pharmacological activity of various analogs of dicoumarol had revealed that only those compounds showed anticoagulant action in vivo that could yield salicylic acid or a derivative thereof during metabolic processing in vivo [27]. On this background, Craven, similarly to Gibson, suggested that aspirin (salicylate) should be considered a less potent – but better tolerated – substitute for dicoumarol. We now know that there is no pharmacokinetic connection between coumarins and aspirin at antiplatelet doses (Section 2.3.1). Thus, the hypothesis of using aspirin as a warfarin replacement ("warfarin-light") for prevention of myocardial infarction was wrong – however, with considerable clinical consequences.

In 1948, Craven started his systemic studies on aspirin as a preventive of myocardial infarction and summarized the findings of his first study as follows:

> . . . during the past two years, I have advised all of my male patients between the ages of 40 and 65 to take from 10–30 grains [650–1,950 mg] of acetylsalicylic acid daily as a possible preventive of coro-

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> nary thrombosis. More than 400 have done so, and of these none has suffered a coronary thrombosis. From past experience, I should have expected at least a few thrombotic episodes among this group. There would appear to be enough evidence of the antithrombotic action of acetylsalicylic acid to warrant further study under more carefully controlled conditions ... [28].

Craven continued these studies and reportedly treated a total of 1,465 patients until 1953 – still without having seen any case of an unstable anginal attack or myocardial infarction. Meanwhile, he had reduced the daily dose to 5 grains = 325 mg, that is, one tablet per day – a dose still frequently used in cardiovascular prevention. Craven was aware of the uncontrolled nature of his studies including missing untreated control patients and stated:

> ... in such a large number of subjects of this type most likely to experience coronary episodes it is – to say the least – remarkable that all remained healthy and active. Such a finding is contrary to statistical expectations as well as to the consistent experience of 36 years in general practice ....

He concluded:

> ... will experimental and clinical research in its slow but steady progress eventually test the observations here presented? Only the future can tell whether they are finally to be substantiated or refuted ... [29].

In the following years, Craven increased the number of patients to about 8,000 – still without having seen any myocardial infarction – and recommended the drug also for prevention of stroke [29, 30]. The recommended daily dose was now 5–10 grains = 325–650 mg = 1–2 tablets per day. Unfortunately, he died himself in 1957, one year after publication of his last study, at the age of 74 years from a heart attack. It is not clear from his records whether or not he has followed his own advices to take one aspirin tablet per day. One reason for *not* taking it could be that according to his opinion, aspirin had a bad benefit/risk profile at the age of over 70 years – he had recommended the drug only for middle-aged (40–65 years) men [31].

These studies of Craven were the first to suggest beneficial effects of aspirin for prevention of myocardial infarction and stroke. However, they were also a stroke of luck for several reasons: Craven treated exclusively males at an age of increased risk for myocardial infarction who, according to current knowledge, benefit most from aspirin prophylaxis. He used a dose of aspirin – 325 mg = 1 tablet per day – that was very low in comparison to antiinflammatory doses used at the time for treatment of pain and inflammatory diseases. Thus, not too many side effects had to be expected, which was also good for the compliance of his patients. Finally – he had no problems with statistics because there were no infarctions in any of the patients' groups.

Consequences of the Craven studies. Unfortunately, these exciting findings did not receive sufficient attention during the following 20 years. This was possibly due to

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the low impact factor of the journals where these studies were published and the fact that Craven himself died from a heart attack. Until the 1970s, the pathology of myocardial infarction was also unclear, that is, whether a coronary thrombosis was cause or consequence of myocardial infarction. *Peter C. Elwood*, *Archibald L. Cochrane* and colleagues from Cardiff (Wales) published in 1974 the first randomized, placebo-controlled trial on aspirin (300 mg/day) in 1,239 men with recent myocardial infarction. They found a reduction in mortality by 25 % after 12 months. This was exciting – but, unfortunately, not statistically significant. Consequently, the authors considered their results as being inconclusive [32]. Until 1988, more than 15,000 patients were studied in seven placebo-controlled trials for secondary prevention of myocardial infarction at the cost of many millions of dollars. None of these studies was significant on its own. Possible explanations from today's viewpoint are: (i) poor study design, (ii) highly variable aspirin doses (300–1,500 mg/day), (iii) the apparently largely if not totally absent control of patient compliance over the months and years of study duration and (iv) a highly variable time point at which aspirin treatment was started, in one study (AMIS) up to 5 years (!) after the acute event [33].

These negative results at first finished the discussion on aspirin use for prevention of myocardial infarction. In addition, infrequent though severe side effects, such as gastrointestinal or cerebral bleeding events, and a suggested, though never really established relationship to Reye's syndrome (Section 3.3.3) have tainted its reputation as a medicine for long-term use in atherothrombosis prevention. Eventually, this resulted in removal of aspirin from the list of essential drugs by the WHO in 1988.

Aspirin revival in thrombosis prevention. Ironically, in the same year, 1988, when the WHO took action to remove aspirin from the list of essential drugs, two large prospective, randomized and placebo-controlled trials were published that convincingly demonstrated a cardioprotective action of regular low-dose aspirin in prevention of ischemic heart disease. In the healthy population of the US Physicians' Health Study (US-PHS), one tablet (325 mg) every second day caused a 44 % reduction in the occurrence of a first myocardial infarction [34, 35]. The ISIS-2 trial on secondary prevention with half a standard tablet, that is, 162 mg daily, showed a 40–50 % reduction of recurrent ischemic events and an impressive and highly significant 23 % reduction in mortality over 5 weeks in patients with acute myocardial infarction (Section 4.1.1) [36]. As a consequence of ISIS-2, additionally supported by the publication of the first of a series of metaanalyses by the Antiplatelet Trialists' Collaboration on secondary prevention of atherothrombotic vascular diseases [37], aspirin became the guideline recommendation for secondary prevention of new atherothrombotic events. It still keeps this position, now frequently in combination with other antiplatelet agents, such as ADP receptor antagonists, for example in patients with acute coronary syndromes (ACSs) and percutaneous coronary intervention (PCI). In contrast, the role of

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aspirin for primary prevention is still under debate mainly due to the poor benefit/risk (bleeding) ratio in persons at low vascular thrombotic risk (Section 4.1.1).

1.1.4.4 Aspirin and tumor prevention

Only three years after John Vane's discovery that aspirin inhibits enhanced prostaglandin biosynthesis, it was found that malignant tumor cell lines produce high amounts of prostaglandins, in particular PGE_2, which promotes tumor cell growth and proliferation [38]. PGE_2 was also found to suppress immune defense mechanisms in amounts that were made by tumor cells (nanomoles). COX inhibitors, including aspirin, had the opposite effect. They blocked immunosuppression in vitro and retarded tumor growth in vivo [39]. Shortly thereafter, PGE_2 was reported to act as a cocarcinogen in experimental skin tumors but not to be carcinogenic by itself [40]. In the 1980s, it was found that aspirin and several NSAIDs exhibit chemopreventive effects in rodent models of colon carcinogenesis [41]. The possible clinical relevance of these experimental findings was first shown in the pioneering epidemiological study by *Gabriel Kune* and colleagues from Melbourne (Australia). They showed in a retrospective case-control trial that regular, long-term aspirin intake reduced the risk of colorectal cancer (CRC) by about 40 % [42].

Similar results were reported in numerous subsequent epidemiological studies (Section 4.3.1). Further experimental and clinical trials have meanwhile confirmed a tumor-promoting action of PGE_2 and (platelet-derived) TXA_2 [43] as well as a CRC-tumor suppressor potential of inhibitors of prostaglandin biosynthesis, such as aspirin and several NSAIDs [44]. These data strongly suggested a pathophysiological relationship between pathogenesis and malignancy of epithelial tumors and the COX/prostaglandin system.

Another, apparently prostaglandin-independent, route to the action of aspirin as a tumor preventive was the discovery that circulating blood platelets contribute to tumor spreading, invasion and metastasis. *Gabriel J. Gasic* and colleagues were the first to show an inhibition of tumor metastasis by experimental thrombocytopenia in animal experiments that was abolished by addition of platelets [45]. Gasic also showed that aspirin inhibited tumor cell metastasis by its antiplatelet effects and that inhibition of … *secretion of platelet products appeare[d], to be heavily involved* … [46, 47]. These and other data [48] strongly suggest platelets and/or platelet-derived products are relevant mediators of tumor spreading, that is, the malignancy of tumors.

Summary

The discovery of a general mode of action of aspirin – inhibition of prostaglandin biosynthesis – had considerable consequences for its clinical use and resulted in the systematic development of new classes of NSAIDs with the common feature of inhibition of prostaglandin biosynthesis. These compounds have largely replaced aspirin in symptomatic long-term treatment of inflammatory diseases, but not as an OTC antipyretic analgesic. Aspirin kept its unique position until today

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– although now in competition with other nonopioid analgesics, such as ibuprofen and the nonantiinflammatory acetaminophen (paracetamol).

In the mid-1960s, the first papers showing prolongation of bleeding time and inhibition of platelet function by aspirin were published. The effects on platelets were irreversible, not seen with other salicylates at comparable doses and (later) explained by inhibition of prostaglandin (thromboxane) biosynthesis. In 1979, the first prospective, placebo-controlled clinical trial in patients at enhanced risk of thrombotic vessel occlusions demonstrated a significant inhibition of av shunt thrombosis by 160 mg/day aspirin in patients on chronic hemodialysis.

Lawrence L. Craven was the first physician to conduct systematic trials on aspirin as a putative preventive of myocardial infarction and stroke. He gave the drug to several thousands of middle-aged male persons, finally at a dose of 325 mg, that is, "one aspirin a day," and reportedly did not see any myocardial infarction in these patients over years. There was, however, no untreated control group. A number of follow-up studies could not confirm these results. It was only in 1988 that the US-PHS in primary and the ISIS-2 study in secondary prevention showed marked reductions of myocardial infarction rates and improved infarct survival, respectively, with only one aspirin tablet (325 mg) every second day or half a tablet every day, respectively.

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1.1.5 Current research topics

The pharmacology and clinical use of aspirin is still subject of intense basic and clinical research. Over the last decade (2010–2020), the publication rate of papers on "aspirin" according to the scientific citation database PubMed amounted constantly to 2,500 and more – every year (!). It will be interesting to see the impact of the new discoveries regarding the pathophysiology of diseases, specifically immunological aspects of inflammation and viral diseases, on this research. Another issue is the detection of new acetylation targets and the expanding knowledge on the key roles of aspirin-sensitive autocrine and paracrine platelet thromboxane-mediated signaling in the natural history of diseases, in particular immunology and tumorigenesis.

1.1.5.1 Clinical research

An increasing number of potential clinical indications for aspirin. Three areas of clinical research are currently of interest for clinical aspirin use, primarily for preventive, long-term use: prevention of venous thrombosis and prevention of preeclampsia in women at elevated risk. Both will be discussed in more detail in Sections 4.1.4 and 4.1.5. Another area of interest are neurological disorders, including cognitive deficits of Alzheimer's disease and their prevention by aspirin, although the evidence is sparse until now [1, 2] and any clinical use may be limited by an increase in harm, in particular bleeding events, in elderly individuals (Section 3.1.3) [3, 4]. Recent clinical data suggest antiplatelet actions of aspirin as possibly important adjunct effects in treatment of sepsis and ARDS. In this context, inhibition of platelet-triggered immunomodulatory actions by aspirin in HIV patients is an issue, as well as most recently the antiviral effects of aspirin [5, 6] in prevention and treatment of flu-related symptoms, including

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aerosolized aspirin for treatment of viral infections of the upper respiratory tract [6] and, perhaps, COVID-19 (Section 4.2.2) [7].

Cardiovascular prevention. The use of aspirin in secondary prevention is firmly established. However, the usefulness of aspirin in primary prevention of cardiovascular events is still under debate because of a poor benefit/risk (bleeding) ratio. This appears to be particularly relevant to the elderly [4]. In this context, a growing number of comedications (lipid-lowering drugs, antihypertensives, antidiabetics) has been introduced and has replaced aspirin as a first-line agent in atherosclerotic vessel diseases. These medications, combined with changes in lifestyle and other environmental conditions, might allow to consider withdrawal of aspirin where appropriate [8]. Most interesting recent data show that aspirin in primary prevention of risk patients as constituent of the polypill has protective effects by its own, that is, in addition to the antilipidemic and antihypertensive components of the drug [9].

Aspirin and tumor prevention. Another interesting field of clinical research is the chemopreventive action of aspirin, in particular with respect to colorectal carcinomas and other solid tumors (Section 4.3.1). An actual metaanalysis of more than 100 observational trials has reported an about 20 % reduction of mortality by aspirin (as an adjuvant) in a number of solid tumors [10]. Current research is focused on the identification of suitable biomarkers to find patients in whom beneficial actions of aspirin on tumor prevention are most pronounced. Interesting new hypotheses, including a role of metabolites of aspirin and salicylic acid generated in the gut through microbial biotransformation, have been developed (Section 2.3.3) [11].

1.1.5.2 Basic research

Inhibition of autocrine and paracrine platelet functions. It is now becoming increasingly evident that antiplatelet actions of aspirin are much more complex than can be seen from measuring inhibition of platelet aggregate formation in vitro. Fresh impact to this issue came from the rediscovery that platelets not only trigger thrombus formation but also inflammatory and immune reactions. These multiple interactions between platelets, white cells and the endothelium [12, 13] are probably of utmost importance to understand the full spectrum of aspirin as an antiinflammatory/antithrombotic/immunomodulatory medicine in vivo. These intercellular interactions of platelets with other cells and the formation of heterotypic platelet–white cell aggregates (neutrophil extracellular traps [NETs]), as well as the identification of new aspirin-sensitive platelet-derived mediators, such as dioxolanes [14] or "high-mobility group box 1 protein (HMGB1)" [15], are currently subject of intense research.

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Acetylation of COX-2. A particular pharmacological property of aspirin that is not shared with traditional NSAIDs or coxibs is the acetylation of COX-2 with subsequent generation of 15-(R)-HETE and ATL, an antiinflammatory and inflammation-resolving mediator [16]. ATL is generated by intercellular interactions with other lipoxygenases. This may help to better understand and to explain clinically well-known phenomena, such as adaptation of stomach mucosa to long-term (high-dose) aspirin use (Section 3.2.1) [17] and the inhibition of white cell recruitment to an inflamed site by aspirin (Section 2.3.2) [16, 18]. In addition, COX-2 has a broad substrate specificity, including also neutral lipids and endocannabinoids. Their generation and action may also become modified after COX-2 acetylation by aspirin but is insufficiently studied yet. All of these activities might be relevant to clinical situations with an upregulated COX-2, including acute and chronic inflammation, immune reactions, tumorigenesis and ischemia.

Acetylation of further molecular targets. Prostaglandins and thromboxane A_2 are the key mediators of interest to understand the biological effects of aspirin via COX inhibition by acetylation of a particular serine residue inside the hydrophobic channel of the enzymes. However, these are not the only acetylation sites. Recent proteomic studies have identified >100 peptide/protein sequences that become (irreversibly) acetylated by aspirin in vitro [19–21]. Another recent "mapping site" analysis has identified more than 500 potential aspirin-modified proteins with high confidence, the majority of them being lysine-acetylated (Fig. 1.1.5-1) [21]. The lowest aspirin concentrations for detectable protein acetylation were in the range of 50–100 µM [19]. These concentrations can be obtained after intake of about 2 g of oral aspirin. If there were no degradation by aspirin deacetylases (aspirin esterases), even higher micromolar concentrations could be obtained by accumulation. Since the acetylation process is long-lasting, the duration of aspirin action is dependent on the turnover rate of the acetylated target, that is, possibly lifelong in acetylated potentially immortal tumor cells [22]. With other words, the half-life of the acetylated protein rather than that of free aspirin will determine the biological response. For example, it amounts to 19 days (!) for plasma albumin. It can, therefore, not be excluded that regular low-dose daily aspirin results in an accumulation of acetylated amino acids, such as lysines or serines, in certain proteins. Although recent work indicated that in the majority of cases aspirin-mediated acetylations do not accumulate to levels likely to elicit biological effects, because deacetylases act to minimize the biological consequences of nonspecific chemical acetylations [22], this is an exciting and largely unstudied area of basic research.

Lysine is the most frequently acetylated amino acid (Fig. 1.1.5-1). The lysine acetylation is a reversible posttranslational protein modification and an important regulator of gene expression [23].

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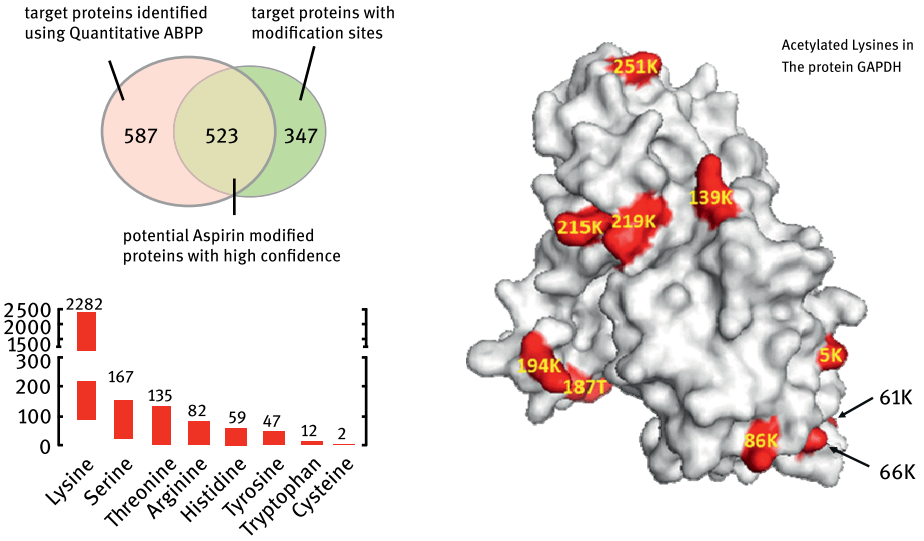

Figure 1.1.5-1: Mapping sites of aspirin-induced acetylation in proteins of human cancer HCT116 cells as detected by quantitative acid-cleavable activity-based protein profiling (QA-ABPP). The right part depicts the acetylated lysine in the reference protein glycerolaldehyde-3-phosphate dehydrogenase (GAPDH) (modified after [21]).

Aspirin analogs. Finally, the discovery of new and real "aspirin-like" drugs, that is, drugs that acetylate molecular targets by covalent binding, is a pharmacological challenge and, eventually, might result in design and development of new class(es) of antiinflammatory, immunomodulatory, anticancer and pain-relieving drugs. One such aspirin analog that binds covalently, i. e., irreversibly, to COX-2 (APHS) is already available [24]. APHS was found to be equipotent to aspirin in stimulating endothelial NO production [25] and to exhibit aspirin-like antitumor effects in certain experimental settings [26]. The recent elucidation of the crystal structure of acetylated COX-2 and the description of a reaction scheme for generation of 15-(R)-HETE by this enzyme [27] will clearly help to better understand the biological significance of these processes and open the door for further design and development of new "aspirin-like" drugs.

Summary

Current research topics of aspirin cover both basic and clinical research. A major topic in clinical research is the usefulness of aspirin as an antithrombotic agent in thromboinflammation and immunothrombosis, including sepsis and ARDS, and as a chemopreventive in colorectal and perhaps other forms of (solid) cancers. Another topic is the identification of biomarkers to improve the efficacy of chemopreventive actions of aspirin. The possible benefits of aspirin in prevention of neurological disorders, specifically cognitive deficits, are also currently being investigated.

In basic research, the aspirin-induced modulation of COX-2 activity, eventually resulting in the generation of new, antiinflammatory compounds such as lipoxins and others is of interest. There is also evidence for transacetylation – mostly lysines and serines – of more than 100 proteins by

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aspirin with yet largely unknown biological consequences and possible accumulation of acetylated proteins dependent on their half-lives and the activity of local deacetylases.

Finally, new aspirin analogs may be designed and developed with more cell-specific action profiles. One compound that selectively and covalently binds to COX-2 has already been designed (APHS). APHS was found in vitro to stimulate endothelial NO production and to have antitumor effects. Others may follow, eventually resulting in the design and development of new and even more effective class(es) of really "aspirin-like" drugs.

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1.2 Chemistry

This section is focused on the pharmaceutical aspects of aspirin and selected salicylates. To these belong physicochemical properties of the compounds as well as methods for determination of aspirin and its metabolites. The section is not written with the intention to provide a complete overview on all pharmaceutically relevant aspects of salicylates but rather to inform about those that are relevant to the understanding of their pharmacology and toxicology in biological systems.

Chemical structures and physicochemical properties of aspirin and other salicylates are discussed in the first part (Section 1.2.1). In this context, the unique mesomeric structure of salicylate, allowing its incorporation and enrichment in cell membrane phospholipids, is of outstanding importance to understand the (nonspecific) actions of salicylic acid on cellular energy metabolism, specifically the uncoupling of oxidative phosphorylation and subsequent depletion of energy-rich phosphates (ATP) (Section 2.2.3). Another aspect is the instability of aspirin at (alkaline) physiological pH (pH 7.4) and the (poor) water solubility of aspirin in acidic media, such as stomach juice, eventually resulting in local tissue irritation but also rapid passage of the unchanged compound into the small intestine (Section 3.2.1). Finally, the particular crystal structure of aspirin and the recent discovery of two polymorphic forms, coexisting in one and the same aspirin crystal, are of considerable pharmacological interest.

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The second part of this section describes analytical methods of salicylate determination in biological media (Section 1.2.2). Several techniques are available, allowing for simultaneous measurement of both aspirin and its major metabolites in biological samples at nanomolar to micromolar concentrations. These concentrations are to be expected after administration of antiplatelet and analgesic/antiinflammatory doses in vivo.

1.2.1 Structures and chemical properties of salicylates

Salicin – the natural salicylate. The β-glucoside salicin with the aglycon saligenin is the natural pharmacologically relevant ingredient of willow bark, isolated for the first time in crystalline form by Leroux in 1830 (Section 1.1.1). Leroux obtained 1 oz (about 28.3 g) salicin from 3 lb of willow bark (*Salix helix*) (cited after [1]). Salicin was later used by Piria as starting material for the preparation of salicylic acid (Section 1.1.1). Salicylic acid (*o*-monohydroxy benzoic acid) is a relatively strong acid with a pK_A of 2.9 and is poorly water-soluble (0.2 %). The solubility can be considerably improved by conversion into the sodium salt, which is approximately 50 % water-soluble. Salicylates for systemic use are either esters with substitutions at the carboxyl group, such as methylsalicylate, or esters of organic acids with substitutions in the phenolic *o*-hydroxyl group, such as aspirin. Aspirin is the acetate ester of salicylic acid (Fig. 1.2.1-1). The crystalline and molecular structures of aspirin have been elucidated [2, 3].

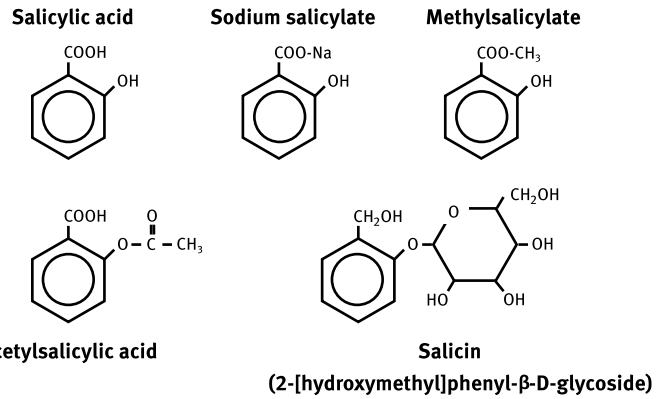

Figure 1.2.1-1: Chemical structure of selected salicylates.

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Computer calculations have suggested another even more stable crystalline isoform of aspirin in addition to the already known isoform I. Experimental studies were able to confirm the real existence of the isoforms I and II and, in addition, also demonstrated a polymorphism between these two isoforms. Importantly, the two different polymorphs can coexist within one and the same crystal . This new and unexpected finding with aspirin as the first compound to show this unique property raises a number of principal physicochemical questions regarding the definition of crystal polymorphism and might also be important for drug development [4, 5].

1.2.1.1 Salicylates in clinical use – chemical properties

Salicylic acid. Salicylic acid (molecular weight, 138.1 Da) in form of its sodium salt (molecular weight, 160.1 Da) was the first entirely synthetic salicylate for medical use (Section 1.1.1). It is no longer used for internal applications. Reasons are the unpleasant, sweaty-bitter taste, caused by direct stimulation of the human bitter taste receptor [6]. In addition, salicylate is a direct irritant of the stomach mucosa. Historically, this was the major reason to search for better tolerable derivatives, such as the acetylated product – acetyl salicylate (aspirin), which was reportedly salicylate-"free" [7] and has replaced salicylate as a medicine for internal use. However, salicylate is still being used as an external medication, for example in ointments, because of its antiseptic and keratolytic properties (corn plaster).

Despite its disappearance from internal use, the pharmaceutical and biological properties of salicylate are still of considerable pharmacological interest. The salicylate component of the intact aspirin molecule is essential for COX(-1) acetylation because it is required for the initial, reversible binding of aspirin inside the COX channel and brings the acetyl group of aspirin in close topographic neighborhood to $serine_{529/530}$, the acetylation target of aspirin (Section 2.2.1) [8]. Salicylate is also the primary metabolite of aspirin and responsible for many of its biological actions on cell function, including most of the symptoms of acute salicylate poisoning (Section 3.1.1). Salicylate shows a peculiar physicochemical behavior because of the formation of a ring structure by hydrogen bridging. This requires a hydroxy group in a close neighborhood to the carboxyl group and is only seen with the *o*-hydroxy benzoic acid salicylic acid (Fig. 1.2.1-2), but not with its *m*- and *p*-analogs. The *o*-position of the hydroxyl group facilitates the release of a proton with decreasing pH by increasing the mesomery of the resulting anion. These properties explain the protonophoric

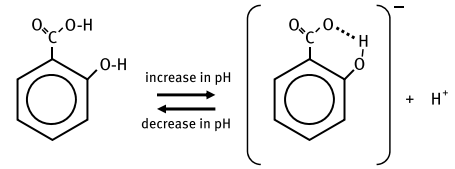

Figure 1.2.1-2: pH-dependent equilibrium of ionized and nonionized forms of salicylate (for further explanation see text).

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actions of salicylates in uncoupling of oxidative phosphorylation by eliminating the impermeability of mitochondrial cell membranes for protons (Section 2.2.3) [9]. In addition, the physicochemical properties of salicylate also help to understand the local irritation of the stomach mucosa as a consequence of direct contact and subsequent pH-dependent uptake into gastric mucosal cells (Section 3.2.1). The *m*- and *p*-hydroxy analogs of benzoic acid do not share these properties with the *o*-analog salicylate and are biologically inert (Fig. 2.3.2-3).

Structure–activity comparisons were made with 80 salicylate analogs in order to clarify the relationship between chemical structure and uncoupling of oxidative phosphorylation. Studies in isolated mitochondria showed that the essential pharmacophore for this activity is a compound with a negatively charged (carboxyl) group at the o-position, that is, acetylsalicylate. The m- and p-hydroxy benzoate analogs of salicylic acid have no effect. This suggested the o-position of the hydroxyl group as an essential steric requirement for its metabolic activity. Mechanistically, this was explained by the unique proton bridging between the oxygen of the carboxyl group and the adjacent proton in the hydroxyl group (Fig. 1.2.1-2). This allows a mesomeric state that promotes a nondissociated configuration and facilitates tissue penetration [10].

Acetylsalicylic acid. Acetylsalicylic acid (molecular weight, 180.2 Da) or aspirin is the acetate ester of salicylic acid. The pharmacological properties are similar to those of salicylate. However, aspirin exhibits additional activities of its own that are due to the reactive acetyl group – the (nonselective) acetylation of multiple cellular targets beyond COXs, including other proteins and DNA. This results in biological effects that are not shared by salicylate (Section 2.2.1).

Aspirin is a white powder with a sour taste. The compound is poorly soluble in water (0.3 %) but somewhat better in ethanol (20 %). The solubility in aqueous media is pH-dependent. It amounts to only 60 µg/ml at pH 2 but increases dramatically with increasing pH (Fig. 1.2.1-3) [11]. Thereby, local pH also determines the "wettability" by aspirin of the stomach mucosal surface [12]. The solubility in aqueous media is also markedly improved after conversion into the sodium salt, specifically at acidic pH. The pH-dependent changes in solubility of aspirin are the reason for its largely unchanged and rapid passage of the acidic gastric juice (Section 3.2.1) as well as the rapid absorption of the dissolved compound inside the upper intestine.

Methylsalicylate. Methylsalicylate is the active ingredient of wintergreen oil from American teaberry (*Gaultheria procumbens*). This oil was used as a natural source of salicylates since the early nineteenth century because it contains up to 99 % methylsalicylate. Methylsalicylate is the active constituent of numerous drug combinations for external use, for example rheumatism ointments and bath salts. It is also of toxicological interest because of its much higher toxicity compared to other salicylates. Particularly dangerous is the erroneous ingestion (by children!) of methylsalicylate-containing ointments and other products for external use (Section 3.1.1).

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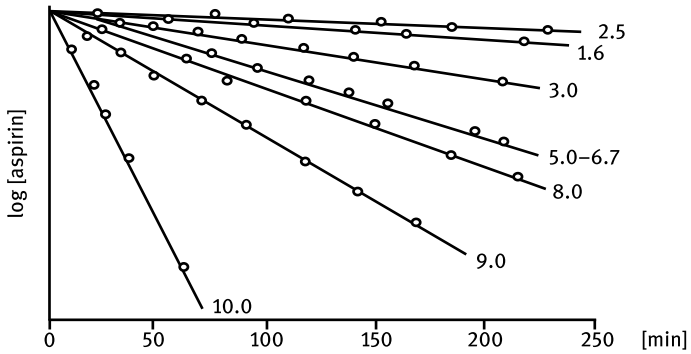

Figure 1.2.1-3: pH-dependent hydrolysis (dissolution) of aspirin (1.5 mM) in aqueous solution at 42 °C. Note the high stability (poor solubility) of aspirin at acidic pH and the significantly improved solubility at increasing alkaline pH (modified after [11]).

1.2.1.2 Aspirin formulations

Galenic formulations. Several galenic preparations of aspirin have been developed for practical use and are discussed in detail in Section 2.1.1. One intention for this was to improve the solubility and gastric tolerance of the compound, another was the adaption of the pharmacokinetics of the drug to the different clinical requirements. Rapid dissolution is particularly useful if short-term or immediate action of aspirin is desired, for example in treatment of acute pain, including migraine- or tension-type headache (TTH), but also for fast inhibition of platelet function – that is, thromboxane formation – in acute coronary syndromes. Alternatively, parenteral application forms were developed for intravenous as well as aerosol (tracheal) application by a nebulizer, for example the well water-soluble lysine salt with basic amino acids such as D,L-lysine (LASAG). This protects aspirin from hydrolysis and can also be safely administered intravenously or as aerosol via the trachea to the lung [13].

Another approach are enteric-coated formulations with retarded release of the active ingredient, predominantly designed for long-term use. The intention was to minimize the direct, physical contact of aspirin/salicylate with the stomach mucosa. The most recent development is a novel micronized, fast disintegrating aspirin formulation with a faster and more complete dissolution compared to standard aspirin tablets (Fig. 1.2.1-4) [14]. A detailed discussion of the pharmacokinetics of these and other formulations in clinical use is found elsewhere (Section 2.1.1).

Quality criteria of generics. In addition to genuine aspirin, there are many generic formulations containing acetylsalicylic acid as the active ingredient on the market. A frequently discussed issue is, therefore, whether all formulations of all manufacturers containing the same active ingredient at the same dose are bioequivalent, with the consequent preference to use the cheapest formulation. In the case of salicylates, this has been refused already many years ago [11]. A more recent study additionally

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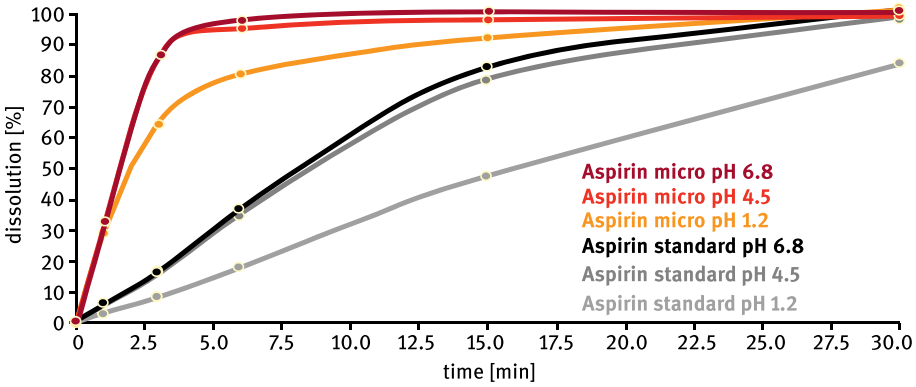

Figure 1.2.1-4: In vitro dissolution vs. time curve of micronized, fast disintegrating aspirin (500-mg tablet) and plain standard aspirin (500-mg tablet) at pH 1.2, 4.5, and 6.8. Note the much faster and largely pH-independent dissolution of the fast disintegrating form [14].

suggested that even aspirin analogs which passed in vitro dissolution specifications may not be bioequivalent in vivo [15]. A comparative study of selected aspirin formulations in Germany has found large differences regarding the pharmaceutical quality of aspirin-containing mono-preparations with suggested use as antipyretic analgesics.

In 1986, a pharmaceutical comparison of all aspirin OTC formulations on the German market was performed. Included were all products containing only acetylsalicylic acid as the active ingredient for suggested use as an antipyretic analgesic. Only tablets were included but no other galenic preparations or tablets for other indications.

All 11 brands fulfilled general pharmaceutical quality requirements, such as the content of the active ingredient. However, marked and, according to the authors, unacceptable differences existed with respect to the individual in vitro release kinetics. These criteria were determined according to a US standard and were not met by five out of the 11 preparations tested (Table 1.2.1-1) [16].

The authors repeated this study 2 years later on 62 different aspirin preparations. They found that 20 (!) of the tested formulations still did not meet the quality standards mentioned above [17]. Thus, not all aspirin preparations might be the same in terms of bioequivalence in vivo, even if they contain the active ingredient (acetylsalicylic acid) in identical amounts.

Quality assessment of generics or biosimilars might be a special problem in developing and threshold countries, where the income is low and OTC compounds are sold in all kinds of supermarkets and discounters without any quality control. A study in Brazil showed that from five tested brands, only genuine Bayer aspirin fulfilled the required pharmaceutical quality standards. In India alone, 77 brands are distributed by 43 different companies; aspirin and other pain-relieving OTC drugs with unknown efficacy and toxicity are exempt from bioavailability studies [18]. Accordingly, the European Medicines Agency (EMA) has raised concerns regarding the quality of medicines

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Table 1.2.1-1: In vitro release kinetics of acetylsalicylic acid (ASA) under standard conditions from different commercial mono-preparations available in Germany. According to predefined quality standards, the content of the active ingredient should be 95–105 % of declaration and at least 80 % of the compound should be released within 30 min under the conditions chosen [16].

ASA preparation	declared ASA content [mg]	% of declaration found	ASA release kinetics [% in 30 min ± SD]
Acetylin	500	98.9	89.6 ± 2.8
Aspirin	500	99.4	100.7 ± 1.0
Aspirin junior	100	102.9	103.5 ± 2.7
Aspro	320	98.1	96.4 ± 4.7
Ass 500 Dolormin	500	98.0	77.2 ± 9.2
ASS-Dura			
Ch.-B. 18613	500	98.2	65.4 ± 16.5
Ch.-B. 074035	500	102.9	72.4 ± 9.5
ASS-Fridetten			
CH.-B. 019026	500	98.3	68.8 ± 10.0
Ch.-B. 020047	500	100.1	75.9 ± 5.7
ASS-ratiopharm	500	96.6	89.3 ± 9.3
ASS-Woelm	500	100.9	77.9 ± 8.8
Temagin ASS 600			
Ch.-B. 212142	600	100.9	50.2 ± 11.4
Ch.-B. 212143	600	100.4	44.0 ± 9.9
Trineral	600	99.2	90.8 ± 3.9

made in India and distributed across the European Union. Findings of noncompliance with good clinical practice are currently investigated.

Summary

Aspirin and the major metabolite salicylic acid are stable compounds and are poorly water-soluble at acidic and neutral pH. The solubility is markedly increased in alkaline pH and is also more than 100-fold higher for the respective sodium salts as compared to the free acids.

Initial, reversible binding of the salicylate portion of unmetabolized aspirin appears to be essential for correct positioning and subsequent serine acetylation of COX(s). In addition, salicylate – in contrast to the unmetabolized aspirin – has unique physicochemical properties. These are due to the small distance from the acetate hydroxyl group to the carboxyl group. This allows for the formation of a chelate ring structure and facilitates the release of protons with decreasing pH. The major functional consequence is the accumulation of salicylate inside the cell membranes, specifically in mitochondrial membranes. There, the compound acts as a protonophore. Consequence is the uncoupling of oxidative phosphorylation because of abolition of the mitochondrial membrane impermeability for protons (Section 2.2.3).

Aspirin is available in hundreds of different medications worldwide. Many of these generics, specifically OTC drugs, with unknown, because uncontrolled, efficacy and toxicity, may not meet pharmaceutical quality standards. A newly developed micronized aspirin formulation that exhibits markedly improved release kinetics might replace standard plain aspirin as an OTC antipyretic analgesic in the near future.

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1.2.2 Determination of salicylates

1.2.2.1 General aspects

Measurement of salicylates in body fluids, mainly plasma and urine, is of interest for several purposes. One reason is the control of plasma levels to verify that these are

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within the therapeutic range. Determination of plasma levels is also necessary in case of intoxication and for controlling the efficacy of detoxification procedures. Plasma or urinary levels of salicylates allow checking for patient compliance, an important issue for long-term aspirin use in cardiovascular prophylaxis and a frequent natural explanation of so-called aspirin-"resistance" (Section 4.1.6). Finally, measurements of salicylates and their metabolites are of interest to study the pharmacokinetics of the compounds in research, in particular in studies on drug metabolism and drug interactions. Measurement of salicylate as a surrogate parameter for its "precursor" aspirin is easier and sufficient in many cases, for example in salicylate intoxication (Section 3.1.1).

The therapeutic plasma levels of salicylate are dose-dependent and vary in dependency on the clinical use. Peak levels in the range of 1–10 µg/ml are obtained with antiplatelet doses, levels of 50–100 µg/ml are analgesic and levels of 100–200 µg/ml and more are conventional antiinflammatory concentrations. The plasma levels of unmetabolized acetylsalicylic acid are considerably lower and largely dependent on the formulation: the more rapid the absorption, the higher the peak level of unmetabolized acetylsalicylic acid in plasma (Section 2.1.1).

1.2.2.2 Methods of determination

Gas-liquid chromatography. Gas-liquid chromatography (GLC) is the reference standard. The technique allows for separate determination of acetylsalicylic acid, salicylic acid and its metabolites. The detection limit is about 1 µg/ml.

High-performance liquid chromatography. High-performance liquid chromatography (HPLC) is an alternative to GLC but more complex and time consuming. Reversed-phase HPLC techniques with photometric detection are the methods of choice [1]. These have the advantage that the complete spectrum of aspirin and its metabolites can be measured simultaneously. However, one problem associated with this type of assay is the spontaneous hydrolysis of aspirin to salicylate in protic solvents, including water and methanol, as well as plasma (Section 2.1.1). Thus, some degradation of aspirin may occur ex vivo during sample processing. A modification of this technique for human plasma, including extraction of salicylates in organic solvents, allows for simultaneous determination of aspirin and its metabolites down to levels of 100 ng/ml with an interassay variation of less than 10 % (Fig. 1.2.2-1). This technique combines simplicity in sample treatment with stability of aspirin over several days (!) without significant decomposition [2].

More recently, another sensitive ultrahigh-performance liquid chromatography has been described, also using methyl-benzoic acid as internal standard. The detection of salicylate and unmetabolized acetylsalicylic acid was linear between 0.2

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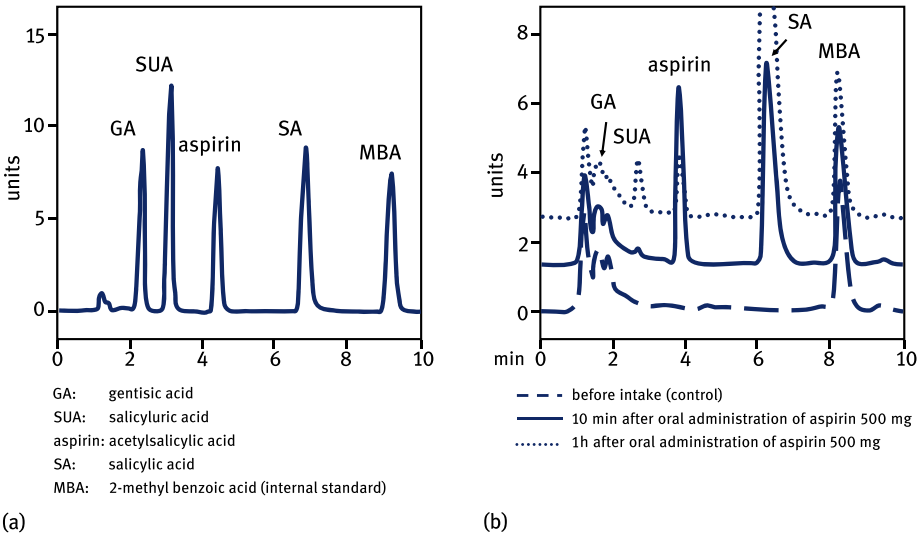

Figure 1.2.2-1: Chromatograms of a standard mixture of acetylsalicylic acid and its major metabolites (50 ng each) (a) and plasma levels in a volunteer before, 10 min after and 1 h after oral administration of 500 mg aspirin (b) (modified after [2]).

and 200 µg/ml (correlation coefficient > 0.999) with a detection limit of 0.17 µg/ml for both compounds [3]. With one exception (tilcitin) the assay was insensitive against more than 60 drugs, possibly coadministered to cardiac patients – however, other antiplatelet drugs were not included.

Spectrophotometry. Spectrophotometry is the earliest and most widely used method for measuring serum salicylate levels. The classical assays are colorimetric assays, taking advantage from the intense red color of salicylate/Fe^{3+} complexes (Gerhardt reaction). The technology is simple and particularly suitable for compliance measurements. Recently, a new spectrophotometric method for determination of aspirin in the presence of salicylic acid and the proton pump inhibitor (PPI) omeprazole has been described [4]: This report presents the first spectrophotometric methods applied for the determination of possible combinations of aspirin, omeprazole, and salicylic acid and poses these methods as valuable analytical tools in in-process testing and quality control analysis.

Trinder method. The Trinder assay [5] is a colorimetric test that determines salicylic acid by measuring the absorbance of the ferric ion–salicylate complex after total serum protein is precipitated by mercuric chloride and allowed to react with ferric iron supplied by ferric nitrate.

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The Trinder method is simple, rapid and inexpensive. This method has been used almost exclusively in the past, when a detection limit of about 100 µg/ml of salicylate was sufficient. The Trinder method measures solely salicylates, *not* acetylsalicylic acid. (False) positive results may be obtained with salicylamide or methyl salicylate. Conversely, the method can also be used to measure these compounds, for example in case of poisoning. However, the Trinder assay is rather nonspecific and sensitive to a large number of other acids and amines [6]. These also include compounds and their metabolites which might be increased in patients with Reye's syndrome, where the Trinder assay was frequently used for the determination of salicylate levels. Interestingly, salicylate levels in liquor and serum of children with Reye's syndrome measured with sensitive and selective HPLC methods where reportedly only 1 % of those measured by the Trinder assay (Section 3.3.3) [7].

Second-derivative synchronous fluorescence spectrometry. Another method that allows for the simultaneous determination of acetylsalicylic acid and its major metabolites in one assay is second-derivative synchronous fluorescence spectrometry [8]. This method appears to be the first nonchromatographic technology for the simultaneous determination of aspirin and its major metabolites in one single serum sample. The technique is not sensitive to several other drugs found frequently in the sera of patients suffering from inflammatory diseases (antipyrine, ibuprofen, indomethacin, theophylline and others).

Summary

Several reliable methods are available to measure aspirin and its major metabolites in biological fluids, including plasma (serum), liquor, synovial fluid and urine. Separate determination of unmetabolized aspirin and salicylate (metabolites) are of interest in studies on pharmacokinetics of several galenic formulations (see below). Most of these assays have detection limits in the nano- to low micromolar range.

HPLC separation and subsequent identification of the spots by appropriate standards is the most frequently used technology. Advantages are the simplicity and reproducibility of the method, high sensitivity and the possibility for simultaneous determination of several aspirin metabolites together with unmetabolized aspirin itself in one and the same sample. Disadvantages of this and some other technologies are the spontaneous (pH-dependent) and enzymatic hydrolysis of aspirin. However, this problem can be solved by appropriate sample processing.

The Trinder method, a colorimetric assay, was historically the most frequently used method for salicylate (not aspirin!) determination. This assay exhibits a number of cross-reactions with other chemicals which might become particularly relevant in hepatic failure and possibly has caused false positive results in Reye's syndrome. Gas chromatography coupled with mass spectrometry is clearly the most reliable technology. However, it needs expensive equipment and experienced investigators.

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2 Pharmacology

The pharmacology of aspirin is complex for several reasons. With respect to pharmacokinetics, the different and independent metabolic pathways of aspirin and salicylate are notable. With respect to pharmacodynamics, it is the mode of action. Aspirin belongs to the rather small group of drugs that do not interact with specific (membrane) target structures, such as receptors, but instead act rather nonspecifically, in most cases by direct chemical modification of macromolecules, via acetylation. This in turn induces a number of follow-up reactions. Affected macromolecules involve proteins and DNA. Importantly, the duration of these effects is *not* determined by the (short) plasma half-life of aspirin, but instead by the turnover rate of the modified (acetylated) target (protein) and the speed of hydrolysis of the bound acetyl group by the action of aspirin esterases that act to minimize the biological consequences of nonspecific chemical acetylations.

Aspirin interacts via its reactive acetyl moiety with many acceptor structures (Fig. 2-1). Functionally most relevant are amino acids in target proteins, such as a serine in COXs or lysines in albumin and the endothelial/platelet NOS (eNOS). The major effect of acetylation of COX-2 serine by aspirin in addition to inhibition of prostaglandin production is the change of enzyme activity towards that of a 15-lipoxygenase which makes 15-(R)-HETE, a precursor of ATL.

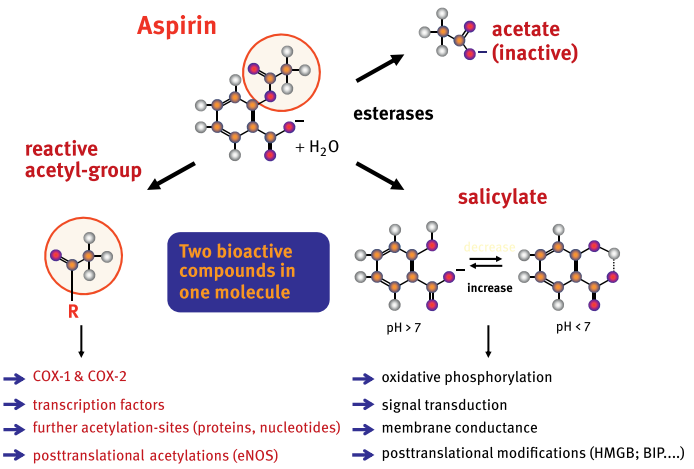

Figure 2-1: The two active principles of aspirin: the reactive acetyl group as part of the intact aspirin molecule and the stable hydrolysis product salicylate. The figure also shows important cellular targets for acetylation (left) and direct actions of salicylate (right) (for further explanation see text) (©Dr. Schrör Verlag, 2016).

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Salicylate has also a broad spectrum of activities. Because of the much longer half-life and high lipophilicity, salicylate tends to accumulate in plasma and tissues, particularly after repeated administration of higher doses of aspirin. This eventually results in uncoupling of oxidative phosphorylation. This is of key importance for many therapeutic (antipyresis) and most of the toxic (hyperventilation, sweating, tinnitus) effects of the compound. However, salicylate at higher concentrations (>1 mM) also contributes to antiinflammatory, antimitogenic and antiviral actions of aspirin. In general, the pharmacodynamic actions of salicylate are similar to but weaker than those of aspirin.

This chapter describes first the *pharmacokinetics* of aspirin and salicylate, focusing on the bioavailability of the active drug(s) and their plasma and tissue distribution, metabolism and clearance from blood and other body fluids (Section 2.1). This is followed by a section on *pharmacodynamics*, describing the broad spectrum of pharmacological actions of aspirin and salicylate at the cellular and subcellular levels (Section 2.2). This includes actions of the compounds on mediator systems, cellular signal generation and transmission pathways as well as on cellular energy metabolism. The discussion is here entirely mechanism-based, without paying too much attention to the question whether the concentrations necessary to obtain these effects can also be achieved in vivo and, therefore, are of potential value for the use of aspirin as a medicine.

Another issue are the functional consequences of these pharmacological actions at the tissue and organ levels (Section 2.3). Here, only those concentrations of the compound which can also be obtained at therapeutic doses in vivo, i. e., help to explain the multiple clinical actions of aspirin discussed in detail in Sections 4.1–4.3, are of interest. Safety aspects and toxicology of aspirin are discussed separately in Sections 3.1–3.3.

2.1 Pharmacokinetics

The pharmacokinetics of aspirin – like that of other drugs – involves all processes between drug uptake and excretion. It starts with drug *absorption*, followed by passage into the blood as the central compartment and subsequent *distribution* throughout the body. This then allows the active compound to reach its cellular targets inside the organs and tissues.

The physical presence and, thus, the interaction of aspirin and salicylate with their biological targets is terminated by removal of the compound from its binding site and release into the extracellular space. In the case of aspirin, this is the fate of the salicylate metabolite, but not of the covalently bound intact aspirin molecules in macromolecules. Here, the duration of biological effects is determined by the action of deacetylases and/or by the half-life of the acetylated macromolecule (protein). In case of irreversible binding, it will be terminated by de novo synthesis of (enzyme)

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protein. Free salicylic acid undergoes several *biotransformations* in the liver, resulting in the generation of phase I and phase II metabolites. These metabolites as well as unmetabolized salicylate are then cleared from the body, almost exclusively by renal *excretion*.

The pharmacokinetic reasons for *generation* of a biological signal, that is, all processes that culminate in binding of aspirin and salicylate to the cellular binding site after absorption and distribution, differ from the pharmacokinetic reasons for the *disappearance* of the biological signal, that is, all processes involved in biotransformation and excretion of the compound. Therefore, absorption and distribution (Section 2.1.1) are discussed separately from biotransformation and excretion (Section 2.1.2), although they pharmacologically represent one functional entity.

2.1.1 Absorption and distribution

2.1.1.1 Absorption and bioavailability

Several factors determine in sequence the speed and extent of absorption as well as the systemic bioavailability of aspirin after oral administration. The first is the solubility of the compound in aqueous media, which is mainly determined by its physicochemical properties (Section 1.2.1), the kind of formulation (tablets, granules, effervescent forms) and the pH of the medium where dissolution occurs. The second is the passage time through the stomach into the upper intestine, the major site of absorption. The duration of contact with the stomach mucosa can vary considerably in dependence on the stomach filling state and gastric pH. After passage through the stomach, aspirin is absorbed in the upper intestine and reaches the presystemic portal circulation, followed by passage through the liver and release into the systemic circulation.

Dissolution of aspirin in aqueous media. The dissolution of a plain aspirin tablet by 50 % in 0.1 N HCl under standard conditions in vitro is quite slow and requires 30–60 min. This indicates a poor solubility and high "wettability" of the drug under the acidic conditions of stomach juice (Section 1.2.1) [1]. The acidic pH in the stomach lumen favors the stability of aspirin and prevents hydrolytic cleavage to the gastric irritant salicylate – one medico-historical reason for the development of "salicylate-free" aspirin as a prodrug for the poorly tolerated salicylate (see also the Aspirin patent in Fig. 1.1.2-3) (Section 1.1.2). One single dose of a 325-mg standard plain aspirin tablet will result in millimolar concentrations of the compound in the 50–100 ml of gastric juice. In case of ingestion of higher (toxic) doses of aspirin, absorption can be additionally retarded by formation of concretions (insoluble aggregates) that might directly injure the gastric mucosa by mechanical irritation [2].

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Absorption of aspirin in the stomach. The stomach is not an important site of aspirin absorption. Only about 10 % of a predissolved standard plain aspirin is absorbed here [3]. This is partially due to the poor solubility of aspirin at strong acidic pH, but also the small absorption surface of the stomach mucosa, amounting to only 0.2–0.3 m^2 or 0.1 % of the resorptive surface of the small intestine. The use of buffered aspirin preparations further reduces the absorption rate because of an increased proportion of the ionized, membrane-impermeable isoform [3]. Use of predissolved preparations, water-soluble salts (lysine, sodium) or the new fast disintegrating aspirin will enhance absorption and increase systemic bioavailability [4], while the use of buffered preparations has little effect on these parameters [5, 6].

Although the absorption of aspirin in the stomach is low, it can nevertheless have clinically relevant consequences, for example in connection with alcohol intake in men (Section 3.2.1).

Human gastric mucosal epithelial cells exhibit significant alcohol dehydrogenase (ADH) activity. The enzyme oxidizes ethanol (alcohol) to acetaldehyde and is inhibited by aspirin – and some other NSAIDs – in a noncompetitive way [7]. Intake of 1 g oral aspirin in men results in a short-lasting but significant increase, by about 15 %, of systemic bioavailability of alcohol in blood. Interestingly, no such effect is seen in women, possibly due to the low or even absent first-pass metabolism of alcohol in the female stomach. This possibly explains the negative finding in another study, containing 50 % women [8]. Social drinkers should be made aware of the possibility that aspirin may potentiate the effects of alcohol consumed postprandially [9].

More recent investigations have additionally identified a pharmacological interaction between aspirin and salicylate: Salicylate may inhibit the hepatic first-pass metabolism of ethanol at the level of the ethanol and aldehyde dehydrogenases. This effect, however, requires plasmatic salicylate concentrations in the millimolar range (1.5 mM). These can be expected after oral intake of 1.5 or more grams of aspirin [10]. These are three or more 500-mg aspirin tablets, taken for example against headache.

The extent and speed of absorption of aspirin in the stomach is critically dependent on the speed of stomach emptying. Addition of antacids or buffering of stomach juice stimulates gastric emptying. This increases initially the plasma levels of aspirin and salicylate. Delayed gastric emptying, for example by comedicated PPIs, has the opposite effect. In this respect, it is interesting that plasma salicylate levels in patients who underwent total gastrectomy (Billroth II) were not significantly different from those in healthy controls [11], confirming the minor role of the stomach in aspirin absorption.

"Ion trapping" of stomach mucosa cells. The penetration of plain aspirin into and out of epithelial cells of the stomach mucosa is strongly dependent on luminal pH. As a consequence of the different pH values between stomach juice and cytosol of the mucosa cells, there is a significant intracellular accumulation with subsequent erosive actions on the mucosa epithelial cells (Section 3.2.1) [12, 13].

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The pK_A for aspirin is 3.5. This means that half of the compound is ionized at this pH and almost all of it at pH 6 is negatively charged within the stomach lumen. In this ionized form, the molecules are lipid-insoluble and cannot diffuse through cell membranes. At pH levels below 3.5, the majority of aspirin molecules is nondissociated, i. e., lipid-soluble, and can penetrate cell membranes by passive diffusion.

At a pH of 2.0 in the stomach lumen, 95 % of aspirin molecules after oral administration are not dissociated. However, the totally dissolved amount is very small because of the poor solubility of the compound in acidic stomach juice. After diffusion into the superficial stomach mucosal cells, there is a dissociation of aspirin within these cells: pH 7 vs. pH 2, equivalent to an ionic gradient of 10^5 (!). This prevents rediffusion of salicylates into the stomach lumen ("ionic trap") and results in intracellular (intramucosal) accumulation of aspirin and subsequent toxic effects on mucosal cells. Similar considerations apply to salicylate with a pK_A value of 3.0 (Fig. 2.1.1-1).

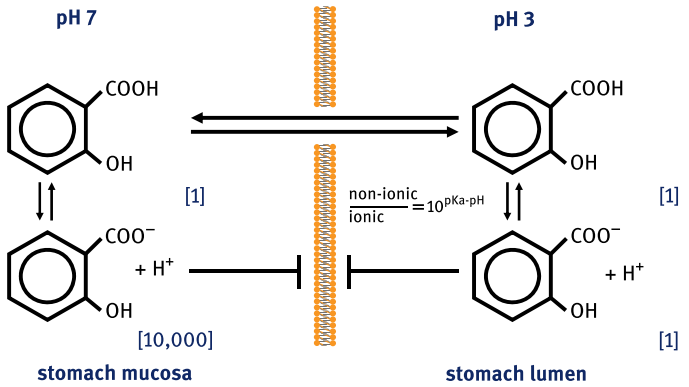

Figure 2.1.1-1: Local accumulation of salicylate (pK_A 3.0) in the stomach mucosa. For further explanation of the "ion-trapping" hypothesis, see text.

A pH-dependent distribution kinetics for aspirin between the extra- and intracellular space is not only relevant for the stomach – although it is here most impressive – but is also true for other compartments of the body. In the kidney it determines the proportion of the nonionized, diffusible form of the salicylates in (tubular) epithelial cells [14]. This is of clinical relevance not only for the acceleration of urinary salicylate excretion after aspirin overdosing by alkalization of the urine (Section 3.1.1), but also for local accumulation of salicylates at sites of inflammation or local ischemia with acidic pH.

Absorption in the intestine. Like most other drugs, aspirin is mainly absorbed in the upper intestine by passive diffusion of the nonionized form. The pH in the duodenum is about 2–4 and then increases gradually towards 7–8 in the distal small intestine and colon. The large resorptive surface of the (small) intestine, amounting to 100–200 m^2, as well as the steadily and markedly increasing solubility of aspirin with

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increasing pH, finally result in a complete intestinal absorption of the compound, despite a higher proportion of the dissociated, ionized fraction. It is an interesting hypothesis that the relatively high local concentrations of salicylates in the intestine after oral intake might influence the gut microbiome, as a key determinant for gut homoeostasis, host immune status and intestinal stem cell proliferation/regeneration. This makes the gut microbiome an interesting target for chemoprevention (Section 4.3.1) [15].

Systemic bioavailability. There is a significant "first-pass" metabolism of aspirin to salicylate during intestinal uptake and subsequent passage to the liver [16, 17]. The duration of passage through the intestine, that is, the duration of exposition of aspirin to esterases of the intestinal wall and inside the presystemic circulation (Section 2.1.2), is critical for systemic bioavailability of the uncleaved compound. These factors are not relevant for the bioavailability of the primary metabolite salicylic acid.

The deacetylation process follows a dose-independent, zero-order kinetics and reduces the systemic bioavailability of aspirin to about 50 % at single of 40–1,300 mg [16, 17, 19, 20]. This applies to standard preparations of plain aspirin but not to formulations with delayed or enhanced release. The high potency and abundance of carboxyl ("aspirin") esterases results in sustained hydrolysis of slow-release, enteric-coated aspirin formulations during the prolonged gastrointestinal passage time. These esterases are nonspecific and are located in the intestinal mucosa, blood of the portal vein (red cells, platelets, plasma) and liver parenchyma (Section 2.1.2). Consequently, the systemic bioavailability ("area under the curve" [AUC]) of standard plain unmetabolized aspirin is markedly reduced after passage of the liver [21, 22], whereas the total bioavailability of salicylates remains unchanged [23, 24].

2.1.1.2 Aspirin formulations and application modes

General aspects. Several galenic formulations and application modes have been developed to adapt the pharmacokinetics of aspirin to its clinical needs. Major objectives were faster dissolution and faster absorption of the active compound, as well as fewer (gastric) side effects. Several new formulations have been designed. These include fast disintegrating aspirin with the major advantages of rapid dissolution and faster intestinal absorption with higher levels of unmetabolized aspirin in the systemic circulation [5, 25], while enteric-coated and phospholipid–aspirin preparations (PL-ASA) might improve gastric tolerance (Section 3.2.1). Nevertheless, the plasma exposure to aspirin and salicylate, as seen from a 500-mg standard oral analgesic dose in all of these different galenic preparations ("area under the curve" = AUC), is apparently the same [25]. Table 2.1.1-1 summarizes some pharmacokinetic parameters of different aspirin formulations.

Several routes of administration are also possible. Clearly, in most cases, aspirin is applied by the oral route. An option for fast onset of action is the intravenous ad-

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Table 2.1.1-1: C_{max} and t_{max} values for different galenic aspirin preparations, each containing 500 mg aspirin. Data are shown as µg/ml or µM (in parenthesis) [5].

Parameter	Standard plain tablet	Dry granules	Effervescent tablet	Micronized, fast disintegrating tablet
mean c_{max} [µg/ml] (µM)				
acetylsalicylic acid	4.4 (30)	6.0 (33)	11.5 (60)	13.8 (74)
salicylic acid	27.0 (173)	29.8 (170)	27.8 (197)	35.1 (221)
mean t_{max} [min]				
acetylsalicylic acid	45.0	25.0	19.8	17.5
salicylic acid	180.0	120.0	49.8	45.0

ministration of aspirin as water-soluble lysine salt. Alternatively, transdermal administration or buccal application as a chewable tablet can be used [26]. These formulations release aspirin directly into the systemic circulation and bypass the liver and other locations of tissue aspirin esterases. A similar effect is obtained by inhalation of soluted aspirin salts by a nebulizer, eventually resulting in high local levels of the unmetabolized drug in lung tissue for treatment of inflammatory/thrombotic pulmonary affections [27].

(Enteric)-coated. Historically the first approach to improve gastric tolerance of aspirin was the introduction of enteric-coated formulations. The theoretical considerations to introduce this formulation were the insignificant (≤10 %) absorption of aspirin in the stomach and the large body of evidence that gastric injury by aspirin requires direct contact of salicylate with the stomach mucosa. Enteric-coated formulations (Aspirin® Protect and others) are largely resistant against stomach juice. This will avoid physical interactions of the drug with the stomach mucosa and results in both slow release of the drug and retarded onset of action. The bioavailability of unmetabolized aspirin in the systemic circulation is also reduced (see above). Enteric-coated formulations are widely used for example in long-term prevention of cardiovascular events. However. low doses, i. e., less than 100 mg of enteric-coated aspirin, might result in insufficient clinical efficacy because of too low systemic bioavailability [28]. One trial found an up to 49 % apparent platelet "resistance" for a 325-mg single-dose aspirin enteric-coated preparation but not for the same dose of plain aspirin, possibly due to delayed and reduced drug absorption in the small intestine [29]. Another issue of concern are increased proportions of immature, reticulated platelets. These are more reactive and less sensitive against aspirin [30, 31]. In patients with a high number of immature platelets, for example in erythrocythemia, enteric-coated aspirin is less active than plain formulations [32], probably because of lower plasma levels of unmetabolized aspirin. The bioavailability of different preparations of low-dose enteric-coated and plain aspirin might vary as well [33]. It is also uncertain to

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what extent gastric tolerance is improved by enteric coating in long-term use – no controlled randomized long-term trials comparing head-to-head plain with enteric-coated aspirin are available so far.

Buffered. In one of the first systematic studies on the influence of galenics on aspirin plasma levels and gastrointestinal blood loss, Stubbé et al. [34] found an apparently complete disappearance of occult blood from stool with an appropriately buffered aspirin preparation (Alka-Seltzer®). In addition, peak plasma levels of salicylate were reached earlier and were also higher than after plain preparations (Stubbé et al., 1962). This was explained by faster emptying of the drug into the intestine and improved solubility. Both assumptions proved to be correct and were later transferred into effervescent formulations, such as Alka-Seltzer® or Aspro® effervescent.

Micronized. A new micronized, fast disintegrating formulation of aspirin (Tarot®, Mille®) was developed to combine fast dissolution and more rapid release from the stomach into the small intestine, i. e., more rapid onset of action, with increased peak concentrations of unmetabolized aspirin in the systemic circulation. In comparison to standard plain aspirin tablets, these micronized tablets are much faster dissolved than standard aspirin at all pH values tested, possibly because of the fixed combination of acetylsalicylic acid with sodium carbonate (e. g., 500 mg + 165 mg) in the Tarot® tablet. The peak aspirin plasma levels after intake of one Tarot® tablet were three times higher than after intake of plain aspirin – 14 µg/ml vs. 4 µg/ml within 20 min as opposed to 45 min with standard plain aspirin (Fig. 2.1.1-2; Table 2.1.1-1) [5]. Similar considerations apply to the salicylic acid metabolite (Fig. 2.1.1-3). The total AUC remained unchanged – as with all other aspirin formulations. Aspirin dry granules (Aspirin® Effect) also have a somewhat higher bioavailability and faster onset than plain aspirin but are inferior in their overall pharmacokinetics in comparison with the fast disintegrating formulation (Figs. 2.1.1-2 and 2.1.1-3, Table 2.1.1-1) [5].

Phospholipid–aspirin (PL-ASA). A novel pharmaceutical formulation of a lipid–aspirin complex was developed to mitigate disruption of the epithelial phospholipid layer of the gastric mucosa by the protonophore salicylate [35] without delaying aspirin absorption. The compound has pharmacokinetic and -dynamic properties similar to those of plain immediate-release aspirin [36]. PL-ASA has also been shown to reduce acute gastric mucosal lesion formation during short-term exposure when compared with standard plain aspirin [37].

Buccal. The administration of aspirin as a chewable tablet allows buccal absorption and subsequent rapid direct access of largely nonmetabolized aspirin to the systemic circulation, avoiding first-step metabolism by the liver. More than 95 % inhibition of

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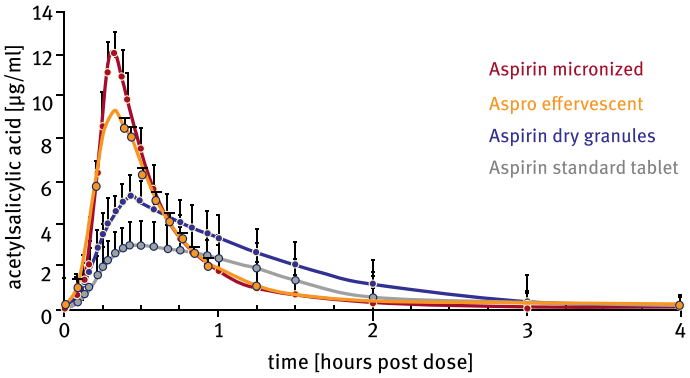

Figure 2.1.1-2: Plasma time course of acetylsalicylic acid concentrations after one single oral dose of 500 mg aspirin in different galenic formulations: standard aspirin tablets (Aspirin®), aspirin dry granules (Aspirin® Effect), micronized, fast disintegrating aspirin (Tarot®) and effervescent aspirin (Aspro® effervescent) [5].

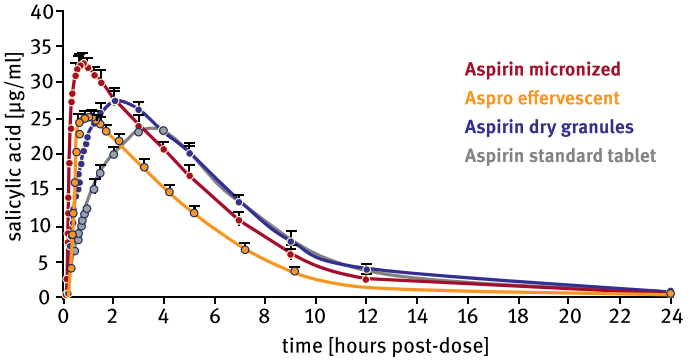

Figure 2.1.1-3: Plasma time course of salicylic acid concentrations after one single oral dose of 500 mg aspirin in different galenic formulations: standard aspirin tablets (Aspirin®), aspirin dry granules (Aspirin® Effect), micronized, fast disintegrating aspirin (Tarot®) and effervescent aspirin (Aspro® effervescent) [5].

platelet thromboxane formation as a biomarker for effective COX-1 inhibition was seen after 15–20 min [38]. This approach is thought to be particularly useful for the rapid onset of pain relief.

Intravenous. Animal experiments have suggested that intravenous aspirin at doses which largely block prostaglandin synthesis of gastric mucosa does not cause gastric injury, because of avoidance of direct contact of the drug (salicylate) with the stomach mucosa (Section 3.2.1) [39]. In practical use, no overt signs of mucosal injury (microbleeding events, gastric potential differences) occurred after intravenous application of 250–500 mg soluble aspirin (Section 3.2.1), although thromboxane formation was

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blocked by >98 % within 5 min [40]. Intravenous aspirin is a widely used first-line measure to achieve fast inhibition of platelet function in ACSs as well as to treat migraine attacks.

Inhaled. Administration of aspirin by a nebulizer is currently under discussion for treatment of pulmonary affections associated with flu or flu-like symptoms. In a molar complex with a basic amino acid such as D,L-lysine, spontaneous aspirin hydrolysis is prevented. Discoloration is prevented when glycine is added. D,L-lysine acetylsalicylate-glycine (BAY 81-8781; LASAG) is licensed as Aspirin i. v. for intravenous medication. It is also known as *Aspirin inhale* since it can be safely administered at antiinflammatory doses (250–750 mg) as aerosol [27]. In comparison with oral application, inhalation has the advantage of direct application of unmetabolized aspirin to the lung and upper airways, thereby reaching high local, antiinflammatory concentrations, without too high active drug levels in the systemic circulation. Because of the unique pharmacodynamic properties of aspirin, including the antiviral action, aerosolized LASAG appears to be a perfect treatment option of pulmonary (viral) affections [41].

Transdermal. Salicylate is a component of many ointments, used for external treatment because of its softening effect on the skin (corn plaster). This stimulated the idea of transdermal administration of aspirin also for systemic use after a significant absorption of salicylates after cutaneous application had been shown [42]. It was suggested that this application will improve gastric tolerance by avoiding gastrointestinal passage, which might be useful for patients at an elevated risk for gastrointestinal complications. In addition, the antiplatelet effect of aspirin was expected to be enhanced by using skin patches as a drug reservoir from which the active compound is slowly released. Avoidance of high peak levels might additionally result in less inhibition of vascular prostacyclin production.

Transdermal aspirin (750 mg/day) was initially reported to inhibit serum thromboxane formation by 95 + 3 % in a small group of healthy volunteers without inhibition of basal or bradykinin-stimulated vascular prostacyclin production [43]. However, a more systematic follow-up study in a larger number of volunteers indicated that this approach may not always work as suggested. In this study, aspirin at the same cutaneous dose (750 mg/day to 29 volunteers for 10 days) had a systemic bioavailability of only 4–8 %, equivalent to salicylate plasma levels of 0.1 µg/ml without any evidence for nonhydrolyzed aspirin in plasma. This dose reduced serum thromboxane by 86 % on average in males and by 32 % in females [44]. This effect is insufficient for safe inhibition of thromboxane production in cardiocoronary prevention, where at least 95 % inhibition of thromboxane forming capacity by aspirin is required.

It was also shown that aspirin applied by skin patches undergoes rapid hydrolysis to salicylate [44], which largely eliminates its antiplatelet activity. The transdermal patch

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is also not free from gastrointestinal side effects and might cause tolerance problems with the skin (maceration) during long-term use. Thus, transdermal aspirin is no option for application of aspirin for systemic use.

2.1.1.3 Distribution and plasma levels

General aspects. Similar to absorption, the distribution of salicylates within body fluids and tissues via the central blood compartment is mainly determined by pH-dependent passive diffusion of the nondissociated free fraction of the compound. As already seen with the stomach, there is a balance between the free, nondissociated acid at both sites of cell membranes. Consequently, any decrease in tissue pH, for example during acute salicylate intoxication, enhances the accumulation of active substance in tissues (central nervous system [CNS]!, kidney tubuli) and increases the symptoms of poisoning (Section 3.1.1).

Distribution volume. The apparent distribution volume of salicylates is dose-dependent. At analgesic doses it amounts to about 0.2 l/kg. This is equivalent to a predominant distribution in the extracellular space, probably because of binding (≥90 %) to plasma albumin, which contains high-affinity binding sites for salicylate (k_D: 25 µM) [45, 46]. This protein (albumin) binding does not affect the pharmacological potency of aspirin as assessed from COX inhibition in the presence and absence of albumin, but reduces that of salicylate by about one order of magnitude [47]. At high aspirin doses or salicylate poisoning, the apparent distribution volume of salicylate is increased to about 0.5 l/kg. This is due to the saturation of salicylate binding sites to plasma albumin, subsequent diffusion of salicylate into the intracellular space and increased binding to tissue proteins with falling tissue pH (Section 3.1.1). These events are additionally enhanced by saturation of phase II metabolic pathways of salicylate and subsequent increase of salicylate plasma levels (Section 2.1.2).

Aspirin and salicylate plasma levels. In human, Ruffin and colleagues found 6.6, 2.9, 1.9 and 1.0 µg/ml aspirin after single oral doses of 648, 324, 162 and 81 mg, respectively [48]. Nagelschmitz and colleagues reported maximum plasma aspirin levels of 1.0, 3.0 and 4.8 µg/ml after single oral doses of 100, 300 and 500 mg, respectively [40]. The salicylate concentrations were about four- to eightfold higher (Figs. 2.1.1-4 and 2.1.1-5). These data and those from others indicate that maximum plasma levels of aspirin around 1 µg/ml (6 µM) are to be expected at a single antiplatelet dose of 100 mg, about 3 µg/ml (15–20 µM) at 300 mg and 5–6 µg/ml (30–35 µM) at 500 mg. Thus, there is a clear dose dependency for plasma levels of both unmetabolized aspirin and salicylate and an expected doubling of these levels to about 10–12 µg/ml (60–70 µM) at an analgesic aspirin dose of 1 g. Application of aspirin in the fast disintegrating form results in marked increases in both aspirin and salicylate (peak) levels to about 14 and

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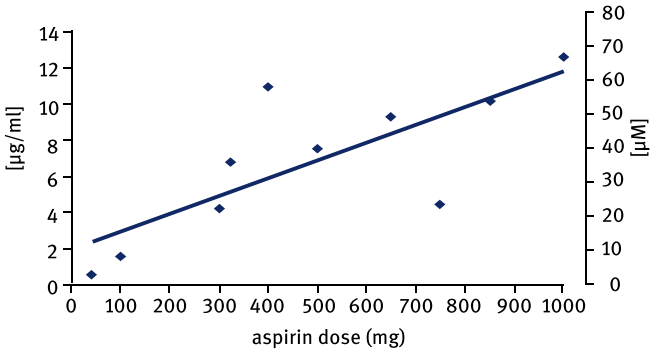

Figure 2.1.1-4: Dose-dependent changes in C_{max} of acetylsalicylic acid (geometric means) in plasma after oral aspirin in different doses [5].

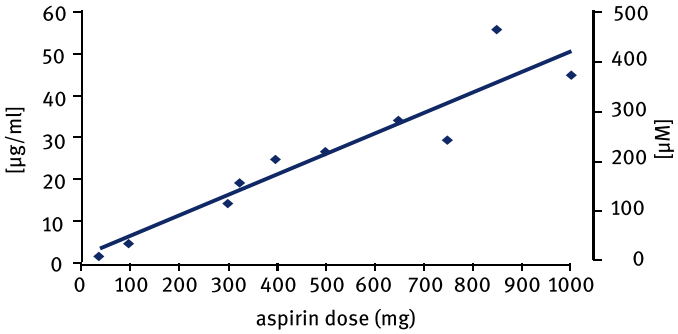

Figure 2.1.1-5: Dose-dependent changes in C_{max} of salicylic acid (geometric means) in plasma after oral aspirin in different doses [5].

35 µg/ml after intake of one 500-mg tablet (Figs. 2.1.1-2 and 2.1.1-3; Table 2.1.1-1). These levels are sufficient to block COX-1 completely and COX-2 largely in vitro, dependent on the particular conditions of COX-2 upregulation (Section 2.2.1).

Because of the short half-life of aspirin, there is no accumulation of unmetabolized aspirin after repeated intake. In contrast, repeated intake of aspirin over several days might cause tissue accumulation of salicylate due to its longer and variable half-life, which increases dose-dependently.

Salicylate levels in selected tissues and body fluids. The maximum tissue levels of salicylates in synovial fluid amount to about 50 % of plasma levels [49]. Salicylate concentrations in the cerebrospinal fluid are about 10–25 % of the plasma level and there is no tight correlation to the plasma level [50]. Similar low percentages of plasma levels, about 30 %, are found in the perilymph. In contrast, salicylate levels in the fetal circulation are similar to those in the maternal circulation ex vivo [51]; however, they

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become very low, amounting to <100 ng/ml shortly after cessation of regular maternal ingestion of low-dose aspirin [52]. This is particularly relevant to the fetus and newborn prior to delivery, whose renal and hepatic clearance systems are not yet fully developed (see Section 3.1.2).

A different issue is the tissue level of salicylates because of the particular physicochemical properties of the compound that allow accumulation inside the cell membranes with marked consequences for cellular energy metabolism (Fig. 2.2.3-3) (Section 2.2.3). Additionally, a higher proportion of the membrane-bound compound is nondissociated and freely permeable, for example in the acidic environment of stomach juice, inflammatory exudates or local ischemia.

2.1.1.4 Modifying factors

Food. Eating will prolong the passage time of drugs through the stomach, allow for adsorption to food particles and reduce the speed of absorption in the small intestine [53]. Total drug bioavailability may not be different between fasted and fed states. The clinical efficacy of aspirin, for example in pain relief, is stronger, is obtained faster and lasts longer with higher early plasma concentrations of the active compound in the fasted state. Thus, taking aspirin with food may make it less effective [54]. However, these salutary effects of more rapid absorption of plain aspirin in the fasting state have to be balanced versus possible individual gastric tolerance problems.

Vegetables and plants as a natural source of salicylates. Many fruits and vegetables contain salicylates, in particular the salicylic acid ester salicin (Section 1.2.1). These salicylates or metabolites thereof may circulate in plasma. Their levels might be increased by an appropriate (vegetarian) diet and, eventually, add to the therapeutic benefit of exogenous aspirin. There is mixed information on whether clinically relevant amounts of salicylates can be obtained in plasma after intake of salicylate-rich diets [55, 56]. One study has provided evidence for a low salicylate level in plasma in the absence of any exogenous salicylate administration. The hypothesis was developed that salicylate might be considered a “biopharmaceutical” by analogy with plants and can be synthesized endogenously from benzoic acid [55].

Plasma salicylate levels can be modestly increased by a vegetarian diet [57]. This last effect has been taken as evidence to explain potential beneficial effects of certain vegetables in chemoprevention of colorectal carcinomas and is discussed in detail elsewhere (Section 4.3.1). While the possibility of “chemo”protective effects of vegetables on tumor prevention exists, the alternative explanation, namely the abdication of (red) meet, the most important natural source not only of cholesterol but also of arachidonic acid (for inflammatory and tumor-promoting prostaglandin formation), by vegetarians, appears at least as likely. Further studies on this interesting issue are likely to follow.

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Sex. The possible influence of sex on the pharmacokinetics of aspirin was studied after single (1,000 mg) oral, intramuscular or intravenous administration of the (water-soluble) lysine salt of aspirin to healthy volunteers. No differences were detected with respect to half-life and distribution volume [58]. The results for low-dose (100 mg) [22] and high-dose (antirheumatic) [59] aspirin were similar for both sexes. Another issue are sex-dependent variations in blood alcohol levels due to sex-dependent differences in gastric mucosal ADH activity after aspirin intake as discussed above.

Age. Bioavailability and metabolism of aspirin in healthy men at the age of 21–40 as compared to men at the age of 55–75 are similar. Age-dependent differences in aspirin bioavailability and metabolism are not considered to play a major role for its therapeutic use [60].

Summary

Standard plain aspirin tablets are poorly soluble in aqueous media at acidic pH. The galenic formulation, speed of tablet dispersion, local pH and velocity of gastric emptying determine the passage time through the stomach and, therefore, gastric tolerance. Absorption of aspirin occurs predominantly in the upper small intestine and is there nearly complete.

Several formulations of aspirin have been developed to improve gastric tolerance and to adapt the systemic bioavailability of the compound to its clinical needs, that is, fast-onset action (headache, acute coronary syndrome) or improved gastric tolerance at long-term use (enteric coating, PL-aspirin). For oral use, there are buffered (i. e., easily soluble or predissolved) formulations as well as micronized, fast disintegrating aspirin. In addition, intravenous administration of aspirin as water-soluble lysine salt or LASAG is possible. LASAG is also an interesting candidate for aerosolic application by nebulizers if high pulmonary levels are desired, for example for treatment of viral/inflammatory infections of the upper airways.

During and after absorption, oral aspirin undergoes hydrolytic cleavage by esterases (deacetylases) in the intestine, portal venous blood and liver. This results in an equimolar transformation into salicylic acid, the primary metabolite. The systemic bioavailability of standard plain aspirin is about 50 %. It is markedly reduced to 15–25 % or less by "controlled-release" formulations. Peak plasma levels of acetylsalicylic acid after 100 and 500 mg standard plain aspirin amount to about 1 and 5 µg/ml, respectively. They are threefold higher with a new micronized, fast disintegrating formulation which also acts about twice as fast as the conventional standard aspirin tablet.

The bioavailability of aspirin is independent of sex and age. Food intake can reduce the bioavailability of aspirin after adsorption to food particles, as does a prolonged exposure against esterases prior to entering the systemic circulation. Otherwise, there are no relevant pharmacokinetic interactions between aspirin and other compounds with respect to drug absorption and distribution. Several fruits and vegetables are natural sources of salicylates. However, it is unlikely that a regular vegetarian diet will markedly increase plasma salicylate levels in men.

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2.1.2 Biotransformation and excretion

General aspects. The biotransformations of acetylsalicylic acid involve two principally distinct events: (i) hydrolysis of the reactive acetyl moiety to inactive acetate and water and (ii) generation of the active metabolite salicylic acid. Both processes are independent of each other, exhibit particular reaction kinetics due to different enzymes engaged and have a different biological significance for the overall pharmacodynamic actions of aspirin (Section 2.2).

2.1.2.1 Biotransformations of aspirin

The short half-life of aspirin in the systemic circulation, amounting to only about 20 min, is due to rapid hydrolytic cleavage to salicylic acid and acetate. This occurs

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either by spontaneous, pH-dependent hydrolysis or by enzymatic cleavage by acetylsalicylate *o*-acetylhydrolase(s) (aspirin esterase[s]; synonyms: acetylsalicylic acid esterase, [aspirin] deacetylase, aspirin hydrolase). The enzyme(s) act specifically on carboxylic *ester* bonds. The resulting acetate then enters the Krebs cycle while the remaining salicylic acid undergoes further phase I and phase II metabolic transformations [1]. The excretion of aspirin occurs almost exclusively (98 %) via the kidney, at single aspirin doses of up to 500 mg mainly (70–75 %) in form of the glycine conjugation product salicyluric acid [2–5]. The metabolic pathways of aspirin and salicylic acid in the human are depicted in Fig. 2.1.2-1.

Sites of biotransformations. After oral intake, enzymatic hydrolysis of aspirin to its primary metabolite salicylate starts in the stomach mucosa [6] and continues in the intestinal mucosa, portal vein blood and liver [7, 8] at zero-order kinetics. Overall, presystemic carboxyl esterases in the intestine and liver reduce the systemic bioavailability of plain aspirin by about 50 %. As a consequence, the circulating plasma levels of unmetabolized aspirin after intake of a standard plain formulation are twice as much after intravenous than after oral administration [9]. The metabolic transformation then continues in the systemic circulation and finally yields salicylate and acetate as stable end products.

Aspirin esterases. There are (at least) three "aspirin" esterases (acetylsalicylate-*O*-acetylhydrolases) in the systemic circulation that hydrolyze aspirin to acetate and salicylic acid: the aspirin esterases of red cells [10–13] and the two aspirin esterases of plasma [1, 14].

The enzymatic activity of erythrocytes probably involves different enzymes, that is, butyrylcholinesterase, carboxylesterase and probably also the pseudocholinesterase of albumin [14–18]. Aspirin is rapidly hydrolyzed within erythrocytes by a heterodimer of the platelet activating factor (PAF) acetylhydrolase (PAFAH1b2/PAFAH1b3). This explains the much faster aspirin hydrolysis in vitro in whole blood as opposed to plasma (Table 2.1.2-1) [10, 18, 19].

Table 2.1.2-1: Hydrolysis half-life of aspirin at 37 °C in different body fluids compared to a physiological buffer solution (pH 7.4) (modified after [20]).

medium	aspirin concentration [mg/ml]	aspirin half-life [h]
Krebs buffer	10	15.5
gastric juice	10	16.0
duodenal juice	10	17.0
blood	13	0.5
plasma	13	1.9

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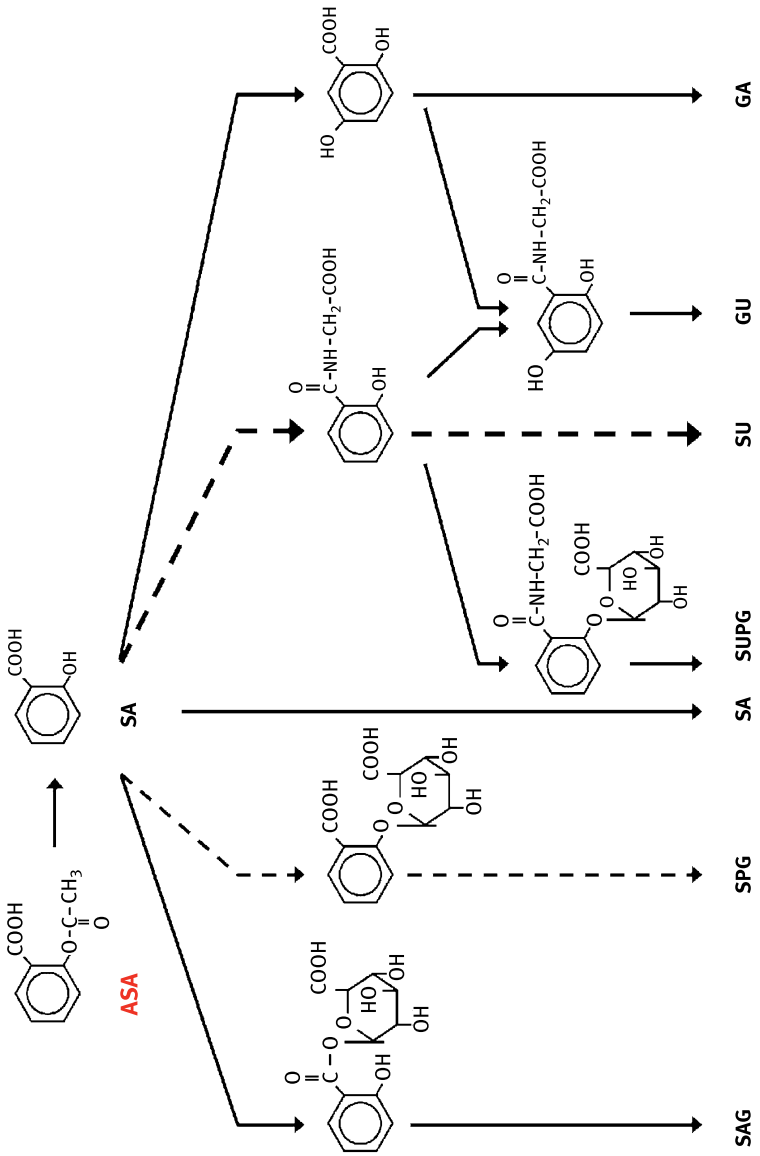

Figure 2.1.2-1: Metabolic pathways of plain aspirin (ASA) after intake of 500 mg as a single oral dose. Acetylsalicylic acid (ASA) is initially hydrolyzed to salicylic acid (SA) as the primary metabolite and the reactive acetyl group (not shown). SA is excreted in urine either unchanged (10 %) or as SA-phenol-glucuronide (SPG) and SA-acyl-glucuronide (SAG) (5–10 %). The dominant metabolic pathway is conjugation with glycine to salicyluric acid (SU) (70–75 %). SU is mainly excreted as such or as SU-phenol-glucuronide (SUPG) (≤1 %). SA can also be hydroxylated to gentisic acid (GA) (<5 %) and gentisuric acid (GU) (<1 %), which can also be formed from SU by glycine conjugation. Dashed lines mark metabolic pathways with limited (saturated) capacity at this dose (≥500 mg). The elimination of SA at higher therapeutic and toxic doses occurs increasingly as unchanged SA with increasing half-life (for further explanation see text) (modified after [5]).

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The aspirin esterase activity in plasma is due to two different enzymes: butyrylcholinesterase (pseudocholinesterase) [21] and a recently detected homomeric PAF acetylhydrolase (PAFAH1b2) [22]. In men, the plasmatic aspirin esterase activity requires Ca^{++} for optimal activity and exhibits a skewed distribution [21].

The Cleveland Clinic Gene Bank cohort has been used to study aspirin hydrolase activity in cell-free plasma samples from 2,226 subjects. All subjects underwent elective diagnostic coronary angiography or elective cardiac computed tomographic angiography combined with extensive clinical and laboratory measurements. Coronary artery disease (CAD) patients – according to conventional classifications – were compared with controls without history of known CAD and <30 % stenosis in any [coronary] vessel.

Aspirin hydrolysis in plasma varied markedly, by up to 12-fold. Interestingly, there was a slight but significantly higher esterase activity in patients with established CAD. This was associated with a reduced antiplatelet effect of aspirin in a subset of participants who had been tested.

It was concluded that the extent of inactivation of aspirin by aspirin esterases in the vascular compartment is highly variable and this might contribute to a variable antiplatelet effect of aspirin in vivo [23].

Despite these interindividual variabilities, there appears not to exist any influence of disease state, drug treatment and comorbidities on aspirin plasma esterase activity and also no ability of aspirin to induce esterase function [18].

Transacetylation targets. Aspirin acetylates albumin by dose-dependent and covalent acetylation of the ε-*N*-amino group of several lysines [24]. In tumor cells in vitro, this action requires aspirin concentrations of about 100–300 μM or more and becomes detectable within hours [25, 26]. Possible consequences of the acetylation are structural changes which may alter the binding affinities of proteins for ligands [27]. Recent work indicated that aspirin has an enormous potential to alter protein function by acetylation. In the majority of cases, aspirin-mediated acetylations do not accumulate to levels likely to elicit biological effects. This is due to hydrolysis of the bound acetyl group by the action of further aspirin esterases that act to minimize the biological consequences of nonspecific chemical acetylations [28].

Nevertheless, acetylation of critical sites in macromolecules might subsequently modify or even abolish their (enzymatic) activity. Well-known examples are the serine acetylation of COX-1 in platelets with subsequent abolition of thromboxane generation and acetylation of COX-2, also resulting in reduced prostaglandin production but mainly in conversion of the enzyme into a 15-lipoxygenase with generation of 15-(R)-HETE, a precursor of ATL (Section 2.2.1). Transacetylation might also modify the activation of transcription factors, most notably NF-κB, but also properties and function of RNA, DNA and low-molecular weight metabolites, such as coenzyme A [29]. A proteomic analysis of living (tumor) cells has identified 120 acetylated proteins, most of them not previously reported, to be acetylated by aspirin [26]. The biological consequences of these transacetylations – as well as their control by deacetylases [28] – are

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still incompletely understood but might be of utmost importance to get fresh insights into aspirin's molecular modes of action in oncology, immunology and inflammation.

Aspirin and hepatic cytochromes. Drugs that induce the cytochrome P450 (CYP) system in the liver will also stimulate aspirin esterase activity. This has been shown for patients treated with phenobarbitone, carbamazepine, phenytoin or valproic acid and was associated with an increase in plasma cholinesterase activity [30].

High-dose aspirin treatment of rats markedly increased CYP2E1 expression, probably caused by transcriptional upregulation of the CYP2E1 gene. The same effect was seen with salicylate, suggesting that it was salicylate-induced [31, 32]. In men, high-dose aspirin (1 g three times daily for 6 days) did not change the activity of CYP2E1 [33]. In healthy males, low-dose aspirin (50 mg for 1–2 weeks) has been shown to increase the activity of CYP2C19 but not of other cytochromes [34]. This is interesting, because CYP2C19 is a critical enzyme for bioactivation of clopidogrel and this interaction might be relevant in dual antiplatelet therapy (DAPT) (Section 4.1.1). Interestingly, most of the variability in clopidogrel active metabolite levels in one clinical trial was found not to be explained by patient characteristics or the individual CYP2C19 metabolizer status [35].

2.1.2.2 Biotransformations of salicylic acid

The biotransformations of salicylic acid are dose-dependent and involve several capacity-limited pathways. This is of importance for the kind and composition of metabolic products as well as the excretion rate of salicylate and its terminal metabolites [36]. A schematic overview of the different metabolic pathways is shown in Fig. 2.1.2-1. Table 2.1.2-2 summarizes important pharmacokinetic parameters of aspirin and salicylate [37].

Table 2.1.2-2: Pharmacokinetic parameters of aspirin and salicylate (adapted from [37]). (a) Dependent on dose and pH of urine. (b) 95 % at 14 µg/ml; 80 % at 300 µg/ml, further decrease at higher doses. (c) ~0.2 at 130–300 µg/ml. (d) ~2 h at 300 mg to 20 and more h in intoxication.

Parameter	aspirin	salicylate
Bioavailability [%]	68	100
urinary excretion [%]	~1	2–30[a]
protein binding [%]	50–60	80–95[b]
clearance [ml/min × kg]	9.3	~0.2[c]
volume of distribution [ml/kg]	~150	150
half-life [h]	0.25	dose-dependent[d]
effective concentrations [µg/ml or mM]	see salicylate	50–≥200 (0.5–≥1.0 mM)
toxic concentrations [µg/ml or mM]	see salicylate	>200 (>1 mM)

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Phase I and phase II metabolism. The plasma half-life of salicylic acid at analgesic doses (0.6–1.2 g) amounts to about 3 h [19]. The major product formed at this dose from salicylic acid via conjugation with glycine is salicyluric acid (70–75 %). A smaller proportion (about 10 %) is conjugated with glucuronic acid to form acyl- and phenolic glucuronides, respectively (5–10 %).

Glucuronidation is a quantitative, capacity-limited metabolic pathway of salicylate and occurs via a polymorphic UDP-glucuronosyltransferase (UGT) [39]. The isoform UGT1A6*2 confers more rapid glucuronidation of salicylic acid than the wild-type UGT1A6 *1/*1, allowing for faster salicylate excretion [40]. This genomic variant of enzyme expression has been brought into connection with the chemopreventive effect of aspirin in colorectal cancer, after the UGT1A6 genotype was found to strongly increase the risk of colorectal cancer (Sections 2.3.3 and 4.3.1) [41]. An extensive genomic analysis was, however, unable to confirm a clinically relevant relationship between aspirin intake, UGT1AG genotype and risk for colorectal cancer [42].

Another metabolite of salicylic acid is the hydroxylation product gentisic acid (<5 %) and its phase II metabolite gentisuric acid (<1 %). In addition, there are some other minor metabolites, amounting to <10 % of total salicylic acid (Fig. 2.1.2-1) [5, 42].

Dose-dependent changes in plasma half-life of salicylate. The formation of the main metabolite salicyluric acid is capacity-limited and becomes saturated already at doses of ≥300 mg. Higher doses lead to accumulation of free salicylate [43]. At intoxications, half-lives of salicylate of more than 20 h have been reported. At acidic pH at inflammatory sites and/or systemic intoxication with metabolic acidosis, free salicylate can penetrate more easily into tissues and amplifies toxicity (CNS!) (Section 3.1.1).

At an analgesic single dose of 0.5–1 g aspirin, the approximate recovery rates of salicylate and its metabolites in urine are as follows: 70–75 % salicyluric acid, including glucuron-conjugated products, 10 % salicylic acid, 1–2 % gentisic acid, <1 % gentisuric acid (Fig. 2.1.2-1) [44–46].

The conjugation of salicylate with glycine to salicyluric acid in liver mitochondria occurs at the following sequence:

$$\text{Salicylate} + \text{ATP} + \text{CoA} \rightleftarrows \text{salicylyl-CoA} + \text{inorganic P (I)}$$

$$\text{Salicylyl-CoA} + \text{glycine} \rightleftarrows \text{salicyluric acid} + \text{CoA (II)}$$

The first step is rate limiting and results in the formation of "activated" salicylic acid (salicyl-CoA) as an intermediate under consumption of ATP. Without availability of ATP as an energy source and glycine as a substrate, this reaction will not take place. Both events are possibly involved in the reduced generation of salicyluric acid at increasing salicylate plasma levels [44]: Depletion of ATP pools because of inhibition of oxidative phosphorylation at salicylate levels of >1 mM and exhaustion of the glycine pool [47] will further reduce the renal clearance of salicylate.

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A similar reaction might also occur in the presence of other glycine-consuming metabolic drug transformations, such as high-dose paracetamol (acetaminophen) – an experimentally shown though clinically not (yet) confirmed explanation of phenacetin-related nephropathy.

2.1.2.3 Excretion of salicylates

The excretion of salicylates occurs almost exclusively (98 %) via the kidney, at single aspirin doses of up to 500 mg mainly (70–75 %) in the form of the glycine conjugation product salicyluric acid [2–5]. The composition of the excreted metabolite spectrum is dose-dependent. At toxic doses, free salicylic acid is the dominating metabolite (Table 2.1.2-3) [48].

Table 2.1.2-3: Metabolites of aspirin (salicylic acid [SA]) recovered in urine after intake of therapeutic or toxic doses. Metabolites are given as percent of the amount of total salicylate (adapted from [49]).

metabolite	therapeutic (aspirin 600 mg) (n = 45)	overdose (plasma SA 240-600 µg/ml) (n = 24)	overdose (plasma SA 715–870 µg/ml) (n = 13)
salicylic acid (SA)	9 ± 1	32 ± 4	65 ± 4
salicyluric acid (SUA)	75 ± 1	47 ± 3	22 ± 4
salicylic acid phenol-glucuronide (SPG)	11 ± 1	23 ± 2	15 ± 4
gentisic acid (GA)	5 ± 1	10 ± 2	7 ± 2
total salicylate recovered [mg, as SA equivalents]	246 ± 8	2999 ± 374	8092 ± 1470

There is a large interindividual variability in urinary excretion of salicylate metabolites. In two studies, the urinary excretion of the main metabolic product salicyluric acid after oral administration of 900 mg aspirin varied between 6–72 % and 1–31 % of the dose within 12 h [36, 50]. It is interesting that despite these large *inter*individual variations, the *intra*individual reproducibility (±12 %) was quite good, suggesting that the metabolic pattern of salicylate metabolism is genetically fixed [50]. This hypothesis is supported by studies on salicylate metabolism in twins [51]. Possible consequences of variable pharmacokinetics of salicylates for the pharmacodynamic actions of aspirin have not been studied systematically.

Age dependency. Total salicylate excretion at therapeutic doses is independent of age [52–55]. This is also true for plasma levels of salicyluric acid, which are modestly higher in the elderly (age ≥ 75 years) than in younger persons [56]. Overall, the changes are small and probably not clinically relevant [57].

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Modification by drugs. The absorption/secretion balance within the kidney tubules depends on the pH in the tubular fluid: The renal clearance is considerably higher at pH 8.0 than at pH 6.0, mainly due to inhibition of tubular reabsorption because of a reduced availability of salicylate in the membrane-permeable undissociated form. It increases the excretion of salicylate (metabolites) 5–10-fold. This effect is used therapeutically in treatment of salicylate poisoning (Section 3.1.1).

Summary

The first step in biotransformation of aspirin is its deacetylation by different deacetylases ("aspirin esterases"). These enzymes are ubiquitously present in blood and other body fluids and tissues, including the intestinal mucosa, the presystemic circulation and the liver. Different forms of this enzyme are present in red cells and plasma. The hydrolysis half-life of standard aspirin in blood is about 20–30 min and is independent of the aspirin dose. Nonmetabolized aspirin binds to a number of macromolecules via covalent acetylation of several amino acids, most notably lysine and serine. This might change enzyme activities. The best known examples are serine acetylation of COXs and lysine acetylation of endothelial/platelet eNOS. Acetylation(s) may persist and even accumulate with repeated dosing for the survival time of the acetylated protein if not terminated enzymatically by deacetylases.

In contrast to aspirin, the metabolism and excretion of salicylic acid is strongly dose-dependent. At low aspirin doses (≤500 mg), renal excretion occurs mainly (70–75 %) as glycine conjugate (salicyluric acid). This transformation is capacity-limited and cannot be further increased at doses above 500 mg. Possible reasons are ATP depletion and exhaustion of the available glycine pool in the liver. Further major metabolites in addition to salicylic acid (10 %) are salicylic acid glucuronides (5–10 %) and gentisic acid (10 %). These enzymatic conversions of salicylic acid are genetically fixed but exhibit a large interindividual variability.

At higher aspirin doses, the capacity-limited phase II salicylate metabolism is saturated, resulting in accumulation of free salicylic acid and an increase of its plasma half-life from 2–3 h to up to 20 h and more at toxic aspirin doses. This is associated with metabolic acidosis and uncoupling of oxidative phosphorylation (Section 2.2.3). Further events are an enhanced volume of distribution of salicylate with increased tissue salicylate levels. The pH dependency of renal salicylate excretion can be utilized for enhanced elimination by alkalinization of urine in case of aspirin overdosing (Section 3.1.1).

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2.2 Cellular modes of action

The pharmacodynamic profile of aspirin is determined by *two* components: the reactive acetyl moiety of the intact aspirin molecule and salicylic acid (salicylate), its primary metabolite. Historically, it was salicylate which was assumed to be the pharmacologically active component and the acetylsalicylate molecule as a whole was just considered as an inactive prodrug (Section 1.1.2). Today, it is the acetylation of proteins and other target structures which is in focus of aspirin action and is most likely responsible for most of its pharmacodynamic effects at therapeutic doses. An overview on these is shown in Fig. 2.2-1. However, the salicylate part of the intact aspirin molecule contributes to this effect by positioning the acetyl moiety to the right place inside the hydrophobic channel of COX-1 and COX-2, respectively, that is, close to the serine-binding acetylation site. Independent of this, free salicylate as the primary metabolite of aspirin has also actions by its own. These relate to its unique physicochemical properties as protonophore. Salicylate-mediated actions are preferentially seen at higher doses, that is, about 2 g or more orally or medium to high micromolar concentrations at its sites of action.

The best known and most intensively studied pharmacodynamic action of aspirin is the inhibition of prostaglandin cyclooxygenases COX-1 and COX-2 by acetylation of a particular serine inside the COX channel. This results in complete suppression of prostaglandin synthesis by COX-1. Aspirin also inhibits prostaglandin production via COX-2. In addition, it changes the enzyme activity towards a 15-lipoxygenase, eventually resulting in biosynthesis of 15-(R)-HETE, the substrate for generation of ATL by white cell lipoxygenases. Further high-affinity substrates for COX-2 are neutral lipids, such as arachidonylglycerols and arachidonylethanolamine (anandamide). Most interesting are the recently discovered dioxolanes, such as DXA_3, a COX-1-derived product of blood platelets that stimulates neutrophils in an aspirin-sensitive but thromboxane-independent manner (Section 2.2.1).

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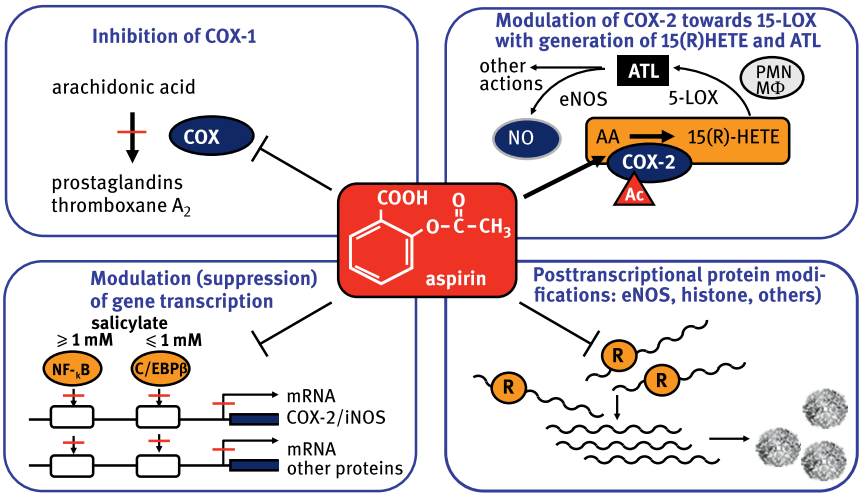

Figure 2.2-1: The multiple pharmacological actions of aspirin. Note the different actions of aspirin on COX-1 and COX-2 activities. Acetylated COX-2 also produces less prostaglandins but mainly acts as a 15-lipoxygenase (15-LOX). 15-LOX produces 15-(R)-HETE, the precursor of "aspirin-triggered lipoxin" (ATL). ATL is generated by intercellular interactions with LOX(s) from white cells. There are numerous effects of aspirin and salicylate on gene transcription, predominantly but not exclusively at higher concentrations as well as posttranscriptional protein modifications (acetylations) (© Dr. Schrör-Verlag, 2018).

Acetylation of molecular targets by aspirin is nonspecific and also seen for transcription factors with possible consequences for gene transcription. Acetylation at the translational and posttranslational levels also affects proteins (enzymes) others than COXs. Histones and eNOS are two important examples for this. Largely prostaglandin-independent are many actions on cellular signaling cascades, specifically via non-selective kinase inhibition by salicylate. These effects are involved in control of pain, inflammation and cell proliferation (tumorigenesis and apoptosis) either directly or via transcription factors, such as NF-κB (Section 2.2.2).

Finally, salicylate at millimolar concentrations exerts nonspecific effects on cell membrane permeability because of its unique physicochemical properties. Most notable in this context is its action as a protonophore on mitochondrial membranes. This eventually results in uncoupling of oxidative phosphorylation with multiple follow-up effects, most importantly the nonselective inhibition of kinases because of lack of ATP (Section 2.2.3).

This chapter provides an overview of the pharmacological profile of aspirin. This involves all biochemical, physicochemical and molecular changes that are produced by aspirin and salicylate at the cellular and subcellular levels, independent of the required doses or concentrations. The consequences of these (sub)cellular actions for tissue and organ function in vivo are discussed in Section 2.3, and the transformation into clinical use as a medicine is discussed in Chapter 4.

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In this context, the differences in the definition of a drug, i. e., a chemical/*pharmaceutical product* as opposed to its use as a *medicine* for treatment of diseases, should be explained. A pharmaceutical is a chemical entity exerting pharmacological actions in biological systems independent of their kind and consequences for this particular system, for example the human organism or a diseased organ. In contrast, a medicinal drug is a compound (pharmaceutical) that is used with the intention to prevent or to treat a disease and to obtain a therapeutic benefit for the patient.

Thus, a chemical is not qualified as a medicinal drug by its pharmacological properties but by its clinical usefulness as determined from the benefit/risk ratio in controlled, randomized clinical trials. In other words, not all pharmaceuticals are medicines. In addition, the pharmacological properties of a drug remain always the same whereas their translation into medical use may change, dependent on scientific knowledge about the pathophysiology of diseases and the availability of (drug) alternatives.

Aspirin, because of its global, nonorgan- or cell-specific actions, including multiple acetylation reactions, uncoupling of oxidative phosphorylation and nonselective kinase inhibition, exhibits many more pharmacological actions than those which are (currently) medically used.

2.2.1 Inhibition of cyclooxygenases

2.2.1.1 General aspects

Inhibition of prostaglandin cyclooxygenase(s), originally described by *John Vane* in 1971 (Section 1.1.3), is probably the most intensively studied pharmacological property of aspirin. However, it is by no means the only one and there are marked differences in the consequences of aspirin binding and subsequent modulation of enzyme activity and product formation between COX-1 and COX-2 [1–5]. In addition to these two genetically defined isoforms of COX, there are several splice variants, specifically, a so-called "COX-3" [6]. "COX-3" is a functionally active splice variant of COX-1 (COX-1b) which, however, is only of limited interest as target of aspirin [7]. In men (in contrast to dogs and insect cells), COX1b mRNA is not transformed to COX-1 mRNA and there is no evidence for generation of COX-1 protein and its enzymatic products [8].

COX isoforms. There are two genes encoding proteins with COX activity: COX-1 and COX-2, both sensitive to interaction (acetylation) with aspirin.

COX-1 is constitutively expressed in about every tissue and organ throughout the organism. It is the dominating isoform in blood platelets but is also expressed in the endothelium and resident inflammatory cells. In these cells, COX-1 expression might become upregulated in inflammatory conditions [9]. Its permanent, constitutive expression in platelets together with the irreversible acetylation of COX-1 is the essential prerequisite for the irreversibility of the antiplatelet action of aspirin in terms of inhibition of platelet-COX-1-dependent TX formation.

COX-2 is also constitutively expressed in some tissues, such as vascular endothelium, kidney and neuronal cells of the CNS. However, in contrast to COX-1, this enzyme mainly synthesizes prostaglandins "on demand" – this in huge amounts – and

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– again in contrast to COX-1 – has a broad substrate specificity. The gene for COX-2 belongs to the group of "early response genes" and encodes a protein with a high turnover rate and short half-life. COX-2 mRNA in serum-treated fibroblasts has a half-life of about 30 min [10]. COX-2-derived prostaglandins are not only involved in several acute pathologies, such as inflammation, pain and acute immune reactions, but also in physiological functions, such as renal perfusion and sodium excretion (blood pressure control!), in pregnancy from fertility to delivery, including the patency of the ductus arteriosus Botalli, and neuronal signal transmission and pain perception in the CNS. A significant proportion or even the majority of endothelial PGI_2 and PGE_2 is generated via COX-2 [11]. PGI_2 contributes to the antithrombotic effects of endothelium, PGE_2 to the regulation of vessel tone. Both functions become relevant in systemic inflammatory/immune reactions [9] as well as in advanced atherosclerosis, a low-grade inflammatory disease, with marked transcriptional upregulation of COX-2 (Section 2.3.1) [12].

Substrates. The classical substrate of COX-1 and COX-2 is arachidonic acid (AA), the natural precursor of all prostaglandins and TXA_2. Arachidonic acid is set free from glycerophospholipids of the cell membrane by phospholipases, specifically a cytosolic phospholipase A_2 ($cPLA_{2a}$). This enzyme is rate limiting for subsequent eicosanoid production, specifically under conditions of high substrate requirements and an upregulated COX-2. These conditions are typical for inflammation and ischemia. Alternative sources for arachidonic acid are 2-arachidonylethanolamine (e. g., anandamide) or 2-arachidonylglycerol. Both are also constituents of membrane lipids from which arachidonic acid is released by the fatty acid amide hydrolase (FAAH). Anandamide and 2-arachidonylglycerol are endogenous ligands of the cannabinoid receptors CB1 and CB2 and specific substrates for COX-2 with an affinity comparable to that of arachidonic acid (Fig. 2.2.1-1). The end products are prostamides and prostaglandin glycerols [3, 13–15]. They are likely to be involved in the analgesic/antiinflammatory actions of the compound and might be modified by aspirin too. However, only limited research data on this issue are currently available (Section 2.3.2).

Regulation. In addition to availability of substrate (arachidonic acid), COX-derived product formation is also regulated by COX protein expression and its enzymatic activity. While the constitutive expression of COX-1 protein does not markedly vary, that of COX-2 as an inducible enzyme is subject to multiple regulatory forces, including not only inflammatory and mitogenic stimuli [16], but also humoral factors, such as cytokines or hormones (estrogens).

Product formation, that is, generation of prostaglandin endoperoxides (PG-EPs), requires substrate binding to the active site at $tyrosine_{385}$ [17, 18]. The enzymatic activity is determined by the local peroxide tone, that is, the presence of a hydroperoxide

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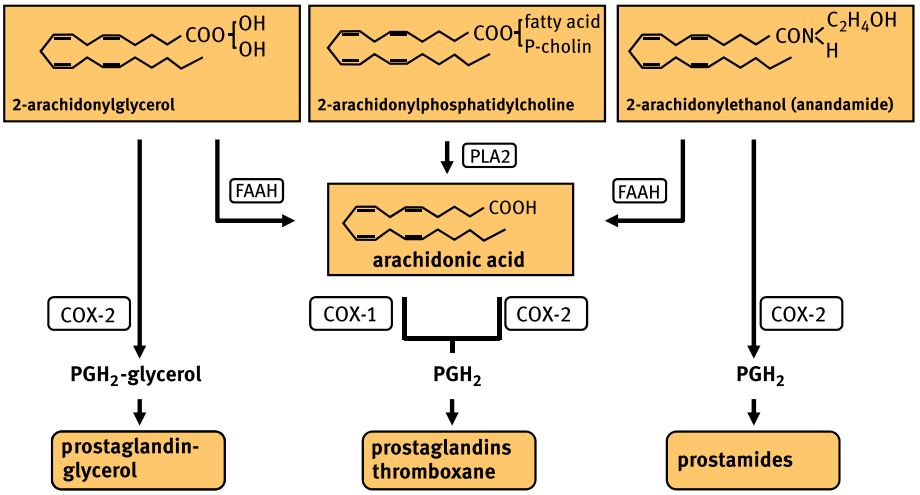

Figure 2.2.1-1: Release of arachidonic acid (AA) from its binding sites in membrane phospholipids (phosphatidylcholine), neutral lipids (arachidonylglycerol) and endocannabinoids (anandamide) by phospholipase A_2 (PLA_2) or fatty acid amide hydrolase (FAAH) and its conversion into several prostaglandins, thromboxane and prostamides. Note the broad substrate specificity of COX-2 at comparable affinities of the enzyme to all substrates.

activator that generates the $tyrosine_{385}$ – radical, necessary for initiation of arachidonic acid oxygenation [2]. The acetylation of COX-2 by aspirin is regulated by the catalytic activity of the peroxidase and inversely related to ambient hydroperoxide concentrations [19, 20]. This is relevant for the antiinflammatory action of salicylates (Section 2.3.2).

The generation of eicosanoids from arachidonic acid through PGHS proceeds via several steps. The first is the introduction of two oxygen functions into arachidonic acid with subsequent cyclization and formation of the prostaglandin hydroendoperoxide PGG_2 (COX reaction). This is followed by the reduction of the hydroperoxide at C_{15} to the corresponding hydroxyl fatty acid, PGH_2 (peroxidase reaction). PGH_2 is then further converted to prostaglandins, prostacyclin and thromboxane A_2 by specific synthases and isomerases (Fig. 2.2.1-2).

2.2.1.2 Inhibition/modulation of cyclooxygenases by aspirin

Molecular structure of the COX proteins and prostaglandin formation. The gene and amino acid sequences of the two COX enzymes are known [2], as well as the crystal structures of the nonacetylated [21, 22] and acetylated [23, 24] enzymes. Both are homodimers and integral membrane proteins. They are attached to the membrane lipid bilayer by means of an array of amphipathic helices along one side of the protein. These also frame the entrance to the COX active site at $tyrosine_{385}$ that catalyzes the first step in the conversion of arachidonic acid to prostaglandin endoperoxides. The

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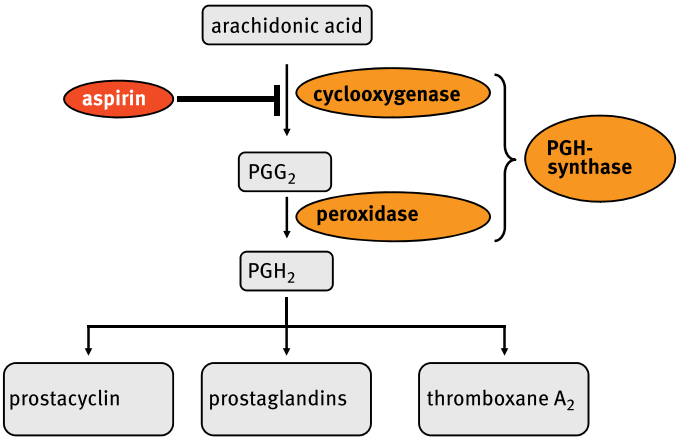

Figure 2.2.1-2: Generation of prostaglandins, prostacyclin and thromboxane A_2 via the COX pathway of arachidonic acid. COX and peroxidase form the PGHS complex. Aspirin inhibits the COX but not the peroxidase activity of PGHS.

entrance to the catalytic site lies at the apex of a long, narrow, hydrophobic channel that runs from the membrane surface into the protein interior, providing direct access for arachidonic acid from the membrane inferior to the active site of the enzyme without traversing the aqueous department (Fig. 2.2.1-3) [23, 25–27].

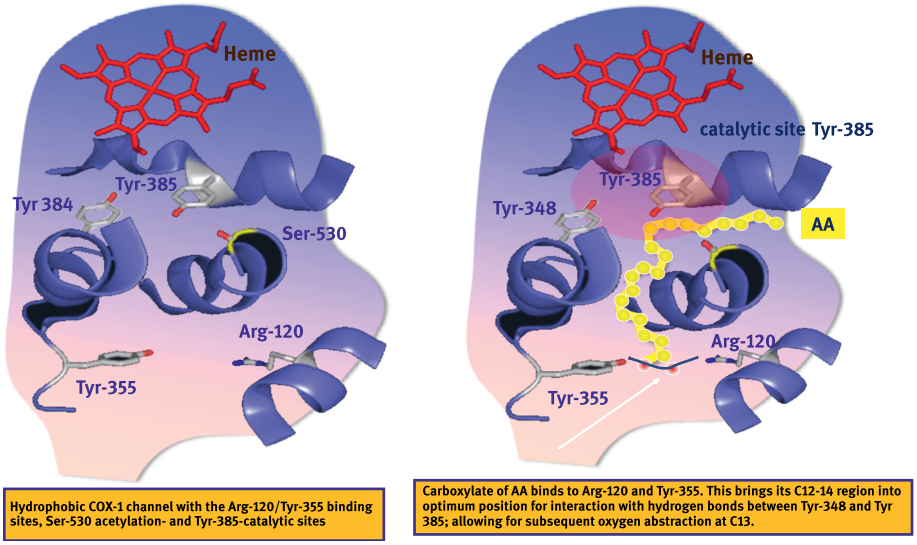

Figure 2.2.1-3: Binding and oxygenation of arachidonic acid (AA) inside the COX-1 channel (adapted from [3, 27]).

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Acetylation of COX-1. *Martin Hemler*, *William E. M. Lands* and *William L. Smith* from the University of Michigan were the first to purify COX(-1) from sheep vesicular glands. The purified enzyme had a molecular weight of 70 kDa. It contained a binding site which was associated with prostaglandin synthetic activity and became acetylated by aspirin [28]. The first step was binding of the salicylate carboxylate group to a binding site close to the substrate binding site of the enzyme inside the hydrophobic COX channel, thereby forming an initial noncovalent enzyme inhibitor complex. This initial binding of the salicylate carboxylate – similar to arachidonic acid – occurs via hydrogen bonds to tyrosine$_{355}$ and arginine$_{120}$ and is a critical determinant of the subsequent aspirin acetylation of serine$_{530}$ [29]. This, initially reversible, low-affinity binding of the lipophilic salicylate moiety is comparable, although not fully identical, with that of NSAIDs [30]. It explains the negative interactions between salicylate and NSAIDs such as indomethacin or ibuprofen, that is, the competition with aspirin binding by high-affinity NSAIDs but also by salicylate itself [30]. It also explains the reversible inhibition of prostaglandin formation by oral sodium salicylate (3 g) [31] and the hindrance of aspirin-induced inhibition of platelet function by salicylate [32, 33], as well as the reversible inhibition of the antiplatelet actions of aspirin by several NSAIDs [34] – one iatrogenic reason for aspirin "resistance" (Section 4.1.6).

Serine$_{530}$ is the acetylation site of aspirin – tyrosine$_{385}$ is the catalytic center. The group around *Philip W. Majerus* was the first to describe the covalent modification (acetylation) of the platelet COX-1 by aspirin. This group also found that the acetylation site was an amino acid at the N-terminus of the enzyme close to the active center [35, 36]. This amino acid was identified as serine$_{530}$, the molecular target of acetylation (Fig. 2.2.1-4) [35–39].

Numbering of the serine acetylation target of aspirin: The cyclooxygenases of different natural sources are minimally different in their primary structure. The acetylated serine of COX-1 in sheep seminal vesicles is in position 530 but the homologous serine of the human platelet enzyme in position 529. In COX-2 with a by 18 amino acids shorter sequence, this homologous serine is in position 516. To avoid confusions, in this paper (and many others) the acetylated serine in COX-1 is exclusively named serine$_{530}$ – independent of kind and source of the COX protein – and the homologous serine of COX-2 is named serine$_{516}$.

The half-life of inactivation of the platelet COX-1 in vitro amounted to 10–20 min at 100 µM aspirin and is similar to that of the COX-1 enzyme from sheep seminal vesicles (Table 2.2.1-1). The acetylation of platelet COX-1 is dose-dependent at single oral doses of 20–650 mg to man and is maintained over 2 days after one single dose of 325 mg aspirin [40]. Further studies indicated that binding of *one* acetyl group per molecule was sufficient for full enzyme inhibition. Replacement of serine by alanine, an amino acid without an acetylation site, prevented the inhibition by aspirin but did not change the catalytic activity of the enzyme [41]. This suggested that serine$_{530}$ was essential

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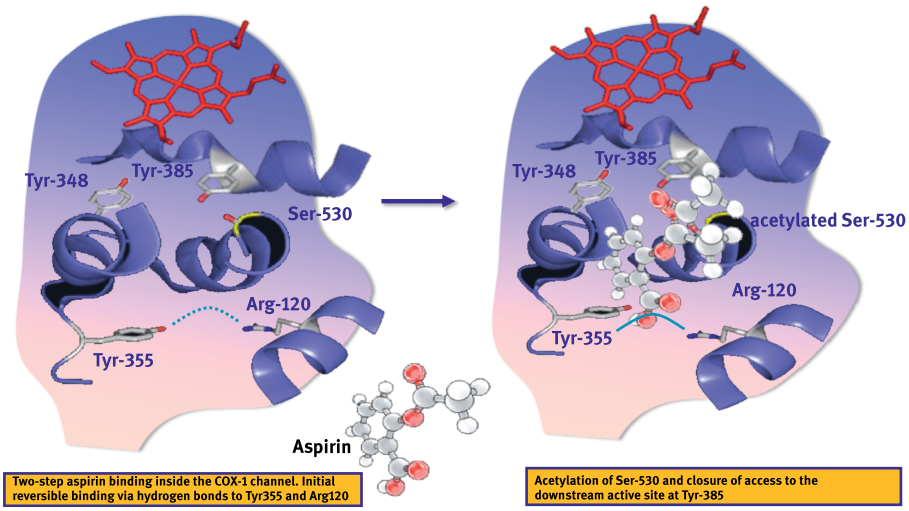

Figure 2.2.1-4: Two-step binding of aspirin inside the COX-1 channel: (1) reversible binding of the salicylate moiety via hydrogen bonds and (2) fixation of the whole molecule in the correct steric position by acetylation of serine$_{530}$ – the active center at tyrosine$_{385}$ remains unattached (adapted from [3, 27]).

for the action of aspirin but not for COX-1 enzymatic activity. Replacement of serine$_{530}$ by asparagine abolished the COX activity but increased the peroxidase activity of the prostaglandin synthase complex (Table 2.2.1-1). This indicated that aspirin was a selective inhibitor of the COX but not of the peroxidase activity of the PGHS complex. It additionally suggested that COX and peroxidase activities of PGHS could be altered independently of each other [42].

Table 2.2.1-1: COX and hydroperoxidase activities of virally transformed mutants of PGG/PGH synthase of sheep seminal vesicles. Acetylation of serine$_{530}$ by aspirin in the wild-type (wt) COX protein results in complete loss of COX activity without inhibition of peroxidase activity of the complex. Replacement of serine$_{530}$ by alanine (no acetylation site) does not change the COX or peroxidase activity of the complex but makes the enzyme resistant against aspirin. Replacement of serine$_{530}$ by asparagine inhibits the COX activity completely but increases the peroxidase activity (after data in Refs. [17, 42, 43]).

mutated amino acids	COX activity [nmol/min × g]	K_m-AA [μM]	enzyme-half life in the presence of aspirin [min]	peroxidase- activity [nmol/min × g]
none (wt)	450	7	30	70
serine$_{530}$ acetylated	0	–	–	70
serine$_{530}$ ↔ alanine$_{530}$	388	8	stable	79
serine$_{530}$ ↔ asparagin$_{530}$	0	–	–	222

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The "active site" of the enzyme, responsible for COX-1 enzymatic activity, was identified at tyrosine$_{385}$ [17, 18, 43]. Its deletion resulted in complete loss of enzyme activity [42]. Aspirin did not directly interact with this active site but rather caused a steric hindrance of access of the substrate arachidonic acid to this active site by introducing a "bulky" constituent after binding to the serine$_{530}$ hydroxyl group [3]. This hypothesis was confirmed after the crystal structure of the enzyme and the acetylation site were elucidated by *Michael Garavito*'s group [23, 27]. They confirmed serine$_{530}$ as the acetylation site and its location at the end of a hydrophobic tunnel which had to be passed by the substrate (arachidonic acid) to reach the more distal active site of the enzyme. In addition, arginine$_{120}$ and tyrosine$_{355}$ at the proximal membrane site of the channel fix the ligands in a particular steric position by hydrogen bonds [3] (Fig. 2.2.1-4).

Dioxolanes. In addition to prostaglandins and thromboxanes, there are other COX-1-derived products that are generated via an aspirin-sensitive pathway of arachidonic acid peroxidation. Stimulation of human platelets by thrombin results in the generation of a new group of eicosanoids, the dioxolanes, the first representative being DXA_3 [44]. This platelet-derived eicosanoid stimulates neutrophils and is possibly involved in platelet–white cell interactions via expression of the integrin Mac-1 [44]. Interestingly, the synthesis of DXA_3 is blocked in man by antiplatelet doses of aspirin but is independent of thromboxane formation (Fig. 2.3.2-2). Dioxolanes amplify immune and inflammatory reactions which are triggered by platelets. Inhibition of their platelet-dependent production subsequent to platelet stimulation by inflammatory stimuli such as thrombin might well contribute to the antiinflammatory and antithrombotic actions of aspirin at antiplatelet doses (Sections 2.3.2 and 4.2.2).

Acetylation of COX-2. The acetylation of COX-2 by aspirin occurs at the same, structurally homologous serine (serine$_{516}$) [45, 46]. In addition, the inhibition of prostaglandin production in vivo is frequently incomplete. Similar to aspirin, salicylate also blocks the enzyme activity but requires substantially higher concentrations. Interestingly, several *in vitro* studies of COX-1 and COX-2 inhibition by aspirin indicated that the inhibitory concentrations of aspirin for COX-1 and COX-2 were similar, both in the low micromolar range [20, 47]. See also Fig. 2.2.1-5.

This suggests that the much lower potency of aspirin to inhibit COX-2 in vivo has pharmacokinetic reasons. One is the much faster turnover and recovery rate of the enzyme as opposed to (platelet) COX-1. Another is the short half-life of unmetabolized, active aspirin which allows acetylation only within a small time window.

As already seen with COX-1, replacement of serine$_{530}$ in COX-2 by alanine inhibited the aspirin acetylation reaction but reduced the enzymatic activity by only 50 %, while replacement of tyrosine$_{385}$ by phenylalanine by site-directed mutagenesis completely prevented any COX activity. All of these procedures left the peroxidase activity unchanged [18]. Overall, the experimental data agree with the clinical observation

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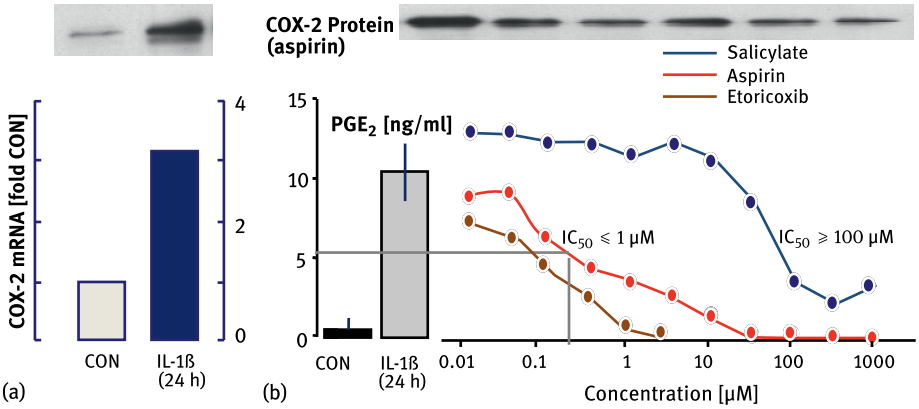

Figure 2.2.1-5: (a) IL-1β-induced stimulation of PGE_2 generation via enhanced expression of COX-2 in vascular cells. (b) Concentration-dependent inhibition of enzyme activity and PGE_2 production by aspirin and salicylate as compared to the COX-2-selective inhibitor etoricoxib. Note the reduced expression of COX-2 protein with increasing concentrations of aspirin, i. e., increasing reduction of the enzyme mass, probably indicating reduced, cAMP-mediated feedback upregulation of COX-2 transcription by PGE_2 (Schrör & Rompel, unpublished data).

that inhibition of COX-2-derived prostaglandin formation by aspirin in vivo is usually incomplete, even at higher doses (cf. Fig. 3.2.1-3).

"Aspirin-triggered lipoxin". In contrast to COX-1, acetylation of COX-2 not only inhibits COX activity, that is, generation of PG-EPs, but also alters the steric structure of the enzyme and its functionality towards that of a 15-lipoxygenase. The modification results in the generation of a new product, 15-(R)-HETE, at at least 10-fold higher amounts than COX metabolites (PGE_2) [45, 46, 48, 49]. This reaction is aspirin-specific and not shared with NSAIDs or coxibs. In contrast to aspirin, the production of 15-(R)-HETE by aspirin-acetylated COX-2 is inhibited by some traditional NSAIDs and selective COX-2 inhibitors (Fig. 2.3.2-6) [43, 49]. Elucidation of the crystal structure of the acetylated enzyme has shown that acetylation of $serine_{530}$ prevents the access of the substrate arachidonic acid to the hydrophobic side pocket of COX-2 and that this acetylation reaction was also preceded by reversible binding of salicylate [24].

This unique interaction of aspirin with COX-2 led to the hypothesis that the generation of 15-(R)-HETE by acetylated COX-2 may not just be a removal of metabolic "waste" but rather might serve specific purposes. *Charles Serhan* and his group were the first to show that 15-(R)-HETE was indeed the precursor of a new class of lipids, the lipoxins. These compounds resulted from a synergistic interaction of the acetylated COX-2 (15-(R)-HETE) with 5-lipoxygenase from white cells (Fig. 2.2.1-6) [50]. ATL not only contributes to the antiinflammatory actions of aspirin (Section 2.3.2) but might

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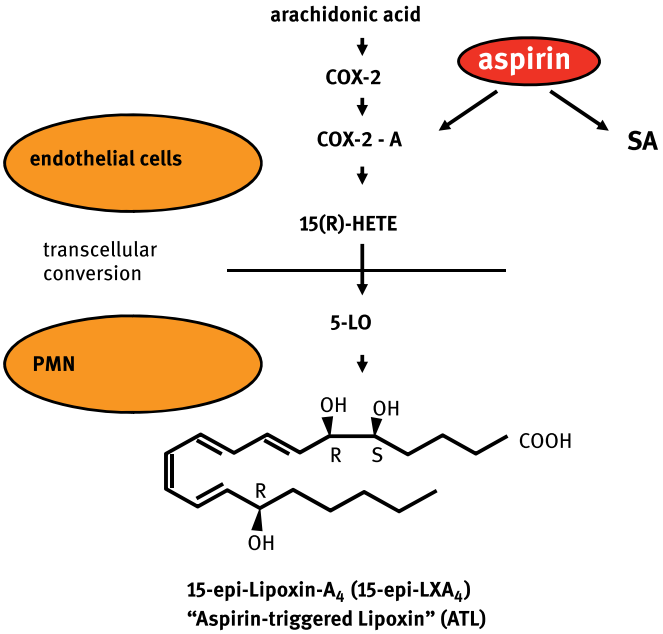

Figure 2.2.1-6: Generation of 15-(R)-HETE by acetylated COX-2 (COX-2-Ac) and its intercellular conversion to 15-epi-lipoxin A_4 (15-epi-LXA_4) or "aspirin-triggered lipoxin" (ATL) by 5-lipoxygenase(s) (5-LO) of polymorphonuclear cells (PMN. SA: Salicylic acid) (after [50]).

also be involved in endothelial protection by stimulation of eNOS and NO formation [51]. Stimulation of eNOS was also shown for the acetylated COX-2 (Fig. 2.3.1-9) [52].

The generation of (R)-precursors of biologically active eicosanoids by aspirin is not limited to arachidonic acid but was also seen with docosahexaenoic acid and eicosapentaenoic acid, two precursors of series "3" prostaglandins and thromboxanes. Interaction of dosahexaenoic acid with aspirin-treated COX-2 in human endothelial cells resulted in the generation of 17-(R)-hydroxy docosahexaenoic acid (17-(R)-HDHA). Human polymorphnuclear leukocytes (PMN) transform COX-2–aspirin-derived 17-(R)-HDHA into two sets of novel di- and trihydroxy products – the resolvins. Analogous pathways exist for eicosapentaenoic acid. Resolvins and the related protectins are potent stereoselective agonists that control the duration and magnitude of inflammation and work in cooperation with ATL. They are generated within the inflammatory resolution phase and down-regulate leukocytic numbers at the inflammatory site to prepare for orderly and timely resolution [53, 54].

Overall, lipoxins are an exciting new class of antiinflammatory and proresolving lipid mediators. However, most data so far come from in vitro or animal studies, and more clinical data are clearly required. Specifically, there is little or no information about the half-lives of acetylated enzymes and other aspirin targets, which is definitely needed to understand the clinical significance of these products in greater detail (Charles Serhan, personal communication).

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15-(R)-prostaglandins. More recent studies indicated that acetylated COX-2 also retains residual COX activity, forming predominantly 15-(R)-configuration prostaglandins, although the catalytic efficiency was reduced 10-fold. Aspirin increased 15-(R)-PGD_2 but not 15-(R)-PGE_2 levels in isolated human leukocytes activated with LPS to induce COX-2. 15-(R)-PGD_2 inhibited human platelet aggregation induced by the TX-receptor agonist U46.619 with half of the potency of 15-(L)-PGD_2, and this effect was abrogated by an antagonist of the DP_1 prostaglandin receptor. Thus, acetylation of $serine_{516}$ in COX-2 not only triggers formation of 15-(R)-HETE and allows for subsequent lipoxin formation but also causes oxygenation and cyclization of arachidonic acid to a 15-(R)-prostaglandin endoperoxide. These 15-(R)-prostaglandins are novel products of aspirin treatment via acetylation of COX-2 and may contribute to its antiplatelet and other pharmacologic effects [55].

2.2.1.3 The different pharmacology of aspirin and NSAIDs

COX inhibition by traditional NSAIDs, coxibs and salicylates. Most traditional NSAIDs inhibit both COX-1 and COX-2 at concentrations that are obtained at therapeutic doses in vivo (Fig. 2.2.1-7) [4, 56, 57]. Paracetamol (acetaminophen) is a selective inhibitor of COX-2 [19, 58, 59], but in contrast to coxibs fails to do so in inflammatory conditions. The likely reason is its markedly reduced antiinflammatory/antiplatelet po-

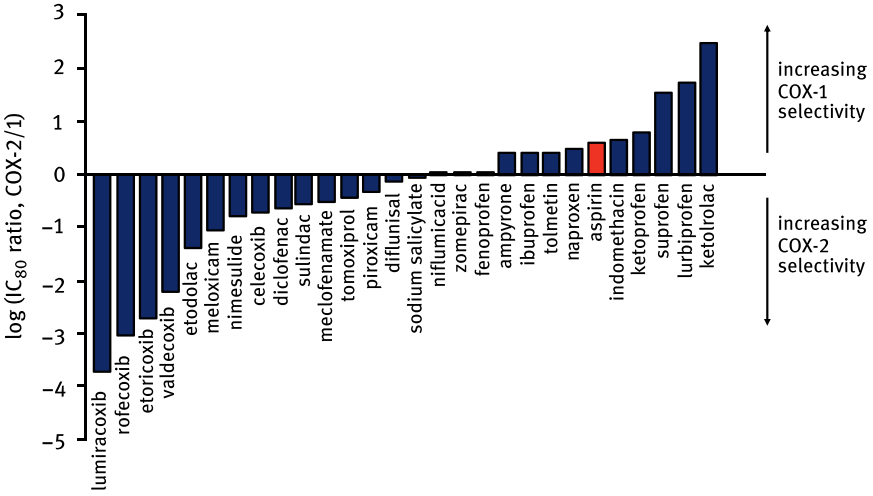

Figure 2.2.1-7: Relative selectivity of agents as inhibitors of human COX-1 and COX-2, displayed as the ratio of IC_{80} concentrations. Inhibitor curves for compounds against COX-1 and COX-2 were constructed in a human-modified whole blood assay and used to calculate IC_{80} concentrations. The IC_{80} ratios are expressed logarithmically so that 0 represents the line of unity, that is, compounds on this line are equipotent against COX-1 and COX-2. Compounds appearing above the line are COX-1-selective, those below the line are COX-2-selective [4].

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tency at high local peroxide tone, for example in inflamed tissue (Section 2.3.2) [60] and platelets [61].

The so-called "selectivity" of traditional NSAIDs for inhibition of COX-1 and COX-2 isoenzymes and the different results reported for this "selectivity" with laboratory assays have raised questions regarding the transfer of these experimental data to clinical use. In many cases, however, quantitatively different data may result rather from different study procedures than from different intrinsic drug properties.

In contrast to the constitutively expressed COX-1, the expression of COX-2 protein in most nucleated cells has first to be induced by appropriate stimulation before inhibition of enzyme activity can be measured. The natural stimuli change (increase) cellular activity, which is associated with upregulation of COX-2. For these stimuli (tumor promoters, inflammatory interleukins, endotoxin and others) upregulation of COX-2 is only one of many biological activities and rather an accompanying phenomenon than a causal factor. In addition, the extent of upregulation and subsequent product formation by COX-2 depend upon the duration and intensity of the stimulus.

It is, therefore, not surprising that the sensitivity of COX-2 to inhibitors varies in dependency on the assay used. Frequently used assays are: purified enzyme preparations, COX-2-transfected cells with constitutive (high) COX-2 activity or transient upregulation of COX-2 expression by chemicals in otherwise normal diploid cells. In this context, the frequently used approach to study COX-2 inhibition after upregulation of the enzyme by a 24-h in vitro incubation of whole blood with endotoxin appears not to be very physiological. However, it is probably closer to real-life conditions (sepsis!) than a constitutive permanent COX-2 activity in genetically manipulated (tumor) cell lines with unlimited survival.

Interaction of NSAIDs with aspirin. The binding of NSAIDs and aspirin (salicylate) to similar sites in the lining of the hydrophobic substrate channel of COX will result in competition for common binding sites. This "fight" is usually won by the NSAIDs since these compounds exhibit a considerably higher affinity (lipophilicity) to these binding sites than salicylate [30, 42]: The IC_{50} values for NSAIDs are usually in the low micromolar range, i. e., the same range as arachidonic acid (8 µM) [42], while the binding affinity of aspirin (salicylate) is two to three orders of magnitude lower and may be further reduced in the presence of competing free arachidonic acid [57]. NSAIDs and salicylate interact with two sites inside the COX channel: the catalytic site and a supplementary site. Salicylate interacts more efficiently with the supplementary site and, therefore, will antagonize the COX inhibition by aspirin as well as that of some other NSAIDs, such as indomethacin [30].

This interaction between NSAIDs and salicylates is particularly relevant to antiplatelet effects of aspirin as a consequence of COX-1 inhibition. This was first shown for indomethacin [30] but later confirmed for a number of other NSAIDs, most notably ibuprofen, both in vitro [62–64] and in vivo [34, 65], diclofenac being a remarkable exception [66]. More recently, pyrazoles, such as dipyrone (metamizol), were also found to prevent aspirin-induced acetylation of COX-1 [67]. If these compounds are given

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shortly before aspirin, they may occupy salicylate binding sites inside the COX protein and prevent the access of aspirin to its acetylation site. Since NSAIDs act reversibly, the duration of this interaction is determined by the half-life of the active compound, in most cases a few hours. This time is sufficient for deacetylation of aspirin by esterases, which occurs within less than 30 min. Thus, aspirin might have lost its antiplatelet activity because the active form is no longer present when the acetylation site at the enzyme becomes free again. Functionally, this results in an abolition of the antiplatelet effects of aspirin (Section 2.3.1) [34] or aspirin "resistance" (Section 4.1.6). Interestingly, inhibition of the platelet COX-1-mediated antiplatelet effects of aspirin is also seen by coadministration of NSAIDs, such as ibuprofen, for some days at conventional analgesic doses (400 mg three times daily or more) despite continuous aspirin treatment (Fig. 4.1.1-10). This might have an impact on aspirin use in cardiocoronary prevention (Section 4.1.1). No such interactions exist for selective COX-2 inhibitors [68].

Synthesis of new "aspirin-like" drugs. Aspirin is the only known compound within the group of NSAIDs and coxibs that covalently modifies COX-1 and COX-2. Attempts have been made to create a real "aspirin-like" drug, i. e., an irreversibly acting compound, with a higher COX-2 selectivity. This was done by modifying the side chain of aspirin, in order to improve its access to the COX-2-specific side pocket. This resulted in the synthesis of APHS, which inhibits COX-2 60-fold more potently and 100-fold more selectively than COX-1 (Fig. 2.3.1-9, Section 2.3.1) [69, 70]. APHS appeared to be the first selective, covalent inhibitor of COX-2 which inhibits COX-2 by serine acetylation like aspirin. APHS qualitatively differs in this mode of action from both reversible-type NSAIDs and coxibs [71, 72]. A model of its reaction kinetics has been developed [72]. However, its efficacy is lower than that of coxibs and no data about clinical testing have been published so far.

2.2.1.4 Further actions of salicylates on lipid mediators

Arachidonylglycerol and anandamide. Neutral lipids such as arachidonyl glycerol and anandamide (Fig. 2.2.1-1) are also high-affinity substrates of COX-2 and, therefore, possible targets of aspirin. Both lipids are also endogenous ligands of cannabinoid (CB) receptors which are relevant to pain and inflammation. Unfortunately, data on these compounds in pain and inflammation are scarce (Section 2.3.2).

Summary

Aspirin inhibits PG and TX biosynthesis primarily by posttranslational acetylation of COX-1 and COX-2 proteins. In addition, both aspirin and salicylate also inhibit COX-2 gene transcription by interaction with the binding of transcription factors to the promoter regions of the genes.

The mechanism of posttranslational COX inhibition by aspirin has been elucidated. Aspirin binds covalently to $serine_{530}$ and the homologous $serine_{516}$ in the substrate channels of COX-1 and COX-2, respectively. This is preceded by an initial reversible binding of the salicylate carboxyl

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moiety inside the COX substrate channel. This binding brings the acetyl group in a steric position at serine$_{530/516}$ that allows for covalent binding and inhibition of access of substrate (arachidonic acid) to the downstream catalytic site of the enzyme at tyrosine$_{385}$. The peroxidase reaction of the enzyme is not affected.

Acetylation of (platelet) COX-1 by aspirin results in concentration-dependent and finally complete inhibition of thromboxane/prostaglandin biosynthesis. Acetylation of COX-2 also inhibits prostaglandin formation. However, it also causes conversion of the COX into a 15-lipoxygenase activity, which generates a new compound, 15-(R)-HETE. This hydro(pero)xy fatty acid is substrate for 5-lipoxygenases of white cells and precursor of 15-epi-lipoxin A_4 or ATL, an antiinflammatory and inflammation-resolving mediator.

Competitive-type, highly lipophilic NSAIDs as well as several pyrazole analgesics (dipyrone) interact with aspirin because of interference with its binding within the COX substrate channel. The short half-life of aspirin in blood (20-30 min) allows for rapid deacetylation by plasma/red cell aspirin esterases and loss of the antiplatelet effect of circulating aspirin. Traditional NSAIDs and COX-2-selective inhibitors antagonize the activity of acetylated COX-2 to synthesize 15-(R)-HETE and subsequent generation of ATL. The potential medical value of newly synthesized, "aspirin-like" noncompetitive COX-2 inhibitors, such as APHS, remains to be determined.

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2.2.2 COX-independent actions of aspirin on cell function

2.2.2.1 General aspects

With the exception of one study, providing evidence for endogenous biosynthesis of salicylate from benzoic acid in men [1], there is general agreement that natural salicylates are solely made by plants. Here, they are considered a cell-based defense system that becomes rapidly upregulated in response to any kind of noxious stimuli and protects plants from injury in the hostile environmental conditions. Inhibition of prostaglandin formation is not involved since plants cannot make them. However,

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plants have a peroxidation pathway of C18 polyunsaturated fatty acids which exhibits a number of similarities to the eicosanoid system in animals and human. This suggests that salicylic acid and aspirin – a chemically "processed" salicylate – might also exhibit pharmacological actions independent of prostaglandins, thromboxanes and other eicosanoids and that these actions are somehow protective for the organism. In fact, a number of COX- and prostaglandin-independent but salicylate-sensitive pathways of tissue protection have also been detected in animals and human. Among them and discussed in more detail below are several transcription factors, kinases and iNOS: NF-κB, C/EBPβ [2], runt-related transcription factor-1 (Runx-1) [3], HMGB-1 [4] and others.

2.2.2.2 Salicylates in the plant kingdom

Salicylates as an integral part of cell defense. The probably most convincing evidence for prostaglandin-independent though biologically most significant protective actions of salicylates is their biosynthesis and function in plants. Salicylates, specifically the salicylate ester salicin, are an integral part of a highly regulated cell-based defense system that protects plants from environmental injuries, including injuries by bacteria and viruses. In response to these noxious stimuli, salicylate formation can be substantially upregulated at the transcriptional level (Section 1.1.1) [5, 6]. Methylsalicylate, a volatile compound made by a number of plants, is even suggested to act as a mobile airborne signal in plant defense which activates systemic resistance against a broad spectrum of pathogens [7, 8]. Experimental knock-down of this system, for example by suppression of upregulation of salicylate biosynthesis in response to injury, results in loss of resistance, subsequent severe cell injury and, ultimately, cell death (Fig. 2.2.2-1) [5, 6].

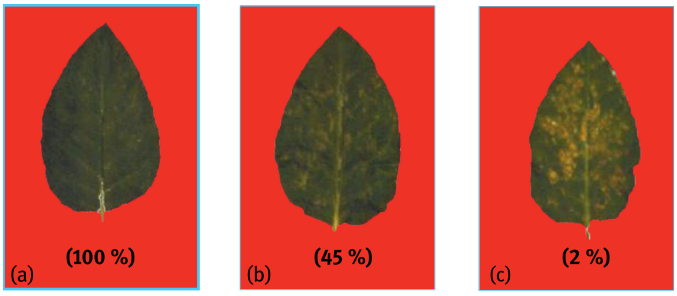

Figure 2.2.2-1: Salicylate-mediated resistance of tobacco leaves against infection by tobacco mosaic virus. (a) Plants with normal salicylate production are resistant. (b and c) Resistance is reduced after reduction of salicylate generation to 45 % (b) and apparently absent after complete prevention (2 %) of salicylate generation (c) (modified after [5] – with kind permission of The American Association for the Advancement of Science).

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Plants can synthesize (polyunsaturated) fatty acids only up to a C18 hydrocarbon (C18) backbone. Thus, neither arachidonic acid (C20) nor prostaglandins or other arachidonic acid-derived lipid mediators can be formed. However, plants can synthesize jasmonic acid from the C18 fatty acid α-linolenic acid via a lipid peroxidation pathway that has much in common with the prostaglandin system of animals. Interestingly, in this system aspirin inhibits allene oxide synthase (AOS), the CYP that initiates plant oxylipin synthesis. This results in irreversible inactivation of this enzyme (Fig. 2.2.2-2).

Functionally, generation of salicylates in plants represents a prostaglandin-independent protective system, acting via modulation of biologically active lipid peroxides (oxylipines) and synergizing with other defense systems which increase plant resistance and improve recovery after injury [10–13]. These and other discoveries on salicylates in plants have also provided several innovative approaches for the development of insecticides and pest management.

2.2.2.3 Non-COX acetylation actions of aspirin

Multiple cellular targets. In addition to effects on COXs as discussed in detail before (Section 2.2.1), aspirin and salicylate also exhibit a broad spectrum of other pharmacological actions on cell functions [2, 12, 15]. These include nonselective and nonspecific transacetylations which may occur at any appropriate molecular site (amino acids) in any macromolecule. Most notable examples are plasma proteins, such as albumin, hemoglobin and fibrinogen [16], red cell membranes [17], DNA, histones and others [18–22]. Further targets are transcription factors as well as low-molecular weight metabolites, such as coenzyme A [23]. Lysine appears to be the most frequently acetylated amino acid in proteins (Fig. 1.1.5-1) [24] at low-to-medium concentrations of aspirin (≥100 μM) [25]. A proteomic analysis of several cancer cell lines has identified 120 proteins which were acetylated by aspirin. Threshold concentrations were around 100 μM [26]. These concentrations can be achieved by oral aspirin doses of 1–2 g. The biological consequences of these multiple transacetylations as well as their control by deacetylases (Section 2.1.2) [27] are incompletely understood but might be considerable. One example is the acetylation of core histone proteins in cancer cells, implicating aspirin as a potential regulator of gene transcription [26].

The duration of biological effects of these covalently, i. e., irreversibly, modified proteins will be determined by their turnover rate and local activity of aspirin deacetylases rather than by the short – about 20 min – half-life of active aspirin in blood. Albumin has a half-life of 19 days, the life span of red cells amounts to 3 months and malignant tumor cells keep dividing endlessly.

2.2.2.4 Aspirin and nitric oxide formation

Salicylates and iNOS. NO is generated by NO-synthases. At least two out of the three NO-synthases are targets of salicylates: iNOS in macrophages, fibroblasts and vascu-

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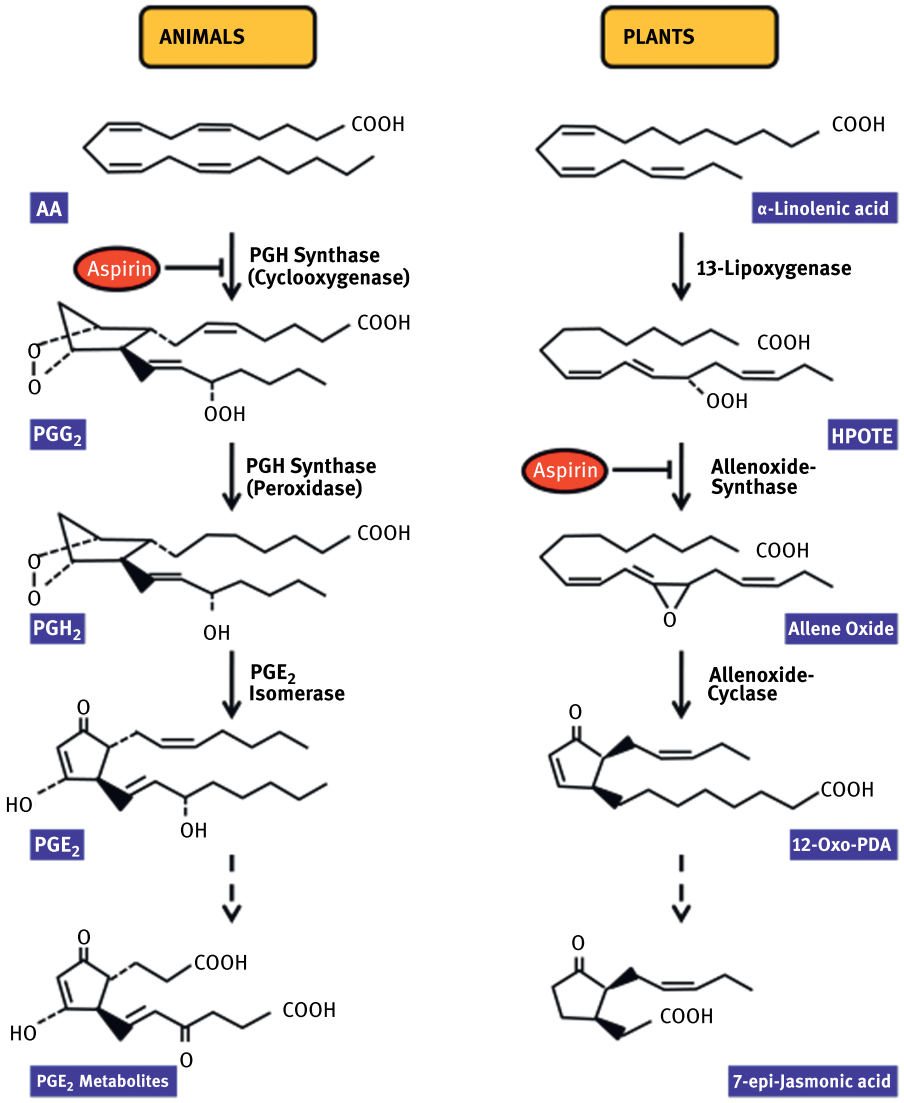

Figure 2.2.2-2: Biosynthesis of biologically active lipid peroxides (oxylipines) from α-linolenic acid plants as compared to lipid peroxide metabolites from arachidonic acid (PGE_2) via the COX pathway. Inhibition of product formation by salicylates (aspirin). Abbreviations: AA: arachidonic acid; PGH: prostaglandin H; HPOTE: hydroperoxy-9(Z),11(E),15(Z)-octadecatrienoic acid, PDA: phytodienic acid (modified after [10]).

lar cells and the constitutive eNOS in endothelial cells and platelets. Transcriptional inhibition of cytokine-induced iNOS expression contributes to the antiinflammatory effects of aspirin [28–31]. Together with inhibition and modulation of COX-2 transformation towards 15-HETE and subsequent lipoxin production, this will enhance the

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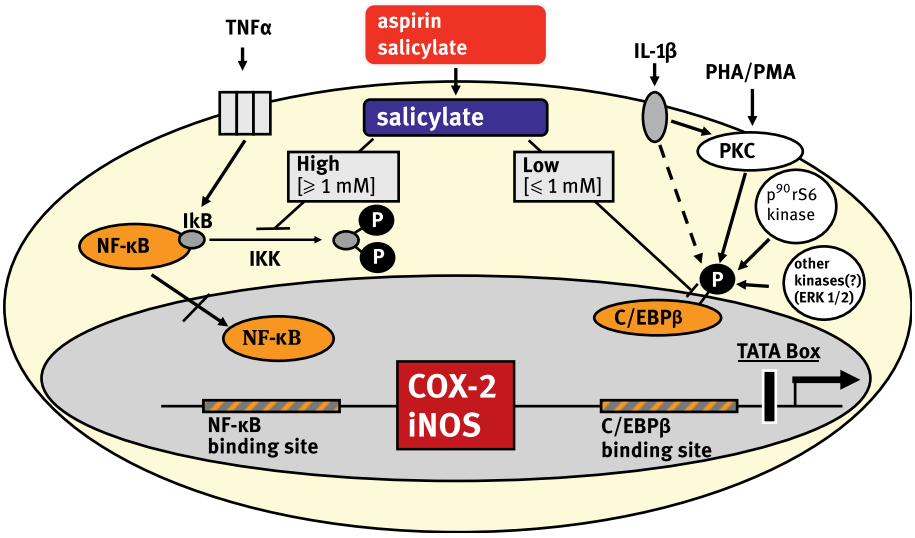

Figure 2.2.2-3: Different control of COX-2 and iNOS promotor activity by salicylates via C/EBPβ and NF-κB. Aspirin at lower concentrations (<1 mM) inhibits phosphorylation of C/EBPβ by inhibition of several kinases, most notably PKC which is stimulated by extracellular activators such as IL-1ß and PHA/PMA. At higher concentrations (>1 mM) salicylate also inhibits IκB kinase (IKK) and subsequent activation of NF-κB (for further explanation see text) (modified after data in [30–33]). Abbreviations: NF-κB: nuclear factor κB; TNFα: tumor necrosis factor α; PHA: phythemagglutinin acetate; PKC: protein kinase C; PMA: phorbolmyristate acetate; IL: interleukin.

antiinflammatory potency of aspirin at concentrations around 1 mM and will functionally synergize with COX-2 inhibition (Fig. 2.2.2-3).

Salicylates and eNOS. In contrast to the inhibition of iNOS by aspirin and salicylate, only aspirin but not salicylate stimulates endothelial NO generation via eNOS by a posttranslational protein modification [34]. The expression of eNOS protein remains unchanged [35]. This effect is seen at low, antiplatelet concentrations (<1 µM) and is due to eNOS acetylation [34], having activation of heme oxygenase-1 (HO-1) as a downstream target. Stimulation of endothelial (and platelet) NO formation is considered vasoprotective [34, 36]. It will not only improve local perfusion and inhibit platelet activation, but also improve the antioxidative potential of affected cells (Section 2.3.1) [37].

Concentration-dependent acetylation of several serine residues and stimulation of platelet NO production were shown in vitro with aspirin concentrations around 10 µM [38]. Human studies additionally showed that acute high-dose intravenous aspirin (800 mg) but not chronic oral treatment (75 mg/day for two weeks) stimulated eNOS activity in platelets. The inefficacy of low-dose aspirin was assumed to be due to the much lower plasma level of nonmetabolized aspirin [39]. Mechanistically, ly-

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sine acetylation of eNOS has been reported [40]. Interestingly, enhanced formation of ATL, an antiinflammatory compound, also induced NO formation via eNOS and iNOS. Aspirin lost its antiinflammatory action in either eNOS or iNOS knockout mice with IL-1β-induced peritonitis [41]. These data collectively suggest that NO-mediated actions of aspirin might contribute to its antiinflammatory activity and oxidative stress at least at higher doses in vivo (Section 2.3.2).

2.2.2.5 Salicylates and kinases

Phosphorylation of proteins by kinases is a central mechanism in cell biology to regulate enzyme activities. To become active, kinases have first to be phosphorylated themselves. This process starts by binding of ATP to an ATP (substrate) binding site of the enzyme. Subsequently, the active site of the enzyme becomes phosphorylated by transfer of energy-rich phosphate. The activated phosphate group is then transferred by the kinase reaction to a target substrate, such as another enzyme or transcription factor, eventually resulting in a biological response.

Modes of salicylate action. Salicylates can interact with these processes at several levels: One is the occupation of the ATP binding site because of structural analogies between the ring structures of the nucleotide and salicylic acid. This reaction is competitive, reversible and stoichiometric at a 1:1 relationship and requires higher concentrations of salicylates (≥1 mM). Another kind of interaction is direct interference with kinase activity by (steric) interaction with the transfer of the energy-rich phosphate from the ATP binding site to the active site of the kinase, for example after binding to another binding site, such as an arginine (by analogy with $arginine_{120}$ in COX-1). This reaction is noncompetitive and nonstoichiometric and probably requires lower concentrations of salicylates (≤1 mM). A hypothetical model of these interactions is shown in Fig. 2.2.2-4.

Nonselective salicylate actions on kinases. Inhibition of kinases by salicylates is a both simple and comprehensive explanation for the diversity of (high-dose) salicylate actions on cell functions. The consequences of kinase inhibition for cell function are then determined by the function of the particular phosphorylated target protein or transcription factor, respectively. In this sense, salicylate actions are cell-specific. Salicylate concentrations of 5–10 mM in vitro are likely to inhibit most if not all kinases that have been investigated so far [42]. Interestingly, a recent study has shown that salicylate but not aspirin *in*creases the activity of adenosine monophosphate-activated protein kinase (AMPK) in several cell lines in vitro at concentrations of 5–10 mM. This kinase is considered a cellular energy sensor that maintains the balance between ATP production and consumption. These findings might have a relationship to salicylate-induced changes in cellular energy metabolism [43] as well as salicylate-related anti-

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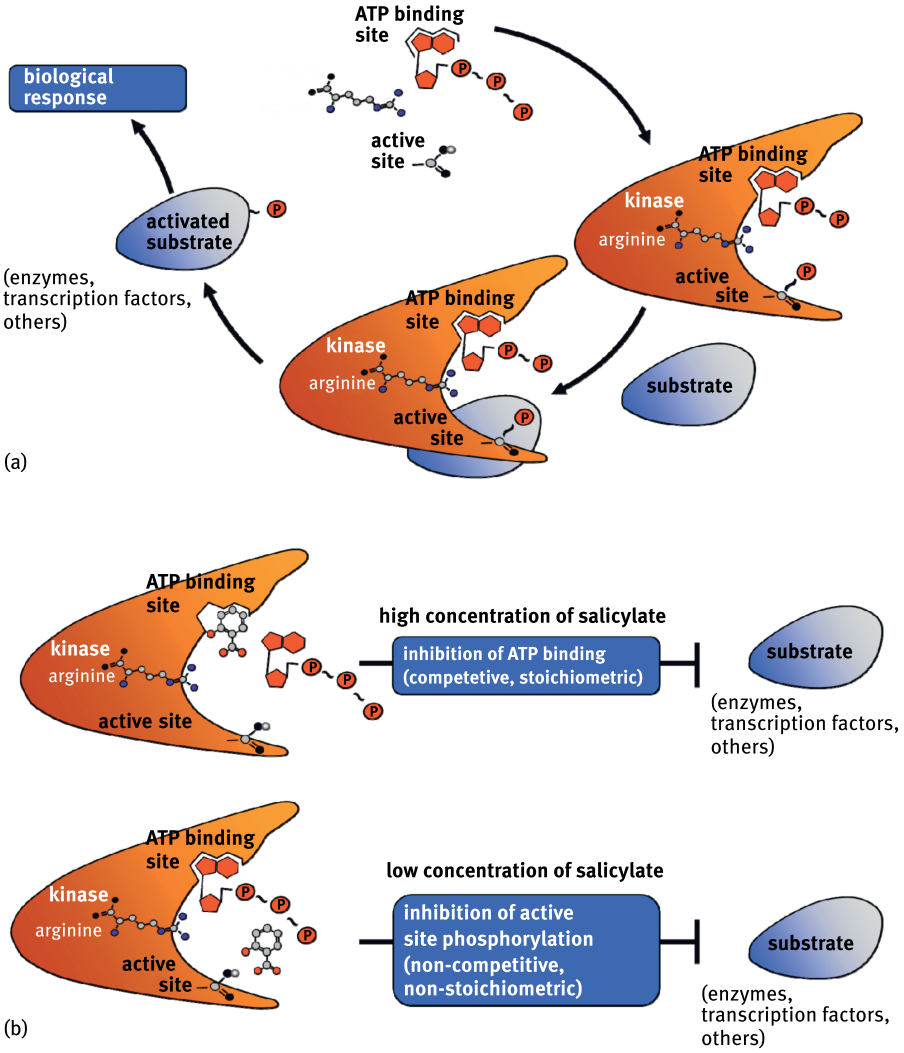

Figure 2.2.2-4: (a and b) Cellular mechanisms of kinase activation and action (a) and possible sites of modulation by salicylates (b) via inhibition of ATP binding (top) or interaction with the active site (bottom) (for further explanation see text).

inflammatory/antitumor actions (Section 2.2.3) [44]. In vivo, plasma salicylate levels of 5 mM and above are considered potentially lethal because of severe disturbances in energy metabolism, resulting from complete uncoupling of oxidative phosphorylation with subsequent collapse of most energy-dependent cellular signaling systems (Section 2.2.3). Uncoupling of oxidative phosphorylation with marked increases in membrane permeability already starts at salicylate levels of >1 mM and is apparently complete at >5–10 mM [45].

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The hypothesis of nonselective inhibition of kinases with subsequent inhibition of activation of multiple kinases as a general mode of action of salicylates was originally put forward by *Frantz* and *O'Neill* (1995) [46]. These authors showed that sodium salicylate caused a concentration-dependent inhibition of a variety of different transcription factors at low-to-medium millimolar concentrations. This effect was probably due to a nonselective inhibition of cellular kinases, necessary for transcriptional activation, since an apparently identical inhibition was seen for three functionally different kinases as well as a cell-free kinase preparation of the same cells (Fig. 2.2.2-5).

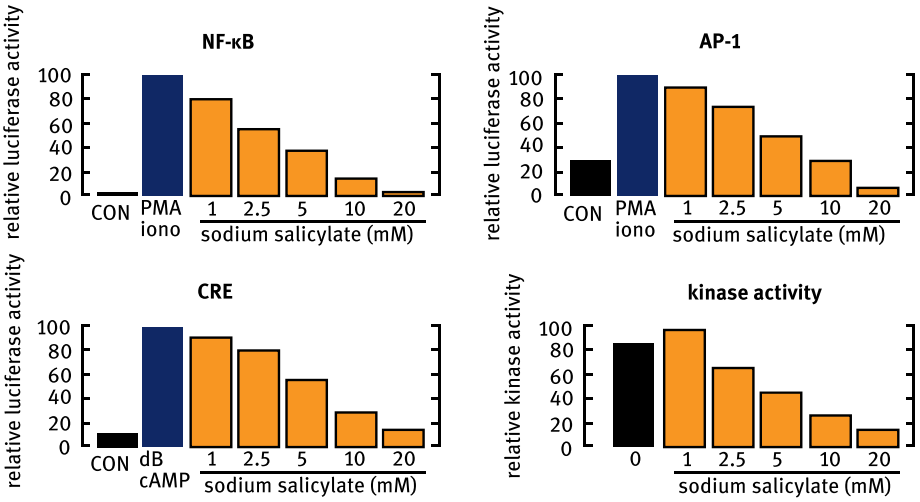

Figure 2.2.2-5: Effects of salicylates on transcriptional activation of transcription factors (NF-κB, activator protein-1 [AP-1], cyclic AMP-responsive element [CRE]) in transfected Jurkat cells. Note the apparently identical concentration–response curves for inhibition of three functionally different transcription factors after stimulation by phorbolester/ionomycin (PMA/iono) or cAMP (CRE) and an identical concentration-dependent inhibition in an acellular, nonselective kinase activity assay (for abbreviations and further explanations see text) (modified after [46]).

Specific kinase inhibition and antiinflammatory actions. Importantly, not all kinases might behave the same, i. e., become inhibited, by salicylates only at millimolar concentrations. An early study demonstrating kinase inhibition at micromolar concentrations of aspirin and salicylate via blockade of peptide-induced ATPase activity came from the group of *Kenneth K. Wu* [47]. These authors made the interesting observation that salicylates not only interact with substrate (ATP) binding to kinases but also with kinase activity, eventually resulting in inhibition of inflammatory signal transduction.

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Specific cellular binding sites of salicylates were identified in homogenates of human foreskin fibroblasts after incubation with ^{14}C-sodium salicylate. The labeled protein fraction was isolated and sequenced. This fraction contained a 15-amino acid sequence and an ATP binding site, identical with a sequence in the heavy chain of human immunoglobulin binding protein (BiP). The k_D values of salicylate binding to the crude extract and to recombinant BiP were low and apparently identical: 45 and 55 µM, respectively, suggesting that salicylates may specifically interact with this sequence. Binding occurred via the o-hydroxy group of salicylate, leaving the carboxyl function free for chemical reactions.

BiP (also known as GRP78) belongs to the heat shock protein 70 (HSP70) family. HSP70 proteins have important chaperone functions and are known as possible target for salicylates. These functions include binding of newly synthesized polypeptides, allowing for appropriate protein folding and transport across the membrane. A synthetic heptapeptide containing this particular sequence displaced salicylate from its binding in a concentration-dependent manner. Binding of the peptide-induced ATPase activity was blocked by both aspirin and salicylate at micromolar concentrations. Neither aspirin nor salicylate inhibited ATP binding or modified BiP protein expression.

It was concluded that salicylates bind specifically to the polypeptide binding site of BiP in human cells, resulting in a disturbed chaperone function of BiP, i. e., a change of its steric structure necessary for activation of specific kinases, such as ribosomal S6 kinase. In this way, salicylates may interfere with the processing of proteins important in inflammation (Fig. 2.2.2-6) [47].

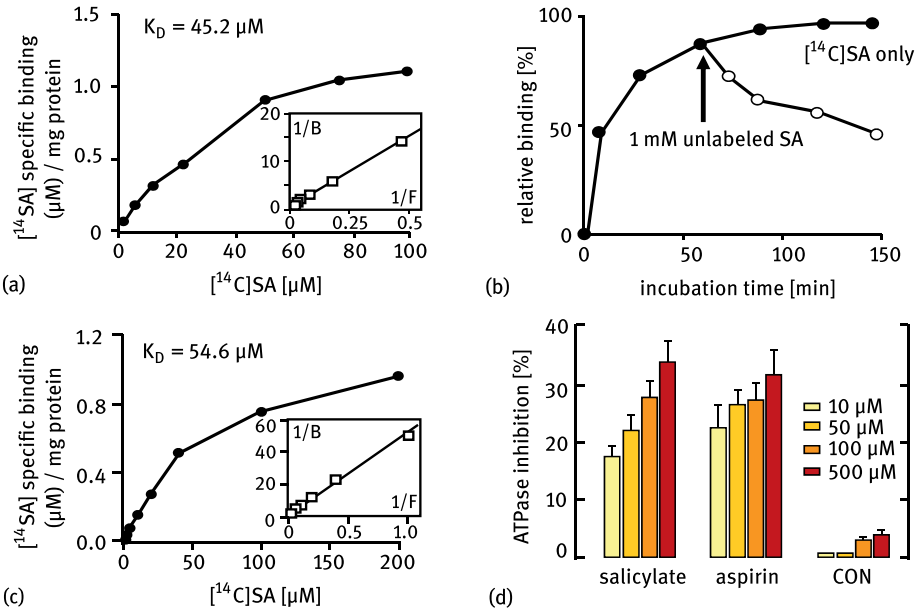

Figure 2.2.2-6: (a and b) Specific binding of [^{14}C] salicylic acid (SA) to whole cell extracts of human fibroblasts (a) and its displacement by addition of unlabeled salicylic acid (b). (c) Binding kinetics of SA similar to the fibroblasts were also found in purified recombinant human immunoglobulin "heavy chain binding protein" (BiP). Aspirin and salicylate inhibit the constitutive ATPase activity of purified recombinant BiP in a concentration-dependent manner. (d) No such effect is seen with the reference compound 3,4-dimethyoxy-γ-benzoic acid (modified after data in [47]).

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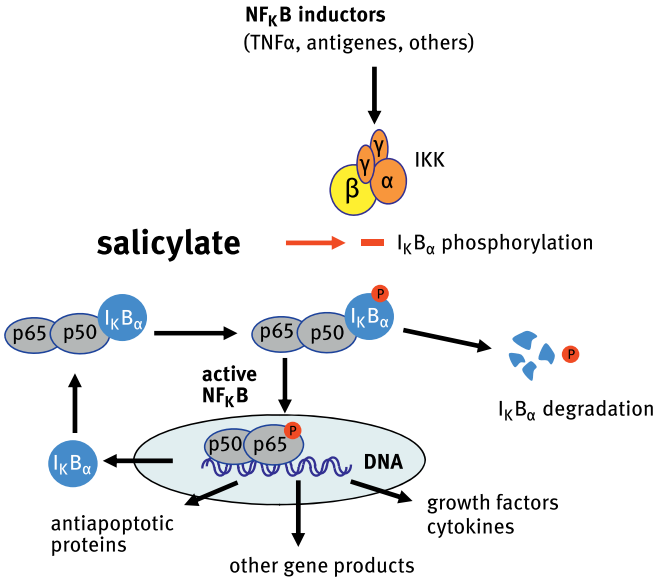

Figure 2.2.2-7: Molecular mode of inactivation of NF-κB by salicylates: Inhibition of IKKβ kinase.

Inhibition of ATP binding to kinases. In addition to inhibition of kinase activation, inhibition of ATP binding to kinases by salicylates was also discussed as an alternative mode of action of aspirin. For example, Yin et al. (1998) showed a competitive and specific inhibition by salicylates of ATP binding to the inhibitory kinase β (IKKβ). This was followed by inhibition of activation (phosphorylation) of the transcription factor NF-κB (Fig. 2.2.2-7). The effect was reversible and could be antagonized by increasing the ATP concentration [48].

IKKβ is probably not the only kinase that becomes inhibited by aspirin or salicylates via inhibition of ATP binding. Another important group of kinases are stress-activated Jun N-terminal kinases from the MAP kinase family [49] that are involved in multiple cell functions. More research in this exciting area of specific kinase–aspirin (salicylate) interactions is urgently needed.

2.2.2.6 Salicylates and transcription factors

In addition to NF-κB, salicylates can modify the binding of a variety of other transcription factors to the promoter regions of genes, eventually resulting in modifications of gene regulation as discussed above. Alternatively, there might be a direct interaction with the binding of transcription factors, such as NF-κB or nuclear factor of activated T-cells (NFAT), to the promoter region, which is kinase-independent [50–52]. In both cases, the result is the same – modulation (inhibition) of gene transcription and subsequent protein synthesis.

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The understanding of the biological significance of modulation of transcription factors by salicylates is often hampered by the fact that these changes are preferentially found in vitro and, in many cases, require high concentrations of free salicylates. Concentrations of 5 mM or above completely uncouple oxidative phosphorylation. This might be fatal for somatic cells, and there is clearly a connection between kinase inhibition and cell injury.

It is also less likely that cell lines in vitro, expressing continuously otherwise inducible genes after gene transfer or stimulation by tumor promoters, can be directly compared with "normal" somatic nontransfected cells, being subject of rather transient stimulation by cytokines or related mediators of inflammation, ischemia or immune reactions. The sensitivity of affected cells and tissues against added salicylates may be changed accordingly.

Two of the most intensively studied salicylate-sensitive transcription factors with possible relevance to salicylate actions in vivo are NF-κB and C/EBPβ, probably the most important factors of transcriptional regulation of iNOS and COX-2, the two key proinflammatory and growth-promoting genes (Fig. 2.2.2-3).

Nuclear factor κB. The nuclear factor κB (NFB)/RelA family of transcription factors regulates the expression of numerous genes involved in the control of immune and inflammatory responses, most notably tumor necrosis factor α (TNFα) and IL-1β [53]. NF-κB also controls cell survival as a regulator of the apoptotic program, either for induction of apoptosis or, more commonly, as its inhibitor. NF-κB also acts as a central regulator of longer lasting changes in cell function, including stress responses and cell survival [53, 54]. In addition, NF-κB is a key player in the interplay of inflammation and thrombosis [55].

Intracellular NF-κB resides inactive in the cytosol of immunocompetent white cells, endothelial cells and vascular smooth muscle cells as a heterotrimeric complex with the inhibitor protein IκB. Stimulation of IκB by IKK kinases results in phosphorylation, cleavage of the inhibitor and translocation of the active NF-κB heterodimer into the nucleus. IKK kinase activity is stimulated by cytokines, reactive oxygen species and numerous other stimuli. The liberated heterodimer p50/p65 activates the genes of IL-1, IL-6, TNFα, "intercellular adhesion molecule"-1 (ICAM-1), ("vascular cell adhesion molecule" (VCAM)-1, "vascular endothelial growth factor" (VEGF) and many others, participating in the regulation of inflammation, immune responses and apoptosis. The net reaction of each particular cell is determined by signaling pathways, distal to NF-κB (Fig. 2.2.2-7).

Salicylates prevent the activation of NF-κB by inhibition of IKKβ kinase-induced phosphorylation of the NF-κB trimer and the subsequent cleavage of the inactive heterotrimer into the active dimer which translocates into the nucleus. Inside the nucleus, NF-κB exerts multiple actions on gene transcription and subsequent protein synthesis.

Aspirin and salicylate inhibit NF-κB activation via inhibition of IKKβ kinase activity in numerous cells and tissues in vitro, predominantly at low millimolar concentrations [56–58]. The effects of aspirin on NF-κB are specific for salicylates and are not seen with NSAIDs. Direct inhibition of IKKβ by salicylates probably contributes to the

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hypoglycemic actions of high-dose salicylate [59], inhibition of transcriptional activation of tissue factor [60, 61], inhibition of "mammalian target of rapamycin" (mTOR) signaling [62], repression of NF-κB-driven transcription events in tumor cells [63] and neuroprotection from the excitatory amino acid glutamate [64]. Interestingly, the antiviral activity of aspirin against human rhinoviruses [65], including the inhibition of influenza virus replication, also involves inhibition of NF-κB-mediated signaling pathways in host cells (Section 2.3.2) [66].

C/EBPβ and other transcription factors. C/EBPβ, another important transcription factor, is phosphorylated by several kinases, in particular p90 ribosomal S6 kinase [32]. C/EBPβ controls transcriptional activation of COX-2, iNOS and probably several other genes which are involved in inflammatory, mitogenic and immune reactions [30, 33].

Further transcription factors which are potential targets of salicylates are "activator protein-1" (AP-1) [46], "signal transducer and activator of transcription 6" (STAT-6) [67] and NFAT. NFAT shares some homologies with NF-κB and becomes activated after *de*phosphorylation by the phosphatase calcineurin. Salicylates inhibit DNA binding and activation of this transcription factor without affecting the phosphorylation status or intracellular localization of NFAT [52]. This effect as well as most of the other actions of salicylates on transcription factors usually require concentrations in the low millimolar range, which might not be available after therapeutic aspirin doses in vivo.

Summary

The hypothesis of inhibition of prostaglandin formation as primary or even only pharmacologically relevant aspirin action in living cells is being increasingly challenged. A most convincing finding in this respect is the fact that salicylates are natural signaling molecules in plants, where salicylate biosynthesis becomes transcriptionally upregulated in response to certain environmental noxes. Salicylates are an essential determinant of plant resistance despite their inability to synthesize prostaglandins and other eicosanoids.

With improved knowledge about control of cell function and its molecular switches, it is now established that aspirin and salicylates interact with numerous cellular targets beyond the eicosanoid pathways and that biologically relevant transacetylation processes are not restricted to cyclooxygenases. At antiinflammatory concentrations of about 200–300 µg/ml (1–2 mM) and more, aspirin has numerous effects on cellular signal generation and transmission, especially in consequence to cell stimulation by inflammatory cytokines, growth factors, oncogenes or immunostimulants. Transcriptional, translational and posttranslational levels of regulation might be affected, which makes the net response difficult to predict. For example, there are different consequences of NF-κB inhibition on cell functionality and survival in neuronal tissue than in tumor cells.

Inhibition of kinases is another general mode of action of high-dose salicylates. Although this effect is rather nonspecific, the sensitivity of different kinases to salicylates may not be the same; for example, ribosomal S6 kinase phosphorylates (activates) the transcription factor C/EBPβ and is inhibited by salicylates. More work is necessary to establish the biological significance of these

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findings in vivo. These effects often require higher concentrations of salicylates than can be safely applied in vivo. They are salicylate-specific and not shared with NSAID-type compounds.

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2.2.3 Energy metabolism

Changes in expression and function of proteins as well as interactions with cellular signal transduction pathways are examples for aspirin-induced biological responses at the cellular level. In most cases, these actions are energy-dependent and will only proceed if sufficient free energy, usually provided by ATP, is available. This is the case under normal situations, but energy supply might become a limiting factor in pathologic conditions. This includes the action of environmental factors, such as drugs, that interact with cellular energy metabolism. These drug-induced metabolic disturbances are less specific than their interactions with particular cellular signaling pathways. However, they are very effective and can markedly affect generation, receipt, processing and dispatch of biological signals. In other words, they can interfere with multiple cell functions even without directly targeting specific signaling pathways. Higher-dose/concentration salicylates are an excellent example for this.

2.2.3.1 General aspects

Different pharmacodynamics of aspirin and salicylates. Mitochondria are the power plants of cells. Salicylates interact with the mitochondrial function at two levels: inhibition of β-oxidation of fatty acids and uncoupling of oxidative phosphorylation, that is, generation of ATP via the energy-providing electron transport system of the respiratory chain. Both actions are dose-dependent and are typically seen at millimolar concentrations of salicylates. Uncoupling of oxidative phosphorylation is due to the unique physicochemical properties of salicylate rather than a particular aspirin-induced acetylation process [1, 2]. The clinical correlate of this is hyperventilation, that is, increased oxygen uptake and increased heat production (sweating). Both are typical features of systemic aspirin (salicylate) overdosing (Section 3.1.1). The pathophysiological reason is the loss of excess energy that cannot be stored, in the form of heat.

Acetylation of mitochondrial proteins. Recent studies have challenged the concept that actions of aspirin on energy metabolism are solely salicylate-mediated. In human liver cells it was shown that aspirin acetylates about every enzyme involved in energy metabolism, including glycolytic enzymes, enzymes of the Krebs cycle and enzymes of fatty acid metabolism. At the molecular level, protein lysine acetylation is a prevalent modification in enzymes that catalyze intermediate metabolism. Virtually every enzyme in glycolysis, gluconeogenesis, the Krebs cycle, the urea cycle, fatty acid metabolism and glycogen metabolism was found to be acetylated in human liver tissue at medium concentrations (≤500 μM) of aspirin [3]. Glucose-6-phosphate dehydrogenase (G6PD), the central enzyme of glycolysis, becomes acetylated (inhibited) at concentrations of 100 μM; 35 % inhibition is achieved at 500 μM (Fig. 2.2.3-1) [4].

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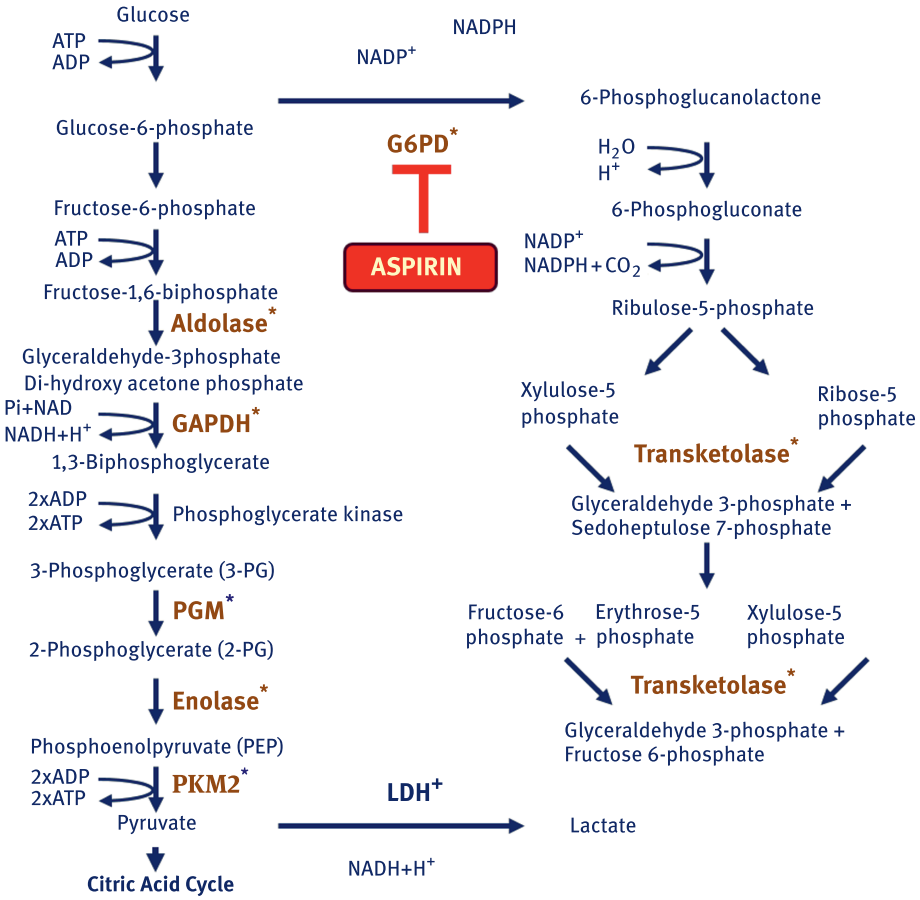

Figure 2.2.3-1: Acetylation targets (*) of aspirin in glycolysis and the pentose phosphate pathway. Aspirin acetylates six enzymes in glycolysis and two enzymes in the pentose phosphate pathway [4].

The implications of these findings for modulation of cell function by aspirin in the whole organism are unknown yet, but might be considerable, for example in cancer chemoprevention by inhibition of ribonucleotide synthesis (Section 2.3.3) [4]. In this context, protein lysine acetylation/deacetylation is an important posttranslational modification of enzyme activities, in the control not only of energy metabolism but also of cellular signaling via histones [5] and nuclear transcription factors [3]. Recent data with aspirin (5 mM in vitro for 24 h) indicated that the compound did not directly alter mitochondrial matrix fatty acid oxidation enzymes, but most likely exerted its effects at the level of long-chain fatty acid transport into mitochondria, possibly related to inhibited electron transport chain function. This acetylation probably occurred via nonenzymatic transfer of the acetyl group to lysines and was resolved within 48 h by protein turnover or mitochondrial deacetylases [6].

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Taken together, the acetylation issue is complex and has been studied so far mostly in vitro at high salicylate concentrations, but not under more reliable in vivo conditions. Therefore, this chapter will be focused on the role of deacetylated salicylates as "established" modulators of cellular energy metabolism, although a contribution of acetylation reactions should not be ignored.

The liver as pharmacological target of salicylates. The metabolic effects of salicylate and their consequences for cell function become most apparent in the liver, the main site of energy metabolism of the body. The extent depends on the concentration of free salicylic acid as well as the duration of drug exposure. The actions of salicylates on energy metabolism are probably due to their unique physicochemical properties, allowing for enrichment in cell membranes and changes in membrane permeability (see below). The subsequent changes in metabolic functions are generally reversible, even at high salicylate concentrations [7].

Salicylates are phenols and, like other phenolic compounds, such as the classical metabolic inhibitor 2,4-dinitrophenol (DNP), interact with mitochondrial proteins which are involved in oxidative phosphorylation. This interaction is due to an allosteric effect which results in changes in mitochondrial protein configuration after salicylate binding and, eventually, results in uncoupling of oxidative phosphorylation. Albumin binds phenols via the phenolic hydroxyl group and restores the capacity for oxidative phosphorylation in isolated mitochondria by removing salicylates from mitochondrial proteins [8]. Consequently, hepatic metabolic failure by salicylate is particularly prominent in vitro in protein-free media and can be antagonized by supplementation with serum albumin [9].

According to these findings, in vitro data obtained at constant levels of salicylates over hours at low or absent protein in the incubation medium cannot be directly transferred to the in vivo situation with high albumin levels and a continuous metabolic degradation, washout and transformation of aspirin and salicylates into inactive metabolites and conjugates thereof.

2.2.3.2 Salicylates and fatty acid β-oxidation

Basic mechanisms. Mitochondrial β-oxidation of fatty acids is the principal source of generation of ATP, the conserved form of energy. Disturbances become evident first in organs with high metabolic rates, such as liver, brain, heart and kidneys. Because of their amphiphilic nature, fatty acids become easily associated with mitochondrial membranes. In order to enter the mitochondrial β-oxidation process, they have to pass the outer and inner mitochondrial membranes prior to further processing in the mitochondrial matrix. Short- and medium-chain fatty acids, such as salicylic acid, can cross the mitochondrial membranes without prior activation. However, they also have to become activated by binding to CoA in an ATP-consuming process (Fig. 2.2.3-2) [10]. Long-chain fatty acids (C14–C18) first require conversion into acyl-carnitine for

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translocation across the inner mitochondrial membrane ("carnitine shuttle"). Within the mitochondrial matrix, carnitine is removed and the resulting acyl-CoA undergoes β-oxidation, resulting in the generation of reducing NADH equivalents that are subsequently stepwise oxidized within the mitochondrial respiratory chain. The energy thus produced is stored in the form of ATP [11].

Actions of salicylates. Inhibition of hepatic mitochondrial β-oxidation of fatty acids, predominantly long-chain ones, is achieved at millimolar concentrations of aspirin or salicylate (≥1.5 mM) in vitro [12]. The explanation is a lack of cofactors that are required for fatty acid transport and metabolization, such as CoA or carnitine due to formation of salicylyl-CoA and, possibly, salicyl-carnitin [12, 13]. This inhibits and finally prevents the passage of long-chain fatty acids through the mitochondrial membranes. Biochemical consequence is the intracellular but extramitochondrial fatty acid accumulation and a number of follow-up effects, including reesterification into triglycerides. The morphological correlate of these biochemical events is microvesicular steatosis.

Marked changes in liver fatty acid metabolism were found in liver biopsy specimens of patients with rheumatoid arthritis after long-term treatment with high-dose aspirin [14].

One study reported:

> The hepatic lipid distribution pattern was studied in liver specimens obtained at autopsy from seven patients with rheumatoid arthritis. All patients had taken 3.25–5.85 g aspirin daily for many years because of arthritic pain. Seven age-matched controls who had not taken aspirin were used as controls. All patients of both groups died from acute myocardial infarction and there was no known functional liver abnormality in any of them at the time of death.
>
> The total lipid content was significantly, >20 %, higher in liver biopsy specimens of aspirin-treated patients as opposed to controls without aspirin intake. Most striking differences were seen in free fatty acids which were more than doubled in aspirin-treated patients while total hepatic phospholipids were reduced by >30 %. The phospholipid depletion was due to a considerable, about 40–50 %, decrease in phosphatidyl ethanolamine, phosphatidyl choline and cardiolipin while other phospholipid classes remained unchanged.
>
> It was concluded that major metabolic impairments of fatty acid oxidation can occur in patients at long-term (years) (very) high-dose aspirin exposure. The increase in neutral lipids and free fatty acids in these patients suggests a reduced β-oxidation, indicating a relationship between abnormalities in fatty acid oxidation and aspirin intake (Table 2.2.3-1) [14].

Unfortunately, this study did not analyze the composition of the free fatty acid fraction, specifically the percentage of long-chain fatty acids, or the occurrence of dicarboxylic acids. Nor were there any morphological data of the liver specimens. Thus, there was no information about microvesicular steatosis. It is also interesting that despite markedly elevated levels of free fatty acids, there was no increased reesterification in triglycerides. These data differ from animal studies with high-dose short-

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Table 2.2.3-1: Liver lipid composition in seven patients treated for years with 3.25–5.85 g aspirin (ASA) daily as compared to seven age-matched controls without aspirin intake. All patients died from myocardial infarction and had no clinical liver pathology. All data are % of total lipids (modified after [14]).

	CON	ASA
neutral lipids		
total neutral lipids	**49.5 ± 1.0**	**65.6 ± 0.7**
free fatty acids	12.6 ± 1.5	27.4 ± 2.4
mono- and diacylglycerols	2.3 ± 0.1	6.4 ± 1.0
triacylglycerols	11.9 ± 0.6	12.0 ± 3.2
fatty acid esters	3.3 ± 0.2	4.8 ± 0.4
cholesterol	8.0 ± 0.4	5.9 ± 0.7
cholesteryl esters	6.8 ± 0.6	5.9 ± 0.6
undetermined	5.3 ± 0.4	3.2 ± 0.4
phospolipids		
total phospholipids	**50.5 ± 1.0**	**34.1 ± 0.6**
phosphatidylinositols	3.0 ± 0.1	2.3 ± 0.2
phosphatidylethanolamines	13.5 ± 0.4	6.0 ± 1.5
phosphatidyserines	4.6 ± 0.4	3.3 ± 0.3
phosphatidylcholines	14.4 ± 1.1	6.0 ± 0.4
lysophosphatidylcholines	1.0 ± 0.1	1.1 ± 0.1
cardiolipins	0.6 ± 0.1	0.3 ± 0.0
phosphatidic acids	9.2 ± 0.8	11.8 ± 1.2
sphingomyelins	2.8 ± 0.3	2.3 ± 0.7
undetermined	1.3 ± 0.4	1.1 ± 0.3

term aspirin treatment where increased triglycerides are a regular finding [12]. Of interest are also the marked reductions in phospholipids, possibly indicating altered lipid signaling related to changes in membrane conductance. Unfortunately, apparently no further studies on the important issue of changes in local lipid distribution pattern after long-term aspirin administration to men – at antiplatelet/analgesic doses – have been conducted. It is therefore unknown whether long-term aspirin-induced alterations in hepatic lipid metabolism are a general phenomenon or superimposed to the altered immunologic status of rheumatic patients studied here (Section 3.2.2).

Disturbed carnitine shuttle. Like other fatty acids, salicylate is activated to salicylyl-CoA in mitochondria by a medium-chain fatty acid–CoA ligase [15]. This activation is also a prerequisite for conjugation with glycine to form salicyluric acid (Section 2.1.2) [16]. Generation of large amounts of salicylyl-CoA in the presence of higher salicylate levels will deplete the cellular stores of CoA and possibly carnitine (Fig. 2.2.3-2) [13]. In addition, there is depletion in ATP pools due to inhibition of oxidative phosphorylation (see below). As a consequence, less carnitine, CoA and ATP are available for

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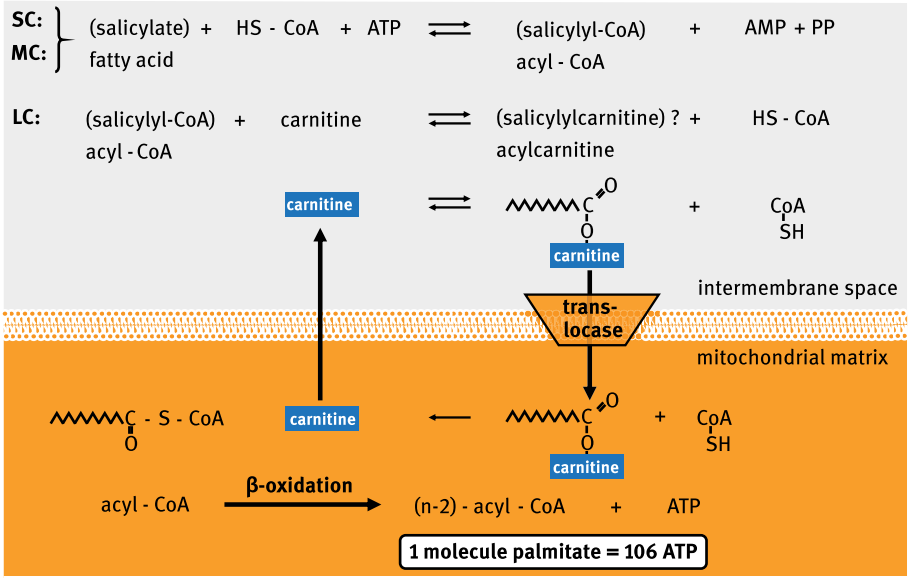

Figure 2.2.3-2: Mitochondrial β-oxidation of short- (SC), medium- (MC) and long-chain (LC) fatty acids and their modification by salicylates (for further explanations see text).

transport of long-chain fatty acids to the mitochondrial matrix and their subsequent β-oxidation. Secondary events of disturbed β-oxidation are changes in gluconeogenesis and ureagenesis.

Appearance of dicarboxylic fatty acids. Another feature of impaired mitochondrial β-oxidation of long-chain fatty acids and their subsequent local accumulation is the appearance of long-chain dicarboxylic fatty acids as products of enhanced extramitochondrial ω-oxidation [17, 18]. These acids, like other long-chain fatty acids, are natural uncouplers of oxidative phosphorylation in mitochondria [19]. Their physicochemical properties [20] allow them to act as protonophores, while short- and medium-chain fatty acids fail to do so (see below). Dicarboxylic acids are not found in blood in healthy conditions but became apparent after excessive high oral doses (600–700 mg/kg) or 1 % of diet (!) (over several days or weeks) of aspirin to rats [21, 22]. Animal data also suggest the induction of a specific CYP isoform in the rat liver that catalyzes ω-hydroxylation of free fatty acids, eventually resulting in the generation of dicarboxylic acids [21]. No comparable studies are available for human. It has been shown that the appearance of dicarboxylic acids in blood may be associated with Reye-like symptoms in men (see below) [12, 18, 23, 24].

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2.2.3.3 Salicylates and uncoupling of oxidative phosphorylation

Basic mechanisms. Energy coupling in the respiratory chain results in generation of ATP from ADP and inorganic phosphate at the expense of energy. This energy is provided by the electron transport chain. The oxidative phosphorylation system is localized in the inner mitochondrial membrane. Uncoupling agents allow electron transport to oxygen to continue but prevent the phosphorylation of ADP to ATP, that is, they uncouple the energy-yielding from the energy-saving process. This results in increased mitochondrial oxygen uptake and reduced ATP levels despite increased ATP synthase activity. Functionally, this indicates a "waste" of energy generated via the respiratory chain as heat instead of generation of ATP.

The energy-yielding and energy-requiring processes are coupled by a high-energy intermediate state. An electrochemical proton gradient across the mitochondrial inner membrane serves as means of coupling the energy flow from electron transport to the formation of ATP. An intact mitochondrial membrane that is impermeable to H^+ ions is essential for maintaining this proton gradient. The electron transport chain pumps H^+ ions outwards while ATP formation is accompanied by an inward H^+ movement.

The system is devised as not to waste energy when this is not needed [11]. When the utilization of ATP is low, there is little ADP in the mitochondrial matrix and little reentry of protons through ATP synthase, and the high proton gradient slows down the activity of the respiratory chain by inhibition of ATP release from the ATP synthase. If ATP is consumed, the concentration of ADP increases, and protons reenter the matrix through ATP synthase and regenerate ATP. The electron transport through the respiratory chain causes H^+ to be pumped outward across the inner membrane of the mitochondrion, building up a gradient of H^+. This gradient is the energy-rich state to which electron transport energy is transformed and is the immediate driving force for the phosphorylation of ADP. The maintenance of this gradient, i. e., the impermeability of the inner mitochondrial membrane for H^+, is essential for functioning of this coupling process (Fig. 2.2.3-3).

Salicylates as protonophores. Uncoupling agents, such as DNP or salicylates, increase the mitochondrial membrane proton conductance (Fig. 2.2.3-4) [1, 2] and reduce or remove the selective impermeability of the membrane for protons in a time- and concentration-dependent manner. This abolishes the energy-conserving proton gradient [25, 26] and results in bypassing the ATP synthase and direct release of protons into the matrix.

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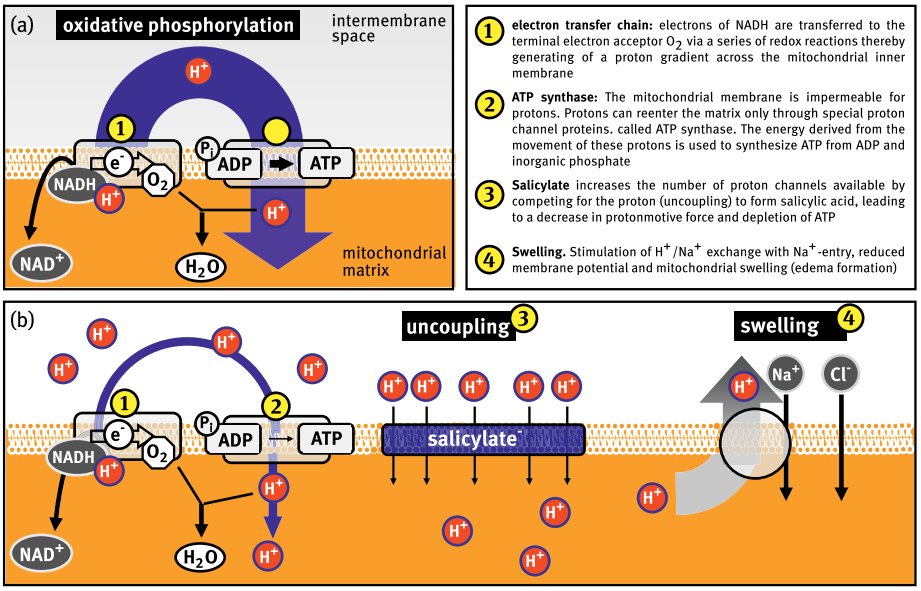

Figure 2.2.3-3: (a and b) Energy production by oxidative phosphorylation (a) and uncoupling of oxidative phosphorylation by salicylate (b). An electrochemical gradient of H^+ ions across the mitochondrial inner membrane couples the energy flow from electron transport to the generation of ATP. An intact mitochondrial membrane that is impermeable to protons is the prerequisite for maintaining the proton gradient. The electron transport chain pumps H^+ ions outwards (1), while ATP formation is accompanied by an inward H^+ movement (2). Uncoupling agents like salicylate work as protonophores by increasing the membrane permeability, that is, the proton conductance, of the mitochondrial membrane (3). As a consequence, the proton gradient and membrane potential decrease and increased oxygen and substrate consumption will be required to maintain the proton motive force at increasingly depleted ATP levels. The increased proton accumulation inside the mitochondrion stimulates H^+/Na^+ exchange and causes mitochondrial swelling (4).

In isolated mitochondria, the mitochondrial membrane proton conductance is increased more than 4-fold in the presence of 1 mM salicylate [27]. This is associated with a time-dependent membrane depolarization, an increase in membrane permeability and finally cell death by apoptosis [28]. The intracellular pH decreases from 7.4. to 7.2, 6.9 and 6.7 at 1, 2 and 4 mM salicylate, respectively [29]. Complete uncoupling occurs in model systems at 2–5 mM salicylate [30, 31] and is associated with the decrease and finally collapse of the mitochondrial membrane functions [32]. The increasing H^+/Na^+ exchange also causes swelling of mitochondria [1, 2]. Swelling of mitochondria and reduced urea generation after aspirin treatment were also found in "primarily living" intact rat hepatocytes [33].

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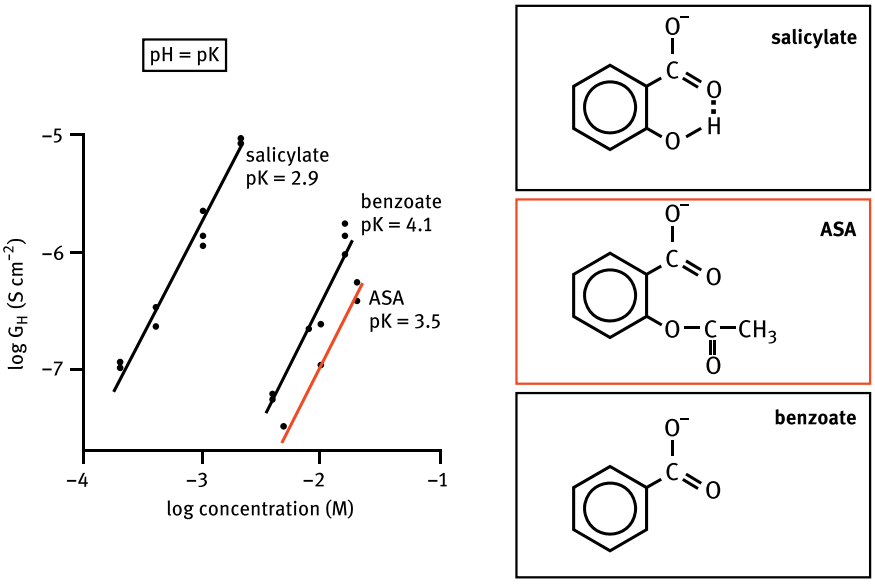

Figure 2.2.3-4: Changes in membrane conductance by salicylate, aspirin (ASA) and benzoate. Proton conductance (G_H) is shown as a function of total weak acid concentration ([HA] + [A^-] when pH = pK). Note the about 10-fold increase in conductance by salicylate at 1 mM (modified after [1]).

At least two factors determine the activity of salicylate in uncoupling oxidative phosphorylation: The first is partition from an aqueous phase into a lipid-rich phase, allowing for penetration through the cell wall and access to the mitochondrion – the ultimate site of action. The second is a specific structural arrangement to be able to act as protonophore. Structure–activity comparisons for uncoupling oxidative phosphorylation in isolated mitochondria of 80 salicylate analogs showed that the essential pharmacophore for uncoupling activity is a salicylate with a negatively charged (carboxyl) group at the o-position, i. e., o-hydroxybenzoate (salicylate) [34]. The m- and p-hydroxybenzoate analogs were inactive. This suggests that the o-position of the hydroxyl group is an essential steric requirement for this protonophoric action. It is unique to salicylate [35] and probably also involved in the primary reversible binding of the salicylate carboxyl group of aspirin inside the COX channel (Fig. 2.2.1-4).

Salicylate metabolites and paracetamol (dipyrone) have no protonophoric properties [1, 36].

Consequences of uncoupling of oxidative phosphorylation by salicylates. Uncoupling of oxidative phosphorylation reduces mitochondrial energy production and all mitochondrial and cell functions that are energy-dependent. The uncoupling is reversible, requires the physical presence of salicylate and is usually followed by complete recovery after salicylate removal [25, 26]. The uncoupling is not restricted to the liver, but has also been found in isolated mitochondria of the kidney, brain and heart at millimolar (2–5 mM) salicylate concentrations [30, 37].

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Metabolic effects are typical for initial stages of salicylate overdosing (Section 3.1.1) [30–32, 38]. Clinically, they present as hyperventilation, i. e., increased oxygen uptake, associated with increased heat production (sweating) [39].

2.2.3.4 Metabolic actions of salicylates and Reye´s syndrome

General aspects. Reye´s syndrome is a hepatic encephalopathy that has been brought into connection with aspirin-induced alterations in the hepatic fatty acid metabolism, gluconeogenesis and urea metabolism (Section 3.3.3). Possible analogies between disturbed mitochondrial functions after high-dose aspirin and the hepatopathy of Reye´s syndrome were taken occasionally as evidence for a causal relationship between the two [40]. There are, however, serious doubts regarding any causal role of salicylates in the disease. This also includes uncertainties about the really taken aspirin doses – if any – and plasma salicylate levels. Reye´s syndrome as a clinical entity and its relation to aspirin – and other hereditary and acquired factors – is discussed in detail in Section 3.3.3. The present subsection compares metabolic alterations in liver metabolism by high-dose salicylate with symptoms of Reye-associated hepatic failure.

Impaired β-oxidation. The free fatty acid content in liver and plasma is markedly increased in patients with salicylate intoxication as well as Reye's syndrome, in one study up to 10-fold the normal value [24]. However, salicylate-induced mitochondrial failure – the reason for this pathology at high-concentration salicylate exposure – is generally transient and fully reversible after salicylate removal [40–42]. Morphologically, high-dose salicylate in animal studies [12] and severe salicylate intoxication in men [42] cause microvesicular steatosis of the liver. Similar alterations were also seen in Reye's syndrome. However, histopathology and ultrastructural pathology of liver biopsy specimens in Reye patients were different from those in salicylate intoxication [43, 44]. Moreover, microvesicular steatosis of the liver is not a unique etiologic entity and is seen in different forms of mitochondrial injury [45]. Inborn errors of ureagenesis may also present with Reye-like microvesicular steatosis but exhibit a morphology that is different from that observed in Reye´s syndrome [46].

Medium-chain acyl-CoA dehydrogenase deficiency is an inherited defect of mitochondrial β-oxidation of fatty acids. It was found to be associated with (early) Reye-like symptoms [47], as were inborn defects in the carnitine shuttle [48]. There is also lipid infiltration of the liver and impaired mitochondrial β-oxidation [11]. This indicates a relation of Reye-like symptoms with hereditary or acquired liver pathologies that have nothing to do with salicylates. Moreover, impaired β-oxidation of long-chain rather than short- or medium-chain fatty acids is a typical feature of high-dose salicylate- (540 mg/kg) induced liver toxicity in mice [12] and the reason for generation of dicarboxylic acids.

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Dicarboxylic acids. A considerable percentage, at least 55 %, of total serum free fatty acids in Reye's syndrome are dicarboxylic acids, the vast majority of them (85–90 %) being long-chain [18, 23, 24]. The appearance of dicarboxylic acids is an index of general disturbances of mitochondrial function and a consequence of disturbed β-oxidation, that is, degradation of fatty acids, allowing for their further extramitochondrial ω-oxidation [18, 24]. Generation of these abnormal fatty acids is seen at high circulating salicylate levels, but in the only study that investigated the relationship between plasma salicylate and Reye disease, no correlation between the two was established [49]. Dicarboxylic acids are also found in patients with inborn errors of metabolism in mitochondria or peroxisomes, such as Zellweger's syndrome or neonatal adrenodystrophy [50]. Thus, the presence of these abnormal fatty acids suggests some general dysfunction of hepatic mitochondria for inborn or acquired reasons rather than being a Reye-specific change.

Plasma salicylate levels and exhaustion of the acyl-CoA-carnitine shuttle. Exhaustion of the carnitine shuttle with subsequent generation and accumulation of long-chain free fatty acids and formation of dicarboxylic acid requires high, toxic concentrations of salicylate [18]. In most reported cases of Reye's syndrome, serum salicylate levels, if measured at all, were not in the toxic range, for example on average 150 µg/ml (range 0–460 µg/ml) in 27 children with Reye´s syndrome who died and 100 µg/ml (range 0–480 µg/ml) in 103 children with Reye's syndrome who fully recovered [49]. In one report on a fatal case, the plasma salicylate level was 120 µg/ml as measured with gas chromatography/mass spectrometry (GC/MS) [18]. Studies on salicylate biotransformations in Reye patients and patients on long-term high-dose salicylate for treatment of juvenile rheumatoid arthritis showed different metabolic patterns for salicylate metabolites in both groups of patients [51]. Finally, there are considerable doubts regarding the reliability of measurement of circulating salicylates in clinical conditions of Reye-like disease(s) by photometric methods like the Trinder assay (Section 1.2.2) [52, 53], which was used in many of these studies (Section 3.3.3).

No proven causal relationship between aspirin-induced changes in liver metabolism and Reye's syndrome. Overall, there are some similarities between symptoms of Reye-like diseases and aspirin-related liver toxicity, but also significant differences. In addition to the points mentioned above, there is definitely more than one reason for Reye syndromes in the clinics – According to Glasgow and Middleton, the diagnosis of the "classical" Reye syndrome must of necessity be a diagnosis of exclusion (!) [54]. The finding that salicylates in certain experimental setups may cause liver injury, specifically in vitro at high, toxic concentrations maintained for many hours without any metabolic degradation or washout in largely protein-free media, is not immediately transferable to the in vivo situation. This becomes particularly evident at the background of genetic defects in fatty acid metabolism of the liver that were

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unknown at the "high season" of Reye syndromes in the mid-1980s, although even at that time only a minority of children had reportedly taken aspirin prior to onset of symptoms. Today, there is no convincing evidence to postulate any causality between aspirin and Reye syndrome (Section 3.3.3).

Summary

Aspirin and salicylates exert a number of actions on cellular energy metabolism that become most prominent in liver mitochondria. Recent studies also indicate a role of aspirin-induced acetylations of target enzymes of glycolysis and the Krebs cycle. However, most of these studies were experimental in nature and the clinical significance of these findings is not sufficiently established yet.

In addition to and apparently independently of acetylation processes, salicylate has metabolic actions by its own. It uncouples oxidative phosphorylation and inhibits mitochondrial β-oxidation of long-chain fatty acids, eventually associated with the generation of dicarboxylic acids. These actions require the physical presence of salicylates and are usually fully reversible after their removal. Metabolic disturbances become detectable at about 1 mM salicylate in vitro and cause typical clinical symptoms (sweating, hyperventilation) of aspirin overdosing or intoxication in vivo (Section 3.1.1).

Salicylate needs to be activated by CoA into salicylyl-CoA for further metabolic processing. Salicylyl-CoA sequesters extramitochondrial CoA. At high salicylate levels, this might result in exhaustion of carnitine, which is necessary for the carnitine shuttle of long-chain fatty acids. In addition, salicylate acts as protonophore. It abolishes the selective impermeability of the mitochondrial membrane for protons and prevents the build-up and maintenance of the H^+ gradient across the mitochondrial membrane, which is essential for energy storage in the form of ATP. The probably most important functional consequence of these changes is the inhibition of all energy-dependent cellular processes – including many kinase-dependent enzymatic reactions.

The metabolic actions of salicylates share some similarities with the liver pathology in Reye syndrome. There are important differences too, and several inherited disorders of fatty acid metabolism in the liver exhibit similar laboratory and clinical features. As discussed in more detail in Section 3.3.3, until now, no causality between the "genuine" Reye's syndrome, that is, hepatic encephalopathy subsequent to a viral infection of the upper airways, and salicylate-induced liver pathology has been established.

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2.3 Actions on organs and tissues

Section 2.2 was focused on cellular and molecular targets of aspirin and salicylate with the intention to cover the spectrum of pharmacological actions as completely as possible. In this context, the selection of experimental conditions (in silico models, cell culture studies, sophisticated in vitro assays without drug kinetics as a variable) and the choice of doses (concentrations) were of minor interest. Often doses (concentrations) were given that would never have been tolerated in vivo but were used by the investigator to generate a biological response that could be measured.

This view changes when the consequences of pharmacodynamic actions are to be transferred to the tissue and organ levels. Here, different cell types with different sensitivities to aspirin and salicylates and a different cellular reaction pattern form one functional unit. Additionally, pharmacokinetics have to be considered, such as blood supply as determinant for drug provision and washout, respectively, as well as (hepatic) drug metabolism and (renal) excretion. All these variables determine the local concentration of the active compound and its interaction with local effector sites.

Three areas of pharmacological actions of aspirin at the tissue and organ levels are of particular significance for its use as a medicine and will be discussed in more detail below: antithrombotic effects (Section 2.3.1), antiinflammatory, antimicrobial, analgesic and antipyretic activities (Section 2.3.2) and immunosuppressive/antitumor effects (Section 2.3.3). These actions differ with respect to the dosing which is necessary to elicit the desired clinical action, e. g., ≤0.1–0.3 g/day is recommended for antiplatelet effects, the principal mechanism for the antithrombotic activities of aspirin (Section 2.3.1), and 1–2 g, mostly single-dose or short-term treatment, is recommended for analgesic/antipyretic effects (Section 2.3.2). This overlaps with full antiinflammatory doses, i. e., 2–4 g/day, if additional effects of the salicylate metabolite are desired. Antiviral actions probably also belong to this category. Interestingly, the chemopreventive action of aspirin appears to be dose-independent and is fully present at antiplatelet doses of around 0.1 g. An overview on therapeutic and toxic actions of aspirin in relation to the plasma level of salicylate is shown in Fig. 2.3-1.

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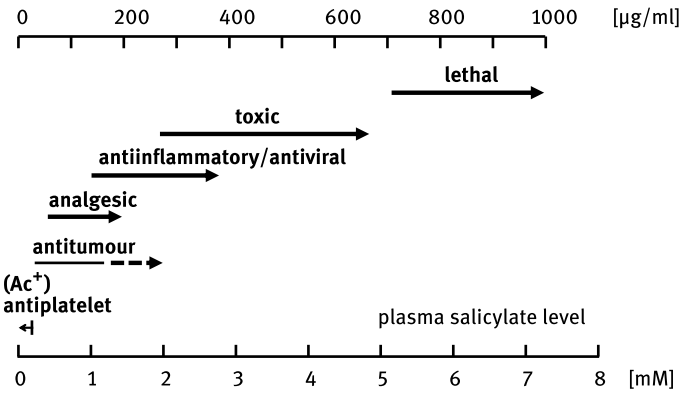

Figure 2.3-1: Therapeutic and toxic actions of aspirin in relation to the total plasma level of salicylate. Note that the plasma level of salicylate is irrelevant for the antiplatelet actions of aspirin because they are entirely due to acetylation (Ac) of target enzymes (here platelet COX-1).

2.3.1 Hemostasis and thrombosis

2.3.1.1 General aspects

Hemostasis. The rapid cessation of bleeding after vessel injury is a vital function of the organism. Numerous chemical products, supported by physical factors (shear stress, etc.), are involved to make sure that this goal will be reached as soon and as completely as possible and that the clot-forming processes will be localized and limited to the site where they are needed. Prostaglandins, prostacyclin and thromboxane A_2 (TXA_2) are tightly involved in all of these processes [1].

Numerous chemicals collectively form the functional unit of the clotting cascade, having the generation of a stable fibrin clot that stops bleeding as the endpoint [2]. In physiological conditions, the system is carefully balanced by a variety of procoagulant and anticoagulant factors. These factors can be rapidly generated from inactive precursors (zymogens) that are available in large excess in circulating blood. The clotting process starts immediately after tissue injury. The decisive event for stopping of bleeding is the generation and release of tissue factor from different sources with subsequent thrombin formation and generation of a platelet-fibrin clot that becomes fixed to the vessel wall [3]. In arteries, clotting starts with targeted adhesion of platelets to the subendothelium in an area of endothelial injury. Activated platelets secrete negatively charged polyphosphates for activation of factor XII [4] and act as a matrix for thrombin formation [5]. Platelet activation involves arachidonic acid release from membrane phospholipids, followed by thromboxane biosynthesis, platelet aggregation and secretion of vasoactive, inflammatory and mitogenic factors. The result is an occluding thrombus that stops bleeding mechanically by “plugging” the “leak” at the site of vessel injury while local vasoconstriction and adherence of the thrombus to the injured vessel wall prevent washout. Tissue factor release, platelet activa-

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tion and generation of TXA_2 act synergistically as both starting and amplifying events for thrombin formation, platelet aggregation, secretion and vessel constriction in an endothelium-injured area.

At a about the same time, platelet inhibitory, antithrombotic mechanisms (prostacyclin, NO, endothelial nucleotidases) become activated in the noninjured endothelium in the neighborhood. These processes limit thrombus growth to the site of vessel injury. Finally, the mature thrombus is stabilized by fibrinogen bridges and covered by a fibrin coat. Activation of the fibrinolytic system (tissue plasminogen activator [tPA] and subsequent clot lysis then allow recanalization of the thrombus, i. e., restitution of blood flow. This initiates the healing phase of the injured vessel wall, which is associated with cell proliferation and, inside the vessel, ends with the formation of a neointima.

Thrombosis. This well-balanced dynamic equilibrium between hemostatic and fibrinolytic factors is disturbed in atherosclerosis, the most frequent cause of atherothrombosis. Atherosclerosis is a chronic low-grade inflammation which is associated with endothelial dysfunction. At advanced stages, there is increasing loss of vasodilatory, antithrombotic and profibrinolytic properties of the endothelium, and its conversion into a prothrombotic surface which expresses adhesion molecules and becomes a target for inflammatory cytokines and growth factors [6]. The extent of platelet adhesion and activation, the initial process of arterial thrombosis, is largely determined by platelet reactivity. Platelet reactivity is also a key determinant of thrombin formation at the membrane of activated platelets which acts as a catalytic surface [5, 7] and markedly further stimulates platelet activation and fibrin formation. Inside the clot, significant thrombin [8] and factor Xa formation [9] are maintained over many hours, while the real clotting process is finished within a few minutes [10]. Consequently, thrombi are not only the result, but – more importantly – also the source of long-lasting thrombus-associated release of clotting factors and inhibitors of fibrinolysis, such as PAI-1 [11]. Blockade of the platelet-specific glycoprotein (GP)IIb/IIIa receptors markedly reduces thrombin formation in platelet-rich but not platelet-poor plasma [12]. GPIIb/IIIa receptor blockade also overcomes the suboptimal if any inhibition of platelet aggregation by ADP antagonists in acute ST elevation myocardial infarction (STEMI) patients in vivo [13]. Both findings indicate the important role of platelet-dependent thrombin formation for the thrombotic process.

Increased platelet reactivity can be demonstrated in patients with stable angina by enhanced expression of platelet adhesion receptors for P-selectin and fibrinogen (GPIIb/IIIa) after ex vivo stimulation by platelet agonists, such as ADP (Fig. 2.3.1-1) [14, 15].

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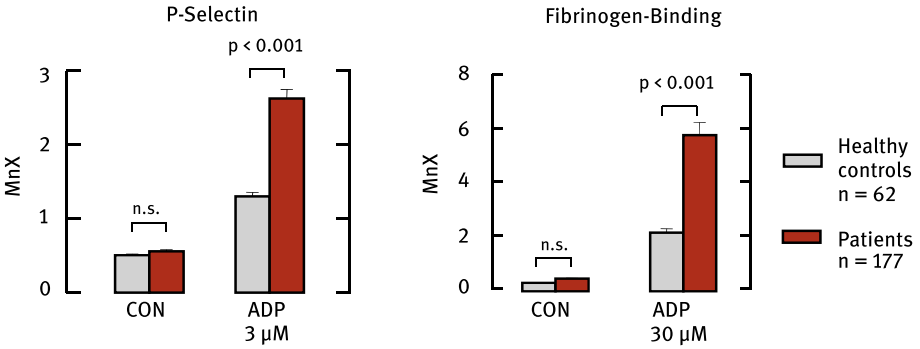

Figure 2.3.1-1: Unchanged platelet reactivity under resting conditions and increased reactivity after stimulation by ADP in patients with chronic coronary vascular disease as opposed to healthy controls. The figure shows expression of P-selectin and active GPIIb/IIIa binding sites for fibrinogen at the platelet surface (flow cytometry) (modified after [14, 15]).

Arterial vs. venous thrombosis. The acute complication of an enhanced thrombosis tendency in both arteries and veins is thromboembolic vessel occlusion, in arteries appearing as acute coronary syndrome, transient ischemic attack and stroke or leg ischemia. In arteries, these events are frequently initiated by erosions or rupture of an atherosclerotic plaque [16]. This exposes tissue factor and other thrombogenic materials from inside the plaque and other sources, such as vascular smooth muscle cells [17] that come in contact with the flowing blood after endothelial injury and initiate thrombin formation [3, 18].

The mechanisms of thrombus formation in the venous circulation are basically the same: local disturbances of hemostasis because of a pathological interaction between blood constituents and the vessel wall. They are also amplified by disturbed local hemodynamics, such as shear stress (arteries) or stasis (veins). However, the pathomechanisms differ: In arterial thrombosis, it is the platelets and their adhesion to the vessel wall under high shear stress conditions that initiate thrombus formation in endothelium-denuded or dysfunctioning areas. In the low-pressure venous system, it is primarily stasis and fibrin formation [19], facilitated by the circulation of (microparticle-bound) tissue factor and accumulation of nondegraded activated clotting factors. There is also a decisive role for platelets, initiated by the formation of heterotypic platelet–white cell aggregates that release a number of bioactive mediators which stimulate thrombus growth (see below). Platelets are important amplifying and disease-relevant factors in both arterial and venous thrombosis and in this respect useful targets for antiplatelet drugs such as aspirin [20–24].

Targets of aspirin. Aspirin can principally modify all three components of the hemostatic system, i. e., platelet function, plasmatic coagulation and fibrinolysis. Inhibition of platelet function(s) is not only the most significant but also the most intensively

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studied component. For formal reasons, actions of aspirin on platelets, plasmatic coagulation and fibrinolysis are discussed separately. However, in vivo they form a functional unit having the control of clot formation and resolution as a common final target.

2.3.1.2 Platelets

Aspirin actions and platelet functions. Pioneering work on this issue came form *Philip W. Majerus* (Section 1.1.4) [25]. His group originally found that aspirin inhibits platelet functions via acetylation of platelet COX [26]. This inhibition of platelet-dependent thromboxane formation is now the generally accepted mode of antiplatelet actions of aspirin [26–30]. The acetylation starts already in the bone marrow megakaryocytes [31]. It probably also involves acetylation of megakaryocyte genes, including those for COX(s) [32]. Acetylation is irreversible for the platelet COX-1, lasts for the lifetime of circulating platelets (7–9 days) and is functionally terminated by the appearance of a sufficient amount of fresh platelets, derived from aspirin "naïve" megakaryocytes in the circulation. These "naïve" platelets can act as seeds for aggregate formation during antiplatelet treatment with aspirin or other antiplatelet agents [33].

Aspirin and TXA_2. TXA_2 holds an outstanding position as an aspirin-sensitive trigger of platelet activation subsequent to stimulation by platelet agonists. Release of arachidonic acid from its binding sites in the cell membrane and subsequent "explosion" of TXA_2 formation – probably due to the high peroxide tone in platelets [34] – initiates Ca^{++} entry and the contraction of the platelet cytoskeleton ("shape change"). This process is synergistically amplified by other platelet agonists, acting via the same $G_{(q)}$ protein, such as ADP ($P2Y_1$), adrenaline, serotonin and thrombin via the protease-activated receptors (PARs) (PAR-1/PAR-4) [35, 36], finally resulting in clustering and activation of the GPIIb/IIIa receptors at the platelet surface. These activated receptors bind fibrinogen and form a platelet–fibrin aggregate.

TX formation is not essential for platelet aggregation but rather an amplification mechanism for weak platelet stimuli to guarantee a full aggregation and secretion response. Consequently, and in contrast to the artificial conditions of ex vivo platelet studies in Ca^{++}-deprived media [37], aspirin will not or only partially inhibit platelet activation, initiated by TX-independent stimuli, such as high-dose thrombin [38], ADP [37, 39], shear stress [40–43], collagen, circulating catecholamines [44] and psychic stress [45, 46] in vivo. Only platelet aggregation induced by adding arachidonic acid (in vitro) is completely blocked by aspirin because it is entirely dependent on TXA_2 formation. This is an in vitro artifact. In vivo, release of endogenous arachidonic acid occurs only as an accompanying phenomenon of cell (platelet) activation and there is little if any free arachidonic acid in the extracellular space. No release of arachidonic acid into the extracellular space is required because arachidonic acid, set free from

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membrane phospholipids by phospholipase(s) (Section 2.2.1), enters directly the hydrophobic channel of cyclooxygenases inside the cell membrane. For these reasons, arachidonic acid-induced platelet stimulation (aggregation) is a useful pharmacological tool to determine the efficacy of TX inhibition, but has no natural correlate in vivo. Aspirin does also not interact with platelet TX receptors. Thus, TXA_2, made by "aspirin-naïve" platelets and agents activating thromboxane receptors, such as isoprostanes, nonenzymatic products of lipid peroxidation [47, 48], can cause and maintain platelet stimulation even in the presence of fully effective blockade of TX biosynthesis by aspirin. This complex interplay between aspirin and stimulation of platelet function by different agonists should be kept in mind for the interpretation of aspirin effects on platelet function in vivo [31, 49]. The general mode of action of currently used antiplatelet drugs is shown in Fig. 2.3.1-2.

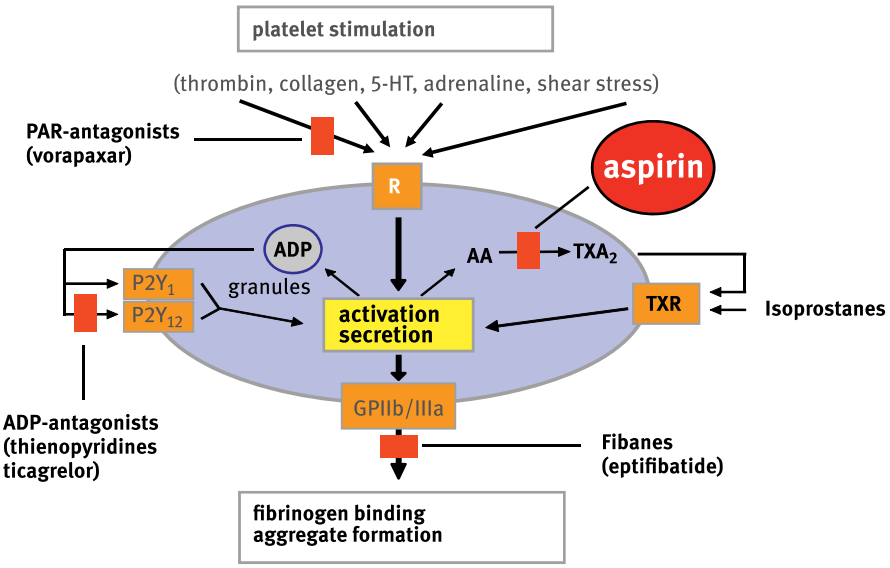

Figure 2.3.1-2: Stimulation of platelet activation, secretion and aggregation by selected agonists and shear stress. Sites of action of aspirin and selected classes of antiplatelet drugs. 5-HT: serotonin; PAR: protease-activated receptor, TXR: thromboxane receptor.

Aspirin and thrombin formation. It is well known that aspirin inhibits thrombin-induced PG/TX formation in platelets without inhibiting thrombin-induced platelet secretion [38]. This suggests that thrombin-induced platelet activation/secretion/aggregation and thrombin-induced prostaglandin/thromboxane production in platelets are two separate events. Another question is whether aspirin can also interact with endogenous thrombin production, that is, activation of the coagulation pathway. The fact that the surface of activated platelets provides the matrix for initiation and po-

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tentiation of thrombin formation during activation of the clotting cascade in blood [50] would suggest this [51]. Possible mechanistic explanations are acetylation of clotting-related proteins, such as prothrombin [52] and fibrinogen [53], in addition to inhibition of platelet activation [5, 54]. It has also been shown that human thrombi contain an abundance of active thrombin and PAI-1, associated with the accumulation of platelets inside the thrombus [8]. Combined inhibition of COX-1-dependent platelet TXA_2 generation and platelet-dependent inhibition of thrombin formation by aspirin might be particularly effective in certain clinical situations of enhanced thrombin formation such as acute coronary syndromes [55], where platelet thromboxane receptors are upregulated as well [56, 57].

Autocrine vs. paracrine functions of TXA_2. In addition to autocrine functions, i. e., the positive feedback of released thromboxane on further platelet activation and thromboxane formation, TXA_2 also exhibits paracrine functions on other cells and synergizes with ADP. This includes the activation (recruitment) of fresh, aspirin-naïve platelets and the recruitment of inflammatory white cells as well as the aspirin-sensitive generation of soluble inflammatory mediators, such as P-selectin and CD40 ligand (CD40L), TX-mediated growth factor release (VEGF, PDGF, TGF-β1) [58] and others [59–61] (Fig. 2.3.1-3). Activated platelets have also been shown to create the

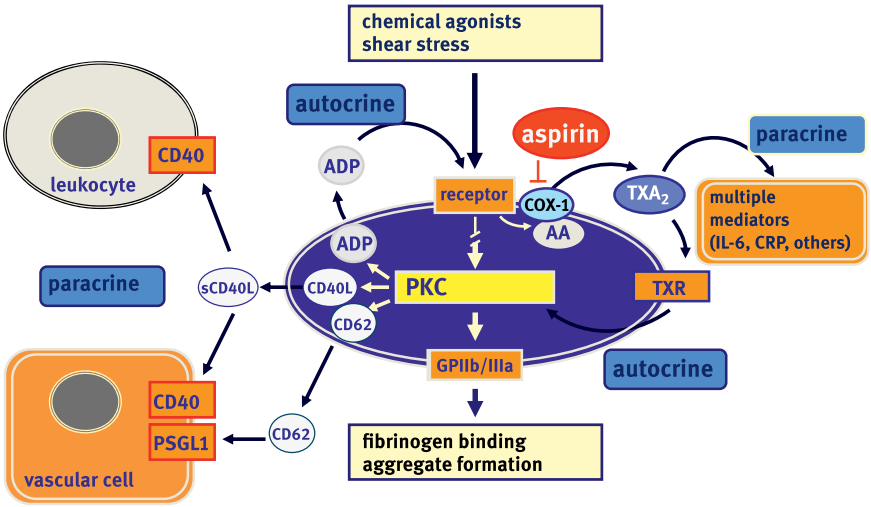

Figure 2.3.1-3: Autocrine and paracrine secretory functions of platelets via thromboxane A_2 + TXA_2) and ADP. Inhibition of COX-1-dependent thromboxane formation in platelets by aspirin results in reduced autocrine and paracrine platelet functions via inhibition of protein kinase C (PKC). CD40L and CD62 (P-selectin) and ADP act independently of TXA_2 but form a synergistic network that is sensitive to inhibition by aspirin with inhibition of subsequent paracrine actions on white cells and cells of the vasculature. For further explanations see text. Abbreviations: CRP: C-reactive protein; PSGL: P-selectin glycoprotein ligand.

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active CRP monomer, a potent inflammatory agent, from the pentamer precursor at their surface [62]. Thus, antiplatelet therapy will also have an impact on inflammation and coagulation and it is quite likely that, in the case of aspirin, TXA_2 is the primary mediator in many cases [63]. Other mediators of interest are stored platelet products, such as serotonin, growth factors or sphingosine-1-phosphate (S1P), a lipid mediator that is stored in platelets and released in a strictly thromboxane-sensitive, that is, aspirin-sensitive, manner (Section 2.2.3) [64]. The clinical significance of these and many other findings regarding "heterotypic" platelet functions [65] is currently under intense investigation. There is also increasing evidence for a decisive role of (platelet) COX-1-dependent prostaglandin formation in tumorigenesis and metastasis and COX-1/TXA_2 signaling as a target for the prevention of metastasis by aspirin [66, 67].

Time-dependent inhibition of platelet functions by aspirin. Maximum inhibition of platelet function in vitro and prolongation of bleeding time by aspirin occur within a few minutes [68, 69]. A nearly complete inhibition of arachidonic acid-induced platelet aggregation and TX formation (>99 %) is seen within 5 min after intravenous application of 250 and 500 mg soluble aspirin [70, 71]. After oral intake of 300–500 mg, it requires about 2 h to obtain nearly complete blockade of collagen-induced platelet aggregation and serum TX [72]. An initial intravenous loading dose of 250–500 mg aspirin is necessary if immediate inhibition of platelet function is required, for example as emergency first-line treatment in ACS.

Inhibition of platelet COX-1 and platelet function by aspirin are functionally antagonized by the 10–15 % fresh platelets that normally enter the circulation every day from the bone marrow and are capable of undamped thromboxane formation. The

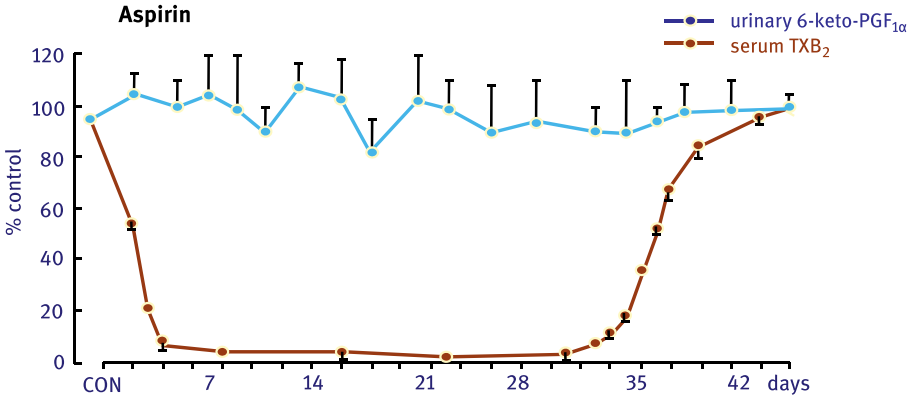

Figure 2.3.1-4: Time-dependent inhibition of platelet COX-1 (serum thromboxane) but not of renal prostacyclin metabolite excretion, presumably generated via COX-2/COX-1 in nucleated cells by low-dose aspirin (0.45 mg/kg per day) [27].

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relationship between TX inhibition and inhibition of the antiplatelet effect of aspirin is nonlinear [29, 73]: The TX-forming capacity has to be reduced by ≥95 % before (arachidonic acid-induced) platelet aggregation is inhibited. With very low-dose aspirin (0.45 mg/kg) this requires about 3 days to obtain a sufficient accumulation of aspirinized platelets in the circulation of healthy volunteers [27]. At the same time, there is no reduced prostacyclin synthesis as seen from an unchanged excretion of a prostacyclin metabolite. Possibly, prostacyclin can still be made by COX-2/COX-1 of nucleated cells which can replace the acetylated enzyme shortly by de novo synthesis (Fig. 2.3.1-4).

Conversely, 3–5 days are required to fully restore platelet function after stopping aspirin. This recovery occurs at 70–80 % inhibited thromboxane formation and is considerably faster than the recovery of inhibition of platelet function by the P2Y12 antagonist clopidogrel (Fig. 2.3.1-5) [74].

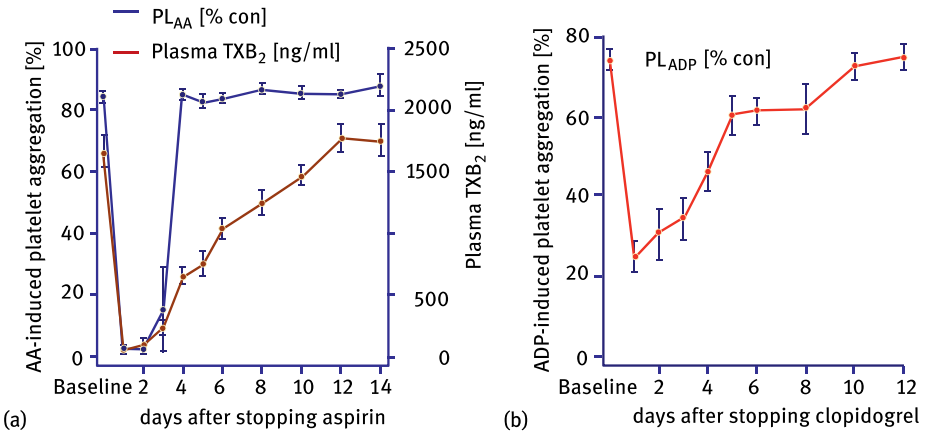

Figure 2.3.1-5: Offset of the effects (a) of aspirin (81 mg/day) on arachidonic acid-induced aggregation (PL_{AA}) and thromboxane formation and (b) of clopidogrel (75 mg/day) on ADP-induced aggregation (PL_{ADP}) after 1 week of treatment of healthy volunteers (modified after [74]).

Platelet turnover rate as a variable for aspirin dosing intervals. The platelet turnover rate is an important variable of aspirin's antiplatelet effect. About 100 billion new platelets are produced from megakaryocytes every day [75]. Of untreated platelets, 10 % may be sufficient to correct the aspirin-induced platelet "abnormalities" [76]. This is roughly equivalent to a normal platelet life span [77].

Essential thrombocythemia patients have an accelerated thrombopoiesis and exhibit an increased number of immature, reticulated platelets, as a consequence of high platelet turnover rates. Immature, reticulated platelets are more reactive and less sensitive to aspirin [78, 79]. Because of this, the standard of 75–100 mg aspirin once daily

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might not be sufficient to obtain a clinically relevant inhibition of platelet TX formation in the vast majority of these patients. Consequently, the antiplatelet response to low-dose aspirin was markedly improved by shortening the dosing interval to twice or three times daily [80] in patients with increased platelet count. This shortening of treatment intervals appears not to be required in patients with normal platelet count [81]. Recent findings from a substudy of the "Aspirin Regimens in EsSential thrombocythemia" (ARES) trial have confirmed this and additionally shown that the platelet count appears to be the strongest determinant for efficacy of thromboxane inhibition and is positively correlated with serum TXB_2 levels [82]. The long-term effects of twice daily aspirin on clinical outcome of patients with thrombocythemia remain to be determined [83].

Enhanced platelet turnover rates have been described in diabetics [84–86]. An increased platelet turnover rate with increased numbers of immature platelets will reduce the duration of sufficient blockade of platelet COX-1 and antiplatelet effects by once daily aspirin [84–87]. Increased platelet turnover was considered as one factor to explain the low efficacy of aspirin as a cardiocoronary preventive in diabetics (Section 4.1.1) [88]. Twice daily aspirin was found to be more effective than once daily administration in diabetics [89, 90]. Clearly, thrombophilia in diabetes is a complex event, characterized by dysregulation of more than one signaling pathway of coagulation [91]. For example, there is not only higher oxidative stress, as seen from elevated levels of malondialdehyde (MDA) in platelets of type 2 diabetes mellitus patients, but also impaired antioxidant defense, as seen from reduced platelet vitamin E and cytosolic glutathione peroxidase concentrations [92].

Platelet aspirin "resistance" is found in a significant proportion of patients with coronary artery disease (CVD). There is reduced inhibition of TX (and enzymatically generated MDA) formation as well as a time-dependently reduced antiplatelet effect of aspirin within the usual 24-h dosing interval (Fig. 2.3.1-6) [93]. In a small study of survivors of acute myocardial infarction there was still a significantly shortened megakaryocyte platelet regeneration time 6 to 12 months after the acute event [94]. Similar results were obtained with patients undergoing coronary bypass surgery. The explanation was a more rapid platelet turnover rate in the postoperative period [95]. This delayed "recovery" is independent of the aspirin dose but possibly related to the inflammatory/prothrombotic processes of advanced atherosclerosis [93].

Dose-dependent inhibition of platelet functions by aspirin. No other issue in aspirin research has been discussed more intensively than the question of the optimal antithrombotic dose. This is frequently, though not necessarily correctly, considered to be equal to the effective antiplatelet dose as determined from platelet function testing – in most cases by measurement of aggregation. There is general agreement that regular daily doses of 75–100 mg plain aspirin are sufficient to inhibit platelet function and platelet-dependent thromboxane formation via acetylation of COX-1 [29, 96].

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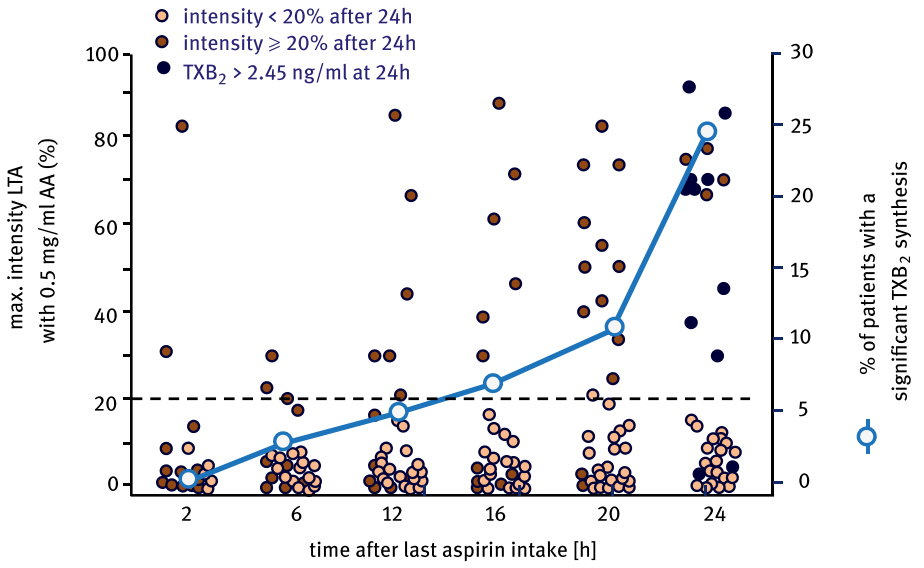

Figure 2.3.1-6: Variable time-dependent inhibition of arachidonic acid (AA)-induced platelet aggregation and thromboxane (TXB_2) formation in 150 aspirin-treated patients with stable angina. Inhibition of aggregation by <80 % (dotted line) or serum thromboxane levels of >2.45 ng/ml were considered insufficient for clinical efficacy. Measurements were done within 24 h after the last aspirin dose (75–250 mg/day) (after [93]).

Somewhat higher doses are under discussion if enteric-coated preparations are used, specifically at increased platelet turnover rates [81], but the discussion is not closed yet [97–99].

Serum thromboxane. The TX level in serum is an estimate of the platelets' TX-forming capacity. It has to be reduced by more than 95 % to indicate a clinically meaningful antiplatelet effect of aspirin [29, 96, 100]. However, this TX-synthesizing capacity, amounting to about 200–500 ng/ml with wide interindividual variations, is an in vitro artifact, describing the pharmacological potency of aspirin without any physiological or clinical correlate to circulating TX levels in vivo. These are four to five orders of magnitude (!) lower. They amount to about 2 pg/ml in plasma in resting conditions [101] and to about 60 pg/ml in bleeding time blood (Section 3.1.2) [102].

A direct pharmacological comparison between single-dose and repeated-dose administration of aspirin to men shows two parallel dose–response curves differing in IC_{50} values of TX inhibition by a factor of 8. This is equivalent to a platelet turnover rate of about 10–15 % per day in healthy volunteers and suggests that one daily maintenance dose of aspirin that compensates for the entry of new platelets into the circulation is sufficient (Fig. 2.3.1-7) [27, 103].

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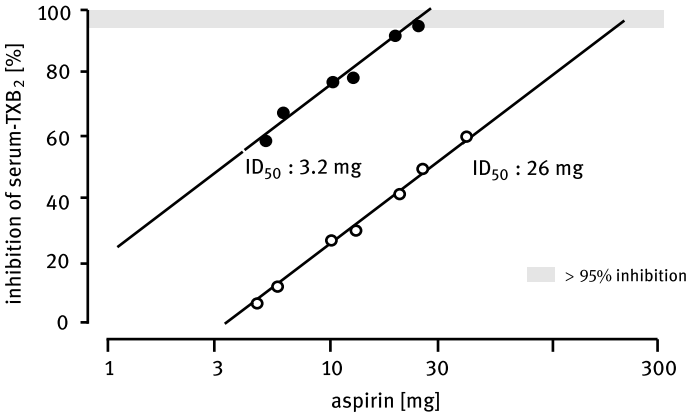

Figure 2.3.1-7: Dose-dependent inhibition of thromboxane B_2 (TXB_2) formation in serum after single (○) and repeated (5 days, ●) oral administration of aspirin in different doses to healthy volunteers.

Inhibition of TX formation in serum by 50 % (ID_{50}) is seen at a single 26-mg aspirin dose. For the same effect only 3.2 mg aspirin is necessary at repeated administration. An about 8-fold lower ID_{50} at repeated dosing corresponds to the maintenance dose of aspirin necessary to acetylate the 10–15 % fresh platelets that enter the circulation every day from the bone marrow. Note that inhibition of TX formation in most cases is <95 % of capacity and, therefore, is not likely to result in inhibition of TX-sensitive platelet functions in vivo [103].

Aspirin vs. other antiplatelet drugs. All currently used inhibitors of platelet function finally inhibit the clustering and activation of the platelet GPIIb/IIIa receptor, that is, they prevent the binding of fibrinogen and subsequent aggregate formation by interplatelet fibrinogen bridges. Aspirin is unique among these compounds because it is the only one that acts primarily by blocking platelet COX-1-mediated TX formation. Therefore, aspirin will act synergistically with other antiplatelet agents, such as ADP receptor antagonists or antithrombins which interact with other pathways of platelet activation (Fig. 2.3.1-2) [96, 104].

Aspirin is *stricto sensu* not a specific antiplatelet agent. It differs from conventional antiplatelet drugs because of missing target selectivity [60]. It exhibits a broad spectrum of biological actions that are not restricted to or even selective for platelets. In contrast, both ADP and GPIIb/IIIa antagonists act largely in a platelet-specific manner because their cellular targets, the P2Y12 and GPIIb/IIIa receptors, are (almost) exclusively expressed there. This does not exclude pleiotropic actions of aspirin and ADP antagonists via modification of generation and release of platelet-derived mediators and their action on nonplatelet targets, for example P-selectin glycoprotein ligand (PSGL) receptors or CD40L.

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Interactions of aspirin with other COX inhibitors and salicylate. Nonaspirin NSAIDs, such as ibuprofen or indomethacin, also inhibit TX formation in platelets via inhibition of COX-1. They cannot replace for aspirin as antiplatelet drugs but interact with aspirin (salicylate) binding and, in consequence, will reduce or even abolish the antiplatelet action of aspirin (Fig. 2.2.1-4) (Section 2.2.1) [105, 106].

Under certain experimental conditions, such as pretreatment with salicylate at a high salicylate/aspirin ratio (≥20:1), this type of substrate inhibition can also be shown for salicylate [107, 108]. However, there is no evidence for any clinically relevant antagonism of antiplatelet actions of aspirin by the salicylate (metabolite) at conventional antiplatelet doses. Even pretreatment with 1,200 mg/day sodium salicylate for 3 days did not antagonize inhibition of platelet function and thromboxane formation after 350 mg intravenous single-dose aspirin in vivo [109].

At this point, it is interesting to speculate whether acetylation of platelet serine$_{529}$ by aspirin is really the reason for its antiplatelet effect, that is, inhibition of COX-1 enzymatic activity. Probably, it is not, since the active center at tyrosine$_{385}$ remains unblocked and other compounds circulating in blood that become enzymatically deacetylated, such as acetylcholine or heroin (diacetylmorphine), do not modify platelet function.

It is probably the initial rapid and reversible binding of the salicylate part of the molecule that closes the COX channel and brings the acetyl group in the correct steric position to serine$_{529/530}$ (Fig. 2.2.1-4). The enzymatic activity of the active center remains untouched, as also seen from increased 15-H(P)ETE production after acetylation of COX-2 – an action not shared with conventional NSAIDs or coxibs.

In other words, irreversible acetylation of serine$_{529}$ in platelets only fixes the salicylate group in the necessary steric position to block access of arachidonic acid to the downstream active center. For these reasons, it is rather the salicylate which is the true antiplatelet component of aspirin than the acetylation process *per se*.

Interactions of aspirin with morphine. Pharmacological interactions of opioid analgesics, such as morphine, and aspirin are of interest in acute treatment of myocardial ischemia. Here, the intestinal absorption of aspirin might be delayed by opioids, eventually resulting in a prolonged exposure against aspirin esterases in the gut and lower systemic plasma levels of unmetabolized aspirin. This kind of interaction was not observed when aspirin (162 mg EC oral) and morphine (5 mg intravenous bolus) were given simultaneously. Morphine increased the total systemic aspirin exposure by 20 % compared to placebo but did not change c_{max}, t_{max} or the antiplatelet effect of aspirin [110] – in contrast to the markedly reduced and delayed antiplatelet action of clopidogrel [111].

2.3.1.3 Endothelial cells

General aspects. Spontaneous platelet aggregation or even clot formation do not occur in the intact circulation because of the antithrombotic properties of healthy en-

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dothelium. Endothelial injury or dysfunction results in loss of these properties and allows for local platelet adherence and subsequent thrombus formation, both being sensitive to aspirin [112]. However, aspirin also reduces prostacyclin (PGI_2) production in vascular endothelial cells, which might result in opposite effects, i. e., prothrombotic and antifibrinolytic actions (see below) and functionally antagonize the antiplatelet effect. In vivo, negative interactions of aspirin with endothelial PGI_2 generation appear to be of minor importance at antiplatelet doses. One reason is the mainly COX-2-dependent PGI_2 formation by endothelial cells associated with a rapid turnover rate of the enzyme. This allows for a rapid replacement of the acetylated enzyme by new enzyme protein. Another is the posttranslational acetylation of eNOS with enhanced formation of endothelial protective NO.

Healthy endothelium. Aspirin inhibits PGI_2 production in cultured vascular endothelial cells at concentrations similar to those which also inhibit thromboxane formation in platelets [34, 113]. However, endothelial cells – in contrast to the anucleated platelets – exhibit a rapid recovery of COX activity by de novo synthesis of enzyme protein [113]. In vivo, at 3 h after aspirin administration to healthy subjects, there was already a 50 % recovery of (bradykinin-)stimulated endothelial PGI_2 production, and after 6 h there was complete recovery [114, 115]. Repeated administration of 500 mg aspirin twice daily reduced the excretion of PGI_2 (metabolite) for only about 3 h while inhibition of thromboxane excretion persisted over days [116]. Repeated administration of aspirin at antiplatelet doses of 100–300 mg reduced vascular PGI_2 production incompletely, by about 70 % [29]. There was a comparable (65 %) inhibition of PGI_2 production 24 h after a 600-mg single dose of aspirin [117]. In this study and another one, repeated higher doses of aspirin, that is, 325 mg/day or 500 mg twice daily, also resulted in only partial and short lasting – about 3 h – inhibition of PGI_2 production and subsequent rapid recovery [116]. Interestingly, inhibition of PGI_2 formation by repeated administration of low-dose aspirin over weeks may become less with time, whereas thromboxane inhibition is maintained [29, 117].

Inhibition of vascular endothelial PGI_2 generation by aspirin is generally transient and incomplete [29, 116–119] and may even be totally absent at very low doses (30 mg/day) if there is sufficient time for enzyme recovery or neosynthesis (Fig. 2.3.1-4). Vascular (endothelial) cells not only have a significant protein turnover rate (in contrast to the platelet), but also generate significant amounts of PGI_2 (more than 50 %) via (constitutive?) COX-2 [120]. A COX-2-dependent and possibly upregulated PGI_2 biosynthesis may contribute to cardioprotection [121]. The question which COX isoform is critical for endothelial PGI_2 production is still under discussion [122, 123].

Endothelium in atherosclerosis. Atherosclerosis alters the antithrombotic/vasodilatory/fibrinolytic properties of the endothelium towards a prothrombotic direction. This includes expression of adhesion molecules at the endothelial surface, migra-

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tion and diapedesis of white cells (monocytes) through the vessel wall as well as the release of proinflammatory cytokines. An upregulated vascular COX-2 in atherosclerotic vessel walls [124, 125] and white cells [126] can synthesize substantially more prostaglandins for control of hemostasis and vessel tone, and their generation is only partially inhibited by aspirin [125, 127] as discussed above. As a net result, the hemostatic balance between platelets and the vessel wall is kept maintained at a stable level. This equilibrium can rapidly become disturbed if COX-2-dependent PGI_2 and PGE_2 formation is reduced, for example by inhibition of the enzyme with traditional NSAIDs or coxibs at undisturbed platelet-dependent thromboxane production (Section 4.1.1).

For practical reasons, that is, antiplatelet treatment of patients at enhanced vascular thrombotic risk, it is of great interest to know whether aspirin at antiplatelet doses solely acts as an antiplatelet, antithrombotic agent via inhibition of thromboxane formation or in addition has prothrombotic effects by blocking COX-2/COX-1-mediated generation of antithrombotic and vasodilatory PGI_2 and PGE_2 in patients with atherothrombotic vessel diseases.

In a pioneering paper, Weksler and colleagues studied the inhibition of vascular PGI_2 formation in vessel segments ex vivo and platelet TXA_2 formation in serum of patients with angiographically documented CAD undergoing elective aortocoronary bypass surgery. Patients were pretreated with different single doses of aspirin 12–16 h prior to surgery.

Aspirin caused a dose-dependent inhibition of TX formation in serum and of PGI_2 generation in specimens of the aorta and saphenous vein, respectively. One single aspirin dose of 325 mg completely prevented TX generation but reduced PGI_2 formation by only 75 %. No significant reduction of PGI_2 generation was seen at lower aspirin doses. There was no difference in intraoperative blood loss at 325 mg aspirin as compared to untreated controls.

The conclusion was that 325 mg single-dose aspirin given to patients at advanced stages of atherosclerosis prior to surgical interventions (CABG) sufficiently inhibits TX-dependent platelet aggregation and TX release but blocks only partially vascular PGI_2 production in arterial and venous endothelium (Fig. 2.3.1-8) [128, 129].

These data confirm the general finding of an only incomplete and transient inhibition of vascular PGI_2 production by aspirin at doses which completely eliminate platelet-derived TX production. It should be noted that inhibition of PGI_2 *generation* is not necessarily paralleled by inhibition of PGI_2 *action*. PGI_2 acts via specific G_s-protein-coupled receptors at the cell membrane which, like all G_s-protein-coupled receptors, are subject to agonist-induced downregulation. Bioassay data convincingly demonstrate that inhibition of endogenous PG synthesis is generally associated with an enhanced sensitivity against the agonist. This has been shown for cultured endothelial cells where continuous basal PGI_2 generation is sufficient not only to completely desensitize G_s-protein via PGI_2 receptor-mediated adenylate cyclase activation [130], but also for inhibition of platelet aggregation by PGI_2 after aspirin pretreatment [131]. Downregulation of PGI_2 receptor-mediated reactions is also seen in vivo in situations of extensive endogenous PGI_2 production, for example during ischemia-induced up-

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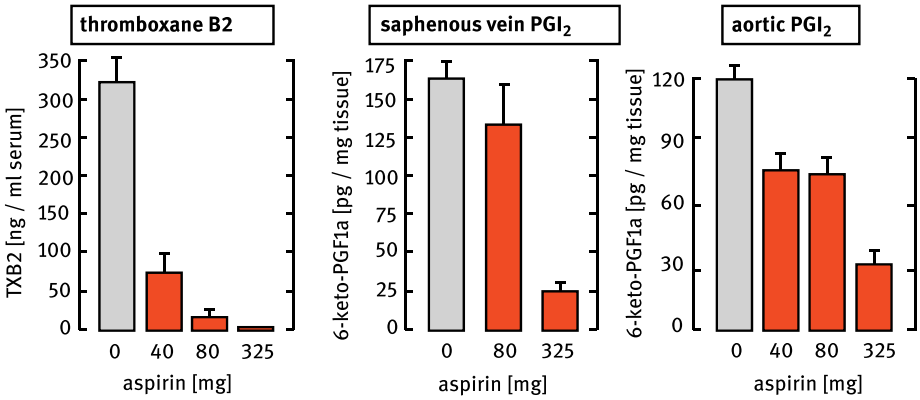

Figure 2.3.1-8: Dose-dependent inhibition of thromboxane (TX) and PGI_2 formation in serum and vessel specimens of patients with ischemic heart disease undergoing elective aortocoronary bypass surgery. All patients received single-dose aspirin at doses indicated. TX and PGI_2 were measured in terms of the stable metabolites TXB_2 and 6-keto-$PGF1_\alpha$. There was no significant inhibition of PGI_2 formation by up to 80 mg aspirin and an only about 75 % reduction at 325 mg but a nearly complete prevention of TX formation at both doses (modified after [128, 129]).

regulation of COX-2 and PGI_2 formation in acute myocardial infarction (Section 4.1.1). This is associated with a marked downregulation of PGI_2 receptor number and sensitivity (Fig. 4.1.1-3) [132, 133]. Even an aspirin-induced reduction of PGI_2 production in myocardial ischemia by 75 % must not result in any clinically relevant inhibition of its antiplatelet effects. In addition, COX-2-derived PGI_2 might well modulate platelet–vessel wall interactions in vivo by limiting the platelet response to TXA_2, as seen from genetically modified animals [134].

Aspirin, eNOS and endothelial protection. PGI_2 is not the only endothelium-derived mediator that inhibits platelet function. Two others are the endothelial cell ADPase (CD39) [135] and NO. Cleavage of platelet-derived ADP with subsequent adenosine formation by the ADPase activity of the 5′-nucleotidase is not changed or only modestly reduced by aspirin [135, 136]. In contrast, aspirin stimulates eNOS and subsequent NO formation and action already at low nano- to micromolar concentrations (3–30 µM) [137]. The more COX-2-specific analog APHS was at least as potent as aspirin (Fig. 2.3.1-9). This effect was independent of inhibition of COX protein expression or activity and seen for eNOS from both endothelium and platelets [138, 139].

Mechanistically, it was shown that aspirin caused posttranslational lysine acetylation of eNOS which is independent of COX-1 [140]. Aspirin also acetylates eNOS in platelets. The reaction was concentration-dependent, started at 5 µM and resulted in enhanced NO production [141]. The functional platelet responsiveness to aspirin was associated with the platelet content of phosphorylated eNOS at $serine_{1177}$ and was reduced in aspirin “resistance” (HTPR) [142]. It has also been shown that homozy-

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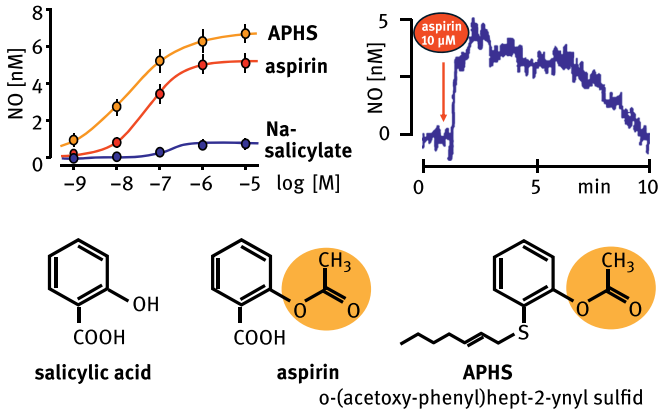

Figure 2.3.1-9: Concentration-dependent generation of NO by aspirin and a more COX-2-specific aspirin analog (APHS) but not by sodium salicylate from vascular endothelium in vitro. Note the low EC_{50} of 50 nM, which is well in the range of antiplatelet concentrations of aspirin (modified after [137]).

gous carriers of a cardiovascular risk variant of the GUCY1A3 genotype, encoding NO-stimulated soluble guanylate cyclase, are at higher risk of cardiovascular death and/or stent thrombosis due to high on-aspirin treatment platelet reactivity ("aspirin resistance") [143].

The group of *Henning Schröder* was the first to show induction of heme-oxygenase-1 (HO-1) as a downstream target of enhanced endothelial NO production by aspirin, suggesting a new prostaglandin-independent vasoprotective action of aspirin [138, 144]. Aspirin would synergize with the iron-binding, endothelial-protective protein ferritin in endothelial cells [145] with subsequent protection of endothelial cells from oxidative stress (Fig. 2.3.2-8) [137, 138, 146]. ATL, an antiinflammatory compound, also stimulates NO formation and induces HO-1 [147]. This suggests an interesting connection between the antithrombotic and antiinflammatory actions of aspirin (Section 2.3.2).

Two observational studies in patients at advanced stages of atherosclerosis and/or hypercholesterolemia have shown that aspirin improves the reduced "endothelium-dependent relaxation," a bioassay equivalent of NO formation in terms of "endothelium-derived relaxing factor" (EDRF). No such effect was seen in healthy subjects [148, 149]. Two further small, but randomized trials in healthy volunteers and patients with coronary heart disease confirmed that aspirin at doses between 81 and 1,300 mg significantly increased HO-1 levels in plasma and at the same time reduced the levels of asymmetrical dimethyl arginine (ADMA), an inhibitor of eNOS. This confirmed HO-1 as a downstream target of aspirin [150, 151]. These clinical data would fit well to the experimental studies mentioned above and overall suggest that acetylation of eNOS by aspirin could add to its antiplatelet/antithrombotic efficacy in cardiovascular prevention.

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2.3.1.4 Plasmatic coagulation

Aspirin can affect plasmatic coagulation via inhibition of thrombin generation. This is partially due to prevention of platelet activation and reduced exposition of the negatively charged membrane surface for the prothrombinase complex. Consequently, there is functional synergism between antiplatelet agents, such as aspirin, and inhibitors of thrombin formation [152]. In addition, aspirin could also inhibit thrombus formation by platelet-independent mechanisms. These involve acetylation of prothrombin [52, 153] and fibrinogen (Section 3.1.2) [53].

In this context there is a nice historical note regarding a suggested, though not real, anticoagulant effect of salicylate as an "active metabolite" of warfarin.

Paul K. Link and his group from Madison (Wisconsin) originally detected the anticoagulant action of dicumarol in the 1940s and also described a fall in plasmatic prothrombin levels, by both high-dose aspirin and sodium salicylate. Salicylate is formed during hepatic metabolism of warfarin (and other coumarins). These findings, a bleeding tendency after intake of about 10 grams of salicylates and the fact that coumarins had no anticoagulant effect in vitro, i. e., without becoming bioactivated by passage of the liver, prompted Link to speculate that the antithrombotic action of coumarins was due to intermediate generation of salicylate as the active metabolite [154].

Later he also showed that only coumarin (derivatives) from whom salicylate was formed as a metabolic intermediate, such as warfarin, exhibited an anticoagulatory action. From these and other data he additionally suggested that the reason for the slow onset of the anticoagulatory action of coumarins after oral intake was the requirement of (hepatic) salicylate formation as the active agent [154, 155].

It is now known that this hypothesis is not the explanation for antiplatelet/antithrombotic effects of aspirin. However, it is interesting from a medical-historical point of view that even a formally absolutely logical concept, apparently verified by well-done experiments providing the correct, expected result, may lead to wrong conclusions. Ironically, this hypothesis had a quite significant clinical impact – the choice of aspirin as a better tolerated warfarin "light" in the first systemic studies on myocardial infarct prevention by Craven (Section 1.1.4) [156].

There is a warfarin-like inhibition of plasmatic coagulation by aspirin at high, anti-inflammatory doses (≥3–4 g). This is due to inhibition of hepatic synthesis of vitamin K-dependent zymogens of clotting factors, predominantly prothrombin, but is not clinically relevant at antiplatelet doses.

2.3.1.5 Fibrinolysis

Fibrinolysis, that is, the reopening of an occluded vessel by dissolution of an occluding thrombus, marks the beginning of repair mechanisms, eventually resulting in complete restoration of the original blood flow prior to injury. Aspirin affects fibrinolysis both directly and indirectly at different levels and acts via different mechanisms. The two most important targets are the platelets and their procoagulant and antifibrinolytic factors and the vascular endothelium, generating anticoagulant and profibrinolytic factors. The net effect depends on the relation between the two.

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Fibrinolysis and platelets. Lysis of an arterial thrombus releases a variety of platelet-activating agents, including TXA_2, from still functioning (over hours!) [157] platelet COX. In addition, there are active thrombin, PAI-1 [11] and other serine proteases, such as factor Xa, and fibrin degradation products [9]. All of them enhance platelet reactivity in vivo and maintain a long-lasting procoagulatory state. Their release during coronary thrombolysis with streptokinase or tPA in patients with acute myocardial infarction stimulates platelet activation and thromboxane formation [157, 158], both being antagonized by aspirin. Mechanistically, it was assumed that aspirin by stimulation of eNOS in platelets might convert the zymogen plasminogen to plasmin [159]. In contrast, aspirin does not directly affect generation of antiplasmin by platelets [160].

Fibrinolysis and the endothelium. Aspirin does not change plasma levels of endothelium-derived t-PA or PAI-1 [161, 162]. Aspirin also does not affect enhanced fibrinolysis after physical exercise [163]. Aspirin inhibits ischemia-induced fibrinolysis in healthy volunteers. The suggested mode of action was inhibition of endothelial t-PA release at unchanged PAI-1 activity [160, 163, 164]. Consequently, inhibition of fibrinolysis by aspirin was prevented by PGI_2 [165, 166]. These data suggest that ischemia-related stimulation of fibrinolysis is at least partially caused by aspirin-sensitive prostacyclin formation.

As a net effect for the clinics, i. e., organ reperfusion after lysis or mechanical removal of a platelet-rich thrombus, there is evidence for a functional synergism between aspirin and fibrinolytics. Aspirin will antagonize platelet-dependent thromboxane and thrombin release during clot lysis. This reduces clot stability and makes it more vulnerable to fibrinolysis by plasmin and endothelium-derived lytic factors. For these reasons, treatment with antiplatelet agents, most notably aspirin, is essential prior to any fibrinolytic treatment – and also to mechanical clot removal by angioplasty. The synergistic effect of aspirin and thrombolysis by streptokinase in prevention of reinfarction has been convincingly demonstrated for the first time in the ISIS-2 trial (Section 4.1.1).

Summary

The main target of aspirin in control of hemostasis and thrombosis is the blood platelet and the inhibition of platelet functions via suppression of platelet COX-1-dependent thromboxane formation. Additionally, aspirin might also act as an antithrombotic via stimulation of eNOS and increased NO formation with subsequent protection of endothelial cells from oxidative stress. Aspirin inhibits (retards) the generation of thrombin, the most potent platelet-stimulating and procoagulatory factor, and enhances fibrinolysis. Thus, aspirin affects all three major components of the hemostatic system in a direction towards endothelial protection, prevention of thrombotic events and facilitation of clot lysis.

The potency of aspirin as inhibitor of platelet functions is dose- and time-dependent and also dependent on the stimulating agent. Regular intake of low-dose aspirin (75–100 mg/day) is sufficient for inhibition of aspirin-sensitive platelet functions and requires an at least 95 % inhibition

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of platelet thromboxane-forming capacity, as seen from the reduction of serum thromboxane levels. The antiplatelet effect of aspirin is irreversible for each platelet and can only be functionally overcome by new platelets that enter the circulation from the bone marrow. An increased platelet turnover rate, including higher proportions of more reactive, immature platelets, is an important variable of platelet reactivity and might require shorter dosing intervals in diseases with increased platelet turnover, such as essential thrombocythemia.

Aspirin is neither a specific nor a selective antiplatelet agent. Its mode of action differs qualitatively from all conventional antiplatelet drugs. This is the rationale for combined treatment with ADP receptor antagonists (thienopyridines, ticagrelor) or GPIIb/IIIa blockers (abciximab, fibans). The antiplatelet actions of aspirin also involve pleiotropic antiinflammatory effects on the vessel wall and the vascular endothelium.

Aspirin might also modify plasmatic coagulation via inhibition of thrombin generation, both by its antiplatelet effect and by acetylation (inhibition) of plasmatic coagulation factors. Consequently, there is a pharmacodynamics synergism of aspirin with all types of anticoagulants.

Fibrinolysis results in release of platelet-activating, prothrombotic factors from the thrombus, including thromboxane and thrombin. Both can be antagonized by aspirin treatment. Aspirin inhibits the release of profibrinolytic factors from the endothelium. As a net result, there is a synergistic action between aspirin and fibrinolytics as originally demonstrated in the ISIS-2 trial (Section 4.1.1).

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2.3.2 Inflammation, microbial infections, pain and fever

2.3.2.1 General aspects

Inflammation is a response to injury, characterized by expression and activation of cellular, humoral and immunological defense mechanisms. The inflammatory reaction is a vital function [1, 2] with numerous parallels – and also evolutionary established connections – to another vital defense system: the blood coagulation cascade. An inflammatory response enables the organism to survive infectious diseases [3] and injuries and to maintain tissue and organ homeostasis under a variety of noxious conditions in hostile environments [4].

Pathophysiology. A *systemic* inflammatory response might develop independently or as a consequence of a *local* inflammatory reaction. Examples for systemic chronic systemic inflammations are not only atherosclerosis [5] and rheumatoid arthritis, but also osteoarthritis as a consequence of chronic degenerative chronic joint affections. A life-threatening disease is SIRS, including sepsis, and ARDS. Enhanced coagulation and a tendency for immunothrombosis is typical and a major determinant for the clinical outcome (Section 4.2.2).

Pharmacologically relevant for all kinds of inflammatory and immune reactions is the generation and release of chemical mediators. *Systemic* inflammations are associated with elevated levels of inflammatory mediators from peripheral blood leukocytes and platelets, elevated C-reactive protein (CRP) levels and enhanced circulating prostaglandin levels as a consequence of COX-2 upregulation [6–10]. *Local* inflammation as a response to local tissue injury (trauma, infections) is associated with the unpleasant symptoms of edema and pain but, in more severe cases, also with systemic reactions like fever or a generalized immune response. In addition, there is always the risk for acute inflammations not to resolve after disappearance of the noxious stimulus but to persist and become chronic due to secondary (auto)immune processes. The therapeutic aim of antiinflammatory medications, such as aspirin and NSAIDs, is to control the inflammatory process, to stimulate repair processes and to reduce or even prevent its generalization and chronification.

The acute local inflammatory syndrome is characterized by its five classical features: heat (calor), redness (rubor), pain (dolor), edema (tumor) and tissue injury

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(functio laesa). These symptoms of acute inflammation are caused by complex interactions between circulating platelets, inflammatory white cells, cell- and tissue-derived mediators, free sensory nerve endings and the vessel wall endothelium. Leukocyte-derived cytokines additionally cause an increase in core temperature (fever) which speeds up the acute inflammatory response while (inflammatory) pain will act as an overall alarming signal, indicating tissue injury and its location. This means that any effective antiinflammatory treatment, including aspirin, in turn will also inhibit the accompanying events fever and pain (Fig. 2.3.2-1).

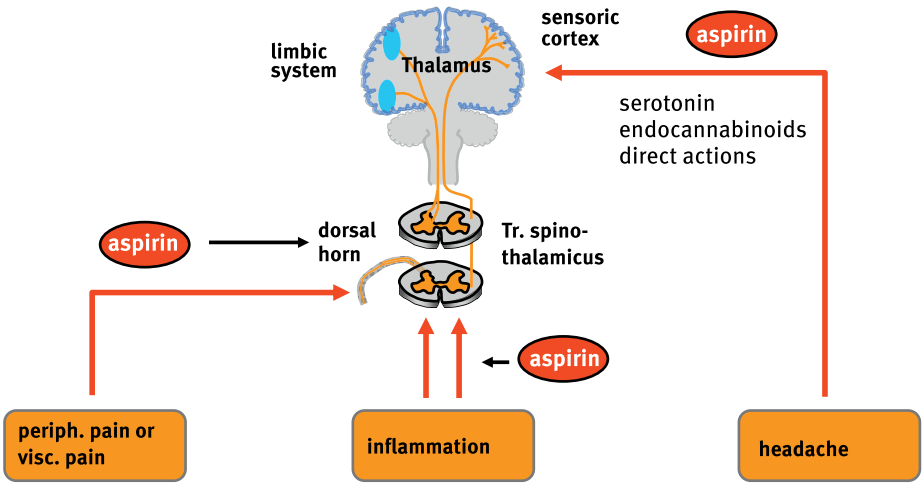

Figure 2.3.2-1: Sites of antiinflammatory/analgesic actions of aspirin.

Inflammatory mediators. The inflammatory reaction is an integrative part of effective host defense. Subsequent to local tissue injury, there is site-specific accumulation and activation of blood cells, associated with the generation and release of inflammatory mediators. The "inflammatory soup" [11] contains inflammatory interleukins (IL-1, IL-6) from leukocytes, kinins, growth factors, amines, protons, peptides and a mix of other chemicals, including adenosine, arachidonic acid, NO and prostaglandins. These chemicals and products thereof are released from broken cells or generated within the inflammatory exudate. Synergistically, they sensitize pain receptors, although prostaglandins have an outstanding, triggering position (see below). In addition, there is enhanced oxidative stress with free radical formation and release of tissue-destructive enzymes, in particular from inflammatory white cells (neutrophils).

In response to injury, inflammation has not only to be induced but also to be finished: This is realized by a process of resolution which limits uncontrolled, excessive tissue injury and minimizes the risk of development of chronic inflammation. Resolution is an active process which also involves lipid mediators: the lipoxins and re-

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solvins [12, 13]. Their antiinflammatory and resolving activity can also be mimicked by aspirin-induced COX-2 acetylation and subsequent production of ATL.

Systemic inflammation and the outstanding role of blood platelets. Platelets are phylogenetically old multipotent inflammatory cells [14, 15]. They also act as starters of clot formation in hemostasis and thrombosis. It is therefore not surprising that many interactions exist between platelets, white cells and the vascular endothelium [16]. These interactions are most relevant in the pathophysiology of (chronic) systemic inflammatory and immune responses. Platelets might interact with each other (homotypic aggregation) or adhere to circulating leukocytes (heterotypic aggregation). This allows for subsequent formation of mixed platelet/white cell aggregates and NETs as a consequence of the release of white cell-stimulating factors from activated platelets [17–19]. These functions are central not only to systemic inflammatory reactions but also to innate immunity [19, 20]. In addition, blood platelets act as pathogen "sensors" and express numerous receptors that are relevant for inflammation but not for hemostasis [21], including all ten known "Toll-like" receptors [22]. Platelets, although anucleated, contain sufficient RNA that can be translated and are also able to release membrane microparticles that can transport inflammatory cargo to inflammatory cells [21]. Of particular actual interest is the intricate relationship between platelet aggregation, secretion and inflammation in viral infections [23].

Taken together, there are tight interactions between platelets, inflammation and immune reactions. Platelets will trigger and enhance these responses, in many cases via aspirin-sensitive pathways. The present antiplatelet therapies which target key pathways of platelet activation and aggregation hold the potential to modulate platelet-derived inflammatory and immune functions by reducing cellular interactions of platelets with white cells and other immune components and by reducing the secretion of inflammatory proteins [14, 16, 24–29].

2.3.2.2 Modes of antiinflammatory actions of aspirin

Metabolic effects. Originally, it was thought that the antiinflammatory effects of aspirin (and its salicylate metabolite) result from their actions on cellular energy metabolism, that is, uncoupling of oxidative phosphorylation. However, other uncouplers of oxidative phosphorylation, such as DNP, have no antiinflammatory effect [30]. Moreover, antiinflammatory actions of aspirin were also described via modulation of platelet-derived mediator release at doses lower than those necessary to uncouple energy metabolism [24, 29]. Thus, the hypothesis of solely metabolic effects as an explanation for the antiinflammatory actions of aspirin, although attractive, was rejected [30]. It was replaced by the prostaglandin hypothesis, where aspirin's antiinflammatory action was explained by inhibition of prostaglandin biosynthesis. While prostaglandins certainly contribute to inflammation, it was already noted by

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Sir John Vane that inhibition of prostaglandin formation may not be the only explanation for the antiinflammatory action of aspirin [31]. There is also substantial evidence that metabolic actions of salicylate, related to kinase inhibition and ATP depletion (Section 2.2.2), will enhance the antiinflammatory actions of aspirin.

Prostaglandins. The finding by Sir John Vane that aspirin and salicylate inhibit prostaglandin biosynthesis provided for the first time a uniform and simple explanation for the well-known antiinflammatory, antipyretic and analgesic actions of salicylates [31]. His discovery was followed by the development of numerous NSAIDs, designed to inhibit inflammatory reactions by reducing local prostaglandin levels via inhibition of injury-induced prostaglandin biosynthesis (Section 2.2.1). Next-generation NSAIDs, the coxibs, are specific inhibitors of COX-2. However, it became clear that inhibitors of injury-induced upregulation of COX-2 will exert many more effects than just affecting inflammatory pain and fever. Additionally, prostaglandins themselves are rather modulators of inflammation with different actions on vascular and white cell-associated signs of inflammation [32, 33]. In addition, a plethora of other chemicals contribute to the inflammatory syndromes and interact which each other in a complex manner. Thus, prostaglandins are important and pharmacologically relevant inflammatory agents but not the only biomolecules that control the inflammatory process. In addition, inhibition of prostaglandin formation might have widespread side effects on physiological signaling pathways, for example renal sodium excretion, blood pressure control or integrity of the stomach mucosa.

Further platelet-derived aspirin-sensitive inflammatory mediators. The mode of antiinflammatory actions of aspirin is also much more complex than originally appreciated. It became evident that nonmetabolized aspirin itself acts as a potent antiinflammatory drug via target protein acetylation. This action of aspirin is probably partially platelet-mediated with platelet-derived thromboxane A_2 (TXA_2) as key mediator of autocrine and paracrine platelet functions. Regular aspirin intake at antiplatelet doses (100 mg/day) upregulates the platelet multidrug resistance protein 4 (MRP4) mRNA levels [34, 35]. This results in extrusion of aspirin from platelets and is one factor for aspirin "resistance" (Section 4.1.6). Dioxolane A_3 (DXA_3) is a new proinflammatory platelet-derived lipid which is generated by COX-1 from endogenous arachidonic acid. Its formation is independent of thromboxane biosynthesis. DXA_3 can activate and prime human neutrophils via Mac-1, suggesting a role in innate immunity and acute inflammation. Its biosynthesis is inhibited by aspirin at antiplatelet doses (Fig. 2.3.2-2) [29].

Another group of platelet-derived inflammatory mediators are stored platelet products, such as growth factors, including platelet-derived growth factor (PDGF) and other growth factors (VEGF, TGF-β1) [36], serotonin and S1P. S1P in blood is released from its storage sites in platelets in a strictly thromboxane-sensitive, that is,

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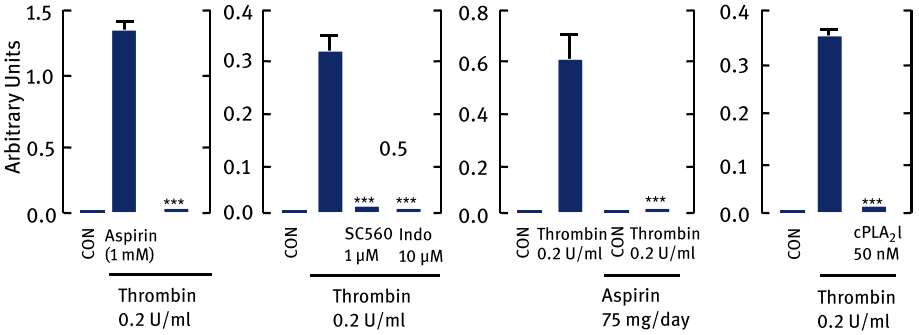

Figure 2.3.2-2: COX-1- and $cPLA_2$-dependent, aspirin-sensitive generation of DXA_3, a neutrophil-activating eicosanoid released by thrombin (0.2 IU/ml)-stimulated human platelets [29]. Abbreviations: SC560: selective inhibitor of COX-1; $cPLA_2I$: inhibitor of cytosolic phospholipase A_2; CON: control; Indo: indomethacin.

aspirin-sensitive, manner (Fig. 2.3.2-3) [37]. S1P is a proinflammatory lipid mediator of the ceramide class that also stimulates cell migration, is considered as a bioregulator in carcinogenesis (Section 2.3.3) [38] and might also play a role in myocardial ischemia and the antiischemic action of aspirin [39].

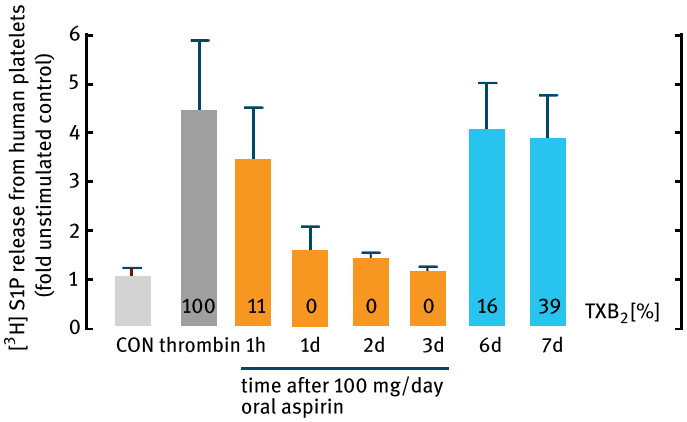

Figure 2.3.2-3: Thromboxane-mediated release of S1P from thrombin-stimulated human platelets and its inhibition by aspirin (100 mg/day for 3 days). The numbers at the columns represent the remaining thromboxane-forming capacity in serum (thrombin = 100 %) (after data in [37]).

A prerequisite for tissue-destructing actions of activated leukocytes in acute inflammation is their adhesion to and transmigration through the endothelial lining of blood vessels. This leukocyte traffic is controlled by numerous signaling and adhesion molecules, a significant proportion of which are aspirin-sensitive. In this context, experimental studies have shown that salicylates inhibit cytokine-induced expression

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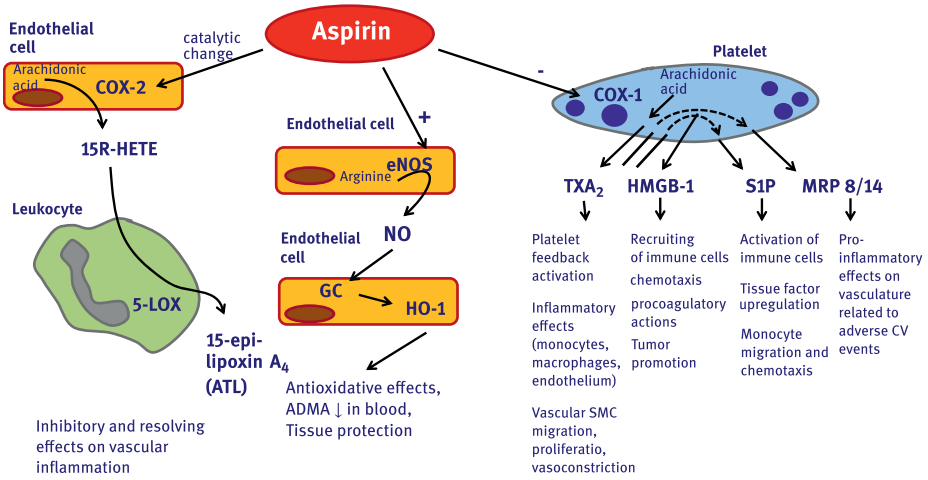

Figure 2.3.2-4: The multiple antiinflammatory effects of aspirin at antiplatelet doses (modified after [44]). Abbreviations: ADMA: asymmetrical dimethyl arginine; HETE: hydroxyeicosatetraenoic acid; MRP: platelet multidrug resistance protein; S1P: sphingosine-1-phosphate [44].

of adhesion molecules on endothelial cells [40], integrin-mediated neutrophil adhesion [41], reduction of specific T-cell recruitment to the inflammatory site [42] and TNFα-induced monocyte adhesion to endothelial cells [43]. However, the clinical relevance of these findings, predominantly shown in vitro, is uncertain because of the high salicylate concentrations, frequently in the millimolar range, that had to be used to see these actions. The clinical significance of these "heterotypic" platelet functions [18] is currently under intense investigation. An overview of possible aspirin-sensitive actions at antiplatelet doses is shown in Fig. 2.3.2-4.

Aspirin and high-mobility group box 1 protein. Of particular significance as a salicylate-sensitive inflammatory mediator is the recently detected HMGB-1. HMGB-1 is a damage-associated molecular pattern that is generated in many cells, megakaryocytes and platelets being major sources. HMGB-1 is becoming increasingly interesting for its outstanding role in inflammation, coagulation and tumorigenesis [45, 46]. For example, HMGB-1 can recruit immune cells to the inflamed synovia, initiating an adaptive immune response and perpetuating disease [47]. Salicylic acid binds to HMGB-1 in submillimolar concentrations (100 µM) and inhibits its chemotactic actions on leukocytes, expression of inflammatory cytokines and expression of COX-2 [48].

Studies in knock-out animals and other in vivo experiments suggest that HMGB-1 causes platelet aggregation and promotes NET formation and (venous) thromboembolism (VTE) (Section 4.1.4) [24, 49]. Circulating levels of HMGB-1 and other so-called "alarmins" are increased in blood of patients with sepsis, thrombosis and several

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inflammatory disorders [47]. Salicylate binds to HMGB-1 and directly suppresses its proinflammatory/procoagulatory activities [48]. This might contribute to beneficial effects of aspirin as an adjunct in treatment of sepsis and ARDS (Section 4.2.2). More recent studies have shown that HMGB-1 mRNA levels can be reduced in human platelets and megakaryocytes by aspirin treatment (100 mg/day), in healthy individuals and also in patients at elevated atherothrombotic risk [50].

The significance of these findings for therapeutic actions of aspirin in treatment of inflammatory diseases is currently unknown. Platelet-derived HMGB-1 (disulfide) is not only a platelet storage product but also a central mediator of platelet-mediated thrombotic/inflammatory processes in high-risk atherothrombosis [50] and venous thromboembolism [24]. Interactions between aspirin-sensitive platelet activation and secretion, NET formation and thromboxane biosynthesis have been established in animal studies on venous thromboinflammation (Fig. 4.1.4-1) [51, 52] and might also be relevant for human [50]. This suggests that HMGB-1 is not only a platelet-related aspirin-sensitive prototypical mediator of sterile inflammation, but also a master regulator of the prothrombotic cascade (Section 4.1.4) [24].

Inhibition of COX-2 by aspirin. COX-2 is the key enzyme for prostaglandin production in inflammatory conditions. In response to injury, the enzyme becomes transcriptionally upregulated in the vascular endothelium, smooth muscle cells and white cells – the dominating cellular source of prostaglandin generation in inflammation. Both aspirin and salicylate inhibit COX-2-mediated prostaglandin production in vitro and in vivo. Inhibitory concentrations of aspirin for COX-1 and COX-2 in vitro are similar, both in the low micromolar range [53–55]. In IL-1-stimulated vascular smooth muscle cells expressing solely COX-2, maximum inhibition of prostaglandin formation was obtained in vitro at concentrations of 30 and 300 µM, i. e., 5 and 50 µg/ml of unmetabolized aspirin and salicylate, respectively (Fig. 2.2.1-5). In vivo, the inhibitory capacity of COX-2 inhibition by aspirin is lower, probably due to the short half-life of non-metabolized aspirin, the significant turnover rate of acetylated proteins and the activity of omnipresent aspirin deacetylases. In addition, the concentrations of plasma salicylate are about 6-fold higher than those of aspirin due to the slower metabolic degradation and prolonged salicylate plasma half-life (Section 2.1.1) from 2–3 to 20 h and more at toxic levels. For these reasons, the salicylate metabolite of aspirin contributes significantly to the antiinflammatory action of the compound at higher doses (about 2 g and more) and repeated administration in vivo reduces the prostaglandin content of inflammatory exudates for a comparable extent. In contrast to salicylic acid (*o*-hydroxybenzoic acid), the *m*- and *p*-analogs of hydroxybenzoic acid are ineffective (Fig. 2.3.2-5) [56], as discussed in more detail in Section 1.2.1.

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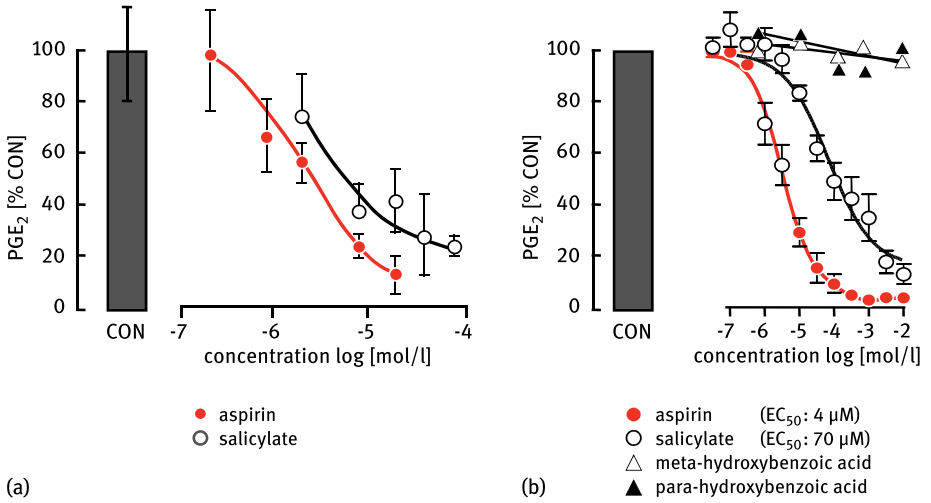

Figure 2.3.2-5: (a) Inhibition of PGE_2 generation in tissue explants of acutely inflamed rat synovia and (b) inhibition of COX-2-dependent PGE_2 generation in cultured human vascular smooth muscle cells by aspirin and salicylate. Note the comparable potency of aspirin and salicylate (*o*-hydroxybenzoic acid) in the inflammation model, a slightly less potent though significant inhibition of COX-2-derived PGE_2 by salicylate and the absence of any inhibition by *m*- and *p*-hydroxybenzoic acid analogs (after [56]; Schrör & Rompel, unpublished).

Lipoxins. The inflammatory process also needs to be terminated by removal of cell debris by tissue-destructive enzymes from white cells followed by a process of resolution, that is, restoration of the situation *quo ante*. COX-2, acetylated by aspirin, changes its functionality from a COX that generates prostaglandins to a 15-lipoxygenase that produces 15-(R)-HETE, a precursor of 15-epi-lipoxin A_4 or ATL (Fig. 2.3.2-6) [13].

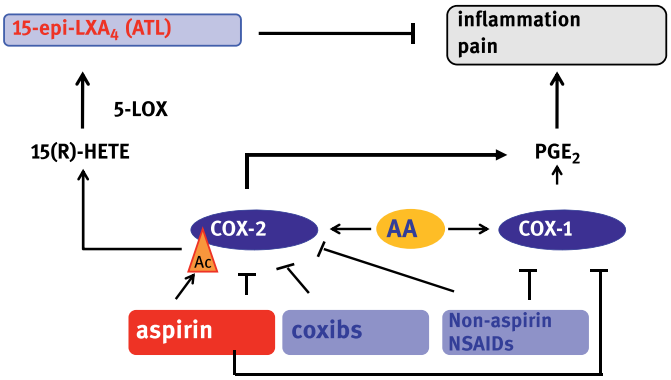

Figure 2.3.2-6: Generation and action of ATL (15-epi-LXA_4) by aspirin but not by nonaspirin NSAIDs or coxibs. Note the synergistic interaction of 15-(R)-HETE made by the acetylated COX-2 and 5-lipoxygenase from white cells. For further explanation see text (© Dr. Schrör Verlag, 2011).

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ATL, like other lipoxins, has dual antiinflammatory and proresolving activities and acts via specific lipoxin receptors (ALX) that are unrelated to those for prostaglandins. ATL and other lipoxins made by intercellular interaction of different cell-based lipoxygenases inhibit cell proliferation and leukocyte recruitment to the inflammatory site, thereby preventing a chronic inflammatory state. Topic ATL antagonizes the neutrophil-induced increase of vascular permeability in an inflamed area [57]. Additionally, lipoxins not only inhibit inflammatory reactions, but also, unlike conventional immune suppressives, stimulate defense mechanisms. For example, ATL was shown to protect mice from endotoxin-induced lung injury by inhibition of NET formation [58]. ATL also inhibits neuroinflammation and neuropathic pain in the spinal cord [59]. Thus, induction of lipoxin formation by aspirin appears to be another attractive new tool to understand the antiinflammatory and inflammation-resolving actions of the compound.

Aspirin has been shown in a randomized controlled trial to (modestly) stimulate ATL formation in man and at the same time to reduce thromboxane biosynthesis [60]. This effect was greatest at low-dose (81 mg/day) aspirin. Importantly, the biosynthesis of 15-epi-LXA_4 (ATL) in an inflamed area of human skin was also shown to be induced by antiplatelet doses of aspirin (75 mg/day for 1 week). This was associated with a reduced accumulation of white cells in the inflammatory exudate, while PGE_2 production was also partially reduced (Fig. 2.3.2-7). At the same time, the number of specific lipoxin receptors was increased [61].

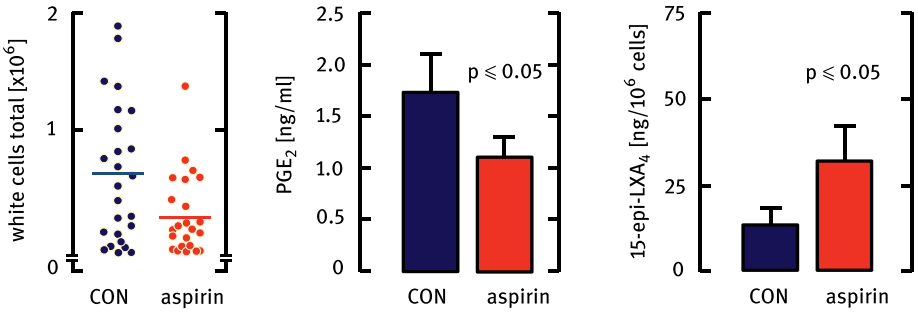

Figure 2.3.2-7: Leukocyte accumulation and local levels of PGE_2 and 15-epi-LXA_4 in a human model of inflammation (cantharidin skin blisters) before (CON) and after 1 week of aspirin treatment (75 mg/day) (modified after [61]).

The same group later extended these studies and demonstrated that aspirin-induced ATL formation acts as an internal breaking signal tempering the severity and longevity of acute inflammatory responses in humans [62].

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Aspirin and endothelial protection via enhanced NO formation. Another aspirin-specific antiinflammatory action which is not shared with conventional NSAIDs is endothelial protection from oxidative stress. This process is initiated by enhanced NO formation via acetylation of eNOS (Section 2.3.1.3). NO, generated by eNOS in vascular endothelium and platelets, improves the oxygen defense of the inflamed tissue and vascular endothelium by upregulation of heme oxygenase-1 (HO-1).

Heme oxygenases are rate-limiting enzymes of heme degradation. HO-1 is the inducible isoform. The enzyme catalyzes the formation of bilirubin, free iron ions and carbon monoxide (CO). Bilirubin is a potent antioxidant. Elevated levels of bilirubin are associated with a reduced atherogenic risk and protect from endothelial injury. Ferritin is the storage protein for iron in blood. Enhanced levels will, therefore, reduce the levels of free iron ions in plasma, eventually also resulting in decreased radical formation and improved oxygen defense (Fig. 2.3.2-8).

Upregulation of HO-1 results in antiinflammatory and antithrombotic actions (Fig. 2.3.2-8) [63–65]. Studies in healthy volunteers subjected to experimental systemic inflammation by *Salmonella typhi* vaccination have shown that inflammation-induced endothelial dysfunction could be prevented by previous aspirin treatment (1.2 g). Aspirin intake *after* vaccination had no effect. It was hypothesized that these actions of aspirin were mediated by improved endothelial defense after inhibition of inflammatory cytokine (IL-1) actions [66]. Similar findings, i. e., improved endothelium-dependent relaxation as surrogate for enhanced endothelial NO formation, were obtained after aspirin treatment of patients with atherosclerosis, a low-grade inflam-

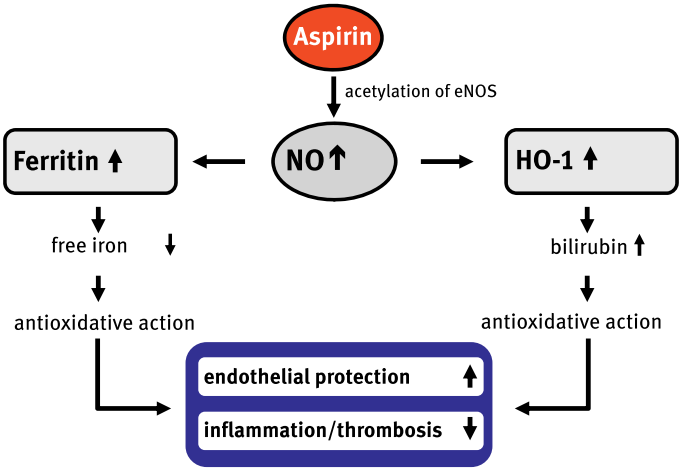

Figure 2.3.2-8: Aspirin (ASA)-induced endothelial protection by enhanced NO formation via acetylation of endothelial NO synthase (eNOS). NO induces the antioxidative enzyme heme oxygenase-1 (HO-1) as well as upregulation of the iron-binding protein ferritin. Both exert antiinflammatory and antithrombotic effects by improving endothelial protection (for further explanations see text) (modified after [63, 64]).

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matory disease with endothelial dysfunction as a general feature [67, 68]. There was a marked increase in HO-1 levels, by about 50 %, and a reduced level of ADMA, an inhibitor of eNOS, by 30 %. Both changes were highly significant and independent of aspirin doses, suggesting HO-1 as a downstream target of aspirin in endothelial dysfunction [69, 70]. Although inhibition of platelet COX-1-mediated thromboxane formation is considered the major mechanism of antithrombotic actions of aspirin, enhanced aspirin-induced generation of NO by endothelial cells – possibly ATL-mediated [71] – might be an important additive factor to improve endothelial functionality [70], thereby connecting the antiinflammatory and antithrombotic pathways of the compound.

Adenosine. An alternative explanation for the inhibition of leukocyte accumulation to an inflamed area by aspirin and salicylates is the local accumulation of adenosine in granulocytes after enhanced ATP breakdown [72] and/or prevention of its rephosphorylation by inhibition of oxidative phosphorylation (Section 2.2.3). In this context, inhibition of leukocyte accumulation by salicylates was shown in an in vivo experimental model to be independent of inhibition of prostaglandin synthesis and was suggested to be adenosine-mediated [73]. This interaction is also interesting because of a possible adenosine-mediated synergism in antiinflammatory actions of aspirin with the disease-modifying antirheumatic drug (DMARD) methotrexate. The antiinflammatory effects of methotrexate are thought to be mediated by adenosine, generated from released and extracellularly converted adenine nucleotides (Section 4.2.2) [74].

Aspirin and NSAIDs. The mode of these multiple antiinflammatory actions of aspirin differs fundamentally from that of COX inhibitors (NSAIDs, coxibs). In contrast to aspirin, these compounds compete specifically and reversibly with the binding of arachidonic acid inside the substrate channel of COXs. They might even negatively interact with aspirin because of their much higher affinities to arachidonate binding sites, with binding affinities in the micromolar (ibuprofen) vs. millimolar (salicylates) range (Section 2.2.1). In addition, NSAIDs cannot acetylate COX-2 and, therefore, are not "aspirin-like" as inductors of a 15-lipoxygenase which generates the 15-(R)-HETE precursor of lipoxins (Fig. 2.3.2-6). Combined use of aspirin and NSAIDs should therefore – if possible – be avoided. This issue becomes clinically relevant in patients with chronic inflammatory pain (osteoarthritis) who also require aspirin prophylaxis because of enhanced risk of atherothrombosis (Section 4.1.1).

2.3.2.3 Modes of antimicrobial actions of aspirin

Many antipyretics, including aspirin, can inhibit growth and replication of bacteria and viruses [3]. This eventually affects motility, adherence and metabolism of mi-

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crobes and their pathogens but might also cause an altered susceptibility of bacteria to antibiotics. A possible consequence is an increased efficacy of antimicrobial treatment by antibiotics [75]. This issue will become particularly relevant in times of rapidly increasing antibiotic resistance [76]. The possible mode of action of salicylate is an improved membrane permeability that causes changes in antimicrobial susceptibility of many pathogens [76]. In addition, aspirin and salicylate also interact with inflammatory cytokines, such as TNFα and IL-1, that are found in significant amounts after viral and bacterial infections and are key players in inflammation and fever.

Aspirin and viral infections. Aspirin has been shown to effectively block influenza virus infections in vitro and in vivo [77, 78]. This was explained by inhibition of virus replication and propagation via the NF-κB signaling pathways in host cells. There were no toxic side effects of aspirin or any drug-related tendency to induce resistant virus variants [78]. The ensuing inflammatory response (cytokine storm) will also be inhibited by this mechanism (Fig. 2.3.2-9) [79].

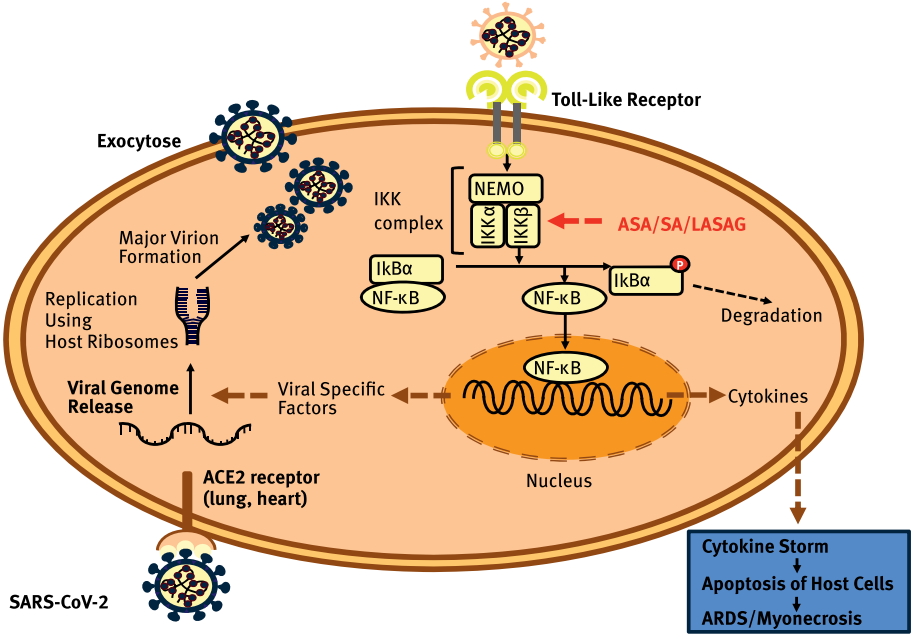

Figure 2.3.2-9: Activation of the NF-κB pathway by SARS-CoV-2 viruses leads to cytokine storm and myonecrosis – aspirin (ASA) inhibits IKK complex phosphorylation and subsequent activation of NF-κB. This results in retention of newly produced viral genomes in the nucleus and inhibits generation of new virus particles (modified after [79]).

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Aspirin demonstrated antiviral activity against all human rhinoviruses [80]. Intravenous application of a soluble aspirin lysine salt (BAY 81-8781 or LASAG) to mice infected with influenza virus reduced mortality by 50 %, even if started as late as 48 h after infection [81]. This was a successful proof of the novel innovative antiviral concept of targeting a host cell signaling pathway that is required for viral propagation instead of targeting viral genetic structures with their high rate of spontaneous mutations. Aspirin acts by blocking IκBα degradation with subsequent inhibition of NF-κB signaling pathways in the host cell and appears to have no effect on other virus-induced host cell kinases [78].

Identical modes of action were found with LASAG in several influenza strains [81]. Interestingly, the presence of LASAG (5 mM) during superinfection of influenza virus-infected lung epithelial cells with *Staphylococcus aureus* in vitro reduced influenza virus titers (replication) and reduced intracellular *S. aureus* loads. This suggests that inhibition of the NF-κB pathway might not sensitize cells against bacterial superinfections but will even inhibit bacterial intracellular invasion [82].

Aspirin with its antiviral potential, added to its clinically established antiinflammatory and antithrombotic actions, is already approved for treatment of flu-like conditions but could also be an interesting candidate for treatment of real flu and COVID-19 (Section 4.2.2) [83]. Aspirin given as aerosolized LASAG reduces viral titers, decreases viral protein accumulation and RNA synthesis and impairs formation of coronavirus replication transcription complexes – all of these actions via a host- and not virus-directed mode of action [84]. Application of the water-soluble lysine salt by a nebulizer would allow for high local salicylate concentrations in the lung, the primary affected organ [85]. It is quite possible that the antiviral activity of aspirin also contributes to the antipyretic actions of salicylates, at least in flu and flu-like conditions.

2.3.2.4 Modes of analgesic actions of aspirin

Different types of pain. Pain is the most unpleasant symptom of inflammation. The injured tissue sends signals, generated locally by chemical mediators, to central areas of pain perception in order to initiate appropriate avoidance reactions (Fig. 2.3.2-1). For these reasons, inflammatory pain relief is a clear and easy readout of the antiinflammatory action of a drug. Aspirin and other COX inhibitors are standard medications to treat inflammatory and ischemic, that is, prostaglandin-related, pain. However, aspirin also affects noninflammatory pain, most notably tension-type headache and migraine (see below). Unfortunately, aspirin is rather ineffective in neuropathic pain syndromes, i. e., pain due to lesions or direct stimulations of somatosensory neurons [86].

Similar to inhibition of platelet functions by aspirin, there is no linear relationship between inhibition of prostaglandin (thromboxane) biosynthesis and the analgesic potency of aspirin. Additionally, there are numerous interactions of aspirin with other nociceptive sites and mediators, including the endocannabinoids [87] and adenosine [73], but also neuronal pain transmission in the spinal cord and other parts of the CNS.

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It is also evident that pain can result from many more reasons than inflammation or ischemia, that is, situations with enhanced local prostaglandin production. Pharmacologically this means that analgesic potency of aspirin will not necessarily be the same in all painful conditions.

Prostaglandins and inflammatory pain. Among the numerous chemicals that are released at a local site of inflammation, vasodilatory prostaglandins, such as PGE_2 and prostacyclin (PGI_2), have an outstanding position as mediators of pain. This was originally described by *Sergio Ferreira* [88] and ultimately confirmed by the disturbed or even missing pain perception in animals with genetically deficient prostaglandin/prostacyclin receptors [89, 90].

The first step in pain perception is the conversion of a chemical signal, generated within the injured area by local mediators, to an electrical signal by nociceptors. Vasodilatory prostaglandins such as PGE_2 or PGI_2 sensitize nociceptors via various EP and IP receptor subtypes [32]. This lowers the activation threshold for nociceptors in the affected area via the nociceptor-specific capsaicin receptors, a voltage-dependent cation channel (TRPV1). The result is an increased excitability of the affected sensory neuron (Fig. 2.3.2-10) [91–94].

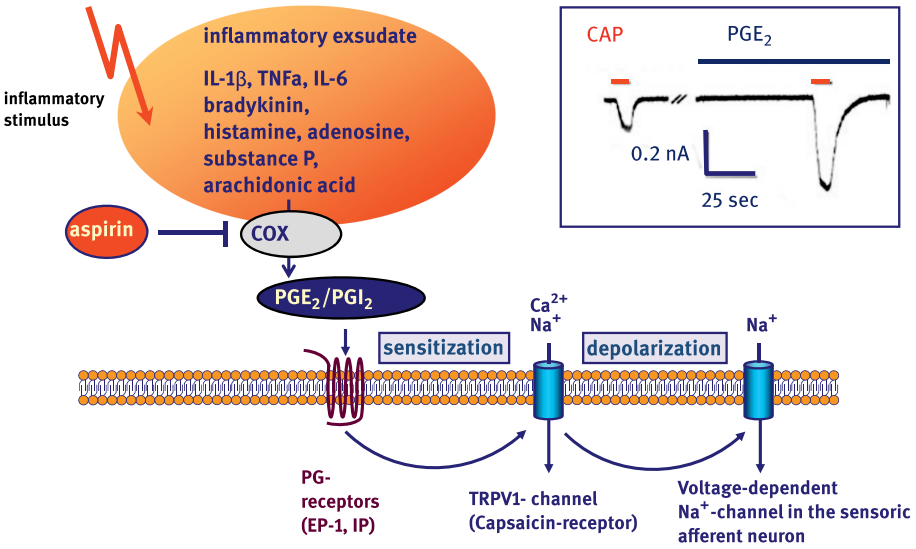

Figure 2.3.2-10: Mediators of pain and inflammation in the inflammatory exudate. The inflammatory exudate contains a plethora of chemical mediators, among them arachidonic acid (AA) from destroyed cell membranes. AA is converted to prostaglandins, including PGI_2/PGE_2, by COXs. This is inhibited by aspirin. Pain mediators activate the capsaicin (CAP) pain receptor, resulting in opening and activation of the TRPV1 cation channel. This action is potentiated in the presence of PGI_2/PGE_2 with subsequent sensitization and activation of afferent sensory neurons (modified after [94]).

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These processes facilitate and stimulate the afferent signal transmission to areas of pain perception in the CNS. The functional consequences are hyperalgesia and allodynia, i. e., the shift of the pain threshold to the left and increased pain perception [95].

The "normal" pain sensation curve in response to painful stimuli is shifted to the left by release of pain mediators from injured tissue. This causes hyperalgesia – increased sensitivity to noxious stimuli – and allodynia – pain sensations in response to stimuli which normally do not provoke pain. Analgesics like aspirin will shift the dose–response curve for pain backwards to the right by peripheral and central modes of action (modified after [95]).

Peripheral analgesic actions of aspirin. Several mechanisms contribute to a peripheral analgesic action of aspirin. Best known is the inhibition of generation of pain mediators, such as prostaglandins. This largely explains the actions of aspirin on pain signal-generating and -processing events at a site of injury. The first experimental evidence in humans for involvement of an arachidonic acid-derived lipid mediator was the appearance of aspirin-sensitive pain after pricking of diluted emulsions of arachidonate into the volar face of the human forearm. This was followed by erythema (Section 1.1.3) [96]. The potentiation of pain responses by threshold doses of other algogens (bradykinin, histamine) or intradermal injection of PGE_1 was not reduced by aspirin [88]. This suggested an action of aspirin on generation but not action of arachidonic acid-derived pain mediator(s), later identified as prostaglandins, as well as a peripheral, prostaglandin-mediated antihyperalgesic (analgesic) site of aspirin action. Changes in pain sensation with associated hyperalgesia and allodynia are depicted in Fig. 2.3.2-11.

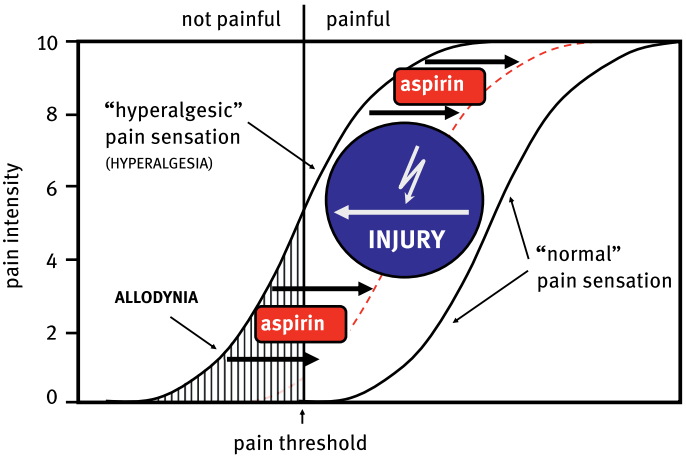

Figure 2.3.2-11: Changes in pain sensation induced by tissue injury: hyperalgesia and allodynia.

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Later experimental and clinical studies showed that inhibition of peripheral prostaglandin synthesis was not the only mechanism of analgesic actions of aspirin [97]. For example, there was no clear correlation between the intensity of analgesia and inhibition of prostaglandin biosynthesis by aspirin. The short-lasting hyperalgesic actions of prostaglandins additionally suggest the contribution of additional factors for longer lasting responses that were released from the inflamed site [98, 99] and/or actions on afferent pain perception at the spinal or supraspinal levels [100, 101]. ATL formation has been shown in animal experiments to attenuate inflammation-induced pain processing [102].

Central analgesic actions of aspirin. Inflammation can also cause cytokine (IL-1β)-induced upregulation of COX-2 in spinal cord dorsal neurons and other regions of the CNS [103], a process which is associated with hyperalgesia [104]. This suggests that in addition to peripheral actions via inhibition of injury-induced prostaglandin release, aspirin might also directly affect signal transduction in afferent nociceptive pathways, perhaps sharing some similarities with coxibs [104, 105]. The central antinociceptive actions of aspirin are not abolished by the opioid antagonist naloxone, suggesting that the endogenous pain control system of endorphins is not involved [106].

Aspirin and endocannabinoids. Another interesting class of mediators that are involved in pain control are endocannabinoids. These are monoacylglycerols, frequently containing arachidonic acid in the 2-position of the glycerol residue analog to phospholipids. The best-known compound is anandamide, an endogenous ligand of cannabinoid receptors (CB1/2) and a selective, high-affinity substrate of COX-2 (Fig. 2.2.1-1) [107]. Appreciable concentrations of anandamide have been found in dorsal horn ganglia and in the spinal cord, suggesting a relationship to pain transmission. Endogenous cannabinoids as well as exogenous cannabis are potent analgesics. A possible functional relationship between aspirin, prostaglandins and endocannabinoids was shown via ATL (Section 2.2.1.2) [108]. Interestingly, desensitization of cannabinoid receptors by long-term treatment with cannabis abolished the analgesic actions of aspirin and modified that of some other NSAIDs but not of paracetamol (acetaminophen) [83]. A causal relationship between cannabinoids and COX inhibition was suggested [87]. A potentiation of subthreshold analgesic doses of aspirin by an agonist of cannabinoid receptors was also shown [109]. These studies provided the first experimental evidence for the possible involvement of the cannabinoid system in the analgesic action of aspirin and several NSAIDs [110] and deserve further investigations.

Aspirin and serotonin. Serotonin (5-HT) is another transmitter for pain sensations, in particular inside the brain, presenting clinically as migraine and tension-type

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headache. An interaction between salicylates and serotonin has been postulated for a long time and is one of the main arguments for a central analgesic effect of aspirin [106, 109, 111, 112]. It has also been suggested that interactions between aspirin and serotonin in the CNS might be relevant not only to headache (Section 4.2.1) [113], but also to cognitive processing [114]. In vitro studies with human platelets, the main storage site of serotonin in blood, indicate that both aspirin and salicylate can inhibit serotonin release from platelets by a thromboxane-independent mechanism (Verheggen & Schrör, unpublished). Another finding are interactions with serotonin synthesis by displacement of the amino acid precursor tryptophan by salicylates from its binding to plasma proteins, eventually stimulating serotonin synthesis in the brain [106, 115]. Elevated brain serotonin was found in cortical and pontine areas of the rat brain, subsequent to parenteral aspirin treatment at antiinflammatory doses [116]. It was suggested that high brain serotonin levels will downregulate serotonin receptors [106, 116] and that this mechanism, possibly interacting with the cannabinoid system [109], might mediate central antinociceptive actions of aspirin.

Direct actions of aspirin on pain transmission and central pain perception. In noninflammatory conditions, nausea, vomiting, tinnitus and dizziness are typical initial symptoms of aspirin overdosing in the CNS (Section 3.1.1). They are mediated by actions of salicylates on particular regions of the brain or inner ear (Section 3.2.4) and suggest specific actions of aspirin in the CNS which are independent of prostaglandins. Experimental studies have shown that intrathalamic injection of aspirin or salicylate depresses C-fiber-mediated nociceptive activity after nerve stimulation, suggesting a central (spinal cord) mechanism of the antinociceptive action of the compound [117]. More recent experimental studies have confirmed an analgesic action of oral aspirin at the spinal cord level and additionally demonstrated a nociceptive processing which was different from that of paracetamol [118]. Studies in men, using a model of mediator-independent evoked pain after direct electrical stimulation of nociceptive sensory nerves, have clearly shown a direct effect of aspirin on pain transmission and reception [119, 120].

Overall, these data show that inhibition of (peripheral) prostaglandin biosynthesis alone does not sufficiently explain the analgesic effects of aspirin – a conclusion already reached by Sir John Vane in his pioneering studies on the mode of action of aspirin [31]. While it is clear that any antiinflammatory action of aspirin will reduce prostaglandin formation and, thereby, remove a pain receptor-sensitizing factor, it is unlikely that this mechanism alone fully explains the reduced pain sensation related to headache or direct electrical stimulation of nociceptors in the absence of tissue injury. Interactions with other mediator systems such as the endocannabinoids or serotonin are likely, as well as direct actions on pain transmission and perception in the CNS.

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2.3.2.5 Modes of antipyretic actions of aspirin

Fever and the mediators of febrile response. A febrile reaction in response to microbial infections is also part of a natural defense reaction. It is caused by a cytokine-induced rise in core temperature and the generation of active phase reactants with the "intention" to speed up the body's own defense systems [121, 122]. The febrile reaction starts with exposition of the organism to exogenous pyrogens, such as viruses, bacterial toxins or other products of microbial origin. These enter the organism and stimulate white cells to phagocytosis and generation of pyrogenic cytokines. The endogenous pyrogens IL-I, TNFα, IFN-γ and IL-6 have the capacity to raise the thermoregulatory center set point in the hypothalamus. They do so by acting directly on thermosensitive neurons after crossing the blood–brain barrier and/or by stimulating the release of other potentially "pyrogenic" mediators, such as PGE_2, in the CNS. This involves up-regulation of COX-2 and results in subsequent increase of PGE_2 in the preoptic region of the hypothalamus. This is an area with high expression levels of prostaglandin EP_3 receptors [123], an established site of fever-inducing PGE (Fig. 2.3.2-12) [32, 124].

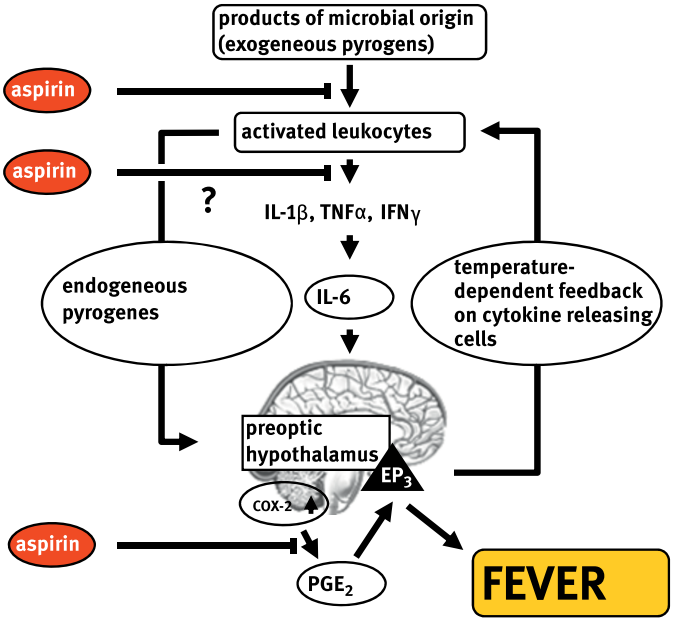

Figure 2.3.2-12: Hypothetical model of genesis of fever and possible sites of antipyretic action of aspirin and salicylate (modified after [122]).

Aspirin versus salicylate. Aspirin does not reduce normal body temperature, nor does it modify an elevated body temperature subsequent to physical exercise [125] or as a result of increased temperature in the environment [126]. Aspirin selectively reduces pyrogen-induced fever [127] by an interaction with the pyrogenic cytokines

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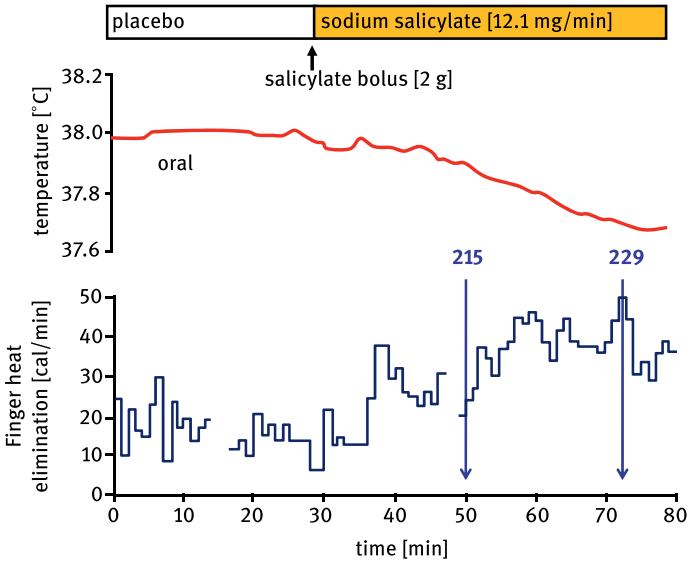

Figure 2.3.2-13: Body temperature and heat elimination in response to intravenous sodium salicylate in a febrile patient. Oral temperature and finger heat elimination were measured during infusion of saline (placebo) and a subsequent bolus injection, followed by intravenous infusion of salicylate. Plasma salicylate levels [µg/ml] are also indicated. Note the fall in body temperature after salicylate administration, which is paralleled by increased heat delivery via the skin (modified after [128]).

IL-1, TNFα, IFN-γ and IL-6 (Fig. 2.3.2-12). The antipyretic response to aspirin is partially salicylate-mediated. It can be obtained after intravenous administration of sodium salicylate at antipyretic salicylate plasma levels of about 1.5 mM (215–230 µg/ml (Fig. 2.3.2-13).

The antipyretic action of aspirin is typically associated with sweating, indicating extra "heat" production and "export" through the skin due to uncoupling of oxidative phosphorylation by salicylate (Section 2.2.3) [128]. This also explains the paradoxical "hyperpyrexia" in children at advanced stages of salicylate poisoning (Section 3.1.1). Thus, aspirin (salicylate) affects both the biochemical and physical sites of temperature control: heat production (reduced) and heat loss (enhanced).

In addition to salicylate-mediated inhibition of endogenous pyrogens, there are the well-known inhibitory effects of aspirin on cytokine-induced stimulation of COX-2 expression [129] and inhibition of its enzymatic activity. COX-2 appears to be the target COX since selective COX-2 inhibition in human reduces fever to the same extent as nonselective COX-1/COX-2 inhibitors [130]. Application of PGE_2 into the hypothalamus or the ventricles of the brain causes fever which, in contrast to fever induced by IL-1 or TNFα, cannot be blocked by aspirin or salicylates. PGE_2 generated via COX-2 appears to determine the febrile response via EP_3 receptors and inhibition of prostaglandin biosynthesis will reduce the febrile response – as already suggested by John Vane [31].

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Summary

Aspirin and salicylate are potent analgesic, antipyretic, antimicrobial and antiinflammatory drugs. Inhibition of prostaglandin biosynthesis via inhibition of COX-1 and upregulation of COX-2 is central to the antiinflammatory actions but does not sufficiently explain all of the multiple actions of aspirin in local and systemic inflammations. Further actions are inhibition of generation and release of platelet-derived inflammatory mediators and their interaction with white cells, which is highly relevant for systemic inflammatory and immune reactions. Inhibition of NF-κB signaling pathways by aspirin and salicylate might also contribute to antiinflammatory and antimicrobial actions at higher doses of the compound.

Acetylation of COX-2 by aspirin – unlike acetylation of COX-1 – causes generation of 15-(R)-HETE, the substrate for subsequent generation of ATL, in the presence of white cell lipoxygenases. ATL, like all active lipoxins, is an antiinflammatory mediator that also stimulates resolution of inflammation. Further actions of aspirin include modulation of inflammatory gene transcription, inhibition of generation of cytokines and other mediators of inflammatory and immune responses. This also involves the posttranslational acetylation of eNOS with subsequently improved oxygen defense via upregulation of HO-1. Many of these effects of aspirin are seen at analgesic (1–2 g) or even antiplatelet (100 mg) doses of aspirin. However, they become amplified in the presence of accumulating local salicylate levels after repeated aspirin intake.

The analgesic effect of aspirin involves peripheral and central sites of action. Aspirin inhibits the functional consequences of enhanced pain sensation, i. e., hyperalgesia and allodynia. Peripheral analgesic actions of aspirin are partially due to inhibition of prostaglandin formation at a site of injury with subsequently reduced sensitization of nociceptive nerve terminals and afferent pain signaling. Central effects involve changes in serotoninergic neurotransmission and, possibly, the endocannabinoid system. In clinical conditions, different sites might be involved, dependent on the kind and intensity of the noxious stimulus.

The antipyretic action of aspirin is primarily due to inhibition of PGE_2 formation in the CNS. In addition, aspirin and salicylate interfere with endogenous pyrogens and their induction of COX-2 expression and activity. In addition, salicylates will also reduce fever by enhanced heat elimination (sweating), possibly via uncoupling of oxidative phosphorylation.

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2.3.3 Aspirin and malignancies

2.3.3.1 General aspects

Malignant tumors result from irreparable gene defects. The reasons can be genetic or epigenetic in nature and mainly affect oncogenes, tumor suppressor genes and DNA repair genes. Malignant cells escape body defense mechanisms and become potentially immortal by defective apoptosis. They digest the intercellular matrix, proliferate, infiltrate the neighboring tissue and spread with formation of distant metastases. These proliferation and invasion processes are supported by tumor-induced angiogenesis. Two pharmacologically modifiable aspects are of particular interest: (i) the transformation of a normally dividing diploid somatic cell with a limited life span into a potentially immortal, endless dividing tumor cell and (ii) pathophysiological mechanisms of tumor growth, spread and invasion into other tissues with particular reference to the chemical mediators that are involved in these reactions and might be modified by drugs.

A piece of history – prostaglandins. Prostaglandins have been brought into connection with this issue after the finding that malignant tumor cell lines produce high amounts of prostaglandins, in particular PGE_2, which promotes cell proliferation [1]. PGE_2, in amounts that are made by tumor cells (nanomoles), was also found to suppress immune defense mechanisms. COX inhibitors including aspirin block immunosuppression in vitro and tumor growth in vivo [2]. Shortly thereafter, PGE_2 was found to act as a cocarcinogen in experimental skin tumors but not to be carcinogenic by itself [3]. In the 1980s, it was reported that aspirin and several NSAIDs exhibit chemopreventive effects in chemical models of colon carcinogenesis [4]. The possible clinical relevance of these experimental data was first shown in the pioneering epidemiological study by *Gabriel Kune* and colleagues in Australia. In a retrospective case-control trial they showed that regular, long-term aspirin intake reduced the risk of

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CRC by about 40 % [5]. Further experimental and clinical trials have meanwhile confirmed a tumor-promoting action of PGE_2 and (platelet-derived) TXA_2 [6] as well as a tumor suppressor potential of inhibitors of prostaglandin biosynthesis, such as aspirin and several NSAIDs (Section 4.3.1) [7]. These data strongly suggested a pathophysiological relationship between pathogenesis and malignancy of solid tumors and the COX/prostaglandin system.

A piece of history – platelets. Another, apparently prostaglandin-independent route to aspirin as a potential tumor preventive was the discovery that circulating blood platelets contribute to tumor spread, invasion and metastasis. *Gabriel J. Gasic* and colleagues were the first to show an inhibition of tumor metastasis by experimental thrombocytopenia in animal experiments that was abolished by addition of platelets [8]. Gasic also showed that aspirin inhibited tumor cell spread and metastasis and stated that inhibition of … *secretion of platelet products appeare[d], to be heavily involved* … [9, 10].

Later work on the role of coagulation factors, namely thrombin, and platelets in tumorigenesis suggested that, upon local injury, activation and aggregation of platelets occurs inside the intestinal mucosa. It was hypothesized that persistent activation of platelets might result in local recruitment of immunocompetent white cells, leading to (chronic) inflammation and, perhaps, formation of adenomas that might progress to carcinomas [11, 12]. Activated platelets provide a procoagulant surface that facilitates cancer cell-induced coagulation processes. Experimental studies have shown that blockade of key stimulatory platelet receptors (GP1b/V/IX; GPIIb/IIIa; GPVI) inhibits tumor metastasis [13]. These and other data strongly suggest activated platelets and platelet-triggered reactions of other cells as relevant factors and mediators of tumor genesis and malignancy (Section 4.3.1).

Gastrointestinal tumors as models for chemoprevention. Evidence for chemopreventive actions of aspirin and nonaspirin NSAIDs has been provided for a number of solid tumors in animal and clinical studies. In both cases, the most convincing data were obtained in prevention (and treatment) of neoplasias in the gastrointestinal tract [14, 15]. The chemoprotective actions of aspirin on the incidence and outcome of other solid cancers, such as those from prostate, lung or breast, are much more variable, although some studies also reported salutary results with aspirin administration [15, 16]. Under the assumption that the pharmacological mode(s) of antitumor action of aspirin are principally comparable in all solid tumors, this section is focused on colorectal tumors (adenomas, cancer) as a "reference" tumor model.

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2.3.3.2 Pathophysiology of intestinal neoplasias

General aspects. Genetic and epigenetic factors are involved in the occurrence, prognosis and clinical outcome of individuals with CRC [17]. These factors initiate a neoplastic transformation of healthy intestinal epithelium and/or determine the progression to higher grades of malignancy. Most common is chromosomal instability (CIN). Another reason are disturbances in the "mismatch repair" (MMR) system with associated microsatellite instability (MSI) [17]. Among the tumor-promoting factors at a cellular basis is a dysfunctioning Wnt/β-catenin pathway [18, 19], associated with defects in the APC tumor suppressor gene (Fig. 2.3.3-1).

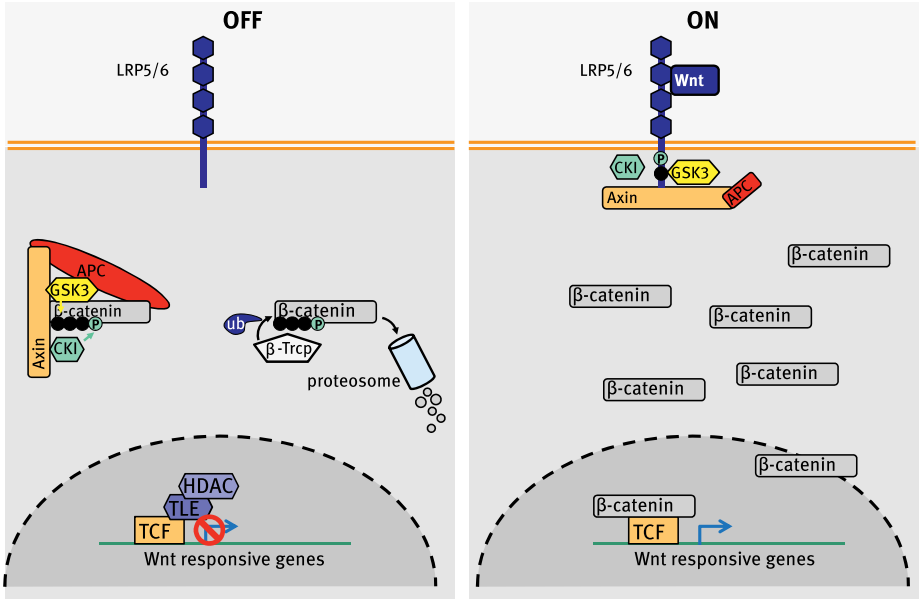

Figure 2.3.3-1: Overview of the oncogenic Wnt/β-catenin signaling pathway in the (a) absence and (b) presence of an activating Wnt ligand (after [18]). If the Wnt pathway is inactive, cytosolic β-catenin is bound in a complex with axin, CK, GSK and APC. Phosphorylation of β-catenin by GSK3 kinase allows its release into the cytosol, ubiquitination and subsequent proteasomal degradation (a). After activation of the Wnt pathway by binding of an appropriate ligand, Wnt signaling is switched on, associated with binding of the APC complex. With absent or dysfunctional (truncated) APC this results in incomplete β-catenin binding and its accumulation in the cytosol. Cytosolic free β-catenin enters the nucleus and acts as a coactivator for TCF with subsequent activation of Wnt-responsive genes (b), among many others also COX-2. Abbreviations: APC: adenomatous polyposis coli tumor suppressor gene; CK: casein kinase; GSK: glycogensynthase kinase; β-TRP: ubiquitin ligase subunit; TLE/HDAC: T-cell factor repressor; Wnt: Wingless & Int-1 (drosophila gene;)β-catenin: coactivator for TCF to activate Wnt-responsive genes after transfer to the nucleus.

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The oncogenic Wnt/β-catenin-pathway in gastrointestinal tumorigenesis. Similar to other solid tumors, mutations at critical sites of oncogenes and/or tumor suppressor genes underlie the pathogenesis of sporadic and hereditary forms of CRC [20]. Probably, cancer stem cells, having the canonical Wnt/β-catenin signaling pathway, act as critical regulators of cancer initiation, progression and invasion [21]. In human CRC, the APC tumor suppressor gene and its product APC appear to be key players for colorectal neoplasias, that is, adenomas and cancer [22].

In virtually all cases of human CRC there is constitutive Wnt-signaling associated with "loss-of-function" mutations at the APC gene [23, 24]. These mutations cause incomplete translation, yielding a truncated APC protein with a reduced number of binding sites for β-catenin. This results in reduced binding and phosphorylation of β-catenin inside the axin–APC complex and subsequently impaired lysosomal degradation. Instead, free β-catenin accumulates in the cytosol with subsequent translocation to the nucleus and activation of the oncogenic Wnt/β-catenin pathway. Here, β-catenin functions as a transcriptional coactivator for the expression of genes that have T-cell factor/lymphoid enhancer family (TCF/LEF) binding sites in their regulatory DNA regions [18, 24, 25]. These processes are amplified by k-ras mutations and DNA methylation [18, 26, 27] and eventually result in the generation of a solid tumor. Affected genes are oncogenes (k-ras), several cell cycle-regulating genes [28], growth factors (EGF, VEGF) and many others, among them COX-2 (Fig. 2.3.3-1) [20, 26].

Cyclooxygenases – COX-1 and platelets. Healthy human colorectal mucosa expresses COX-1 but not COX-2 [29, 30]. The expression level of COX-1 remains unchanged in human CRC and adenoma cells [31]. Studies in the genetically modified Min/Min mice suffering from intestinal adenomatosis coli confirmed that only COX-1 was expressed in healthy intestinal tissue, whereas both COX-1 and variable levels of COX-2 protein were detected in polyps. This suggested that both COX-1 and COX-2 contribute to tumorigenesis and enhanced PGE_2 production [32]. In human, in addition to blood platelets with high COX-1 expression, intestinal (colorectal) mucosa cells are likely candidates [33].

Platelet–white cell interactions are another possible source of tumorigenesis. Platelet-derived TXA_2 and other COX-1-dependent, white cell-stimulating mediators such as dioxolanes [34] and platelet storage products such as S1P [35, 36] or "vascular endothelial growth factor" (VEGF) [37] might contribute to tumorigenesis and spread. S1P stimulates prostaglandin (PGE_2) production and tumor malignancy, possibly by induction of COX-2 (see below). An (indirect) stimulation of PGE_2 production by tumor cells might also result from direct interactions between platelets and tumor cells. Exposure of tumor cells to platelets in vitro increases generation of TXA_2 and PGE_2 formation with an associated increase of their metastatic potential [38–40]. Platelet-derived TGFβ and direct platelet–tumor cell contacts also synergistically activate the TGFβ/Smad and NF-κB pathways in cancer cells, resulting in their transition to an invasive mesenchymal-like phenotype [41]. Thus, there is a bulk of preformed and cir-

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culating mediators, in many cases platelet-derived and this in a COX-1-related manner, that contribute to tumorigenesis [42].

Finally, in addition to signal generation, platelets also express numerous receptors that mediate inflammatory and immune reactions and are considered pathogen "sensors" [43]. Although without a nucleus, platelets contain sufficient RNA that can be actively translated into several proinflammatory and promitogenic products [43, 44]. Thus, there is evidence that platelets together with COX-1-derived platelet products such as dioxolanes [34] might have a trigger function for tumor growth and spread.

Cyclooxygenases – COX-2. Transcriptional upregulation of the COX-2 gene is seen in about half of human colorectal adenomas and most (80–90 %) carcinomas [31, 45]. This upregulation is limited to tumor tissue and probably causally related to the disturbed function of the tumor suppressor APC. COX-1 gene and protein expression are not affected [29, 31, 46–48]. There is also an unchanged prostaglandin production in nearby healthy colonic mucosa [49].

The best known – but not the only – consequence of COX-2 upregulation in colorectal tumors is markedly enhanced prostaglandin production, PGE_2 being the dominating product [49, 50]. Interestingly, there is a two to three orders of magnitude higher content of endogenous arachidonic acid in CRC cells with a minimal conversion rate into PGE_2 and other eicosanoids (Fig. 2.3.3-2) [51].

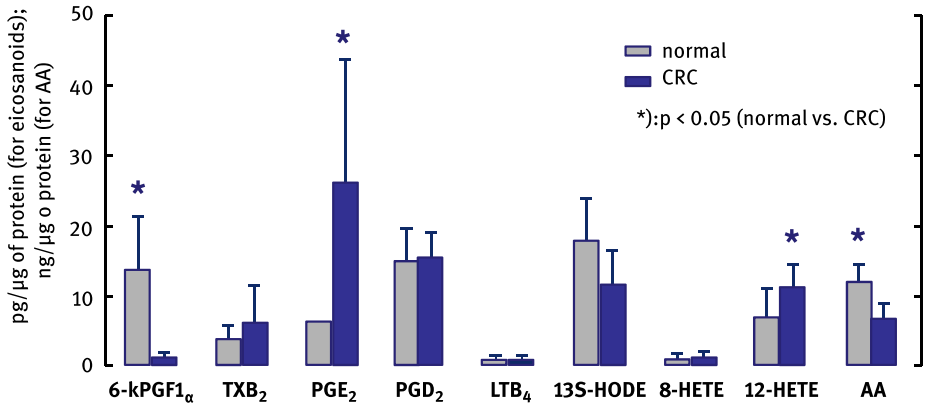

Figure 2.3.3-2: Tissue levels of eight major eicosanoids and the precursor arachidonic acid (AA) in matched pairs of cancerous and normal colon mucosae obtained from colorectal cancer patients. Note the significant increase in PGE_2 and decrease in 6-keto-$PGF1_\alpha$ as well as the different ordinates for AA and eicosanoids, suggesting that only a minority of total AA is converted into these eicosanoids (modified after [51]).

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Exogenous arachidonic acid causes apoptosis in colon cancer cell lines. These cells, in addition to COX-2, overexpress another arachidonic acid-utilizing enzyme, fatty acid-CoA ligase-4 (FACL4). This led to the interesting hypothesis that the cellular level of unesterified free arachidonic acid is a general regulator of apoptosis in colon carcinoma cells and that COX-2 and FACL4 promote carcinogenesis by lowering this level, thereby removing a proapoptotic signal [52].

An upregulated COX-2 protein will generate large amounts of PGE_2, which maintains a positive feedback loop to enhance nuclear actions of the oncogenic β-catenin/Wnt pathway on gene transcription [53] and COX-2 expression [54]. PGE_2 accounts for many effects associated with promotion of tumorigenesis and metastasis. These include transition from the epithelial to the mesenchymal phenotype, angiogenesis [55], invasiveness, stimulation of tumor growth [56] and immunosuppression of monocytes/macrophages [4]. Following disruption of the intestinal epithelium, PGE_2 drives a differentiation state via the prostaglandin EP4 receptor which is also triggered by nuclear β-catenin signaling [57]. Consequently, deletion of the COX-2 gene and COX-2-associated PGE_2 production in a mouse model of adenomatosis polyposis coli resulted in considerable reduction of tumor number and size (Table 2.3.3-1) [58].

Table 2.3.3-1: Number and size of intestinal polyps in a mouse model of familial adenomatous polyposis coli (FAP). Animals bear a defective (heterozygous) deletion (APCΔ716; +/–) of the APC gene and express a huge amount of tumors in the presence of active COX-2. Tumors are markedly reduced in size and number after partial (heterozygous) inactivation of COX-2 and absent after complete silencing of the COX-2 gene (modified after [58]).

Parameter	COX-2-genotype		
	homozygote (COX-2 –/–)	heterozygote (COX-2 +/–)	CON (COX-2 +/+)
total number of polyps	93 ± 98	224 ± 123	652 ± 198
reduction [%]	(86 %)	(66 %)	(0 %)
total number of large polyps in colon (>2 mm diameter)	none	1.5 ± 1.9	6.8 ± 7.2
reduction [%]	(100 %)	(78 %)	(0 %)

Enhanced COX-2-activity in colonic cancer cells is also associated with changes in their phenotype, including increased cell adhesion, resistance to apoptosis and stimulation of tumor angiogenesis. All of these data strongly support the hypothesis that the extent of COX-2 expression and the associated PGE_2 production determine the clinical degree of malignancy. This involves tumor size, invasiveness, metastatic potential and, finally, clinical outcome [4, 59–61].

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15-Hydroxyprostaglandin dehydrogenase. The high local levels of PGE_2 in the vicinity of tumor cells by an upregulated COX-2 might be further increased by downregulation of the major PGE-metabolizing enzyme, 15-PGDH [62]. This enzyme is highly active in healthy intestinal mucosa. It prevents accumulation of active PGE_2 by conversion into the inactive 15-keto metabolite. In colon adenomas, CRC and several other human carcinomas, the enzyme is markedly downregulated [63, 64], as is the prostaglandin transporter (PGT) [65], which is necessary for cellular uptake of the active prostaglandin and subsequent inactivation by 15-PGDH in the cytosol. Suppression of 15-PGDH expression is caused by β-catenin and occurs in very early stages of colorectal neoplasias [62, 66]. In consequence, local PGE_2 levels accumulate.

Non-COX/prostaglandin-related mechanisms. There is a number of further mechanisms of CRC tumorigenesis that are not directly connected to the prostaglandin pathway [67]. Those with a relation to aspirin are discussed below in more detail.

2.3.3.3 Modes of aspirin action

General aspects. Aspirin has multiple actions on cell function that are relevant to malignancies. Most important is its enormous, nonselective acetylation potential. More than 100 different proteins have been found to become acetylated in colorectal tumor cell lines by low-to-medium aspirin concentrations (≥100 μM) (Fig. 1.1.5-1) [68–70]. If there were no degradation by aspirin deacetylases, micromolar concentrations of aspirin and salicylate could be easily obtained by local accumulation. The duration of aspirin-induced acetylation is dependent on the turnover rate of the acetylated protein, that is, possibly lifelong in potentially immortal tumor cells. However, in the majority of cases aspirin-mediated acetylations are nonspecific and do not accumulate to levels likely to elicit biological effects. This is due the action of several aspirin esterases (deacetylases) (Section 2.1.2) [71]. It has also been suggested that chemoprevention of CRC by aspirin (and NSAIDs) might be caused by apoptotic elimination of stem cells that become inappropriately activated by oncogenic stimuli [19, 72].

It has been speculated that the preferential protective effect of aspirin on colorectal neoplasias might be due to the fact that the intestinal mucosa is the first site to become physically exposed to aspirin after oral intake – prior to any larger metabolization [11]. However, the majority of aspirin has already been absorbed in the small intestine and may not be available in sufficient amounts in the colon and rectum, the intestinal locations of the tumors.

For formal reasons the numerous possible antitumor actions of aspirin might be divided into two groups: those that are COX-related and those that are not. Both may also act together. An overview on selected targets of aspirin as a chemopreventive drug in CRC is shown in Table 2.3.3-2.

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Table 2.3.3-2: Possible modes of chemopreventive action of aspirin in colorectal cancer (for details see text).

COX (PGH-Synthase)-related	Non-COX (PGH-Synthase)-related
Inhibition of COX-1-dependent formation of arachidonic acid metabolites (PGE_2, TXA_2, DXA_3, others) in platelets and platelet-stimulated nucleated cells (white cells, tumor cells)	Inhibition of oncogenic gene expression via interaction with transcription factors (NF_kB, others) and kinases (rS6-kinase)
Inhibition of COX-2/peroxidase – mediated PGE_2 formation and activation of (co)carcinogens	Modulation of oncogen-induced expression of transcription factors (EGRF, VEGF, others)
Generation of "Aspirin-triggered lipoxin" (ATL) by interactions with white cell-lipoxygenases	Interaction with DNA mismatch-repair genes
Antagonism of PGE_2-mediated stimulation of β-catenin and immunosuppression via EP_2/EP_4-PGE receptors	Restauration of apoptosis by "sensitizing" tumor cells to apoptotic stimuli (TRAIL)
Inhibition of generation/release of non-prostanoid lipid mediators from platelets (sphingosine-1 phosphate, others)	Energy depletion by uncoupling of oxidative phosphorylation with nonselective kinase inhibition
Inhibition of tumor-angiogenesis by inhibition of generation and release of angiogenic factors (VEGF, TGFβ)	Expression of aspirin-sensitive phenotypes of tumor relevant enzymes: G316A genotype of ODC; PIK3CA genotype of antiapoptotic PI3K-signalling, others
	Acetylation of numerous cellular proteins, including enzymes of glycolysis, cytoskeleton proteins, histones and others.

2.3.3.4 COX-related actions of aspirin

Inhibition of COX-1. A reduced nonvascular (cancer) mortality after regular long-term intake of low-dose aspirin (in many studies 100 mg EC aspirin became apparent after at least 5 years of treatment) was not dose-dependent and tended to further increase with a prolonged observation period (Section 4.3.1) (Fig. 4.3.1-3) [73]. Inhibition of colorectal mucosal PGE_2 formation by repeated low-dose aspirin for 3 months was a regular finding [74, 75] and was associated with a significant, by 30 %, reduced histochemical expression of the tumor marker TGFα [76] in rectal biopsy tissue of patients with a history of adenomas. Patrignani et al. also reported that low-dose aspirin (100 mg/day for 1 week) produced long-lasting acetylation of COX-1, inhibited prostaglandin production and downregulated p-S6 kinase in human colorectal mucosa [33]. Inhibition of platelet COX-1 by low-dose aspirin (81 mg/day for 2 weeks) reduced nonplatelet-derived PGE_2 production (metabolite excretion) by about 40–55 % [40]. All of these findings, the absence of COX-2 expression in healthy colon mucosa epithelial cells and the finding that COX-1 similarly to COX-2 also contributes to intestinal adenomatosis in the genetically modified Min/Min mice [32] suggest that at least part of the chemoprotective action of aspirin on intestinal neoplasias is due to inhibition of COX-1 and/or COX-1-derived product formation in the intestinal mucosa.

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Platelets and platelet-derived mediators. Platelets and platelet-derived mediators such as TXA_2 are of key pharmacological interest as aspirin-inhibitable factors in gastrointestinal tumorigenesis. In addition to thromboxane biosynthesis, this covers also the release of platelet storage products such as S1P [77] and proangiogenic mediators such as VEGF [42]. Platelet-derived TXA_2 might orchestrate the generation of a favorable intravascular metastatic niche that promotes tumor cell seeding and identifies COX-1/TXA_2 signaling as a target for the prevention of metastasis by aspirin [40, 78]. Locally reduced prostacyclin levels in tumor tissue may additionally facilitate platelet activation and adhesion.

Sphingosine-1-phosphate. Sphingolipids, including ceramide, sphingosine and S1P, are bioregulators of carcinogenesis [79, 80] and were also suggested to link chronic intestinal inflammation – an established risk factor for CRC – to intestinal carcinogenesis [81]. S1P is the major product of sphingosine kinase-1, which mediates angiogenesis, metastasis and resistance of tumor cells to drug-induced apoptosis [82]. About 90 % of human colon cancer samples stained positively for sphingosine kinase-1 [80]. Inhibitors of this enzyme exhibit antitumor activity [83]. S1P stimulates COX-2 expression and PGE_2 synthesis in CRC cells, suggesting a relationship between sphingolipids, carcinogenesis and upregulation of COX-2 [84]. About 50 % of S1P in blood is stored in circulating platelets. TXA_2 stimulates release of S1P from platelets in an entirely aspirin-sensitive manner. Complete inhibition of (thrombin-induced) release can be obtained by regular intake of 100 mg aspirin/day (Fig. 2.3.1-3) [36] and might activate multiple inflammatory/oncogenic pathways, including expression of COX-2 [85]. A hypothetical scheme of platelet TXA_2-dependent stimulation of S1P release and its possible function in the complex interplay of carcinogenesis is shown in Fig. 2.3.3-3. Thus, aspirin-induced inhibition of S1P release from human platelets might well contribute to possible anticancer effects of aspirin in vivo.

Inhibition (modulation) of COX-2. Aspirin also inhibits platelet-induced COX-2 expression and PGE_2 biosynthesis in tumor cells [38–40, 77]. Boutaud and colleagues from John Oates' group have shown that the interaction of activated platelets with adenocarcinoma cells results in upregulation of COX-2 expression and enhanced PGE_2 biosynthesis. In vitro, aspirin inhibits COX-2 from lung and colon adenocarcinoma cells to the same or even greater extent than COX-1 in platelets (Fig. 2.3.3-4) [40]. From these data it was suggested that the antimetastatic potency of aspirin might be related to the high sensitivity of COX in tumor cells and their subsequent PGE_2 production against aspirin, combined with inhibition of platelet-promoted PGE_2 biosynthesis in cancer cells [40].

Human recombinant COX-2 exposed in vitro to an excess of aspirin was acetylated by approximately 40–50 %. This was associated with an 80–90 % inhibition of COX-2

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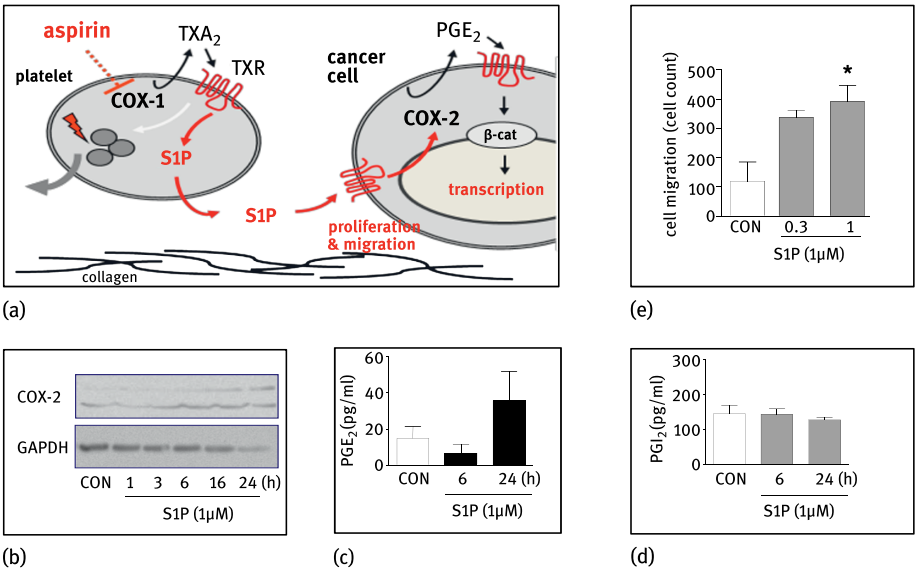

Figure 2.3.3-3: Hypothetic mode of action of platelet-derived TXA_2 on gene transcription in CRC via S1P. (a) Platelet activation by thrombin results in activation of the arachidonic acid cascade with subsequent COX-1-mediated TXA_2 formation and secretion of storage products such as S1P. (b–d) S1P in turn stimulates COX-2 expression in nucleated cells (b) and subsequent generation of PGE_2 (c) but not of PGI_2 (d). (e) In case of an activated Wnt/β-catenin pathway in tumor cells this will enhance malignancy, including tumor cell migration. Aspirin inhibits this signaling cascade by inhibition of COX-1-mediated TXA_2 formation and TXA_2-induced release of S1P (Rauch & Schrör, unpublished).

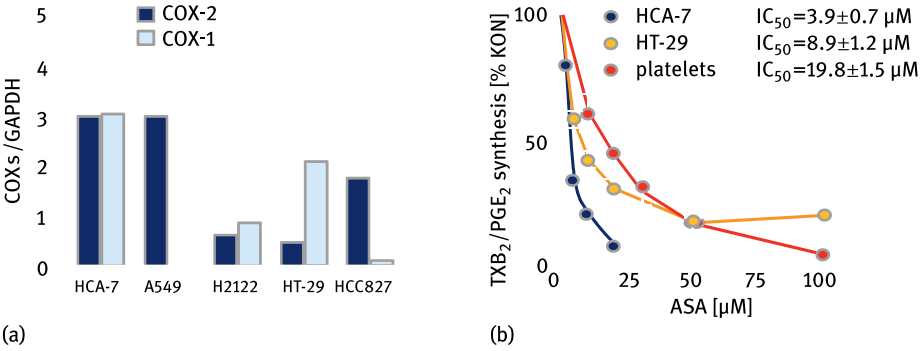

Figure 2.3.3-4: (a) Expression of COX-1 and COX-2 in adenocarcinoma cell lines of the lung (H2122, HCC827, A549) and colon (HCA7, HT-29). (b) Aspirin inhibits COX-derived PGE_2 synthesis in adenocarcinoma cells as potently as it inhibits COX-1-dependent thromboxane (TXB_2) formation in washed platelets. Protein expression levels are expressed as a ratio against the housekeeping protein GAPDH. For further explanations see text [40].

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activity. In three different cell types, including tumor cells and macrophages, expressing COX-2, the extent of COX-2 acetylation and reduction of PGE_2 biosynthesis by aspirin was concentration-dependent with comparable EC_{50} values in the low micromolar range. The maximal acetylation of COX-2 at 1,000 µM aspirin was associated with a virtually complete prevention of PGE_2 biosynthesis [86]. These data together with those of Boutaud [40] collectively suggest a clinically relevant inhibition of (COX-2-derived) PGE_2 formation in CRC by aspirin which, in contrast to inhibition of platelet (COX-1-dependent) thromboxane formation, must not necessarily be complete to become biologically effective. This is relevant on the background of the variable (with respect to time) expression of COX-2, due to the variable activities of mitogens and tumor promoters and short availability of nonmetabolized aspirin in vivo.

Actions on 15-hydroxyprostaglandin dehydrogenase. The colonic 15-PGDH activity in healthy individuals is very stable at a genetically fixed expression level and is not affected by aspirin intake [87]. Interestingly, the chemoprotective efficacy of aspirin in two large clinical trials was restricted to those individuals who exhibited high 15-PGDH expression in normal mucosa of CRC resections [88]. The adenoma-preventive action of celecoxib was abrogated in mice genetically lacking 15-PGDH [63]. These data suggest 15-PGDH as a marker for tumor expression, but without clear evidence for a prognostic value regarding the chemopreventive effects of aspirin [87].

Generation of "aspirin-triggered lipoxin". A unique property of aspirin which is not shared by any NSAID is the acetylation of COX-2. This switches COX-2 from synthesizing (tumor-promoting) prostaglandins (PGE_2) to a 15-lipoxygenase that generates 15-(R)-HETE, a precursor of ATL (Fig. 2.2.1-6). ATL, like all lipoxins, is a tumor-suppressive/antiinflammatory compound [89]. For these reasons, generation of ATL could well contribute to the chemoprotective action of aspirin in CRC [90].

Activation of cocarcinogens. In addition to stimulation of prostaglandin formation, COX-2 can also promote carcinogenesis via the peroxidase activity of the PGHS complex. The peroxidase has a broad substrate specificity and can use many substrates for cooxidation. This eventually results in the generation of free radicals which bind to DNA and might alter gene transcription [91]. On this background, polycyclic aromatic hydrocarbons and amines, such as azoxymethane or dimethylhydrazine, are frequently used to induce colon cancer in rodents. In these animals, both aspirin and nonaspirin NSAIDs (sulindac, celecoxib) reduced the number of colon tumors when treatment was started early after exposure to the carcinogen [92]. One study in azoxymethane-treated rats showed that aspirin even at a relatively low dose (6 mg/kg per day) reduced PGE_2 production by 50 % and colonic tumors by 80 %, and this was associated with reduced inflammatory gene expression [93]. A mechanistic study

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on chemically indued carcinogenesis in mice confirmed that aspirin-treated animals developed fewer colon tumors and had reduced levels of proinflammatory cytokines. In addition, transcriptomic and proteomic analyses indicated that these actions of aspirin involve inhibition of the Wnt/β-catenin pathway (Fig. 2.3.3-1). For aspirin's mode of action, inhibition of Wnt production was suggested, possibly by suppressing its transcription factor NR4A2, which in turn is regulated by PGE_2 [94].

It is difficult to decide whether chemically induced carcinogenesis with a rather short exposure time to high-dose carcinogens in animals – weeks to months vs. years and decades in human – allows firm conclusions for their clinical significance. However, these studies open interesting views on pharmacological modes of action of tumor-suppressive agents, including aspirin.

2.3.3.5 Non-COX-related antitumor actions of aspirin

General aspects. Several lines of evidence suggest that aspirin and salicylate may also affect apoptosis and cell proliferation in CRC by COX-2-independent mechanisms. This might also be caused by apoptotic elimination of stem cells that become inappropriately activated by oncogenic stimuli [19, 72]. Not all human colon cancer cell lines express COX-2 [4, 95]. This allows to study the tumor-preventive action of aspirin in the absence of this enzyme. Experimental data indicate that the potency of aspirin, salicylate and NSAIDs to inhibit cell proliferation and to induce apoptosis in COX-2-negative colon cancer cells is similar to that in COX-2-expressing cells (Fig. 2.3.3-5) [96, 97].

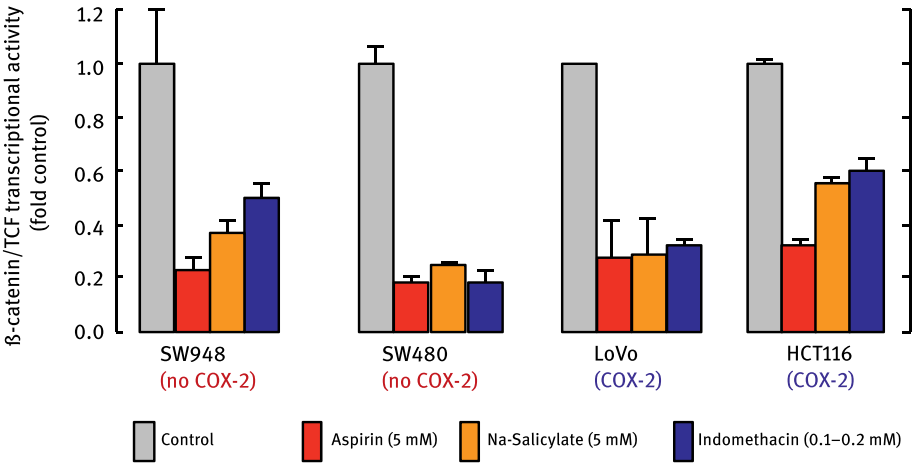

Figure 2.3.3-5: Inhibition of oncogenic gene transcription (β-catenin/TCF-mediated transcription activity) by aspirin, salicylate and indomethacin in four different cancer cell lines with and without COX-2 expression. The inhibitory potency of the compounds was comparable in all cell lines and independent of the expression of COX-2 (modified after [97]).

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Conversely, induction of apoptosis by aspirin and nonaspirin NSAIDs is not reversed by prostaglandins [95]. These findings suggest that antiapoptotic/antitumor actions of aspirin – and NSAIDs – do not necessarily require inhibition of COX-2 or prostaglandin formation [95]. However, aspirin and salicylate concentrations in the millimolar range are necessary to see this.

Aspirin-sensitive genotypes of tumor-relevant enzymes. The genotype of some enzymes has been suggested to be related to the clinical efficacy of aspirin in chemoprevention of CRC. In addition to 15-PGDH expression levels, mentioned above, the ornithine decarboxylase (ODC) G316A genotype in the "United Kingdom Colorectal Adenoma Prevention" trial with aspirin (Section 4.3.1) was found to be associated with a 50 % lower rate of adenoma recurrence [98]. A reduced mortality from CRC was found in aspirin-treated patients with enhanced COX-2 expression and a mutation in the antiapoptotic PI3K signaling pathway (PIK3CA) [99]. There are also genetically determined alterations in the salicylate metabolism, such as a polymorphism in the UGT gene. Individuals who carry the isoform UGT1A6*2 exhibit a more rapid glucuronidation and excretion of salicylate than the wild-type UGT1A6*1/*1 (Section 2.1.2). It has been suggested that polymorphisms in the UGT1A6 gene increase the cancer risk by enhanced generation of carcinogens [100].

In a genome-wide investigation of gene × environment interactions, use of aspirin and/or NSAIDs was associated with lower risk of CRC, and this association differed according to genetic variation at two SNPs at chromosomes 12 and 15 [30, 101]. Another recent large trial studied 42 candidate SNPs in 15 genes whose association with CRC risk was putatively modified by aspirin use according to literature data. No evidence of interactions between genetic variants in genes involved in aspirin pathways, regular aspirin use and CRC risk was found [102]. More work is necessary to establish a relationship between CRC, the antitumor efficacy of aspirin and genome variations.

Apoptosis and cell cycle. Tumor cells are largely resistant to apoptosis. Numerous experimental studies, mainly cell culture experiments, have shown that aspirin and salicylate might restore apoptosis and inhibit cell proliferation in tumor cells by modification of expression of cell cycle-regulating genes [28, 103, 104] and that these effects are independent of COX-1, COX-2 and prostaglandin formation [4, 95, 97, 105]. These inhibitory actions on tumorigenesis in vitro, for example by inhibition of oncogenic gene transcription via the Wnt/β-catenin pathway, require comparably high concentrations of salicylates in both COX-2-positive and COX-2-negative cell lines (Fig. 2.3.3-5) [97].

NF-κB transcription factor and cell signaling. NF-κB regulates the expression of many genes which are involved in not only the control of cell division and apoptosis but

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also immune/inflammatory processes (Section 2.3.2) [106]. Data on modifications of NF-κB by aspirin in CRC are controversial. Aspirin has been shown to activate NF-κB in colorectal carcinoma cells and other tumor cells, inducing apoptosis by stimulating signal-specific IκB degradation, NF-κB nuclear translocation and repression of NF-κB-driven transcription [107–109]. The aspirin concentrations were high, in one study comparable with 600 mg aspirin orally four times daily [107]. Doubling of the apoptosis rate in tumor cell lines required 3–5 mM aspirin [108, 110]. Others have reported the opposite finding, that is, inhibition of NF-κB activation by aspirin and salicylate [109, 111]. It has been suggested that the different effects of salicylates might be due to a heterogenous, cell-specific gene expression in different CRC cell lines [112]. The in vivo relevance of these findings is uncertain. In addition, there are complex interactions between epithelium and stroma cells, including differential regulation of gene expression which are missing in pure CRC tumor cell lines in vitro [30].

Runx. A new possibly aspirin-sensitive transcription factor for colorectal tumors is Runx-1. Runx-1 is a regulatory gene for differentiation of hematopoietic stem cells but possibly also for malignant transformations of epithelial cells in the gastrointestinal tract. Deletion of the Runx-1 gene increased significantly the number of tumors in Min/Min$^-$ mice with a "loss-of-function" mutation in the APC gene. The absence of Runx alone was sufficient for tumorigenesis in wild-type APC mice and in addition also induced a number of changes in other genes involved in inflammation and intestinal metabolism, as well as the metastatic phenotype of colorectal tumors. It was suggested that Runx-1 is a novel tumor suppressor gene for gastrointestinal tumors that maintains the balance between the intestinal stem/progenitor cell population and epithelial differentiation of the gastrointestinal tract [113]. Megakaryocytic cells exposed to aspirin showed upregulation of the Runx-1 signaling pathway and it was also shown that this was associated with colon cancer-free survival. These studies reveal an effect of aspirin on Runx-1 gene expression that might be relevant for CRC [114].

mTOR, TRAIL and kinase inhibition. Aspirin can inhibit cell signaling in CRC tumor cells via inhibition of mTOR and activation of AMPK. Both actions contribute to the chemopreventive action of aspirin (salicylate) in CRC cells [115]. In vitro, the inhibition of these effects required high salicylate concentrations (5 mM). In vivo, some kinase inhibition, among them S6 kinase, was found in rectal mucosa specimens from aspirin-treated individuals (600 mg/day for 1 week) in the same study. The ribosomal S6 kinase is a known target of aspirin and salicylate and activates several transcription factors that are relevant for tumor (cell) growth and spread (Fig. 2.2.2-3) [116]. These effects are salicylate-specific and not seen with NSAIDs. A recent study has shown that low-dose aspirin (100 mg/day for 1 week) not only inhibits COX-1 and PGE_2 production in human intestinal mucosa but also downregulates p-S6 phosphorylation. From

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these data, it was speculated that long-lasting acetylation of COX-1 and downregulation of p-S6 by aspirin may interfere with early colorectal carcinogenesis [33]. For these reasons, aspirin-induced kinase inhibition remains an interesting research topic, not only in inflammation and immunology (Section 2.3.2) but also in tumorigenesis.

Bax. Bax is another proapoptotic gene that is induced by aspirin in colon adenocarcinoma cells at concentrations of ≤1 mM [117], as is the "tumor necrosis factor-related apoptosis-inducing ligand" (TRAIL) [118, 119]. Interestingly, in one of these studies, downregulation of TRAIL-induced apoptosis by aspirin (1 mM) was associated with a reduction of the mitochondrial membrane potential [119]. This suggests uncoupling of oxidative phosphorylation by the protonophoric actions of salicylate (Section 2.2.3), that is, a nonspecific, salicylate-related effect that depletes cellular ATP stores as a contributing mechanism. The implications of these exciting findings for modulation of cell functions by aspirin in the total organism are unknown yet, but might be considerable, for example in cancer chemoprevention by inhibition of ribonucleotide synthesis (Section 2.3.3) [68]. Disturbed energy supply, although nonspecific in nature, is likely to become particularly effective in fast proliferating tumor cells. Similar considerations may apply to the salicylate (10 mM)-induced inhibition of Toll-like receptor-4 on cancer cell lines [120].

Taken together, there are numerous transcription factors that have been brought into connection with antiinflammatory/antitumor actions of aspirin and it is difficult to decide which is the most important and therapeutically relevant "switch" between aspirin and tumorigenesis.

Microsatellite instability and mismatch repair. MMR genes and proteins are important for the correction of DNA instabilities. They remove defect genes and prevent their translation into proteins. Aspirin was found to promote genetic selection for MSI in human CRC cells, deficient for a subset of MMR genes. This effect was COX-independent, suggesting that aspirin might protect from hereditary nonpolyposis CRC where MSI is frequent. Aspirin treatment of CRC cells also reduced DNA instability in nonapoptotic cells where some of these MMR genes were missing. This resulted in a genetic selection of stable genes [121], suggesting a possibly reduced rate of spontaneous tumors in nonpolyposis hereditary forms of CRC, such as Lynch syndrome (Section 4.3.1). However, a long and constant exposure of the cells to aspirin was necessary (12 weeks) and a significantly reduced MSI required aspirin concentrations of at least 2.5 mM. Other investigators have shown that aspirin stabilized DNA by prevention of oxidative DNA damage (strand breaks) [122]. These are interesting pharmacological data that deserve further evaluation.

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Summary

For antitumor actions of aspirin, most information and most promising clinical data are available for colorectal malignancies, that is, carcinomas (CRC) and adenomas. For these reasons, CRC was used as a reference to describe the modes of action of aspirin on tumor cells. CRC, like other solid tumors, results from hereditary or (more frequently) acquired mutations in tumor-relevant genes. Of particular significance for colorectal neoplasias is a dysfunctional APC suppressor gene. After activation of the oncogenic Wnt/β-catenin pathway, its defect results in incomplete β-catenin binding and inactivation, its cytosolic accumulation and translocation to the nucleus. There, β-catenin acts as a cofactor for stimulation of Wnt-inducible gene transcription, including COX-2.

Upregulated COX-2 in tumor tissue synthesizes large amounts of PGE_2. PGE_2 promotes the transition of epithelial cells into a mesenchymal, invasive phenotype, inhibits apoptosis and acts in a proinflammatory and immunosuppressive manner. PGE_2 stimulates tumor angiogenesis and proliferation. In addition, COX-2 causes oxidation (activation) of cocarcinogens. These actions are potentiated by β-catenin-induced downregulation of 15-PGDH, the major PGE_2-inactivating enzyme. Aspirin inhibits COX-2- and COX-1-dependent PGE_2 and TXA_2 formation and actions at comparable molar potencies. This includes inhibition of generation and release of platelet-derived mediators such as S1P and tumor-promoting platelet–white cell interactions. Aspirin-induced acetylation of COX-2 additionally generates ATL, a potential antiinflammatory/antitumor compound.

Experimental evidence further suggests COX-independent actions of aspirin and salicylate on oncogenic signaling and tumorigenesis. This includes inhibition of transcription factors (NF-κB) and kinases (ribosomal S6 kinase), induction of apoptosis and DNA stabilization by interactions with MMR genes. There are also genetic variations in certain tumor-relevant enzymes that might contribute to the efficacy of aspirin in chemoprevention. The recently described acetylation of COX-1 associated with downregulation of S6 kinase in human colorectal mucosa cells by antiplatelet doses of aspirin is an interesting new finding that deserves further investigation.

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3 Toxicity and drug safety

Drug safety is a key issue for both drug manufacturers and drug consumers and, therefore, subject of detailed and sophisticated legal regulations. Safety aspects are particularly important for "over the counter" (OTC) medications that are used by medical lays without prescription by a doctor and where dosing and applicability are the patient's responsibility. In this situation, the diagnosis is made by the patient, and unwanted side effects are usually only considered as much as they affect the (subjective) well-being. Antipyretic analgesics, including aspirin, paracetamol (acetaminophen) and ibuprofen, belong to this category of drugs. Notably, salicylates are also ingredients of a large variety of fixed nonprescription drug mixtures. In many cases it is not immediately apparent from the phantasy names of these products, e. g., Soma Compound, Norgesic, Darvon, Percodan and others [1], that these preparations contain aspirin as an active constituent. Addition of codeine and other potentially habit-forming or allergenic components is another issue of concern. Some of these combinations became "popular" in connection with chronic misuse of analgesics ("analgesic nephropathy") which, fortunately, has now largely disappeared after the removal of phenacetin from analgesic mixtures (Section 3.2.3).

These days, many millions doses of antipyretic analgesics are taken daily worldwide for self-medication of acute and chronic pain and feverish diseases. However, not all users are aware of the fact that the desired actions of drugs, such as disappearance of headache or inflammatory pain, might also be associated with unwanted side effects that will not always cause subjective symptoms. This makes professional information about safety aspects an extremely relevant issue, specifically for persons who take their information solely from advertisements of drug companies or the internet.

For formal reasons, unwanted side effects of aspirin might be divided into three categories: (i) Systemic effects due to acute and chronic overdosing or intoxication (Section 3.1). The aspirin-related bleeding tendency as well as toxic effects in particular life situations, such as pregnancy or older age, also belong to this category. In addition to systemic effects, some organs, in particular those with a preexisting injury or an increased sensitivity to aspirin and/or salicylates, might be affected even at therapeutic doses in the absence of signs of systemic intoxication (Section 3.2). This involves the gastrointestinal tract, liver, kidney and the audiovestibular system. Finally, there are not dose-related side effects which are due to a particular predisposition of the patient (Section 3.3). These "hypersensitivities" might be inherent or acquired. Examples are "*A*spirin-*e*xacerbated *r*espiratory *d*isease" (AERD or "aspirin-sensitive asthma") and allergic reactions at the skin or mucosa (urticaria), that is, "*A*spirin-*e*xacerbated *c*utaneous *d*isease" (AECD). Finally, Reye's syndrome is also placed here, because of a possible but uncertain relationship to aspirin use that needs a more detailed discussion.

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3.1 Systemic side effects

Systemic toxic effects of aspirin may result from acute or chronic overdosing. They become clinically symptomatic at plasma salicylate levels of about 300–400 µg/ml (≥2 mM) and rise in number and severity with increasing plasma levels. Systemic toxic effects can affect every organ and tissue in the body because of the diversity of pharmacological actions of aspirin and salicylates, respectively. The ubiquitous distribution of salicylates within the body and their accumulation in active form within cells (membranes) at increasing doses additionally aggravates tissue toxicity. With exception of a prolonged bleeding time – which does not parallel the clinical severity of salicylate intoxication – most if not all of the other toxic side effects of aspirin are primarily caused by salicylate (Section 3.1.1).

Prolonged bleeding events after aspirin intake are major unwanted side effects in long-term use, for example in cardiocoronary prevention. However, severe or even life-threatening bleeding events, for example in the gastrointestinal tract or the CNS, are rare and it is also not clear whether the bleeding risk remains the same during regular use over years. In short-term or single use, for example as antipyretic analgesic, aspirin-induced bleeding events in most cases are not a clinical problem. Nevertheless, aspirin-induced bleeding events may become a clinically very relevant side effect in particular life situations. This includes surgical interventions on aspirin-treated patients who take the compound because of an elevated risk for atherothrombotic events. The decision will be made by balancing the benefits (thrombosis prevention) versus risk (bleeding) and has to be made individually. In addition, minor gingival or nose bleeding events are not life-threatening but may reduce patient compliance, for example for continuous log-term cardiovascular prophylaxis (Section 3.1.2).

Bleeding and other side effects are of particular significance in particular life situations, such as (late) pregnancy and older age. In patients at older age (≥75 years), the risk of morbidity (tinnitus, malignancies) is increased and bleeding problems may also be more frequent due to comedications and drug interactions. For example, NSAIDs or oral anticoagulants including NOACs may interact with the pharmacokinetics and pharmacodynamics of aspirin, and there are also age-dependent changes in drug metabolism (Section 3.1.3).

In spite of legitimate concerns about the consequences of uncontrolled use and possible side effects, aspirin is a remarkably safe drug when used circumspectly [2]. The rate of serious side effects at single or short-term use is low and might be further reduced by use of appropriate, treatment-adapted galenic formulations (Section 2.1.1). Increased bleeding in long-term use for cardiovascular prevention is the iatrogenic consequence of the therapeutic strategy of blood "dilution." Unwanted side effects frequently result from unnecessary or careless use of the compound. *All* effective drugs have unwanted side effects, and aspirin is no exception from this rule.

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3.1.1 Acute and chronic toxicity

Systemic intoxications with aspirin occur relatively seldom. In public understanding aspirin used to be considered a harmless household remedy because of its apparently unrestricted use by both healthcare professionals and medical lays ("take an aspirin"). This is clearly an underestimation of the pharmacological potential of this agent at both sides [2] and possible misuse is facilitated by the easy access to the drug. Although alternative OTC drugs for treatment of pain and feverish diseases are meanwhile available and have replaced aspirin in acute painful/feverish conditions to a significant extent, in particular as a consequence of the intense discussions – but no confirmation – of a causal relationship between aspirin and Reye's syndrome (Section 3.3.3), aspirin still keeps an outstanding position as one of the most widely used drugs worldwide and – accordingly – can be misused for multiple reasons. This might be associated with systemic toxic events.

3.1.1.1 General aspects

Occurrence. Acute aspirin intoxication results from suicide attempts and accidental (toddlers!) or iatrogenic overdosing. Iatrogenic overdosing, that is, acquired intoxication during therapeutic use, occurs predominantly in the elderly, due to overloading of the body's clearing capacity ("salicylism") in long-term treatment. Most fatal cases of chronic salicylate poisoning occurred in toddlers or the elderly, the two patient populations in which mental deteriorations (confusion, lethargy) are particularly difficult to identify [3]. An actual review focused on risk factors for salicylate poisoning, the pathophysiology of both acute and chronic toxicity and management of aspirin intoxication is available [4].

Case reports. In the past, aspirin used to be a popular remedy for suicide attempts in teenagers and young adults. However, in the meantime it has been replaced worldwide for this "indication" by other (OTC) compounds, most notably acetaminophen (paracetamol) [5–7]. In the older literature, there is an interesting Hungarian report on aspirin poisoning which also might explain the reason for preference of aspirin in suicide attempts at the time.

During an observation period of 7 years (1923–1929) Balazs reported 792 cases of acute aspirin poisoning in Budapest (Hungary). The vast majority of overdosing (590) were suicide attempts in young adults. The average amount taken was 20–30 g (5–95 g). Only four cases of them, i. e., less than 1 %, terminated fatally. In these cases, the minimum fatal dose of aspirin amounted to 30–40 g [8].

This number of overdosing reports corresponded to about 1 case of clinically treated aspirin intoxication every third day in Hungary. Balazs explained this unusually high figure by the fact that aspirin was used as an *ultimum refugium* in partnership problems. For this purpose, aspirin used

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to be very popular because the clinical picture of salicylate poisoning was quite impressive to lay persons and at the same time not associated with a too high risk really to die [9].

According to these data, oral aspirin appears not to be an "effective" suicide drug, also because nausea and/or vomiting belong to the first clinical symptoms after acute overdosing (Table 3.1.1-1).

Table 3.1.1-1: Clinical symptoms of aspirin intoxication as seen 6 hours after intake of the plain drug in a standard formulation in relation to plasma salicylate levels (modified after [10, 11]).

severity of intoxication	serum level of salicylate		symptoms
	[µg/ml]	[mM]	
mild / early	300–600 (adults)	2.2–4.3	nausea / vomiting, abdominal pain, tinnitus
	200–450 (children / elderly)	1.4–3.2	dizziness, lethargy
moderate	600–800 (adults)	4.3–5.8	all of the above plus: tachypnoea, sweating, hyperpyrexia
	450–700 (children / elderly)	3.2–5.0	dehydration, loss of coordination, restlessness
severe	>800 (adults)	>5.8	all of the above plus: hypotension
	>700 (children / elderly)	>5.0	severe metabolic acidosis (after rehydration), bleeding tendency CNS symptoms: hallucinations, stupor, coma renal insufficiency: oliguria, uremia pulmonary edema

It is known from self-experiments and patient studies that up to 20 g aspirin/day (!) can be taken over a longer period of time without significant toxicity [12]. Eight to thirty grams per day (!) were given, probably over many days or even weeks, to American soldiers suffering from Spanish flu in American Army hospitals. Unfortunately, this was not well tolerated by a significant proportion of patients (see below). In the literature, there is one case of a suicide attempt with aspirin after self-administration of approximately 700 (!) aspirin tablets dissolved in water and applied as an enema (!). The patient survived with chronic hypoxic encephalopathy after severe acidosis and transient cardiac arrest [13]. However, there are also reports that "lower" doses of 65 g [14] or 130 g aspirin [15], the last being equivalent to 400 standard tablets (!), were fatal. A comprehensive historical overview on salicylate intoxications, including aspirin, is available [8].

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More frequent than overdosing by suicide attempts is accidental salicylate poisoning due to (erroneous) ingestion of salicylate-containing products, directed solely to topical or external use. To these belong salicylic acid (corn plaster and wart remover) and wintergreen oil or methyl salicylate (one teaspoon = 5 ml of wintergreen oil contains 7,000 mg salicylate, which is equivalent to 22 standard plain aspirin [325-mg] tablets [!]) [1, 16–18]. Methyl salicylate appears to be the most toxic salicylate, possibly due to its rapid uptake, wide tissue distribution and local accumulation [9]. In addition, even topical use of salicylate might cause toxicity or even death. It has been reported that toxic symptoms ("salicylism") can occur with topical use of 6 % of salicylic acid over 40 % of the body surface area [19].

Aspirin use in American soldiers during the "Spanish flu" epidemics. A particular noteworthy event in medical history of iatrogenic aspirin overdosing is its large-scale use as antipyretic during the Spanish flu epidemics of 1918/1919 in US-American soldiers.

After entry of the USA into World War I, in 1917, all branches, patents and trade names of German firms in the USA were considered enemy property. They were confiscated by the Alien Property Custodian and sold to American companies. At the same time, German firms were no longer allowed to sell their products in the USA (Section 1.1.2) [20, 21]. In the case of aspirin, Sterling Company of New York bought the rights (trademark) of "aspirin" and soon started producing "Genuine Bayer aspirin" under the Bayer cross. Thus, it was not the German Bayer Aspirin which was then (after 1917) used in enormous amounts in the USA, including US military physicians to treat American soldiers suffering from Spanish flu.

The recommended single aspirin dose according to the "Journal of the American Medical Association" (JAMA) in 1918 was 1.0–1.3 g with application frequencies ranging from hourly to every 3 hours, eventually resulting in daily doses of 8–31 g (!). These doses were extremely high and probably toxic to (m)any person(s) [22]. The ill soldiers received huge amounts of aspirin. In 12 US Army Camps with more than 10,000 cases of flu or flu-associated pneumonias, there was a remarkable case fatality rate ranging from 0.58 % to 3.3 % and from 2.1 % to 10 %, respectively. In addition to flu, the patients also suffered from unsatiable sweating and frequently died from pulmonary edema – typical symptoms of severe salicylate poisoning. About 100,000 tablets of aspirin had been ordered by the US Army camp with the highest mortality rate. Aspirin sales in the USA were more than doubled between 1918 and 1920 [22].

These high doses of aspirin might have been particularly dangerous for the ill, flu-affected patients. Specifically, the marked tissue accumulation of salicylate at repeated high dosing probably resulted in potentiation of toxicity. The overdosing of aspirin was obviously a consequence of the limited knowledge of the prescribers about the pharmacology of the compound and probably not the result of difficulties in manufacturing the compound by the new owners.

3.1.1.2 Pathophysiology and clinical symptoms of acute toxicity

Pathophysiology. The symptoms of intoxication are determined by the accumulation of salicylate and its action at the cell and tissue levels. Because the major excretion pathway of salicylate via salicyluric acid by glycine conjugation becomes rapidly sat-

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urated, salicylate accumulates in plasma, as also seen from the increasing proportion of unmetabolized salicylate in urine (Table 2.1.2-3). This is associated with a drastic prolongation of salicylate plasma half-life from about 2–3 h at therapeutic doses to 20–30 h and more at massive overdosing (Section 2.1.2). Hyperpnea is caused by direct stimulation of the respiratory center in the medulla oblongata. This effect is amplified at higher salicylate levels by dose-dependent disturbances of cellular energy metabolism due to uncoupling of oxidative phosphorylation [23, 24]. This uncoupling is associated with increased tissue oxygen demand and CO_2 production. Elevated CO_2 levels in plasma further stimulate the respiratory center with subsequently enhanced exhalation of CO_2 (hypercapnia). The pCO_2 in plasma remains unchanged because of simultaneously enhanced renal bicarbonate excretion.

The symptoms of intoxication (see below) are further enhanced by the generalized metabolic acidosis, eventually resulting in a higher percentage of nonprotonized salicylate, which penetrates cell membranes and accumulates inside the cells, eventually resulting in severe and long-lasting metabolic disturbances because of uncoupling of oxidative phosphorylation (Section 2.2.3).

Clinical symptoms. Clinical signs of acute aspirin intoxication become detectable in most individuals at serum salicylate levels above 300–400 µg/ml (≥2 mM) [1, 25–27]. Gastrointestinal symptoms (nausea and vomiting) and tinnitus are frequent initial symptoms. Vomiting occurs in about 50 % of patients when salicylate plasma levels exceed 300 µg/ml (Table 3.1.1-1). Life-threatening intoxications after acute ingestion of aspirin in adults start at doses above 12–15 g but at about 3 g in children [28]. There is a large interindividual variability. Some patients have been relatively asymptomatic with salicylate levels of 500–600 µg/ml [29], while others reportedly died at plasma salicylate levels of less than 150 µg/ml [14]. One reason for these variations might be the different start of treatment, with better prognosis at early beginning.

In a retrospective observational trial, Thisted and colleagues studied the clinical course of 177 consecutive patients with severe salicylate self-poisoning in an intensive care unit in Copenhagen (Denmark) during an observation period of 15 years (1969–1983). All patients were initially treated with gastric lavage and received symptomatic treatment of respiratory and cardiovascular failure.

On admission, cerebral depression (lethargy) was seen in 61 % of patients, respiratory failure in 47 %, acidosis in 37 % and cardiovascular dysfunctions in 14 %. The in-hospital mortality rate was 15 % and proportionally higher in patients aged more than 40 years and patients with delayed diagnosis. Disturbed acid-base balance was found in 50 % of cases and pulmonary complications (edema) in 43 %. Artificial ventilation was performed in 166 patients (94 %). Coagulation disturbances (low plasma prothrombin, prolonged bleeding time, thrombocytopenia) were seen in 38 % of cases. Gastrointestinal bleeding events occurred in 14 % of cases, fever in 20 % and hypotension in 14 % of cases.

An autopsy was performed in 26 of the 27 patients who died. The main findings were: ulcers of the gastrointestinal tract in 46 %, pulmonary edema also in 46 %, cerebral edema in 31 % and cerebral hemorrhage in 23 % of patients.

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The conclusion was that the main toxic effects of severe salicylate poisoning are the disturbed acid-base balance with severe metabolic acidosis, coagulation disturbances, CNS symptoms with depressed consciousness and cardiovascular and renal failure. Death was usually due to cardiopulmonary arrest subsequent to cardiac failure unresponsive to treatment [30].

Salicylate-induced noncardiogenic pulmonary edema can occur in both severe acute but also long-term overdosing of the drug, usually at advanced stages of intoxication, and may be fatal [31]. The incidence amounted to 35 % of salicylate-intoxicated patients who were over 30 years old [32]. At the same time, there might be proteinuria, indicating a generally increased vascular permeability [32–36]. Renal failure is rare and usually restricted to patients with preexisting renal diseases, specifically elderly persons with hypoalbuminemia (Section 3.2.3).

Toxic symptoms of the CNS dominate the clinical picture in later stages, that is, increasing severity of poisoning. The initial cerebral excitation is converted into increased cerebral depression. Finally, there is stupor, coma with cardiovascular failure and death from respiratory arrest.

Mortality. In otherwise healthy persons, the mortality from salicylate overdosing is low. According to an US survey, ingested aspirin amounts equivalent to less than 0.125 g/kg are harmless, a moderate risk exists at 0.15–0.30 g/kg, a severe and prolonged risk at 0.30–0.50 g/kg, while doses greater than 0.50 g/kg are considered potentially lethal [10]. According to a Canadian survey, fatal salicylate plasma levels are in the range of 6–8 mM [37]. The mortality rate in individuals with clinical features of severe salicylate poisoning amounts to 5 % (see below) but can increase to 15 % if treatment is started (too) late, frequently because of delayed diagnosis [11].

Laboratory findings. Laboratory findings are mainly the consequence of uncoupling of oxidative phosphorylation and inhibition of β-oxidation of (long-chain) fatty acids by high-level salicylates (Section 2.2.3). In this situation, metabolic CO_2 production exceeds its respiratory elimination. This effect is further enhanced by the depressive action of high salicylate levels on the respiratory center. With increasing inhibition of oxidative phosphorylation, there is also increasing accumulation of acids (lactate, pyruvate and others) with further aggravation of acidosis and dehydration. Eventually, this results in anion-gap acidosis [38]. Salicylate itself contributes only minimally to the anion gap: about 3 mval/l at serum levels of 500 µg/ml [1]. There is an increased renal excretion of bicarbonate (followed by K^+ and Na^+) and impaired kidney function, possibly also related to disturbed energy metabolism within the renal tubular cells. Water and electrolyte imbalance as well as sweating because of increased heat production may cause dehydration.

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Another symptom of salicylate overdosing are changes in blood glucose levels. Originally, hypoglycemia was the main finding . This was later explained by enhanced insulin secretion, due to interaction of salicylates with the NF-κB signaling pathway in the pancreatic β-cells (Section 2.2.2). This might also lower the glucose levels in liquor to critical levels, eventually requiring glucose substitution. Though hypoglycemia was a frequently reported finding, not all studies could confirm this [39].

Salicylate intoxication in children and Reye's syndrome. A possibly aspirin-induced hepatic failure became of particular interest after the discussion on a possible relationship between aspirin and Reye's syndrome. The question was whether Reye's syndrome in children is causally related to a (particular) aspirin-specific sensitivity of these patients, which is discussed in detail in Sections 2.2.3 and 3.3.3. Overall, there is no evidence for a causal relationship between the two.

Hyperpyrexia in children. Uncoupling of oxidative phosphorylation is compensated for by an increase in metabolic turnover. This is associated with increased oxygen consumption, depletion of liver glycogen and increased production of heat. This increased heat production is responsible for the dangerous hyperpyrexia which is a prominent symptom of salicylate poisoning in infants [40].

*Hyper*pyrexia in salicylate poisoning is somehow difficult to understand since salicylates were frequently and effectively used as antipyretic analgesics in children until they were largely eliminated because of the Reye discussion. The possible explanation is that salicylates "reset" the disturbed temperature regulation in the hypothalamus via their interaction with endogenous pyrogens (Section 2.3.2) but are unable to block the production of "extra" heat as a consequence of uncoupling of oxidative phosphorylation in peripheral organs (Section 2.2.3).

Physical temperature control functions by the production of large quantities of sweat as long as enough fluid for sweat production is available. When this mechanism becomes exhausted because of large water losses and dehydration, unbalanced hyperpyrexia develops because the "upregulation" of core temperature in the hypothalamus is normalized but the production of extra heat via the uncoupled oxidative phosphorylation in peripheral organs persists [40].

These metabolic disturbances, including respiratory alkalosis and metabolic acidosis, are the most important life-threatening effects of salicylates [3, 10, 41].

3.1.1.3 Pathophysiology and clinical symptoms of chronic toxicity

Chronic salicylate intoxication in adults ("salicylism") frequently results from iatrogenic overdosing during long-term aspirin treatment and is frequently overlooked because of the absence of specific symptoms [34]. Typical symptoms of chronic overdosing are tinnitus, multiple neurological deficits, including headache (!), confusion and central excitation, sweating, and hyperventilation, gastrointestinal bleeding and ulcers. Gastrointestinal side effects appear to dominate at a younger age while tinnitus

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and other audiovestibular toxicities (Section 3.2.4) are more frequent in the elderly. (Reversible) hepatic injury was seen in patients with a pathologic immune status, for example rheumatoid arthritis, who required long-term aspirin treatment at high doses (Section 3.2.3) [42]. However, because of available therapeutic alternatives in this indication, this finding is today solely of historical interest.

3.1.1.4 Treatment

Severe salicylate poisoning is an acute life-threatening, although rarely fatal, medical emergency condition. The treatment is entirely symptomatic because no specific antidote is available. As with other systemic intoxications, there are two basic therapeutic strategies: reduction or prevention of absorption and stimulation of excretion of salicylates. Both measures are combined with symptomatic treatment of the functional and metabolic disturbances [11, 43–45].

Inhibition of absorption. Treatment of the "conventional" oral intoxication starts with interruption of further drug uptake from the gastrointestinal tract. Intestinal absorption in the presence of toxic doses will continue for several hours and might be considerably longer in case of enteric-coated preparations because of their retarded absorption [46]. Usual standard procedures are gastric lavage and administration of activated charcoal [47]. As has to be expected, the earlier they start, the more effective are these procedures. An optimum time frame would be within 1 h after ingestion with an expected 30–50 % reduced salicylate absorption with 50 g of oral charcoal [48]. In addition, charcoal may recoat the surface of aspirin concretions within the stomach [49] and thus retard ongoing absorption. Administration of repeated doses of charcoal is recommended in patients who have ingested overdoses of enteric-coated or other slow-release formulations [46, 50]. This procedure (4×50 g charcoal in 1-h intervals to adults or 1 g/kg body weight to children) is recommended until the plasma salicylate reaches peak levels [11]. In the postabsorption phase, there is no accelerated clearance of plasma salicylate by charcoal [51, 52] and no reduction of the prolonged salicylate half-life [53].

Stimulation of elimination. Determinations of salicylate plasma levels by any suitable method (Section 1.2.2) and of the acid-base equilibrium to detect ionic gaps are essential because most of the clinical symptoms of salicylate poisoning are well correlated with these parameters. Measurements of salicylate plasma levels should be done initially and should be repeated at appropriate time intervals until peak plasma levels are obtained. The combined metabolic/respiratory acidosis should be corrected by appropriate treatment with sodium bicarbonate under control of kidney function and rehydration [11]. This procedure works by several mechanisms: inhibition of reabsorption of salicylate in the kidney by alkaline diuresis and improvement of the

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acid-base equilibrium in blood with normalization of plasma pH. This facilitates the rediffusion of (acetyl) salicylic acid from tissues in the blood. Particularly important is rediffusion from the CNS. A urinary pH of 7.5 or higher is suggested while the pH of blood should not exceed 7.55. Renal salicylate clearance is stimulated about 20-fold when the urinary pH increases from 6.1 to 8.1 [54], indicating that renal clearance of salicylates depends much more on urinary pH than on the renal flow rate. An additional approach to stimulate salicylate clearance is conversion into salicyluric acid by substitution of glycine [55].

Severe acute poisoning at plasma salicylate levels above 1,200 µg/ml or 1,000 µg/ml 6 h after ingestion, refractory acidosis or other symptoms of severe intoxication (Table 3.1.1-1), volume overload and renal failure are indications for hemodialysis. In chronic overdose, hemodialysis may be considered in symptomatic patients with serum salicylate levels above 600 µg/ml [1] and has been shown to reduce both morbidity and mortality of salicylate poisoning [56]. An excellent flowchart on algorithms for treatment of acute salicylate poisoning was published by Dargan and colleagues (Fig. 3.1.1-1) [11]. Further details and dose recommendations can be found in their original publication.

Further measures. Hyperthermia and dehydration require immediate cooling and fluid supply, respectively. Ketoacidosis and hypoglycemia (if present) additionally require the administration of glucose [27]. Administration of dextrose may help to avoid low cerebrospinal glucose levels [1]. Pulmonary edema usually resolves quickly with standard supportive therapy, although it might also be lethal (see above) [33].

3.1.1.5 Habituation

The many tons of aspirin consumed every year worldwide have occasionally led to the opinion that the drug may be habit forming. However, antipyretic analgesics, such as aspirin or paracetamol (dipyrone), in contrast to morphine-type analgesics, do not cause physical dependence. This is also confirmed by the scarcity of reports on "addiction" or "habituation" to salicylates. There might be some psychological desire for drug intake, for example regular use for pain relief (headache!), but only to the extent that frequent use of any substance which gives relief, real or imaginary, from pain is a habit [57].

Another issue are the few reports on abuse of aspirin at high doses when toxic effects, such as salicylism with exaltation and deafness, were desired for "therapeutic" purposes.

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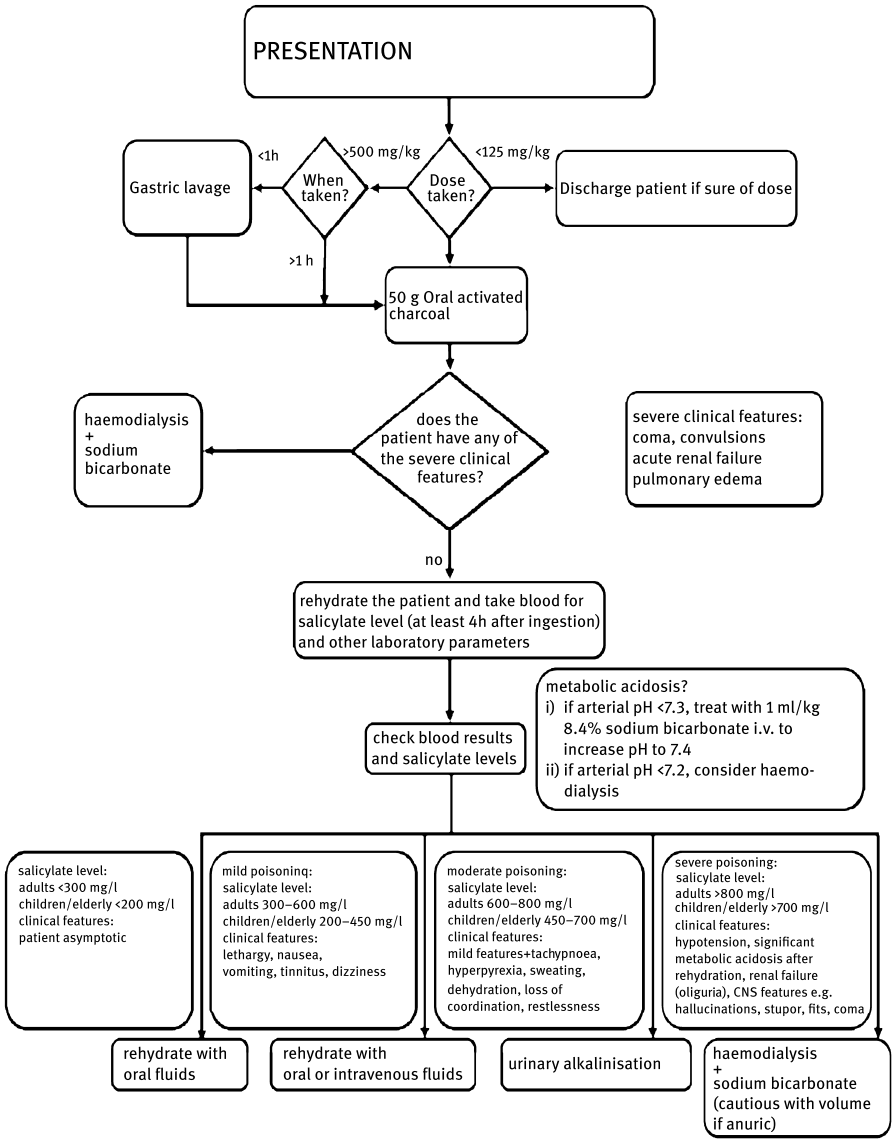

Figure 3.1.1-1: Flowchart with algorithms for treatment of acute aspirin (salicylate) poisoning. For more details and practical recommendations see the original publication [11].

A 59-year-old man took about 100 tablets of aspirin within 2 weeks (about 2.3 g per day) for "encouragement." A 30-year-old epileptic and alcoholic took 20–30 tablets of aspirin within 1 hour for the same purpose and a 58-year-old female alcoholic took up to 100 aspirin tablets against crapulousness and because she was unable to tolerate the noise at her working place [58].

Taken together, there is no evidence that aspirin has a habit forming potential.

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Summary

Acute life-threatening salicylate intoxication in adults occurs at doses of about 12–15 g and above and at 3 g and above in children. This is equivalent to plasma levels of ≥300 µg/ml or ≥2 mM. Initial clinical symptoms are nausea and vomiting, tinnitus, tachypnea with respiratory alkalosis and central excitation, eventually resulting in combined respiratory/metabolic acidosis. With increasing severity of intoxication, there are increasing CNS dysfunctions (hallucinations, stupor, coma) and finally death from pulmonary edema or respiratory arrest can occur. All of these symptoms are caused by salicylate accumulation in organs and tissues, most notably in the CNS, and probably largely due to salicylate-induced disturbances of membrane functions and energy metabolism. Despite a considerable interindividual variability, the salicylate plasma levels in general correlate well with clinical symptoms.

The clinical outcome is critically determined by an early diagnosis, i. e., start of treatment. Under optimum conditions, mortality of severe intoxications in otherwise healthy individuals amounts to ≤5 % but may increase to 15–20 % if the beginning of treatment is delayed and/or comorbidities exist. There is no specific antidote; therefore, the treatment of salicylate poisoning is symptomatic. Inhibition of absorption by (repeated) administration of activated charcoal in sufficient doses is an effective measure in early stages of intoxication, i. e., as long as absorption from the gastrointestinal tract is not completed. Renal salicylate excretion can be considerably enhanced by correction of acidosis and alkalinization of urine by sodium bicarbonate. Disappearance of tissue acidosis also allows redistribution of salicylate from tissues into plasma and facilitates renal excretion and recovery. Hemodialysis is an alternative in case of severe intoxication and/or renal failure.

There is no evidence for addiction or habituation with salicylates, even at long-term use. The risk of persistent injuries of liver (Section 3.2.2) or kidney (Section 3.2.3), the main sites of salicylate metabolism and excretion, respectively, is small if any. It may clinically only become relevant at high-dose, long-term treatment of inflammatory disorders in patients with an altered immune status, such as rheumatoid arthritis. However, in this indication aspirin is no longer used (Section 4.2.2).

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3.1.2 Bleeding time and bleeding risk

3.1.2.1 General aspects

It is not surprising that inhibition of platelet aggregation also bears a bleeding risk. However, bleeding was not considered to be a serious clinical problem in the early days of high-dose but short-term aspirin use – as opposed to problems with aspirin's gastric tolerability (Section 1.1.4). A large Cochrane analysis from 2012, including 68 studies on treatment of postoperative pain with single-dose aspirin (300–1,200 mg), did not even mention bleeding as a noticeable side effect [1]. Serious problems with blood coagulation were only found in a minority (38 %) of patients who were hospitalized because of severe, life-threatening salicylate poisoning. Dominating symptoms in these patients were a disturbed acid-base balance (50 %) and respiratory complications (pulmonary edema) in 43 % of cases (Section 3.1.1) [2].

In real life with frequent OTC aspirin use, single doses of 0.5–1.0 g, taken for acute treatment of pain or feverish disorders, gastrointestinal intolerance is the dominating side effect of aspirin [1]. If (minor) bleeding occurs, this is rather a subjective disturbing and possibly compliance-affecting event (gingival bleeds, nose bleeds, etc.) but not a clinically relevant issue. However, in the presence of synergistically acting factors, such as alcohol [3] or comedication with other antiplatelet agents or anticoagulants [4], there might be a significant bleeding risk. This is also true for certain clinical conditions, such as persons at older age with multiple comorbidities (liver diseases, *H. pylori* infections) [5]. The elderly are in general also at higher risk of drug-related side effects because of frequent polypharmacy (NSAIDs!), as are individuals with a history of aspirin-induced gastrointestinal bleeding [6, 7]. Some nutrients may also act synergistically with aspirin on bleeding time, most notably fish oil [8].

Another issue related to aspirin-induced bleeding is regular, low-dose, long-term use, for example in cardiovascular prevention. Here, an aspirin-related enhanced bleeding risk may become a clinical problem, especially at particular localizations, such as the (upper) gastrointestinal tract (Section 3.2.1) or the CNS (Section 4.1.2). However, "blood dilution" by aspirin in these individuals is part of the clinical treatment strategy of thrombosis prevention and, therefore, has to be balanced against the risk of atherothrombotic events in the absence of aspirin protection. This becomes a critical issue if decisions have to be made on whether or not to continue prophylactic aspirin intake in atherothrombotic risk patients who have to undergo acute surgical interventions. There is also a risk of an aspirin "withdrawal syndrome" with an increased vascular "rebound" risk of cerebral [9] and coronary [10] thrombotic events in risk patients.

3.1.2.2 Bleeding time

Definition and measurement of bleeding time. Bleeding time, frequently determined as capillary bleeding time after a standardized skin injury, is an estimate of primary

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hemostasis, i. e., cessation of local blood loss after vessel injury by clot formation [11]. Bleeding time is also a most popular parameter to estimate the individual bleeding risk. However, hemostasis after capillary injury does not solely result from the formation of platelet/fibrin clots. A linear correlation between bleeding time and platelet count only exists in thrombocytopenia, that is, circulating platelet numbers between 10,000 and 100,000 per microliter, but not with higher, normal platelet counts [12]. This suggests additional hemostatic components in addition to platelets, such as plasmatic clotting factors, most notably thrombin, changes in vascular permeability and an altered endothelial function at the site of injury. Probably all these major constituents of the hemostatic system are involved in control of bleeding time [13]. Considering these multiple interacting – and partially even counteracting – variables, it is not surprising that the interindividual variations of bleeding time (standards) are very high. Despite its considerable relevance as an integral parameter of hemostasis, data on bleeding time, even if determined under well-standardized laboratory conditions by well-trained experts, are not a useful predictor of the individual thrombotic risk [14] or even the efficacy of treatment by antiplatelet drugs [14, 15].

Formation of a platelet/fibrin clot is the ultimate and only functional relevant result of the clotting process. Thrombin and fibrin production still continue for a long time after the clotting process is completed. Thus, conventional laboratory ex vivo/in vitro methods of measuring blood clotting, such as thrombin time (Quick) or platelet aggregation, provide no information about the functionality of the real end product of the clotting process – the platelet/fibrin clot. In contrast, it are the mechanical properties of the fibrin clot (elasticity) that determine the efficacy of thrombus formation and separate normal from disturbed hemostasis (bleeding disorders). In addition to aggregation, platelet granule secretion products contribute to the functionality of the fibrin clot [16].

Bleeding events due to insufficient thrombin generation, clot formation and/or platelet functions are interrelated processes but without any linear correlation between them. For these reasons, thrombocytopathies exhibit a more or less normal clotting time, as long as platelet-dependent thrombokinase (factor X) activation is sufficient. Bleeding time, however, is prolonged in all cases because of insufficient regular thrombus formation as a consequence of an insufficiently stable fibrin network.

In this reaction chain, (weak) inhibition of platelet function – at antiplatelet doses of aspirin – is probably entirely restricted to reduced thromboxane formation and its role for platelet functions, including thromboxane-dependent platelet aggregation [17], while higher initial doses (500 mg) [18] are required to additionally inhibit thrombin production. This means that aspirin-induced antiplatelet effects are not paralleled by changes in bleeding time. This should be considered by interpreting any increased bleeding tendency after aspirin treatment at antiplatelet doses as an index of efficacy for aspirin-induced inhibition of platelet function.

Time dependency of aspirin-related bleeding. The maximum prolongation of bleeding time after a single standard dose of aspirin is about 2–3-fold and is seen at about 2–3 h after aspirin intake [11, 19]. After offset of repeated aspirin administration, bleeding time returns to normal within 3–4 days. At this time, platelet thromboxane for-

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mation is still significantly reduced, by >50 %, although thromboxane-dependent platelet aggregation has already returned to control levels (Fig. 2.3.1-5) [20].

Dose dependency of aspirin-related bleeding. *John Quick* (1966) published one of the first mechanistic studies describing the effects of aspirin on bleeding time. He found that single-dose aspirin to healthy subjects prolonged bleeding when given at high doses (1,300 mg) but not at a medium analgesic standard dose (650 mg). No such effect was seen by salicylate at comparable doses and there was a large interindividual variability (Fig. 3.1.2-1) [21]. In an earlier study he had already found a significant prolongation of the prothrombin time at high-dose (6 g) aspirin but not high-dose salicylamide [22]. Thus, aspirin, unlike other salicylates, could enhance bleeding at analgesic doses while a comparable effect by salicylate required toxic plasma levels of about 500 µg/ml (3 mM) [23]. This suggested some specificity for aspirin which was possibly related to its acetylation potential with platelet COX-1 and prothrombin being a candidate (Section 1.1.4).

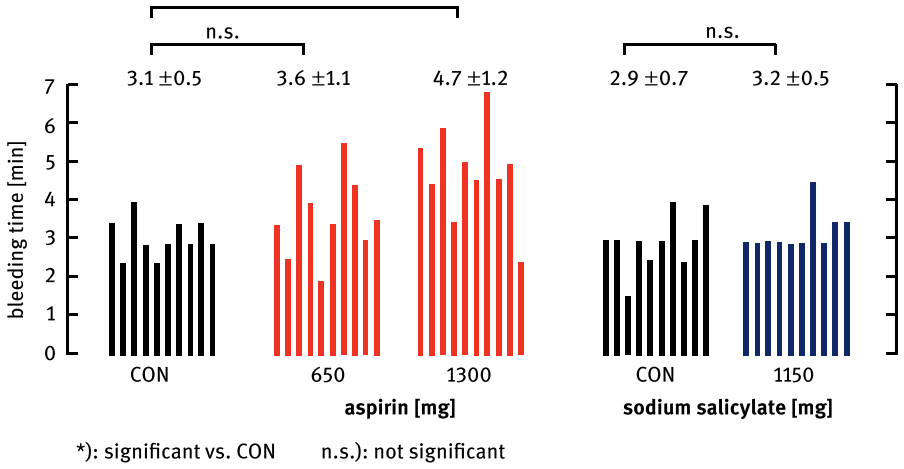

Figure 3.1.2-1: Dose-dependent changes in bleeding time in 10 healthy subjects before (CON) and after oral ingestion of 650 or 1,300 mg aspirin. There is no significant change at 650 mg, a significant increase (by 50 %) at 1,300 mg at high interindividual variability and no change after sodium salicylate at the same doses. There were no changes in clotting time, prothrombin time, clot retraction time and prothrombin consumption (not shown) (modified after [21]).

These observations of Quick of a dose-dependent (80–1,300 mg) prolongation of bleeding time after single-dose aspirin as well as a high interindividual variability, only about 60 % of individuals being responders, was confirmed by later investigators [19, 24]. It also became increasingly evident that platelets and their thromboxane formation are the main aspirin-sensitive players (Section 1.1.4). Nevertheless, the vari-

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abilities in bleeding time despite a (probably) complete inhibition of platelet thromboxane formation at these aspirin doses in each person clearly demonstrated that inhibition of platelet thromboxane formation by aspirin will not explain its effects on bleeding time [13].

Platelet thromboxane and bleeding time. Further arguments for this hypothesis are the very low thromboxane levels in circulating blood, amounting to only 1–2 pg/ml but are increased about 1,000-fold, to 3–5 ng/ml, in blood samples taken from skin incisions [25]. However, this still amounts to only about 1% of thromboxane forming capacity of about 200–300 ng/ml serum [8]. There is no clear correlation between thromboxane levels in blood taken from skin incision for determination of bleeding time and the amount of blood loss (Fig. 3.1.2-2). This suggests further aspirin-sensitive modifying factors such as thrombin generation in the early stages of hemostasis influence bleeding time [18].

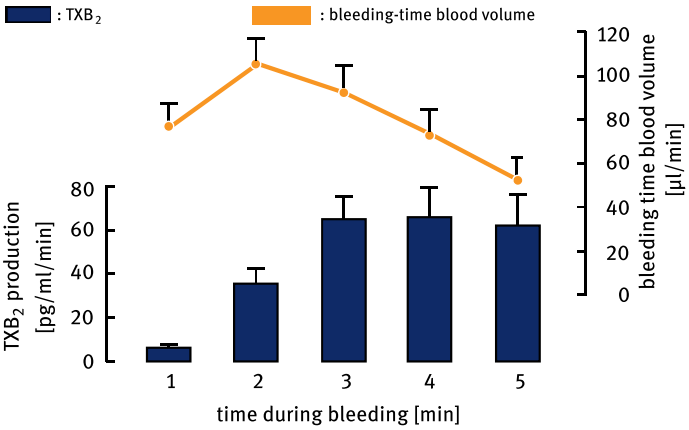

Figure 3.1.2-2: Time-dependent changes of thromboxane B_2 (TXB_2) levels in venous blood taken from bleeding skin incisions (capillary bleeding time) in successive 1-min intervals. Although the rate of blood loss fell with time, the rate of thromboxane production remained constant. There was no relation between bleeding time (blood volume) and TXB_2 content of blood with increased time of bleeding (modified after [8]).

The EC_{50} for stimulation of human platelets by genuine (TXA_2) amounts to about 20 ng/ml (66 nM) [26]. Thus, thromboxane levels of 3-5 ng/ml in blood taken from skin incisions [25] are in the lower threshold range of stimulating platelet aggregation. This suggests a biological function of (TXA_2) for blood clotting primarily in combination with other procoagulatory factors, such as thrombin and disturbed endothelial function.

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In this context some comments should be made regarding the qualitative differences and information content taken from serum and plasma thromboxane level determinations.

Serum thromboxane measurement, frequently done in clotting blood kept in glass vials for about 2 h at 37 °C, reflects a time-independent determination of thromboxane forming capacity of platelets with continuous accumulation of the stable hydrolysis product thromboxane B_2. This thromboxane level differs quantitatively markedly (200 ng/ml and more vs. several pg/ml) from the natural in vivo situation of injury-induced thromboxane formation. The local concentration of the active thromboxane A_2 within the circulation or at a site of injury is determined by the relationship between synthesis and washout as well as spontaneous hydrolysis and/or enzymatic degradation of the active thromboxane A_2. No maximum capacity can be obtained under these dynamic conditions.

For these reasons, serum thromboxane B_2 is a useful parameter for determination of the pharmacodynamic efficacy of aspirin regarding platelet COX-1 inhibition but is useless for description of in vivo hemostasis and/or its alterations by aspirin treatment.

3.1.2.3 Modes of aspirin action

Determinants of aspirin-induced bleeding. Mechanistically, aspirin most likely prolongs bleeding by acetylation of proteins involved in the vascular and blood components of hemostasis. In addition to platelet COX-1, these factors are the zymogens of plasmatic clotting factors, such as prothrombin [27] and fibrinogen [28, 29]. Another variable is enhanced NO production via acetylated eNOS [30], eventually resulting in vasodilation and improved antihemostatic/antithrombotic properties of the endothelium (Section 2.3.1) (Fig. 2.3.1-9). Consequently, aspirin-associated changes in bleeding time are no equivalent for the efficacy of antiplatelet treatment [15] and do also not allow any reliable prediction of the aspirin-related perioperative bleeding risk. With other words, prolongation of bleeding time and antiplatelet/antithrombotic actions of aspirin are no synonyms [27].

Aspirin and inhibition of thrombin formation. Acetylation of prothrombin as well as acetylation of fibrinogen [28] and of proteins at the platelet surface membranes [31] will retard thrombin generation at the platelet surface, the major site of its formation inside the circulation in vivo [32]. This will also alter the mechanical properties of fibrin clots (see above). No such effects were seen for P2Y12 inhibitors [33]. Mechanistic studies on the effects of aspirin on plasmatic coagulation were mainly done with blood with increased bleeding time as a model of clinically relevant thrombin formation [34]. Using this model, it was originally shown by *Paul A. Kyrle* (Wien, Austria) that low-dose aspirin (ca. 35 mg/day for one week) not only inhibited thromboxane formation and platelet secretion but also significantly retarded thrombin generation and action at a site of plug formation. This suggested that thrombin formation in an area of vessel injury was promoted by activated platelets in an aspirin-sensitive manner [18]. After an original report by *Andrzej Szczeklik* (Fig. 3.1.2-3) [27] a number of follow-up investigations was performed by *Anetta Undas* and her group [34–36] from Kraków (Poland).

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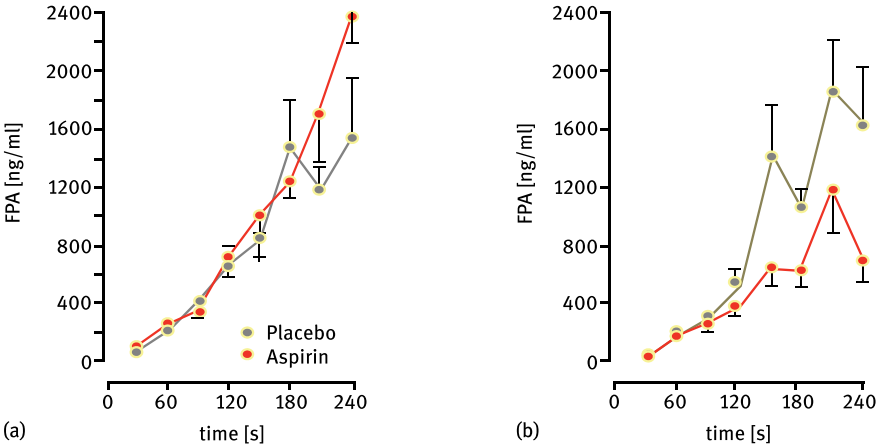

Figure 3.1.2-3: Thrombin generation in whole blood ex vivo (accumulation of the cleavage product fibrinopeptide A [FPA]) after standardized skin incision in healthy volunteers before (a) and 2 h after (b) oral ingestion of 500 mg aspirin (red) or placebo (gray) [27].

In a series of elegant trials, they studied the mechanistic details of aspirin-induced inhibition of thrombin formation, also using the capillary bleeding time model.

The time-dependent sequence of events in tissue factor-dependent activation of the clotting system and its modifications by aspirin were studied ex vivo in capillary blood of healthy volunteers subjected to vessel injury by skin incision. Measurements were performed before and one week after daily intake of 75 mg aspirin. Blood was collected in 30-s intervals. Activation of clotting factors was determined by quantitative immunoassays.

Vascular injury was followed by an immediate, continuous fall of prothrombin levels, approaching less than 10 % of the initial value at the end of bleeding, i. e., thrombus formation. This prothrombin was converted to thrombin, reaching peak values of 38 nM (!). These amounts of thrombin were much higher than those which were required for maximum platelet activation. Fibrinogen levels fell and became undetectable at about 3 min of bleeding, indicating maximum fibrin formation. (Thrombin-induced) activation of clotting factor V to factor Va (amplification mechanism for thrombin formation) was detected after thrombin generation had started and was later followed by the inactivation of FVa by activated protein C. This indicated thrombin-induced stimulation of anticoagulant factors.

Aspirin treatment markedly reduced all of these activation markers, on average by about 30 %. This was associated with a significant retardation of the clotting process.

It was concluded that aspirin at antiplatelet doses not only inhibits thromboxane formation but also impairs thrombin generation and all follow-up reactions catalyzed by thrombin at the site of tissue injury. In a follow-up trial the authors did not find any clear dose dependency at 75–300 mg aspirin/day for thrombin inhibition although the bleeding time was significantly prolonged: 165 s vs. 102 s [35, 37].

These findings of inhibition of thrombin formation by aspirin at antiplatelet doses were basically confirmed in several later trials [36, 38, 39]. They strongly suggested that antithrombin effects significantly contribute to aspirin-related inhibition of the

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clotting process as well as its antithrombotic activity. Interestingly, aspirin-induced inhibition of thrombin formation is largely lost in case of aspirin "resistance" [40]. Platelet activation appears to be a starting and most relevant amplification event for thrombin formation [32]. Inhibition of thrombin formation by aspirin could become clinically relevant in antiplatelet treatment of patients with ACSs, specifically during the first hours after the acute event (Sections 4.1.1 and 4.1.6) [41]. At this time, there is a marked release of tissue factor and increased thrombin generation after plaque rupture which probably also causes the initial "resistance" of activated platelets against oral ADP antagonists, while high-dose aspirin (500–900 mg) was found to significantly reduce thrombin formation in these conditions (Section 4.1.1) [41, 42].

3.1.2.4 Aspirin-related bleeding in long-term prevention

Long-term prevention. Serious bleeding events, presumably in the gastrointestinal tract and the CNS, are the most dangerous side effects of long-term aspirin use in cardiovascular prevention (Section 4.1.1). A large metaanalysis of 35 randomized trials with low-dose aspirin (75–325 mg/day), mostly long-term cardiovascular prevention trials, showed a moderately increased risk of severe bleeding events in aspirin users (odds ratio [OR]: 1.54; 95 % confidence interval [CI]: 1.34–1.74), mainly gastrointestinal and cerebral bleeding events, but no increase in the number of fatal bleeds (OR: 1.22; 95 % CI: 0.78–1.89) (Fig. 3.1.2-4) [43]. Similar results were obtained by analysis of 39 observational trials, suggesting that the risk of major bleeding events with long-term aspirin is similar in real-world settings as compared to that reported in randomized trials [44].

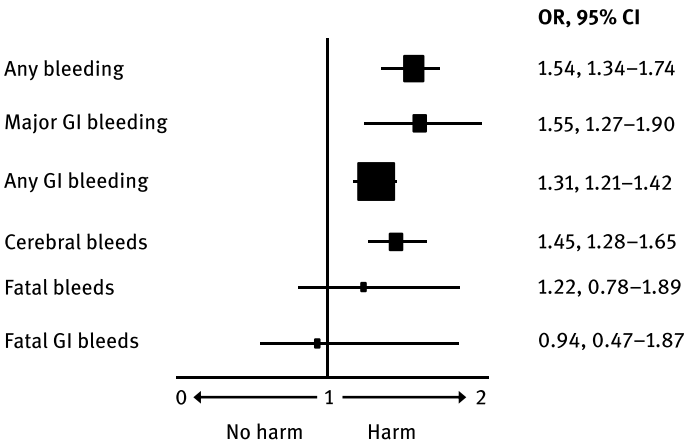

Figure 3.1.2-4: Bleeding events associated with low-dose (75–325 mg/day) aspirin vs. nonaspirin-treated controls. Metaanalysis of 35 randomized prevention trials. In this metaanalysis, aspirin alone decreased the risk for all-cause mortality (risk ratio [RR]: 0.93; 95 % CI: 0.87–0.99), largely because of effects in secondary prevention populations [43].

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A more recent analysis of the incidence of upper gastrointestinal bleeding in 40,000 aspirin-treated patients compared regular aspirin users with those who discontinued prescribed aspirin for secondary prevention of myocardial infarction for different reasons, mainly (70 %) (!) because of compliance problems. During the first year after the acute event, nonusers suffered five more cardiac and three more cerebrovascular events per 1,000 patients and had only 0.4 more upper gastrointestinal bleeding events (Fig. 4.1.1-8) [45]. Another metaanalysis on bleeding in long-term (primary) prevention by aspirin reported that the incidence of bleeding events was low and tended to become smaller with longer use (Fig. 4.3.1-2). The number of fatal extracerebral bleeding events after use for ≥5 years was even significantly reduced [46].

Studies on the bleeding risk of aspirin in long-term prevention of recurrent thrombosis in patients with previous unprovoked venous thromboembolism (VTE) are also available. The INSPIRE collaboration, reevaluating the data of the placebo-controlled WARFASA and ASPIRE trials on an individual patient basis, found an annual incidence of severe bleeding events of 0.5 % for aspirin vs. 0.4 % in the placebo group (P: n. s.) as opposed to the benefit of a 42 % reduction in recurrent VTE by aspirin treatment (hazard ratio [HR]: 0.58; 95 % CI: 0.40–0.85; $P = 0.005$) (Section 4.1.4).

Taken together, there is a bleeding tendency for long-term aspirin users. In most cases this is not dramatic and is counterbalanced by the desired antithrombotic effect. In addition, withdrawal of regular aspirin in subjects with a moderate-to-high risk for CAD might have ominous prognostic implications (see below) [10, 45, 47]. There is a risk for severe bleeding events, predominantly in high-risk patients including the elderly according to the ASPREE trial [48].

3.1.2.5 Aspirin-related bleeding in surgery and withdrawal

Aspirin and periprocedural bleeding risk after minor surgical interventions. Because of the risk of uncontrolled periprocedural bleeding, patients receiving long-term aspirin prophylaxis are frequently asked to discontinue use of the drug for 5–7 days before surgery because of bleeding risk. Aspirin use at antiplatelet doses (100 mg/day) can cause significant, though small increases in bleeding after minor dento-alveolar surgery, such as tooth extraction [49]. A local hemostatic procedure is usually sufficient to control for bleeding if necessary [50], even in patients under dual antiplatelet treatment [51] or combined treatment with oral anticoagulants [52]. Aspirin, taken at antiplatelet doses for atherothrombotic prophylaxis, needs not to be discontinued for minor surgery, including oral surgery, because of an elevated risk of acute vascular events [53]. Similar considerations appear to apply for minor surgery in dermatology [54].

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Aspirin and periprocedural bleeding risk after major surgical interventions. Bleeding problems become an issue if patients at enhanced atherothrombotic risk who regularly use aspirin have to undergo a major surgical intervention. Here, the thrombotic risk is further enhanced by the proinflammatory and prothrombotic conditions of the acute operative procedure [55]. It is, therefore, a clinically highly relevant issue whether withdrawal of aspirin in these patients, specifically in disease-related operations, such as coronary artery bypass surgery or carotid endarterectomy, improves the clinical outcome by reducing perioperative blood loss or rather increases the risk of atherothrombotic vessel occlusions (Section 4.1.1).

A by 50 % increased risk of enhanced periprocedural blood loss is to be expected if aspirin treatment is not discontinued prior to surgery. However, this extra blood loss is usually small to moderate and is not associated with an increased severity of bleeding complications or even bleeding-related death [56–58]. With a few exceptions, including intracranial surgery, middle ear surgery and, possibly, transurethral prostatectomy [59], withdrawal of aspirin before surgical interventions in patients at risk of atherothrombotic events is currently not recommended.

The aspirin "withdrawal syndrome." Ischemic events (stroke, [re]infarctions) were repeatedly described in patients with previous myocardial infarction (Section 4.1.1) [60, 61] or stroke (Section 4.1.2) [9] when aspirin prophylaxis was discontinued because of an elective major surgical intervention. In cardiovascular risk patients with previous stents, aspirin should not be withdrawn because of a possible increase in cardiac events without a major protection from bleeding [62–64]. An overview of retrospective trials came to the conclusion that discontinuation of aspirin in high-risk cardiac patients may cause acute thromboembolic vessel occlusion in up to 10 % of patients. Importantly, atherothrombotic events usually do not occur immediately but only about 1 (myocardial infarctions) to 2 (stroke) weeks after the surgical intervention and, therefore, may not have been seen by the surgeon [58].

Despite this apparently general agreement that aspirin prophylaxis – with a few exceptions – should not be interrupted in patients at elevated cardiovascular risk because of an elective surgery, a large prospective, placebo-controlled randomized trial, the "PeriOperative ISchemic Evaluation 2 (POISE-2)" study, came to the conclusion that administration of aspirin prior to noncardiac surgery in patients at elevated atherothrombotic risk is useless but rather increased bleeding.

A total of 10,010 patients at elevated atherothrombotic risk undergoing elective noncardiac surgery were included and randomly stratified using a two-by-two factorial design to receive aspirin or placebo or clonidine or placebo. For the aspirin part of the study, patients were separated into those with no previous aspirin intake (initiation stratum, 5628 patients) and those who were already on aspirin (continuation stratum, 4382 patients). Patients in the continuation stratum did discontinue aspirin intake on average 7 days before surgery. All patients received 200 mg aspirin

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just before surgery and continued this at 100 mg/day for 30 days in the initiation stratum and for 7 days, followed by the previous regular dosing, in the continuation stratum. Primary outcome was a composite of death or nonfatal myocardial infarction at 30 days. Important safety endpoints were severe and life-threatening bleeding events and clinically relevant hypotension.

The primary outcome occurred in 7.0 % of the aspirin group and in 7.1 % of the placebo group (HR: 0.99; 95 % CI: 0.86–1.15; $P = 0.92$). Major bleeding was more common in the aspirin groups: 4.6 % vs. 3.8 % ($P = 0.04$), while life-threatening bleeding and mortality were unchanged. The overall primary and outcome results were similar in the two aspirin strata, suggesting the absence of an aspirin "withdrawal" effect.

The conclusion was that administration of aspirin before noncardiac surgery and throughout the early postsurgical period did not improve clinical outcome in these patients but increased the risk of bleeding. These findings apply to both aspirin-pretreated and aspirin-naïve patients [65].

This study is one of the few large prospective, randomized, placebo-controlled trials on the benefit/risk ratio of prophylactic aspirin in cardiovascular risk patients undergoing elective noncardiac surgery. It, therefore, requires particular attention. As a net result, it does not recommend prophylactic perioperative aspirin use in the cardiac risk patients studied. However, there are also a number of limitations [66]. Specifically, the study did not include patients already on prophylactic aspirin where aspirin was to be continued during the perioperative time. It is also questioned whether this particular patient population is relevant to other cardiovascular risk patients. For example, the POISE-2 study contained only 4.3 % of stent patients (recent stent was an exclusion criterion). Overall, 4,239 participants, i. e., about 42 % of the included patients in both the aspirin and placebo groups, suffered from a clinically relevant hypotension (systolic blood pressure < 90 mmHg) (safety outcome!). This number is clearly overproportional, possibly due to the particular study design (clonidine arm in the study). The selected loading dose of 200 mg oral aspirin is rather unusual and was unexplained by the authors. It is not sure whether this dose is sufficient for a significant antiplatelet effect of aspirin in terms of inhibition of thromboxane formation in aspirin-naïve patients [67] undergoing major surgery.

Taken together, there is no reason to change the current practice that the perioperative management of antiplatelet therapy should be based individually on the balance between the thrombotic and hemorrhagic risk that characterize each patient and each surgical procedure [68, 69]. In most cases this means that an antiplatelet treatment with aspirin will be maintained throughout the perioperative time.

3.1.2.6 Prevention and treatment of bleeding

Aspirin-related perioperative bleeding events, with few exceptions, such as severe gastrointestinal bleeding (Section 3.2.1) and cerebral bleeding (Section 4.2.1), are not life threatening in most cases and usually do not require particular therapeutic measures. However, in case of severe and/or life-threatening bleeding in aspirinized patients, the bleeding problem is aggravated by the absence of a specific antidote and the irreversibility of aspirin-induced inhibition of platelet function. In noncardiac pa-

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tients, it is sufficient to skip aspirin about 1 week prior to elective surgical interventions. For reversible COX inhibitors, 5 half-lives are sufficient, that is (with the exception of naproxen), one day [70]. However, in patients taking antiplatelet agents, perioperative correction of platelet malfunction might be considered. Two drugs have been frequently used as functional antagonists of (excess) bleeding by antiplatelet drugs: desmopressin (1-deamino-8-D-arginine-vasopressin [DDAVP]) and tranexamic acid. Platelet infusions might also be considered as *ultima ratio* in particular conditions.

Desmopressin (DDAVP). DDAVP is a vasopressin analog that improves platelet adhesion to the vessel wall by generation of abnormally large factor VIII/von Willebrand factor multimers. These multimers bind platelets particularly effectively to subendothelial collagen, resulting in large amounts of platelets at the injury site. The action is specific for platelets and independent of the kind of antiplatelet treatment [71]. DDAVP is well tolerated [72]. The effect on bleeding time is maximum 1–2 h after intravenous administration and lasts for about 4 h [73, 74].

Ten trials with a total of 596 participants were identified in a recent metaanalysis of randomized, controlled trials on desmopressin and perioperative bleeding, all in the setting of cardiac surgery. Platelet dysfunction was due to antiplatelet agents or cardiopulmonary bypass in four trials.

Patients treated with desmopressin received fewer red cell transfusions, lost less blood and had a lower risk of reoperation due to bleeding problems. There were too few events to determine if there was a change in the risk of thrombotic events.

The conclusion was that desmopressin may be a useful agent to reduce bleeding and transfusion requirements for people with platelet dysfunction or with a history of recent antiplatelet drug administration undergoing cardiac surgery [75].

Tranexamic acid. Tranexamic acid is a synthetic analog of the amino acid lysine and an improved follow-up to the older compound ε-aminocaproic acid. It inhibits fibrinolysis by reversibly binding to specific binding sites on plasminogen and preventing plasmin (antiplasmin) from binding to and degrading fibrin. This maintains fibrin's matrix structure and will improve clot stability. The compound was found to correct aspirin-induced defects in arachidonic acid- and ADP-induced aggregation without changing platelet aggregation ex vivo in patients without antiplatelet treatment [76].

In the "Aspirin and Tranexamic Acid for Coronary Artery Surgery" (ATACAS) trial, patients undergoing coronary artery bypass surgery received tranexamic acid (plus placebo) or aspirin (100 mg, enteric-coated 1–2 h prior to surgery). The primary outcome was a composite of death and thrombotic complications (nonfatal myocardial infarction, stroke, pulmonary embolism, renal failure or bowel infarction) within 30 days after surgery.

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In comparison to placebo, tranexamic acid was associated with a lower risk of bleeding and without higher mortality or thrombotic complication rates within 30 days after surgery but with a higher risk of postoperative seizures. A primary outcome event occurred in 19.3 % of patients in the aspirin group and in 20.4 % of patients in the placebo group ($P = 0.55$). Major hemorrhage requiring reoperation occurred in 1.8 % of patients in the aspirin group and in 2.1 % of patients in the placebo group ($P = 0.75$), and cardiac tamponade occurred at rates of 1.1 % and 0.4 %, respectively ($P = 0.08$).

The conclusion was that tranexamic acid was associated with a lower risk of bleeding than placebo and did not cause higher mortality or thrombotic complication rates. The administration of preoperative aspirin resulted in neither a lower risk of death or thrombotic complications nor a higher risk of bleeding than that with placebo [77, 78].

According to this study, tranexamic acid treatment appears to be a useful approach for reducing excess bleeding in coronary artery bypass surgery patients. However, it is seriously questionable whether the 100-mg single-dose enteric-coated aspirin tablet used in this study was sufficient to exert any relevant inhibition of platelet function or thromboxane formation in these aspirin-naïve patients [79]. Experimental data from others do suggest this [67].

Platelet transfusions. Platelet transfusions could be a final option to compensate for platelet dysfunctions. They are the really last chance in critical cases because they can increase the risk for adverse outcomes, specifically new thrombotic events [80]. A particular protocol has been developed for transient "reversal" of antiplatelet treatment in patients requiring urgent surgery and being on dual antiplatelet treatment with aspirin and clopidogrel. This protocol is based on timed platelet transfusion based on the pharmacokinetic profile of aspirin and ADP antagonists and was successfully used in a small group of patients. Mechanistically, it was assumed that supply of aspirin-naïve platelets with intact thromboxane formation could improve or even restore primary hemostasis [81]. However, a larger study is clearly necessary to establish this and to generalize this finding to other antiplatelet regimes.

Summary

A bleeding tendency and an about twofold prolongation of bleeding time are frequently seen after regular aspirin intake, predominantly during continuous long-term use in cardiovascular prevention. Increased bleeding is no clinically relevant event in acute single-term use of aspirin, for example as antipyretic analgesic.

Aspirin-induced bleeding cannot be solely explained by inhibition of platelet thromboxane formation and does also not correlate with the antithrombotic efficacy of the compound in long-term cardiovascular prevention. Mechanistically, acetylation of clotting factors (fibrinogen, plasminogen, prothrombin) with subsequent inhibition of thrombin formation in addition to acetylation of COXs and reduced platelet-dependent thromboxane and thrombin formation are likely to be involved. Another, though less well-studied factor is enhanced endothelial NO formation after acetylation of eNOS.

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Increased aspirin-related bleeding is an important side effect in long-term vascular prophylaxis and has to be balanced against the individual benefits of thrombosis prevention. Aspirin increases the risk of periprocedural bleeding events in major surgical interventions by about 50 %. Current data suggest that this is an inconvenient but not a life-threatening issue. Any withdrawal of aspirin prior to surgery has to be balanced against an enhanced thrombotic risk, due to the proinflammatory and prothrombotic conditions of the operative procedure. Similarly, withdrawal of aspirin (and other antiplatelet agents) during long-term secondary prevention might induce rebound effects, that is, recurrent myocardial infarction or stroke. Actual guidelines (USPSTF) recommend aspirin for long-term primary prevention (if appropriate) only to medium-aged (40–59 years) individuals and those with low bleeding risk.

There is no specific antidote to antagonize an aspirin-induced bleeding disorder. Therefore, treatment or prevention of aspirin-induced bleeding events is symptomatic. Desmopressin is one option to prevent excessive bleeding events, tranexamic acid is an alternative. Infusions of aspirin-naïve platelets, competent of full thromboxane formation, in patients on antiplatelet treatment who need a rapid reversal of platelet dysfunction is an interesting experimental approach but probably not useful in cardiovascular surgery and other major surgical interventions with a considerably increased risk of acute thrombosis.

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3.1.3 Safety pharmacology in particular life situations

3.1.3.1 General aspects

Data on pharmacological properties of aspirin are frequently derived from healthy middle-aged individuals. They may not apply to all individuals, for example those in particular life situations. Pregnancy and older age belong to these particular life situations in which pharmacological actions of aspirin require particular attention. In early pregnancy, it is the possible interference of aspirin-mediated COX inhibition with the natural function of prostaglandins in fertility and embryogenesis. In late pregnancy, it is the transplacental passage of aspirin into the fetal circulation with possible consequences for fetal blood flow and maternal and fetal hemostasis.

A different though not less particular issue is the use of aspirin in people at older age (≥75 years). Biotransformations and renal excretion of salicylates may be reduced in these persons. This increases the systemic bioavailability of the active compound(s), eventually resulting in too high plasma levels and toxic side effects, such as bleeding events or tinnitus and other disturbances of the audiovestibular system (Section 3.2.4). Additionally, drug interactions have to be considered because of frequent comorbidities and multiple comedications. The elderly are also frequently long-term users of aspirin, for example as a preventive of atherothrombotic vascular events or incident cancer. This might be associated with an elevated incidence of (gastrointestinal) bleeding. The ASPREE study in the elderly has provided new information about the benefit/risk ratio of aspirin and has shown that people at older age might differ from younger ones, even when they are free from known cardiovascular diseases and physical or mental disabilities. However, a possibly increased mortality in the aspirin group of ASPREE should definitely be an issue of concern and requires further investigations [1].

3.1.3.2 Pregnancy and fetal development

Aspirin bears potentially positive (prevention of preterm preeclampsia, reduction of preterm delivery, possibly improved in vitro fertilization) and negative (enhanced bleeding risk) actions in pregnant women. Overall, aspirin is considered to be safe throughout pregnancy [2, 3], even in risk pregnancies, such as pregnancies at increased risk for preeclampsia [4]. Nevertheless, no drug should be used in this particular life situation without a clear therapeutic need that outweighs possible risks. Agents that interact with the prostaglandin system are here of particular concern, since prostaglandins are involved in every stage of pregnancy and fetal development.

Aspirin, NSAIDs and fertility. Normal implantation starts with release of the mature oocyte from the luteinized follicle. If the first step of this process, that is, rupture of the follicular wall, does not occur, the cycle remains unovulatory, eventually resulting in "luteinized unruptured follicle" (LUF) syndrome with possible consequences for

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female fertility. Mechanistically, rupture of the follicular wall is mediated by proteases that in turn are activated by prostaglandins. Consequently, inhibition of prostaglandin synthesis might prevent follicle rupture.

Inhibitors of prostaglandin biosynthesis, such as indomethacin and diclofenac, have been shown to inhibit ovulation and to delay follicle rupture, that is, to induce LUF [5–7]. The cycle remains unovulatory. Delay or prevention of follicle wall rupture was discussed as a form of nonhormonal oral contraception since steroid genesis is not affected by inhibition of prostaglandin synthesis [8]. Female mice with disturbed COX-2 expression have deficits in all components of early pregnancy and are infertile [9]. Whether this is also relevant for humans and (planned) pregnancies is uncertain [10].

There are no definite reports that indicate that aspirin negatively interacts with these processes. This might be due to the short plasma half-life of unmetabolized aspirin or the only transient inhibition of COX-2 in vivo, the dominating COX isoform involved in these processes. Alternatively, there might be pharmacokinetic reasons, such as the low lipophilicity of aspirin as opposed to NSAIDs or coxibs.

Another question is whether (OTC) analgesic and aspirin use at ovulation and implantation might influence human fertility.

According to a large prospective cohort study in more than 800 women (30–44 years of age), intake of paracetamol (dipyrone) or NSAIDs around the time of implantation did not influence the probability of conception (fecundability). In contrast, aspirin taken at the time of implantation was associated with considerably, about 2-fold, increased fecundability. This suggested the time point of implantation as a relevant target for aspirin actions on conception. Aspirin might increase fecundability regardless of a possible history of pregnancy loss [11].

It is desirable to know whether these findings can be confirmed in another prospective randomized trial. These results would be of considerable interest in birth control.

Teratogenicity. Animal studies have shown that aspirin may increase the risk of congenital abnormalities when given at high, toxic doses. It is, however, questionable whether these animal data can be transferred to humans [12, 13]. No increased rate of malformations was found in the large cohort study of the "Collaborative Perinatal Project" (CPP) including more than 44,000 pregnancies [14]. In 2,000 children with congenital heart malformations, there was no increased risk for cardiac malformations if the mothers had taken aspirin in early pregnancy [15]. No increased risk of malformations by aspirin was also seen in two smaller randomized trials [16, 17] and confirmed in a large metaanalysis on aspirin consumption in early pregnancy and the risk of teratogenicity [18]. The only malformation with a possible relation to aspirin was gastroschisis. However, its relation to aspirin use is uncertain [18, 19]. Overall, fetal exposure to aspirin taken during early pregnancy (first trimester) by the mother at

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therapeutic doses appears not to be associated with a higher risk of congenital abnormalities in otherwise healthy individuals [18].

Risk of miscarriage and abortions. Two large population-based cohort studies in Denmark and California have addressed the question for a relationship between use of NSAIDs and the risk of miscarriage and abortions. In the Danish study, prenatal use of NSAIDs was associated with an enhanced risk of miscarriage but appeared not to increase the risk of adverse birth outcome. The use of aspirin was not specified [20]. In the Californian study, the association of prenatal aspirin with risk of miscarriage was similar to that of other NSAIDs but generally lower and the estimates were considered unstable because of the small numbers of aspirin users [21].

The "Effects of Aspirin in Gestation and Reproduction" (EAGER) trial was designed to elucidate whether preconception-initiated daily low-dose aspirin would increase the live birth rate in women with one to two prior pregnancy losses and no infertility diagnosis and attempting unassisted conception.

Low-dose aspirin was associated with an increased live birth rate among the 1,088 randomized women who completed the study. When stratified by terciles of CRP levels, a biomarker of inflammation, treatment with aspirin restored a decrement in the live birth rate in women in the highest CRP tercile (RR: 1.35; 95 % CI: 1.08–1.67), increasing to similar rates as women of the lower and mid-CRP terciles.

The conclusion was that inflammation plays an important role in reproduction and that chronic, low-grade inflammation may be amenable to aspirin treatment [22].

There are only few randomized trials studying the role of aspirin in prevention of abortion. These failed to show a relationship between aspirin use and miscarriage rates [23–25]. There was also no significant difference in perinatal mortality or the rate of "small-for-gestational-age" infants. Whether aspirin really improves the outcome of pregnancy in women with recurrent abortions is controversial [26–33]. Women on aspirin had a significantly lower risk of preterm deliveries, possibly due to inhibition of prostaglandin synthesis (see below) [13]. There is no convincing evidence that aspirin – possibly in contrast to the more lipophilic NSAIDs – is a risk factor for mother or fetus in early pregnancy. It appears to be safe also in women with moderate- and high-risk pregnancies. The ASPRE trial has taken advantage of the safety of aspirin also in risk pregnancies and has shown that aspirin can reduce the risk of preterm preeclampsia in pregnancies at high risk for this disease (Section 4.1.5) [34].

A metaanalysis of selected randomized trials compared the efficacy of heparin plus aspirin versus aspirin alone in the prevention of unexplained recurrent spontaneous abortions in women at elevated risk for pregnancy loss.

From a total of 232 published studies from major databases, eight were selected which fulfilled the postulated high-standard inclusion criteria. These included women of childbearing age

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with at least two previous consecutive abortions. Women received either heparin plus aspirin (n = 493) or aspirin alone (n = 501). The primary outcome was the rate of live births.

The number of live births was significantly higher in the aspirin plus heparin group: 70.6 % vs. 55.9 % (OR: 2.09; 95 % CI: 1.29–3.40; P = 0.003). No differences in birth weight, premature delivery, congenital abnormalities or intrauterine growth retardation were observed. In addition, there was a beneficial tendency to prolong gestation in women taking aspirin only. Adverse effects were sporadically reported.

The conclusion was that among women with unexplained recurrent spontaneous abortion, heparin combined with aspirin increased the live birth rate as compared with aspirin alone [31].

Similar results, that is, an increased life birth rate and reduced pregnancy loss, were also reported in a recent Cochrane analysis on women with pregnancy loss because of antiphospholipid antibodies. Combined treatment with heparin plus aspirin in the course of pregnancy increased the life birth rate and was superior to treatment with aspirin alone [33].

According to a previous metaanalysis of 17 studies, no substantial effect was seen with aspirin on in vitro fertilization [35]. Thus, the data are controversial and more high-quality studies are required.

Ductus arteriosus Botalli. Vasodilatory prostaglandins contribute to the low vascular resistance in the fetal circulation [36] and probably also to the low incidence of thrombosis within the placental circulation (Section 4.1.5). In this respect, vasodilatory prostaglandins also play a key role in the maintenance of blood flow through the ductus arteriosus, i. e., blood supply to the fetus *in utero*.

Reversible, transient reductions of ductal blood flow have been reported for aspirin in early studies, however, at much higher doses than are used today [37]. Changes were fully reversible within 12 hours after removal of the substance. In contrast, lipophilic NSAIDs, such as indomethacin and ibuprofen, are effective vasoconstrictors and are being used for pharmacological closure of the ductus postpartum.

In a placebo-controlled double-blind trial, 43 pregnant women at risk for preeclampsia or intrauterine growth retardation received 100 mg/day aspirin or placebo. Doppler measurements of the uterine artery, several fetal arteries and the ductus arteriosus Botalli were performed at 2-week intervals from the 18th gestational week until delivery.

No differences in any of the parameters were seen between the aspirin and placebo groups.

The conclusion was that low-dose daily aspirin during the second and third trimesters of pregnancy does not alter uteroplacental or fetoplacental hemodynamics and does not cause detectable constriction of the ductus arteriosus Botalli [38].

Aspirin is not likely to cause functionally relevant reductions in ductal blood flow. This means that aspirin is also not effective as a pharmacological inducer of postnatal closure of a patent ductus, for example in preterm infants, as opposed to ibuprofen, which is currently the drug of choice for this indication [39].

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Maternal and fetal bleeding risk. Aspirin rapidly passes the placenta and enters the fetal circulation, approaching about 50 % of the plasma level in this postsystemic circulation [41, 42]. Aspirin in platelets significantly reduces thromboxane formation by the fetus and the neonate [42]. This effect is dose-dependent and might also be associated with reduced prostacyclin formation, in particular at higher aspirin doses [41].

Intake of low-dose aspirin (100 mg/day) in the last trimester of pregnancy will neither reduce the placental weight nor retard fetal growth and differentiation [43]. Similar results were obtained in an older cohort study, including more than 40,000 pregnancies. No relation was found between aspirin intake (high, medium, low) and the rates of stillbirth, perinatal mortality and mean birth weights [25]. Thus, aspirin appears to be safe also in advanced stages of pregnancy.

Platelets of neonates (umbilical cord blood) behave similarly to those of the mother with respect to adhesion, aggregation and granule secretion [44, 45]. Whether platelets from newborns are more susceptible to aspirin than those of the mother is unclear [42, 44, 46]. There is, however, no doubt with respect to the significantly enhanced bleeding risk of the newborn if the mother took aspirin shortly prior to labor. This bleeding risk might be markedly greater in premature newborns with immature drug clearance systems.

Ingestion of aspirin during the last week of pregnancy by the mother was associated with a remarkable risk of severe bleeding disorders in premature newborns (<34 weeks of gestation or body weight 1,500 g or less) associated with intracranial hemorrhage. Computer tomography showed that 53 out of 108 infants (49 %) exhibited intracranial hemorrhage within the first week postpartum. The incidence of hemorrhage in the infants whose mothers had ingested aspirin was significantly greater than in infants whose mother did not take either aspirin or paracetamol.

The conclusion was that the use of aspirin is associated with an increased risk of intracranial hemorrhage in premature newborn. Therefore, aspirin should not be taken within the last 3 months of pregnancy [47].

Despite formal criticisms on this study, mainly regarding the uncertainty about aspirin doses and the duration of use, similar results were also seen in a prospective case-control trial on full-term pregnancies [48]. Intake of aspirin by the mother should be avoided during late pregnancy, in particular shortly prior to delivery, because of the increased risk of bleeding.

3.1.3.3 The elderly patient

Elderly patients, at the age of ≥75 years, are frequently multimorbide and multidrug users – on average about six to seven different medicines per day. This allows for numerous drug interactions. Elderly patients also suffer frequently from chronic inflammatory/degenerative diseases of the musculoskeletal system (Section 4.2.2) which require regular intake of antiinflammatory agents that might interfere with the an-

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tiplatelet effects of aspirin (Section 4.2.2). In addition, the elderly are more prone to compliance problems and age-related changes in pharmacokinetics [49] as well as to increased gastrointestinal bleeding problems, for example because of more frequent *H. pylori* affections (Section 3.2.1). This requires particular attention in drug prescription, also because the consequences may be more dramatic in the elderly than in middle-age individuals.

Drug metabolism in the elderly. Age-related changes in pharmacokinetics [49] and in some cases also in pharmacodynamics of drugs are frequent. For example, restricted renal function (Section 3.2.3) and changes in hepatic clearance (Section 3.2.2) may influence the pharmacokinetics of long-term intake of aspirin (Section 2.1.2).

Typical early symptoms of (relative) aspirin or salicylate overdosing in the elderly are those of the CNS, such as bilateral hearing disorders or tinnitus (Section 3.2.4). Further disturbances involve dizziness, loss of motoric speech control, hallucinations, and changes in mood [50].

The generation of protective prostaglandins (PGE_2) inside the stomach mucosa is age-dependent and is reduced by more than 50 % in the elderly. The reduced prostaglandin level in stomach juice is probably related to the doubling in basal acid output in the elderly population [51, 52]. This will reduce the resistance of the stomach mucosa against all kinds of noxious stimuli, including aspirin. Together with a higher rate of *H. pylori* infections in the elderly this might also contribute to the higher rate of peptic ulcers which are frequently asymptomatic [53].

Cerebral hemorrhages. Antiplatelet agents including aspirin enhance the risk of intracranial hemorrhages in the elderly subsequent to traumatic head injury. Whether this is also correlated with enhanced mortality is controversial but certainly unwanted [54, 55]. Although this finding is not totally surprising, the elderly should be made aware of this increased risk by their physicians. In general, the individual risk status, including the risk of major bleeding events and risk of cardiovascular problems, should be known for decision making [56].

Clinical trials – ASPREE. The first large randomized, placebo-controlled prospective study on primary prevention with aspirin in persons at advanced age (median: 74.9 years) was the Australasian/US-American ASPREE trial (see also Sections 4.1.1 and 4.3.1) [1, 57, 58]. This trial and a number of subgroup analyses provide today the standard reference for long-term aspirin use in the elderly *without* known cardiovascular disease, dementia, physical disability or elevated bleeding risk.

A total of 19,114 persons aged 70 years or older (or ≥65 years among African-American and Hispanics in the US) were enrolled and randomized to receive 100 mg/day enteric-coated aspirin or

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placebo for 5 years. The primary endpoint was a composite of death, dementia or persistent physical disability and mortality. Secondary endpoints included major hemorrhage and cardiovascular disease (myocardial infarction, stroke and hospitalization for heart failure).

After a median follow-up of 4.7 years the trial was stopped prematurely, because, according to an interim analysis, it appeared to be unlikely to reveal a significant treatment effect of aspirin on the primary endpoint. At this time, the rate of the composite primary endpoint was 21.5 events per 1,000 person-years in the aspirin group and 21.2 in the placebo group (HR: 1.01; 95 % CI: 0.92–1.11; $P = 0.79$). The rate of cardiovascular events was 10.7 per 1000 patient-years in the aspirin group and 11.3 in the placebo group (HR: 0.95, 95 % CI: 0.83–1.08). All-cause mortality was 12.7 events per 1000 patient-years in the aspirin group and 11.1 events per 1000 person-years in the placebo group (HR: 1.14; 95 % CI: 1.01–1.299). Interestingly, cancer was the major contributor to the higher mortality in the aspirin group. The rate of major hemorrhages was 8.6 and 6.2 events per 1000 person-years in the aspirin and placebo groups, respectively (HR: 1.38; 95 % CI: 1.18–1.62; $P < 0.001$). The adherence to study medications during the last year of the trial was 62 % and 64 %, respectively.

The conclusion was that low-dose aspirin as a primary prevention strategy caused a significantly higher risk of major hemorrhage and all-cause mortality but did not result in a significantly reduced risk of cardiovascular diseases. The increase in mortality appeared to be due to an unexpected higher rate of cancer-related deaths and should be interpreted with caution [1, 57, 58].

These data suggest that aspirin intake, started by the elderly at the age of around 70 years or more, is not associated with any improved cardiovascular or mental outcome but an increase in severe bleeding events and increased mortality. These data are important new findings that deserve documentation but also critical discussion. In this context, several limitations of the trial have to be considered.

The trial was finished prematurely because of an expected negative outcome. This was mainly due a much smaller risk of 0.78 % and 0.88 % for the aspirin and placebo groups, respectively. Instead of the calculated 2.24 % cardiovascular events, there were only 1.13 % and 1.07 % in the two treatment groups. According to the investigators, this low event rate was due to the generally good health status of the participants without any known increased cardiovascular risk despite a mean age of 74.9 years at the beginning of the study. The short duration of 4.7 years and the fact that the majority of participants (89 %) never had used aspirin regularly before enrollment to the study should also be considered. A total of 34 % of participants received statins at the beginning of the study but only 25 % of participants received a PPI, which might have affected the gastrointestinal bleeding risk. The compliance rate of about 60 % in both groups was not high. Finally, the increased total mortality in the aspirin group was primarily due to an increased rate of cancers: 3.1 % gastrointestinal cancer in the aspirin group vs. 2.3 % gastrointestinal cancer in the placebo group (HR: 1.31; 95 % CI: 1.10–1.56). There was a trend toward increased all-cause mortality (HR: 1.14; 95 % CI: 1.01–1.29) that was driven by cancer death. A more detailed, later analysis of the effect of aspirin on cancer incidence in the ASPREE trial did not find an increase in overall cancer incidence (HR: 1.04; 95 % CI: 0.95–1.14) and incidence of CRC (HR: 1.02; 95 % CI: 0.81–1.30) [59]. Despite these criticisms, the finding remains that aspirin had

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apparently no beneficial actions in elderly persons but rather promoted tumor growth and spread with a trend for increased overall mortality [59].

Similar results were obtained in the nonrandomized NHS/HPFS study, where initiating aspirin use at or after 70 years of age did not reduce the risk of CRC [60], and also in the randomized "Japanese Primary Prevention Project" (JPPP). This trial studied primary prevention with low-dose aspirin in 14,466 elderly Japanese patients (60–85 years) with atherosclerotic risk factors. Aspirin (100 mg/day enteric-coated vs. no aspirin) did not improve the risk of vascular events in the total patient group but increased the number of severe bleeding events (cerebral, gastrointestinal). The authors concluded that aspirin for prophylactic purposes in these elderly subjects should only be given with caution or not recommended because of the risk of severe bleeding events [61].

Interestingly, similar conclusions regarding an enhanced (bleeding) risk in the elderly was already reached by Craven, the most creative person among the inventors of aspirin prophylaxis of myocardial infarction and stroke. He reportedly might not have taken aspirin himself above the age of 70 because of a poor risk/benefit ratio (bleeding!) [62] – however, unfortunately he died from myocardial infarction only a few years later at the age of 74 years (Section 1.1.4).

Summary

Pregnancy and older age are two particular life situations with peculiarities regarding the efficacy and safety of aspirin. In pregnancy, possible actions of the drug on the mother and fetus have to be considered. In older age, there are general alterations in pharmacokinetics, possibly also associated with altered pharmacodynamics because of frequent comorbidities and polypharmacy.

Most available clinical data suggest that aspirin, in contrast to traditional NSAIDs or coxibs, does not interfere with ovulation and early or later stages of pregnancy. It rather might improve fecundability. Although aspirin passes the placental barrier and enters the fetal circulation, there is no conclusive evidence that aspirin causes malformations or growth retardation of the fetus. There is also no evidence that aspirin increases the rate of miscarriages. Overall, low-dose aspirin seems to be safe throughout pregnancy. This is one precaution to consider aspirin for prevention of preterm preeclampsia in high-risk pregnancies (Section 4.1.5). Whether aspirin as a single medication or in combination with heparin significantly improves the efficacy of in vitro fertilization is under discussion.

Intake of aspirin shortly before delivery is associated with a significant bleeding risk not only for the mother, but also for the fetus and newborn and should be avoided. There is no conclusive evidence that aspirin at therapeutic doses causes functionally relevant constriction of the ductus arteriosus Botalli. Because of bleeding risks, aspirin should not be used in late pregnancy or shortly before delivery.

Aspirin in the elderly is frequently used in combination with other drugs. In the elderly, drug clearance may be retarded, eventually resulting in toxic symptoms, specifically at long-term use for prevention. At conventional antiplatelet doses, the risk of aspirin overdosing is low, although the risk of side effects, mostly symptoms in the CNS, such as tinnitus, is higher. Prophylactic use of aspirin for long-term prevention in the elderly (>70 years) might cause more problems than benefits. It requires a critical, individual decision, specifically with respect to the risk of severe bleeding events, and it possibly promotes cancer incidence and mortality (Section 4.3.1).

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3.2 Organ toxicity

Systemic manifestations of salicylate overdosing or intoxications result from disturbed energy supply and ionic homeostasis. These finally will result in collapse of (energy-dependent) cellular signaling systems and cell death because of metabolic failure (Section 3.1.1). The severity of symptoms is critically determined by the plasmatic salicylate levels, while the functional consequences for the individual organs rather depend on the local tissue level and the individual sensitivity of the respective organ or tissues against salicylates. For example, hair cells from the cochlea or other neuronal cells behave differently from hepatocytes, kidney tubular cells or cells of the stomach mucosa, although the cause of salicylate-induced dysfunction – metabolic failure – is essentially the same.

Organs of particular interest are the gastrointestinal tract (Section 3.2.1), being directly exposed to the full aspirin dose after oral intake, the liver (Section 3.2.2) and the kidney (Section 3.2.3) as the two major organs of drug metabolism and excretion, respectively. The audiovestibular system of the inner ear is a unique example for disturbed reception and transmission of neuronal signals (Section 3.2.4). With the exception of the hearing cells in the cochlea, where the negatively charged salicylate anion competes with prestin for binding to chloride channels, organ- and tissue-specific toxicity is largely determined by the protonized compound, that is, the free, nondissociated salicylic acid, and in most cases is fully reversible after drug removal.

3.2.1 Gastrointestinal tract

3.2.1.1 General aspects

The stomach is clearly in primary focus of aspirin-related side effects. However, it is important to distinguish between subjective symptoms of gastric intolerance, such as dyspepsia, nausea and vomiting, and the – more important – objective changes, such as gastric erosions, peptic ulcers and overt bleeding.

A piece of history. One major historical reason to chemically process salicylate by acetylation was to improve gastrointestinal tolerance. This was based on the concept that salicylate is the gastric mucosal irritant but not the uncleaved acetylsalicylic acid (Section 1.1.2). Initially, the producer advertised aspirin as a replacement (prodrug) for salicylate which did not contain free salicylate (see the excellent review of McTavish for more historical details [1]). It took about 40 years before the first gastroscopic study showed that oral aspirin at an analgesic dose (975 mg) caused gastric mucosal injury and local bleeding events. This was explained as a consequence of the direct contact of the compound with the stomach mucosa [2]. It took another 20 years before a controlled trial in 180 individuals convincingly demonstrated that aspirin at 0.75

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to 3.0 g/day markedly increased the appearance of "occult" blood in stool, indicating aspirin-induced microbleeding events inside the gastrointestinal tract [3]. Meanwhile, it is general knowledge that aspirin can cause irritations of the upper gastrointestinal tract, most notably of the gastric mucosa, bleeding and ulcers, the latter two being important, dose-related side effects of aspirin intake.

Occurrence of gastrointestinal injuries. The stomach is the primarily affected organ, but also the distal esophagus in case of gastric acid reflux [4, 5]. Aspirin might also, although to a lesser extent, affect the duodenal mucosa and cause or perpetuate duodenal ulcers [6, 7]. The lower toxicity in the intestine is possibly due to the improved solubility of aspirin with increasing pH in the duodenum and upper small intestine [8]. The overall relative risk of serious upper gastrointestinal complications associated with aspirin exposure was increased 2–3-fold as compared to aspirin nonusers in a review of 17 epidemiological studies [9].

Symptoms. Typical early aspirin-induced signs of stomach mucosal injury are superficial erosions. Their incidence is approximately doubled by regular daily intake of antiplatelet doses (75–160 mg) of plain aspirin [10, 11]. More severe and serious but less frequent complications are *p*erforations, *u*lcers and *b*leeding (PUBs). In addition to but independent of these objective changes are subjective symptoms of gastric intolerance, mainly after oral intake of plain, undissolved standard aspirin. These include dyspepsia, nausea and "heartburn." Side effects of this kind are observed in about 5–25 % of aspirin users and are important determinants for patient adherence to drug treatment. However, they do not necessarily indicate an endoscopically visible gastric mucosal injury [7, 12]. As many as 50 % of patients with dyspepsia exhibit a normal-appearing gastric mucosa while up to 40 % of individuals with endoscopic evidence of erosive gastritis are asymptomatic [13]. Consequently, dyspepsia and "heartburn" do not correlate with fecal blood loss due to microbleeding events in the gastrointestinal tract (see below) [14].

3.2.1.2 Pathophysiology of gastrointestinal (stomach) injury

Gastric mucosal barrier. The healthy stomach mucosa is resistant to the high concentrations of HCl and pepsin in gastric juice. This resistance is due to a "mucosal barrier" [15] which maintains a unique proton gradient of about 100,000:1 between the gastric lumen and gastric mucosal cells (Fig. 2.1.1-1). This barrier prevents back-diffusion of protons into mucosal cells and subsequent autodigestion of the stomach wall by pepsin. Any (sub)cellular gastric mucosal injury can only occur after the mucosal barrier does not function normally and/or has been physically disrupted.

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Mucosal injury subsequent to disturbed barrier function. The term "mucosal barrier" describes functional properties of the borderline membranes between the gastric lumen and mucosal cells. It cannot be ascribed to one particular, anatomically defined structure. However, hydrogen carbonate-containing gel-mucus and epithelial phospholipids are likely constituents [16]. Another determinant is mucosal blood flow [17]. Aspirin, salicylate or other lipophilic agents, such as bile acids or ethanol, but also mechanical and thermic stimulation, associated with food intake, may disturb this barrier function and allow for penetration (back-diffusion) of protons into the mucosal cells with the consequence of intracellular acidosis [18]. This process can be quantified by measuring the gastric transmucosal potential difference. Changes (reductions) of this gradient by aspirin are transient, concentration-dependent and probably caused by the protonophoric properties of salicylate (Section 2.2.3) [19]. After ingestion of one 600-mg single dose of predissolved plain aspirin, maximum decreases in gastric transmucosal potentials are obtained at about 10 min, and recovery starts within 30–60 min and is complete within 6 h [20].

Longer lasting or more intense irritations of the stomach mucosa will result in morphologically detectable defects of the mucosal epithelium that require repair processes. Restitution of the surface epithelium starts with migration of epithelial cells from gastric pits to cover the area of damage, later followed by cell division for complete coverage. There is no correlation between the severity of mucosal injury and the reduction of the potential difference, i. e., the total amount of rediffused protons [7, 17]. It has been speculated that aspirin-induced changes of the transmucosal potential difference probably reflect damage to the oxyntic glands and not the breaking of the surface and pit cell mucosal barrier [21]. Thus, not all cells in the stomach mucosa might be affected by aspirin to the same extent.

Adaption and repair of gastric mucosal injuries. Gastric mucosa has the unique property to become tolerant against noxious stimuli of any kind after repeated or continuous challenging. This long-known phenomenon of "adaption" has been ascribed to morphological alterations in the gastric mucosal epithelium. This, eventually, results from the emergence of a new cell population with an increased rate of cell turnover and replacement and/or greater resistance to noxious stimuli, including aspirin [7, 22, 23]. In human, adaption, that is, resolution of mucosal injury by cell regeneration, starts within one week of continuous aspirin exposure and presents clinically as (chronic) gastritis. The process is stimulated by enhanced generation of growth factors, such as TGFβ [24, 25]. The stomach mucosa appears then morphologically normal [24, 26]. These events appear not to require prostaglandins [23, 27] but are accelerated in their presence [28]. This suggests that inhibition of (mucosal) prostaglandin biosynthesis per se does not cause mucosal injury but can amplify it, in particular by retarding the healing process of reepithelization.

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Prostaglandins and cyclooxygenases. Prostaglandins can modulate virtually every aspect of mucosal defense and repair. Therefore, insufficient stimulation of their biosynthesis in response to irritation or injury by prostaglandin synthesis inhibitors, such as NSAIDs or aspirin [16], is the most popular explanation for gastrointestinal-related side effects of these compounds. Since the first description by *André Robert* [29] and further work of his group, prostaglandins are endogenous protective factors made inside the stomach mucosa. PGE_2 appears to be the most relevant compound and has been found to protect the stomach mucosa not only by its still somehow mysterious "cytoprotective" action, but also by inhibition of acid secretion, enhanced secretion of bicarbonate and mucus and improved mucosal blood perfusion [30, 31]. Much effort has been made to elucidate the mode of prostaglandin-related "cytoprotection," not only seen in the stomach, but also in other organs, with a synthetic PGE analog, misoprostol [32]. The detection of two genetically different COX isoforms with different regulation and localization as well as the identification of about 10 prostaglandin receptors with different distributions and functions within organs, including the stomach, has generated new concepts of "cytoprotection" and has also extended the conventional concept that aspirin-induced gastric injury is mainly or even solely due to inhibition of prostaglandin production.

Originally, it was assumed that COX-1 is the only COX isoform expressed constitutively within the stomach mucosa. Later studies identified constitutive COX-2 mRNA expression in the stomach wall mucosa [33, 34] as well as its translation into COX-2 protein even under "resting" conditions [35]. In the human stomach, COX-2 is constitutively expressed mainly in the mucosa of the antrum, and there in the muscularis mucosae and mucosal endothelial cells (Fig. 3.2.1-1).

COX-2 expression in the gastric mucosa becomes upregulated not only by physiological stimuli (mechanical, thermic, osmotic, chemical) associated with food uptake, but also by chemical mediators such as gastrin or inflammatory cytokines (TNFα) [38]. Together with a differentially expressed and regulated COX-1 [39], the synthesized prostaglandins will improve the natural resistance of the stomach mucosa. Severe mucosal injuries because of insufficient prostaglandin formation probably require (complete) inhibition of both isoforms [40]. Similar results have been obtained in genetically modified animals (mice) (see below) [41, 42].

Prostaglandin production in the stomach is age-dependent and is reduced by more than 50 % in the elderly. This reduced prostaglandin formation is probably related to the doubling in basal acid output in the elderly population [43, 44] and will reduce the resistance of stomach mucosa against environmental stimuli [45, 46].

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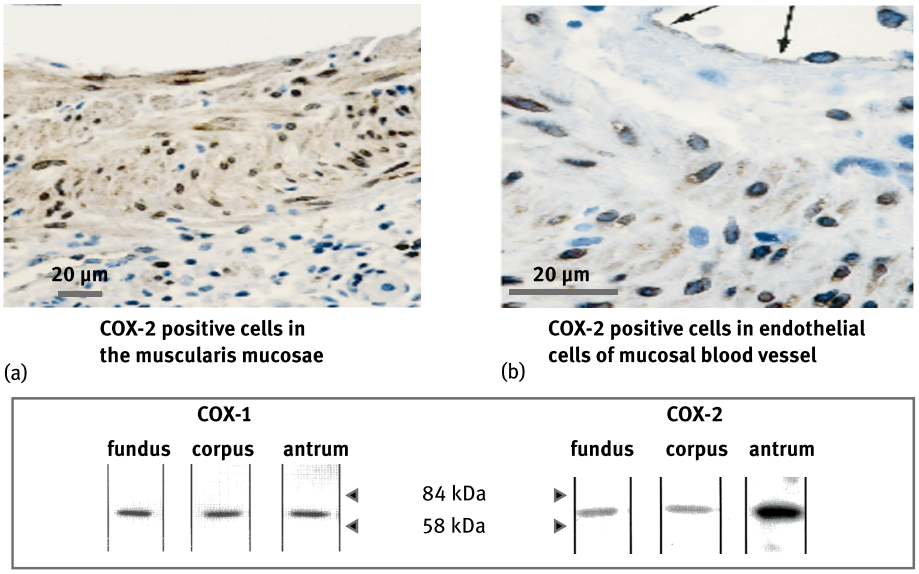

Figure 3.2.1-1: Immunohistochemical localization of COX-2 in human gastric mucosa. COX-2 positive cells (brown) were detected in the mucosal layer, muscularis mucosae (a) and endothelium of mucosal blood vessels (b) (arrow). COX-1 and COX-2 protein were identified by Western blot in homogenates of different sections of the stomach mucosa with highest expression of COX-2 in the antrum (c) (modified after [36, 37] – photos with kind permission of the American Society for Pharmacology and Experimental Therapeutics).

3.2.1.3 Modes of aspirin action

General aspects. Pharmacological actions of aspirin in the gastrointestinal tract, most notably the stomach, involve different mechanisms: (i) direct contact of the agent (salicylate) with the stomach mucosa and subsequent penetration (sequestration) into the mucosa cells and (ii) inhibition of prostaglandin biosynthesis. Another, although less well understood issue is the specific function (inhibition/modification) of COX-2, possibly associated with generation of ATL. Aspirin-induced bleeding events present as (frequent) microbleeding as well as (dangerous) serious bleeding events from peptic ulcers. An overview of these mechanisms is shown in Fig. 3.2.1-2.

Mucosal injury by physical contact and penetration of salicylate into mucosal cells. Intake of oral plain standard aspirin may cause endoscopically visible acute gastric mucosal injury, presenting as mucosal and submucosal hemorrhages (petechiae). These injuries are seen within 1 h after drug ingestion. Probably, salicylate is the main determinant of this gastric injury by aspirin. One reason is its long half-life in stomach juice due to the poor solubility at acidic pH (Fig. 1.2.1-3) [47], another the unique ability of salicylate to accumulate inside cell membranes to destabilize the

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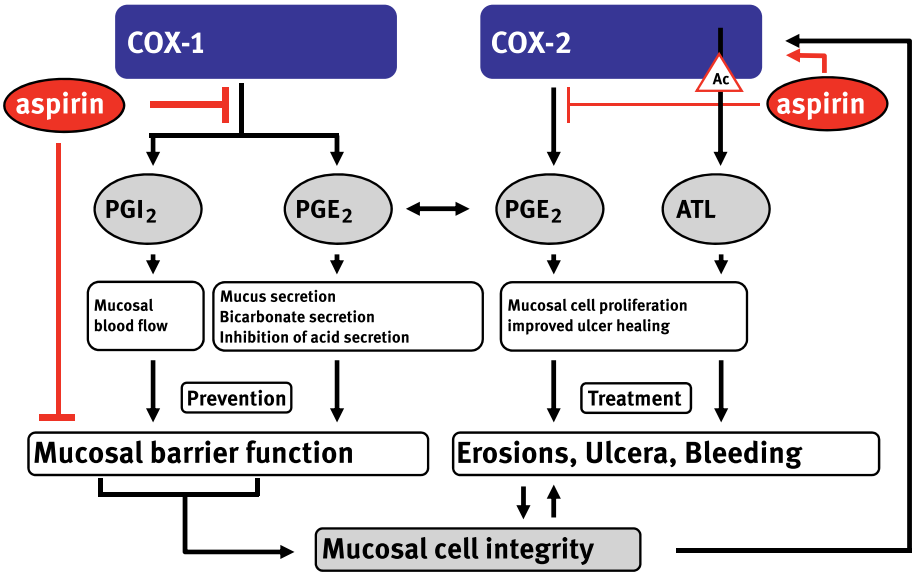

Figure 3.2.1-2: Actions of aspirin, prostaglandins and "aspirin-triggered lipoxin" (ATL) on gastric mucosal cells (for further explanation see text).

proton gradient of mitochondria and prevent ATP storage (Section 2.2.3, Fig. 2.2.3-3). As a consequence, aspirin-induced gastric injury is more pronounced at acidic pH (fasting) and can be reduced if the compound is applied in galenic formulations that either avoid or shorten direct contacts of "naked" aspirin with the stomach wall and/or facilitate gastric emptying (Section 2.1.1). Mucosal injury can also be reduced by increasing surface hydrophobicity by administration of exogenous phospholipids [48], which are natural constituents of the mucosal barrier [16]. Based upon this concept, Phospholipid-Aspirin (PL-ASA) was developed (Section 2.1.1), a novel pharmaceutical formulation of a lipid–aspirin complex with pharmacokinetic and -dynamic properties similar to those of plain aspirin [49] but a reduced acute gastric mucosal lesion potential [50].

These data collectively suggest that aspirin and/or salicylate cause mucosal injury mainly by direct physicochemical actions that disrupt the mucosal barrier function [19, 51]. This is confirmed by experimental and clinical data. In animal studies, aspirin at doses that inhibit prostaglandin production of stomach mucosa by about 95 % only caused gastric injury if given intragastrically but not if given parenterally [52]. In human, gastric mucosal injury was reduced with increasing gastric pH, that is, improved gastric solubility, and apparently absent at alkaline pH [53].

Inhibition of prostaglandin synthesis. Originally it was thought that inhibition of prostaglandin biosynthesis by aspirin offers a simple and logical explanation to un-

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derstand the gastric side effects of the compound. After detection of COX-2, this hypothesis was modified to a more or less selective inhibition of COX-1 by aspirin that removes gastroprotective prostaglandins and in addition causes gastrointestinal bleeding which is facilitated by its antiplatelet actions (Section 3.1.2). Experimental studies in wild-type and COX knockout animals have now clearly shown that it may be too simple to refer the complex actions of aspirin – and other NSAIDs and gastric irritants on the stomach mucosa – solely to inhibition of COX-1-mediated prostaglandin synthesis. Most convincing is the fact that COX-1 knockout mice are *less* and not *more* prone to inflammation and stomach ulcers, although the remaining PGE_2 levels in the gastric mucosa were reduced to about 1 % of that in wild-type animals [54].

On the other hand, there is no doubt that aspirin inhibits prostaglandin synthesis (PGI_2, PGE_2) in the stomach mucosa with subsequently reduced mucosal defense, impaired cell renewal (PGE_2) and reduced mucosal blood flow. However, clinical studies have unequivocally shown that inhibition of gastric PGE_2 formation by aspirin in vivo is incomplete [6] and not really dose-dependent. Even 2.6 g aspirin per day for 1 week reduced PGE_2 generation by only about 50 % while inhibition of platelet-dependent thromboxane formation in the same study was complete already at regular daily intake of 30 mg (Fig. 3.2.1-3) [6, 55].

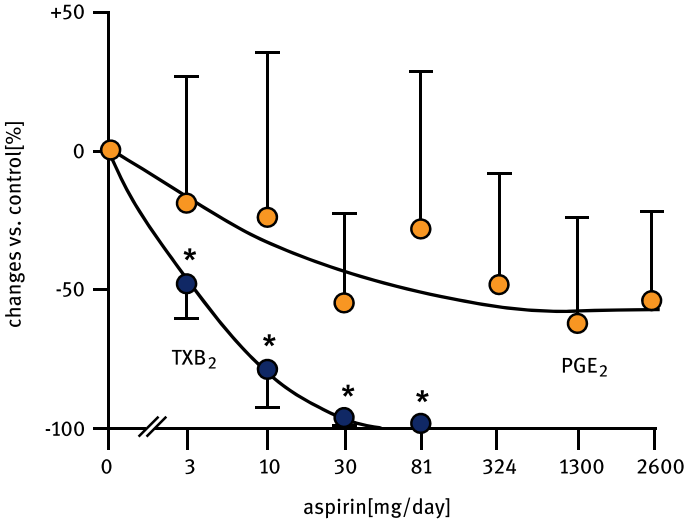

Figure 3.2.1-3: Serum levels of thromboxane (TXB_2) and gastric juice PGE_2 levels after oral intake of aspirin. Healthy subjects received aspirin at the doses indicated or placebo in a double-blind crossover design, each for one week, followed by a two-week washout. The 50 % inhibition (ID_{50}) for reduction of thromboxane formation by aspirin was 3 mg/day. Inhibition was complete at 30 mg/day. The ID_{50} for reduction of PGE_2 formation was about 30 mg/day. Inhibition did not become stronger with increasing doses. *$P < 0.05$ (PGE_2 vs. TXB_2 at the same aspirin dose) (modified after [55]).

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Recovery of gastric prostaglandin biosynthesis after cessation of aspirin at antiplatelet doses (81 mg/day) was linear and apparently complete at 5 days. Fifty percent recovery was already seen at 2 days after withdrawal. At this time platelet-dependent thromboxane formation was still completely blocked (Fig. 2.3.1-5) [56]. These data suggest that inhibition of PG(E)-synthesis in gastric mucosal cells by aspirin, as opposed to inhibition of thromboxane formation by platelets, is incomplete, and rapidly reversible. Possible explanations are (i) prostaglandin (PGE_2) production mainly via COX-2 with a rapid protein turnover rate as opposed to COX-1-mediated inhibition of platelet thromboxane formation, (ii) less effective and short-lasting aspirin action on COX-2 in gastric mucosal in vivo. In any case, these data suggest that depletion of gastric prostaglandin synthesis by aspirin is not the only cause of gastric mucosal injury but rather an aggravating factor which reduces resistance and delays mucosal recovery from injury. In consequence, the appearance of chronic ulcers subsequent to aspirin, NSAIDs and other noxious stimuli might primarily reflect a focal failure of adaption, that is, disturbed healing processes, rather than a direct effect on tissue integrity. It is also likely that COX-1- and COX-2-derived PGE_2 serves different purposes, and the action of generated PGE_2 on mucosal cells depends on the local distribution of the prostaglandin (EP1–4) receptors [57].

Aspirin and COX-2. In the presence of aspirin, the enzymatic activity of COX-2 is converted into a 15-lipoxygenase that generates 15-(R)-HETE as a major product in COX-2-bearing cells, including the human stomach mucosa [35]. 15-(R)-HETE could then be utilized by white cell-derived 5-lipoxygenase as a precursor to synthesize ATL (Sections 2.2.1 and 2.3.2) (Fig. 2.2.1-6) [58]. Aspirin, despite (partial) inhibition of prostaglandin formation, will trigger the generation of the antiinflammatory gastroprotective ATL via an intercellular interaction with white cells (Fig. 2.3.2-6) [59, 60]. Gastric endothelial cells are a rich source of COX-2 in the human stomach mucosa (Fig. 3.2.1-1) and may be the site where these reactions mainly occur [35–37, 58, 61]. Thus, there is a complex interaction between aspirin, (inducible) COX-2, COX-1 and direct irritation of the stomach mucosa.

An elegant technology to evaluate the relative contributions of COX-1 and COX-2 to mucosal cell integrity in the stomach is the use of gene-manipulated animals. These studies nicely demonstrated the complexity of beneficial and deleterious actions of aspirin actions on the stomach mucosa [41].

Deletion of the COX-1 gene in mice did not cause spontaneous gastric ulcerations [54]. However, in COX-1-deleted animals, the gastric mucosa became more severely injured by HCl, both as single application and in the presence of a high concentration (20 mM) of intragastric aspirin. No such changes were seen in wild-type and COX-2 knockout mice, respectively [41].

The mucosal injury by aspirin in COX-1 knockout mice could be avoided by using phosphatidylcholine-bound aspirin instead of the plain compound [62]. This suggested that appropriate “coat-

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ing" of aspirin will reduce or avoid mucosal contacts of the compound and prevent subsequent mucosal injury.

Intragastric application of the aspirin/HCl combination induced a 4–6-fold increase in gastric mucosal PGE_2 levels in COX-1 knockout animals as opposed to saline- or HCl-treated controls. This was explained by an aspirin-induced transcriptional upregulation of the COX-2 gene. In contrast, PGE_2 levels in wild-type and COX-2 knockout mice were reduced. The gastric lesion score appeared to be directly related to alterations in mucosal surface hydrophobicity by HCl but not to mucosal PGE_2 levels.

The conclusion was that aspirin causes gastric injury predominantly by prostaglandin-independent mechanisms, such as an attenuation of mucosal surface hydrophobicity after local contact. However, COX-1 may play a permissive role in maintaining gastric mucosal barrier integrity via PGE_2 formation. Independent of this, aspirin can increase gastric mucosal PGE_2 levels even in the absence of COX-1, possibly by transcriptional upregulation of COX-2 (Fig. 3.2.1-4) [41].

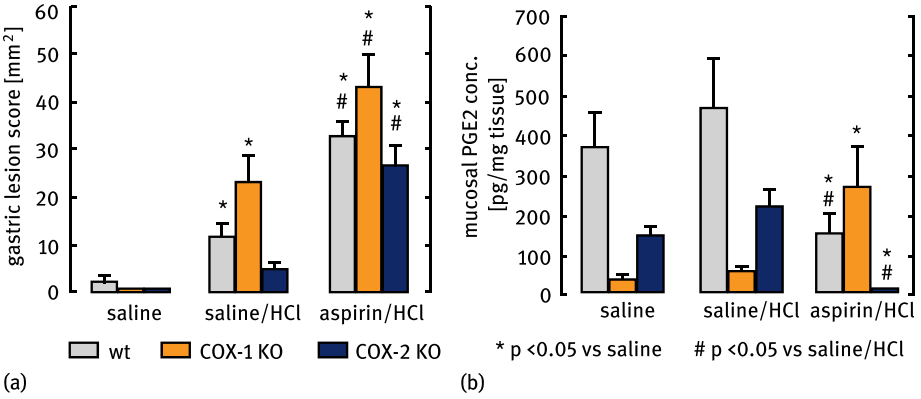

Figure 3.2.1-4: (a) Gastric lesion score and (b) gastric mucosal PGE_2 biosynthesis in wild-type (wt) as compared to COX-1 and COX-2 knockout (KO) mice. Animals were treated orally with saline, diluted HCl or aspirin (20 mM) + HCl. *$P < 0.05$ vs. NaCl; #$P < 0.05$ vs. NaCl/HCl (for further explanations see text) (modified after [41]).

Upregulation of COX-2-dependent prostaglandin production and enhanced generation of ATL and (maintained) formation of prostaglandins (PGE_2) is the consequence of enhanced "need" under conditions of tissue injury. Inhibition of COX-2 under these conditions might aggravate the injury and retard the healing process. This has also been shown for combined treatment with aspirin and NSAIDs or coxibs in both experimental studies and clinical trials [61, 63, 64].

Aspirin and gastric mucosal blood flow. Another aspirin-sensitive variable that determines gastric mucosal integrity is mucosal blood flow. As in other organs, gastric mucosal blood perfusion is mainly regulated by NO, synthesized by eNOS. Gastric adaption to aspirin in human is associated with enhanced expression of mucosal eNOS

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within a few days and a subsequent increase in mucosal blood flow despite reduced prostaglandin formation [65]. ATL has been shown to induce NO formation and to improve oxygen defense in endothelial cells (Section 2.3.2) [66]. This might explain the reduced gastric injury by NO-releasing aspirin formulations as opposed to standard plain aspirin [67]. Although the clinical benefits of "nitro-aspirin" are not established yet, there is clinical evidence that nitrovasodilators, such as organic nitrates, may reduce upper gastrointestinal bleeding in high-risk patients to a similar extent as PPIs [68].

Aspirin and gastrointestinal microbleeding events. A frequent but uneventful sign of disturbed mucosal integrity by aspirin are gastrointestinal microbleeding events, eventually appearing as "occult" blood in the feces [69]. The average, spontaneous gastrointestinal blood loss with the feces amounts to about 1 ml/day and is increased to about 2–6 ml/day by aspirin with marked interindividual variations [7, 69, 70]. The aspirin-induced blood loss is in the same range as the natural blood loss during menstruation and is not accompanied by any complaints [3]. There is no direct correlation between aspirin-induced gastrointestinal microbleeding events and the prolongation of skin bleeding time (Section 3.1.2) [14] or morphologically visible gastric mucosal injury [71]. Though microbleeding events should be facilitated by inhibition of platelet function, their intensity rather correlates with the pH of gastric juice, that is, local aspirin effects on the stomach mucosa, than with its antiplatelet action [72]. In agreement with this, there is significantly less gastrointestinal bleeding after parenteral (intravenous) administration of aspirin [52, 73]. Aspirin-induced gastric microbleeding events are local events, requiring the physical presence of aspirin/salicylate, but without any direct relation to bleeding time or antiplatelet effects.

Aspirin and major gastrointestinal bleeding events. Increased gastrointestinal microbleeding events are more or less regular events. In contrast, in very few cases aspirin may also cause overt, severe bleeding events, for example from perforated gastric ulcers (PUBs). A metaanalysis of 35 randomized aspirin trials estimated the risks for a major gastrointestinal bleeding at 1–2 events per 1,000 person-years [74]. These are serious, life-threatening side effects of aspirin and possibly involve a combination of at least two mechanisms: (i) local mucosal injury with ulcer formation due to retarded or insufficient tissue repair during the gastric mucosal adaption process and (ii) inhibition of blood clotting by the antiplatelet effects of the compound. These processes will be aggravated by inhibition of gastroprotective prostaglandin formation and perhaps by further factors, such as older age, *H. pylori* infections, alcohol [75], comorbidities and comedications such as NSAIDs [76]. In the elderly (>70 years) there is about 50 % reduced mucosal PGE_2 biosynthesis (mucosal biopsies), as well as a significant about 2-fold increase in basal gastric acid production [43, 44]. These changes among oth-

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ers might contribute to the increased bleeding tendency in the elderly, as shown for example in the ASPREE trial [77, 78].

Helicobacter pylori. A significant proportion of patients with gastrointestinal pathologies, including peptic ulcers, are infected with *H. pylori*. The proportion is age-dependent and is increased to up to 80 % at the age of 80 years. NSAIDs, aspirin and *H. pylori* are considered independent risk factors for gastrointestinal ulcer formation and bleeding and act synergistically [76, 79]. *H. pylori* eradication reduces aspirin-induced gastric injury and ulcer recurrence. Eradication was also shown to be equieffective to comedication of a PPI in patients with previous gastrointestinal bleeding who required long-term treatment with low-dose aspirin for cardiocoronary prevention [80]. Mechanistically, it is thought that *H. pylori* interacts with HSP70, which is involved in adaptive reactions of stomach mucosa to aspirin [81]. Any potential harmful effects of PPIs on gastric cancer development appears to be limited to nonaspirin users [82]. Coprescription of PPIs is, therefore, recommended for *H. pylori*-eradicated patients who are at risk of aspirin-induced upper gastrointestinal bleeding events.

Aspirin can also reduce the risk of gastrointestinal cancer in H. pylori-eradicated subjects (HR: 0.30; 95 % CI: 0.15–0.61). This effect was related to frequency, dose and duration of aspirin intake after eradication and most prominent in subjects who used aspirin daily or for five or more years [83].

This suggests that eradication of *H. pylori* might be a useful preventive measure in all positive patients to improve gastric tolerance to aspirin users, in particular during long-term administration [46]. Long-term use of aspirin in *H. pylori*-eradicated persons might be associated with the risk of gastric cancer [83].

3.2.1.4 Clinical studies

General aspects. Gastrointestinal side effects of aspirin limit its clinical use, both short-term use for pain relief and long-term use in cardiocoronary prophylaxis. PUBs in the gastrointestinal tract are serious clinical complications and, together with hemorrhages in the CNS, the most dangerous side effect of aspirin, in particular in regular, long-term use of aspirin for cardiovascular protection. This is particularly true for elderly patients with age-related disturbances of upper gastrointestinal function. Thus, an individual benefit/risk calculation is necessary in particular in the elderly and subjects with an increased bleeding risk. Consequently, a history of gastrointestinal bleeding and ulcers are contraindications for aspirin.

Two different study designs. There are two different types of clinical studies, designed to evaluate aspirin-related side effects in the gastrointestinal tract: (i) safety

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studies which address the occurrence of aspirin-induced gastrointestinal injury as a study endpoint and (ii) studies that investigate the clinical benefits of aspirin with a clinical efficacy endpoint, such as prevention of myocardial infarction or stroke, where gastrointestinal problems are a safety endpoint. The gastrointestinal side effects of these primary and secondary prevention studies are outlined in Sections 4.1.1, 4.1.2, 4.1.3 and 4.3.1 together with the efficacy endpoint, that is, prevention of myocardial infarction, stroke and (gastrointestinal) cancer as well as the other major aspirin-associated bleeding risk: cerebral hemorrhages.

Gastrointestinal side effects as study endpoints – observational studies. Numerous observational studies on gastrointestinal side effects with aspirin are available. The following three large retrospective and frequently cited studies from three different countries are discussed in more detail.

A British case-control study was conducted in subjects who were hospitalized for upper gastrointestinal bleeding. The objective was to determine the risk of hospitalization for bleeding peptic ulcers in patients with current prophylactic aspirin use at antiplatelet doses of 300 mg/day or less. A total of 1,121 patients with gastric or duodenal ulcer bleeding (cases) were included and matched with 1,126 hospital and 989 community controls.

A total of 144 (12.8 %) of the cases had been regular users of aspirin (taken at least five days a week for at least the previous month) as compared to 9.0 % and 7.8 % of the hospital and community controls, respectively. ORs were increased for all doses of standard aspirin and amounted to 2.3 (95 % CI: 1.2–4.4) at 75 mg, 3.2 (95 % CI: 1.7–6.5) at 150 mg and 3.9 (95 % CI: 2.5–6.3) at 300 mg. Thus, 75 mg aspirin had a 40 % lower risk than 300 mg. There was a clear dose-dependent increase in the risk of peptic ulcer bleeding from 75 to 300 mg plain aspirin (tablets or solutions) but no increased risk with enteric-coated formulations (OR: 1.1; 95 % CI: 0.2–6.1). The risks seemed particularly high in patients who took nonaspirin NSAIDs concurrently with aspirin.

The conclusion was that no conventionally used prophylactic aspirin at antiplatelet doses seems to be free of the risk of peptic ulcer complications. However, the on average much lower incidence of ulcers with the enteric-coated preparation also indicated a better tolerability of this formulation [10].

An US-American retrospective case-control study investigated drug-related gastrointestinal bleeding in 550 incident cases, admitted to Massachusetts hospitals because of acute upper gastrointestinal bleeding (confirmed by endoscopy). Controls were 1,202 persons, identified from population census lists. Cases and controls were asked for the use of aspirin, including the kind of aspirin formulation (source not specified), and nonaspirin NSAIDs during the last week before the bleeding event (cases) or interview (controls).

The ORs for risk of drug-related bleeding were similar, 2.6–3.1, between the different treatment groups and were also not different between different aspirin preparations, including enteric-coated aspirin.

The conclusion was that enteric-coated aspirin also carries a 3-fold increased risk in major upper gastrointestinal bleeding and that this formulation is not less harmful than plain aspirin [84].

A Scandinavian retrospective cohort study also investigated the relationship between upper gastrointestinal bleeding and aspirin intake in Denmark. The data of 27,694 users of low-dose as-

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pirin (100–150 mg/day) were compared with the incidence rate of upper gastrointestinal bleeding in the general population in the same region. Gastrointestinal bleeding was 2.6-fold more frequent in aspirin users (95 % CI: 2.2–2.9) and there was no difference between plain and enteric-coated preparations. However, the combined use of aspirin and traditional NSAIDs increased the incidence rate to 5.6 (95 % CI: 4.4–7.0).

The conclusion was that regular low-dose aspirin is associated with an increased risk of upper gastrointestinal bleeding which is about doubled when combined with nonaspirin NSAIDs. Enteric coating of aspirin appears to not to reduce the risk (Sorensen et al., 2000).

These and further nonrandomized observational trials clearly demonstrate an increased risk of gastrointestinal intolerance by (regular) aspirin intake, which might also be affected by the galenic preparation. Observational trials are not randomized and suffer from the inherent difficulties of all observational studies, specifically the unknown aspirin dosage, the exact duration of treatment, morbidities of the participants and their comedications. Further confounding factors, such as age, smoking, *H. pylori* infections or (additional) use of OTC aspirin and/or NSAIDs. Alcohol in combination with aspirin also has a marked synergistic effect on overt gastric hemorrhages [75, 85].

A metaanalysis of 17 epidemiological studies on the association between aspirin use and serious upper gastrointestinal complications (PUBs) found an overall enhanced risk of 2.2 (95 % CI: 2.1–2.4) for cohort studies and nested case-control studies and 3.1 (95 % CI: 2.8–3.3) for nonnested case-control studies. The overall risk was 2.6 (95 % CI: 2.3–2.9) for plain, 5.3 (95 % CI: 3.0–9.2) for buffered and 2.4 (95 % CI: 1.9–2.9) for enteric-coated aspirin formulations [9]. A later metaanalysis of this group determined the RR of gastrointestinal bleeding with low-dose (75–325 mg/day) aspirin versus nonuse. This amounted to 2.3 for upper and 1.8 for lower gastrointestinal bleeding, respectively [86].

Gastrointestinal side effects as study endpoints – randomized trials. Serious gastrointestinal bleeding after aspirin in a small but significant proportion of patients in observational trials prompted the design of randomized studies including supposedly safer aspirin formulations. Among those were different enteric-coated formulations. One of the first systematic investigations of the relationship between enteric-coated aspirin and gastrointestinal mucosal injury in men was from Stubbé et al. (1962).

The authors compared stomach-resistant enteric-coated preparations (“home-made” in the hospital-own pharmacy) with an aspirin standard formulation, both preparations containing 500 mg aspirin. In all individuals studied, there was an increase of occult blood in stool with plain aspirin, but this was only observed in four out of 30 subjects with the enteric-coated formulation. This suggested for the first time that the increased gastrointestinal blood loss subsequent to aspirin intake was mainly from the stomach and could be largely reduced or even avoided by appropriate coating of the preparation.

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The conclusion was that enteric-coated formulations cause no or minor gastrointestinal injury, in many cases being in the range of placebo effects [69].

Similar results, that is, less stomach injury by the coated, acid-resistant aspirin preparation as opposed to standard plain aspirin, were also obtained by Lanza and colleagues [8]. The first controlled, prospective randomized trial on low-dose enteric-coated aspirin was conducted by Hawthorne and colleagues (1991).

The authors compared the gastrointestinal tolerance of different doses of plain and enteric-coated aspirin (300 mg each). A total of 20 healthy subjects were treated for 5 days in a placebo-controlled, double-blind crossover design.

Plain aspirin caused significant increases in gastric mucosal injury (Lanza-Score) as compared to placebo and enhanced mucosal bleeding. Enteric coating apparently eliminated this type of gastric injury at the same dosage. Both formulations caused comparable but incomplete inhibition of (stimulated) gastric mucosal PGE_2 formation in homogenates of gastric mucosa ex vivo and suppressed nearly completely (>99 %) serum thromboxane generation.

The conclusion was that enteric-coated aspirin should be considered a useful approach for long-term use, specifically in cardiovascular prophylaxis (Table 3.2.1-1) [87].

Table 3.2.1-1: Effects of plain and enteric-coated (EC) aspirin (300 mg/day for 5 days) as compared to placebo on prostaglandin/thromboxane biosynthesis and gastric mucosal injury in 20 healthy volunteers. Data are means ± CI (quartiles). All differences between plain aspirin and EC aspirin/placebo were significant except serum thromboxane levels (for further explanations see text) (data from [87]).

parameter	placebo	ASA 300 plain	ASA 300 EC
hemorrhage erosion score	0	2	0
	(0–0.3)	(0–5)	(0–1.3)
visual analogue injury-score	0	5	0
	(0–8)	(0–31)	(0–8)
mucosal bleeding [µl/10 min]	0.9	2.8	1.0
	(0.6–1.3)	(1.6–4.8)	(0.6–1.5)
mucosal PGE_2 synthesis [pg/mg]	18	0.7	1.8
	(1–51)	(0.4–11)	(0.5–9.2)
mucosal TXB_2 synthesis [pg/mg]	19	1.4	2.5
	(4.1–37)	(1.1–1.9)	(1.1–5.2)
serumTXB_2 [% of placebo]	100	0.4	0.3
		(0.2–1.1)	(0.2–0.9)

These data on improved gastric tolerance of enteric-coated formulations were confirmed in several other but small controlled endoscopic studies in man [8, 88–92] and endoscopically in a metaanalysis for a wide range of doses – 75 mg to 3.9 g [93]. However, the duration of treatment in these studies was short, mostly 1 week or less, and the studies were conducted in healthy, middle-aged volunteers. Thus, they might not

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be applicable to long-term prevention trials and there is no head-to-head comparison between enteric-coated and plain aspirin preparations in long-term trials, but there is evidence that the efficacy of aspirin to inhibit COXs might change, that is, be reduced, with time [94] and might be lower in enteric-coated preparations due to incomplete absorption in the intestine [95, 96].

A metaanalysis on side effects of aspirin in all randomized controlled trials on secondary prevention listed in the Anti-platelet Trialists' Collaboration suggested that regular intake of standard aspirin at doses between 75 and 1,500 mg for one year will double the risk of gastrointestinal bleeding and increase the number of peptic ulcers 1.5-fold [97]. The absolute risk of endoscopically visible injuries in the upper gastrointestinal tract for regular aspirin intake is equivalent to 5 cases per 1,000 aspirin users [98]. This incidence is currently decreasing: The incidence of gastrointestinal bleeding with low-dose aspirin was 0.48–3.64 cases per 1000 person-years in a more recent metaanalysis [86]. Probable reasons are an earlier diagnosis of stomach problems, including eradication of *H. pylori* and the comedication of PPIs, especially in long-term prevention of cardiovascular events in subjects at elevated gastrointestinal risk. Interestingly, there is even evidence for a reduced gastrointestinal bleeding risk after regular aspirin intake for more than 5 years in cardiovascular prevention trials (Fig. 4.3.1-3) [99]. The placebo-controlled ASPREE trial in elderly persons has shown that aspirin (100 mg/day) over a median follow-up of 4.7 years increased gastrointestinal bleeding events. The HR was 1.36 (95 % CI: 0.96–1.94; $P = 0.08$). Age, smoking, hypertension, chronic kidney disease and obesity also increased bleeding risk. The absolute 5-year risk of bleeding was 0.25 % (95 % CI: 0.16–0.37 %) for a 70-year-old not on aspirin and was increased to 5.03 % (95 % CI: 2.56–8.73 %) for an-80-year old with additional risk factors taking aspirin [100].

3.2.1.5 Aspirin and other drugs

Proton pump inhibitors. The most convincing data for protection of the stomach from aspirin-induced mucosal injury are available for proton pump inhibitors (PPIs), such as omeprazole, pantoprazol and its analogs. These compounds have a long duration of action, can be given orally once daily and have been shown to protect from aspirin- and NSAID-induced gastric ulcer formation [101–103]. The risk of interference with the antiplatelet actions of aspirin is low; the reason is that antiplatelet effects of aspirin are largely determined by the amount of active drug absorbed in the small intestine, while PPIs act selectively on the acid-producing oxyntic cells in the stomach mucosa [104]. Accordingly, there is no protection by PPIs against aspirin-induced lower gastrointestinal tract bleeding. Data from a phase III clinical trial on PA32540 (a coordinated-delivery tablet containing 325 mg enteric-coated aspirin and 40 mg omeprazole) vs. 325 mg enteric-coated aspirin alone showed improved gastric protection in subjects at risk for aspirin-associated gastric ulcers, a similar cardiovascular event profile and

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markedly improved adherence to drug treatment because of less upper gastrointestinal tract adverse effects [105].

Nonsteroidal antiinflammatory drugs. NSAIDs are the standard treatment for pain and inflammation. They bear a risk of gastrointestinal side effects. Concomitant treatment of aspirin and NSAIDs – occurring in about 20 % of patients taking aspirin for cardiocoronary prophylaxis – increases the risk of serious gastrointestinal events [64]. In addition, NSAIDs might antagonize the antithrombotic actions of aspirin (Section 4.1.1). For these reasons, simultaneous use of NSAIDs and aspirin, if necessary, should be done with consideration of the different pharmacokinetics of the two classes of compounds (Section 4.1.1).

COX-2 inhibitors. Selective inhibitors of COX-2, such as celecoxib and rofecoxib, were originally introduced because of less expected gastric injury than with conventional NSAIDs as a consequence of the absent inhibition of COX-1. Clinical data confirm a significantly reduced risk of gastrointestinal bleeding and ulcer formation with these compounds. These benefits may be lost in patients, requiring additional aspirin cotreatment for cardiocoronary prevention, in particular those suffering from rheumatoid arthritis. These patients are at a 32–55 % higher risk for cardiocoronary events than patients with osteoarthritis or healthy individuals [106]. This might explain why participants of the CLASS study, containing 28 % patients with rheumatoid arthritis, who were allowed to take low-dose aspirin in addition to a coxib, did not have improved gastrointestinal safety with the COX-2 inhibitor [64]. They rather exhibited a higher frequency of upper gastrointestinal ulcer complications (RR: 4.5; $P = 0.01$) than patients receiving celecoxib alone [9]. Today, the use of coxibs instead of traditional NSAIDs might be an alternative in short-term analgesic use but not for long-term treatment, in particular not in patients at enhanced cardiovascular risk.

Summary

Gastric intolerance is a typical and frequent side effect of oral aspirin intake. Gastrointestinal intolerance usually presents with subjective symptoms such as dyspepsia, nausea and heartburn as well as increased fecal blood loss from gastrointestinal microbleeding events. Intake of standard aspirin at doses between 75 and 1,500 mg for one year will double the risk of gastrointestinal bleeding events and increases the number of peptic ulcers 1.5-fold. Subjective and objective symptoms of gastric intolerance are not interrelated.

Aspirin has different and partially antagonistic effects on the stomach mucosa. It directly injures the mucosal lining by disturbing its barrier function and morphological integrity. In addition, aspirin may stimulate the more chronic (inflammatory) event of mucosal cell adaption. The role of COX inhibition and prostaglandins in these processes is still a matter of debate. Aspirin-induced expression of COX-2 and subsequent generation of gastroprotective lipoxins (ATL) is currently an issue of great scientific interest, specifically with respect to mucosal adaption to ulcerogenic actions of aspirin.

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The incidence and severity of gastric injury by aspirin in clinical trials was dose- and time-dependent. Randomized, short-term trials showed that gastric mucosal injury is more severe after direct contact of the active compound (salicylate) with the stomach mucosa. In long-term prevention, there is an increased risk of gastrointestinal bleeding and ulcers. This risk tends to become lower with longer lasting (>5 years) regular intake and becomes increased in the elderly, by smoking, hypertension, chronic kidney disease and obesity.

Aspirin-related gastric injuries can be treated or even prevented by eliminating gastric acid secretion, for example by appropriate comedications such as PPIs, eradication of *H. pylori* or (better) both. For OTC or short-term (analgesic) use, predissolved or soluble preparations are available as well as a new micronized, fast disintegrating aspirin formulation. In this indication, aspirin is at least as well tolerable as conventional OTC analgesics, such as paracetamol (acetaminophen) and ibuprofen (Section 4.2.1).

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3.2.2 Liver

3.2.2.1 General aspects

Salicylate-induced hepatopathy may occur as a consequence of repeated high-dose aspirin treatment. This hepatopathy is typically associated with increases in liver enzymes and a dose-dependent impairment of β-oxidation of long-chain fatty acids related to the uncoupling of oxidative phosphorylation. These metabolic effects are physicochemical in nature and caused by the salicylate moiety (Section 2.2.3) rather than a specific pharmacodynamic effect of aspirin on the hepatocyte.

Occurrence. The liver, located between the sites of drug absorption in the gastrointestinal tract and drug targets in the systemic circulation after its "first pass," is central to the metabolism of virtually every drug and xenobiotic [1]. It is, therefore, not surprising that drug-induced liver injuries are among the most frequent reasons not only for stopping the further development of new drugs, but also for withdrawing already "established" drugs from the market [2]. Some OTC analgesics, in particular paracetamol (acetaminophen), have a worldwide leading position in this respect [3, 4]. In the US, paracetamol mis- or overuse causes more than half of drug-induced liver injuries and is the reason for 20 % of all liver transplantations [5]. By comparison, clinically relevant toxic liver injury by aspirin requiring hospitalization or even ending fatally is extremely seldom [6]. Toxic liver injury is also not a typical symptom of acute salicylate overdosing [7]. Changes in laboratory tests, if any, are transient and generally subside after withdrawal of aspirin [8], sometimes even if the treatment was continued [9]. A probably different etiology has the liver injury of Reye's syndrome subsequent to viral infections and, perhaps, other infections as well [10], as discussed in detail elsewhere (Sections 2.2.3 and 3.3.3).

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Symptoms. Long-term use of aspirin in high, anti-inflammatory doses, resulting in plasma levels of at least 200–350 µg/ml (>1 mM), can cause transient liver injury. This injury presents with elevated transaminases but not jaundice. Intoxications are clinically asymptomatic, in most cases not even diagnosed [8]. The biochemical and functional disturbances are caused by the salicylate component of the drug and are fully reversible within a few days [11].

3.2.2.2 Pathophysiology and mode of aspirin action

Major determinants of aspirin-induced hepatic injury are the dose, i. e., the plasma levels of salicylate, and duration of treatment. Additional determinants are underlying diseases, specifically immune diseases, such as chronic rheumatoid diseases [11] and preexisting hepatic or renal failure, allowing for higher accumulation of the salicylate inside tissues [8].

Salicylate accumulation. Laboratory signs of hepatotoxicity can develop at high salicylate plasma levels (200 µg/ml or 1 mM and more). A regular intake of high aspirin doses appears to be necessary because even huge single overdoses of aspirin, although causing significant general toxicity and metabolic failure (Section 3.1.1), are not associated with any overt hepatic failure [12–14]. Regular intake of high doses will also result in significantly increased plasma levels of salicylate even at medium doses because of the reduced clearance and marked prolongation of the salicylate half-life (Section 2.1.2).

Inflammatory components. Other factors relevant to salicylate toxicity are preexisting diseases with an immunological background. Almost all reported cases occurred in patients with rheumatoid diseases who had taken the drug for a long time at high doses. Rheumatic diseases are known to be associated with the generation of inflammatory cytokines, such as TNFα or IL-6 (Section 2.3.2). Similarly to other organs, these cytokines might induce and maintain immune-inflammatory processes in the liver. Consequently, after repeated high-dose aspirin serum transaminase levels were higher in rheumatics, when patients were in an active stage of the disease [15]. Today, other anti-inflammatory agents, such as NSAIDs or DMARDs (Section 4.2.2), have replaced aspirin in this indication. Interestingly, at least some of these compounds, including diclofenac and sulindac, also bear a hepatotoxic potential, possibly by impairment of mitochondrial ATP synthesis and production of hepatotoxic reactive metabolites [16].

3.2.2.3 Clinical studies

Aspirin-related liver injury. First reports about a possible hepatotoxic potential of aspirin appeared after more than half a century of extensive clinical use of the compound

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as an anti-inflammatory analgesic and this at doses of several grams per day. Possible explanations for this somewhat surprisingly late finding are the absence of typical clinical symptoms of liver toxicity, such as jaundice, and the inability to measure liver enzyme activities in routine laboratory settings at the time. After these techniques became available, several studies reported elevated serum transaminase levels after repeated aspirin intake, preferably at high doses. For example, about one third or more of patients treated with salicylates because of rheumatic diseases had elevated serum levels of liver enzymes [9, 17, 18]. In these and some other studies, the serum salicylate levels were related to serum transaminase activity, suggesting a relationship between the two [8].

Severity of liver injury by salicylates. In about 3 % of reported cases, injury has been more severe. All of these patients received high, anti-inflammatory doses of aspirin over longer periods of time. There are five case reports about a possible relation between salicylate-induced hepatic injury and encephalopathy. Plasma levels of salicylate in these patients ranged between 270 and 540 µg/ml (1.9–3.8 mM) [8, 19]. There is only one report of a fatal case in a 17-year-old girl suffering from rheumatoid arthritis who received aspirin combined with paracetamol. The girl died from liver necrosis [20], possibly due to paracetamol rather than aspirin. For these reasons, salicylate-induced hepatopathy is not an issue of concern.

Hepatoprotective actions of aspirin? Actual epidemiological studies have suggested a protective effect of regular aspirin intake on liver function. The "Third *N*ational *H*ealth *a*nd *N*utrition *E*xamination *S*urvey (NHANES III) in more than 1,000 adults indicated that regular aspirin use (≥15 times per month) is associated with an overall 38 % lower prevalence of nonalcoholic fatty liver (OR: 0.62; 95 % CI: 0.51–0.72; $P = 0.04$). This effect was limited to men (OR: 0.32) and persons above the age of 60 years (OR: 0.21) [21]. Even more exciting were data from a prospective register study on more than 300,000 men and women from the US National Institutes of Health (NIH). This study showed that aspirin users taking the drug regularly had an about 50 % reduced risk of both chronic liver disease and incident hepatocellular carcinoma (HCC) (RR: 0.55; 95 % CI: 0.45–0.67) as compared to nonusers [22]. Similar results were obtained in a population-based cohort study from Korea which also showed a lower risk of HCC for aspirin users as compared with non-users (HR: 0.87; 95 % CI: 0.50–0.85) [23]. A metaanalysis of eight studies with a total of 2,604,319 participants confirmed a significant reduction in the risk of HCC in participants who used aspirin (HR: 0.59; 95 % CI: 0.47–0.75). Similar results were obtained in another more recent metaanalysis which additionally showed that the benefits of aspirin appeared to increase with increasing doses and duration of aspirin use [24]. A linear dose–response model also showed a significant inverse association between aspirin dosing and risk of HCC [25]. These findings, although all from epidemiological observational trials,

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are clear arguments against any relevant liver toxicity of aspirin even at long-term use in therapeutic doses but rather suggest remarkable beneficial effects in the prevention of liver injury and hepatocellular cancer.

Summary

Aspirin is well tolerated and does not cause any liver injury at single analgesic or repeated antiplatelet doses. Repeated administration of high antiinflammatory doses in the past has been shown to increase serum transaminase levels but not to induce overt liver failure or clinical problems (jaundice). The symptoms were transient and reversible after withdrawal of the drug and probably salicylate-mediated.

Liver toxicity of aspirin, if any, is probably related to impaired hepatic oxidation of free fatty acids associated with salicylate-induced uncoupling of oxidative phosphorylation. Both require repeated treatment with the drug at high doses (Section 2.2.3). These metabolic changes are also generally reversible.

The finding of a chemopreventive effect of aspirin on the incidence of HCC, increasing with dose and duration of aspirin treatment in several large observational trials and metaanalyses, suggests a remarkable beneficial effect of aspirin on the liver and urgently needs confirmation in randomized controlled trials.

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3.2.3 Kidney and heart failure

3.2.3.1 General aspects

The excretion of salicylates and their several phase I and phase II metabolites (Section 2.1.2) occurs exclusively through the kidney. It is, therefore, to be expected that the kidney is also a major target of salicylate toxicity. This is not true. With the possible exception of the (multimorbide) elderly with impaired renal function and hypoalbuminemia, severe renal failure is neither typical of acute salicylate poisoning nor of chronic "abuse" of salicylates, for example as OTC preparation for analgesic use and the so-called "analgesic nephropathy."

A piece of history. Toxic renal failure with transient shedding and excretion of renal tubular cells and albuminuria after long-term, high-dose salicylate intake has been

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described for the first time in 1917 [1] and was basically confirmed in subsequent publications [2]. The patients presented with disturbed acid-base balance (metabolic acidosis) as well as Na^+ and water retention [3, 4]. The changes were transient, even if salicylate intake was continued, and in most cases fully reversible. However, there was a large interindividual variability as exemplified by some impressive case reports about aspirin abuse and kidney function:

One report was on 17 patients who were treated with high-dose aspirin because of rheumatoid arthritis. The overall cumulative aspirin intake over the years of each of them was 5–20 kg (!) aspirin. There were only small abnormalities in kidney function testing and none of them exhibited any clinically significant impairment of renal function [5].

Another report was on a healthy, 21-year-old male who was hospitalized a few hours after oral intake of 125 grams of aspirin. Clinically, there was massive polyuria, which was restored to normal levels within one week [6].

Another case report described a 16-year-old man who had taken 135 g aspirin in a coated formulation. At 4 h after intake, the plasma level of salicylate amounted to 920 µg/ml (6.4 mM). Clinical symptoms were cramps, pulmonary edema and acute renal failure. The plasma salicylate levels were reduced to 374 µg/ml (2.6 mM) and 113 µg/ml (1.9 mM) after 10 h of hemofiltration. The patient survived and was discharged from the clinics a few days later without any symptoms remaining [7].

An acute intoxication in a 35-year-old man who had taken about 400 standard aspirin tablets (130 g) and had comparable initial salicylate plasma levels, 896 µg/ml (6.3 mM) 7.5 h after intake of aspirin, terminated fatally. Neither initial standard treatment with charcoal plus bicarbonate nor two subsequent hemodialyses improved the clinical symptoms (central excitation, cramps, delirium, hyperthermia) or reduced the plasma salicylate levels significantly. The patient died 40 hours after drug intake [8].

In most cases of intoxication, the kidney excretion mechanisms remained functioning, and this is the prerequisite for successful standard treatment of intoxications, eventually resulting in full recovery (Section 3.1.1).

3.2.3.2 Mode of aspirin action

The central biochemical process in the kidney which might be affected by aspirin (and NSAIDs) even in low therapeutic doses is renal prostaglandin biosynthesis [9]. Vasodilatory prostaglandins, generated in the kidney, predominantly PGE_2, serve several physiological functions: They regulate renal hemodynamics and blood flow as well as water and sodium exchange by stimulating sodium excretion in the medulla [10]. Renal prostaglandin biosynthesis is low in healthy individuals. However, it is increased in situations of volume overload in the course of systemic diseases and congestive heart failure. This increment of renal prostaglandin synthesis is important, since PGI_2 and PGE_2 act as modulators of renal perfusion. This involves a negative feedback loop through which PGE_2 and PGI_2 reduce the vasoconstrictor action of agonists, such as angiotensin II or norepinephrine [11]. Interestingly, COX-1- and COX-2-derived prostaglandins might exert opposite effects on systemic blood pressure and renal function, as seen in COX knockout animals [12]. COX-2 inhibitors reduce re-

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nal medullary blood flow, decrease urine flow and enhance the pressor effect of angiotensin II. Inhibition of enhanced prostaglandin formation by NSAIDs might cause imbalances in the regulation of blood pressure and sodium excretion, eventually resulting in edema formation. Aspirin – in contrast to nonselective NSAIDs and coxibs – has only weak effects on these mechanisms, probably because the stimulated renal prostaglandin formation is mainly COX-2-mediated and aspirin is not a potent COX-2 inhibitor in vivo (Section 2.2.1).

3.2.3.3 Clinical studies – individuals without kidney diseases

Aspirin as monopreparation. No renal side effects of aspirin were reported in a post hoc analysis of the more than 11,000 participants of the American Physicians' Health Study who took a total of at least 2,500 aspirin tablets (812 g) over 5 years [13]. Life-time analgesic consumption, including aspirin, several nonaspirin NSAIDs and paracetamol, was also not associated with any dose-dependent decline in kidney function in the "National Health Nutrition Examination Survey" (NHANES). This study included more than 8,000 habitual analgesic users who regularly (daily) over at least 5 years took aspirin or other analgesic monopreparations [14]. Further observational trials confirmed an absent or low renal toxicity of aspirin and traditional NSAIDs even during life-long use. Only paracetamol (acetaminophen) exhibited an increase in renal dysfunction within 11 years at higher doses in the participants of the Nurses Health Study, an observational trial in female American health care providers (Table 3.2.3-1) [15].

Analgesic mixtures. In contrast to aspirin or paracetamol monopreparations, there is evidence for nephrotoxicity of phenacetin/paracetamol-containing analgesic mixtures ("analgesic nephropathy"). Some of them also contain aspirin [16–21]. The nephrotoxicity of mono- vs. mixed analgesic preparations was studied in a retrospective trial in patients with end stage renal failure, undergoing hemodialysis.

The risk of long-term intake of antipyretic analgesics for terminal kidney failure was studied in a case-control trial of end-stage renal disease patients undergoing renal replacement therapy as compared to 517 matched controls. The study contained all 921 patients undergoing chronic hemodialysis during 1984–1986 in (West) Berlin. Control subjects, matched to patients by age, sex and nationality, were 517 patients in outpatient clinics from university hospitals. The life-long analgesic history was recorded by interview. Regular analgesic intake was defined as continuous consumption of 15 or more analgesic units (tablets, liquids, suppositories) per month for at least one year.

There was a clear dose- and time-dependent relation between drug intake and terminal kidney failure for combined analgesic mixtures with a particular high risk for combinations, especially those containing caffeine; the relative risk for high-dose (>1250 g) lifelong use was 52.6 as opposed to 4.1 with high-dose (>1000 g) paracetamol and as opposed to 2.4 at high-dose (>1000 g) aspirin. No increased risk was seen with any of the single ingredients.

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The conclusion was that an increased risk of end-stage renal failure is related to both dose and exposure time for mixed analgesics, but not for the single-ingredient analgesics. No such risk exists for analgesic monopreparations [22, 23].

Table 3.2.3-1: Lifetime consumption of nonopioid analgesics (aspirin, paracetamol [acetaminophen], NSAIDs) in women and the consequences for changes (relative decline [OR]) in glomerular filtration rate (GFR) in 11 years (reference = 1). Data refer to the absolute number of participants with the percentage of participants showing change in brackets (data from the Nurses' Health Study) (modified after [15]).

lifetime intake [g]	participants (n = 1697)	participants with change [%]	odds ratio (OR) (95 % CI)
Aspirin			
<100	608	53 (9)	1.0 (reference)
100–499	176	16 (9)	0.7 (0.4–1.3)
500–2999	403	46 (11)	0.8 (0.5–1.3)
>3000	455	49 (11)	0.9 (0.6–1.4)
NSAIDs			
<100	790	67 (8)	1.0 (reference)
100–499	181	24 (13)	1.3 (0.8–2.2)
500–2999	376	41 (11)	1.1 (0.7–1.7)
>3000	292	31 (11)	1.1 (0.7–1.8)
Paracetamol			
<100	819	56 (7)	1.0 (reference)
100–499	186	21 (11)	1.80 (1.0–3.2)
500–2999	288	40 (14)	2.23 (1.4–3.6)
>3000	352	45 (13)	2.04 (1.3–3.2)

3.2.3.4 Clinical studies – individuals with preexisting kidney diseases

Chronic kidney disease (CKD) is frequently associated with other chronic systemic diseases, such as coronary heart disease, hypertension and diabetes. These diseases might also be treated with aspirin as an adjunct to more disease-related treatment options. It is therefore of interest to know whether the pharmacological (antiplatelet) effect of aspirin is influenced by a concomitant renal failure or whether aspirin itself might influence (aggravate) any preexisting CKD. This last effect appears to be negligible, independent of the reason for CKD [24, 25].

Coronary heart disease. There appears to be no clear benefit of aspirin in a meta-analysis of primary prevention of cardiovascular events in CKD patients but rather an increased risk of bleeding events – similarly to non-CKD individuals [26, 27]. However, the number of randomized controlled trials is insufficient and there are occasional reports from small but randomized trials indicating that aspirin might reduce

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coronary events in CKD patients and possibly retard renal disease progression even without an increased bleeding tendency [28]. Aspirin is a recommended medication for most patients with CKD and elevated cardiovascular risk [29]. A metaanalysis of 14 randomized trials of antiplatelet drugs in secondary prevention of cardiovascular ischemic events including more than 2,600 hemodialysis patients showed that antiplatelet treatment (mostly aspirin) was associated with a significant 41 % reduction of new severe atherothrombotic events. There were only 46 major bleeding events in this large population, suggesting that aspirin-induced bleeding is not more frequent in patients even with end-stage renal failure than in others [30].

Another large randomized cohort study on more than 28,000 hemodialysis patients provided further information on the risk/benefit ratio of aspirin. Aspirin use was associated with a decreased risk of stroke in all patients, including those with coronary artery disease (CAD) (RR: 0.82; $P < 0.01$), but an increased risk of myocardial infarction (RR: 1.21; $P = 0.01$) and cardiac events (RR: 1.08; $P < 0.01$). Aspirin did not increase gastrointestinal or general bleeding. The authors concluded that these data do not support the notion that prescribing aspirin to hemodialysis patients decreases cardiovascular disease risk but rather might decrease cerebrovascular events. However, even data from large observational studies always bear a risk for bias and need to be confirmed by randomized controlled studies [31].

One of the first prospective, placebo-controlled randomized studies on the effects of long-term aspirin on the progression of CKD was the "First United Kingdom Heart and Renal Protection" (UK-HARP-I) study [32].

The UK-HARP-I study was designed as a feasibility study to investigate the efficacy and safety of simvastatin and aspirin vs. placebo on renal function in a prospective randomized trial. The study included 448 patients with advanced CKD (predialysis, dialysis, kidney-transplanted patients) who were treated with aspirin (100 mg/day retarded-release formulation) or a matching placebo for a median observation period of 1 year.

There was no aspirin-related progression of kidney dysfunction nor increased urate levels or acute gout. The use of aspirin was also not associated with a significant increase in major bleeding events as compared to placebo. There was an about 3-fold increase in the risk for minor bleeding events (HR: 2.8; 95 % CI: 1.5–5.3; $P = 0.001$).

The conclusion was that aspirin at 100 mg/day is well tolerated in patients with CKD. Its use is associated with a 3-fold increase in minor bleeding. Overall, aspirin appears to be safe also in long-term use in patients with CKD. However, a much larger trial is required to reliably determine whether low-dose aspirin has indeed no clinically significant effects on renal function in predialysis and dialysis patients [32].

The efficacy of aspirin for patients with CKD presenting with ACS is well established. For example, the CCP showed that aspirin reduced in-hospital mortality by 64–80 % across all quartiles of (reduced) creatinine clearance [33]. These and other studies [34] convincingly demonstrated a positive benefit/risk ratio for aspirin also for ACS patients with CKD [34].

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Diabetes. Diabetics, in particular of type 2, need thrombosis prevention because of their enhanced risk of atherothrombotic vessel occlusions. This is the reason for long-term treatment with antiplatelet drugs, including aspirin (Section 4.1.1). Disturbed kidney function, that is, diabetic nephropathy with reduced glomerular filtration and albuminuria, is frequent in advanced diabetics and might complicate the clinical outcome. Renal prostaglandin and thromboxane excretion appear to be increased in these patients, suggesting a potential contribution of (extra)renal prostaglandins to kidney function, that is, regulation of renal blood flow and sodium excretion [35, 36], possibly via vascular COX-2 upregulation [37]. Aspirin might not interfere with these processes because of its low COX-2 inhibitory capacity in vivo.

This agrees with a prospective, randomized, double-blind crossover trial which indicated that low-dose aspirin (150 mg/day for 4 weeks) had no effect on microalbuminuria, glomerular filtration, blood pressure or HbA1c in type 1 diabetics [38]. Similar results were obtained with the same aspirin dose in type 2 diabetics [39] as well as in a subgroup analysis of the JPAD2 cohort study (Section 4.1.1) after treatment for 8.5 years with low-dose aspirin [40].

These data and those from epidemiological trials suggest that regular use of aspirin by diabetics at antiplatelet doses for cardiovascular prevention does not have any negative impact on renal function in type 2 diabetics. However, large, placebo-controlled prospective trials are missing. The first human study, the double-blind "Renal Disease Progression by Aspirin in Diabetic Patients" (LEDA) trial, is currently underway. This study will investigate whether aspirin treatment may beneficially affect kidney function in patients with type 2 diabetes by reducing the decline in glomerular filtration [36].

Hypertension. In essential hypertension, enhanced renal generation of vasodilatory prostaglandins is an important blood pressure-regulating factor. Inhibition of (renal) prostaglandin biosynthesis by COX inhibitors such as NSAIDs and coxibs might result in an amelioration of potency of antihypertensives, including ACE inhibitors, sartans, β-blockers and diuretics. The blood pressure-lowering effect of these agents involves stimulation of renal vasodilator prostaglandin biosynthesis. Inhibition of this reaction by coxibs and several NSAIDs, such as ibuprofen, might increase blood pressure [41] by inhibition of COX-2-dependent vascular prostaglandin production [42]. Aspirin is only a weak inhibitor of COX-2 in vivo. Therefore, low-dose aspirin is not expected to cause hypertensive effects, fluid retention or edema. Aspirin does also not interact with the blood pressure-lowering action of antihypertensives. These theoretical considerations agree with clinical reality, as shown for example by the absence of negative interactions of aspirin with the blood pressure-lowering effects of losartan [43] and other antihypertensives in the HOT trial (Section 4.1.1) [44].

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A post hoc subgroup analysis of the randomized HOT study (Section 4.1.1) studied the cardiovascular risk reduction by aspirin in hypertensive patients with and without CKD who were successfully treated with antihypertensives.

Aspirin (75 mg/day) reduced the cardiovascular risk in CKD patients stronger than in patients without renal failure. There was a significant trend towards reduced total mortality in the aspirin group with increasing severity of CKD. After a total observation period of 3.8 years, there was (per 1,000 patients) a reduction of severe cardiovascular events by 76 and of mortality by 54 patients at the price of 27 additional severe aspirin-induced bleeding events in the subgroup with most severe renal failure (glomerular filtration rate [GFR] < 45 ml/min per 1.73 m^2).

The conclusion was that aspirin therapy produces a greater absolute reduction in major cardiovascular events and in mortality in hypertensive patients with CKD than in those with normal kidney function. An increased risk of major bleeding appears to be outweighed by these substantial benefits [45].

These data and most of the other available studies in CKD patients do not suggest any clinically relevant negative interaction of aspirin with the blood pressure-lowering effects of antihypertensives, specifically ACE inhibitors and sartans, at daily aspirin doses below 300 mg/day [46, 47].

3.2.3.5 Heart failure

Hypertension, diabetes and renal failure are also frequent causes of heart failure. Although the positive effects of aspirin in patients with CAD are well documented, it is questioned whether there is a comparable benefit in patients with heart failure without coronary heart disease. Two randomized trials compared aspirin with warfarin in patients with chronic heart failure: The "Warfarin/Aspirin Study in Heart failure" (WASH) trial [48] and the "Warfarin and Antiplatelet Therapy in Chronic Heart failure" (WATCH) trial [49]. Both studies suggested a rather negative effect of aspirin on the progression of the disease and clinical outcome. However, the number of patients was small: 146 hospitalizations for about 600 patients in both trials, and one of these studies (WATCH) had to be stopped prematurely because of difficulties in patient recruitment.

Two further, large observational trials on the safety of aspirin in heart failure patients treated with ACE inhibitors are available. The first was conducted in more than 24,000 Medicare beneficiaries (≥65 years) who were hospitalized because of heart failure with CAD. Only 54 % of them had received aspirin prior to hospitalization. The patients on aspirin had a slightly lower mortality (RR: 0.94; 95 % CI: 0.90–0.99). This effect was independent of previously existing hypertension, renal failure or treatment with ACE inhibitors [50].

Another large prospective cohort trial studied the safety of aspirin in heart failure patients treated with ACE inhibitors, including those without coronary heart disease but with renal dysfunction.

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The study group included 7,352 patients who were discharged alive from the clinics after a first hospitalization for heart failure. The mean age was 75 years, 56 % of them had an ischemic pathology, 48 % had systolic dysfunction and 29 % had renal dysfunction (comorbidities frequent); 44 % of the patients were without coronary heart disease; 38 % of the patients died or required readmission because of heart failure within the first year. The question of the study was whether comedication of aspirin at "regular" or "high" (>325 mg/day) doses had any influence on the outcome and the treatment efficacy of ACE inhibitors.

About 40 % of patients had an aspirin prescription, mainly 325 mg/day. Compared to non-aspirin users, these patients were no more likely to die or to require heart failure readmission (HR: 1.02; 95 % CI: 0.91–1.16), even patients without coronary heart disease (HR: 0.98) or patients with renal dysfunction (HR: 1.13). Patients with ACE inhibitors were less likely to die or to be rehospitalized (HR: 0.87; 95 % CI: 0.79–0.96), and these beneficial effects were not reduced by aspirin cotreatment (HR: 0.86; 95 % CI: 0.77–0.95). There were no dose-dependent interactions between aspirin and ACE inhibitors.

The conclusion was that aspirin in heart failure patients did not attenuate the beneficial effects of ACE inhibitors, even in patients without coronary heart disease or kidney dysfunction. There were no differences between high- and low-dose aspirin [51].

According to these data from nonrandomized trials, aspirin appears not to negatively interact with other drugs, specifically ACE inhibitors, in patients with heart failure. A similar conclusion was reached from a large, community-based cohort trial in elderly persons. Regular aspirin use was even associated with a significant reduction in mortality and morbidity [52]. However, more prospective randomized trials are required to establish the role of aspirin in patients with heart failure.

The UK Health Improvement Network, a multicenter prospective primary care database, recently published retrospective data on aspirin use in diabetics with heart failure but no previous history of myocardial infarction or coronary heart disease.

The study cohort contained 5,967 individuals on aspirin as compared to 6,567 individuals who were not, the mean age being 75.3 years and the mean follow-up 5 years. The primary endpoint was a composite of all-cause death and hospitalization for heart failure.

The mean estimated GFR was 58–62 ml/min per 1.73 m^2, and the mean HbA1c level was 7.5–7.6 in all groups. Aspirin was associated with a significant decrease in the primary outcome and all-cause mortality (HR: 0.88; 95 % CI: 0.82–0.93; HR: 0.88; 95 % CI: 0.83–0.94), but an increased risk of nonfatal myocardial infarctions (HR: 1.66; 95 % CI: 1.49–1.85) and nonfatal stroke (HR: 1.23; 95 % CI: 1.01–1.05). Major bleeding events and hospitalization for heart failure were not significantly higher with aspirin and there was no additional benefit at aspirin doses above 75 mg/day.

The conclusion was that these data support aspirin use in primary prevention of patients with type 2 diabetes and heart failure. The increased risk of nonfatal myocardial infarctions might reflect a shift from fatal to nonfatal events and the increase in strokes might reflect the same and/or an excess of nonfatal strokes of unknown etiology [53].

The data are interesting. However, this study was a retrospective analysis from a nonrandomized epidemiological trial. There were a lot of comedications, specifically diuretics (75–78 %), ACE inhibitors (67–70 %), sartans (22–23 %), β-blockers (50–51 %), statins (63–68 %), and several oral antidiabetics. Protection obtained by aspirin was less in patients with hypercholesterolemia and/or hypertension.

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The randomized but open "Aspirin for primary prevention of cardiovascular disease and renal disease progression in chronic kidney disease patients" (AASER) trial was conducted to study whether aspirin is able to decrease the cardiovascular risk and to slow renal disease progression in patients with chronic kidney failure but without preexisting cardiovascular events. There was no effect on the combined cardiovascular/renal endpoint after 65 months of treatment with aspirin in addition to standard therapeutic measures [28]. Unfortunately, the number of participants was small (111) and larger prospective randomized trials on this important issue are clearly needed.

Summary

Any aspirin-induced alteration in kidney function is probably associated with its ability to inhibit (renal) prostaglandin biosynthesis. Renal vasodilator prostaglandins (PGE_2, PGI_2), possibly made via COX-2, are involved in control of renal blood perfusion and blood pressure regulation. They also regulate volume homeostasis by stimulating sodium excretion. Repeated or long-term use of aspirin as single drug or in analgesic mixtures in individuals with normal kidney function is not associated with any elevated risk of nephropathy or renal failure. There is also no established relationship between the progression of preexisting renal diseases and aspirin intake, including patients with diabetic nephropathy who regularly have to take aspirin for cardiovascular prevention.

Inhibition of vascular (renal) prostaglandin biosynthesis by aspirin at single analgesic doses or long-term treatment at antiplatelet doses appears not to be relevant for kidney function. Theoretically, inhibition of prostaglandin biosynthesis might interfere with antihypertensive agents or other compounds for which stimulation of (vasodilatory) renal prostaglandin formation is part of their clinical efficacy. There is no convincing evidence that aspirin at antiplatelet doses (ca. 100–300 mg/day) negatively interacts with antihypertensives or induces or aggravates preexisting kidney dysfunction, including high-risk groups of patients, such as diabetics or hypertensives. Long-term use of aspirin will probably also not aggravate heart failure, due to ischemic or nonischemic conditions in patients with CKD. More randomized controlled clinical trials are necessary to establish this.

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3.2.4 Audiovestibular system

3.2.4.1 General aspects

A piece of history. Hearing loss, tinnitus and vestibular dysfunction were about the first known side effects of salicylates after their introduction into the clinics [1]. Ototoxicity was later also seen with aspirin and appeared to be correlated with the plasma level of salicylates [2]. Plasma levels of salicylate were high in the past because of the high doses used for treatment of inflammatory diseases. Accordingly, tinnitus and deafness are also typical early symptoms of acute aspirin poisoning (Section 3.1.1).

Symptoms of ototoxicity. Salicylate-related ototoxicity is typically bilateral symmetric. It is associated with a low- to medium-degree severity hearing loss and usually completely reversible within 2–3 days after salicylate withdrawal. In addition to hearing disturbances, vestibular disturbances including nystagmus, vertigo and imbalance are further manifestations of salicylate ototoxicity [3, 4].

Hearing loss. Salicylate-induced hearing loss presents with loss of absolute acoustic sensitivity and changes in sound perception. The reasons for this are functional disturbances in the cochlea, possibly amplified by a disturbed signal transduction via the statoacoustic nerve. In addition, there is loss of spontaneous, otoacoustic activity of the cochlea [5–7]. These changes are due to the salicylate metabolite of aspirin.

Tinnitus. Tinnitus (Latin “tinnere” = “ringing in the ear”) is a subjective and, with respect to appearance, highly variable sound sensation (“phantom sound”), mostly 5–15 dB above the hearing threshold. Typical for tinnitus is the absence of any foreign sound source. There appears to be a direct relationship between the (subjective) occurrence of tinnitus and the (objective) hearing disturbances above a certain threshold concentration (≥200 µg/ml) of salicylates [8]. Tinnitus is a typical side effect of as-

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pirin at salicylate plasma levels of ≥200 µg/ml and is also an early symptom of salicylate overdosing (Section 3.1.1). There is experimental evidence that salicylate-induced tinnitus-like behavior in animals can be antagonized by glutamate receptor (NMDA) antagonists, suggesting a possible activation of cochlear NMDA receptors by salicylates [9–11]. The complex neuronal network that is involved in tinnitus and hyperacusis, both consistently induced by high-dose salicylates, probably contains both auditory and nonauditory structures [12].

Vestibular disturbances. Imbalances, vertigo and dizziness are another kind of ototoxic side effects of aspirin. Probably, the imbalance is also related to functional disturbances in the inner ear (labyrinth) [13]. There are no detailed mechanistic studies available.

3.2.4.2 Pathophysiology of aspirin-induced hearing loss

Inner ear and cochlea. The primary site of salicylate action is the cochlea, and here the outer hair cells [14, 15] with subsequently disturbed outgoing signals to the sensory cortex via the statoacoustic nerve. *Post mortem* studies of hearing bones and the inner ear (Corti's organ, cochlea, hair cells) in patients with known regular high-dose aspirin consumption – 5–10 g/day over several months – did not show any morphological abnormalities. In guinea pigs, sodium salicylate at high doses (375 mg/kg for 1 week) did also not cause any marked morphological alterations of the cochlea, except some deformation of the outer hair cells [16]. In cultured explants of the rat cochlea, degenerations of nerve cells were detected in the presence of high salicylate concentrations (3 mM and more for at least 2 days) but no hair cell necroses [17]. Thus, the available morphological data do not support the concept of salicylate-induced, morphologically detectable injury of hair cells, neurons or blood vessels in the cochlea but rather a functional disturbance.

Because of the low and usually reversible toxicity of salicylates, the reliability of their ototoxic effects, that is, hearing loss and tinnitus, and the excellent solubility of salicylate sodium salts, salicylates have become a widely used tool to study signal perception and dispatch in the auditory system. In vitro assays with salicylate concentrations between 3 and 10 mM were the preferred setting [18]. Unfortunately, these salicylate levels are not only toxic for the inner ear but also potentially fatal for the organism (Fig. 2.3-1). Thus, actions of salicylates obtained at these disproportional high salicylate levels might be of pharmacological interest to study toxic actions of xenobiotics on the function of the inner ear but of limited significance to understand the ototoxic side effects of aspirin in men in vivo.

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Prestin as the molecular salicylate target. The cylindric outer hair cells of the cochlea are no conventional neuronal cells but mechanotransducers that act as amplifiers of acoustic waves and translate them into electrical signals ("voltage sensors"). Mechanical stimulation by incoming sound waves deflects the ciliary bundles and thereby triggers the opening and closing of mechano-sensitive ion channels in the stereocilia membrane [19]. These unique mechanical properties are due to a particular membrane protein that is specifically expressed in outer hair cells – prestin. Prestin is a contractile protein. Changes in its size translate the sound pressure-induced vibrations of the cochlea into electrical signals [19]. Prestin is essential for hearing in all mammals. It belongs to a family of anion (chloride) transporters and is the molecular target of salicylate-related ototoxicity.

The effective form of salicylate is the negatively charged anion, generated inside the cytosol of hair cells after penetration of the nondissociated form through the cell membrane. The anion acts as a competitive antagonist at the anion (chloride)-binding site of prestin. It inhibits mechano-electrical sound transmission (otoacoustic emissions) of hair cells and subsequent generation of an action potential in a concentration-dependent manner [20].

As a result, there is reduced transmission of sound signals, arriving at the cochlea and translated into otoacoustic emissions. The spontaneous electrical activity of outer hair cells disappears [21]. This molecular mode of action of salicylate agrees well with the clinical symptoms of aspirin-induced hearing loss, specifically the dose dependency of action and its reversibility after drug removal [22–24].

These data suggest disturbed mechano-electrical sound transmission (otoacoustic emissions) by outer hair cells inside the cochlea as the primary site of salicylate-induced hearing disturbances. In vivo, medium to high aspirin doses also reduce motility and frequency selectivity of outer hair cells. Reduction in their mechano-sensory functions is particularly prominent at low sound pressure and might even result in complete disappearance of spontaneous emissions. The functional correlate of this salicylate-induced reduced hair cell motility, including increases in cell volume [25], is hearing loss after suprathreshold sound stimuli, associated with disturbed resolving power and localization of the sound source.

In vitro studies on isolated inner ear preparations confirmed that the mechanical properties of outer hair cells were reversibly impaired by salicylates in a concentration-dependent manner. As mentioned above, very high concentrations (3–10 mM) of salicylate were used in many of these settings that are unlikely to be tolerated in vivo. Alternatively, there might be a contribution of additional factors, such as inhibition of prostaglandin formation, enhanced synthesis of permeability-increasing leukotrienes or reduced cochlear blood flow as well as events in central auditory pathways. These modifying factors are missing in experiments on isolated hair cells.

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Inner hair cells. The inner hair cells transform mechanical oscillations in the cochlea into electrical signals via release of the neurotransmitter glutamate. Glutamate binds to specific glutamate (NMDA) receptors and generates electrical signals in the distal acoustic neurons, eventually resulting in sound perception in the CNS. In this context, it is interesting to note that arachidonic acid itself but not arachidonic acid metabolites potentiate NMDA-mediated signaling in neuronal cells. This suggests that arachidonic acid released by activation of NMDA (or other) receptors will potentiate NMDA receptor currents and this does not require its conversion into lipoxygenase or COX metabolites [26].

Prostaglandins and cochlear blood flow. In addition to alterations in the mechanical properties of outer hair cells, reduced cochlear blood flow is another side effect of aspirin that might reinforce hearing loss and tinnitus [4]. Inhibition of prostaglandin biosynthesis by vascular structures of the inner ear after aspirin administration augmented functional disturbances, possibly due to reduced cochlear blood flow, suggesting prostaglandins as humoral mediator of the cochlear microcirculation homeostasis [27]. Experimental studies in rabbits have shown a salicylate-induced reduction of cochlear blood flow by 30–40 % [28]. Interestingly, intracochlear perfusion with aspirin or sodium salicylate caused comparable decreases in cochlear function and blood perfusion, while traditional NSAIDs (indomethacin) failed to do so [29]. This is an argument against prostaglandins as the only pathological factor in disturbed cochlear function and suggests a salicylate-specific component, most likely the interaction of salicylate with prestin.

Independently of the role of prostaglandins, COX inhibition by aspirin could increase the synthesis of potentially ototoxic leukotrienes, such as LTC_4 [28, 30]. As a pharmacological "proof of concept" it has been shown experimentally that salicylate-associated hearing loss can be prevented by treatment with a leukotriene antagonist [28, 31]. Thus, reduced cochlear blood flow, possibly related to disturbed generation of arachidonic acid COX metabolites, might contribute to salicylate-induced ototoxicity.

3.2.4.3 Clinical studies

In subjects with normal hearing sensation, salicylate-induced hearing disturbances are bilaterally symmetric and seen at all auditory frequencies. The maximum hearing loss is about 40–50 dB [4] and similar in individuals with normal hearing and those with preexisting hearing disturbances. Hearing loss is preferentially seen at high-dose aspirin intake, i. e., 3–4 g over several days [32, 33], particularly in the elderly. This is also associated with loss of the spontaneous otoacoustic activity of the cochlea [5–7]. The intra- and interindividual variabilities are considerable. Salicylate-induced hearing disturbances are fully reversible and are correlated with the plasma salicylate level: In 16 different studies involving more than 100 individuals, there was a remark-

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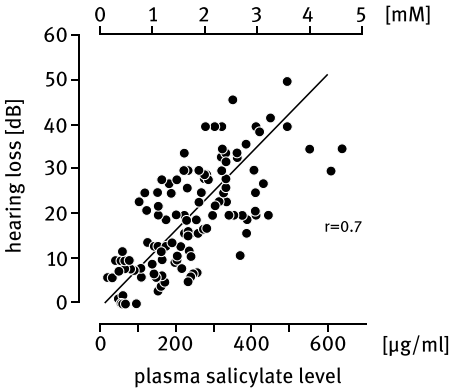

Figure 3.2.4-1: Absolute hearing loss in correlation to the plasma salicylate level of patients in 16 clinical trials. There is an approximately linear correlation between plasma salicylate level and the loss of hearing sensitivity (modified after [4]).

able linear correlation ($r = 0.7$) between absolute hearing loss and plasma salicylate levels (Fig. 3.2.4-1) [4].

Today, aspirin-associated hearing disturbances and tinnitus may become an issue for patient compliance to long-term aspirin use in primary or secondary prevention, especially in the elderly [34]. Interestingly, repeated or long-term use of OTC analgesics might also be associated with hearing disturbances. A recent post hoc analysis of the Nurses' Health Study (Section 4.3.1) indicated that regular (more than twice a week) and longer lasting (>6 years) use of NSAIDs (ibuprofen) or paracetamol increased slightly but significantly the risk of hearing disturbances in elderly female participants – not aspirin [35], confirming earlier data from the same study on women at younger age [36].

A systematic review of 37 clinical trials including a total number of >18,000 individuals showed that high-dose aspirin ingestion (≥1.95 g/day) was associated with worse audiometric results (4–112 dB threshold shift). The effect was dose-dependent and reversible in short-term use. Unfortunately, there are no audiometric data that investigated whether long-term antiplatelet doses of 81 mg or 325 mg daily had no hearing consequences [37]. Interestingly, this review even found some protective effect of aspirin when coadministered with intravenous gentamicin, although aspirin alone was rather detrimental [37], confirming earlier data from others [38].

These possibly beneficial actions of aspirin will be studied in more detail for age-related hearing loss in the ASPREE-Hearing study, a subanalysis of the ASPREE trial. This is a 3-year double-blind, randomized controlled trial of oral 100 mg/day EC aspirin versus placebo in the elderly with normal cognitive functions and no overt cardiovascular disease (Section 4.1.1). One outcome is the change in mean pure tone average hearing threshold (decibels) [39], another whether aspirin slows development or progression of age-related hearing loss, and the study will interrogate the relation-

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ship between inflammatory and microvascular mechanisms that may underlie these effects of aspirin.

Summary

Aspirin bears an ototoxic potential which is salicylate-mediated. Clinical features are hearing loss, tinnitus and imbalances. All of these disturbances are dose-dependent and consequently appear predominantly at high-dose treatment or overdosing. They are usually fully reversible and disappear within 2–3 days after drug withdrawal.

The site of salicylate ototoxicity is the cochlea and here in particular the outer hair cells. Salicylates impair the mechanical properties of hair cells and the subsequent mechano-electrical conversion of sound waves into electrical currents (otoacoustic emissions) by a specific interaction with the cochlear motor protein prestin. Additional mechanisms include interactions with arachidonic acid metabolism, eventually resulting in accumulation of free arachidonic acid and leukotriene formation, as well as a reduction of cochlear blood flow. The net response is a disturbed sound perception and localization and phantom sound (tinnitus). The interesting hypothesis that tinnitus might be due to activation of cochlear NMDA receptors needs further experimental support.

The clinical significance of salicylate-related ototoxicity is steadily decreasing after the replacement of high-dose aspirin as an antiinflammatory analgesic by NSAIDs and other nonopioid analgesics. Nevertheless, tinnitus might still occur in long-term prevention, even at low-dose aspirin, in particular in the elderly as a sign of individual (relative) overdosing, and might negatively influence patient compliance.

In addition to these toxic effects of salicylates on the inner ear at high doses, aspirin might also have possible protective effects on hearing functions at low doses because of retardation of “natural” age-related hearing loss. Studies on this issue are underway.

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3.3 Hypersensitivity to aspirin and Reye's syndrome

Typical unwanted side effects of aspirin are gastrointestinal intolerance and an increased bleeding tendency. These side effects are dose-dependent and have to be balanced individually vs. the expected therapeutic benefits. A different kind of side effects are (hyper)sensitivity reactions to aspirin. These are typically unexpected and not dose-dependent. Hypersensitivity reactions to aspirin require a particular individual (inherent or acquired) predisposition and are detected or demasked after (repeated) aspirin challenge. Three different phenotypes of these reactions can be distinguished: (i) the respiratory type, associated with eosinophilic chronic rhinosinusitis with nasal polyposis and asthma, also known as AERD, "aspirin-sensitive asthma" or Widal's disease, (ii) AECD, that is, the urticaria/angioedema type with dominant reactions at the skin and mucosae, and (iii) the systemic type with hypotension, angioedema, tachypnoea and lapses in consciousness. The systemic type is the most serious and can be even fatal, while local respiratory and cutaneous reactions are more frequent but in most cases less severe and reversible [1].

Hypersensitivity reactions to aspirin are not caused by a pathological immune reaction against aspirin as a chemical agent. Although aspirin hypersensitivity is only seen with acetylated salicylate(s), it is also most likely not the consequence of aspirin-induced acetylation of (non-COX) proteins with structural changes and their subsequent function as haptens or direct immunogens that induce generation of specific antibodies [2, 3]. Instead, there is a frequent cross-reactivity of aspirin with structurally unrelated drugs and chemicals. These chemicals, such as NSAIDs, share with aspirin the ability to block COX-1 and COX-1-dependent prostaglandin biosynthesis. Thus, the most likely explanation for aspirin hypersensitivity reactions are aspirin-induced changes (shifts) in the spectrum of eicosanoid biosynthesis, associated with reduced generation of bronchodilatory prostaglandins via the COX-1 pathway. This

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eventually also shifts the balance of local eicosanoid production towards an increased generation of leukotrienes (LTs) by enhanced precursor availability and upregulation of the LT biosynthesis pathways. Probably, all of these changes act in concert and cause the multiple clinical symptoms of aspirin hypersensitivity. This is best studied in AERD (Section 3.3.1) [4, 5].

The most frequent manifestation sites of aspirin hypersensitivity are the respiratory tract and the skin. The "Widal triad" of the respiratory tract ("aspirin-induced asthma," AERD) (Section 3.3.1) is the most intensively studied form of aspirin hypersensitivity. Manifestations of aspirin intolerance at the skin, that is, AECD, are urticaria and/or angioedema. More severe anaphylactic reactions, such as acute toxic epidermolysis (Lyell's syndrome) or Stevens–Johnson syndrome, are systemic manifestations of drug intolerance, but have no causal relationship to aspirin (Section 3.3.2).

Another disease with a possible pathological hypersensitivity to aspirin (and a number of other chemicals), specifically in small children, is Reye's syndrome (Section 3.3.3), a noninflammatory hepatoencephalopathy with severe CNS injury and even fatal outcome. However, Reye's syndrome is a descriptive term for a group of heterogenous diseases with a different etiology, induced or amplified by viral, genetic or environmental factors. The last also include a plethora of toxins and drugs, among them aspirin [6].

3.3.1 Aspirin-exacerbated respiratory disease ("aspirin-induced asthma," Widal syndrome)

3.3.1.1 History and epidemiology

A piece of history. In 1902, that is, only 3 years after the clinical introduction of aspirin as an antipyretic/antiinflammatory analgesic, *G. Hirschberg*, a General Practioner from Posen (Posen), published the first case report of a hypersensitivity reaction(dyspnoea, urticaria) occuring within 3 h after intake of 1 g of aspirin [7]. Twenty years later, *Fernand Widal* and colleagues [8] in Paris were the first to identify aspirin-induced hypersensitivity reactions as part of a new clinical entity ("Anaphylaxie et Idiosynkrasie") consisting of chronic rhinosinusitis with nasal polyposis and bronchial asthma that could be successfully treated by desensitization with aspirin. This syndrome, subsequently named the "aspirin triad" or "Widal syndrome", was later described in its clinical course by *Max Samter* and Ray F. Beers from Chicago. They named the disease "Aspirin disease" and not only confirmed the known clinical symptoms, but also detected a cross-reactivity of aspirin with other anti-inflammatory analgesics, such as indomethacin and pyrazolones. Interestingly, there was no cross-reactivity with salicylic acid and a number of other carboxylic acid esters of salicylates [9, 10]. The real breakthrough in the understanding of the pathophysiology of the disease came from the work of *Andrzej Szczeklik* and coworkers

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from Kraków (Poland). These authors showed for the first time that precipitation of an asthmatic attack by aspirin in sensitive persons (asthmatics) is related to inhibition of prostaglandin biosynthesis [11]. They also confirmed a cross-reactivity between aspirin and other NSAIDs but not with paracetamol, salicylamide and other analgesics that did not block prostaglandin biosynthesis at therapeutic doses [10, 11]. After the detection of LTs and further metabolites of arachidonic acid, such as the lipoxins (LXs), this "prostaglandin" hypothesis of "aspirin-induced asthma" was extended in the direction of a more general, aspirin-induced disturbance of eicosanoid formation and action. Reduced generation of PGE_2 via the COX-1 pathway is probably critical, specifically in conditions when the "normally" induced enhanced PGE_2 production via an upregulated COX-2 does not occur in affected tissues for yet unknown reasons [12]. Inhibition of COX-1 – the principal producer of PGE_2 under these conditions – eventually results in a shift of the spectrum of local eicosanoid production away from prostaglandins towards an increased generation of proinflammatory and spasmogenic eicosanoids, such as the cysteinyl-leukotrienes (Cys-LTs). This will be particularly effective on the background of preexisting chronic inflammatory/immunological conditions of the upper airways, associated with an activated 5-lipoxygenase pathway and upregulated Cys-LT receptors.

Epidemiology. According to epidemiological surveys, the prevalence of AERD amounts to about 0.5–2 % [13, 14] in the general population but to 10–20 % in adult asthmatics [4, 5, 15]. This suggests that AERD occurs in a measurable proportion of the population and is a relevant disease also from an epidemiologic point of view with a comparable distribution worldwide [13, 16]. However, some 15–20 % of affected persons are not aware of their disease or predisposition, respectively. In these individuals, ingestion of aspirin and other COX-1 inhibitors might induce precipitations of the airway disease that may be life threatening [13, 17].

Typical reactions in aspirin-sensitive individuals are those of an acute inflammatory immune reaction, including profuse rhinorrhea, eosinophilic rhinosinusitis and bronchospasm. The symptoms usually start subsequent to a viral infection of the upper airways. Within the following 2–5 years there are the first asthma attacks and a developing aspirin hypersensitivity [18].

3.3.1.2 Pathophysiology of AERD

General aspects. The pathophysiology of the disease is complex and multifactorial. Involved are many different cell types, mostly those that are known as sources of mediators of inflammation and immune reactions [5], most notably eosinophils and mast cells. It appears to be certain that aspirin and other COX-1 inhibitors do not initiate but rather precipitate the hypersensitivity reaction, this on the background of a preexisting inflammation of the upper and lower respiratory tract and upregulated 5-lipoxygenase/LT pathways [13, 19], that is, Cys-LTs and their receptors [20].

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The clinical symptoms upon exposition to aspirin in aspirin-sensitive persons are those of an antigen/antibody-like allergic response of the immediate type. Although IgE levels may be elevated in some of these patients, no specific antibodies against aspirin and related compounds have been found and skin tests with soluble aspirin were negative [21]. This and the cross-reactivity between aspirin and nonselective NSAIDs are arguments for pharmacological rather than immunological causes of attack precipitation, possibly related to critically reduced local PGE_2 levels subsequent to inhibition of COX-1. This will reduce antiinflammatory, spasmolytic and immunosuppressive actions of PGE_2 that otherwise would antagonize inflammatory functions of white cells [22, 23], which are the major players in AERD: Unopposed, enhanced generation of LTs, specifically Cys-LTs such as LTC_4 and LTD_4, with spasmogenic and permeability-increasing actions would then cause the symptoms of AERD [19, 24]. In addition, there are upregulated Cys-LT receptors and, possibly, also an enhanced generation of LTB_4, a (polymorphonuclear) leukocyte-"recruiting" LT [25]. Typical changes of representative eicosanoids in biopsy specimens of the nasal mucosa are shown in Fig. 3.3.1-1 [26].

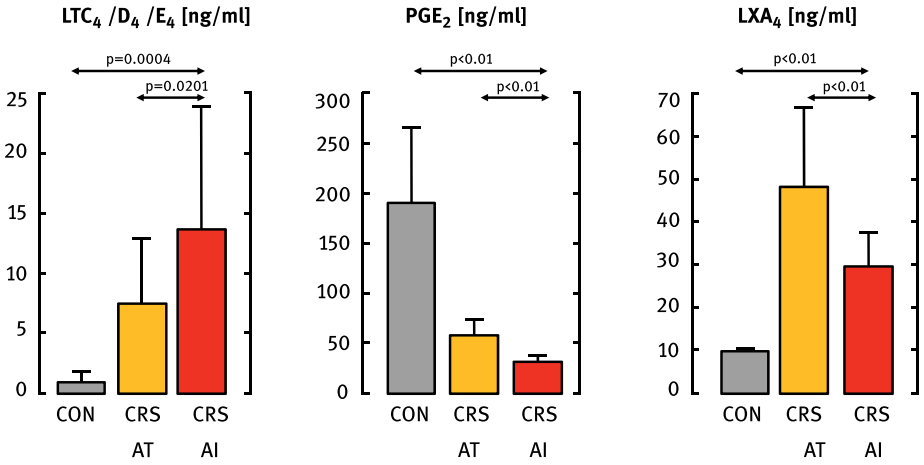

Figure 3.3.1-1: Levels of cysteinyl leukotrienes ($LTC_4/D_4/E_4$), PGE_2 und lipoxin A_4 (LXA_4) in homogenates of naso-mucosal tissue of aspirin-intolerant (AI) and aspirin-tolerant (AT) patients with chronic rhinosinusitis (CRS) and nasal polyps as compared to nasal mucosal tissue from healthy controls (CON) (modified after [26]).

Leukotriene synthesis. Cys-LTs (LTC_4, LTD_4 and LTE_4) induce bronchoconstriction, edema formation and bronchial mucus secretion. LTs are well-known mediators of asthmatic attacks, also in nonaspirin-induced asthma [25, 27]. Cys-LTs are synthesized via the intermediate LTA_4 by LTC_4 synthase, predominantly in mast cells and eosinophils [4]. The enzyme is rate limiting for synthesis of Cys-LTs (Fig. 3.3.1-2).

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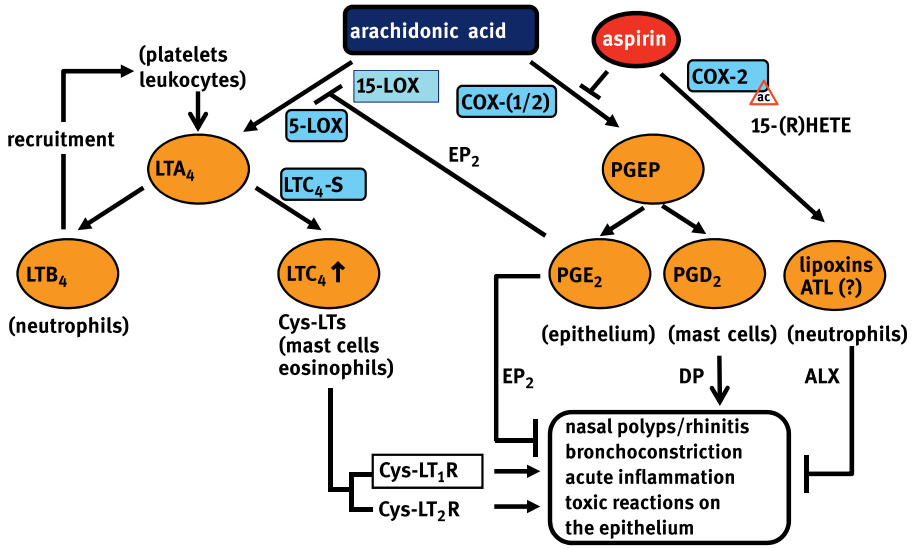

Figure 3.3.1-2: Arachidonic acid metabolism in the respiratory system via lipoxygenases (LOXs), LT synthases (LT-S) and COXs (COX-1/2) in AERD ("aspirin-sensitive asthma") – Mode of action of aspirin. Upregulation of LTC_4 synthase (Cys-LT_1) receptors enhances both LTC_4 production and action. This is further amplified by reduced generation of antiinflammatory LX. PGE_2 formation via COX-1 is inhibited by aspirin, resulting in reduced inhibition of LT formation and action. The efficacy of PGE_2 might further be reduced by downregulation of (inhibitory) EP_2 receptors. Whether there is generation of antiinflammatory lipoxin via the acetylated COX-2 (ATL) in effective concentrations is uncertain (© Dr. Schrör-Verlag, 2020). Abbreviations: ATL: aspirin-triggered lipoxin; ALX: lipoxin receptor(s); DP: PGD_2 receptor; EP2: prostaglandin-E_2 receptor; Cys-LT: cysteinyl-leukotriene; LTR: leukotriene receptor; LTC4-S: leukotriene-C_4 synthase; 15-(R)-HETE: 15(R)-hydroxyeicosatetraenoic acid; LX: lipoxin.

LTC_4 synthase but not 5-lipoxygenase is constitutively overexpressed in nasal polyps, eosinophils and mast cells of patients with AERD and accounts for enhanced LT production in the upper airways in these patients [5, 27–29]. There is evidence for a genetic upregulation of this enzyme [30]. The increased levels of Cys-LTs and PGD_2 are associated with reduced levels of PGE_2 in the sputum of AERD patients after aspirin challenge [31]. The urinary levels of the final metabolite, LTE_4, are significantly higher in aspirin-sensitive than in nonaspirin-sensitive subjects under resting conditions and are markedly increased after aspirin challenge [32–34]. These effects as well as enhanced PGD_2 formation were significantly reduced by selective Cys-LT receptor antagonists, suggesting some feedback interaction between the two [34, 35]. For these reasons, both urinary LTE_4, the end product of this pathway, and PGD_2 -(metabolites) are considered biomarkers for detection of AERD [32, 36–38].

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Cys-LTs – Cys-LT_1 and Cys-LT_2 receptors. The hyperreactivity of bronchi to these mediators in aspirin-sensitive asthmatics is likely due to an increased Cys-LT receptor density [39, 40]. There are two LT receptor subtypes in the airways and inflammatory cells. The Cys-LT_1 subtype mediates airway smooth muscle contraction, mucus hypersecretion and microvascular leakage and the Cys-LT_2 subtype mediates inflammatory reactions in glands and the epithelium, including changes in vascular permeability and tissue fibrosis [25, 27, 39]. One study found that the percentage of inflammatory cells expressing the Cys-LT_1 receptor was 5-fold higher in nasal biopsies of aspirin-sensitive patients compared with controls. This hypersensitivity could be antagonized by aspirin desensitization (see below) [40]. Animal experiments have additionally shown that aspirin-induced Cys-LT generation and mast cell activation depend on platelet-adherent granulocytes and thromboxane receptors [41], suggesting an interrelationship between platelet activation and white cell functions, as also seen in other inflammatory conditions (Section 2.3.2) [42]. Although Cys-LT_1 receptors predominate on inflammatory leukocytes in aspirin-sensitive patients, Cys-LT_2 receptors have also been brought into connection with AERD and receptor polymorphisms were shown to be associated with aspirin intolerance in asthmatics [39, 43, 44]. Thus, blockade of only one receptor subtype by appropriate drugs might not be sufficient for prevention and/or treatment of AERD (see below).

Prostaglandin E_2. In the airways, PGE_2 is regarded the dominating prostaglandin with the most significant immunomodulatory, antifibrotic and bronchodilating properties. PGE_2 is synthesized preferentially by epithelial cells, fibroblasts and smooth muscle cells of the airways and prevents and reverses clinical features of aspirin-induced asthma. Most important is probably the inhibition of enhanced LT formation [13, 26, 45]. Consequently, inhibition of COX-1 activity and subsequent PGE_2 production by aspirin or related compounds might cause the precipitation of an acute attack in sensitive individuals. Several in vitro studies in homogenates of nasal polyps or other isolated cells and tissues have found reduced PGE_2 formation and enhanced production of LTs (Fig. 3.3.1-1) [26, 45]. PGE synthase-deficient mice develop an AERD-like phenotype in a model of eosinophilic pulmonary inflammation. Aspirin challenge of these animals causes sustained increases in airway resistance, along with lung mast cell activation and Cys-LT overproduction. A stable PGE_2 analog and a selective E prostanoid (EP_2) receptor agonist blocked these responses by approximately 90 % [41]. All these and further data confirm the original hypothesis of Szczeklik that the key event in aspirin-induced asthma is (local) PGE_2 deficiency in sensitive individuals [5, 24, 45, 46]. However, aspirin-precipitated asthmatic attacks are not necessarily paralleled by changes (reductions) in the systemic levels, that is, plasma levels, of PGE_2 in these patients. Whether this is only due to the local increase of eicosanoid formation (respiratory system) with subsequent dilution in the systemic circulation or additionally modified by COX-2-dependent PGE_2 produc-

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tion due to the underlying (chronic) inflammatory process remains to be determined [13, 47].

Prostaglandin D_2. Mast cells are the natural source of PGD_2 and histamine and, similarly to eosinophils, they generate huge amounts of the spasmogenic and permeability-enhancing Cys-LTs LTC_4 and LTD_4 [48]. In addition, mast cell-released preformed granules contain many inflammatory mediators, including chemokines and cytokines that promote inflammation in AERD [49]. Aspirin challenge of sensitive individuals is associated with mast cell activation and eosinophilia in sputum and blood as well as elevated plasma and urinary levels of metabolites of PGD_2 [29, 50]. Inhibition of COX-1 by aspirin should also block generation of PGD_2 and granule-derived inflammatory mediators from mast cells. Interestingly, PGD_2 levels are reduced by aspirin only in aspirin-tolerant individuals but not in subjects with aspirin-sensitive asthma [37, 51–53].

An interesting new finding was transcriptional upregulation of the 15-lipoxygenase gene in nasal polyps of patients with AERD, predominantly in epithelial cells. It was suggested that epithelial and mast cell interactions in AERD cause synthesis of 15-oxo-eicosatetraenoic acid. This might contribute to the dysregulation in arachidonic acid metabolism and the severity of asthma in AERD [54].

Lipoxins. LXs are antiinflammatory eicosanoids that can be generated by interaction of leukocytes with endothelial/epithelial cell lipoxygenases. 15-epi-LXA_4, that is, ATL, is the product of the intercellular interaction between white cell lipoxygenases and 15-(R)-HETE from acetylated COX-2 (Fig. 2.2.1-5). ATL has antiinflammatory and inflammation-resolving properties (Section 2.2.1). ATL generation was diminished in whole blood of AERD patients in the presence of aspirin but was increased in samples from aspirin-tolerant asthmatics [55]. Similar results were reported for the nasal lavage fluids after aspirin challenge [56] and homogenates of nasal polyps of aspirin-sensitive patients (Fig. 3.3.1-1). A reduced formation of antiinflammatory LXs after aspirin challenge might additionally aggravate the inflammatory process in the airways of AERD patients.

3.3.1.3 Mode of aspirin action

The prostaglandin hypothesis of "aspirin-induced asthma." This hypothesis of COX inhibition as explanation for the AERD, first formulated by Szczeklik and colleagues, has two aspects: (i) aspirin-induced inhibition of prostaglandin (PGE_2) production and (ii) activation of the LT biosynthesis pathways [19]. The last mechanism on the background of elevated LT receptor expression would then cause the typical symptoms of AERD. Downregulation of Cys-LT_1 receptors after repeated exposure to aspirin, that is, enhanced generation of Cys-LT, in desensitization procedures is also the likely

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mode of action of desensitization (see below) [40]. Today, it is generally accepted that prostaglandins which inhibit white cell function, such as PGE_2, are also natural inhibitors of white cell-derived generation of inflammatory mediators, including LTs, PGD_2, histamine and others. The efficacy of PGE_2 might additionally be reduced by downregulation of prostanoid EP_2 receptors the major target of bronchoprotective actions of PGE_2 [57–59].

COX-1 vs. COX-2. Both COX-1 and COX-2 isoforms are expressed to a similar extent in normal respiratory epithelium and bronchial tissue. Neither isoform is upregulated in bronchi of aspirin-intolerant patients as compared with aspirin-tolerant individuals [29]. Aspirin will preferentially inhibit COX-1 and has only weak inhibitory action on COX-2 in vivo. Compounds that preferentially (nimesulide) or selectively (rofecoxib) inhibit COX-2 do not induce hypersensitivity in aspirin-sensitive individuals [60, 61]. This suggests that COX-2 and/or COX-2-derived products are of minor significance in the pathophysiology of the disease. Interestingly, several experimental studies even suggested a significant transcriptional downregulation of COX-2 in fibroblasts and tissue samples (nasal polyps) prepared from patients with AERD [12, 46, 62].

Roca-Ferrer and colleagues investigated the cytokine (IL-1β)-induced expression of COX-1 and COX-2 as well as PGE_2 production in cultured fibroblasts prepared from nasal polyps of aspirin-sensitive (AERD) and not aspirin-sensitive patients.

There was a marked downregulation – or missing upregulation – of COX-2 and COX-1 in nasal polyp fibroblasts of aspirin-sensitive patients. This was associated with considerably reduced PGE_2 biosynthesis. No such effect was seen in fibroblasts from healthy controls but rather the expected marked upregulation of both COX-1 and COX-2 associated with an about 8-fold increase in PGE_2 formation after stimulation by the inflammatory cytokine IL-1β. This was associated with a doubling of PG-EP_2 receptor density in healthy controls but not in subjects with AERD (not shown).

The conclusion was that missing or insufficient upregulation of COX-1 and COX-2 might at least partially explain the predisposition of asthmatics for AERD (Fig. 3.3.1-3) [46].

These data show that local upregulation of COX-2 subsequent to stimulation by inflammatory cytokines such as IL-1β that is seen in fibroblasts of nasal mucosal tissue from healthy individuals (septal deviation) but does not occur to a comparable extent in patients with nasal polyposis, independently of whether they are aspirin-sensitive or not. The reasons for this are unknown, and more detailed studies of COX gene regulation have just been started. It would be very interesting to know whether, and if so, which transcriptional changes occur in COX-1 and COX-2 gene regulation and the mechanisms behind.

Working hypothesis. Inhibition of COX-1-dependent, PGE_2-mediated control of inflammatory/immunological processes by aspirin and related compounds in aspirin-intolerant persons will further reduce PGE_2 generation at a time when it is particularly

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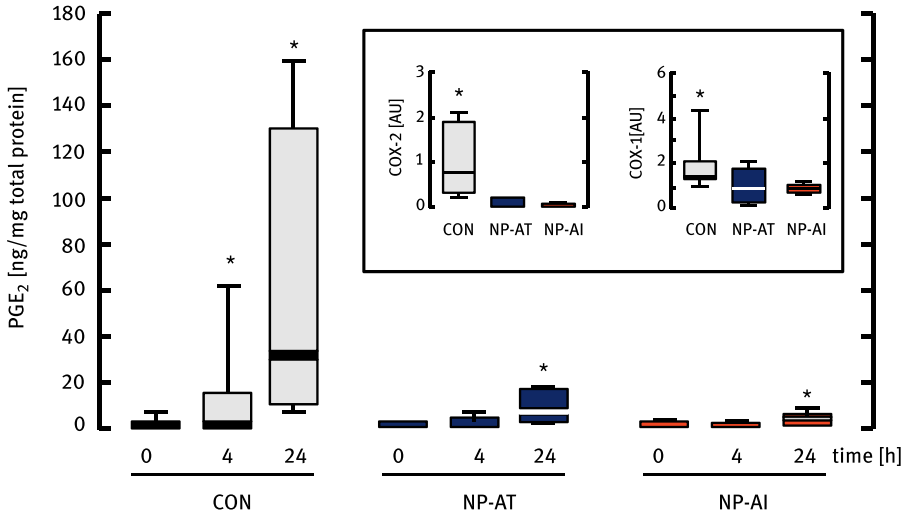

Figure 3.3.1-3: Cytokine (IL-1β)-induced biosynthesis of PGE_2 and expression of COX-2 and COX-1 (insert) in cultured nasal mucosa fibroblasts of patients with nasal polyps (NP). Patients were aspirin-intolerant (AI) or -tolerant (AT) and were compared to healthy controls (CON). Note the significant expression of COX-2 and enhanced PGE_2 biosynthesis in controls after cytokine challenge, the lower increase in aspirin-tolerant individuals and the complete absence of COX-2 induction, associated with reduced increase in PGE_2 biosynthesis in aspirin-intolerant subjects (for further explanation see text). *$P < 0.05$ vs. CON or time 0 [46].

needed to antagonize enhanced LT production and actions [47]. The situation might be further aggravated by enhanced availability of the arachidonic acid precursor for Cys-LT formation after COX inhibition, downregulation of EP_2 receptors [46, 63], reduced generation of LXs [29] and a stronger action of LTs on the (hyperreactive) bronchial epithelium of asthmatics, due to upregulation of Cys-LT receptors [39].

3.3.1.4 Clinical studies

Diagnosis. The diagnosis of aspirin intolerance can be validated by aspirin challenge. This can be done by oral ingestion or inhalation of aspirin via the nasal route in escalating doses. Oral tests are most common and are done with 30–150 mg of aspirin as a starting dose. In inhalation and nasal provocation tests, solute aspirin in the form of the water-soluble lysine salt administered as aerosol is a reliable and comparably safe procedure for diagnosis of aspirin intolerance [64].

Symptoms and clinics. AERD is an acute, inducible disease that often occurs at the background of (chronic) rhinitis, nasal polyposis and/or preexisting (severe) bronchial asthma in adults at 30–40 years of age. A genetic predisposition is likely. Typical pulmonary reactions to aspirin in intolerant individuals are bronchospasm,

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profuse rhinorrhea, conjunctival injection, periorbital edema and scarlet-like flushing of head and neck. The patients exhibit the first symptoms such as perennial rhinitis in their third decade of life, frequently subsequent to a viral upper respiratory tract infection. During the following 2–5 years, the first asthma attacks follow and an aspirin hypersensitivity develops. About 50 % of patients with aspirin intolerance already suffer from chronic severe asthma requiring steroid treatment and 30 % have moderate asthma [5]. Several actual reviews on this issue are available [4, 13, 65].

The clinical symptoms of the asthmatic hypersensitivity reaction usually begin within the first hour after aspirin intake. The asthma attacks might even be life threatening – about 25 % of asthmatic patients with intolerance against aspirin or other NSAIDs require emergency mechanical ventilation [66, 67]. Systemic corticoids are required in about half of patients.

Treatment. Desensitization to aspirin is the treatment of choice. Clinical experience shows that most if not all aspirin-sensitive patients can be desensitized by increasing doses of aspirin after repeated challenge over several days. The procedure should be performed by experts inside a specialized hospital. Specific desensitization protocols are available [1, 64, 68–70]. A frequently used technique is an aspirin inhalation test. This procedure is safer and faster to perform than the oral test. Aspirin solution is prepared as crystalline lysine-Aspirin (Aspisol®) and applied by a dosimeter-controlled nebulizer. Unlike oral challenge, inhalation of soluted aspirin does not produce systemic reactions at incremental cumulating doses from 0.18 up to 218 mg given over 3 h. These doses are only about one fourth of the cumulative oral dose, suggesting a higher efficacy of inhalation vs. oral application [69].

After successful desensitization, a maintenance dose of aspirin is recommended for at least 6–12 months or even life-long, if necessary. The duration and recommended daily doses vary between 100 and 1,300 mg/day, usually escalating doses of aspirin are used over 2–5 days until 325–650 mg of aspirin twice daily is tolerated [13]. The main effect is prevention or reduction of sinonasal symptoms and reduced requirements for corticosteroids in amelioration of asthma attacks [71]. The cellular targets of desensitization are probably the Cys-LT_1 receptors in inflammatory cells, which are downregulated in response to repeated agonist (Cys-LT) stimulation [40]. This would also explain the transient nature of this process and the necessity of further daily aspirin intake to maintain the refractory state.

Aspirin hypersensitivity and cardiocoronary prevention. One question with respect to hypersensitivity reactions is whether long-term regular aspirin intake will increase the occurrence of hypersensitivity reactions in otherwise healthy subjects. According to data from the Womens' Health Study with aspirin administration over 10 years (Section 4.1.1), this appears not to be the case [72]. A special subgroup of AERD patients

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are those who require regular long-term aspirin treatment for cardiocoronary prevention. The efficacy, safety and possible problems with desensitization protocols across a broad spectrum of patients with CAD and aspirin or NSAID hyperreactivity have been described in several recent observational trials [73–75] and metaanalyses [76]. Interestingly, it has recently been shown that patients with a self-reported allergy to aspirin or NSAIDs were at a significantly increased risk for venous thromboembolism if they received nonaspirin thromboprophylaxis following total joint arthroplasty [77].

3.3.1.5 Aspirin and other drugs

In addition to glucocorticoids as systemically active compounds in acute asthmatic attacks, pharmacological interest has been focused on compounds that more specifically interfere with the LT pathways, i. e., inhibitors of LT biosynthesis via 5-lipoxygenase or antagonists of Cys-LT receptors.

Leukotriene modifier drugs. After PGE mimetics such as misoprostol appeared not to be succesful [78], the therapeutic interest was focused on drugs interacting with the LT system. Two major classes of compounds have been introduced into the clinics: (i) antagonists of the Cys-LT_1 receptor such as montelukast [79, 80] and (ii) several other compounds interfering with leukotriene formation and action [20]. These compounds are effective against some symptoms in a majority of patients but not against all [20, 65]. The recent demonstration that mice lacking both known Cys-LT receptors exhibit a full/augmented response to Cys-LTs points to the existence of additional subtypes of Cys-LT receptors that have not been identified so far [20]. In addition, there is no LTB_4 antagonist available in the clinics to date. So far, LT modifier drugs have not fulfilled the great expectations originally associated with their design and development. Possibly, this has also to do with the complexity of the disease as well as the fact that enhanced LT production and enhanced receptor sensitivity are secondary events and, therefore, might not modify the underlying chronic (genetically determined?) inflammatory conditions of the airways but only some of their consequences.

COX inhibitors and paracetamol. All inhibitors of COX-1 appear to cross-react with aspirin and should be avoided in AERD patients. Selective COX-2 inhibitors can be used in these individuals as well as paracetamol at low doses since the compound is only a very weak inhibitor of COX-1. It has been shown, however, that about one third of patients with AERD can cross-react with paracetamol (acetaminophen) at conventional analgesic doses of 1–1.5 g [81]. Although the reactions are mild in most cases, a maximum dose of 1 g paracetamol should not be exceeded.

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Summary

A syndrome of aspirin intolerance ("Widal triad," "aspirin-sensitive asthma," "aspirin hypersensitivity," AERD) occurs with an overall incidence of ≤1 % in the general population but about 10–15 % in asthmatics. Although a genetic predisposition is likely, AERD is an induced disease after appropriate challenge and presents typically with bronchospasm, nasal polyps and profuse rhinorrhea. The symptoms start about 1 h after aspirin challenge in sensitive individuals. There is cross-reactivity with other NSAIDs which inhibit COX-1 but not with selective inhibitors of COX-2.

The disease is probably due to a pathology of eicosanoid metabolism that becomes manifest predominantly in the respiratory tract. There is increased biosynthesis of Cys-LTs in leukocytes, predominantly mast cells, but also other cells inside the respiratory tract. There is also an increased sensitivity of the upper airways against these mediators, probably related to an upregulation of Cys-LT_1 receptors and reduced LX formation. PGE_2 biosynthesis inside the affected tissues is reduced. Importantly, there is no upregulated COX-2, absolutely contrary to what would be expected in inflammatory conditions. Production of bronchoprotective PGE_2 under these conditions becomes critically dependent on COX-1. Prevention of COX-1-mediated PGE_2 biosynthesis in these patients will evoke acute attacks which might be life threatening in sensitive individuals.

In addition to treatment of symptoms, treatment of choice is controlled desensitization by aspirin challenge. This is done by repeated, incremental increase in dosing (oral or by inhalation) over several days to weeks. A maintenance dose is then necessary to keep the aspirin-tolerant state. Several treatment protocols are available for patients, including those who require rapid desensitization because of regular long-term aspirin treatment in primary prevention.

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3.3.2 Urticaria/angioedema, Stevens–Johnson syndrome and Lyell syndrome

In addition to AERD ("aspirin-induced asthma") (Section 3.3.1), AECD is the aspirin-induced hypersensitivity reaction of the skin [1]. Angioedema occurs when urticaria is located within the subcutis. In contrast to AERD, AECD is relatively rare and amounts to only 0.1–0.2 % in the general population [2]. In extremely rare conditions, more severe anaphylactic reactions can develop. Similarly to AERD, some genetic markers of Cys-LT overproduction have been identified and might be useful for improved diagnostic and therapeutic interventions [3].

3.3.2.1 Urticaria/angioedema

Pathophysiology and mode of aspirin action. Similarly to AERD, AECD can also occur on the background of chronic idiopathic inflammation, here urticaria, and is exacerbated by aspirin-specific modifications of eicosanoid pathways. Urticaria is associated with angioedema in 40 % of cases. Similarly to AERD, there are also certain genetically defined preconditions, especially in leukotriene-related genes and genes associated with immune functions [4] that might cause a hypersensitivity phenotype. Acute and chronic forms can be separated [5]. The symptoms last for <6 weeks and have an identifiable cause, such as exacerbation of symptoms after challenge with aspirin or nonselective NSAIDs [2, 6]. In contrast, the etiology of chronic or idiopathic urticaria is seldom identified and frequently (40–60 %) associated with elevated IgE

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levels. The syndrome is often the cutaneous manifestation of an autoimmune disease without any causal relation to aspirin and related compounds [5].

About 20–30 % of chronic urticaria patients experience symptom aggravation when exposed to aspirin or NSAIDs [4, 7]. A recent studied identified one quarter of children and adults with chronic urticaria as being aspirin-hypersensitive [8]. The pathophysiological reason is overproduction of leukotrienes in the skin and subcutaneous tissue after inhibition of COX-1-mediated PGE_2 formation [6, 9]. Leukotrienes increase vascular permeability and enhance local inflammatory skin reactions including urticaria [10]. In addition, there is increased PGD_2 formation by mast cells. Thus, the pathophysiology appears to be similar to AERD (Section 3.3.1) [11] and differs mainly in the manifestation site.

Clinical trials. Patients usually develop symptoms of urticaria and/or angioedema within 1–4 h after aspirin exposure [12]. Provocation tests confirm the diagnosis, including cross-reactivity to NSAIDs that inhibit COX-1 [13]. In a majority of patients, the hypersensitivity persists over years [12].

The possibility of desensitization to aspirin in individuals with the cross-reactive type of aspirin-induced urticaria/angioedema is controversial [14] and requires careful monitoring of the skin eruptions. Treatment protocols are available [15]. Treatment of the chronic form should be conducted according to the actual guidelines [14].

3.3.2.2 Stevens–Johnson syndrome and Lyell syndrome

Epidemiology. Stevens–Johnson syndrome and toxic epidermal necrolysis (Lyell syndrome) are extremely rare but life-threatening immune reactions at the skin. These reactions can be caused by various medications, including NSAIDs [16]. A large epidemiological trial in the USA including about 260,000 patients who were treated with several drugs including NSAIDs and aspirin over 16 years did not detect any increased incidence of these diseases in relation to aspirin use [17]. Similar results were obtained in a retrospective epidemiological trial in France. There were 333 cases of Lyell syndrome which were seen over an observation period of 5 years. The incidence was up to 1.3 cases per 1 million inhabitants and year. This confirms that Lyell syndrome is an extremely rare disease, although fatal in 30 % of cases. Aspirin as a possible risk factor could be excluded: The risk estimate for occurrence of the syndrome in aspirin-treated subjects was 1.1 as opposed to 1.9 for diclofenac, 4.0 for piroxicam, 13 for fenbufen and 18 for oxyphenbutazone [18].

Similar results were obtained in a European case-control study conducted between 1989 and 1995 in Germany, France, Italy and Portugal. This study searched specifically for Stevens–Johnson syndrome and Lyell syndrome and their possible relation to salicylates, including aspirin and salicylate combinations. Among 373 cases and 1,720 controls, the multivariate RR estimate for any salicylate use was 1.3 and not

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different from controls (95 % CI: 0.8–2.2). This suggests that aspirin and other salicylates are not associated with any measurable increase of these severe anaphylactic events [19]. A recent review on the incidence of severe adverse skin reactions to NSAIDs, including also COX-2-selective inhibitors, came to similar conclusions [20].

Summary

AECD, that is, aspirin-induced urticaria/angioedema, occurs in about 0.1–0.2 % of the healthy population, and about 20–30 % of patients with chronic idiopathic urticaria might suffer an exacerbation of the disease when challenged with aspirin or nonselective NSAIDs.

The pathophysiology of the disease is not uniform and involves pharmacological and immunological mechanisms, including a genetic background. Acute aspirin-exacerbated cutaneous reactions have probably a similar pathophysiological background as aspirin-induced hypersensitivity in the respiratory system (AERD): inhibition of COX-1-dependent prostaglandin formation, allowing for uncontrolled overproduction of leukotrienes because of an upregulated 5-lipoxygenase/leukotriene pathway (Section 3.3.1).

Lyell syndrome and Stevens–Johnson syndrome are extremely rare, but severe and life-threatening diseases with manifestations at the skin. These diseases might be caused by intolerance to several drugs, including nonselective NSAIDs in very rare cases. Available epidemiological evidence does not suggest that aspirin, either alone or in combination with other drugs, is a causal factor in the pathogenesis of the disease.

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3.3.3 Reye's syndrome

3.3.3.1 History and epidemiology

A piece of history. In 1963, *Ralph Douglas Kenneth Reye*, pathologist from Sydney (Australia), and his colleagues *Graeme Morgan* and *Jim Baral* described a hitherto unrecognized disease in small children, morphologically presenting as noninflammatory encephalopathy associated with fatty degeneration of the liver [1]. The disease was clinically preceded by an initial period of "malaise," mostly associated with upper airway infections and flu-like symptoms that then proceeded to the real disease. The 21 children of his report presented to the hospital with hyperpnea, severe protracted vomiting, hypoglycemia and elevated liver enzymes. There were deteriorations in consciousness, including stupor or coma, sometimes followed by convulsions. Seventeen of these children died within the first 3 days after admission, exhibiting signs of severe encephalopathy; the surviving children recovered completely. Necropsy showed a fatty degeneration of the liver and other viscera as well as a noninflammatory cerebral edema with cell degeneration, presumably in the cortex. Reye considered this disease, which was later named after him, a

> clinicopathological entity of unknown etiology

but also stated that [he] was

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. . .not convinced that the etiology was identical in every case. . . .

Another report on 16 fatal cases of a similar disease was published a few months later in a community of North Carolina (USA) during an outbreak of influenza B [2]. Because of clinical symptoms and autopsy data, the authors speculated that this disease might be identical to that described by Reye. However, the majority (66 %) of Reye's cases was below the age of 2 years, i. e., much younger than those described in the American study, and there was no seasonal association, i. e., no preceding flu [2]. Reports on patients with similar encephalopathies had already been published sporadically since 1929 [3]. Thus, a low number of patients (children) suffering from this or a similar (hepato)encephalopathy obviously already existed, and a relation to an antecedent (viral) infection as "primer" of the disease appeared not unlikely. No relation to any drug intake was reported or even suggested. Interestingly, in Australia – very much in contrast to the USA and other countries – aspirin has reportedly not been used as a medicine for children since the 1950s [4].

Epidemiology. Besides a few single cases, there were no reports on Reye-like diseases until the 1960s – or they were not published. This changed dramatically in the 1970s. The clinical incidence of Reye's syndrome in children reached epidemiological dimensions until about 1980 but afterwards fell dramatically [4, 5]. It had been speculated that this sudden "rise and fall" of Reye's syndrome was caused by a precipitous mutation of a virus [4, 6–8]. In this context, the virulence of a particular influenza strain is not a constant, but quite complex and involves host adaptation, transmissibility, tissue tropism and replication efficacy. Different recombinations of viral genes may considerably change their behaviour, eventually resulting in a marked gain in virulence. One example could be the 1918 "Spanish Flu" pandemic [9, 10], resulting in the deaths of about 30–50 million of people. These were considerably more deaths than altogether during World War I.

The reasons for the transformation of a more or less trivial and frequent viral upper airway infection into a life-threatening follow-up disease remain unexplained, and the fact that this only occurred in a very low proportion of patients has been a matter of dispute. The primary affection of (small) children might be related to their unprotected exposure to viruses. As already suggested by Reye, the pathogenesis of the syndrome may be different with the (final) common feature of a noninflammatory hepatoencephalopathy. This points to a decisive role of disease-modifying factors.

Many of those factors have been brought into connection with Reye's syndrome: toxins, environmental factors and many kinds of drugs, presumably those that are frequently used for symptomatic treatment of febrile infections. This also includes aspirin, a drug which is subject to hepatic metabolism and potentially hepatotoxic by its actions on fatty acid metabolism and mitochondrial function (Section 2.2.3). At a first view, there may also be some parallels between Reye's syndrome and acute

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aspirin poisoning since aspirin-induced hepatic changes are preferentially found at high, toxic concentrations of the agent (Section 3.1.1). However, there is no evidence for an increased use of aspirin in the late 1970s and early 1980s, when several thousand cases of Reye's syndrome were identified in voluntary notification schemes [11] and the search for a (causal) association between Reye's syndrome and aspirin became an issue of epidemiological dimensions. A statistical correlation (with a number of limitations, see below) between the two was deducted from several, mostly retrospective, observational trials. A causal relationship, however, has never been established and probably never will, because of the current scarcity of the disease.

The "genuine" Reye syndrome (in children), subsequent to a viral infection of the upper airways or the gastrointestinal tract, is nowadays extremely random. Clinical cases of a Reye-like syndrome that are still occasionally described are frequently rather the consequence of inborn errors of metabolism (IEM), i. e., a disease of a completely different etiology and pathogenesis. Nevertheless, aspirin use in children is a matter of considerable concern and each aspirin package throughout the world contains a Reye's syndrome warning label from the FDA. This label, existing in the USA since 1986, says in its edition from April 2017:

> ...The labeling of orally or rectally administered over-the-counter drug products containing aspirin or nonaspirin salicylates as active ingredients.... is required to prominently bear the following warning: "Reye's syndrome [subheading in bold type]: Children and teenagers who have or are recovering from chicken pox or flu-like symptoms should not use this product"....

All clinical evidence for a connection between aspirin and Reye's syndrome so far comes from single case descriptions and (retrospective) case-control studies. This type of observational studies, by definition (Section 4.1), cannot determine or even prove any causal relationship between aspirin intake and Reye's syndrome. The different opinions of whether the warnings of the FDA, subsequently adopted by most other health authorities worldwide, are (still) sufficiently supported by data, is discussed in greater detail in several overviews [4, 11–16].

3.3.3.2 Clinics, laboratory and morphological findings

Clinics. The syndrome in its advanced stages is clinically dominated by a severe, acute noninflammatory encephalopathy. It is typically preceded by a prodromal viral infection, most frequently influenza B, influenza A or varicella (chicken pox), affecting the upper respiratory (more than 70 %) or the gastrointestinal tract. The symptoms last for 3–5 days and are followed by a recovery phase of another 1–3 days. In a very low number of cases, there is an abrupt onset of encephalopathy, dominated by pernicious vomiting associated with varying degrees of neurological impairment and cerebral edema. There are no focal neurological signs. Deterioration in consciousness is followed by delirium, alternating with stupor or lethargy and convulsions in 30 % of cases. The patient either recovers or proceeds to coma. Death occurs in about 30–40 %

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of cases due to brainstem dysfunction. Recovery may be complete; however, persistent neurological deficits can remain. The clinical picture is similar in children and adults [11, 17].

Laboratory findings. The laboratory findings typically indicate massive tissue breakdown with enormous losses of protein and nitrogen, associated with a severe liver pathology. In serum, alanine aminotransferase, aspartate aminotransferase and ammonia levels are manifold elevated. Pathognomonic for the disease are marked elevations in plasma free fatty acid levels with the occurrence of long-chain dicarboxylic acids. There is hypoglycemia with plasma glucose levels of <40 mg/dl but no signs of inflammation in the cerebrospinal fluid.

The primary cause of these alterations is a severe disturbance of hepatic mitochondrial function (Section 2.2.3). A defect in mitochondrial β-oxidation of free fatty acids [18] causes depletion of mitochondrial ATP storage sites [17] with subsequent impairment of all energy-dependent (hepatic) functions, including gluconeogenesis and urea synthesis. The uncoupling of oxidative phosphorylation and subsequent depletion of ATP is a general finding in all cells and tissues of the organism and markedly affects their function. The most dramatic changes are seen in neurons, which are particularly dependent on sufficient energy supply. The symptoms are aggravated by starvation and insufficient alimentary (glucose) uptake by food – events, which frequently occur during febrile viral infection, specifically in children.

Morphological findings. The major morphological alterations were already in detail described by Reye and colleagues [1]. There is glycogen depletion and marked microvesicular steatosis (extracellular fat deposition) in the liver and other organs. In electron microscopy hepatic mitochondria appear enlarged and pleiomorphic. There are proliferations of smooth endoplasmic reticulum and peroxisomes but no hepatocellular necroses. Microvesicular steatosis and depletion of hepatic glycogen stores were also seen in a retrospective trial by light microscopy of livers of children who died from salicylate poisoning [19]. These data and the "gross cerebral findings," mainly cerebral edema, identified retrospectively in this study from the patients' records were taken as evidence for a *causal* relationship between salicylates and Reye's syndrome (title of the paper!) [19]. However, ultrastructural studies were not performed. Others have reported that ultrastructural changes of liver biopsy specimens in patients with Reye's syndrome differ from those with salicylate intoxication and have strongly recommended liver biopsy with electron microscopic examination for definitive diagnosis of Reye's syndrome [20]. Finally, patients with inborn (hepatic) metabolic defects may also present with Reye-like manifestations; in these patients, the mitochondria are dysfunctional as well, but normal in size and appearance [21].

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3.3.3.3 Etiology and pathogenesis

Etiology. Reye's syndrome is a descriptive term. It covers a group of etiologically heterogeneous disorders caused by infectious, metabolic, toxic or drug-induced alterations that are characterized by the combination of liver dysfunction and noninflammatory encephalopathy [14, 21–23]. A high index of suspicion is critical for diagnosis and the "genuine" Reye syndrome must of necessity be a diagnosis of exclusion [14, 23–25]. A primary genetic background, i. e., inborn errors of metabolism (IEMs) as cause of Reye's syndrome, was not diagnosed or suffciently considered in the 1970s and 1980s, when the majority of Reye syndromes was reported. In a retrospective analysis of Reye cases from the British Isles, published in 1992, IEMs were found to account for 43 % of "revised" Reye diagnoses [24]. This leads to the question whether the "changing clinical pattern of Reye's syndrome" [25], including its today extremely rare appearance, is primarily due to changes in the virulence of viruses as mentioned above [4], the regulatory activities of the (US) health authorities [26, 27] or a more critical diagnosis [14], taking advantage of the improved laboratory approaches to identify IEMs. There is no doubt that medical progress has significantly contributed to the positive identification of (the many) factors that can induce Reye-like syndromes. A hypothetical scheme on the multifactorial etiology of Reye's syndrome is shown in Fig. 3.3.3-1.

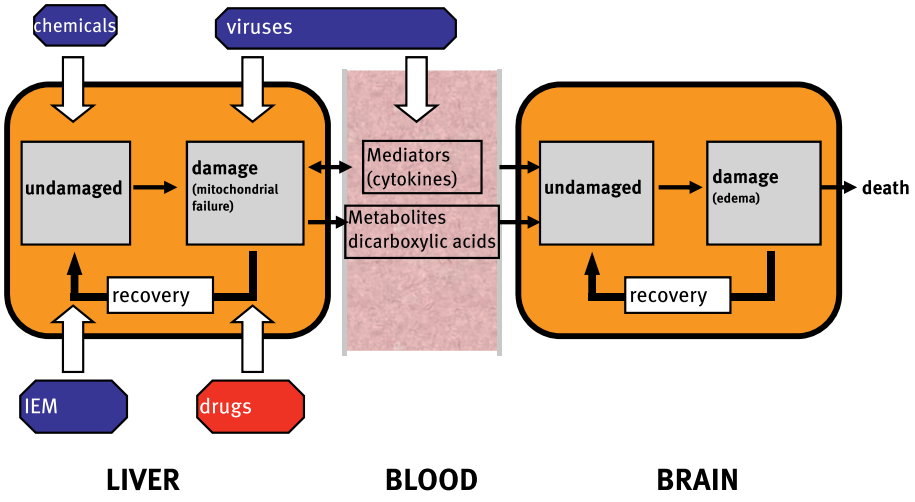

Figure 3.3.3-1: Etiology and pathogenesis of Reye's syndrome.

An altered immune response, possibly caused by viruses, initiates the release of inflammatory cytokines and other chemicals (xenobiotics) with subsequent mitochondrial injury. In the liver, this results in metabolic failure (impaired β-oxidation of long-chain fatty acids, generation of dicarboxylic acids and disturbances in carbohydrate

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and ammonia metabolism. These reactions may become aggravated by environmental factors (chemicals and toxins, pesticides, certain drugs – including aspirin). Alternatively, genetic abnormalities in the mitochondria, i. e., IEMs, with similar consequences may already exist. Hypoglycemia, hyperammonemia and dicarboxylic acids in plasma cause encephalopathy with cerebral edema and neurological deficits. Complete or partial recovery may follow or the disease progresses to death from brainstem dysfunction.

Pathogenesis – inborn errors of metabolism. Various IEMs can present clinically with Reye-like symptoms and became increasingly apparent with improved diagnostic facilities. Of particular interest in this context are inherent disorders of mitochondrial fatty acid metabolism which disturb the energy supply for cell functions via oxidative phosphorylation [28–36]. All of them might cause Reye-like symptoms, specifically after anteceding infections, and may worsen or even become fatal if exogenous alimentary energy supply is restricted for longer times than permitted by available (hepatic) glycogen stores [37].

Inborn mitochondrial failure usually becomes clinically symptomatic prior to the third year of life. Already in studies published in 1998/1999, it has been suggested that about 10–20 % of children diagnosed initially with Reye's syndrome in fact had suffered from hitherto unknown inherited metabolic disorders (mainly fatty acid oxidation or urea cycle defects) [38, 39]. An inborn disturbance in fatty acid metabolism of the liver was diagnosed in 21 out of 38 children aged <2 years as cause of an acute encephalopathy or Reye's syndrome in an observational study in Japan [30]. It is to be expected that further IEMs associated with clinical Reye symptoms will be identified in the future.

Pathogenesis – infections and immune responses. An antecedent respiratory or gastrointestinal illness – usually influenza – or chickenpox – ≤3 weeks prior to hospitalization was part of the clinical definition of Reye's syndrome in the main epidemiological trial on Reye's syndrome and medications in the US [27]. Viral infections and/or mediators, generated and released as a result of these infections, might sensitize organs such as the liver for subsequent injury by environmental factors (Fig. 3.3.3-1) [15]. Activation of systemic "host defense" reactions by inflammatory cytokines subsequent to virus infection(s) is well known and has been shown to cause depression of various CYP isoforms in the liver and many other organs. In (genetically) predisposed individuals even small doses of a drug may result in serious clinical mishaps in the presence of concomitant risk factors, such as viral infections [40]. At least 19 different viruses have been brought into connection with Reye's syndrome [11]. A number of them disturb Kupffer cell function, eventually resulting in the release of inflammatory hepatotoxic cytokines such as TNFα [41]. Cytokines such as TNFα and IL-1 cause

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metabolic alterations similar to those in Reye's syndrome. They are found in significant amounts after viral and bacterial infections (Section 2.3.2). High levels of an endotoxin-like activity were also found in the serum of patients with Reye's syndrome [42]. Thus, viral infections per se could well induce the clinical picture of Reye's syndrome. In this context, it is interesting that aspirin has even been shown to efficiently block influenza virus infections in vitro and in vivo [43, 44]. This was explained by inhibition of virus replication and propagation via inhibition of NF-κB activation in host cells in the absence of any toxic side effects of aspirin or even a drug-related tendency to induce resistant virus variants [44]. Aspirin demonstrated antiviral activity against all human rhinoviruses (HRVs) [45]. It is therefore quite unlikely that aspirin causes Reye-like symptoms via negative interactions with viruses. Rather the opposite might be the case and is currently under discussion with respect to treatment of COVID-19 infections [46].

Pathogenesis – xenobiotics and salicylates. Many external factors have been described that were brought into a connection with Reye's syndrome. These include environmental toxins, such as aflatoxin, DDT, organophosphates, toximul, polyoxyethylene, insecticides, solvents and numerous drugs, among them salicylates, phenothiazines, zidovudine, valproic acid, metoclopramide and others [14]. These agents might cause disturbances in mitochondrial metabolism, eventually resulting in mitochondrial failure with subsequent Reye-like symptoms. These symptoms might become aggravated by potentially neurotoxic drugs, such as phenothiazine-type antiemetics [14].

Salicylates at high and toxic concentrations (>1–2 mM) can alter liver mitochondrial function. Specifically, they uncouple oxidative phosphorylation and impair mitochondrial fatty acid β-oxidation, allowing for subsequent extramitochondrial ω-oxidation and generation of long-chain dicarboxylic acids (Section 2.2.3). The mitochondrial oxidation of long-chain fatty acids (palmitate) in skin fibroblasts from children who had recovered from Reye's syndrome was more sensitive to inhibition by salicylate (1–5 mM) than that in healthy controls [41] and there appeared to be some dose dependency between with aspirin intake and Reye syndrome. In most studies on Reye's syndrome, no reliable data on aspirin doses or even plasma levels of salicylates were available. There appears to be only one trial which has been conducted in a larger number of children with a clinical Reye syndrome which tried to correlate salicylate plasma levels with the severity of the disease:

The possible relationship between systemic salicylate levels and Reye's syndrome was studied in 218 children, diagnosed between 1963 and 1980 in Cincinnati. In 130 cases, the diagnosis was confirmed by liver biopsy. Question was the relationship of appearance and severity of clinical Reye's syndrome with salicylate plasma levels.

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Mean salicylate serum levels in 27 children who died or survived with neurological deficits were 150 µg/ml (range 0–460 µg/ml) but only 100 µg/ml (range 0–480 µg/ml) ($P = 0.01$) in the 103 patients who recovered completely without neurological deficits. The initial serum salicylate levels in 130 bioptically confirmed cases amount to 110–130 µg/ml. There was no correlation with the severity of the diseases (stages I–V; III–V = coma). In contrast, the serum salicylate levels in a group of 27 age-matched, untreated controls were less than 20 µg/ml.

The conclusion was that increased salicylate concentrations at admission in Reye patients could result from a higher ("excessive") dosage because of a greater severity of the prodromal illness or of diminished salicylate clearance because of an impaired hepatic function. Reye's syndrome was also identified in children without salicylate intake and in the absence of measurable salicylate in serum. The authors found it. . . . impossible to determine from these data whether salicylates are involved in the etiology of or in determining the outcome of Reye's disease [47].

Unfortunately, no information was provided in this important study about the individual aspirin dosage and the interval between the last administration of the drug and the time when the blood sample for salicylate determination was taken. The interindividual variability was considerable, and the blood level of salicylate at the time of outbreak of the disease remained unknown. The method of salicylate determination is also an issue of concern. An automated version of the colorimetric Trinder assay (Dupont-Autoanalyzer) has been used which reportedly overestimated the real salicylate levels [48]. In addition, the Trinder assay (Section 1.2.2) is not specific for salicylates in the presence of a number of chemicals: 63 (!) organic acids and amines – some of them also elevated in Reye's syndrome – may interact with the assay and can cause false positive results [49].

A comparison of the Trinder assay with more sensitive and specific HPLC technologies has shown that salicylate levels in liquor and serum of children with Reye's syndrome are not only much lower, ca. 1 % of the Trinder data, but also did not correlate with the severity of the disease [50]. In another study no increased serum salicylate levels in Reye patients were seen although salicylate-induced changes in oxidative metabolism (HPLC technology) were detectable [51]. Although a dose–response relationship between plasma salicylate levels and the severity of Reye's syndrome has been occasionally reported [52], most other published studies could not confirm this [51, 53–55].

3.3.3.4 Clinical studies

General aspects. A Reye-like disease, except for a few sporadic reports, did not exist (or was not reported) for any significant extent before 1950 and disappeared in the late 1980s [4, 5]. The reasons for this are a matter of heavy controversies and this in particular with respect to the role of aspirin as a potentially contributing or even initiating factor.

Historically, an association between salicylates and Reye's syndrome arose from the recognition that the symptoms of salicylate poisoning are often similar to the clinical manifestations of Reye's syndrome [56]. It is now known that the typical symptoms

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of salicylate poisoning, in particular those in the CNS, have a different pathophysiological background. Thus, while some patients in the early epidemiological report of Linnemann et al. in 1975 [6] had a history of excessive aspirin use, it was evident already at that time that aspirin intake alone could not explain all manifestations of Reye's syndrome and that a preceding viral infection might be a particular risk factor [6, 8, 14, 57]. Today, with markedly improved diagnostic facilities, many Reye-like syndromes can be explained by IEMs without any causative role for aspirin, while the "genuine" Reye syndrome subsequent to a viral infection apparently disappeared [4]. Any causal relationship between aspirin and Reye's syndrome has never been established. Nevertheless, it were the early observational studies, conducted in the USA and UK, which eventually resulted in the practical removal of aspirin as an antipyretic analgesic in pediatrics – with the exception of Kawasaki disease (Section 4.2.3) – and therefore require particular attention.

Studies from the USA. The first clinical data on a possible relationship between aspirin intake and Reye's syndrome came from epidemiological studies in the USA [27, 58–60]. The four initial case-control studies, also named after their respective localizations as Arizona, Ohio and Michigan 1 and 2, were widely cited by the lay and medical media and have markedly stimulated the hypothesis of a (causal) association of Reye's syndrome with salicylate intake.

In the first study from *Arizona*, a total of seven children hospitalized with Reye's syndrome in 1978 were compared with 16 control individuals. All children with Reye's syndrome had influenza A and had taken aspirin but only 50 % of controls had taken aspirin. In contrast to the Reye patients, no attempt was made in the controls to identify a viral background of the disease. Therefore, it is unknown whether the controls had the same prodromal illness as the cases [59].

The two studies from *Michigan* (1 and 2) had similar diagnostic weaknesses in terms of Reye syndrome patients and controls and also involved small numbers of patients. In addition, in the first study (Michigan 1) the final diagnosis was based upon interviews with the parents, conducted on average 6–8 weeks after the child had been diagnosed with possible Reye's syndrome. In these two studies, 30 of 46 patients with Reye's syndrome and 13 of the 29 control individuals had received aspirin. The controls did not develop Reye's syndrome, despite a similar preceding illness, mostly upper respiratory tract infections [60].

The *Ohio* study (1978–1980) was at the time both the largest and the most controversially discussed investigation [4]. In total, 94 of the 97 patients with Reye syndrome (97 %) and 110 of 156 controls (71 %) had taken aspirin. The questionnaire was revised after it became evident that not only aspirin but also other drugs, most notably phenothiazines, had been taken more frequently by Reye patients than by controls: 22 % of Reye's syndrome patients as opposed to only 4 % of controls. Phenothiazines and other antiemetics could contribute to an escalation of the viral disease and specifically to extrapyramidal reactions [61] which might have an influence on clinical outcome. Only 97 out of the total 227 cases of Reye's syndrome that were reported to the Ohio Department of Health were included in this study. Interestingly, 10 % of the included cases but 25 % of the excluded patients had varicella [58].

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Each of these studies found a statistically significant association between aspirin intake and Reye's syndrome, suggesting a "possible link" between the two. In the conclusions of the Arizona study, it was even postulated that salicylate, operating in a dose-dependent manner, represents a primary causative agent of Reye's syndrome [59]. However, all of these studies have been subject of considerable criticism, regarding the way the study was published (only the Arizona study was originally published with a detailed description of methods), the results obtained and their interpretation [4, 56, 62, 63]. All of these studies were retrospective case-control trials with small numbers of patients and three different definitions of cases. Thus, it was not proven whether the patients suffered from the "true" Reye syndrome, and bioptic confirmation of the diagnosis of Reye syndrome was rare. There were significantly fewer individuals who took aspirin in the control groups of all of the studies. Insufficient, if any, data on salicylate plasma levels and practically no data on the duration and dosage of aspirin treatment were published. Information about aspirin ingestion was usually obtained from interviews of the parents. The time interval between the presumed drug exposure and the interview varied from a few days to 3 months (!) and, in the Ohio study, these time intervals were even different between patients and controls. These and other limitations might have caused considerable bias. This includes selection bias in both the patients with Reye's syndrome and controls and confounding bias after the parents were informed in the meantime by the lay press about a possible relationship between aspirin and Reye syndrome and became uncertain about a possible aspirin treatment of their children.

With reference to these trials, a National Consensus Conference in the USA identified a strong statistical association between Reye syndrome and aspirin use in 1981 and recommended caution in the use of salicylates in children with influenza or varicella. However, it was also concluded that the role of aspirin in the development of the disease remains controversial and that salicylates are not likely to be the only factor for the development of Reye's syndrome. As a consequence, the US Public Health Service (PHS) conducted two studies: a pilot feasibility study, also in order to identify the requested number of cases and a main study [27]. The main study was among the largest epidemiological trials searching for an interaction between salicylate intake and Reye syndrome and will be discussed in more detail.

The main study was designed as a case-control study and was conducted in 1985/1986 for 17 months, including a large seasonal influenza B epidemic. Throughout the USA, initially 50 and finally 70 pediatric centers participated. Inclusion criteria were: a diagnosis of (a uniformly defined) Reye syndrome by a physician, an antecedent respiratory or gastrointestinal illness within 3 weeks before hospitalization or chicken pox and an advanced (stage II or more) degree of encephalopathy (cases). The cases were matched to randomly selected controls.

Initially, 53 patients were enrolled as cases by attending physicians. Seven of them were subsequently reclassified to another diagnosis. Further 13 cases that had been enrolled by the attending physician were later excluded by the physician review panel because other diagnoses appeared

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more likely. Of the remaining 33 cases, six patients who had Reye's syndrome as confirmed by the expert panel were not included because an antecedent illness (as defined by the inclusion criteria) was not identified. Thus 27 patients with Reye's syndrome and 140 matched control individuals were available for analysis. Of these 27 patients, three (11 %) died. Bioptic evidence for Reye's syndrome was reported for eight patients.

This mortality rate was "suspiciously" low. The number of Reye cases was also much lower than the "desired" 100–200 cases which were specified in the protocol for an appropriate analysis. However, a "strong association between salicylates (specifically aspirin) and Reye syndrome" in a (planned) midpoint analysis was found. As a result of this and the increasing rarity of the disease, the study was finished prematurely at this time point.

Fifteen (!) different chemicals were given to at least 20 % of the study participants. In total, 26 of the 27 patients with Reye syndrome (96 %) and 53 of 140 control individuals (38 %) had taken salicylates, mostly aspirin, while 30 % of cases and 86 % of controls had taken paracetamol (acetaminophen). There was a highly significant difference in total and average doses of salicylates between cases and controls, both being almost 3-fold higher in the former group. The median total salicylate dose in patients with Reye's syndrome was 74 mg/kg (26 mg/kg per day, range 4–89), that is, slightly more than 5 g per one 70-kg adult.

The conclusion was that >90 % of patients with Reye's syndrome received salicylates, suggesting a strong statistical association with the ingestion of salicylates during the antecedent illness (OR for all salicylates: 40; OR for aspirin: 26). Moreover, the risk of Reye's syndrome was also related to the quantity of salicylates ingested. The overall recommendation was to "limit" the use of aspirin (and other salicylates) for the treatment of children with chicken pox and influenza-like illnesses [27].

However, essential biases also remain in this study [64, 65], most notably information (reporting) bias because of prior public knowledge about a possible link between aspirin and Reye's syndrome. This was taken as an explanation for the much lower number of cases than expected. Parents or other caregivers who were interviewed could have reported aspirin ingestion because of prior knowledge of aspirin as a risk factor. There were multiple medications in about all patients and highly variable aspirin doses prior to the onset of the disease. All information about medication came from interviews, mostly of the parents with the main health care provider. In no case blood salicylate levels were determined (compliance!). Moreover, the diagnostic criteria were relatively nonspecific and could have led to the inclusion of children with distinct genetic metabolic disorders [11]. Liver biopsies to provide morphological support for the diagnosis were only available in a minority (27 %) of patients. Another important question that could not be adequately addressed in this study was whether an increased risk of Reye syndrome was specific for aspirin or also seen with other salicylates [27]. The considerably higher OR for total salicylates vs. aspirin alone – 40 vs. 26 – would suggest a salicylate- rather than aspirin-related risk. However, the number of cases was too small to analyze this separately.

To confirm the validity of the aspirin–Reye syndrome association, another large epidemiological case-control study was undertaken to minimize possible sources of bias and, in essence, provided similar results [66]. From 129 children found eligible, 24 were classified as definite Reye's syndrome. In total, 21 of these cases (88 %) had taken

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aspirin in the prodromal illness, in contrast to only eight (17 %) of the matched controls (n = 48), resulting in a calculated OR of 35 for the relationship between aspirin and Reye syndrome that was increased to 106 in the eight children receiving high-dose aspirin (>70 mg/kg). However, from the 24 cases, only eight had liver biopsies and 12 had urinary samples collected early during the disease. It is unclear whether the 12 urinary samples included samples of the eight children who had liver biopsies and how many of the electron microscopic and enzymatic studies confirmed the diagnosis of Reye syndrome [11].

Between 1980 and 1997, a total of 1,207 cases of Reye syndrome were reported to the US Center of Disease Control. The annual peak correlated with the seasonal occurrence of viral upper respiratory tract infections. A maximum was obtained in 1980 with 555 cases reported. The number steadily declined thereafter. In 1994, the hospitalization rate for Reye syndrome in the US was estimated at 0.06/100,000 persons aged <18 years and even this low number was considered to be an overestimate. The incidence of Reye's syndrome was higher in years with epidemics of influenza B than in years with influenza A. This association was not found subsequently. Two or fewer cases have been reported per year in the US since 1994 [21]. In 2001, the incidence was estimated to be <0.03–1/100,000 individuals aged <18 years [22] and has probably not much changed since then.

Studies from the British Isles. The etiology of Reye's syndrome in the British Isles differed from that in the USA. In the early 1980s, the "high season" of the disease, the median age of patients with Reye's syndrome in Britain was lower (14 months) than that in the USA (9 years), and there was no seasonal peak in winter, that is, no clear association with seasonal influenza waves. However, an association between Reye's syndrome and preadmission aspirin was also reported in some children presenting with flu-like febrile conditions [65].

From January 1979 to December 1982, a total of 23 children aged <6 years with a diagnose of Reye's syndrome were treated in Belfast (Northern Ireland). All participants fulfilled the laboratory and clinical requirements of Reye's diagnosis. Eighteen out of these patients had received one or more drugs, 14 of them salicylate, usually soluble aspirin tablets, 75 mg each, up to four times in all.

No statistically valid relation was found between salicylates and any of the clinical or laboratory parameters studied. Follow-up at 1 to 5 years showed that 17 (74 %) of children had a complete neurological recovery, five died and one survived with severe neurological deficits.

The conclusion was that referring to the total population of Northern Ireland, amounting to 1.5 million people, among them 300,000 children aged <13 years, Reye's syndrome is a less than rare disorder with a local incidence similar to that of phenylketonuria. There is no statistical association between the severity or outcome of Reye syndrome and aspirin intake during the prodrome [67].

A total of 264 cases of Reye's syndrome were reported to the "British Reye's Syndrome Surveillance Scheme" between 1981 and 1985, when recruitment to the risk factor study for Reye's syndrome was terminated. From these, 106 patients were included

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into the study and compared with 185 control children with febrile illnesses. Within 3 weeks prior to admission, a similar overall proportion of cases and controls – 72 % vs. 68 % – had taken antipyretics, but 59 % of cases as opposed to 26 % of controls had taken aspirin ($P = 0.0005$), suggesting a significant correlation between Reye's syndrome and aspirin (but not paracetamol) exposure [65]. However, as already seen with the US-American trials, a number of inherent biases were present in this and other studies from the British Isles as well.

The incidence and possible causes of Reye's syndrome between 1982 and 1990 in the UK were studied in more detail. The authors divided their study into two parts: 4.5 years prior to and after the aspirin warnings by the British Health Authorities (June 1986).

During this time, 445 cases of Reye syndrome were reported; 91 (20 %) of those were misdiagnoses. Interestingly, 16 % of diagnoses were revised in the first period but twice as much (34 %) in the second. An explanation for this was possible misclassification and correct (re)identification as a "Reye-like" inherited metabolic disorder (of the liver). Only 33 % of Reye patients with the most severe disease but none of the patients with the lowest Reye score had reportedly taken aspirin.

According to the wide distribution of severity scores, the reported cases were considered a heterogeneous group of patients and not all cases even of the classical Reye's syndrome were considered to be aspirin-related [14, 24, 25].

According to these data, the number of misdiagnoses is not seldom but appeared to become lower by time with improved diagnostic facilities and knowledge about inherited metabolic diseases. It has been suggested by expert panels in two studies from the USA and Canada that one third [68] and up to three quarters [69] of cases definitely or probably did not have a genuine Reye's syndrome. In addition, only one third of patients with severe Reye's disease in the study of Hardie et al. [25] had taken aspirin, as opposed to the >90 % in the American case-control study and 59 % in the British risk factor study [66].

Studies from Continental Europe. Reye's syndrome has always been very rare in Continental Europe. A survey of 99 children's hospitals in Germany indicated an incidence of 0.04–0.05 cases per 100,000 children and adolescents aged <18 years between 1983 and 1985. The disease was fatal in about half of the cases [70]. Ten years later during a one-year observation period (1997) of all severe complications of varicella infections in 485 German pediatric hospitals, there was not one single case of Reye syndrome [71].

In Denmark, all pediatric departments were asked to report all cases of Reye's syndrome for the year 1979, a year of epidemic influenza B (182,500 cases between January and April). None had to report. However, there was one case (autopsy report) of a girl with a Reye-like syndrome with measles but without any further information on treatment. From this one case, an incidence of 0.09/100,000 children up to the age of 14 years was estimated for Denmark (!) [72].

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In France, 0.08/100,000 children aged <15 years were hospitalized for Reye's syndrome in 1995/1996. Of the 46 suspected cases, 14, i. e., less than one third, were classified as Reye's syndrome, five had a metabolic disorder and 27 were probably misdiagnosed. A total of eight children were exposed to aspirin, alone or in combination with other drugs [73].

In Switzerland, seven fatal cases of Reye's syndrome were diagnosed between 1971 and 1984; aspirin intake was reported in one of them [74].

In Spain, a total of 57 cases of Reye's syndrome were reported between 1980–1984. This is equivalent to an incidence of 0.12/100,000 children at the age up to 15 years. Of these cases, 40 % was reportedly pretreated with salicylates.

Thus, in Continental Europe [70, 73–76] and many other countries worldwide [16, 77–80], a majority of children diagnosed with Reye's syndrome did not take aspirin: on average <30 % (range: 0–71 %) in 11 different countries as opposed to 94 % and 88 % in the two large epidemiologic case-control studies in the USA (Table 3.3.3-1) [27, 66].

Table 3.3.3-1: Reye syndrome and aspirin intake (alone or in combination with other drugs) throughout the world. *Not all cases originally considered to be "Reye's syndrome" were positively confirmed in all studies. **Only fatal cases. ***Possible relationship to aflatoxin intake with contaminated food. ****Alone or combined with other drugs. ****50 % of patients with highest disease scores (14/28) but 0 % of patients with the lowest score (0/8) reportedly took aspirin (0/8) (modified from [15]). See also [16, 25, 27, 65–67, 69, 70, 74, 77–81].

country # of	cases*	# taking aspirin****	%	reference
Australia	49	4	8	16
France	14	8	57	69
(West) Germany	15	3	20	70
Great Britain	106	63	59	81
Great Britain	354	–	0–50****	25
Hong Kong	27	3	11	80
India	71	none	–	79
Northern Ireland	23	14	61	67
Japan	30	7	23	78
Thailand	73	52	71***	78
South Africa	21	5	22	77
Spain	57	23	40	75
Switzerland	7	1	14**	74
USA	27	26	94	27
USA	24	21	88	66

Kawasaki's disease. Another feverish disease of small children is Kawasaki's disease (Section 4.2.3), especially occurring in Japan. There, up to 200,000 children have received aspirin for treatment of the disease and to prevent disease-related coronary aneurysms. Until 2004, one case of Reye's syndrome associated with Kawasaki's dis-

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ease has been reported (and only in the Japanese literature), yielding an incidence of <0.005 % [82]. Today, 30–50 mg aspirin per day are recommended initial doses during the acute phase of the illness. It is interesting to note that in a British guideline for clinical management of Kawasaki's disease, published in 2014, that is, many years after the publication of the first US and British safety statements, a possible risk of Reye's syndrome in these (small) children is not even mentioned [83], although the British Isles used to be a hotspot of the disease. In addition, since 2002, the British "Committee on Safety on Medicines" permits the use of aspirin for children below the age of 16 years "if prescribed by a doctor."

3.3.3.5 Actual situation

Although some epidemiological data, preferentially from the USA and UK, might suggest a correlation between salicylate intake and the occurrence of Reye's syndrome, most other countries in the world did not find convincing evidence for this relationship (Table 3.3.3-1). A causal relationship has never been established and probably will not be established in the future because of the rarity of the disease and the fact that many patients who present in the clinics today with Reye-like symptoms probably suffer from genetically defined IEMs. According to the "Oxford Centre for Evidence-Based Medicine" definitions, the grade of "recommendation" between aspirin intake and the occurrence of the "classic" Reye syndrome is "C" (extrapolation from level 2/3 [retrospective] observational studies and single case reports) – not one prospective randomized trial. Certainly, among the many million children being infected worldwide with viruses of all kinds every day, there might be a few cases of Reye-like syndromes without any clear etiologic reason [4].

Benefit/risk calculations. Independently of the unclear relationship between salicylates and possible subsets of patients with Reye's syndrome sensitive to them, the question arises whether the benefits of salicylate removal – and replacement by other drugs, originally largely acetaminophen (paracetamol) – outweigh the possible risks of aspirin use [84]. It might be added that in many countries of the world aspirin and paracetamol as OTC drugs can be bought by anybody in any supermarket. In the US there was a statistical relationship between reduced sales of "baby aspirin tablets" (81 mg aspirin per tablet) and a decreased incidence of Reye's syndrome over 5 years (1980–1985) while the sales of paracetamol increased accordingly (Fig. 3.3.3-2) [85].

Inflammation of the upper airways and larynx. Aspirin possesses antiinflammatory properties which may be useful for prevention of bacterial superinfections and febrile inflammatory diseases subsequent to viral infections of the upper airways. Paracetamol at antipyretic doses does not have antiinflammatory properties but is hepatotoxic even at minor overdoses given during a few days [86, 87]. Aspirin can also cause toxic

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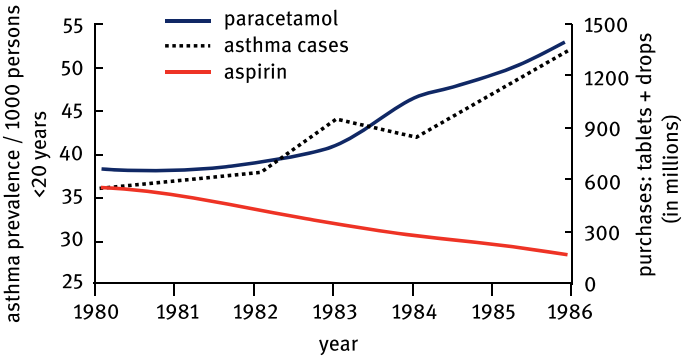

Figure 3.3.3-2: Prevalence of asthma in children and adolescents (aged <20 years) and total over-the-counter purchases of pediatric aspirin (tablets) and acetaminophen (paracetamol) (tablets + drops) in the US from 1980 to 1986 (adapted from [93]).

liver injury. However, in contrast to paracetamol, these injuries are rare, seen exclusively at high (toxic) doses and generally reversible (Section 2.3.3). In contrast, toxic liver injury by paracetamol (metabolites) is largely irreversible, frequently fatal (Section 3.1.1) and the most frequent reason of acute hepatic failure (Section 3.3.2) [88].

In infections frequently occurring in children aged between 4 and 10 years, such as laryngitis/pharyngitis and otitis/sinusitis, the decision to replace aspirin by paracetamol is the decision for a less potent medication [89]. Alternatively, overstating the risk of aspirin use may cause a compensatory increase in the use of other NSAIDs which also have adverse effects and are not better tolerated [90]. In this context, it has even been warned not to use aspirin in African children for treatment o feverish diseases because of a hypothetical Reye syndrome or even to replace aspirin by potentially hepatotoxic agents such as paracetamol [91]. Currently, ibuprofen and paracetamol are the most frequently used alternatives to aspirin for treatment of fever in children [92]. However, these drugs are also not free from severe side effects (see below).

Asthma. One long-term risk of replacing aspirin by paracetamol is the possible facilitation of allergic sensitization (asthma) in genetically predisposed children. The prevalence in childhood asthma in the US increased by 23 % from 1970 to 1980 but nearly twice as much, i. e., by 40 %, from 1980 to 1986. Among other environmental factors, the nearly complete interruption of the use of aspirin in children with febrile respiratory infections and its replacement by paracetamol has been discussed as a possible explanation [93]. There was a close linear correlation observed in the US between the increasing use of paracetamol and the prevalence of asthma in children and adolescents at the same time that aspirin use declined (Fig. 3.3.3-2).

Meanwhile, it is well known that frequent paracetamol use can cause asthma attacks [94]. Moreover, there is a significant correlation and even dose dependency be-

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tween paracetamol use in early childhood [95] and the risk of asthma and other allergies according to the epidemiologic "International Study of Asthma and Allergies in Childhood" (ISAAC) trial, including >200,000 children and >320,000 adolescents [96, 97], as well as the prospective birth cohort study of >263,000 children in Taiwan [98]. There might even exist a dose dependency between asthma and other allergic diseases and the use of paracetamol in early childhood [96, 97]. There appears to be a clear need to establish causation for children at risk of asthma [99]. Considering the fact that paracetamol is so commonly used from a young age to adulthood, there is a demand for large prospective epidemiological trials, ideally focused on paracetamol exposure vs. placebo and asthma and other outcomes of interest [100]. Interestingly, among 45 NSAIDs consumed by 1.3 million children in four European countries, only ibuprofen showed a weak (doubled) risk for asthma exacerbation [101]. A prospective randomized double-blind trial on short-term use of ibuprofen vs. paracetamol in children with asthma and febrile illnesses did not find an increased risk with ibuprofen vs. paracetamol but rather the opposite [102], while no difference between the two was seen in another study in small children [103]. Deeper knowledge about the safety of NSAIDs and paracetamol in children is required [99, 100]. Regarding pediatric aspirin, its replacement by paracetamol in the early days of aspirin-focused Reye research may be an unrecognized but important contributor to the increase in asthma prevalence in adolescents and adults [96, 97].

Summary

Reye's syndrome is an extremely rare but severe and then often fatal hepatoencephalopathy. It presents clinically with protracted vomiting and hepatopathy with signs of diverse hepatic dysfunctions, indicating mitochondrial failure and disturbed mitochondrial energy metabolism. The consequences of hepatic failure are particularly dramatic for the CNS and include several noninflammatory neurological deficits, eventually even fatality, because of brainstem dysfunction. However, in many cases there is (complete) recovery.

The etiology of Reye-like syndromes is multifactorial. Genetic defects with IEMs are a frequent reason. The "genuine" form of Reye's syndrome is typically preceded by a viral infection of the upper airways with an intermediate disease-free interval of 3–5 days before, in extremely rare cases, further progression into severe liver injury and CNS dysfunction occur. The syndrome might also result from an unusual response of the organism to a viral infection which is determined by host genetic factors but modified by environmental factors. These include a number of pesticides, solvents, toxins and at least ten different drugs, aspirin being one of them.

The "rise and fall" of the Reye syndrome "pandemic" between the late 1970s and early 1980s is still poorly understood and finally remains unexplained. With a few exceptions, most Reye syndromes published thereafter were either due to IEMs or misdiagnoses. Thus, the "fall" of the Reye syndrome also reflects scientific progress, namely improved diagnostic procedures, the advances in molecular biology and a deeper knowledge of the pathophysiology of (hepatic) diseases.

Whether the benefit/risk ratio of drug treatment in febrile children was really improved by replacing aspirin with paracetamol in the early days of aspirin-focused Reye discussions needs to be established – not one Reye-related comparative prospective trial with these drugs is available. The missing antiinflammatory action of paracetamol for treatment of laryngitis/pharyngitis and otitis/sinusitis, frequently associated with upper airways infections, makes this compound less

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suitable for treatment of these disorders. Paracetamol may also have favored the occurrence of asthma and other allergic diseases in adolescence and adulthood. In addition, paracetamol bears a significant hepatotoxic potential even at slight overdoses in children. Ibuprofen has become another alterative to aspirin with the advantage of a potent antiinflammatory action as opposed to paracetamol but also bears a number of NSAID-typical side effects.

No causal but only a statistical relationship has been provided between aspirin intake and Reye's syndrome in epidemiological trials. These trials suffer from a number of limitations and biases. The current grade of "recommendation" is "C." Only a minority of children affected by the disease worldwide (<30 %, except the USA) had reportedly taken aspirin prior to the disease. There is no effective drug without a risk of side effects. A carefully balanced decision whether a certain medication justifies the risk of unwanted reactions is always required. This is also true for aspirin and the critical evaluation of available alternatives.

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4 Clinical applications of aspirin

General aspects. The potential clinical applications of aspirin are wide, as is its spectrum of biological activities. These include both nonselective acetylation reactions and the salicylate-related effects on cellular signaling and metabolism. However, not all of the multiple pharmacological actions of a drug are also applicable for medical purposes. This becomes immediately clear after reading the patient instructions for its use. With respect to the therapeutically recommended application(s), there are many more undesired actions of a drug which have to be considered, too. This does also apply for the therapeutic use of aspirin. High-dose effects, such as hypoglycemic, uricosuric and tocolytic actions of the compound as well as its original use for long-term treatment of rheumatic diseases, are not used anymore in clinical medicine. The main reason for this is the availability of more effective and safer therapeutic alternatives – a frequent fate of innovative, pioneering drugs in medicine.

Another issue is the selection of appropriate dosing because of the different cellular targets for aspirin and salicylate: ≤100 mg for antiplatelet/antithrombotic actions, 0.5–1.0 g for analgesic actions and doses of 1–2 g and above for stronger analgesic/antiinflammatory effects that also include metabolic actions and a contribution of the salicylate metabolite. Doses of 3 g and more are probably needed for antiviral effects. The most effective doses for antitumor actions of aspirin are not yet known. Current evidence suggests no clear dose dependency with efficacy (gastrointestinal neoplasias) starting in the range of antiplatelet doses (Table 4-1).

Table 4-1: Pharmacological actions of aspirin and their clinical application.

action	effective dose [g]	in clinical use
antiplatelet	≤0.1*–0.3	yes
analgetic	0.5–2.0	yes
antipyretic	0.5–2.0	yes
anti-inflammatory**	2.0–4.0	(yes)
metabolic / antiviral	>1.0–3.0 (?)	no
antitumorigenic	?****	(no)***
hypoglycemic	4.0–6.0	no
tocolytic	1.0–2.0	no
uricosuric	?	no

*): recommended maintenance dose for long-term use

**): 30–60 mg/kg in children (initial acute treatment of children at high vascular risk (Kawasaki disease)) ~2–4 g/70 kg adults

***): in discussion as part of primary prevention in selected persons

****): no clear dose-dependency

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Current clinical applications. The current medical interest in aspirin is focused on three areas. The first is the primary and secondary prevention of arterial and venous thrombosis, that is, prevention of (recurrent) myocardial infarction, ischemic stroke, peripheral arterial vessel occlusions and VTE. This is mainly obtained by inhibition of platelet-dependent thromboxane formation and platelet activation, secretion and aggregation. A similar mode of action probably also accounts for prevention of PIH (preeclampsia). Inhibition of platelet functions, including inhibition of generation and release of multiple autocrine and paracrine mediators with secondary actions on inflammation, immunothrombosis and other immune reactions, currently dominates the clinical use of aspirin and is also in focus of experimental-clinical research (Section 4.1).

The second, although historically first indication for aspirin is its use as antipyretic/antiinflammatory analgesic for treatment of pain, inflammation and febrile disorders. In this indication, aspirin is still among the most popular nonprescriptional drugs worldwide. The antiinflammatory effects of aspirin have found renewed interest after the detection of ATL formation by acetylated COX-2. 15-(R)-HETE, the main product of acetylated COX-2, acts in concert with white cell lipoxygenases and generates ATL with potent antiphlogistic, inflammation-resolving and tissue-protective properties. These also include improved vascular oxygen defense by stimulation of endothelial NO formation via acetylated eNOS. In addition to these "established" uses, further clinical indications for aspirin as an antiinflammatory drug are currently being investigated. Most interesting is the inhibition of NF-κB-dependent signaling pathways in the crosstalk between inflammation and vascular diseases [1]. This is of clinical interest for adjunctive treatment of severe SIRS, sepsis and ARDS and the prevention of immunothrombosis and thrombotic complications of acquired immune deficiencies (HIV) and Kawasaki disease (Section 4.2.3). In this context, the antiviral effects of aspirin have gained interest, specifically with respect to flu-like conditions and, perhaps, COVID-19 (Section 4.2).

The third area of research is focused on aspirin and tumor prevention and treatment. More than 100 studies, although mainly nonrandomized, are meanwhile available which document a reduced incidence and an overall on average by 20 % reduced cancer mortality. Whether this applies to all cancers or predominantly to those of the gastrointestinal tract is currently under intense study. Even more important is the still unsolved question, whether cancer protection by aspirin is clinically relevant. Finally. Alzheimer's disease and perhaps other forms of degenerative cognitive diseases are further potential options but currently without sufficient clinical support for beneficial actions of aspirin (Section 4.3).

Evaluation of therapeutic efficacy – risk reduction vs. event reduction. The evaluation of drug efficacy in clinical trials requires information about both efficacy and safety. This benefit/risk ratio can be calculated by determining the ratio of the num-

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ber of patients who have been successfully treated ("number needed to treat" [NNT]), referred to as the (untreated) control group, and the "number needed to harm" (NNH), that is, the inverse of the proportion of patients in the study group suffering unwanted side effects. For example, effective analgesia by aspirin, that is, clinically meaningful *reduction* of a painful *event*, can be expected in about each second treated patient, equivalent to an NNT of 2. This has to be compared with an NNH of about 40, here treated patients who suffer from (mostly) subjective intolerance symptoms, such as gastric intolerance, dizziness or fatigue, resulting in a ratio of 1/20 (Section 4.2.1).

A different issue is the evaluation of efficacy and safety in clinical prevention trials. Here, efficacy is usually expressed in terms of absolute or relative *risk reduction* (RR), referring to an untreated control group. Another expression for the relative risk is the *hazard ratio* (HR). Alternatively, the term *odds ratio* (OR) is used if the real basic risk is unknown and the efficacy of treatment is quantified by referring to a defined control group, for example in epidemiological case-control trials (see below). *Risk* and *event* are two different terms and an RR can only be determined with reference to individuals who had an event. With other words, the RR will be higher if the event rate in the particular patient population is higher and vice versa. The efficacy of cardiovascular primary and secondary prevention with aspirin is a nice example to explain this:

According to the metaanalyses of the ATTC on secondary prevention of patients with previous vascular events (myocardial infarction, stroke and/or transient ischemic attacks), the risk of a recurrent serious vascular event without aspirin prophylaxis amounted to about 2 % per year. There was a relative RR by aspirin in these patients of about 20 %: 6.7 % vs. 8.2 % per year as compared to the nonaspirin-treated patients ($P < 0.0001$).

This means that 82 out of 1,000 patients with previous cardiovascular events suffered a serious new vascular event without aspirin prophylaxis, and 67 out of 1,000 patients suffered a serious new vascular event with aspirin prophylaxis, mostly myocardial infarctions. Out of 1,000 patients, 15 were protected. In other words, 67 of 1,000 patients suffered a thrombotic event despite taking aspirin and 15 patients were protected. Thus, 1,000 patients had to be treated to avoid 15 serious vascular events, that is, NNT = 66.

In primary cardiovascular prevention, there is a reduction of (nonfatal) myocardial infarctions by 0.06 % per year ($P < 0.01$). This is an event reduction in less than 1 person per year while about 999 persons are taking aspirin, and this at the expense of an increased risk of severe bleeding events by 0.03 %. The NNT is 999. Thus, the risk (bleeding) in relation to the benefit of preventing ischemic events by aspirin in primary prevention is considerably worse than in secondary prevention.

This benefit/risk ratio (NNT/NNH) of aspirin is considered positive according to most actual guidelines on aspirin and secondary prevention. For primary prevention, the NNT at the same NNH is too high but can possibly be reduced with increasing vascular risk in certain individuals (diabetes, hypertension, hypercholesterolemia, etc.). Most actual guidelines do not generally recommend aspirin for primary prevention. (Section 4.1.1).

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Types of clinical trials. The clinical efficacy of aspirin – as of all other drugs – is determined by the individual benefit/risk ratio. The assessment of clinical efficacy is a dynamic, self-correcting process according to the development of new alternative treatment options. In contrast, the pharmacological properties of compounds will never change, just new modes of action might be detected with increasing scientific knowledge and progress in basic research. The clinical usefulness is determined by the clinical outcome in terms of clinical parameters but not in terms of pharmacological mechanisms of drug action.

Clinical trials of new drugs or drugs to be used in new indications start with estimation of safety (phase I) and feasibility (phase II), both having mechanism-based endpoints. The following phase III clinical trials determine the efficacy/safety in a prospective, predefined, randomized, controlled patient population in terms of clinical endpoints. After introduction to the market, clinical endpoints and the benefit/risk ratio in "real-life" situations are determined in phase IV clinical trials. In any case, clinical decisions should be based on the totality of the best evidence and not the results of individual studies [2].

Two main categories of clinical trials can be separated: randomized, controlled trials (RCTs) and several forms of observational trials which could be done both in a prospective or retrospective manner [3]. Another increasingly popular form of investigations are metaanalyses of earlier trials including the Cochrane database. Major advantages and disadvantages of these study designs as well as some examples from aspirin trials are summarized in Table 4-2.

Table 4-2: Types, properties and examples of clinical aspirin trials. For explanations for acronyms see "Acronyms of clinical trials."

type of trial	important advantages	important disadvantages	examples
randomized controlled (RCT)	only study type with predictive information	possible underestimation of risk because of patient selection	US-PHS WHS ISIS-2 CLASP
epidemiological observational case-control	real life conditions high number of patients	no randomization no control group no clear information about doses, duration of treatment and comedications	NHS GRACE PHS CPS-II
meta-analysis	helps to address clinical questions in the absence of data from large RCTs	pooled estimate of data no inclusion of "missing studies" study bias passed on to the meta-analysis and might affect conclusions	Antiplatelet Trialists (APT) CPS-II PARIS

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The *RCT* is the "golden standard" of data generation for drug action in the selected study population. If conducted in a double-blind prospective manner vs. placebo or another standard medication, RCTs are the only form of a trial that can prove a treatment hypothesis. Phase III RCTs stand at the end of preclinical research and, if positive, result in introduction of a new drug on the market. CAPRIE (clopidogrel), TRITON-TIMI-38 (prasugrel) and PLATO (ticagrelor) are examples of phase III clinical trials of ADP-P2Y12 antagonists that resulted in their introduction for clinical use. These trials include many patients, in most cases 10,000–20,000 or more. They are expensive and require very detailed protocols, including predefined subgroups and supervision. For these reasons, most clinical trials are *observational*, that is, hypothesis generating. Here, *cohort trials* define certain patient groups (cohorts) before the onset of a disease (myocardial infarction, cancer) and follow the further disease developments usually over many years, for example in relation to the duration of drug intake. *Case-control trials* compare the efficacy of treatment in a treated patient (case) as opposed to untreated control persons who are identical to the treated patient, except the missing treatment.

Observational trials such as case-control or cohort trials are more rapid (and cheaper!) to perform than RCTs. In addition, they can be done in both a prospective and retrospective manner and are well suited for the tailoring of future design of a prospective randomized trial, for example, the estimation of required case numbers per study group. Continuation of an RCT as an observational study has been done with the early cardiovascular prevention trials on aspirin. The randomized part was finished, as scheduled, after about 5 years. The studies were then continued as open trials and are currently, more than 30 years after the beginning, extremely useful for evaluation of the long-term benefit/risk ratio in cancer studies (Section 4.3.1).

Epidemiologic registry trials are noninterventional and retrospective trials. The requested information is obtained by questionnaires at predefined timepoints. Although these observational studies are nonrandomized and subject to bias because of many uncontrolled variables, they are valuable because they are closer to real-life and everyday practical medicine than RCTs with predefined patient populations and numerous inclusion and exclusion criteria. The duration is principally unlimited and the evaluation can be repeated as often as necessary. Phase IV registry trials belong to this group. They have no statistical power; however, they might be useful to detect new therapeutic – and toxic – drug actions because of the nonselected nature of data accumulation.

Metaanalyses are becoming increasingly popular in evidence-based medicine. With the increased availability of advanced computer technologies, they are also suitable for post hoc evaluation of older data. This allows for data accumulation and the evaluation of drug effects in multiple patient populations. Major advantages are the large number of cases resulting in a higher statistical power for hypothesis generation, including also more random events. In addition they have the advantage of generalization to a larger population. However, study selection and careful editing

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of data and weight of individual studies, i. e., quality and size, of included data is essential to make different studies intercomparable. Another issue is study duration and possible changes in drug efficacy/safety with time. For example, the antithrombotic efficacy of aspirin in prevention appears to be largest immediately after an acute event and more or less disappears after a treatment period of >5 years according to the large metaanalyses of the ATTC [4] and Rothwell and colleagues [5]. However, the most popular and about 3,000 times cited metaanalysis of the ATTC on primary and secondary prevention trials by aspirin in 2009 was standardized to a 2-year observation period, independent of the real study duration, which in many cases was much longer [6]. Finally, only methodologically sound studies should be included. This might cause selection bias. In the cardiovascular field, a recent study demonstrated that 56 metaanalyses reporting relationships between biomarkers and cardiovascular events exhibited considerable heterogeneity and only 13 were not affected by selection bias [7]. Similar considerations are probably also valid for the metaanalysis of studies on aspirin use and cancer mortality [8]. In addition, publication bias may arise in favor of the drug being tested if not all negative trials with this drug were also considered. This possibility exists, as well as premature finishing of studies if the results tend to become not the expected ones or in case of changes in (primary) endpoints for similar reasons. Because of this, metaanalyses are only secondary sources of information. They are hypothesis generating but do not define any causality and their messages need to be confirmed in prospective randomized trials.

The probably most real and reliable estimates of drug efficacy and safety are *Cochrane* analyses. These are systematic reviews of primary research in human health care and are internationally recognized as the highest standard in evidence-based medicine. This type of evaluation was initiated by the Scottish physician Dr. *Archibald L. Cochrane* and his book "*Effectiveness and Efficiency: Random Reflections on Health Services*" (1972). Cochrane analyses review the drug effects in published studies on interventions for prevention and treatment of diseases, according to predefined evaluation criteria. The results are published – in most cases with regular updates – by a changing authorship, in the Cochrane Library with open public access. In the case of aspirin, Dr. Cochrane was also coauthor of the first randomized, placebo-controlled trial on the efficacy of aspirin in secondary prevention of acute myocardial infarction (Section 4.1.1).

The clinical efficacy/safety endpoint. Most important for the interpretation of the study results is the choice of an appropriate clinical study endpoint. A negative primary endpoint, seen in several large clinical trials (WHS, AAAT, ProFESS), changes of the primary endpoint because of possibly negative results of the study (US-PHS) and premature stop of the study because of expected "futility" (JPPP, ASPREE) make a study difficult to interpret. In many cases, a combined vascular endpoint is chosen to increase the likelihood of significant changes in the efficacy parameters by (drug)

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treatment. Another important issue is the definition of the primary clinical endpoints, in cardiovascular studies in many cases a mix of myocardial infarction, stroke/TIA, (cardiovascular) death and severe bleeding events. Clearly, death from any cause is not equivalent to the occurrence of a first nonfatal myocardial infarction or even transient ischemic attacks. A critical review discussing the issue of "composite outcome" and the role of "funding sources" was written by Cordoba and colleagues:

> In this overview, a total of 40 randomized clinical trials published in 2008 and studying a binary composite outcome were systematically reviewed. The majority of them was cardiovascular (73 %) and 24 (60 %) of them entirely or partly industry funded.
>
> The "Composite outcome" had a median of three components (range 2–9). Death or cardiovascular death was the most important component in 33 trials (83 %). The components were not of similar importance in 28 trials (70 %); in 20 of these, death was combined with hospital admission. Other major problems were changes in the definition of the composite outcome between the abstract, methods, and results sections (13 trials); missing, ambiguous, or uninterpretable data (9 trials); and post hoc construction of composite outcomes (4 trials). Only 24 trials (60 %) provided reliable estimates for both the composite endpoint and its components, and only 6 trials (15 %) had components of similar, or possibly similar, clinical importance and provided reliable estimates. In 11 of 16 trials with a statistically significant composite, the abstract conclusion falsely implied that the effect applied also to the most important component.
>
> The conclusion was that the use of composite outcome endpoints in clinical trials might be problematic, in particular for subsequent metaanalyses. Components are often unreasonably combined, inconsistently defined, and inadequately reported. These problems will leave many readers confused, often with an exaggerated perception of how well interventions work [9].

4.1 Thromboembolic diseases

Arterial and venous thromboses. Thromboembolic vessel occlusions result from intravascular formation of a thrombus. "Thrombosis" was the term introduced by *Rudolf Virchow* in 1845 to describe this phenomenon as result of an abnormal interaction between the vessel wall and circulating blood. Cellular blood constituents, including blood platelets, were not particularly mentioned in the "Virchow-triad" of thrombosis. This view about a negligible role of platelets has changed. In addition to mechanically "plugging" a site of vessel injury as "bricks" in the building of a platelet fibrin thrombus, they also initiate the propagation phase of thrombin formation at their (activated) surface and can generate and release a bulk of chemical mediators. Many of these mediator-driven actions result from intercellular interactions, most notably of platelets with white cells and the endothelium.

Antithrombotic action of aspirin. Aspirin will modify primarily platelet-dependent events of thrombus formation via inhibition of platelet COX-1-dependent thromboxane generation. This appears to be the most relevant site of action in prevention and treatment of coronary vascular disease (CVD), i. e., myocardial infarction (Section 4.1.1) and

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(ischemic) stroke (Section 4.1.2). There is no convincing evidence that aspirin will be effective in primary prevention of peripheral arterial occlusive diseases (Section 4.1.3). However, these patients have an elevated risk for myocardial infarctions, due to comorbidities and a generalized small and large vessel disease (atherosclerosis). Therefore, antiplatelet agents, such as aspirin, have their place as supportive treatment too. Aspirin was not considered for a long time as a useful preventive of primary or recurrent venous thrombosis. This has changed after detection of the important role of platelets for growth and stability of venous thrombi (Section 4.1.4). Another relatively new clinical indication is aspirin use for reducing the risk of preeclampsia (Section 4.1.5). All these actions of aspirin are seen at antiplatelet doses of 75–325 mg/day).

Aspirin "resistance" (High on [aspirin] treatment platelet reactivity). All the prophylactic and therapeutic uses of aspirin as an antiplatelet/antithrombotic can only be effective if aspirin-sensitive inhibition of platelet-dependent thromboxane formation is relevant to the clinical outcome. A missing or insufficient inhibition of platelet function by aspirin, the so-called aspirin "resistance" or "high on treatment platelet reactivity" (HTPR), due to an insufficient pharmacological action of aspirin is rare. Treatment failure with aspirin is more frequent and in most cases a disease-related clinical condition. The most likely explanations for this phenomenon are platelet COX-1-independent pathways of platelet activation in vivo (Section 2.3.1), negative interactions of aspirin with other drugs such as several NSAIDs (ibuprofen) and increased platelet turnover rates, for example in myeloproliferative diseases, but also – in many cases – compliance problems (Section 4.1.6). Unfortunately, (frequent) clinical treatment failures with aspirin and the (random) pharmacological inability of the drug to act are terms which are often mixed up in an inappropriate manner, and this has caused much confusion [10].

4.1.1 Coronary vascular disease

4.1.1.1 General aspects

A piece of history. The clinical use of aspirin for prevention of myocardial infarction started with the Craven trials in the early 1950s. Craven originally reported that regular use of aspirin in middle-aged males prevented myocardial infarction and stroke and strongly suggested further larger controlled trials to test aspirin systematically as an antithrombotic for these indications (Section 1.1.4). One of these first follow-up trials was the "Boston Collaborative Drug Surveillance Group Study," reporting an inverse relationship between (regular) intake of aspirin and the occurrence of reinfarctions in patients after an acute myocardial infarction [11]. The same issue of the *British Medical Journal* also published the first randomized, prospective, placebo-controlled trial on aspirin intake and the prevention of reinfarctions in a group of 1,239 men who had

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taken 300 mg/day aspirin for prevention of reinfarctions. The authors *Peter C. Elwood*, *Archibald L. Cochrane* and coworkers found that aspirin reduced the total mortality by 12 % after 6 months and by 25 % after 1 year [12]. Unfortunately, these figures were not significant in comparison to nonaspirin-treated controls and the data were considered to be "inconclusive." Therefore, the authors recommended further prospective, randomized clinical trials on aspirin and secondary prevention, preferentially in high-risk patients. The "International Study on Infarct Survival-2" (ISIS-2) has then for the first time confirmed a significant survival benefit for aspirin-treated patients with acute myocardial infarction [13]. This was confirmed in numerous follow-up trials. Since then, aspirin alone or in combination with other antiplatelet/antithrombotic drugs became the "golden standard" in secondary prevention and treatment of ACS as well as long-term prevention of recurrent coronary vascular events in patients with chronic CVD. The usefulness of aspirin in primary prevention of cardiovascular events is less clear and needs, in any case, an estimation of the individual benefit/risk ratio [14, 15].

Etiology and pathophysiology. CVD is usually a consequence of generalized atherosclerosis. The most frequent complication is myocardial infarction, subsequent to a critical thrombotic occlusion of a large coronary artery. Thrombus formation most frequently results from the rupture of an atherosclerotic plaque [16, 17]. One of the earliest events associated with plaque rupture is availability of tissue factor and subsequent thrombin generation. Thrombin generation "explodes" in the propagation phase of coagulation at the surface of activated platelets. This platelet procoagulant response is related to platelet reactivity [18, 19]. The higher fibrin content of thrombi in STEMI as opposed to non-STEMI (NSTEMI) or unstable angina indicates that the coagulation cascade, including thrombin formation, is activated to a greater degree in the former as opposed to NSTEMI or unstable angina [17]. (Autocrine) platelet activation by "exploding" platelet-dependent thromboxane formation is another trigger of initial thrombus formation. Thrombin formation and thromboxane biosynthesis are the two key, synergistically acting trigger and amplification events of arterial thrombus formation. They are both aspirin-sensitive, although inhibition of thrombin formation might require somewhat higher doses [20].

Epidemiology. Cardiovascular diseases including myocardial infarction and stroke remain the leading cause of death and a major contributor to disability worldwide [21], although with large variations between different countries [22, 23]. According to an epidemiological study in the US for the year 2015, involving about 440,000 individuals above the age of 18 years, almost 16 % of this population had angina, myocardial infarction or stroke [24]. In 2019, in the US one out of three adults aged ≥40 years reported aspirin use for cardiovascular disease prevention and approximately 46 % of adults aged ≥70 years reported primary prevention aspirin use, although there was a

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slight decrease during the last decade [25]. Similar figures appear to exist for Europe. In contrast, China's (PRC) mortality rate for cardiovascular disease is high and shows a clearly rising trend [23]. Improved drug treatment protocols, for example by introducing statins and other lipid-lowering drugs and new antidiabetics in addition to aspirin and nonaspirin antiplatelet agents, have improved the clinical outcome. A further step forward was a more intense antiplatelet treatment, that is, dual antiplatelet therapy (DAPT), mostly with aspirin and an ADP antagonist [26, 27]. While this improved the clinical efficacy, it was also associated with an increased risk of bleeding events. The introduction of new procedures for prevention and treatment of ACS, such as PCI and stenting, were further important and successful steps forward for better clinical outcome. However, these procedures were invasive and associated with endothelial injury and activation of the clotting system. Therefore, they required a more intense antiplatelet thrombosis prophylaxis, such as DAPT prior to coronary interventions. Despite these advantages, there is still a 10 % recurrence rate of atherothrombosis during the first year after the acute ischemic event [28]. Whether this can be reduced by addition of a directly acting new oral anticoagulant (NOAC) (triple therapy) at an acceptable risk of side effects is currently under discussion but appears not to be the case.

It is beyond the scope of this chapter to present and discuss all studies on aspirin and cardiocoronary prevention. This chapter is focused on a selection of historically important and clinically particularly relevant trials. Excellent reviews on the current status are available, for example the large metaanalyses of the ATTC [4, 29]. The 2002 edition contains results of all randomized trials on antiplatelet agents in secondary prevention of death, myocardial infarction and stroke until 1997. For primary prevention, there was a more reluctant view on aspirin because of a disappointing benefit/risk ratio [6]. The European Society of Cardiology (ESC) and the American Heart Association (AHA) publish the actual treatment recommendations in regular intervals.

For a more critical view, in particular on the results of randomized aspirin prevention trials, one should take into account the enormous variations in study protocols. This includes the presence or absence of CVD and its severity, accompanying other vascular and metabolic diseases, such as hypercholesterolemia, hypertension and diabetes, vasoprotective comedications such as ACE inhibitors and angiotensin receptor blockers, statins, LDL receptor-protective agents such as PCSK9 inhibitors and new oral antidiabetics. A number of excellent reviews on this issue is available [30–33].

4.1.1.2 Thrombotic risk and mode of aspirin action

Aspirin, platelets and thrombosis. In 1991, prospective evidence of an association between platelet number and reactivity with long-term incidence (13.5 years) of fatal coronary heart disease was provided for the first time in a population of 487 apparently healthy middle-aged men. Interestingly, there was no association between platelet counts and the development of angina pectoris. This suggested that the role

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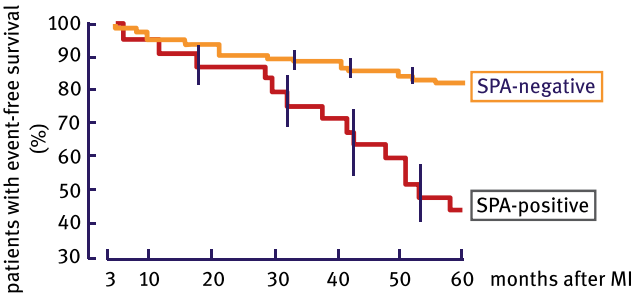
of blood platelets was rather to precipitate complications of stenotic coronary arteries but not the progression of the atherosclerotic disease itself [34]. Others have shown that the platelet-inherent capability of spontaneous aggregate formation might be also a valuable long-term predictor of vascular risk in patients at elevated vascular risk, such as patients who already had suffered a myocardial infarction but did not receive aspirin prophylaxis (Fig. 4.1.1-1) [35].



patients at risk

SPA-negative	94	92	89	85	84	81	79
SPA-positive	26	25	23	21	19	16	12

Figure 4.1.1-1: Event-free survival during 5 years of follow-up after acute myocardial infarction (MI), according to the platelet reactivity state as seen from spontaneous platelet aggregation (SPA) ex vivo without addition of platelet stimulating agents. Measurements were started at 3 months after the acute event. None of the patients received any antiplatelet treatment [35].

A significant positive interaction between ADP- and thrombin-induced platelet aggregation and electrocardiographic evidence of postmyocardial ischemia was also found in the 1,811 men who did not receive antiplatelet treatment in the CAERPHILLY study of Elwood et al. [36]. The pathogenesis of platelet hyperreactivity, the consequences for thrombotic vessel occlusions and the efficacy of aspirin in patients with stable angina are complex and variable [37, 38]. However, enhanced circulating levels of thrombin – the most potent stimulus of platelet activation – have been found in patients for at least months after an acute heart attack [28, 39], as well as elevated levels of CRP and impaired fibrinolysis. These findings strongly suggest an ischemia-induced long-lasting activation of the clotting system.

Platelets, aspirin and thromboxane. It is unlikely that blood platelets of healthy men or women in the large cardiovascular primary prevention trials had any increased "resting" activity or even increased spontaneous, platelet-related thromboxane biosynthesis. Similarly, physical exercise of healthy individuals only causes minor, aspirin-sensitive changes in platelet function despite complete inhibition of thromboxane formation [40]. Plasma thromboxane levels are also largely unchanged

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in patients with chest pain due to psychical stress. There are variable data on circulating thromboxane levels in patients with stable CVD [41–43]. In contrast to stable conditions, circulating thromboxane levels become dramatically increased in ACS – in parallel with enhanced vascular PGI_2 formation (Fig. 4.1.1-2) [43, 44].

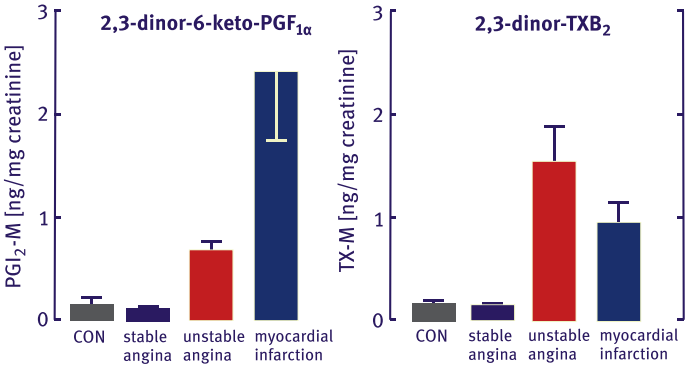

Figure 4.1.1-2: Urinary thromboxane (TX-M) and prostacyclin (PGI_2-M) metabolite excretion in non-cardiac patients (CON) as compared to patients with acute myocardial infarction (MI) and unstable or stable angina. There is a marked increase of excretion of both TX and PGI_2 metabolites in patients with ACS (unstable angina and MI) but no enhanced excretion in patients with stable angina or non-cardiac chest pain (CON) (modified after [43]).

Interestingly, aspirin treatment has no effect on the chronic recurrent form of angina pectoris due to vasospasm (Prinzmetal angina) despite inhibition of thromboxane formation, suggesting that thromboxane is unlikely to cause vasospastic angina [45].

Permanent blockade of platelet COX-1 by aspirin, associated with permanent inhibition of thromboxane formation, will protect from sudden platelet-dependent "explosion" of thromboxane formation, for example as a consequence of rupture of an atherosclerotic plaque. The proportionally higher clinical efficacy of aspirin with increasing atherothrombotic risk could then be explained by a higher probability of such sudden events in these patients. The analogy to β-receptor antagonists and their antiischemic action by preventing the deleterious effects of ischemia-induced cardiac catecholamine overflow is obvious. In addition, there is evidence for a circadian rhythm in the onset of thrombotic coronary occlusions:

> In a total of 2,999 patients admitted to the hospital with myocardial infarction, a marked circadian rhythm in the frequency of onset of ischemia was detected, with a peak from 6 a. m. to noon ($P < 0.01$). CK-MB-estimated timing confirmed the existence of a circadian rhythm, with a 3-fold increase in the frequency of onset of myocardial infarction at peak (9 a. m.) as compared with trough (11 p. m.) periods. The circadian rhythm was not detected in patients receiving β-adrenergic blocking agents before myocardial infarction. If coronary arteries become vulnerable to occlusion when

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an intima-covering atherosclerotic plaque becomes disrupted, the circadian timing of myocardial infarction may result from a variation in the tendency to thrombosis [46].

Whether this has an impact on cardiocoronary prevention by aspirin has not been studied systematically so far. However, evidence for a circadian rhythm that markedly determines the blood pressure-lowering activity of aspirin was provided for women with pregnancy induced hypertension (PIH) (Section 4.1.5) [47] and has also been shown for other cardiovascular drugs, such as statins.

Platelets, aspirin and prostacyclin. The increased vascular PGI_2 formation at advanced stages of atherosclerosis [48] is probably a consequence of COX-2 upregulation in nucleated cells [49]. This might also be the reason why high levels of PGI_2 are found in patients with ACS (Fig. 4.1.1-3) [43, 50]. This has consequences for platelet function, here, inhibition of platelet aggregation by endogenous PGI_2. High local PGI_2 levels are associated with an agonist-induced downregulation of PGI_2 receptors and subsequent resistance of platelets against inhibition by PGI_2 [51, 52]. Accordingly, PGI_2 infusion to patients with acute myocardial infarction did not reduce systemic cardiocoronary thromboxane production – in contrast to aspirin [50]. This also means that there is no reason to assume that even high-dose intravenous aspirin for first-line treatment of ACS will reduce the antiplatelet effects of endogenous PGI_2 – rather the opposite might be the case (Section 2.3.1) [53], but this has not been studied in more detail.

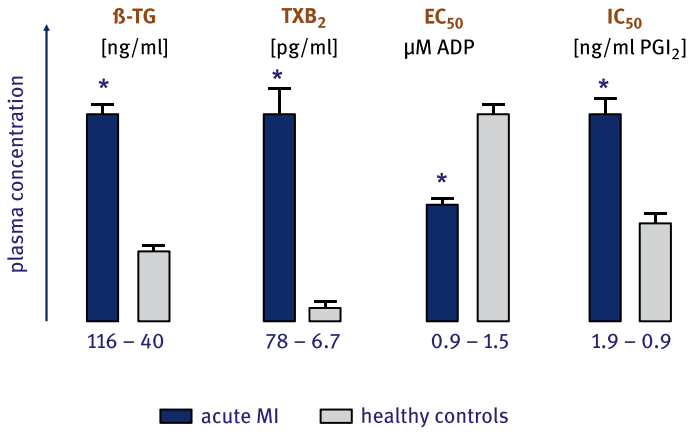

Figure 4.1.1-3: Platelet function profile in 37 patients with acute myocardial infarction (MI) as compared to 20 healthy controls. Note the increased plasma levels of β-thromboglobulin (β-TG) and TXB_2 in MI. This was associated with an increased platelet aggregation to ADP but also a reduced platelet inhibition by prostacyclin (PGI_2), possibly due to downregulation of platelet PGI_2 receptors by the acute ischemic event. *$P < 0.05$ compared with controls. None of the patients or controls was treated with aspirin or any other antiplatelet agent [51].

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Aspirin, platelets and inflammation. Platelets are not only a source of mediators that favor clot formation but also potent stimulators of inflammatory processes in the vessel wall [54–57]. In addition to promoting vessel occlusion by clot formation, platelets also prime other cells, such as monocytes/macrophages or endothelial cells, to express adhesion molecules and to participate in the inflammatory and matrix-modifying processes of vascular remodeling [58].

Several aspirin-sensitive markers of inflammation, such as CRP, fibrinogen and certain cytokines, are associated with the cardiovascular risk not only in healthy individuals but also in individuals with coronary heart disease. These are independent predictive biomarkers of acute cardiovascular events [59–63]. Many of these markers are aspirin-sensitive [64] and are significantly reduced by aspirin at antiplatelet doses (Fig. 4.1.1-4) [61].

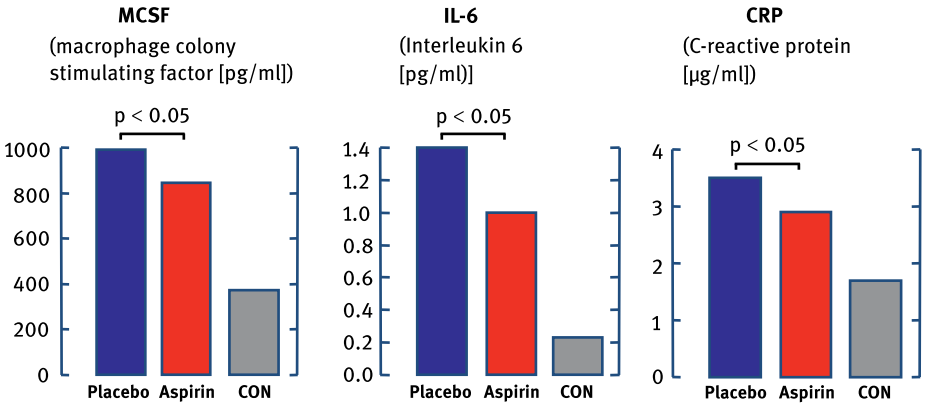

Figure 4.1.1-4: Elevated inflammatory markers in plasma of patients with chronic CVD ($n = 40$) and their reduction by aspirin treatment (300 mg/day) for 6 weeks in a randomized, double-blind crossover study versus placebo. CON: healthy untreated controls ($n = 24$) (modified after [61]).

Aspirin also exerts antiinflammatory actions via acetylation of COX-2 and subsequent generation of "aspirin-triggered lipoxin" (ATL) by interaction with white cell lipoxygenases (Fig. 2.3.2-3). ATL has been suggested to be involved in stimulation of NO formation by eNOS [65]. Aspirin induced generation of ATL [66], and improved antioxidative defense by NO formation subsequent to acetylation of eNOS has also been shown in clinical trials [67, 68].

4.1.1.3 Clinical trials – primary prevention in apparently healthy individuals

General aspects. The clinical efficacy of regular use of prophylactic aspirin in prevention of cardiovascular events is generally appreciated. Disputable is, however, the price that has be to paid, that is, the risk of bleeding, and it is also certain that there are clinically relevant differences in the benefit/risk ratio between primary and secondary

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prevention. In any case, an estimation of the individual benefit/risk ratio is strongly recommended [14, 15]. A metaanalysis of the ATTC has analyzed this benefit/risk ratio in primary and secondary prevention using an individualized approach. In addition, the incidence of serious vascular events (myocardial infarction, stroke or vascular death) was calculated on the basis of a normalized observation period of 2 years, independent of the real (in most cases much longer) study period. This is not trivial, since the antithrombotic efficacy of aspirin appears to be most pronounced in the first year(s) of administration in both primary (Fig. 4.3.1-2) [5] and secondary [29, 69] prevention. Therefore, the efficacy of aspirin in cardiovascular prevention, recalculated on an annual basis, might be apparently lower in a 10-year study (for example WHS) as opposed to a 5-year trial (US-PHS).

The ATTC study was a metaanalysis of serious vascular events (myocardial infarction, stroke or vascular death) and major bleeding events in six primary prevention trials (95,000 individuals at low average risk, 660,000 person-years, 3,554 serious vascular events) and 16 secondary prevention trials (17,000 individuals at high average risk, 4,000 person-years, 3,306 serious vascular events) that compared long-term aspirin use versus control (nonuse).

In the primary prevention trials, aspirin allocation yielded a 12 % proportional reduction in serious vascular events (0.51 % for aspirin vs. 0.57 % for controls per year; $P = 0.0001$), due mainly to a reduction of about a fifth of nonfatal myocardial infarction (0.18 % vs. 0.23 % per year; $P < 0.0001$). The net effect on stroke was not significant (0.20 % vs. 0.21 % per year; $P = 0.4$; hemorrhagic stroke: 0.04 % vs. 0.03 %; $P = 0.05$; other stroke: 0.16 % vs. 0.18 % per year; $P = 0.08$). Vascular mortality did not differ significantly (0.19 % vs. 0.19 % per year; $P = 0.7$). Aspirin allocation increased the incidence of major gastrointestinal and extracranial bleeding events (0.10 % vs. 0.07 % per year; $P < 0.0001$), and the main risk factors for coronary disease were also risk factors for bleeding.

In the secondary prevention trials, aspirin allocation yielded a greater absolute reduction in serious vascular events (6.7 % vs. 8.2 % per year; $P < 0.0001$), with a nonsignificant increase in hemorrhagic stroke but reductions of about a fifth in total stroke (2.08 % vs. 2.54 % per year; $P = 0.002$) and in coronary events (4.3 % vs. 5.3 % per year; $P < 0.0001$).

The conclusion was that in primary prevention without previous disease, aspirin is of uncertain net value as the reduction in occlusive events needs to be weighed against any increase in major bleeding events (Table 4.1.1-1) [6].

Thus, there was a 10–15-fold higher efficacy for aspirin in secondary prevention at an about 10-fold higher incidence of vascular events as opposed to primary prevention with myocardial infarction as the main risk determinant. This indicates a significant benefit of aspirin on prevention of vascular events in both conditions, although the absolute risk reduction by aspirin in primary prevention was rather small and was additionally halved by a significant increase in severe bleeding events.

Another interesting and previously unknown finding of this analysis was the practically identical *relative* reduction of event rates in primary and secondary prevention by aspirin. This suggests that the individual risk profile and not the pharmacological properties of aspirin determine the absolute clinical benefit.

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Table 4.1.1-1: Comparison of proportional and absolute effects of aspirin in primary and secondary cardiovascular prevention trials [6].

Event	Number of patients with events (aspirin / no aspirin)		Rate ratio (aspirin / no aspirin)		absolute difference [% per year]	
	Primary prevention	Secondary prevention	Primary prevention	Secondary prevention	Primary prevention	Secondary prevention
Major coronary event	934 /1115	995 / 1214	0.82	0.80	−0.06	−1.00
Non-fatal MI	596 / 756	357 / 505	0.77	0.69	−0.05	−0.66
Cardiovascular mortality	372 / 393	614 / 696	0.95	0.87	−0.01	−0.34
Stroke	655 / 682	480 / 580	0.95	0.81	−0.01	−0.46
Hemorrhagic	116 / 89	36 / 19	1.32	1.67	0.01	n.d.
Ischemic	317 / 367	140 / 176	0.86	0.78	−0.02	n.d.
Unknown	222 / 226	304 / 385	0.97	0.77	−0.001	n.d.
Vascular death	619 / 637	825 / 896	0.97	0.91	−0.01	−0.29
Any serious vascular event	1671 / 1883	1505 / 1801	0.88	0.81	−0.07	1.49
Major extracranial bleed	335 / 219	23 / 6	1.54	2.69	0.03	n.d.

However, there are also limitations of this analysis in addition to the standardized evaluation time of 2 years. For example, the event rates in the original studies that were included in this metaanalysis varied by about 10-fold and two thirds of all data were from two studies on healthy individuals without known risk factors and the – by far – lowest incidence of myocardial infarctions of all studies (US-PHS, WHS). The quality of included studies and the aspirin doses were different [70] and the available data on stroke and extracerebral bleeding events in some studies on secondary prevention were incomplete [6].

Randomized studies. Two large randomized, placebo-controlled double-blind trials on the benefit/risk ratio of long-term aspirin prophylaxis in apparently healthy, middle-aged individuals without overt risk factors are available. The "US- Physicians´ Health Study" (US-PHS) in men and the "Womens´Health Study" (WHS) in women. Another early report was the "British Male Doctors" study (BMDS).

The US-PHS was a double-blind placebo-controlled prospective trial in 22,071 apparently healthy male physicians, 40–84 years of age at entry. Exclusion criteria included a preexisting coronary heart disease, gastric intolerance to aspirin or preexisting ulcers as well as missing drug adherence during an 18-week run-in period. Eligible participants received 325 mg aspirin, 50 mg β-carotene or placebo every other day in a 2×2 factorial design. Primary study endpoint was cardiovascular death. The total study period was scheduled to 8 years.

The trial was stopped prematurely after an average treatment period of 5 years and the primary endpoint changed to "occurrence of a first myocardial infarction" because of a too low number of

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events. At this time, the total incidence of a first myocardial infarction was reduced from 189 in the placebo to 104 in the aspirin group ($P < 0.0001$). This corresponded to a relative RR by 44 %. In absolute numbers, this was equivalent to a reduction in the event rate from 0.4 % to 0.1 % per year. *Significant* protection from a first myocardial infarction was only seen in men who were 50 years of age or older. The cardiovascular mortality results were inconclusive. Regarding side effects, there was a nonsignificant, 2-fold increase in hemorrhagic strokes ($P = 0.06$) while the total stroke rate remained unaffected due to a tendency in reduction of ischemic strokes. The number of severe (blood transfusion required) bleeding events was 48 in the aspirin group (one fatal) and 28 in the placebo group (OR: 1.71; 95 % CI: 1.09–2.69; $P = 0.02$). Although this trial was not assigned to assess *gastrointestinal* effects, there was a small though significant increase in gastrointestinal hemorrhage requiring transfusion, 0.5 % in the aspirin vs. 0.3 % in the placebo group [71], and, interestingly, also an increased incidence of duodenal ulcers [72].

The conclusion was that aspirin was an effective measure to prevent a first myocardial infarction in men and that the compound should be used for this purpose if the benefit/risk ratio is appropriate [72–74].

Of interest in this context was the correlation of levels of plasmatic inflammation markers (CRP, D-dimer, fibrinogen, Lp_a) with the infarct risk, as was the reduction of these markers by aspirin treatment also in apparently healthy individuals. This was a first hint to an aspirin-sensitive platelet-mediated antiinflammatory action as an explanation for its cardioprotective effects (Fig. 4.1.1-5) [59].

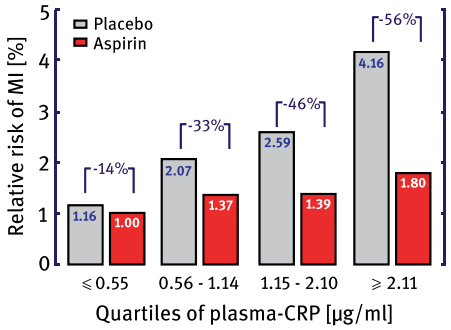

Figure 4.1.1-5: Relative risk of a first myocardial infarction (MI) in relation to the plasma level of CRP in participants of the US Physicians' Health Study (US-PHS) and the effects of aspirin. The risk, as well as the cardioprotective action of aspirin, increases in proportion to the plasma CRP levels (modified after [59]).

These pioneering findings on cardioprotective actions of aspirin were subject of numerous comments and also criticisms, mainly regarding the transfer of a 44 % prevention rate of a first myocardial infarction in the selected study population of American physicians to the nonselected populations of daily life. Also, less than 10 % of the about 260,000 doctors who were originally found eligible for the trial really entered the study [75]. This extremely healthy participating study population had an annual coronary event rate of only 0.4 %, which was markedly lower than the average rate of

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the general US population, although this number was used for calculation of the study size. It was estimated that a sufficient number of cardiovascular deaths (the original endpoint) for calculation of mortality benefits by aspirin would require to prolong the study at least for another 10 years. An unrepresentative good health status was also confirmed by the low overall mortality rate, amounting to only 15 % of the general US population. Explanations given by the investigators included high motivation of the participating doctors including personal lifestyle, educational status and a higher individual willingness to accept side effects. This is also reflected by the unusually high compliance rate of 80 % after 5 years [76]. By definition, the study could also not exclude that aspirin every day instead of every other day might have yielded higher benefits [76]. An issue of concern was the increased rate of severe bleeding events, most notably hemorrhagic strokes. Preexisting gastrointestinal intolerance to aspirin was an exclusion criterium and, therefore, the real number of patients at gastrointestinal (bleeding) risk might have been underestimated. However, a similar incidence of gastrointestinal side effects was also noted in the open British Male Doctors' trial [77] and the randomized Dutch TIA-Trial [78].

Several post hoc subgroup analyses of this and other primary prevention trials in healthy volunteers provided further interesting results. The beneficial effect of aspirin was seen shortly after start of treatment and then remained unchanged during the 5-year observation period. This suggests prevention of acute thromboembolic occlusions rather than retarded progression of atherosclerotic changes in the vessel wall as the underlying mechanism of myocardial infarction prevention [79]. In agreement with this, there was no reduced relative risk of later coronary heart disease (confirmed angina pectoris) by aspirin intake in the 331 affected study participants [80]. This also agrees with experimental findings on P2Y12 receptor antagonists, such as clopidogrel and ticagrelor. Both compounds did not retard the development of atherosclerotic lesions in mice fed a high-fat diet at doses that exerted a full antiplatelet effect. This confirmed that antiplatelet effects *per se* apparently play no role in early atherogenesis [81]. The beneficial effects of aspirin required close adherence of the participants to the study medication and disappeared if aspirin was taken less frequently than every other day (less than 150 tablets/year) [76]. Aspirin "resistance" was seen for combined intake with the intake of NSAIDs that could antagonize the antiplatelet effect of aspirin (Fig. 4.1.6-2) (Section 2.3.1) [82]. Unfortunately, in this and most of the other large randomized cardiovascular trials, no pharmacological determinations of aspirin action and patient compliance such as serum thromboxane determinations were performed or only separately in very small subgroups [83].

Two days after the first report of the US-PHS [75], the BMDS was published. In contrast to the American counterpart, no beneficial effect of aspirin on the incidence of major cardiovascular events was observed in this study [77].

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A total of 5,139 apparently healthy British male doctors were randomized to take 500 mg/day aspirin in different galenic preparations (plain, soluble, effervescent, or 300 mg enteric-coated) or no drug. Study endpoints were the incidence of and mortality from stroke, myocardial infarction and other vascular conditions. The study design was open.

After a 6 year follow-up, there was no significant difference in the rates of fatal and nonfatal myocardial infarctions between the 3,429 doctors who took aspirin and the controls who did not. The total mortality was 10 % lower in the treatment group, possibly related to other diseases than atherothrombotic events. The reduction of a first myocardial infarction was 3 % and also nonsignificant ($P = 0.889$). Similar to the US-PHS, in the British study, the number of cerebral ischemias (TIA) was reduced by about one half ($P < 0.05$) while the total number of strokes remained unchanged. The number of peptic ulcers in the aspirin group was significantly increased by 58 %. There was a tendency for an increased number of noncerebral bleeding events (43 %) in the aspirin group while the rate of fatal bleeding events was unchanged.

The conclusion was that only for patients with an appropriate history of vascular disease there appears to be clear evidence that antiplatelet treatment reduces the overall incidence of fatal or disabling vascular thrombotic disease [77].

This study also suffered from several limitations. The number of participants was much smaller – less than one quarter of that of the US-PHS – and the aspirin dose was considerably higher than in the US-PHS study. The study design was open, there was no placebo-treated control group and the definition of endpoints was less restrictive than in the American trial. About 75 % of the 20,000 doctors approached by the British investigators were judged ineligible for the trial. Thus, similar to the US-PHS, the BMDS also studied a selected population. The adherence of the study participants to treatment was quite different: 70 % for the aspirin group but 98 % for controls. The number of dropouts during the trial periods was only about 5 % in the US-PHS but 24 % in the BMDS. This was associated with a better compliance to aspirin treatment in the US-PHS as opposed to the multiple changes in the BMDS [84]. Although the participants were declared to be "apparently healthy," they obviously had a worse baseline health status than their American colleagues: 8.8 % of participants in the BMDS deceased during the trial period, as opposed to only 2.1 % of participants in the US-PHS trial. In addition, the participants were allowed to change the medication group during the treatment period if they wished to do so. At the end of the trial, 86 % of physicians in the treatment group and 14 % in the control group were taking aspirin or another antiplatelet medication [77].

The different results between the US-PHS and the BMDS were extensively discussed, because, taken together, they did not answer the question whether regular aspirin intake over years will provide protection from cardiovascular events in a low-risk population at an acceptable risk of side effects (bleeding). On the other hand, both studies showed an increased incidence of severe side effects, most notably hemorrhagic stroke [75, 84].

Neither the US-PHS nor the BMDS included women. Ischemic heart diseases in middle-aged persons are more frequent in men than in women: Below the age of 60 years, 1 out of 17 women and 1 out of 5 men suffer a myocardial infarction [85]. How-

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ever, coronary heart disease becomes an equally important cause of death and disability among women above the age of 60 years [85]. The possible benefits of aspirin in prevention of vascular events in women was investigated in the prospective, placebo (vitamin E)-controlled randomized trial on primary prevention in women, the WHS [86].

A total of 39,876 initially healthy female health care providers (>45 years of age) were randomly assigned to receive 100 mg aspirin each second day or placebo (vitamin E). Primary endpoints were cardiovascular death, nonfatal myocardial infarction and nonfatal stroke. The secondary endpoint was the individual risk in several predefined subgroups. The total observation period was 10 years.

During the follow-up, 477 major cardiovascular events occurred in the aspirin group as opposed to 522 in the placebo group. This corresponded to a nonsignificant reduction of primary endpoint events by 9 % (OR: 0.91; $P = 0.13$). Thus, the predefined primary endpoint was missed and the study as such was negative. Nevertheless, a number of subgroup analyses was done. Regarding the individual risk profiles, there was a significant reduction of ischemic stroke in the aspirin group by 24 % (OR: 0.83; $P = 0.04$) but no change in the risk of fatal or nonfatal myocardial infarctions (OR: 1.02; $P = 0.83$) or cardiovascular mortality. However, further subgroup analyses showed a 26 % reduction of major cardiovascular events, including a 22 % reduction in total stroke and a 30 % reduction in ischemic strokes among women at 65 years of age and older. There was a nonsignificant increase of hemorrhagic strokes and a significantly increased risk of severe gastrointestinal bleeding (OR: 1.40; $P = 0.02$). A total of five bleeding events in the aspirin group and three in the placebo group terminated fatally.

The conclusion was that aspirin lowered the risk of stroke in women aged ≥45 years without affecting the risk of myocardial infarction or cardiovascular death [86].

These data were at variance with the US-PHS findings in males, although they also suggested a significant protection from acute vascular ischemic events in women, here a 24 % reduction in ischemic strokes, by regular, long-term aspirin intake. In comparison to the US-PHS, where a 44 % reduction of a first myocardial infarction was found, the rate of myocardial infarctions in the WHS was much smaller: only 97/100,000 patient-years, as opposed to 440/100,000 patient-years in the US-PHS, i. e., 0.1 % vs. 0.4 % per year, at a comparable incidence of strokes in both groups [87]. This number was extremely low, amounting only to one third of the already low risk – 15 % of that of the normal American population – in the US-PHS (Table 4.1.1-2). At this low risk level, it will be very difficult or even impossible to further reduce the incidence of cardiovascular events by any preventive measure within a technically acceptable time frame. If the vascular risk becomes increased with increasing age, beneficial effects become apparent. This was seen in the US-PHS for men above the age of 50 [72] and in the subgroup of elderly women (≥65 years) in the WHS, where both ischemic strokes and myocardial infarctions were significantly reduced.

Several hypotheses were developed to explain the negative findings on cardioprotection by aspirin in women in the WHS. In addition to possible sex-specific variations, the most likely explanation is the very low basic vascular risk in medium-aged women. Another could be an insufficient compliance in the aspirin group, considering the two-day dosing interval and this over 10 years. An experimental post hoc analysis of the

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Table 4.1.1-2: Design and results from prospective randomized primary prevention trials with aspirin [6]. *325 mg (US-PHS) or 100 mg (WHS) each other day. **Start of the aspirin part only after successful reduction of diastolic blood pressure to ≤85 mmHg. ***Prematurely finished. ****Significant reduction of cardiovascular mortality ($P = 0.049$). #All patients on sotalol (for explanation of acronyms see Table "Acronyms of clinical trials").

Study	#	mean duration of follow-up [years]	Target population	Annual MI-rate in the control group [%]	Aspirin dose [mg/d]	Placebo-controlled	MI-risk reduced ($p < 0.05$)	Total mortality reduced ($p < 0.05$)
WHS	39,876	10.0	female health professionals	0.1	50*	yes	no	no
US-PHS	22,071	5.0	male doctors	0.4***	162*	yes	yes	no
BMDS	5,139	5.6	male doctors	1.0	300–500	no	no	no
PPP	4,495	3.7	men and women with ≥1 risk factor for CHD	1.2***	100	no	yes	no****
TPT	5,085	6.7	men with risk factors for CHD	1.6	75	yes	yes	no
HOT	18,790	3.8	men and women with diast. BP 100–115 mm Hg	1.6	75	yes	yes	no
SAPAT	2,035	4.2	men and women with stable CHD	3.0	75	yes#	yes	no

WHS on cardiovascular prevention has raised doubts whether the original protocol may have underestimated both the efficacy and the toxicity of aspirin [88]. A separate pilot study in a small group of 22 individuals of either sex (!) showed that aspirin given according to the WHS protocol did inhibit platelet function ex vivo but reduced thromboxane levels to only 7.5 % of control levels, with wide interindividual variations [83]. A follow-up experimental study raised doubts whether this protocol, that is, 100 mg aspirin each second day, may have underestimated both the efficacy and the toxicity of aspirin [88]. Nevertheless, the WHS has provided important new information, also with respect to (inflammatory) markers of cardiovascular risk, confirming the US-PHS data: The 122 women in the WHS who suffered a first cardiovascular event during a 3-year follow-up period had significantly higher baseline CRP levels than a matched control cohort from the trial [60] and in this respect was similar to the male counterpart of the US-PHS (Fig. 4.1.1-5).

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The currently last available large randomized study on primary prevention with aspirin in apparently healthy persons was focused on the elderly, that is, persons at advanced age (median: 74 years): the US/Australasian ASPREE trial [89–91].

A total of 19,114 persons of either sex at 70 years of age or older (or ≥65 years of age among blacks and Hispanics in the United States) who did not have known cardiovascular disease, dementia or disability were enrolled and randomized to receive 100 mg/day enteric-coated aspirin or placebo. The primary endpoint was a composite of death, dementia or persistent physical disability and mortality. Secondary endpoints included major hemorrhage and cardiovascular disease (myocardial infarction, stroke and hospitalization for heart failure). The planned study duration was 5 years.

After a median follow-up of 4.7 years the trial was stopped prematurely, because, according to an interim analysis, it appeared to be unlikely to reveal a significant treatment effect of aspirin on the primary endpoint. At this time, the rate of the composite primary endpoint was 21.5 events per 1,000 person-years in the aspirin group and 21.2 per 1,000 person-years in the placebo group (HR: 1.01; 95 % CI: 0.92–1.11; $P = 0.79$). The rate of cardiovascular events was 10.7 per 1,000 patient-years in the aspirin group and 11.3 per 1,000 patient-years in the placebo group (HR: 0.95; 95 % CI: 0.83–1.08). All-cause mortality was 12.7 events per 1,000 patient-years in the aspirin group and 11.1 events per 1,000 person-years in the placebo group (HR: 1.14; 95 % CI: 1.01–1.29). Interestingly, cancer was the major contributor to the higher mortality in the aspirin group. There were 137 upper gastrointestinal bleeding events (89 in the aspirin arm and 48 in the placebo arm (HR: 1.87, 95 % CI 1.32–2.66; $P < 0.01$) and 127 lower gastrointestinal bleeding events (73 in the aspirin arm and 54 in the placebo arm, HR: 1.36, 95 % CI: 0.96–1.94; $P = 0.08$) reflecting a 60 % increase in bleeding overall. The rate of major hemorrhages overall was 8.6 and 6.2 events per 1,000 person-years in the aspirin and placebo groups, respectively (HR: 1.38; 95 % CI: 1.18–1.62; $P < 0.001$). The adherence to study medications during the last year of the trial was 62 % and 64 %, respectively.

The conclusion was that low-dose aspirin as a primary prevention strategy caused a significantly higher risk of major hemorrhages and all-cause mortality but did not result in a significantly reduced risk of cardiovascular diseases. The increase in mortality appeared to be due to an unexpected higher rate of cancer-related deaths and should be interpreted with caution [89–91].

The study was terminated prematurely and the primary efficacy endpoint was not reached. Nevertheless, according to the available data, aspirin increased the overall gastrointestinal bleeding risk in these elderly persons by 60 %. The 5-year absolute risk of serious bleeding is modest in younger, healthy individuals. Multivariable analyses also indicated that age, smoking, hypertension, CKD and obesity might have increased the bleeding risk. For example, the absolute 5-year risk of bleeding was 0.25 % (95 % CI: 0.16–0.37 %) for a 70-year-old person not on aspirin and was increased to 5.03 % (95 % CI: 2.56–8.73 %) for an 80-year-old taking aspirin with additional risk factors. This frames convincingly the individual risk profile for bleeding. In addition, there is no information of possible measures to reduce the (gastrointestinal) bleeding risk, including eradication of *H. pylori*, regular cotreatment with PPIs or both. It is also questioned whether an apparently healthy person without cardiovascular problems should really start cardiovascular prophylaxis at a medium age of about 75 years – Craven, the pioneer in myocardial infarction prophylaxis with aspirin (Section 1.1.4), would probably not have recommended this and definitely not in the current presence

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of multiple medical and nonmedical options for treatment of concomitant diseases and avoidance of environmental factors. These might also have reduced the cardiovascular risk in the ASPREE trial. The increase in mortality was mainly driven by an increased cancer mortality as originally reported by the investigators. However, a later more detailed analysis of the effect of aspirin could not demonstrate an increase in overall cancer incidence (HR: 1.04; 95 % CI: 0.95–1.14) and CRC incidence (HR: 1.02; 95 % CI: 0.81–1.30) (Section 4.3.1) [92]. Thus, the ASPREE data may not assist patients and their clinicians to make informed decisions about prophylactic use of aspirin in the general population [93].

4.1.1.4 Clinical trials – primary prevention in individuals with vascular risk factors

General aspects. The benefit/risk ratio for aspirin as an antiplatelet agent is improved with increasing vascular risk and exposure time to risk factors. This suggests that prevention studies on older persons with preexisting risk factors will provide more convincing data than those in apparently healthy, middle-aged individuals. Importantly, multiple risk factors potentiate the vascular risk rather than acting additively. Some important studies on primary prevention in patients with risk factors as compared to those without are summarized in Table 4.1.1-3 [94].

Hypertension. Hypertension is an independent risk factor for stroke (Section 4.1.2) and myocardial infarction. On the other hand, treatment of hypertensive patients with aspirin might overproportionally increase the risk of cerebral hemorrhages. This raises the question whether hypertensives will benefit from the antiplatelet/antithrombotic actions of aspirin and whether the disease-related increased risk of (hemorrhagic) stroke persists in conditions of adequate blood pressure control. This issue was addressed in the "Hypertension Optimal Treatment" (HOT) trial [95, 96].

The study population included 18,790 patients with severe arterial hypertension, with a diastolic blood pressure between 100 and 115 mmHg (mean: 105 mmHg). All patients were treated with antihypertensives until a diastolic blood pressure of 85 mmHg or less was obtained. Afterwards, patients additionally received either aspirin (75 mg/day) or placebo. The study was aimed to assess the optimum target diastolic blood pressure and the potential additional benefit of low-dose aspirin in adequately treated hypertensives. The average observation period was 3.8 years.

The desired decrease in diastolic blood pressure was obtained in all treatment groups. Intensive lowering of blood pressure in patients with hypertension was associated with a lower rate of cardiovascular events. In comparison to the antiplatelet placebo group, there was a significant relative RR of myocardial infarctions by 36 % ($P = 0.002$) and of major cardiovascular events by 15 % ($P = 0.03$) in the group subsequently treated with aspirin, while the numbers of fatal and nonfatal strokes remained unchanged. There was a significant reduction of myocardial infarctions in men ($P = 0.001$) but not in women ($P = 0.38$). Cardiovascular and total mortality rates were also unchanged. Similar effects were seen in the subgroups of diabetics and in elderly subjects. There was no increase in fatal bleeding events (including cerebral hemorrhages) in the aspirin group but

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about twice as many nonfatal severe bleeding events – 129 vs. 70 – in the aspirin group, mainly from the gastrointestinal tract. The estimated compliance rate for aspirin was 78 %.

The conclusion was that intensive lowering of blood pressure in hypertensives is associated with a reduced rate of cardiovascular events. Aspirin treatment of these patients significantly reduces the incidence of myocardial infarctions but doubles nonfatal major bleeding events without changing cardiovascular or total mortality. Aspirin did not change the incidence of strokes or fatal bleeding events and there was no difference between diabetics and nondiabetics. Thus, daily aspirin is recommended for cardiocoronary prophylaxis to well-treated hypertensives, including elderly patients and diabetics [95, 96].

Table 4.1.1-3: Variability in baseline characteristics, compliance (compl.) and efficacy outcome of selected randomized, placebo-controlled primary prevention trials with aspirin where diabetics were included (modified from [94]).

Trial	Total study population	DM (%)	Age (years)	Men (%)	Aspirin (mg/day)	Follow up (y)	Compl. (%)	Prim. eff. EP reached?
US-PHS	22,071 healthy men	4	40–84	100	325	5.0	–	yes
ETDRS	3,711 type 1 & 2 diabetics	100	18–70	56	650	5.0	92	no
HOT	18,790 hypertensives	8	50–80	53	75	3.8	78	yes
PPP	4,495 pat. with risk factors	23	64 (average)	48	100 EC	3.6	81	yes
WHS	39,876 healthy women	3	≥45	0	100	10	–	no
POPADAD	1,276 type 1 & 2 diabetics	100	≥40	44	100	6.7	50	no
JPAD	1,235 type 2 diabetics	100	60–85	42	81 or 100	4.4	90	no
ASCEND	15,480 type 1 & 2 diabetics	100	≥40	62	100 EC	7.4	70	yes
ASPREE	19,114 elderly with no known cv disease	11	≥70	44	100 EC	4.7	73	no

Similar results were obtained in male hypertensives of the US-PHS. No *relative* RR was seen in the about 2,000 subjects with a diastolic blood pressure of >90 mmHg as compared with the total study population. However, the *absolute* RR was twice as much: 4.4 % versus 2.5 % in hypertensives as compared to 2.2 % versus 1.3 % RR in the total study population [72]. These findings also agree with the Medical Research Council's "Thrombosis Prevention Trial" (TPT, 1998; see below), where beneficial effects of aspirin were predominantly seen in the individuals with the lowest blood pressure and

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there was no significant reduction of myocardial infarctions in women, by analogy with HOT [95, 96] and the WHS [97].

Multiple risk factors. There are two large, prospective randomized trials on primary prevention with aspirin in individuals with multiple risk factors: The "Thrombosis Prevention Trial" (TPT) and the "Primary Prevention Project" (PPP). Another trial is the CHARISMA study [98], using aspirin alone and in combination with clopidogrel. In addition to patients with preexistent vascular events (secondary prevention), this trial also included a group of patients with multiple cardiovascular risk factors without a preceding vascular event (see below).

The TPT was a prospective comparison of aspirin and warfarin alone and in combination vs. placebo in men at markedly elevated cardiovascular risk (top 20–25 % according to a risk scale for myocardial infarctions) because of preexisting risk factors [99].

A total of 5,499 men aged 45–69 years with elevated vascular risk because of preexisting risk factors (smoking, hypercholesterolemia, hypertension, positive family history of ischemic coronary heart disease, elevated BMI) were initially recruited. After a pilot phase, the trial was expanded into a factorial comparison of low-intensity oral anticoagulation by warfarin (INR: 1.5) and microencapsulated aspirin (75 mg/day). The four treatment groups were warfarin + aspirin, warfarin + placebo, aspirin + placebo and placebo + placebo. The median duration of the study was 6.8 years. Primary endpoints were the total number of cardiovascular deaths and fatal and nonfatal myocardial infarctions.

The main effect of aspirin in comparison to placebo was a reduction of the primary endpoints by 20 % ($P = 0.04$). This was almost entirely due to the 32 % reduction of nonfatal myocardial infarctions while the number of fatal events was modestly increased (not significant). The main effect of warfarin was a reduction of primary endpoints by 21 % ($P = 0.02$). This was mainly due to a 39 % reduction in fatal vascular occlusive events. Overall, warfarin reduced the death rate from all causes by 17 % ($P = 0.04$). There was a significant 20 % increase in minor bleeding events by aspirin and a tendency but no significant increase in intermediate or major bleeding events. Warfarin significantly increased the number of hemorrhagic strokes: seven vs. zero in the placebo group ($P = 0.009$). Combined treatment with warfarin and aspirin was more effective in reducing the risk of cardiac events than either agent alone but further increased the risk of bleeding.

The conclusion was that aspirin reduces nonfatal coronary events, while warfarin reduced all cardiovascular events, chiefly because of an effect on fatal events. The combined treatment with both agents was additive for both the reduction of cardiovascular events and the increase in bleeding [99].

The PPP also studied the efficacy and safety of aspirin in patients at elevated cardiovascular risk. In contrast to the TPT, this study also included women and differed slightly from the TPT in the inclusion criteria [100].

In a controlled, randomized, prospective but open trial, 4,495 men and women (about half each, mean age 64 years) with multiple preexisting cardiovascular risk factors (older age, i. e., ≥65

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years, hypertension, hypercholesterolemia, diabetes, obesity, family history of myocardial infarction) were randomly allocated to four treatment groups: enteric-coated aspirin (100 mg/day) or no aspirin and vitamin E (300 mg/day) or no vitamin E, according to a 2 × 2 factorial design. Efficacy endpoint was the cumulative rate of cardiovascular deaths, nonfatal myocardial infarctions and nonfatal strokes.

After an interim analysis, the study was stopped prematurely for ethical reasons at a mean follow-up of 3.6 years. At this time, there was a statistically significant benefit in the aspirin arm. In addition, two other primary prevention trials with aspirin in similar risk groups (TPT, 1998; HOT, 2000) had been published in the meantime and had demonstrated beneficial effects of aspirin. When the study was stopped, aspirin had significantly reduced the incidence of all endpoints. There was a reduction of cardiovascular deaths by 44 % from 1.4 % to 0.8 % (OR: 0.56; 95 % CI: 0.31–0.99) and of total cardiovascular events from 8.2 % to 6.3 % (OR: 0.77; 95 % CI: 0.62–0.95). The cardiovascular mortality was reduced but the overall mortality remained unchanged: 2.8 % vs. 3.4 %. There were significantly more severe bleeding events in the aspirin group: 1.1 % vs. 0.3 % ($P = 0.0008$). No effect of vitamin E treatment on any of the parameters was seen.

The conclusion was that low-dose aspirin in men and women with at least one major risk factor given in addition to appropriate treatment of other existent risk factors contributes an additional preventive effect at an acceptable risk profile [100].

One of the latest cardiovascular prevention studies in persons with risk factors was "A Study to Assess the Efficacy and Safety of Enteric-Coated Acetylsalicylic Acid in Patients at Moderate Risk of Cardiovascular Disease" (ARRIVE). This study was designed as the first large prospective randomized, placebo-controlled primary prevention trial with aspirin in nondiabetic patients at estimated moderate risk of a first cardiovascular event [102].

The study included patients aged ≥55 (men) or ≥60 years (women) who had an average cardiovascular risk, deemed to be moderate on the basis of the number of specific risk factors. Patients at high risk of gastrointestinal bleeding, other bleeding or diabetes were excluded. Patients were randomly assigned to receive enteric-coated aspirin tablets (100 mg/day) or placebo. The primary efficacy endpoint was a composite outcome of cardiovascular death, myocardial infarction, unstable angina, stroke or transient ischemic attack. Safety endpoints were hemorrhagic events and incidence of other adverse events.

A total of 12,546 patients were enrolled and randomly assigned to receive aspirin or placebo. Median follow-up was 60 months. The primary endpoint in the intention-to-treat (ITT) analysis occurred in 4.29 % of patients in the aspirin group versus 4.48 % in the placebo group (HR: 0.96; 95 % CI: 0.81–1.13; $P = 0.60$). In the per-protocol but not the ITT analysis, there was a lower rate of myocardial infarctions in the aspirin group. Gastrointestinal bleeding events (mostly mild) occurred in 0.97 % of patients in the aspirin group versus 0.46 % in the placebo group (HR: 2.11; 95 % CI: 1.36–3.28; $P = 0.0007$). The overall incidence of treatment-related adverse events was low: 16.8 % vs. 13.5 % in the placebo group ($P < 0.0001$). Total mortality was unchanged: 2.55 % vs. 2.57 % in the placebo group.

The conclusion was that the findings are consistent with earlier primary prevention trials of aspirin in patients at low vascular risk. The role of aspirin in primary prevention among patients at moderate risk could not be addressed [102].

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Despite several amendments to the study protocol in order to increase the number of events (extended study duration, more predefined study endpoints, higher number of included patients) there were still marked differences between the estimated and the real vascular risk of the participants: The estimated 10-year vascular risk in the ARRIVE population was 17.5 %; however, the real risk was only 4.29 % and 4.48 % in aspirin- and placebo-treated individuals, respectively. In other words, the risk was not elevated, that is, "moderate," but "low". Thus, the study protocol was not representative of individuals at elevated vascular risk.

A total of 43 % of patients in the ARRIVE trial received statins and two thirds of patients received antihypertensive medications. This might have reduced the number of modifiable environmental risk factors to levels lower than in the times of Framingham or PROCAM (1960s–1970s) (used in modified form for basal vascular risk calculation). The HR for the occurrence of myocardial infarctions was 0.85 ($P = 0.2325$) in the conventional ITT analysis but 0.53 ($P = 0.0014$) in the "per-protocol" aspirin-treated patients. This points to possible differences in the self-reported compliance rate, amounting to only about 60 % in the per-protocol group (when, how and how often determined?). There was a withdrawal of study medications by 30 % of individuals in both treatment groups, but only 2 % of them because of side effects. The main reason was a (patient-related) uncertainty about the therapeutic benefit of the active study medication, forced by negative press releases on aspirin in Great Britain (40 % of the study population) in 2009, that is, ca. 2 years after the study had begun.

Taken together, this study did not address the study population of interest and, in essence, was largely confirmative of earlier trials.

4.1.1.5 Clinical trials – primary prevention in patients with diabetes

General aspects. The "classical" study of Haffner and colleagues from 1998 in diabetics showed that patients suffering from type 2 diabetes without preceding myocardial infarction had the same risk for a myocardial infarction as nondiabetics who already suffered a myocardial infarction. The annual infarct risk of patients with diabetes in this study amounted to 3.2 % versus 0.5 % in nondiabetics and the cardiovascular mortalit to 0.3 % vs. 2.5 %. This was equivalent to a 6–8-fold increased vascular risk in diabetics. Accordingly, diabetes was considered a (cardio)vascular disease and these diabetic patients died from vascular problems and their complications (large and small vessel diseases, renal failure) rather than from acute derailments of blood sugar control, such as diabetic coma [103].

Aspirin, platelet reactivity and clot formation in diabetes. The major target of thrombosis prevention by aspirin in diabetics is the platelet. A specific problem of diabetics, in addition to a possibly reduced acetylation of COX-1 [104, 105], is the increased platelet turnover rate. At increased turnover, an increased proportion of platelets will

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be aspirin-naïve and can act as seed for aggregate formation [106]. This is associated with a lower sensitivity to and shorter duration of aspirin action [107–110] and a higher proportion of young immature platelets with elevated COX-2 expression [111, 112]. Thus, a higher proportion of these immature platelets might contribute to or even explain any reduced efficacy of aspirin as a cardiocoronary preventive in diabetics [113]. In agreement with this hypothesis, twice daily aspirin (at the same total dose) was found to be more effective than the same dose once daily in diabetics with CAD (Fig. 4.1.1-6) [114, 115]. Thus, in a significant proportion of patients with CAD, there is clinical aspirin "resistance," i. e., too early recovery of the reduced platelet-dependent thromboxane formation (see also Fig. 2.3.1-6) [116].

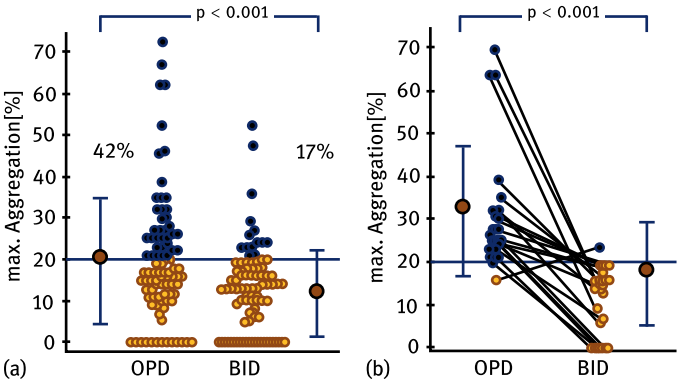

Figure 4.1.1-6: Maximum arachidonic acid-induced platelet aggregation in diabetics ex vivo after once per day (OPD) 150 mg or twice per day (BID) 75 mg of aspirin ($n = 92$). Blue circles represent patients resistant to aspirin. Yellow circles represent patients responding to aspirin. Patients were considered being resistant to aspirin treatment when maximal aggregation (mean ± SD) was still ≥20 % after aspirin treatment. A total of 42 % of patients were resistant to aspirin at OPD but only 17 % of patients at BID application of the same daily aspirin dose (a). After BID application of the same total dose to the nonresponders, about all of them became responders (b) [115].

In addition, platelets are also important determinants of clot structure (density) as originally described by thromboelastography [117]. In healthy men, aspirin did not influence fibrinogen levels but increased fibrin permeability, most significant at low-dose aspirin (Section 2.3.1). Interestingly, the opposite, that is, a stronger increase of fibrin permeability, was seen in (type 1) diabetics: Only 320 but not 75 mg/day aspirin for 4 weeks (crossover design) significantly increased fibrin network permeability, suggesting that diabetics might need higher doses of aspirin than nondiabetics [118]. However, the number of patients was small (24) and no type 2 diabetics were included.

Taken together, there are multiple reasons for a generally elevated thrombotic risk in diabetics. There are procoagulatory changes in the clotting and fibrinolytic systems with generation of tissue factor and thrombin as well as proinflammatory transforma-

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tions of the vessel wall, predominantly in the context of enhanced oxidative stress. At the same time, antioxidative defense is reduced, eventually resulting in the formation of "advanced glycation endproductgs" (AGE) proteins in the vessel wall [119–121]. Thus, diabetics are a high-risk population for atherothrombotic events. Although the situation in Europe and the US has been improved by introducing new therapeutic and prophylactic measures, the situation might still be worse in countries like China (PRC) with an estimated total number of 10.9 % diabetics – only 4 % of them diagnosed – and 35.7 % prediabetics [122].

Aspirin, which modifies both platelet function and plasmatic coagulation, most notably thrombin formation, and in addition has antiinflammatory actions via its antiplatelet effects (Section 2.3.1), appears to be an attractive medication for primary and secondary prevention in diabetics [123]. While there is no doubt about the efficacy of aspirin as antiplatelet agent in secondary prevention in diabetics [124], which is now also recommended in guidelines (level A) [125], the situation in primary prevention is more complex (level C) and the currently available studies in Western societies in general failed to demonstrate a clearly positive benefit/risk ratio for aspirin [126].

Clinical trials. One of the first large placebo-controlled, randomized double-blind trials on the cardioprotective actions of aspirin in primary prevention of diabetics was the randomized, placebo-controlled "Early Treatment Diabetic Retinopathy Study" (ETDRS), including 3,711 patients with both type 1 and type 2 diabetics. The relative risk for total mortality – primary endpoint – for aspirin-treated patients compared with placebo-treated patients for the entire study period was 0.91 (99 % CI: 0.75–1.11). Larger reductions, by 17 %, were noted for the occurrence of a first fatal and nonfatal myocardial infarction. The estimate of relative risk was 0.83 (99 % CI: 0.66–1.04). There was no evidence of harmful effects of aspirin. The overall conclusion was to recommend aspirin to diabetics at increased risk of cardiovascular disease [127]. Nevertheless, the cardioprotective effects of aspirin were largely variable and the aspirin dose was high (325 mg twice daily). In addition, the about 50 % participants with cardiovascular disease – a frequent comorbidity of advanced diabetes – were not formally excluded. Thus, this study might not be representative of primary prevention trials of diabetics with aspirin.

Meanwhile, three more randomized trials are available, using low antiplatelet doses of aspirin: the "Prevention of Progression of Arterial Disease and Diabetes" (POPADAD) trial, the "Japanese Primary Prevention of Atherosclerosis with Aspirin for Diabetes" (JPAD) trial and "A Study on Cardiovascular Events in Diabetics" (ASCEND).

The POPADAD study was a randomized, double-blind, placebo-controlled trial in 1,276 adults (>40 years) suffering from type 1 or type 2 diabetes and asymptomatic peripheral arterial disease (ankle/brachial index [ABI] < 1.00) but no symptomatic cardiovascular disease. Patients were treated

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with aspirin (100 mg/day) and/or an antioxidant or placebo using a 2 × 2 factorial design. Primary endpoint was the composite of cardiovascular ischemic events, stroke, cardiovascular death or amputation.

After an average observation period of about 6 years, there were no significant differences between aspirin and placebo with respect to myocardial infarction and death. No interaction was found between aspirin and antioxidant. There were 18.2 % primary events in the aspirin group as compared to 18.3 % in the placebo group (HR: 0.98; 95 % CI: 0.76–1.26).

The conclusion was that aspirin is not useful for primary prevention of cardiovascular events in diabetics with asymptomatic peripheral arterial occlusive disease [128].

Negative results were also obtained in the JPAD trial:

The JPAD trial was also a prospective, randomized but open trial including 2,539 Japanese patients with type 2 diabetes and no known coronary heart disease. Patients were treated with aspirin (81 or 100 mg/day). For patients of the control nonaspirin group, other, not well-controlled medications, including aspirin and antithrombotics, were allowed "if needed." The combined primary endpoint was complex and included all types of atherosclerotic vascular events, newly diagnosed angina, aortic dissection and peripheral vascular disease.

After an average observation period of 4.4 years, there was a nonsignificant overall RR by 20 % (HR: 0.80; 95 % CI: 0.58–1.10; $P = 0.16$) in the aspirin group. There were 12 gastrointestinal bleeding events in the aspirin group and four in the control group, at a comparable number – six vs. seven – of hemorrhagic strokes in both groups. Interestingly, there was only one fatal ischemic event (stroke) in the aspirin group as opposed to 10 events (five fatal myocardial infarctions and five fatal strokes) in the control group (HR: 0.10; 95 % CI: 0.01–0.79; $P = 0.0037$).

The overall conclusion was that aspirin did not reduce the risk of cardiovascular events in type 2 diabetics [129].

Both studies have been controversially discussed and had a number of weaknesses [130]. The POPADAD trial was underpowered because of an only 2.9 % annual event rate instead of the calculated, desired annual risk of 8.0 %. The chosen confidence limit (95 %) for the primary endpoint included a possible 24 % RR [131]. This unexpectedly low event rate was possibly due to the beneficial effects of frequent cotreatment, for example with statins, that have been shown to markedly enhance antiplatelet effects of aspirin in diabetics [130, 132, 133]. In addition, the ABI threshold value as an index for increased cardiovascular risk is smaller (<0.90) [134, 135] than that used here (≤1.00). Finally, the compliance rates of the aspirin groups in the diabetes studies at the end of the study period were quite different: only 50 % in the POPADAD trial as opposed to the 92 % in ETDRS and 90 % in the JPAD trial [133].

In the JPAD trial, it was the open design and the poorly controlled medications in the treatment groups that raised concerns. Furthermore, events such as the development of stable exertional angina, peripheral vascular disease and atherosclerosis progression were included as primary study endpoints although it is known from other primary prevention trials that they are not aspirin-sensitive. This might have diluted the statistical power [79, 80]. It was suggested that the observed trend of a 20 % RR would have become significant if nonaspirin-sensitive endpoints were excluded [130].

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These limitations also apply to the recent 10-year results from JPAD. These confirmed that aspirin did not affect the risk for cardiovascular events but increased the risk for gastrointestinal bleeding [136].

Much was expected from the actually last but also currently largest available study on primary prevention by aspirin in diabetics, ASCEND [137].

The study was conducted to analyze the benefits and hazards for the prevention of a first cardiovascular event in patients with diabetes in a prospective, double-blind manner. A total of 15,480 participants with diabetes but no evident cardiovascular disease were randomized to 100 mg/day enteric-coated aspirin or a matching placebo. Primary efficacy outcome was a first serious vascular event (myocardial infarction, stroke or TIA or vascular death [excluding intracerebral hemorrhages]), and primary safety outcomes were major bleeding events (intracranial hemorrhage, eye and gastrointestinal bleeding events or other serious bleeding events). The mean follow-up was 7.4 years.

Serious vascular events occurred in a significantly smaller proportion of participants in the aspirin group than in the placebo group: 8.5 % vs. 9.6 % (HR: 0.88; 95 % CI: 0.79–0.97; $P = 0.01$). Major bleeding events (mostly gastrointestinal and other extracranial bleeding events) occurred in 4.1 % of the aspirin group and 3.2 % of the placebo group (HR: 1.29; 95 % CI: 1.09–1.52; $P = 0.003$). There was no difference between the two groups in the incidence of gastrointestinal cancer.

The conclusion was that aspirin prevented serious vascular events in these individuals with diabetes, but the absolute benefits were largely counterbalanced by the bleeding hazard [137].

This study was the first to show a significant antithrombotic efficacy of low-dose aspirin in diabetics in a prospective, large-sized, placebo-controlled, double-blind randomized trial. The efficacy appeared to be greatest in myocardial infarction prevention in the per-protocol analysis. However, despite some amendments to the study protocol in order to broaden the risk level, the annual vascular event rate was only 1.2 % and 1.3 % in both groups, respectively. That is equivalent to an only modestly elevated vascular risk, as also evidenced by the only 12 % relative RR by aspirin. This is exactly in the range of other primary prevention trials (Table 4.1.1-1). A possible explanation for the low efficacy of aspirin in absolute terms is the generally low vascular risk in these diabetics who, in addition, were also apparently well treated by antidiabetic drugs, according to an HbA1c value of 7.0 % at the beginning and 7.4 % at the end of the study, corresponding to a total duration of the (known) diabetes for 13–14 years. Another aspect are the cardiovascular comedications, such as statins (7.5 %) or ACE inhibitors/sartans (60 %). Of some concern is the low compliance rate, amounting to only 60 % even in the "per-protocol"-treated patients at the end of the study according to information by the patients. Objective confirmation of aspirin compliance was only done once after 2 years of treatment and in only 1 % of participants by measuring a nonplatelet-specific thromboxane metabolite in urine [138]. This appears to be insufficient as a compliance control. A total of 25 % of patients was on PPIs at the end of the study but only 14 % at the beginning. The average BMI of the participants was between 30.6 and 30.8. At an estimated average body length of 180 cm, this corresponds to a body weight of >100 kg (!). According to a recent body weight-based metaanaly-

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sis of the cardioprotective actions of aspirin in clinical prevention trials, the participants of ASCEND should exhibit an *increased* cardiovascular mortality [32]. Surprisingly, rather the opposite was found. Taken together, the ASCEND study did not add much to the benefit/risk calculation for aspirin and primary prevention in diabetics but rather confirmed the already known findings.

The ACCEPT-D trial, investigating the prevention of cardiovascular events in diabetics by aspirin (100 mg/day) with and without comedication of simvastatin with over 5,000 diabetics, is currently underway [139].

4.1.1.6 Clinical trials – patients with chronic coronary vascular disease

General aspects. Chronic CVD (coronary vascular disease = stable angina pectoris) is a functional consequence of atherosclerosis. It is first clinically evident as exertional angina pectoris. Anginal symptoms of ischemic pain occur if narrowing of the coronary arteries becomes critical for myocardial oxygen supply, for example after psychological or physical stress. In stable angina, platelets also circulate as singular elements in their discoid resting state or as (reversible) aggregates [140, 141]. However, they are more sensitive to stimulation by platelet agonists, as seen from the enhanced platelet responses to ex vivo stimulation by ADP (Fig. 2.3.1-1) [142, 143]. There is also a tendency for formation of platelet–white cell aggregates [143]. Plasma and urinary thromboxane levels vary, but are usually not critically elevated, even under conditions of exercise-induced angina pectoris or noncardiac chest pain (Fig. 4.1.1-2). For these reasons, ex vivo studies of platelet aggregation have probably no prognostic value for the further clinical outcome in patients with chronic CVD. This agrees with clinical studies (Fig. 4.1.6-5) [37, 144, 145].

Randomized clinical trials. The "Swedish Angina Pectoris Aspirin Trial" (SAPAT) was the first large prospective, double-blind primary prevention trial of low-dose aspirin in patients with stable angina, demonstrating a beneficial effect of the compound in patients with stable angina versus placebo.

A total of 2,035 patients with stable angina were randomized in a double-blind manner to treatment with aspirin (75 mg/day) or no aspirin. The inclusion criterion was exertional chest pain for at least 1 month. All patients received sotalol, a nonselective β-blocker, for control of symptoms. The primary endpoint was myocardial infarction and sudden death. Secondary endpoints were other vascular events, including vascular death, all cause mortality and stroke. The median duration of follow-up was 50 months. There was no systematic control of compliance.

Compared with the sotalol (control) group, the aspirin + sotalol group had a 34 % (81 vs. 124 patients) reduction in primary outcome events ($P = 0.003$). The reduction of secondary outcome events ranged between 22 % and 32 %. There was no significant difference between the two groups in treatment withdrawal due to adverse events or in major bleeding events including hemorrhagic stroke.

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The conclusion was that treatment with low-dose aspirin (in addition to sotalol) shows a significant benefit in terms of prevention of cardiovascular events, including a significant reduction in the incidence of a first myocardial infarction in patients with symptomatic stable angina. Therefore, it should be recommended for this indication [146].

Similar results were obtained in a subgroup of patients with stable angina pectoris in the US-PHS. In comparison to placebo, there was an 87 % (!) reduction of the incidence of a first myocardial infarction by aspirin ($P < 0.001$). The cardiovascular mortality remained unchanged. This was probably due to an increased number of strokes in the aspirin group [147, 148].

These data suggest that aspirin has probably no effect on the natural history of the pathophysiology of atherosclerosis. However, it protects from the acute, catastrophic event of sudden platelet activation, for example after plaque rupture.

4.1.1.7 Clinical trials – patients with acute coronary syndrome (ACS)

General aspects. Stable angina pectoris evolves in a chronic, progressive manner and causes ischemic symptoms if cardiac oxygen demand exceeds the capacity of cardiac oxygen supply. In contrast, in ACS, that is, unstable angina, myocardial infarction and sudden cardiac death, usually are nonpredictable events. ACS is caused by rupture of an atherosclerotic plaque inside a large coronary artery [16, 17, 149]. Consequences are tissue factor release with subsequent thrombin formation, platelet activation and aggregation inside the coronary circulation [150] with subsequent morphological or functional ("no reflow") [151] vessel occlusion. If coronary occlusion persists for a critical period of time, myocardial tissue distal to the occlusion site becomes increasingly injured, eventually resulting in tissue necrosis, that is, Q-wave elevation myocardial infarction or STEMI.

These processes are accompanied by an immediate, massive increase of thromboxane generation inside the coronary circulation, resulting in 3–5-fold higher levels than in the systemic circulation [51, 152]. Thrombin formation at the surface of activated platelets further stimulates platelet activation and secretion. There is ischemia-related leukocytosis, which is due to the formation of platelet–platelet and platelet–white cell (monocytes, neutrophils) aggregates [43, 44, 140]. Histological examinations of coronary thrombectomies taken from patients with ACS (STEMI) undergoing primary PCI showed activated neutrophils that underwent NETosis at the culprit lesion site [153]. This ischemia-induced leukocytosis and generation of platelet–white cell aggregates appears to be at least partially triggered by platelet-dependent thromboxane formation since they can be largely inhibited by a thromboxane receptor antagonist [154] and the platelet-specific GPIIb/IIIa antagonist tirofiban [155]. In addition, platelet thrombi can fragment and cause dynamic coronary obstructions [17] that can be prevented by aspirin [156].

Even after successful removal of the occluding thrombus by PCI or thrombolysis, the ruptured plaque still remains a culprit lesion and site of local platelet adhesion

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and aggregation [149]. This, eventually, results in reocclusion of the coronary artery by generation and release of procoagulatory products, most notably again thrombin and TXA_2. Elevated numbers of circulating (reversible) platelet aggregates and elevated thrombin levels can persist over months in patients with ACS and manifest as a persistent hypercoagulable state, although with minimal generation of fibrin [39]. The outstanding role of activated platelets in the natural history of thrombus formation was shown for the first time by the tight correlation between "spontaneous platelet aggregation" ex vivo (an index for platelet hyperreactivity) and the appearance of a new vascular event (cardiovascular death or recurrent myocardial infarction) over an observation period of 5 years [35]. Taken together, platelet hyperreactivity, increased thromboxane synthesis, platelet–white cell coaggregation and NET formation as well as thrombin generation and stimulation of plasmatic coagulation form a positive feedback loop for further platelet activation and secretion, thrombus formation and vasoconstriction. The prevention of the priming function of platelet-derived thromboxane release, otherwise "exploding" after vessel injury or plaque rupture, is the rationale for continuous, long-term prevention of recurrent atherothrombotic events by aspirin and other antiplatelet/antithrombotic agents.

A piece of history. The "Veterans Administration Cooperative Study" [157] was the first randomized, placebo-controlled, double-blind trial to establish a protective action of aspirin on the incidence of and mortality from myocardial infarction in patients with unstable angina. Aspirin (325 mg/day) reduced this combined endpoint from 17.0 % to 8.6 %, that is, by 51 %, during a 3-month observation period. Similar results were obtained in three further randomized, double-blind, placebo-controlled trials [158–161], collectively suggesting an about 50 % protection from a recurrent acute ischemic vascular event in this group of high-risk patients. The maximum therapeutic effect was obtained early, within one month, and there was no further improvement up to one year thereafter [160, 161].

Aspirin and acute myocardial infarction – the ISIS-2 trial. Antiplatelet treatment of ACS should prevent further thrombus growth inside the occluded coronary artery and inhibit the formation of new thrombi. The clinical significance of antiplatelet treatment of ACS with aspirin alone and in combination with thrombolysis has been studied for the first time in the ISIS-2 trial [13].

A total of 17,187 patients with clinical symptoms of acute myocardial infarction were randomized to receive enteric-coated aspirin (162 mg/day = half a 325-mg tablet), with the first dose crushed, sucked or chewed after admission to the hospital for a rapid effect, intravenous infusion of streptokinase (1.5 million IU/h), both active treatments or neither treatment (placebo) within 5 h after the acute event. Of all patients, 56 % had a transmural infarction (STEMI). The primary endpoint was cardiovascular mortality at 5 weeks.

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At the end of this observation period, there were 804 cases of vascular death in the two aspirin (streptokinase) groups as opposed to 1,016 fatality cases in the placebo group. This was equivalent to an absolute reduction of vascular mortality from 13.2 % in the placebo group to 8.0 % in the group with combined treatment ($P < 0.001$). Aspirin alone caused a relative decrease in mortality by 23 %, streptokinase by 25 %. Both treatments were additive and together reduced mortality significantly more, by 38 %. There was also a significant reduction of the number of reinfarctions and ischemic strokes by 50 % and 40 %, respectively. Aspirin alone reduced the number of reinfarctions from 284 in the placebo group to 156. This was equivalent to an absolute RR from 3.3 % to 1.8 %. The number of nonfatal strokes was reduced from 0.6 % to 0.3 % and was not associated with an increase in cerebral hemorrhages or bleeding events requiring transfusions. There was a tendency for increased severe bleeding events in both treatment groups: 0.4 % and 0.5 % as compared to 0.2 % in the placebo group. That, however, was only significant in the streptokinase group. Importantly, there was no additive effect of the combined treatment on bleeding.

The conclusion was that this study had shown for the first time the utility of low-dose aspirin for treatment of acute myocardial infarctions as a first-line treatment, to be provided as early as possible. Aspirin significantly reduced reinfarctions and, most importantly, improved survival after 5 weeks. The study also showed a synergistic effect of aspirin with streptokinase, i. e., a doubling of efficacy, but no synergistic effects on bleeding (Fig. 4.1.1-7) [13].

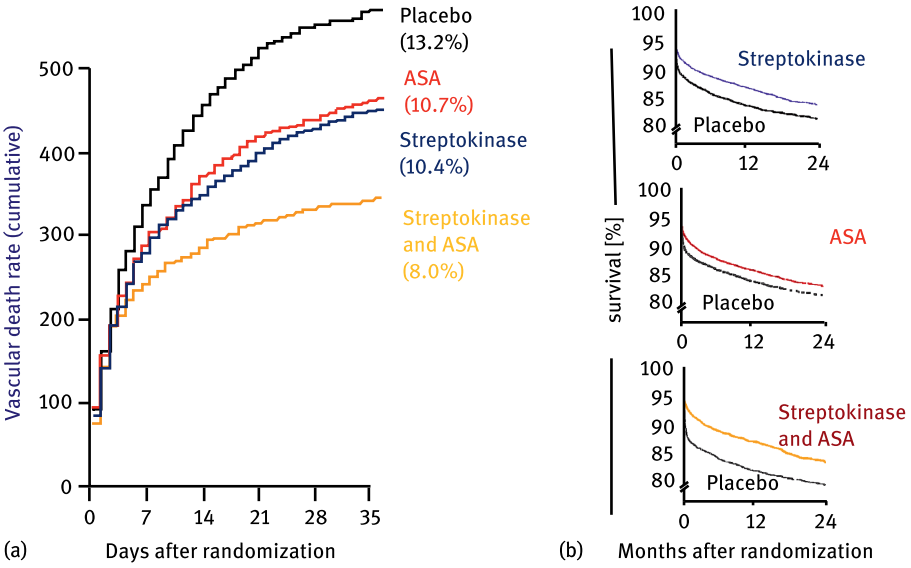

Figure 4.1.1-7: The ISIS-2 trial. Shown is vascular mortality after treatment with aspirin (ASA) (162 mg/day), streptokinase (SK) (1.5 million U/h), the combination of both or placebo in patients with acute myocardial infarction. Treatment was started within 5 h after the qualifying acute event [13].

These dramatic effects of aspirin were unexpected but basically confirmed in many subsequent trials, including those where thrombolysis was replaced by PCI, the current standard procedure for treatment of ACS. A post hoc analysis of ISIS-2 showed

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that the early survival benefit in the active treatment groups was maintained over at least 10 years [69].

In this context, it is important to note that aspirin not only inhibits platelet-associated thromboxane formation in ACS [162] but also platelet-associated thrombin biosynthesis [20] and other platelet-dependent inflammatory reactions (Section 2.3.1) [163]. Interestingly, most thrombin (96 %) is generated in tissue injury well after clot formation has been finished [164]. Thus, thrombin, generated by a fresh platelet–fibrin clot, specifically in STEMI, could be an important platelet-activating factor during the first 2–4 hours of ACS. At this time, platelets are hyperaggregable to ADP (Fig. 4.1.1-3) [51]. This might contribute to a refractory state of these platelets against inhibition by oral ADP antagonists from both the thienopyridine type as well as ticagrelor, as seen from the FABOLUS-PRO [165] and PRIVATE ATLANTIC platelet substudies [166], respectively. This "resistance" to ADP antagonists could be overcome by direct blockade of the platelet fibrinogen receptor by a GPIIb/IIIa antagonist (tirofiban) [165], suggesting that this is a platelet-specific reaction. Importantly, there are no pharmacokinetic interactions (reduced absorption) of aspirin with morphine (given as an analgesic to about one third of myocardial infarction patients in some studies). In contrast, thienopyridine-type PY12 antagonists, such as prasugrel and clopidogrel, might undergo delayed absorption which might reduce the plasma level of their active metabolites. In case of clopidogrel, but not prasugrel, this is associated with reduced antiplatelet efficacy [167–169].

An ISIS-2-specific, additional antithrombotic effect of aspirin is the inhibition of lysis-induced (streptokinase) platelet activation and thromboxane biosynthesis as well as streptokinase-induced bleeding. Fibrinolysis enhances platelet aggregation by exposing thrombin, which is abundantly present inside the clot (Section 2.3.1). At the same time there is enhanced thromboxane formation which continues for hours within the thrombus and is potentiated by lysis as seen from a >20-fold increase in thromboxane metabolite excretion [170]. In addition, aspirin might inhibit clot-stabilizing actions of thrombin by acetylation of fibrinogen, making polymerized fibrinogen more susceptible to fibrinolysis (Section 3.1.2) [171]. Whatever the final contributions of these multiple factors to the clinical outcome were, it was the ISIS-2 study which resulted in introduction of aspirin as guideline-recommended standard medication in patients with ACS and PCI.

The choice of aspirin as a first-line antiplatelet agent in ACS is based upon its inhibition of platelet-dependent thromboxane formation and its follow-up events. Recommended doses are 250–500 mg (intravenous administration), given as a well water-soluble salt. This will inhibit thromboxane formation and associated platelet functions completely within about 5 min (Fig. 2.3.1-4) [172, 173]. At the same time, there is also inhibition of PGI_2 formation (serum ex vivo) by 80–90 % [172]. This is not necessarily paralleled by a loss of platelet-inhibitory actions of PGI_2 because there is an agonist-induced downregulation of PGI_2 receptors and "resistance" of platelets against inhibition by PGI_2 at high local levels [51, 52], such as under the conditions of

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COX-2 upregulation in ACS (Fig. 4.1.1-3) [43]. Thus, there is no reason to assume that high-dose aspirin for immediate treatment of ACS will reduce the antiplatelet effects of endogenous PGI_2. Rather the opposite might be expected as it is well known that aspirin treatment of platelets will enhance their sensitivity against PGI_2 (Section 2.3.1) [53].

ACS – dual antiplatelet therapy (DAPT). PCIs are associated with an additional platelet stimulation due to procedure-related injury of the vascular endothelium and exposure of the thrombogenic subendothelium to the circulating blood. This causes significant thrombin [174] and thromboxane formation [175] with further platelet stimulation. Although aspirin still remained the "golden standard" basal medication (1A recommendation) and an integral part of the combined antithrombotic treatment, additional and, perhaps, more potent platelet inhibition became necessary. The "Clopidogrel in Unstable angina to prevent Recurrent Events" (CURE) trial in NSTEMI patients investigated combined treatment with aspirin plus clopidogrel. During a 12-month follow-up the incidence of recurrent vascular events or death was reduced from 11.4 % to 9.3 % as compared with aspirin alone (OR: 0.80; 95 % CI: 0.72–0.90; $P < 0.001$). However, there were also significantly more patients with severe bleeding, 3.7 % vs. 2.7 % ($P < 0.001$), and there was no change in mortality [26]. Currently, clopidogrel is only second-choice antiplatelet treatment in ACS and has been replaced by more advanced third-generation oral ADP antagonists, such as prasugrel or ticagrelor. The optimum duration of DAPT after coronary interventions (stenting) is still under discussion (see below) [176]. Interestingly, preexisting treatment with aspirin and/or statins or the combination of both was found to be associated with a lower incidence of STEMI and less reduced CK levels and myocardial contractile function in patients with a first ACS enrolled in the "Swiss Program University Medicine" (SPUM)-ACS study [177]. Similar results were obtained in another observational study in patients with acute coronary events [178].

4.1.1.8 Clinical trials – long-term secondary prevention

General aspects. In a large epidemiological study in the USA from the year 2015, involving about 440,000 individuals above the age of 18 years, almost 16 % of participants had angina, myocardial infarction or stroke [24]. Of them, 65 % and 71 % regularly took aspirin for secondary prevention of heart attacks or stroke [24]. According to data of the ATTC, long-term aspirin treatment of patients who already suffered a myocardial infarction or another thromboembolic vascular event reduced the annual risk of a new, severe vascular event from 8.2 % to 6.7 % ($P < 0.0001$). At the same time, there was a nonsignificant increase in hemorrhagic strokes but a significant reduction of total strokes (2.08 % vs. 2.54 %; $P = 0.002$) and coronary events (4.3 % vs. 5.3 %; $P < 0.0001$) [6]. The protective effect is greatest within the first year after the acute event (28 % odds reduction) and decreases thereafter to 24 % and 12 % in the second

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and third year, respectively. No positive effect on cardiovascular events was seen at later times [29] (see also Fig. 4.3.1-2 [5]) and there was also no further improvement by aspirin in the ISIS-2 trial in ACS patients beyond the 35 days of initial treatment over 10 years [69]. Regular aspirin intake does probably not induce more rapid hydrolysis by aspirin esterases [179], although some relationship between increased plasmatic aspirin esterase activity and the incidence of coronary heart disease has been suggested in a large genomic study [180]. In absolute terms, the efficacy of aspirin is 5–10-fold higher in secondary than in primary prevention (Table 4.1.1-1). This resulted in the recommendation of aspirin intake for secondary cardiocoronary prevention by about every guideline worldwide.

A piece of history. One of the first long-term studies addressing the issue of secondary prevention of myocardial infarctions by aspirin in a controlled, prospective study was the "Cottbus Reinfarction Trial" by *Werner Förster* and colleagues from Halle/Saale (Germany).

The prospective population-based cohort study investigated whether regular very low-dose aspirin (30 mg/day) according to data of the Patrono group [181] was as effective in secondary prophylaxis of reinfarction patients with acute myocardial infarction as higher doses (1 g/day) and whether the incidence of side effects was lower.

Included in this study were *all* patients less than 70 years of age in the district of Cottbus (former East Germany) who had suffered an acute myocardial infarction in the years 1981–1983. After initial application of $2 \times 5{,}000$ IU heparin for the first 4 days after the acute event, treatment was continued with 1,000 mg/day aspirin for the first 2 weeks. The 701 eligible patients were afterwards randomized to treatment with either 30 mg (once or twice daily) or 1,000 mg aspirin daily according to the location of their hospital ("quasi-random" design). The question was whether the efficacy of the 1,000 and 30 (60) mg daily aspirin was comparable and whether there were fewer side effects in the low-dose aspirin group(s). There was no placebo control.

After 2 years, there was a significant reduction of reinfarctions in the 30 mg aspirin group, by 58 % as compared to the group receiving 1,000 mg aspirin per day. These beneficial effects of low-dose aspirin were still evident 4 and 6 years after the end of the controlled trial (the study was then continued as an open trial). At this time, the incidence of nonfatal myocardial infarctions was reduced by 50 % in patients previously treated with 30 mg aspirin as opposed to those who had received 1,000 mg per day.

The conclusion was that 30 mg aspirin per day is sufficient for secondary prevention of myocardial infarction. Higher doses are not necessary and might even be dangerous because of an increased incidence of side effects [182–184].

This study was the first suggesting beneficial effects of lowest-dose aspirin in secondary prevention of myocardial infarction. However, as many other innovative studies, the Cottbus trial has also caused a number of controversial discussions. These included the study design (initial 2-week treatment with high-dose aspirin [1 g/day!]), patient selection (no "random" randomization but hospital-specific distribution of the patients to the treatment groups), heterogeneity in both entrance criteria and con-

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comitant treatments (linseed oil in some subgroups!), possible compliance problems and the fact that no placebo group was included [185]. The absence of placebo at the time when the study was conducted was not a matter of ethical concerns since the efficacy of long-term aspirin treatment in secondary prevention was unknown at the time. While the possibility exists that 30 mg aspirin is effective, as suggested by the authors, it can also not be excluded that the 1,000 mg dose was less effective or not effective at all (see below) [186]. It is also questioned whether 30 mg aspirin per day is sufficient to obtain an optimum cardioprotective effect [29]. Importantly, *all* patients, independently of their later randomization, had initially received 1,000 mg aspirin daily during the early postinfarction period (days 5–14 after the acute event). Thus, while the study has merits, it did not answer the question of the optimal aspirin regime for long-term reinfarction prophylaxis in secondary prevention.

Further early studies. Two other early trials investigated the efficacy of low-dose (100 mg/day) aspirin vs. placebo [187] or high-dose aspirin (1,000 mg/day) [186] in postinfarction patients in a prospective, randomized manner. Treatment was started within 4–7 hours after the acute event. In both studies, there was a significant reduction of the incidence of reinfarctions, by 44–55 %, respectively, within 3 months with the 100 mg dosage but not with placebo or the 1,000 mg dose. Infarct size at 3 days was unaffected, as was the cardiovascular mortality at 3 months [187]. Husted and colleagues [186] additionally measured platelet aggregation and thromboxane formation in their patients and found a comparable inhibition of aggregation and complete inhibition of thromboxane formation by both the 100 mg and 1,000 mg aspirin doses. This suggested that the worse clinical outcome with 1,000 mg aspirin could not be simply explained by a different inhibition of platelet function. Other (negative) effects of aspirin may be involved that only become apparent at higher doses.

The metaanalyses of randomized trials of the ATTC have convincingly documented that aspirin is an effective drug for long-term secondary prevention of myocardial infarction and stroke [29]. The last available edition, from 2009, this time performed on the basis of individual patient data on a standardized 2-year observation basis, was restricted to patients with previous myocardial infarction, stroke and/or transient ischemic attacks. This analysis also included 16 studies comparing aspirin to placebo. The results confirmed an about 20 % RR of serious vascular events by aspirin: 6.7 % vs. 8.2 % per year ($P < 0.0001$) (Table 4.1.1-1) [6, 188].

Nevertheless, there are large disease-related differences in clinical outcome at the most conventional doses of 75–325 mg/day aspirin. This indicates a considerable heterogeneity between studies, that is, disease-related variability of vascular protection by aspirin, and also suggests that the about 20 % RR by aspirin in secondary prevention [29] might be a too general statement and not applicable to single patients.

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Further clinical trials. Several randomized but small trials in secondary prevention have shown that higher doses of aspirin, that is, 325 mg/day, cause more potent inhibition of thromboxane formation and platelet function than lower ones (75–81 mg/day). In addition, there were time-dependent and patient-dependent variations in platelet sensitivity to aspirin [107, 113, 189–192]. A large although not randomized post hoc observational study in secondary prevention of more than 20,000 patients with ACS (GUSTO IIb and PURSUIT) showed no effect of aspirin dose on a composite vascular endpoint at 6 months (HR: 0.92; 95 % CI: 0.79–1.07; $P = 0.28$) but fewer reinfarctions (HR: 0.79; 95 % CI: 0.64–0.98; $P = 0.03$) and more strokes within 6 months in surviving patients at medium (≥150 mg/day) aspirin doses as compared to low doses (<150 mg/day). Total mortality was unchanged [193]. Similar positive results for higher-dose aspirin (>162 mg/day) in a similar patient population were also found in the BRAVO trial [194] although the study was stopped prematurely for safety reasons [195]. However, in DAPT studies with an ADP antagonist and aspirin, the aspirin component was never randomized [196]. Frequently, a certain "tradition" already starting with CURE [26] is to name the aspirin group in comparative studies with competitor drugs "placebo." This is misleading and ignores the well-established fact that aspirin is a clinically effective standard treatment by its own at least in secondary prevention.

The "Aspirin Dosing: A Patient-centric Trial Assessing Benefits and Long-term Effectiveness" (ADAPTABLE) trial was designed to determine the appropriate dose of aspirin to lower the vascular risk in patients with established atherosclerotic cardiovascular disease [196].

Using an open-label pragmatic design, patients with established atherosclerotic cardiovascular disease (36 % previous myocardial infarction, 53 % previous coronary revascularization, 23 % congestive heart failure, >80 % hypertension and/or dyslipidemia, 38 % diabetics, etc.) were randomly assigned to a strategy of 81 mg or 325 mg aspirin/day. Primary efficacy endpoint was a composite of total mortality and hospitalization for myocardial infarction or stroke. Primary safety outcome was hospitalization for major bleeding.

A total of 15,076 patients were followed for a median of 26.2 months. Before randomization, 96 % of patients were already taking aspirin, and 85 % of them were taking 81 mg/day. The primary endpoint occurred in 7.28 % in the 81 mg group and 7.51 % in the 325 mg group (HR: 1.02; 95 % CI: 0.91–1.14). The safety endpoint occurred in 0.63 % of the 81 mg group and in 0.60 % of the 325 mg group (HR: 1.18; 95 % CI: 0.79–1.77). Of patients assigned to the high-dose aspirin group, 42 % switched to the low-dose group during the study, but only 7 % switched from the low-dose to the high-dose group.

The conclusion was that there were no significant differences in cardiovascular or major bleeding events between 325 and 81 mg of aspirin daily. However, there was a substantial dose switching to the 81 mg dose, which needs to be considered when interpreting the study data [197].

ADAPTABLE was unique due to its remote enrollment, the short study duration and outcome assessments (no assessment of minor bleeding events!). Noteworthy is also the fact that participants had to purchase (!) their OTC study medication. An important limitation is the large number of participants who switched from high-dose to low-

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dose aspirin. For statistical evaluation, the trial used an ITT analysis, that is, patients were analyzed in the assigned group, even if they switched doses during the trial. In a prespecified on-treatment analysis, patients who received 81 mg aspirin, regardless of their assigned group, had an increased risk for the primary outcome compared with patients who actually received the 325 mg dose (HR: 1.25; 95 % CI: 1.10–1.43). This finding reduces confidence in the ITT analysis and the result of the 81 mg dose group [198].

Aspirin-induced bleeding and rebound phenomena after withdrawal. An increased rate of perioperative thrombotic events (myocardial infarction, stroke) has been described in patients at elevated vascular risk shortly after withdrawal of aspirin because of surgical interventions [199, 200]. The opposite, that is, improved graft patency, was seen with maintained preoperative aspirin medication [201]. A survey of retrospective studies suggested that aspirin withdrawal in these patients increases the incidence of acute embolic vessel occlusions by up to 10 %. These occlusions occur on average about one week after withdrawal [202]. Specifically patients with coronary stents, who undergo an invasive procedure, are at high risk of perioperative myocardial infarction and stent thrombosis. In these patients interruption of oral antiplatelet treatment for more than 5 days prior to (non)cardiac invasive procedures is a key factor affecting cardiac and cerebrovascular events [203]. Perioperative aspirin use should be balanced on an individual basis, considering the benefits of preventing the risk for thrombotic graft occlusion, in particular in cardiac surgery [204, 205].

Several studies have addressed the issue of a withdrawal syndrome after aspirin removal in patients who continuously took the drug for thrombosis prevention. A large metaanalysis of more than 50,000 patients at elevated risk who took aspirin for secondary thrombosis prevention reported that aspirin withdrawal was associated with a 3-fold higher risk of major adverse cardiac events (MACE) (OR: 3.14; 95 % CI: 1.75–5.61; $P = 0.0001$) [200]. Similar results were obtained in a small but placebo-controlled double-blind study in 156 high-risk patients who had (endoscopically treated) peptic ulcer bleeding. Among aspirin (80 mg/day plus PPI)-treated patients there were significantly fewer cardiovascular complications than in patients in the placebo (plus PPI) group, 1.3 % vs. 10.3 %, and in addition a reduced mortality, 1.3 % vs. 10.3 %, despite an increased rate of recurrent bleeding events [206]. A large metaanalysis of 39,315 patients confirmed that withdrawal of aspirin within the first year after the acute ischemic event was associated with a marked increase in myocardial infarctions and strokes but only a minor increase in bleeding events. Reasons for discontinuation were mainly nonadherence (“saw no effect,” “forgotten,” etc.); only <10 % discontinued aspirin use because of safety reasons (!) (Fig. 4.1.1-8) [207].

Most impressive results about the negative consequences of early aspirin withdrawal were obtained in a recent Swedish registry trial.

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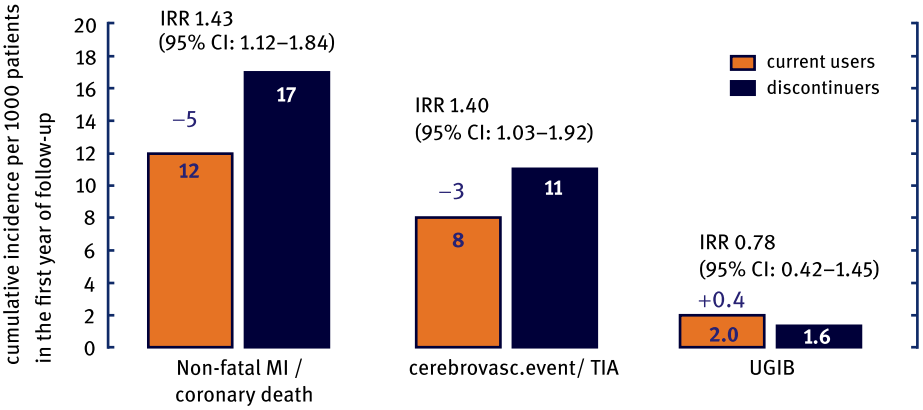

Figure 4.1.1-8: Absolute risk and incidence ratios (IRRs) of vascular events and upper gastrointestinal bleeding events (UGIBs) in aspirin-treated patients in secondary prevention of cardiovascular and cerebrovascular events. Data are incidence rates for the first year after the acute event. Discontinuation caused five more cardiovascular and three more cerebrovascular events and avoided 0.4 UGIBs in the 30 % of patients who discontinued aspirin intake [207].

A total of 601,527 users of low-dose aspirin for primary or secondary prevention according to the Swedish prescription register between 2005 and 2009 were included. Eligible individuals were >40 years of age, were free from previous cancer and had at least 80 % adherence during the first observed year of treatment. Cardiovascular events were identified with the Swedish inpatient and cause-of-death register. The mean study period was 3 years. The study was begun 1 year after the start of aspirin intake.

Of all patients, 31 % discontinued regular aspirin intake without replacement by another antiplatelet medication (not because of severe bleeding complications!). This withdrawal resulted in a markedly increased risk for a new vascular event (myocardial infarction, stroke) or death (HR: 1.37; 95 % CI: 1.34–1.41) as opposed to those patients who continued or used other forms of antiplatelet drugs. This corresponded to one additional cardiovascular event observed per year in 1 of every 74 patients who discontinued aspirin. The risk increased shortly after discontinuation and did not appear to diminish over time.

The conclusion was that discontinuation of low-dose aspirin in long-term users in the absence of major surgery or bleeding was associated with a >30 % increased risk of cardiovascular events. Adherence to low-dose aspirin treatment in the absence of major surgery or bleeding is likely an important treatment goal [208].

Thus, noncompliance or withdrawal from aspirin treatment without replacement by other antiplatelet medications has ominous prognostic implications, specifically in subjects with elevated risk for vascular thrombotic events [209].

Aspirin and (PPIs). Aspirin as long-term treatment is freqently combined with a PPI because of gastric protection. PPIs have a long duration of action, can be given orally once daily and have been shown to markedly protect from aspirin-induced gastric injury [210–212]. This also includes protection from upper gastrointestinal bleeding

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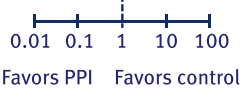
Table 4.1.1-4: Efficacy and safety of PPIs in long-term aspirin users: metaanalysis of randomized cardiovascular prevention trials (mod. after [210]).

Study or subgroup	PPI total	Control total	# of studies	HR mean (95 % CI)	Risk ratio	*P*
All cause mortality						
Number (events)	3,991 (14)	3,105 (5)	7	1.72 (0.61–4.87)		0.31
Cardiovascular mortality						
Number (events)	3,991 (10)	3,105 (3)	7	1.80 (0.59–5.44)		0.30
Nonfatal myocardial infarction/ischemia						
Number (events)	1,533 (6)	1,490 (13)	5	0.56 (0.22–1.41)		0.22
Ischemic stroke/TIA						
Number (events)	3,915 (7)	3,031 (5)	6	1.09 (0.34–3.53)		0.89



but not from bleeding in the lower gastrointestinal tract [211]. The risk of interaction with the antiplatelet actions of aspirin is low or absent (Table 4.1.1-4) [210]. The reason is that antiplatelet effects of aspirin are largely determined by the amount of active drug absorbed in the small intestine, while PPIs act selectively on the acid-producing oxyntic cells in the stomach mucosa [213]. A large metaanalysis clearly demonstrated that PPIs are effective in preventing gastrointestinal symptoms (peptic ulcers, erosive esophagitis) and resolution of the dyspeptic symptoms without increasing adverse events, cardiac risks or mortality in long-term aspirin users. PPIs did also not increase the risk of a new vascular event in individuals on DAPT after PCI, despite reducing inhibition of platelet aggregation by ADP [214].

For East Asian patients, PPIs with a weaker affinity to the CYP2C19 bioactivating isoenzyme, such as pantoprazole, have been recommended [215]. Protection by PPIs appears to be independent of the aspirin dose [216]. Data from a phase 3 clinical trial on PA32540 (a coordinated-delivery tablet containing 325 mg enteric-coated aspirin and 40 mg omeprazole) vs. 325 mg enteric-coated aspirin alone showed improved gastric protection in subjects at risk for aspirin-associated gastric ulcers, a similar cardiovascular event profile and markedly improved adherence to drug treatment because of less upper gastrointestinal tract adverse effects (see also Section 3.2.1) [217].

4.1.1.9 Clinical trials – coronary artery bypass graft surgery and other surgical interventions

General aspects. Surgical revascularization of coronary arteries by inserting a bypass vessel is the surgical alternative to the PCI of an interventional cardiologist. However, this cardiosurgical intervention, like other surgical procedures, is also associated with

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significant platelet activation and thromboxane formation inside the (coronary) circulation [218]. This is partially due to the surgical procedure itself, including extracorporeal circulation during open-heart surgery. This will expose platelets to artificial surfaces, causing platelet adhesion, activation, secretion of storage products and formation of coaggregates with white cells. There is a significant drop of circulating platelet count because of aggregate formation. Another issue is the release of inflammatory mediators during cardioplegia and/or reperfusion of the heart [219] which appears to be sensitive to aspirin treatment, at least in reperfused ischemic rat hearts in vivo [220]. Thus, thrombotic reocclusion of successfully transplanted vessels is an inherent problem of bypass surgery and an appropriate antithrombotic regime is strongly recommended. The price to be paid is an elevated risk of periprocedural bleeding events.

Aspirin and thrombotic vessel occlusions. Treatment with antiplatelet drugs, such as aspirin or ADP antagonists, is commonly employed in patients subjected to coronary artery bypass surgery (CABG), except those at a particular risk constellation (Section 3.1.2). Low-dose aspirin is well known to protect from early arterial graft occlusions [221–224]. Regular postoperative aspirin intake has been shown to improve 5-year survival from 66 % (no aspirin) to 79 % (aspirin) in an early observational trial on more than 5,600 CABG patients [225]. Interestingly, a metaanalysis of randomized early trials indicated that medium aspirin doses (300–325 g/day) might be more effective to prevent graft occlusion than lower ones (75–100 mg/day) [226]. Neither improved clinical outcome nor increased bleeding was seen with 100 mg enteric-coated aspirin vs. placebo, given as a single dose 1–2 hours prior to surgery in aspirin-naïve patients in the randomized, placebo-controlled ATACAS trial [227]. This is not totally surprising since the selected doses might have been too low in many patients to exert any significant antiplatelet effect during the surgical intervention [172].

There are two large observational trials on aspirin and CABG in cardiac patients. One was a case-control study in more than 8,600 patients that showed a lower in-hospital mortality of CABG patients who were given preoperative aspirin within 7 days before surgery (OR: 0.55; 95 % CI: 0.31–0.98) without increased severe bleeding events or increased bleeding-related morbidities [228]. The other was a large prospective although not randomized trial designed to evaluate the benefit/risk ratio of patients who received aspirin (up to 650 mg/day) within 48 hours after revascularization and the 1-month survival of more than 5,000 CABG patients [229].

A total of 5,065 patients undergoing coronary bypass surgery (cardiopulmonary bypass) who survived the first 48 hours after surgery were included in a prospective multicenter study to discern the relation between early aspirin use and clinical outcome after 30 days. Patients received a total dose of 80–650 mg aspirin, according to the hospital recommendations, within the first 48 h after surgery or received no aspirin.

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There were significantly more patients on aspirin on admission – 52 % vs. 39 % ($P < 0.001$) – however, only 1.3 % of patients on aspirin (40 out of 2,099) died 48 h after surgery or later, as opposed to 5.0 % of patients on placebo (81 out of 2,023) ($P < 0.001$). In addition, in comparison to patients without aspirin, treatment caused a 48 % reduction in the incidence of myocardial infarctions (2.8 % vs. 5.4 %), a 50 % reduction in the incidence of strokes (1.3 % vs. 2.6 %), a 74 % reduction in the incidence of renal failure (0.9 % vs. 3.4 %) and a 62 % reduction in bowel infarctions (0.3 % vs. 0.8 %). There was no increased risk for hemorrhage by aspirin, nor an increased risk for gastritis, infections or impaired wound healing. There was also no dose dependency of these aspirin actions.

The conclusion was that early aspirin after CABG is safe and reduces mortality and ischemic complications, involving the heart, brain, kidneys and gastrointestinal tract. There is no evidence of aspirin-induced severe bleeding complications [229].

These are remarkable findings, suggesting a significant beneficial effect of aspirin that was at least 2-fold higher than in "conventional" secondary prevention and was not obtained in these patients at the price of increased severe bleeding. However, the study was also criticized for several reasons. The treatment group assignment was nonrandomized and the aspirin doses were quite variable at the 70 centers in 17 countries where the study was conducted. Since aspirin was the only antithrombotic treatment, this could have resulted in an overestimation of its efficacy. The study did also not address the outcome of these patients later than 1 month after surgery. Nevertheless, this trial suggested that early aspirin (antiplatelet) treatment is associated with a remarkable 68 % (!) reduction in overall mortality and substantial and comparable reductions of ischemic complications, affecting the heart and other organs. Importantly, there was no increased risk of bleeding, possibly because of the marked inflammatory response associated with the surgical procedure. Accordingly, there appears to be no reason to increase the coagulation potential by infusion of platelets, clotting factors or antifibrinolytic drugs (tranexamic acid); these procedures might rather aggravate ischemia-induced organ failure.

In an editorial to this paper, it was discussed whether the efficacy of aspirin might even have been underestimated because only 48 h survivors were included into the study. It was also suggested that in addition to antiplatelet effects, other pharmacological properties of aspirin might have been involved, for example its antiinflammatory actions, including inhibition of platelet-induced activation of white cells (monocytes) [230]. Another observational study investigated the effects of aspirin intake on blood loss during surgery. Of the CABG patients, 63 % were identified as aspirin users. Among these, 23 % required postoperative blood transfusions compared with 19 % for the nonusers, indicating increased blood loss during surgery [231]. A large metaanalysis of 17 studies on preoperative aspirin in CABG patients including more than 9,000 patients showed that aspirin increased the amount of chest tube drainage. However, this was not associated with an increased risk of reoperation for bleeding. In addition, the early postoperative all-cause mortality and myocardial infarction incidence were not reduced by using preoperative aspirin [232].

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Coronary artery bypass surgery and dual antiplatelet therapy. Like in other prevention studies (see below), the question arose whether DAPT, that is, combined use of aspirin and an ADP antagonist, will improve the outcome or only increase bleeding.

The outcomes of aspirin monotherapy versus DAPT following CABG over a median follow-up of 4.9 years were studied in a propensity-matched retrospective study from a large, multihospital healthcare system. Patients prescribed aspirin monotherapy started on 81 mg aspirin daily, and patients on DAPT were prescribed 81 mg aspirin daily and 75 mg clopidogrel daily. Primary outcomes included overall survival and major adverse cardiac and cerebrovascular events (MACCE), defined as a composite of death, myocardial infarction, stroke or repeat revascularization.

A total of 3,562 patients were included, 35 % receiving aspirin monotherapy and 65 % receiving DAPT. DAPT was associated with a higher rate of postoperative blood transfusions (30.7 % vs. 25.4 %; $P = 0.001$). Overall survival was comparable between groups (1-year aspirin 95.9 % versus DAPT 97.2 % and 5-year aspirin 86.3 % versus DAPT 87.8 %; log-rank $P = 0.194$). Rates of MACCE were also similar (1-year aspirin 9.4 % versus DAPT 8.7 % and 5-year aspirin 26.7 % versus DAPT 24.7 %; $P = 0.798$).

The conclusion was that DAPT did not confer any advantage in terms of improved survival or freedom from MACCE compared to aspirin monotherapy following isolated CABG, and was associated with a higher postoperative transfusion rate [233].

Similar results were obtained in the randomized, double-blind, placebo-controlled POPular CABG trial:

This study investigated whether ticagrelor added to standard aspirin (80 mg or 100 mg/day) improves graft patency at one year after CABG. The indication for CABG was ACS in 31.3 %, and 95.2 % of procedures used cardiopulmonary bypass. The primary outcome was graft occlusion at 1 year.

The graft occlusion rate among 499 randomized patients amounted to was 10.5 % in the aspirin/ticagrelor group, as opposed to 9.1 % in the aspirin alone group (OR: 1.29; 95 % CI: 0.73–2.30; $P = 0.38$). Graft failure occurred in 14.2 % of patients in the aspirin/ticagrelor group versus 11.6 % in the aspirin alone group (OR: 1.22; 95 % CI: 0.72–2.05).

The conclusion was that addition of ticagrelor to standard aspirin reduce neither graft occlusion nor graft failure at 1 year after CABG [234].

More large randomized, prospective trials on DAPT in CABG are required. Until those are available, preoperative aspirin at a dose of ≤160–325 mg has been recommended in patients undergoing CABG surgery [235, 236] and DAPT appears not to have significant benefits over aspirin alone.

4.1.1.10 Aspirin and other drugs

General aspects. Aspirin for cardiocoronary prophylaxis in secondary prevention, the most significant indication for long-term administration, is frequently used in combination with other drugs. In addition to PPIs (see above) these include not only compounds that enhance the antiplatelet and antithrombotic actions of aspirin, such as ADP antagonists (prasugrel, clopidogrel, ticagrelor) and oral anticoagulants (coumarins, NOACs), but also statins and PCSK9 inhibitors because of frequently

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coexisting hypercholesterolemia. On the other hand, aspirin might cause unwanted side effects because of general inhibition of prostaglandin biosynthesis, if this is part of the clinical action of drugs taken as comedications. This is particularly relevant to ACE inhibitors (Section 3.2.3). Another issue are drug interactions between aspirin and other inhibitors of COXs, such as traditional NSAIDs and coxibs.

Aspirin and ADP antagonists. Aspirin inhibits platelet function by inhibition of thromboxane formation, thienopyridines (clopidogrel, prasugrel) and ticagrelor by blockade of the ADP receptor subtype P2Y12. These compounds act synergistically with aspirin (Section 2.3.1). The overall efficacy of clopidogrel in cardiocoronary prevention in the CAPRIE study was modestly stronger than that of aspirin: 8.7 % relative vascular RR for clopidogrel vs. aspirin ($P = 0.043$). Compared with clopidogrel, there was an increase in the number of gastrointestinal bleeding events in the aspirin group, 0.71 % vs. 0.49 % ($P < 0.05$), but no increase in the number of total bleeding events, 1.55 % vs. 1.38 % [237].

The CAPRIE trial was done in the pre-PCI era when with the exception of ticlopidine none of the more advanced ADP antagonists such as prasugrel or ticagrelor were available. Recently a second head-to-head comparison of aspirin and clopidogrel was published: The "Host-extended antiplatelet monotherapy" (HOST-EXAM) trial [238].

The HOST-EXAM trial was an investigator-initiated, prospective, randomized, open trial in South Korea. In total, 5,438 post-PCI patients (mean age 64 years), three quarters after an ACS who were event-free during 6–18 months of DAPT, were randomly designed to receive a monotherapy of clopidogrel 75 mg once daily or aspirin 100 mg once daily for 24 months. The primary endpoint was a composite of all-cause death, nonfatal myocardial infarction, stroke, readmission due to ACS and bleeding.

During 24-month follow-up, the primary outcome occurred in 5.7 % of patients in the clopidogrel group and in 7.7 % in the aspirin group (HR: 0.73; 95 % CI: 0.59–0.90; $P = 0.0035$). This difference in favor of clopidogrel was mainly driven by readmission due to ACS: 2.5 % in the clopidogrel group and 4.1 % in the aspirin group (HR: 0.61: 95 % CI: 0.45–0.82; $P = 0.001$). Total mortality was not different: 1 % in the clopidogrel group and 1.3 % in the aspirin group (HR: 1.43; 95: CI: 0.93–2.19; $P = 0.10$).

The conclusion was that clopidogrel monotherapy was superior to aspirin monotherapy in preventing future adverse clinical events [238].

This study was subject to a number of comments. In addition to the rather short treatment period of slightly more than 1 year and the open design, there was no change in mortality despite the significant reductions in ischemic and bleeding events [239]. In addition, the observed event rate of the primary endpoint was 36 % lower than expected: 7.7 % vs. 12 %, respectively, in the aspirin group, suggesting a possibility of underreporting [240]. Finally, the study was conducted entirely in an East Asian population with about 50–60 % carriers of loss-of-function mutations of the CYP2C19 gene [241, 242] associated with an attenuated or reduced antiplatelet effect of clopidogrel

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[241, 242]. While the genetic predisposition needs to be noted [240], it should have resulted in a reduced antiplatelet effect of clopidogrel, which is somehow contrary to the results. According to a recent metaanalysis, P2Y12 inhibitor monotherapy compared with aspirin monotherapy is associated with an RR for myocardial infarction and a comparable risk of stroke in the setting of secondary prevention. The benefit of P2Y12 inhibitor monotherapy is of debatable clinical relevance in view of the high NNT to prevent a myocardial infarction and the absence of any effect on all-cause and vascular mortality [243]. A paradigm shift requires randomized comparisons of aspirin monotreatment vs. P2Y12 monotreatment after PCI with appropriate stratification of subgroups. Of interest are also not only the first 1–3 months after the index event but also next years' results [244].

Dual antiplatelet therapy. The combination of aspirin with ADP-P2Y12 antagonists appears to be a useful strategy in long-term DAPT. This was shown originally in the CURE trial [26]. Here, combined use of aspirin plus clopidogrel was superior to aspirin alone in patients with ACS (NSTEMI). DAPT reduced the absolute risk of a combined vascular endpoint (myocardial infarction, stroke, cardiovascular death) by 2 % as compared to aspirin alone but increased the rate of severe bleeding events by 1 %. Based on these data, the combined use of aspirin and an ADP antagonist (today preferentially prasugrel or ticagrelor) became guideline-recommended DAPT in ACS.

The "Organization to Assess Strategies for Ischemic Syndromes" (OASIS)-7 trial studied the effect of standard and double-dose clopidogrel combined with low- and medium-dose aspirin on clinical outcome (cardiovascular death, myocardial infarction or stroke) at 30 days after PCI because of ACS. There was no difference in efficacy between higher (300–325 mg/day) and lower (75–100 mg/day) aspirin doses but a significantly improved clinical outcome at the double-dose clopidogrel. Interestingly, in the subgroup with double-dose clopidogrel, only the combination of double-dose clopidogrel with higher-dose aspirin improved the clinical outcome (HR: 0.82; 95 % CI: 0.69–0.98; $P = 0.03$) – at the price of significantly increased major bleeding events – while the subgroup of clopidogrel combined with lower aspirin dose did not [245]. The mechanisms and the clinical significance of this finding are unclear. However, a pharmacokinetic interaction between aspirin and the active metabolite of clopidogrel was excluded [246].

Another question is whether combined use of aspirin and clopidogrel also results in an improved clinical outcome during long-term prophylaxis of patients at elevated atherothrombotic risk but without previous cardiovascular event. In other words, is it useful to generally replace the currently practiced monotherapy with aspirin in these patients by the combination of aspirin with an ADP antagonist, here clopidogrel. This issue was studied in the "Clopidogrel for High Atherothrombotic Risk and Ischemic Stabilization Management and Avoidance" (CHARISMA) trial.

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CHARISMA was a prospective, double-blind randomized study in 15,603 patients (age > 45 years) at high atherothrombotic risk. Three quarters of the patients (12,153) already had suffered an atherothrombotic event (myocardial infarction, cerebrovascular ischemic event) or had a symptomatic peripheral arterial occlusive disease. One quarter (3,284) of the patients was asymptomatic but had multiple risk factors. Patients received aspirin (75–162 mg/day plus placebo or aspirin plus clopidogrel (75 mg/day). Primary efficacy endpoints were myocardial infarction, stroke and cardiovascular death. Primary safety endpoints were severe bleeding events. Secondary endpoints were similar but additionally included hospitalization because of vascular problems. The study endpoint was set at 1,040 events.

The predefined primary endpoint was obtained at about 30 months in the total population. The cumulative event rate of primary events at this time was 7.3 % in patients with aspirin and 6.8 % in patients with the combined treatment. This was equivalent to a relative RR of 7.1 % with wide variations (95 % CI: −4.5 to 17.5) and not different from the treatment with aspirin alone ($P = 0.22$). There was a slightly improved efficacy in secondary endpoints in the group with combined treatment: 16 % vs. 17.9 % ($P = 0.04$). The number of severe bleeding events was not different. However, there were significantly more "moderate" bleeding events (including those which required transfusion) in the combined treatment group. A later evaluation of the study data [247] indicated that the increase in moderate bleeding was associated with a significant about 3–4-fold increased risk of myocardial infarction and stroke and also a significant increase in all-cause mortality (HR: 2.55; 95 % CI: 1.71–3.80; $P < 0.001$).

According to a subgroup analysis of primary efficacy endpoints of symptomatic and asymptomatic patients, there was a slight but significant ($P = 0.046$) benefit in favor of the symptomatic patients, i. e., secondary prevention, but no benefit or rather a tendency for a deleterious effect in asymptomatic patients with multiple risk factors without preceding vascular event ($P = 0.20$). Of concern in this group was a significant increase in cardiovascular ($P = 0.01$) and total ($P = 0.04$) mortality after combined treatment.

The conclusion was that combined use of clopidogrel and aspirin tends to improve the efficacy of secondary prevention in symptomatic patients, that is, those who already had suffered a vascular event, although at the price of increased bleeding. The combined use of aspirin and clopidogrel cannot be recommended for primary prevention in patients with risk factors but without preceding vascular event. In these patients, addition of clopidogrel to aspirin causes an increase in cardiovascular mortality as well as an increased risk of bleeding (Fig. 4.1.1-9) [98].

Thus, enhanced antiplatelet treatment did not result in improved clinical outcome in asymptomatic patients without previous vascular event.

After the introduction of the more advanced ADP antagonists prasugrel and ticagrelor, there were also studies on their combined use with aspirin in ACS, that is, the TRITON-TIMI 38 study with prasugrel [248] and the PLATO study with ticagrelor [249]. Both studies showed reduced cardiocoronary events, however at the expense of additional severe and fatal bleeding events as opposed to the combination of aspirin with clopidogrel. Both studies are not directly comparable for several reasons. However, it appears that any more intense antiplatelet treatment with DAPT not only prevents more thrombotic events but also causes an elevated risk of severe bleeding events.

The recent "Prevention with Ticagrelor of Secondary Thrombotic Events in High-Risk Patients with Prior Acute Coronary Syndrome – Thrombolysis In Myocardial Infarction Study" (PEGASUS-TIMI 54) trial investigated the efficacy and safety of DAPT

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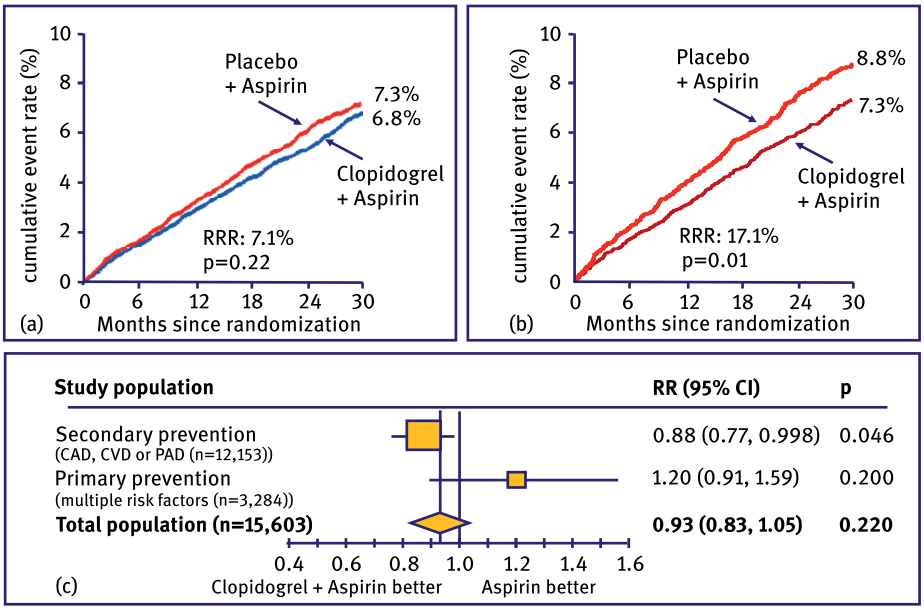

Figure 4.1.1-9: The CHARISMA trial. Cumulative event rate of primary MACE endpoints (myocardial infarction, stroke, cardiovascular death) in the overall population (a), the subpopulation with a previous qualifying event (b) and in patients with multiple risk factors without a prior qualifying vascular event (c). There was no difference in the overall population between the two treatment groups and rather a tendency in favor of aspirin alone in the multirisk factor group, while the opposite was seen in the patients with a previous qualifying event (for further explanation see text) [98].

with ticagrelor plus aspirin in patients who had suffered a myocardial infarction 1–3 years earlier vs. aspirin alone. Similar to CHARISMA, there was also an improved outcome at 33 months in the groups with combined treatment (60 or 90 mg ticagrelor twice daily) as compared to aspirin alone: 7.85 % in DAPT (90 mg) vs. 9.04 % in aspirin alone (HR: 0.85; 95 % CI: 0.74–0.95; $P = 0.004$). However, this was again associated with a marked increase in severe bleeding events: 2.60 % vs. 1.06 % ($P < 0.001$) [250]. In addition, a large number of patients of the two ticagrelor groups stopped treatment prematurely. Main reasons were bleeding events and dyspnea, a typical side effect of ticagrelor [251].

The GLOBAL-LEADERS trial was a randomized open-label prospective trial aimed to show the superiority of ticagrelor vs. aspirin monotherapy in long-term secondary prevention of stented patients with atherosclerotic CVD.

Ticagrelor in combination with aspirin was given for 1 month, followed by ticagrelor alone or aspirin alone, in 15,968 stented PCI patients. Patients had stable CAD or ACS. Patients received 75–100 mg aspirin daily plus 90 mg ticagrelor twice daily for 1 month, followed by 23 months of ticagrelor alone, or standard DAPT with 75–100 mg aspirin daily plus either 75 mg clopidogrel daily (for pa-

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tients with stable CVD) or 90 mg ticagrelor twice daily (for patients with ACS) for 12 months, followed by aspirin monotherapy for 12 months. The primary endpoint at 2 years was a composite of total mortality or nonfatal new Q-wave myocardial infarction. The secondary endpoint was bleeding.

At 2 years, 3.81 % of participants in the ticagrelor group had died or had a nonfatal myocardial infarction, as opposed to 4.37 % of participants in the control group (HR: 0.87; 95 % CI: 0.75–1.01; $P = 0.073$). No difference in treatment effects was also obtained in prespecified subgroups of ACS and stable CAD patients ($P = 0.93$). There were no differences in severe bleeding: 163 participants in the experimental group and 169 in the control group (2.04 % vs. 2.12 %; HR: 0.97; 95 % CI: 0.78–1.20; $P = 0.77$).

The conclusion was that ticagrelor in combination with aspirin for 1 month followed by ticagrelor alone for 23 months was not superior to 12 months of standard DAPT followed by 12 months of aspirin alone. Rates of severe bleeding events were similar between the groups. The current recommendations for stented patients remain the same, that is, 6–12 months DAPT after PCI, followed by aspirin monotherapy [252].

The study missed the primary endpoint; the expected superiority of ticagrelor over conventional treatment after 2 years has not been shown. There was also a time-dependent decrease in patient compliance throughout the study period. Adherence to study medication in the first year was 82 % in the experimental group and 85 % in the control group, at 2 years it was only 78 % in the experimental group and 93 % in the control group. The most frequent reason for nonadherence in the experimental group was dyspnea ($P \leq 0.005$). Incidence rates of 20–30 % and withdrawal from the study were also seen in previous ticagrelor trials (PLATO, PEGASUS).

The "Ticagrelor with aspirin or alone in High-Risk patients after coronary intervention" (TWILIGHT) study was conducted to compare the safety of ticagrelor vs. aspirin after a minimum period of DAPT in high-risk patients after PCI.

In a randomized, double-blind trial, the effect of ticagrelor alone as compared with ticagrelor plus aspirin with regard to clinically relevant bleeding was studied among patients who were at high risk for bleeding or an ischemic event and had undergone PCI and stenting. All enrolled patients received treatment with ticagrelor (90 mg twice daily) plus enteric-coated aspirin (81–100 mg/day) for 3 months. Patients who had not had a major bleeding event or ischemic event continued to take ticagrelor and were randomly assigned to receive aspirin or placebo for 1 year. The primary endpoint was superiority of the ticagrelor group with respect to clinically relevant bleeding. The secondary endpoint was a composite efficacy endpoint of death from any cause, nonfatal myocardial infarction or nonfatal stroke, using a noninferiority hypothesis with an absolute margin of 1.6 percent points.

The study enrolled 9,006 patients; 7,119 of them underwent randomization after 3 months. Between randomization and 1 year, the incidence of the primary endpoint was 4.0 % among patients assigned to receive ticagrelor plus placebo and 7.1 % among patients assigned to receive ticagrelor plus aspirin (HR: 0.56; 95 % CI: 0.45–0.68; $P < 0.001$). The incidence of the secondary endpoint was the same, 3.9 % in both groups.

The conclusion was that among high-risk patients who underwent PCI and completed 3 months of DAPT, ticagrelor monotherapy was associated with a lower incidence of clinically relevant bleeding than ticagrelor plus aspirin, with no higher risk of death, myocardial infarction or stroke [253].

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According to the authors, these data cannot be generalized to all cardiac patients at high risk, for example patients with STEMI or nonacute coronary syndromes. Consequently the clinical effects of ticagrelor monotherapy should not be extended to all PCI patients, especially those with stable disease. Clearly, to continue with aspirin alone after the 3 months of DAPT treatment instead of ticagrelor is another alternative. Of the initially enrolled patients, 13 % did not tolerate DAPT during the first 3 months (side effects of ticagrelor?) and there was a tendency for more ischemic cerebrovascular events in patients receiving ticagrelor monotherapy. It is also unclear why the primary endpoint (safety) was statistically evaluated according to ITT procedures but the secondary endpoint (efficacy) by on-treatment analysis. This is quite unusual and might cause evaluation bias. Taken together, to switch to monotreatment with aspirin or ticagrelor after the end of guideline-recommended DAPT remains in the discretion of the physician's clinical judgement and the patient's specific baseline characteristics [253].

A metaanalysis of 32,145 PCI patients with ACS or stable angina from five randomized trials where aspirin was discontinued 1–3 months after PCI but P2Y12 inhibitor monotherapy was maintained and compared to traditional DAPT has basically confirmed these conclusions: During a follow-up of 12–15 months post-PCI, discontinuation of aspirin therapy in both patient groups significantly reduced the risk of major bleeding by 40 % compared to DAPT (1.97 % vs. 3.13 %; HR: 0.60; 95 % CI: 0.45–0.79), with no change in the risk of MACE (2.73 % vs. 3.11 %; HR: 0.88; 95 % CI: 0.77–1.02), myocardial infarction (1.08 % vs. 1.27 %; (HR: 0.85; 95 % CI: 0.69–1.06) or death (1.25 % vs. 1.47 %; HR: 0.85; 95 % CI: 0.70–1.03). These data suggest that discontinuation of aspirin with continued P2Y12 inhibitor monotherapy 1–3 months after PCI reduces the risk of bleeding but has no effect on MACE [254].

Duration of DAPT. Yet another question is the optimal duration of DAPT, i. e., aspirin in combination with an ADP antagonist in stented patients. Most guidelines recommend DAPT treatment up to 12 months. However, a randomized, double-blind, placebo-controlled trial reported lower numbers of stent thromboses and myocardial (re)infarctions after 30 months vs. 12 months of DAPT treatment, and for patients with previous myocardial infarction an impressive reduction from 5.2 % to 2.2 % (HR: 0.42; $P < 0.001$) was found – although at an increased rate of bleeding events (1.9 % vs. 0.8 %; $P = 0.005$). Similar though smaller effects were seen in patients without previous myocardial infarction [255]. Thus, the situation is not entirely clear and more randomized controlled trials are required.

GPIIb/IIIa antagonists. Parenteral (intravenous) GIIb/IIa antagonists, that is, direct inhibitors of fibrinogen binding and platelet-dependent clot formation, such as abciximab, tirofiban or eptifibatide, have still a position as adjunct medication in certain cardiovascular interventions. However, these compounds have a narrow therapeutic

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range, that is, they might cause severe, life-threatening bleeding events, and, in addition, do *not* inhibit platelet functions but rather might enhance them by amplifying outside-in signaling, according to their nature as integrin ligands [256, 257]. This particular pharmacology also strictly requires comedication of inhibitors of platelet functions or thrombin formation/action, such as aspirin/ADP antagonists and/or antithrombins such as heparin for intravenous administration. Oral compounds were found to even increase the cardiovascular thrombotic risk and are now outdated. For example, oral lotrafiban induced a 33 % increase in death rates in patients with cardiovascular/cerebrovascular disease in the "Blockade of the Glycoprotein IIb/IIIa Receptor to Avoid Vascular Occlusion" (BRAVO) trial. This excess mortality was associated with enhanced severe bleeding events, was vascular in origin and was not affected by the type of atherosclerotic involvement at entry to the trial. Consequently, the study was stopped prematurely because of safety concerns. Of interest, however, were the data from the aspirin arm. Although the dose of aspirin was not randomly assigned, there was an increase in bleeding events with aspirin doses (>162 mg/day) associated with a lower mortality (1.7 %) as opposed to a higher mortality (2.8 %) at low-dose (75–162 mg/day) aspirin [195].

Coumarins. Despite the use of antiplatelet drugs and DAPT, if indicated, the recurrence rate of cardiovascular ischemic events remains high. In secondary prevention, there is also still a 10 % recurrence rate of atherothrombosis during the first year after the acute event [28]. This persistent risk may be in part attributed to a sustained activation of the coagulation cascade leading to generation of thrombin, with its key role in thrombus formation [28, 258]. Oral anticoagulants of the coumarin type reduce thrombin levels and thrombin activity, both being elevated over months to years in patients after acute myocardial infarction [39, 259]. No such affect was seen with aspirin [259]. This, as well as the long duration of action of warfarin, suggested a synergistic action between the two.

The use of anticoagulants such as warfarin in treatment and secondary prevention of acute myocardial infarction is not new. An impressive 30–50 % reduction of reinfarction rates was reported already more than 60 years ago [260]. The efficacy of full-range anticoagulation (INR: 2.8–4.2) in secondary prevention of cardiovascular (and cerebrovascular) events was confirmed in two large randomized, double-blind, placebo-controlled trials in the 1990s – at the expense of a markedly increased risk of serious bleeding events [261, 262]. The OASIS-2 trial studied the efficacy of moderate-intensity oral anticoagulation (INR: 2.0–2.5) in comparison to standard treatment, mostly aspirin, in patients with unstable angina or NSTEMI. There was a small, non-significant overall thrombotic RR after 5 months by the anticoagulant treatment as opposed to standard treatment but a significant increase in major bleeding: 2.7 % vs. 1.3 % ($P = 0.004$). The efficacy of anticoagulation on both protection from recurrent vascular events and bleeding was dependent on compliance and significant in good

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but not in poor compliers. Thus, compliance problems might limit the efficacy of oral anticoagulants [263]. However, with the exception of stroke prevention in atrial fibrillation (Section 4.2.1), the use of warfarin-type anticoagulants in prevention of atherothrombotic events was and is limited because of the high risk of severe (cerebral) and fatal bleeding events. Additionally, only about 60 % of warfarin-treated patients are in the desired therapeutic range of INR. This is less than expected for effective prevention of a life-threatening thromboembolic vessel occlusion. Thus, the idea came up to combine aspirin as an antiplatelet with (reduced-dose) coumarins (warfarin) to increase the antithrombotic efficacy at reduced bleeding risk.

The data on combined use of aspirin and coumarin-type anticoagulants in cardiovascular prevention are conflicting. An add-on effect of the combination of low-dose (INR: 1.5) warfarin and aspirin was reported in primary vascular prevention in the "Thrombosis Prevention Trial" [99]. The "Coumadin Aspirin Reinfarction Study" (CARS) in patients with previous myocardial infarction, low, fixed-dose warfarin (INR: 1.2–1.5) was not found to provide any additional clinical benefit in combination with aspirin (160 mg/day) as compared to aspirin alone but doubled the risk of major bleeding events [264]. Similar results were obtained in the open randomized "Combined Hemotherapy and Mortality Prevention" (CHAMP) study: Low-INR (1.8) warfarin plus aspirin (81 mg/day) did not provide any additional benefit in postmyocardial infarction patients treated with aspirin (162 mg/day) daily but caused twice as much severe bleeding events [265]. In patients with unstable angina and/or NSTEMI who had prior CABG, combined treatment with aspirin (80 mg/day) and warfarin (INR: 2.0–2.5) was not superior to low-dose aspirin alone in the prevention of recurrent ischemic events but also tended to produce more bleeding events [266]. Interestingly, according to the "Antithrombotic Therapy in Acute Coronary Syndromes" (ATACS) trial, aspirin alone (162 mg loading, starting 9.5 h after start of symptoms and continued with 162 mg/day) vs. combination with heparin/warfarin (target INR: 2.0–3.0) reduced recurrent ischemic events (ACS, death) at 30 days by about half as compared to aspirin alone ($P = 0.03$) at only small increases in bleeding [267]. Similar positive results were reported in a Norwegian randomized trial in postinfarction patients [268]. In this study, high-dose warfarin (target INR: 2.8–4.2) in combination with aspirin (160 mg/day) was superior to aspirin alone but caused significantly more nonfatal major bleeding events: 0.57 % vs. 0.17 % per year (RR: 0.25; 95 % CI: 0.10–0.60).

Thus, the available studies on warfarin as an antithrombotic in cardiovascular prevention provided mixed data. Medium (INR: 2–3) warfarin doses are generally effective, however, at the price of an about 2–3-fold increase in severe and life-threatening bleeding events. The combined use of aspirin and dose-adapted warfarin has been discussed as an option under conditions of well-controlled INR [269, 270]. After the availability of NOACs as alternative, that is, NOACs that do not require monitoring, the situation has been considerably changed.

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New oral anticoagulants. Nonvitamin K antagonist oral anticoagulants (NOACs) inhibit only one single protease of the clotting cascade, that is, factor Xa or thrombin (factor IIa), as opposed to the multiple-step warfarin-type anticoagulants. The biochemical properties of NOACs contribute to their suitability for use in conditions that require a predictable moderate degree of anticoagulation [271]. However, NOACs have also the side effect of bleeding, including gastrointestinal bleeding. Results from both randomized clinical trials and observational studies suggest that high-dose dabigatran (150 mg twice daily), rivaroxaban and high-dose edoxaban (60 mg daily) were associated with an even higher risk of gastrointestinal bleeding than warfarin [272]. In addition, firm platelet adhesion and thrombus formation on human atherosclerotic plaques were increased in the presence of oral thrombin inhibitors in comparison to warfarin-type anticoagulants, and the increased, platelet-mediated thrombus formation in injured carotid arteries of mice was abrogated by the presence of aspirin [273]. This suggests a certain prothrombotic, aspirin-sensitive activity by this type of thrombin inhibitors that might contribute to an increase in myocardial ischemia [273].

Acute coronary syndrome. Rivaroxaban was the first NOAC that was approved by the EMA in 2013 for antithrombotic treatment of ACS on top of guideline-directed DAPT. A recent metaanalysis of antiplatelet treatment alone and in combination with NOACs has shown that single antiplatelet treatment with aspirin plus NOAC vs. aspirin alone reduced the rate of MACE by 30 % (HR: 0.70; 95 % CI: 0.59–0.84) but also increased clinically significant bleeding (HR: 1.79; 95 % CI: 1.54–2.09) [274]. Addition of an oral anticoagulant to DAPT with aspirin and clopidogrel decreased the incidence of MACE modestly (HR: 0.87; 95 % CI: 0.80–0.95) but increased bleeding more than 2-fold (HR: 2.34; 95 % CI: 2.06–2.66) [274]. The ATLAS-ACS-2-TIMI-51 trial on rivaroxaban in ACS showed a significant survival benefit with the lower rivaroxaban dose of 2.5 mg twice daily, but not with the higher dose of 5 mg twice daily. The prize was again a 3-fold higher major bleeding rate including increased intracranial bleeding. Although the investigators [275] and even an expert review [276] stated that this study was a comparison of a NOAC versus "placebo," it was in fact a trial on guideline-directed DAPT in ACS using aspirin plus clopidogrel with and without additional rivaroxaban. A similar head-to-head comparison of rivaroxaban plus ADP antagonist (clopidogrel or ticagrelor) versus rivaroxaban (2.5 mg twice daily) plus aspirin (100 mg/day) in the GEMINI-ACS-1 study showed similar rates of clinically significant bleeding with rivaroxaban plus ADP antagonist versus aspirin plus ADP antagonist treatment, 5 % in each group within 6–12 months after randomization ($P = 0.584$). The study was not designed to detect differences in efficacy, which also apparently did not exist. However, it should be noted that all included patients had to be on aspirin plus clopidogrel/ticagrelor for at least 48 h by the time of randomization and were kept on aspirin for the first 5 days of treatment after the index event [277].

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Thus, in patients with ACS, the addition of a NOAC to standard DAPT may result in some reduction of cardiovascular events but also a substantial increase in bleeding. In addition, oral thrombin inhibitors might have a prothrombotic activity that facilitates coronary thrombus formation and ACS [273]. However, this problem might be (partially) overcome by newly developed, selective thrombin inhibitors, such as BMS-986120 – a selective antagonist of the thrombin receptor PAR-4, which according to phase I clinical trials substantially reduces platelet-rich thrombus formation under conditions of high shear stress but did not prolong coagulation time [278]. There are still open questions regarding the advantages of NOACs vs. DAPT in cardiocoronary indications, including possible pharmacological differences between the available NOACs [279].

Atherosclerotic vascular disease (stable angina). Of particular interest is the question whether NOACs (rivaroxaban) alone or in combination with aspirin will improve the clinical outcome in patients with stable angina. This was studied in the "Cardiovascular outcomes for people using anticoagulation strategies" (COMPASS) trial [280].

The study population included 27,395 patients with stable angina or peripheral arterial occlusive disease (mean age 68 years). High bleeding risk, previous recent stroke and lacunar and hemorrhagic strokes were exclusion criteria. The patients were treated with rivaroxaban (2.5 mg twice daily) plus aspirin (100 mg/day), rivaroxaban (5 mg twice daily) or aspirin (100 mg/day) in a double-blind randomized approach. Primary endpoint was a combination of myocardial infarction, stroke or cardiovascular death. The planned duration of the study was 3–4 years.

The study was terminated prematurely after 23 months when about half of the clinical endpoints were reached. The reason was a possible superiority of the rivaroxaban plus aspirin arm. At this point, 4.1 % of the patients of this group had reached the primary endpoint as opposed to 5 % in the group on aspirin only (HR: 0.76; 95 % CI: 0.66–0.86; $P < 0.001$). This benefit was driven by a significant reduction in strokes: 0.9 % in the combined treatment group as opposed to 1.6 % in the aspirin alone group ($P < 0.001$). The number of myocardial infarctions was not different between these groups: 1.9 % vs. 2.2 % ($P = 0.14$). Most important was a tendency for reduced mortality in the combined treatment group (3.4 %) as compared to aspirin alone (4.1 %) (HR: 0.82; 95 % CI: 0.71–0.96; $P = 0.01$; threshold P value for significance with the statistical model used here: 0.0025). There was a significantly higher number of severe bleeding events in the combined treatment group (3.1 %) compared to the aspirin group (1.9 %) (HR: 1.7; 95 % CI: 1.40–2.05; $P < 0.001$), predominantly bleeding events in the gastrointestinal tract (1.5 % vs. 0.7 %; $P < 0.001$), while the number of fatal bleeding events was not different (0.2 % vs. 0.1 %; $P = 0.32$). The high-dose rivaroxaban alone treatment did not show any differences to aspirin alone with respect to the primary efficacy endpoint (4.9 % vs. 5.4 %; $P = 0.12$), but resulted in significantly more bleeding events (2.8 % vs. 1.9 %; $P < 0.001$).

The conclusion was that the combined use of rivaroxaban plus aspirin in patients with stable atherosclerotic vascular disease is more effective than aspirin alone. Rivaroxaban at higher doses (5 mg twice daily) is not superior to aspirin alone but causes significantly more severe bleeding events than aspirin alone [280].

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This study was the first head-to-head comparison of a NOAC with aspirin in patients with stable atherosclerotic vessel disease. Interestingly, the combination of rivaroxaban plus aspirin did *not* reduce the incidence of myocardial infarctions. The tendency for reduced mortality was mainly driven by a reduced number of cardioembolic and atherosclerotic strokes. The price to pay was a considerable increase of severe although not of life-threatening bleeding events.

Similar results were obtained in a subgroup analysis of the COMPASS trial including only patients with stable CVD. Rivaroxaban alone did not improve the primary outcome when compared to aspirin alone (5 % vs. 6 %; HR: 0.86; 95 % CI: 0.78–1.02; $P = 0.094$), but increased (major) bleeding (3 % vs. 2 %; HR: 1.66; 95 % CI: 1.7–2.03; $P < 0.0001$). Combined rivaroxaban plus aspirin also caused more bleeding events than aspirin alone (3 % vs. 2 %), but also reduced the primary outcome in terms of the combined endpoint by 26 % (4 % vs. 6 %; HR: 9.74; 95 % CI: 0.65–0.86; $P < 0.001$). Again, the number of myocardial infarctions was not reduced by the combined treatment. However, the patients with atrial fibrillation were excluded in this analysis [281]. From a mechanistical point of view this is interesting – rivaroxaban alone even at a high dose did not reduce the incidence of myocardial infarctions although it caused more bleeding events.

Another substudy of COMPASS was conducted in patients with recent coronary bypass grafts. The combination of 2.5 mg rivaroxaban twice daily plus 5 mg aspirin or rivaroxaban twice daily alone compared with aspirin alone did not reduce graft failure in patients with recent CABG surgery, but the combination of 2.5 mg rivaroxaban twice daily plus aspirin was associated with similar reductions in MACE, as observed in the complete COMPASS trial [282].

Taken together, current evidence suggests that the combination of a factor Xa inhibitor and an antiplatelet agent such as aspirin might be a promising therapeutical approach in patients with stable angina, that is, dual pathway inhibition, for example with the combination of low-dose rivaroxaban (2.5 mg twice daily) to attenuate thrombin generation and aspirin (100 mg once daily) to reduce platelet activation [283]. In this respect, the COMPASS trial has provided new facts and, together with other upcoming trials, might change the guideline recommendations in the future [284].

ACE inhibitors. Aspirin and ACE inhibitors are frequently used in combination to treat coronary heart disease, hypertension and chronic heart failure. ACE inhibitors stimulate prostaglandin formation via inhibition of bradykinin breakdown and this is probably part of their clinical efficacy. Thus, COX inhibition by aspirin might reduce this pharmacological action of ACE inhibitors. The clinical consequences would depend on the significance of (stimulated) prostaglandin biosynthesis for the particular disease and patient, respectively, specifically in heart failure.

Whether aspirin at low doses interferes with the treatment effect of ACE inhibitors in heart failure is controversially discussed [285–288] and obviously also determined

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by the kind and severity of heart failure (Section 3.2.3). Prospective randomized trials with hard endpoints (mortality) are definitely needed to answer the question of a clinically relevant ACE–aspirin interaction and its possible relation to the aspirin dose.

Statins. One of the most frequent and important cardiovascular risk factors is hypercholesterolemia. Therefore, appropriate combined treatment with lipid-lowering agents, such as statins, is guideline-recommended with a well-established clinical benefit, i. e., plaque stabilization. In addition, there is an attenuation of the inflammatory state of atherosclerosis, as seen from lowered circulating CRP levels [289], especially in secondary prevention. There is also a "withdrawal" syndrome with increased vascular event rates when statin treatment of ACS patients was discontinued [290]. It would be of considerable interest to know whether statin cotreatment, which is performed in the majority of ACS patients according to guideline recommendations, does have an impact on the antithrombotic effect of aspirin and clinical outcome. There is, however, limited evidence on the effectiveness of statins for primary prevention with mixed findings from participants with widely ranging baseline risks [291]. More advanced LDL receptor-preserving drugs, such as the PCSK9-inhibitors evolocumab and alirocumab have shown promising results in high-risk cardiovascular patients in the FOURIER- and ODYSSEY OUTCOMES studies, respectively. From a pharmacological point of view, synergistic effects will be expected between these agents and antiplatelet compounds that offer new, exciting perspectives for further improved antithrombotic treatment.

NSAIDs and coxibs. In 1985 it was shown for the first time in a placebo-controlled trial that indomethacin reduced thromboxane formation but also coronary perfusion in patients with coronary heart disease and that the reduced perfusion could be antagonized by aspirin cotreatment [292].

Pharmacological studies have meanwhile provided clear evidence for a negative drug interaction between some NSAIDs, such as indomethacin and ibuprofen, with the antiplatelet effects of aspirin (Section 2.2.1) [293, 294]. An original experiment is depicted in Fig. 4.1.1-10 [56].

The possible clinical significance of this finding was first demonstrated in a retrospective observational trial in patients hospitalized because of CVDs. A total of 7,107 patients that were included had received ibuprofen in addition to aspirin (<325 mg/day) after discharge from the hospital. Compared with those who used aspirin alone, patients taking aspirin plus ibuprofen had an increased risk of all-cause mortality (HR: 1.93; 95 % CI: 1.30–2.87; $P = 0.0011$) and cardiovascular mortality (HR: 1.73; 95 % CI: 1.05–2.84; $P = 0.0305$) [295, 296].

No such effect was seen with the combined use of aspirin and diclofenac [295]. Diclofenac in contrast to ibuprofen has been shown not to interact with the antiplatelet

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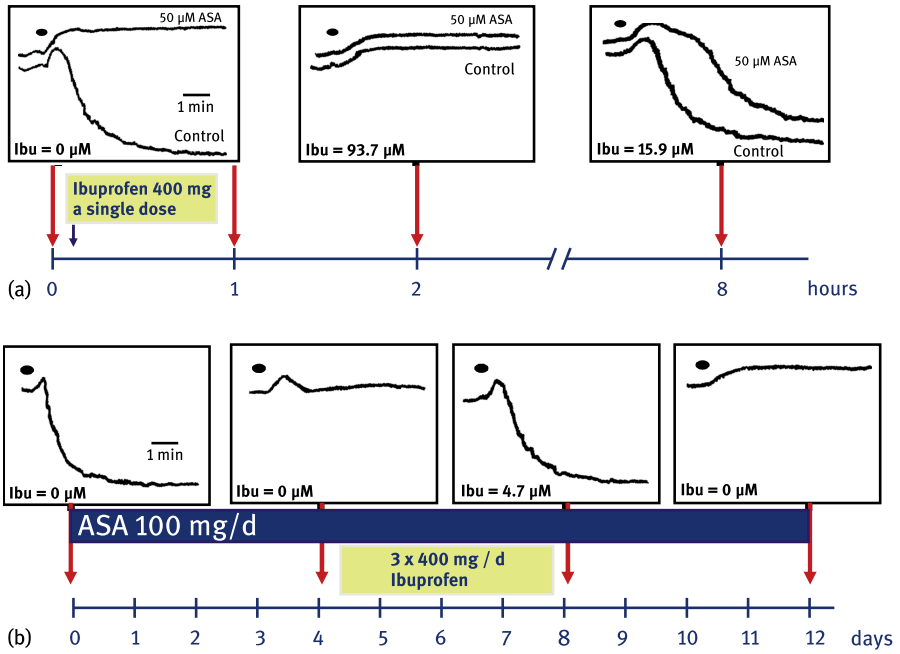

Figure 4.1.1-10: Interaction of oral ibuprofen with aspirin (ASA) in a healthy subject (T. H.), as demonstrated by arachidonic acid-induced light transmission aggregometry in two settings. (a) Light transmission aggregometry before (0) and 2 hours and 8 hours after an oral single dose of 400 mg ibuprofen. Before ibuprofen, 50 µM aspirin in vitro completely inhibits aggregation. Two hours after ibuprofen, platelets are still inhibited by the high plasma concentration of ibuprofen. Eight hours after ibuprofen the aggregation has partially recovered. (b) Oral administration of 100 mg/day aspirin achieves complete inhibition of platelet aggregation within 4 days. Subsequent cotreatment with ibuprofen (three times 400 mg over 4 days) abolishes the platelet inhibition by aspirin. Four days after discontinuation of ibuprofen, platelet inhibition by aspirin is fully restored. Black dots mark the addition of 1 mM arachidonic acid. Actual ibuprofen plasma concentrations (HPLC) are also indicated [56].

effects of aspirin (Fig. 4.1.6-4) [297]. This and other studies suggested that a combination of ibuprofen and aspirin might be deleterious specifically in patients at elevated cardiovascular risk by antagonizing the antiplatelet actions of aspirin [293, 296, 297]. Similar data were obtained from a post hoc subgroup analysis in the US-PHS. The cardioprotective action of aspirin was abolished by regular intake of NSAIDs (nonspecified) (Fig. 4.1.6-2) [82, 298]. A pharmacological inhibition of aspirin's antiplatelet effect by comedication of dipyrone (metamizole) has also been reported [299] and might be significant in case of postoperative analgesic treatment of patients who undergo cardiocoronary surgery as well of patients with acute myocardial infarction [300]. Mechanistically, this interaction is possibly caused by competition of the highly lipophilic NSAIDs with the low-lipophilic salicylate part of aspirin for binding sites in the substrate channel of COX-1 (Section 2.2.1; Fig. 2.2.1-4).

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According to these data, warning labels regarding the simultaneous use of aspirin and NSAIDs or coxibs have been placed on drug boxes for information of costumers. However, more prospective randomized trials are definitely needed to determine the individual cardiovascular risk of combined treatment. In patients at elevated atherothrombotic risk who also suffer from arthritic pain, there is certainly a need for analgesic/antiinflammatory drugs which do not interfere with aspirin's antiplatelet effects. Whether celecoxib could be such an analgesic replacement to ibuprofen or naproxen was studied in the PRECISION trial [301]. However, the data were inconclusive and the study as such was subject to substantial criticism [302].

4.1.1.11 Actual situation

General aspects. Aspirin is still the golden standard antiplatelet drug for prevention of atherothrombotic vessel occlusions. Recommendations on doses and duration of use for prevention of acute vascular events can be found in the actual, regularly updated guidelines of the European Society of Cardiology (ESC), the American Heart Association (AHA) and other health authorities.

Primary prevention. In primary prevention, there is a modest but significant ($P < 0.001$) reduction of (nonfatal) myocardial infarctions at the expense of an increased risk of extracranial and gastrointestinal bleeding events. Based on this benefit/risk ratio, the actual guidelines are very restrictive regarding aspirin prophylaxis in persons without cardiovascular and cerebrovascular diseases and remains a "matter of balance" [303]. This balance might change, also with the introduction of new vasoprotective and antilipid drugs, such as PCSK9 or SGLT2 inhibitors which might directly affect the atherosclerotic process. In addition, a more personalized, safer allocation of aspirin in primary prevention might be provided by improved biomarkers, such as determination of coronary calcium. The individual plaque burden, rather than clinical risk estimations, were suggested as a predictor for the aspirin benefit/risk ratio in primary prevention [304, 305] and there might be more in the future.

A different situation might exist in developing countries and countries with a high incidence of cardiovascular events, for example by coexisting vascular risk factors. For example, in China the annual cardiovascular event rate in apparently healthy individuals amounts to 2.6–3.0 % per year [23]. Here prophylactic use of aspirin appears to be clearly indicated. In this context, there is also the polypill.

The polypill. In 2003, Wald and Law developed the concept of a polypill, containing a mixture of established – and cheap – cardioprotectives, including aspirin, for primary prevention [306]. This strategy was heavily discussed but meanwhile has resulted in introduction of polypills on the market (for example in India). The International Poly-

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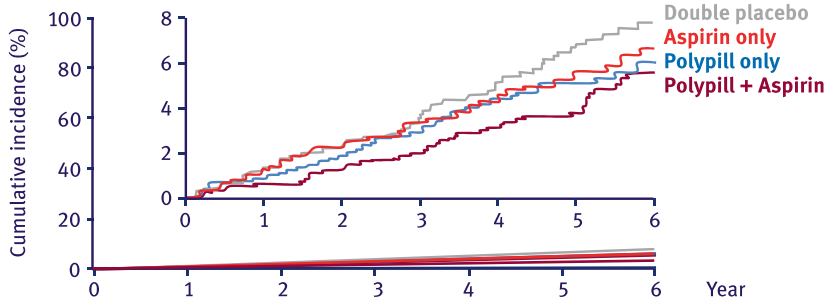
cap Study 3 (TIPS-3) has studied a polypill with and without aspirin versus placebo in a large primary prevention trial [307].

The TIPS-3 study was designed to compare the efficacy of aspirin and a polypill in cardiovascular prevention in persons at elevated cardiovascular risk but without known cardiovascular disease. A total of 5,713 participants (mean age 64 years) were randomized to one of the following groups: polypill (statin and multiple antihypertensives) without and with aspirin (75 mg), aspirin alone and placebo. Treatment lasted for 4.6 years. Primary endpoints were cardiovascular events, including cardiovascular death, stroke and myocardial infarction.

The primary outcome for the polypill group vs. placebo in the ITT analysis due to nonmedical reasons (drug supply, COVID-19 restricted mobility) was 4.4 % vs. 5.5 % (HR: 0.79; 95 % CI: 0.63–1.00). The outcome for the aspirin alone group vs. placebo was 4.1 % vs. 4.7 % (HR: 0.86; 95 % CI: 0.67–1.10) and the outcome for the polypill plus aspirin group vs. placebo was 4.1 % vs. 5.8 % (HR: 0.69; 95 % CI: 0.50–0.97). There were no differences in major bleeding events between the experimental groups and the placebo group. There was a high overall incidence of discontinuation of the trial regimen in both the polypill and aspirin groups, 40–42 %, mainly due to nonmedical reasons (inadequate drug supply, reduced return to study sites because of COVID-19 mobility restrictions).

The conclusion was that combined treatment with a polypill plus aspirin reduced the incidence of cardiovascular events by 29 % within an observation period of 4.6 years in comparison with placebo among participants without CVD who were at an intermediate cardiovascular risk (Fig. 4.1.1-11) [307].

Despite the limitations mentioned by the authors, this study is of considerable clinical and populationwide importance. The number of side effects was low. However, there was a 3–4-week run-in phase which resulted in exclusion of 715 eligible participants (9.5 %) because of side effects and another 560 persons (7.4 %) were excluded be-



 # at risk

Double placebo	1421	1348	1358	1239	767	493	317
Aspirin only	1431	1397	1367	1260	785	491	317
Polypill only	1432	1409	1381	1268	790	511	340
Polypill + Aspirin	1429	1405	1378	1268	791	509	336

Figure 4.1.1-11: Effects of the polypill alone and the polypill plus aspirin, as compared with double placebo and aspirin alone, on clinical outcomes in primary cardiovascular prevention. The hazard ratio (HR) of polypill plus aspirin vs. placebo was 0.69 (95 % CI: 0.50–0.97) [307].

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cause of a less than 80 % adherence to the trial drug regime – here not related to drug distribution issues. For these reasons, side effects might have been underestimated among eligible persons. Nevertheless, this study is a milestone for primary prevention, specifically if one also considers the low costs – for the Indian polypill actually 15$ per month [307].

Secondary prevention. In contrast to the controversially discussed issue of primary prevention, aspirin, in the absence of contraindications, is generally recommended as the drug of first choice in long-term secondary prevention. The recommended daily maintenance dose is between 75 and 325 mg/day, with an increasing preference for lower doses. The most recent COMPASS data in patients with stable angina have shown that factor Xa inhibition alone has only minor cardioprotective effects. However, factor Xa inhibition might substantially improve the total vasoprotective action of aspirin at the price of also significantly increased (severe) bleeding events [281].

Acute coronary syndrome. Since CURE [26], aspirin is guideline-recommended and an integral part of the standard DAPT in ACS. The immediate application of a "loading dose" of 250–500 mg intravenous aspirin as water-soluble (lysine) salt is now strongly recommended for initial, that is, immediate, (pre)hospital treatment in the absence of contraindications and might also have detectable additive beneficial effects even in subjects who already were on aspirin before the acute event [308]. Percutaneous intervention with stenting is the standard treatment for reopening of occluded coronary arteries. This is accompanied by DAPT with aspirin plus prasugrel/ticagrelor or clopidogrel. After stent implantation and in the absence of contraindications, the duration of DAPT should be 6–12 months and could then be continued life-long with aspirin alone. Whether prolonged DAPT provides additional benefits is under discussion [309–311].

Summary

Thromboembolic complications of atherosclerotic CVD (ASCVD) are ACSs, that is, unstable angina, STEMI, NSTEMI and sudden cardiac death. The syndrome results most frequently from atherosclerotic plaque rupture and generation and release of plaque material, including tissue factor, associated with thrombin generation, thromboxane A_2 synthesis and thrombus formation at the affected site. Aspirin prevents platelet-dependent thromboxane formation and all thromboxane-related secondary autocrine and paracrine events. It might also reduce tissue factor-induced thrombin formation by inhibiting platelet function. This is the rationale for its prophylactic use in cardiocoronary prevention.

Low-dose aspirin (75–100 mg/day) is the 2021 ESC guideline-recommended drug of primary choice for secondary prevention of cardiocoronary events (evidence level IA) [312]. This results in an overall about 20 % RR of recurrent vascular events in secondary prevention, however with marked disease-related variations in efficacy, dependent on the etiology of platelet hyperreactivity. These beneficial effects have to be balanced individually against side effects, most notably an increased bleeding tendency.

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Most guidelines currently do not recommend aspirin for primary prevention in subjects without preexisting cardiovascular and cerebrovascular diseases as well as diabetics except those at high/very high vascular risk. Several large prospective randomized trials have recently been published (ASCEND, ARRIVE) to define the individual risk profile more precisely. Unfortunately, the vascular risk of the study participants was not "moderate" (as planned) but "low" and, thus, just confirmatory of earlier trials. No benefits but even an increased mortality were found for regular aspirin intake in the elderly in the ASPREE trial.The situation may be different in China and developing countries with annual cardiovascular event rates of 2.6–3.0 %. Here, combined approaches such as an aspirin-containing polypill might also be considered. In addition, any positive chemopreventive effects of aspirin in CRC might change the guideline policy in the future. Actually, this indication was removed from the list of possible uses by the US Preventive Services Task Force (USPSTF) in 2021.

Long-term treatment of patients at enhanced cardiovascular risk frequently requires comedication of other drugs that might interact with aspirin. In addition to ADP antagonists, this refers to synergistically acting lipid-lowering and antihypertensive drugs (SGLT-2 inhibitors), lipid-lowering drugs (statins, PCSK9 inhibitors) and ACE inhibitors/angiotensin receptor blockers. Their frequent comedication might have contributed to the low event rates in recent clinical primary prevention trials. Comedication of PPIs and eradication of *H. pylori* will reduce the (gastrointestinal) bleeding risk. The COMPASS trial has set the stage for further therapeutic strategies regarding the combined use of antiplatelet and anticoagulant agents (NOACs). Negative interactions have been described for several NSAIDs, most notably ibuprofen, with respect to inhibition of platelet functions and clinical outcome.

In case of acute surgical interventions, specifically CABG, but also major noncardiac surgery, any elevated bleeding risk by perioperative aspirin as well as aspirin withdrawal has to be balanced versus the risk of increased periprocedural thrombotic complications. In most cases of cardiac patients on long-term aspirin prevention this means that interruption of aspirin intake is not recommended but might even induce adverse "rebound" effects after withdrawal.

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4.1.2 Cerebrovascular diseases

4.1.2.1 General aspects

Etiology. Cerebral ischemia is the consequence of impaired cerebral blood perfusion due to arterial obstructions inside the cerebral circulation. Consequences of critical ischemia are inflammatory alterations in the microcirculation, involving increased endothelial permeability with edema formation. There is platelet and white cell activation and adhesion to the endothelium as well as a "no reflow" phenomenon in the ischemic area [1]. This neuroinflammatory response causes cerebral dysfunctions in the area distal to the affected site. The kind and severity of these dysfunctions are determined by the kind, localization and size of the obstruction as well as the duration of ischemia. Clinically, cerebral ischemia presents with TIAs, transient, reversible, nondisabling strokes ("minor strokes") and (irreversible) disabling strokes ("major strokes").

There are two principally different categories of stroke: hemorrhagic stroke after intracranial bleeding (10–15 % of strokes) and ischemic stroke subsequent to critical cerebral vascular obstruction (80–90 % of strokes). Both are mostly caused by hypertensive angiopathy and/or cerebral amyloid angiopathy and are also related to aspirin. Intracranial hemorrhages and hemorrhagic stroke are important aspirin-related side effects while prevention of ischemic strokes is the therapeutic goal of aspirin treatment.

In contrast to the monocausal etiology of myocardial ischemia (infarction), usually resulting from thrombotic occlusion of a large coronary artery, the etiology of ischemic stroke is multifactorial [2]. The major types of ischemic stroke are large artery atherosclerosis and thrombosis, microatheromas and other small artery occlusions (lacunar stroke). In addition, there are cryptogenic strokes with unclear etiology, that is, "embolic stroke of undetermined source" (ESUS). Lacunar stroke results from a specific small vessel disease of cerebral arteries (lipohyalinosis). Its etiology is different from that of other types of ischemic stroke and probably more complex [3–5]. Atrial fibrillation is the most frequent cardiac reason of ischemic (cardioembolic) stroke and accounts for about 25 % of strokes at the age of 75–84 years [6, 7].

Pathophysiology. This heterogeneity in the etiology of ischemic stroke is also reflected by the variable role of platelets and their activation and secretion products in the pathophysiology of the disease. Human cerebral arteries are extremely sensitive to thromboxane and serotonin, the two major platelet-derived products which are not only potent platelet activators but also potent vasoconstrictors of cerebral arteries (Fig. 4.1.2-1) [8]. There might also be a role for more stable eicosanoids, such as $PGF2_{\alpha}$ [9], or (nonenzymatically generated) isoprostanes. Both can accumulate at a site of intracerebral bleeding and cause long-lasting constriction of cerebral arterioles [10]. Furthermore, activation of the coagulation cascade with thrombin formation and

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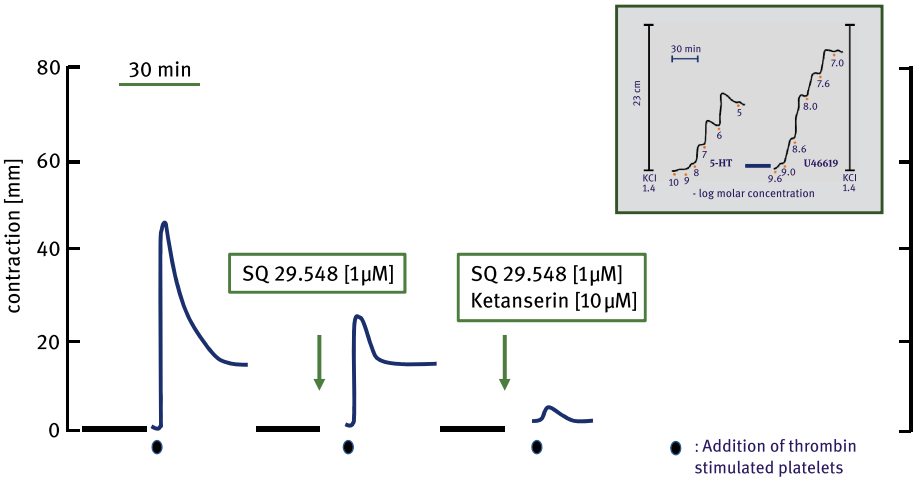

Figure 4.1.2-1: Contraction of an isolated postmortem (13 h) prepared human basilar artery by thrombin-stimulated human platelets and its inhibition by a thromboxane receptor antagonist (SQ 29,548) and a serotonin (5-HT) antagonist (ketanserin). Note the potent contractions induced by 5-HT and a thromboxane mimetic U46619 as compared to the maximum KCl-induced contraction (insert) [12].

other inflammatory processes might amplify (i) the ischemic process by promoting edema formation and (ii) damage of neuronal tissue [11].

Antiplatelet drugs such as aspirin will only act if an aspirin-sensitive, platelet-mediated event triggers cerebral ischemia or critically amplifies and maintains the local ischemic process. In this context, stroke subsequent to atherothrombotic occlusion of a large cerebral (carotid, basilar) artery is the only subtype of stroke with a pathogenesis comparable to myocardial infarction while lacunar stroke is not a platelet-triggered event [3] and cardioembolic stroke subsequent to atrial fibrillation is the domain of anticoagulants rather than antiplatelet agents (see below). It is, therefore, not surprising that the efficacy of antiplatelet treatment in prevention of noncategorized ischemic strokes, that is, ischemic stroke including all subtypes, is highly variable and, in general, considerably less than that of protection from myocardial infarction.

Epidemiology. Stroke is typically a disease of the elderly (≥75 years) with frequent comorbidities and comedications. It is also the most important thrombotic complication of atrial fibrillation, whose incidence also increases considerably at advanced age. There might also be sex-related and racial differences. According to data of the WHS and US-PHS primary prevention trials, aspirin is more effective in preventing strokes in women than in men [13]. In (East) Asian populations (Japan, China, Taiwan, Korea) there is a higher risk for strokes than for myocardial infarctions, in contrast to Europe and the US. In China and Japan, stroke is the leading cause of mortality with

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an approximately 3-fold higher risk for hemorrhagic strokes as compared to Europe and the US [14, 15]. This has to be considered when transferring data of clinical trials conducted entirely in (East) Asian populations to the rest of the world and vice versa. Considering the fact that Europeans only represent 11 % of the worldwide population but people from Asia more than 60 %, one third of them being Chinese, this has also led to discussions by Asian scientists whether Europe/US-based guidelines on vascular protection by antiplatelet/antithrombotic drugs can be directly transferred to (East) Asian countries [16].

Major stroke, if not resolved, is a most disabling disease with individual and social consequences that are much more aggravating than critical ischemias in other circulations, such as myocardial infarction or claudication. Patients with cerebrovascular events are also more likely to suffer a new cerebrovascular event than a myocardial infarction [17]. This points to a specific risk profile of the cerebral circulation. The annual stroke rate increases markedly not only with increasing age but also with increasing numbers of risk factors, most notably hypertension, diabetes and hypercholesterolemia. This is the reason for intense preventive measures which, by comparison with the efficacy of existing strategies, could clearly be improved.

4.1.2.2 Thrombotic risk and mode of aspirin action

Platelet reactivity and thromboxane A_2. Wu & Hoak [18] originally demonstrated enhanced numbers of circulating platelet aggregates in patients with TIA. This aggregate formation was significantly reduced by aspirin treatment. Further studies showed that platelets become activated in TIA and stroke patients during passage of the cerebral circulation, suggesting that this process is ischemia-induced. Platelet hyperfunction is associated with enhanced thrombin generation and thromboxane formation over weeks after the acute event [18–24]. These mediators along with platelet-derived serotonin are potent vasoconstrictors for cerebral arteries as exemplified in Fig. 4.1.2-1.

A consequence of platelet activation and secretion – in addition to enhanced thromboxane biosynthesis – is secretion of platelet storage products into the blood stream. There are increased circulating levels of platelet activation markers, such as P-selectin, CD63, serotonin and β-thromboglobulin [25–29]. Platelet-derived products also mediate inflammatory responses [30]. They are likely to contribute to neuroinflammation and neuronal injury, in particular at the high local concentrations within a thrombus inside an occluded cerebral artery [31]. Recent experimental studies of the group of *Bernhard Nieswandt* from Würzburg (Germany) suggested that ischemic cerebral infarction also differs from myocardial infarction by a different contribution of leukocytes, specifically T-cells, to the local ischemic thromboinflammatory process. Leukocytes are stimulated by activated platelets and in turn initiate cerebral ischemia/reperfusion injury, eventually resulting in growth of the irreversibly injured ischemic area (penumbra). This process is triggered by activated platelets. It possibly can be reduced by inhibition of platelet adhesion and subsequent activation by

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blockade of platelet-specific GPIb and GPVI receptors – without increased bleeding [32, 33]. Clinical studies to confirm this exciting concept and to transfer it into new therapeutic approaches are urgently needed.

Mode of aspirin action. The (elevated) levels of platelet activation markers and secretion products as well as platelet aggregate formation can be reduced by aspirin. However, the sensitivity of platelets to aspirin varies considerably (see below). High-dose (500 mg) intravenous aspirin has been shown to rapidly redissolve cerebral microemboli in patients with recent stroke of atherothrombotic origin [34]. However, different oral maintenance doses of aspirin may be necessary to prevent platelet aggregation and thrombogenesis induced by different triggers of platelet activation at different strengths [35].

Weiss and colleagues were the first to show in a placebo-controlled animal study that aspirin largely prevented the thrombotic occlusion of endarterectomized or chemically injured carotid arteries while dipyridamole had no effect [36]. Another striking evidence for a role of aspirin-sensitive platelet activation in cerebral ischemia was the occurrence of recurrent ischemic strokes as a "rebound" phenomenon within the first (two) weeks after aspirin withdrawal as well as a higher efficacy of aspirin as a preventive for recurrent stroke during the first 3 weeks after the index event in the IST and CAST trials (see below). These data confirm a key role of platelet hyperreactivity and aggregate formation in the pathogenesis of ischemic stroke which is at least partially aspirin-sensitive. They also show that the cerebral circulation of the affected side is the site of activation.

The clinical situation is more complex. This is largely due to the heterogeneity in the etiology of stroke which might not have been sufficiently considered in several early large stroke trials. This might be one explanation for the different outcome. The repeated finding that platelets of patients with cerebral ischemia appear to be less sensitive to aspirin than those of patients without cerebral ischemia [37] and the high inter- and intraindividual variability of platelet activation and its inhibition by aspirin are remarkable.

Grotemeyer and his group were the first who described a reduced antiplatelet effect of aspirin (200–500 mg single dose) ex vivo in about one third of 180 patients ("nonresponders") who had suffered an acute stroke (nonclassified) within the last 12 hours [38]. In a clinical follow-up study they studied high-dose aspirin (500 mg three times daily) in these stroke patients over 2 years and found that 24 out of 60 "nonresponders" (40 %) but only five out of 114 (4 %) "responders" suffered a new vascular event (stroke, myocardial infarction, vascular death) within a 2-year observation period ($P < 0.0001$). The conclusion was that early identification of aspirin nonresponders is a clinically useful tool to classify patients at high risk for recurrence of vascular events [39].

Most notable in this study is the finding of a correlation between inhibition of platelet function by aspirin and its predictive value for future cerebrovascular events – similar

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results were also described for prevention of reocclusion of reopened peripheral arteries (Fig. 4.1.3-2) [40]. Further studies indicated that the large interindividual variability could partially be overcome by increasing the aspirin dose. For this hyperreactivity or HTPR the term "resistance" was introduced by Helgason and colleagues and was considered as one explanation for the frequent treatment failure with the compound in stroke prevention (Section 4.1.6) [41]:

Helgason and coworkers studied the dose dependency and time-dependent reproducibility of inhibition of platelet function by aspirin in patients with previous ischemic stroke. The etiology of ischemic stroke was not specified. They reported an incomplete inhibition with 325 mg aspirin in these patients and a dose-dependent increase in efficacy with 650, 925 and 1,300 mg aspirin per day. In a follow-up study on 306 patients, they investigated the reproducibility of these variations during a longer period in stroke patients. The data showed a large intra- and interpatient variability over 33 months, indicating that the antiplatelet effect of a fixed aspirin dose was not constant and "resistance" could be largely abolished by increasing aspirin doses; however, 8 % of patients still remained nonresponders, even at 1,300 mg aspirin per day.

The conclusion was that the potency of aspirin as an antiplatelet (and antithrombotic) agent is highly variable – over time and in the same subjects. This "resistance" in stroke patients can be partially overcome by higher aspirin doses for reasons which are unknown [41, 42].

The variability of platelet reactivity as well as inhibition of platelet function and thromboxane formation by aspirin was also seen in other human studies (Fig. 4.1.2-2) [43]. One disease-related explanation for this phenomenon are the different subtypes of stroke (see below). As mentioned above, aspirin is less effective in lacunar stroke [44] and there is a higher degree of aspirin "resistance" [45]. Thus, the efficacy of aspirin-induced platelet inhibition for the clinical outcome, in most cases secondary

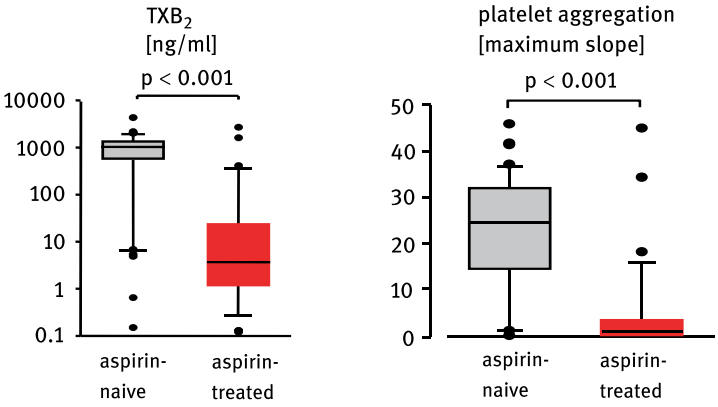

Figure 4.1.2-2: Variable arachidonic acid-induced platelet aggregation and thromboxane formation ex vivo in 90 patients with acute ischemic stroke. Note the high variation in both aggregation and thromboxane formation in stroke patients. Patients were either "aspirin-naïve" or on treatment with aspirin at antiplatelet doses prior to stroke [43].

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stroke prevention, depends on the subtype of ischemic stroke as outlined in more detail below.

Aspirin "resistance" in ischemic stroke is also correlated with higher levels of the inflammatory cytokine IL-6 [45] and worse clinical outcome [37, 46, 47]. For these reasons, the prognostic value of platelet function testing for clinical outcome and secondary prevention of stroke patients could be improved by measurements of surrogates of thromboinflammation, such as platelet and/or white cell activation products and circulating inflammatory markers (cytokines, chemokines and others) [48].

Similar limitations apply to the measurement of antiplatelet actions of aspirin in terms of thromboxane formation. The excretion of a thromboxane metabolite (11-DH-TXB_2) is elevated in TIA and stroke patients and significantly but incompletely reduced by aspirin treatment [27, 49]. Measurement of urinary thromboxane metabolite excretion provides no information about the site of its formation within the circulation. The relation to clinical outcome, that is, reoccurrence of cerebrovascular events, is also uncertain. A subgroup study of aspirin-treated hypertensives in the "Heart Outcomes Prevention Evaluation" (HOPE) study found no increased risk for stroke but an increased risk for myocardial infarctions in "aspirin-resistant" patients based on elevated thromboxane metabolite (11-DH-TXB_2) excretion (Section 4.1.6) [50]. Similar findings were obtained in the HOT trial, which demonstrated that normalizing diastolic blood pressure in hypertensive patients by antihypertensive treatment results in a significant reduction of cardiovascular events. Comedication of aspirin to these blood pressure-normalized patients protected from myocardial infarction but not from stroke (for details see Section 4.1.1) [51]. The reason for the failure to show an association between aspirin treatment and stroke in these studies is unclear. However, neither HOPE nor HOT have determined serum thromboxane (TXB_2) formation, the best estimate for the efficacy of aspirin to inhibit platelet COX-1 and thromboxane production. Taken together, available data suggest that platelet hyperreactivity and enhanced thromboxane formation are typical for stroke patients. They appear to be related to clinical outcome but are in general more variable in response to aspirin treatment than in other atherosclerotic diseases.

Aspirin doses. There are no clear data about the dose dependency of aspirin and clinical outcome in stroke patients. Two larger clinical trials, the Dutch-TIA trial and the UK-TIA trial (see below), did not find any difference in outcome between 30 and 283 mg/day and between 300 and 1,200 mg/day. In both studies, there were more bleeding events with the higher dose. In addition to the "Aspirin Carotid Endarterectomy" (ACE) trial in patients with carotid stenosis (see below) [52], the placebo-controlled Danish very low-dose aspirin trial also reported a satisfactory platelet inhibition of low-dose (50–70 mg/day) aspirin in almost all of the 301 patients (97 %) who had undergone carotid endarterectomy for extracranial arterial stenosis. During a 25-month follow-up period this was not associated with any significant reduction in

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new cerebral or coronary vascular events (RR: 11 %; 95 % CI: –38 % to 48 %; $P > 0.1$) [53]. Studies of aspirin as an antiplatelet drug additionally suggested that the optimum individual antiplatelet dose might change with time even in the same patient and that higher doses might be more effective than lower ones [41, 54]. Disease-related factors such as kind, strength and duration of platelet stimulation might be additional variables [35].

Aspirin withdrawal. The relevance of platelet function for aspirin-induced inhibition for the clinical outcome of stroke patients also becomes evident from recurrence of thrombotic cerebrovascular events after aspirin withdrawal [55–58]. Retrospective investigations suggested that aspirin withdrawal precedes up to 10 % of acute recurrent cerebrovascular events, occurring on average about two weeks after withdrawal [58, 59]. In high-risk cardiac patients, interruption of oral antiplatelet treatment for more than 5 days prior to (non)cardiac invasive procedures appears to result not only in cardiac (Section 4.1.1) but also in cerebrovascular events [60].

4.1.2.3 Clinical trials – primary prevention

General aspects. Stroke is predominantly a disease of the elderly (≥75 years). These individuals are frequently multimorbide and multidrug users. With the increasing life expectancy in industrialized societies, both the percentage and the absolute number of elderly persons will increase, eventually resulting in an increased risk of atrial fibrillation and stroke: Each fourth stroke at the age of 75–84 years is caused by atrial fibrillation [7]. This cardioembolic stroke due to atrial fibrillation is the only subtype of ischemic stroke where anticoagulants rather than antiplatelet drugs are the treatment of choice. Whether this also applies to other forms of "embolic stroke of undetermined source" (ESUS) is currently under study.

Randomized prospective primary prevention trials in healthy individuals. The first large prospective randomized trials, the US-PHS [61] and the BMDS [62], did not have stroke as a separate study endpoint. Moreover, the study populations were middle-aged healthy men at a very low risk of ischemic stroke. It is, therefore, not surprising that the overall stroke incidence was unchanged. There was, however, a trend for increased hemorrhagic strokes in both studies, amounting to 0.2 % in the aspirin group vs. 0.1 % in the placebo group at an unchanged number of ischemic strokes in the aspirin group in US-PHS. A cohort study in the United Kingdom with about 200,000 participants at the age of ≥64 years at entry studied the incidence rates of intracerebral bleeding events among new users of low-dose aspirin over a median follow-up of 5.58 years. The incidence of intracerebral bleeding events was 0.08 per 100 person-years, among them about one half, 0.04, being hemorrhagic strokes [63].

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In the WHS [13] there was an unchanged risk for myocardial infarctions among aspirin users – albeit at an annual event rate of only 0.1 % – the lowest number that has ever been reported in a controlled clinical trial – but a significant reduction in the secondary endpoint of ischemic strokes in the aspirin group, by 24 %. The number of hemorrhagic strokes was unchanged. The reasons for this surprising finding are unclear and the study as such has been discussed in more detail elsewhere in this book (Section 4.1.1). Probably, the overall vascular risk in these young healthy women was so small that treatment effects became only visible for the most frequent event, here stroke. It however indicates that aspirin-sensitive risk factors that determine the incidence of stroke in primary prevention of apparently healthy women might be different from those for myocardial infarction and there might also exist differences between men and women.

An interesting issue is the risk of stroke in women aged ≤60 years with previous pregnancy induced hypertension (PIH). In a prospective cohort study in 83,749 women, 4,070 (4.9 %) had PIH. Women with prior PIH had an increased risk of all stroke (adjusted HR: 1.3; 95 % CI: 1.2–1.4) but no increased risk of stroke before age 60 (adjusted HR: 1.2; 95 % CI: 0.9–1.7). There was an interaction ($P = 0.18$) between (i) aspirin use and PIH history and (ii) the risk of stroke before age 60: Nonusers of aspirin had a higher risk (adjusted HR: 1.5; 95 % CI: 1.0–2.1), while aspirin users did not (adjusted HR: 0.8; 95 % CI: 0.4–1.7). According to these data, women with prior PIH had an increased long-term stroke risk, which was reduced by aspirin use and needs to be studied in more detail in randomized trials [64].

Taken together, current evidence suggests that aspirin has a very low preventive power in primary prevention of stroke (and myocardial infarction) in women and men. This has to be balanced against the increased risk of bleeding, including cerebral bleeding events. Most health authorities find this ratio insufficient to recommend low-dose aspirin for primary prevention of stroke. This was recently confirmed in a large metaanalysis with 157,054 persons. In this metaanalysis, aspirin was not associated with a significant reduction of total or cardiovascular mortality or any reduction of nonfatal strokes (OR: 0.94; 95 % CI: 0.85–1.04) but was associated with a significant reduction of nonfatal myocardial infarctions (OR: 0.80; 95 % CI: 0.69–0.94) and an increased risk of major gastrointestinal bleeding (OR: 1.83; 95 % CI: 0.69–0.94). The conclusion was that aspirin has no benefit for primary stroke prevention [65].

4.1.2.4 Clinical trials – secondary prevention

General aspects. A significant proportion of patients with TIA or stroke will suffer new atherothrombotic events, in most cases stroke, within the next 5 years.

Another issue is progression of acute stroke subsequent to the acute cerebrovascular event. Here, antiplatelet treatment could, perhaps, have a beneficial, retarding effect.

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The prevention of stroke progression was studied in a randomized, double-blind, placebo-controlled trial in Sweden. Patients with ischemic stroke but not complete paresis were included. No antiplatelet drugs were allowed within the last 72 h before onset. Delay until first trial dosage was maximized to 48 h.

A total of 441 patients completed the trial. Aspirin (325 mg) or placebo was given once daily for five consecutive days. Neurological assessments were carried out three times daily to detect progression of stroke according to the Scandinavian Stroke Supervision Scale. Patient outcome was recorded at discharge and at 3 months.

Amongst aspirin-treated patients, clinically relevant stroke progression occurred in 15.9 % in the treatment group as compared with 16.7 % in the placebo group (HR: 0.95; 95 % CI: 0.62–1.45; $P > 0.05$). Patient outcome at discharge and after 3 months was also not different between the aspirin and placebo groups.

The conclusion was that aspirin did not show any clinically relevant effect on the frequency of stroke progression or patient outcome [66].

Randomized prospective trials. Early randomized, placebo-controlled prospective trials on aspirin prophylaxis in patients with TIA and cerebral ischemic infarction yielded different and mostly negative results. Only the Canadian Cooperative Study Group (CCSG) showed a significant protective action of aspirin with a reduction in incidence of stroke and mortality by 31 %, but only in men [67]. The aspirin doses in these older studies were high (1.3–1.5 g/day), as were the dropout rates. The number of included patients as well as their compliance was low. This stimulated several large prospective, randomized multicenter trials with stroke as a primary endpoint. The first of them, comparing different doses of aspirin with placebo, was the UK-TIA trial [68, 72], the second, comparing high- and low-dose aspirin, the Dutch-TIA trial [69].

The UK-TIA trial included a total of 2,435 patients with known recent transient ischemic attacks (TIA) or minor ischemic stroke (medium age at entry: 60 years). The observation period varied between 1 and 7 years (average 4 years). There was no differentiation of stroke subtypes, and patients with cardiac sources of embolism (not treated with anticoagulants) were also included. The patients (73 % male, about 600 patients per group) were randomized to receive "blind" (pills not with neutral taste) treatment with low-dose aspirin (300 mg/day), high-dose aspirin (600 mg twice daily) or placebo. All medications were given twice daily. Primary study endpoints were disabling stroke or vascular death. The compliance rate was estimated with 75 %.

Neither of the two aspirin groups alone showed a reduced incidence of strokes or vascular death as compared to placebo. There was a small, nonsignificant reduction of vascular events (death, nonfatal stroke and nonfatal myocardial infarction) in the combined aspirin groups by 15 %. No difference was seen between men and women. However, significant dose-dependent differences existed with respect to bleeding: The number of gastrointestinal bleeding events (per 1,000 patient-years) was 11 for 600 mg aspirin twice daily, seven for 300 mg aspirin and three for placebo. There were conflicting reports regarding prolongation of bleeding time, which was measured in two different follow-up trials in selected subgroups of these patients [70, 71], confirming the observa-

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tion of several other studies that the strength of the antiplatelet/antithrombotic actions of aspirin is not paralleled by prolongation in (skin) bleeding time.

The conclusion was that there is no significant reduction in major stroke or vascular death by aspirin in these high-risk, stroke-prone populations but a dose-dependent increase in gastrointestinal bleeding. As a possible reason for the therapeutic failure of aspirin, a statistical type II error was not excluded (too low patient numbers per group) [72].

The Dutch-TIA trial was also scheduled to compare two different though smaller doses of aspirin (carbasalate): 30 mg/day vs. 283 mg/day in 3,131 patients in a double-blind, randomized design. About half of the patients was >65 years of age at entry. Inclusion criteria were previous TIA or "minor stroke" within the last 3 months due to arterial thrombosis or thrombembolism (no atrial fibrillation!). The mean follow-up period was 2.6 years. Primary endpoints were vascular death, nonfatal stroke and nonfatal myocardial infarction.

The vascular death rate was reduced by 14.7 % in the "low-dose" aspirin group and by 15.2 % in the "high-dose" group (HR: 0.91; 95 % CI: 0.76–1.09). This difference was not significant (HR: 0.91; 95 % CI: 0.76–1.09). Despite the significantly lower rate of minor bleeding events with the lower aspirin dose, neither the number of major bleeding events (40 vs. 53) (nonsignificant trend in favor of 30 mg) nor gastrointestinal intolerance (164 vs. 179 patients) was different between the two doses of aspirin.

The conclusion was that 30 mg aspirin was no less effective than the 283-mg dose in the overall prevention of vascular events in patients with TIA or minor stroke but had fewer adverse effects [69].

The numbers of nonfatal strokes as a study endpoint was small in both treatment arms of the Dutch-TIA trial: 6–7 % per year. A low stroke incidence was also seen in the UK-TIA trial: Only 6 % instead of the expected 10 % of strokes per year that were used for calculation of patient numbers. Due to the low number of events, the studies also had low statistical power and the possibility of a statistical type II error (wrong negative results) was discussed by the authors [35, 73]. Importantly, the Dutch-TIA trial had no placebo arm. The reason for waiving a placebo group were (positive) interim results from the 300 mg/day aspirin group in the placebo-controlled UK-TIA trial [68], which, however, could not be confirmed after the trial was completed (see above). The Dutch-TIA study is often cited as evidence for the efficacy of 30 mg/day aspirin in stroke prevention and sometimes even generalized to indicate protection from all kinds of atherothrombotic events, including myocardial infarctions. This has *not* been shown. In fact, it has never been shown until now in any randomized, controlled trial that daily doses of 30 mg aspirin are effective at all in primary or secondary prevention of stroke or myocardial infarction. Interestingly, a later Dutch trial of aspirin (carbasalate) doses in a similar TIA/stroke population indicated that carbasalate at a dose equivalent to 30 mg of aspirin showed a 3-fold higher urinary thromboxane excretion ($P = 0.05$) than a dose equivalent to 75 or 325 mg aspirin/day [74] at 1–2 weeks, indicating insufficient inhibition of thromboxane production. One to two weeks after the acute event is exactly the time when most recurrent cerebral events occur after discontinuation of (standard-dose) aspirin treatment [58].

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The important issue of the dose dependency of aspirin effects for clinical outcome in large cerebral (carotid) artery stenosis was studied in the randomized, double-blind ACE study.

A total of 2,849 patients with carotid stenosis scheduled for carotid endarterectomy were included. Patients were randomly assigned to 81 mg, 325 mg, 650 mg or 1,300 mg aspirin daily, starting before surgery. Treatment was continued for 3 months. Primary endpoint was the combined rate of stroke, myocardial infarction and death.

The primary endpoint was lower in the two low-dose groups than in the high-dose groups at 30 days (5.4 % vs. 7.0 %; $P = 0.07$) and at 3 months (6.2 % vs. 8.4 %; $P = 0.03$). In an efficacy analysis which excluded patients taking 650 mg or more acetylsalicylic acid before randomization and patients randomized within 1 day of surgery, combined rates were 3.7 % and 8.2 % at 30 days ($P = 0.002$) and 4.2 % and 10.0 % at 3 months, respectively ($P = 0.0002$).

The incidence of this combined endpoint was somewhat lower in the low-dose groups than in the high-dose groups at 30 days (5.4 % vs. 7.0 %; $P = 0.07$) and at 3 months (6.2 % vs. 8.4 %; $P = 0.03$).

The conclusion was that the risk of stroke, myocardial infarction and death within 30 days and 3 months of endarterectomy is lower for patients taking 81 mg or 325 mg acetylsalicylic acid daily than for those taking 650 mg or 1,300 mg [52].

This study indicates that a stronger and more reliable platelet inhibition seen in previous platelet studies with aspirin at higher doses ex vivo does not necessarily translate into improved clinical outcome. Interestingly, some beneficial effect of low-dose aspirin was shown here for the atherosclerotic subtype of stroke. This subtype is considered most sensitive to aspirin. However, the study only investigated perioperative strokes in surgically treated patients, predominantly at 1 month after surgery. Their risk must not necessarily be identical with that of patients with long-term use and there was also no placebo group.

The "Swedish Aspirin Low-Dose Trial" (SALT) [75] was the first randomized, placebo-controlled trial with low-dose aspirin (75 mg/day) in patients with cerebrovascular disease. It was also the first study with a cerebrovascular primary endpoint that clearly demonstrated the efficacy of low-dose aspirin for stroke prevention and mortality in nonatrial fibrillation stroke.

A total of 1,360 patients (65 % men, mean age 67 years), were randomized 1–4 months after TIA, minor stroke or retina thrombosis and received 75 mg/day enteric-coated aspirin or placebo for a total period of 32 months. Patients with a cardiac source of emboli, including those with atrial fibrillation or recent (within 3 months) myocardial infarction, were excluded. Primary endpoints were stroke or death from any cause, secondary endpoints were other vascular events.

Aspirin significantly reduced the incidence of primary endpoints by 18 % (HR: 0.82; 95 % CI: 0.67–0.99; $P = 0.02$). Interestingly, the prevention of myocardial infarctions (secondary endpoint) by aspirin was about twice as high: 36 % (!). The rate of side effects was 22 % in the aspirin group and 18 % in the placebo group. However, all five fatal hemorrhagic cerebral infarctions ($P = 0.03$) and nine out of 13 severe gastrointestinal bleeding events requiring stop of treatment occurred in the aspirin group. The compliance rate (pill count) was estimated at >90 %.

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The conclusion was that 75 mg/day aspirin significantly reduces the risk of stroke and death in patients with preexisting cerebrovascular diseases. However, it was also stated that a (total) risk reduction of vascular events by 17–25 % is substantial but far from being the ultimate therapeutic approach since a large proportion of subsequent atherothrombotic events could not be prevented. It was also not excluded that higher doses may be more efficient than the low dose of 75 mg used in this study [75].

The low, although significant efficacy of aspirin in secondary stroke prevention might be related to low aspirin dosing – as suggested not only by the authors, but also by the fact that most patients of the SALT trial were only randomized some weeks after the acute event had occurred. For a critical assessment of the study, one might also consider that all five fatal hemorrhagic infarctions and 9 out of 13 severe gastrointestinal bleeding events occurred in the aspirin group.

The two probably largest randomized trials on recurrent stroke prevention in patients with acute stroke were the "Chinese Acute Stroke Trial" (CAST, 1997) and the "International Stroke Trial" (IST, 1997), each including about 20,000 nonselected patients. These trials studied whether aspirin protects from early recurrent stroke in real life in an unselected patient population if treatment is started immediately after the suspected stroke and whether this is also accompanied by a significantly increased number of bleeding events.

The diagnosis of stroke was confirmed by CT-scanning in 88 % of CAST and 67 % of IST patients. Patients of the CAST trial received 160 mg aspirin/day for 4 weeks after the acute event and patients of the IST trial received 300 mg aspirin/day for 2 weeks. Treatment was started within the first 48 h, on average 24 h, after the suspected cerebral insult. The design of the two trials was similar. However, in the CAST trial, patients of the control group were given placebo while in IST they were untreated, that is, IST was an open trial.

A preplanned metaanalysis of the two studies showed that aspirin caused a significant reduction in recurrent ischemic strokes during the treatment period in comparison to controls (1.6 % vs. 2.3 %; $P = 0.00001$) and a modest reduction of mortality (5.0 % vs. 5.4 %; $2P = 0.05$). There was a tendency for an increased number of hemorrhagic strokes (1.0 % vs. 0.8 %; $2P = 0.07$).

The conclusion was that early aspirin is of benefit for a wide range of stroke patients. Its prompt use should be considered for all patients with suspected acute ischemic stroke, mainly to reduce the risk of early recurrence (Fig. 4.1.2-3) [76].

These positive data were also confirmed in an actual metaanalysis. The strongest protective action was seen within the first 6 weeks after the acute event [77].

Effects of a "restarted" antiplatelet therapy on the risk of recurrent strokes and intracerebral hemorrhages in stroke patients after withdrawal because of previous intracerebral hemorrhage were studied in the "Restart or Stop Antithrombotics Randomized Trial" (RESTART) trial, a randomized, open-label study in patients with previous hemorrhagic stroke.

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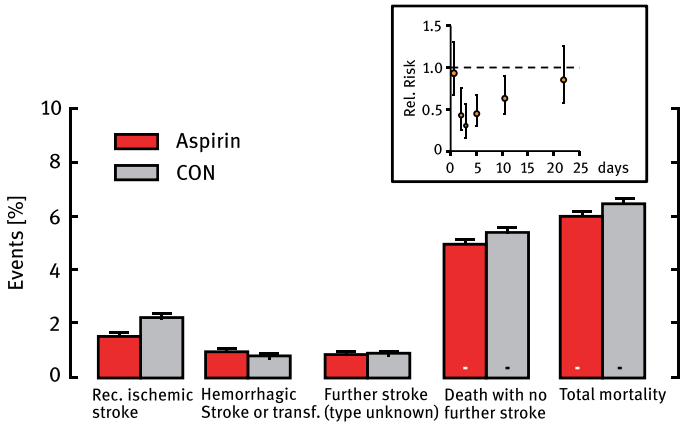

Figure 4.1.2-3: Effect of early aspirin on stroke and death in 40,000 randomized patients with suspected acute ischemic stroke (combined data from the CAST [160 mg/day for 4 weeks] and IST [300 mg/day for 2 weeks] trials). Note the early benefit of aspirin treatment within the first two weeks (insert) [76–78].

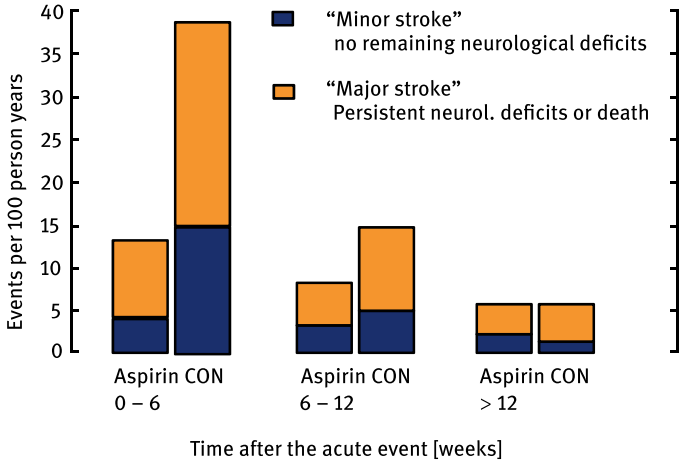

Figure 4.1.2-4: Early protective action of aspirin on the absolute risk for recurrent stroke in a meta-analysis of 12 secondary prevention trials including 15,778 patients after TIA and/or ischemic stroke. The strongest protective action was seen within the first 6 weeks after the acute event. CON: not aspirin-treated patients [77].

The study aimed to investigate the effects of antiplatelet therapy on recurrent intracerebral hemorrhage and its relation to any reduction in occlusive vascular events. A total of 537 participants were recruited a median of 76 days after intracerebral hemorrhage onset. Half of them were assigned to start and half of them to avoid antiplatelet treatment, most of them on aspirin, clopidogrel or both. Primary endpoint was fatal or nonfatal morphologically proven recurrent symptomatic intracerebral hemorrhage. The initial median follow-up was 2 years, later extended to 4 years.

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At the end of the study, a total of 4 % (12/268) participants allocated to the antiplatelet treatment group and 9 % (23/268) of the avoidance group had recurrent intracerebral hemorrhages (HR: 0.51; 95 % CI: 0.15–1.03; $P = 0.060$). There was no difference in total hemorrhagic events (7 % on antiplatelet vs. 9 % without; $P = 0.27$) and also no difference in major occlusive vascular events between the groups (15 % on antiplatelet vs. 14 % without; $P = 0.92$).

The conclusion was that these data exclude all but a very modest increase in the risk of recurrent intracerebral hemorrhage with antiplatelet therapy for patients on antithrombotic therapy for the secondary prevention of occlusive vascular disease when these developed intracerebral hemorrhage. The risk of recurrent intracerebral hemorrhage in these patients is probably too small to exceed the established benefits of antiplatelet therapy for secondary prevention [78].

This study is of considerable interest since it appears to be the first randomized prospective trial, although open and only single-blinded, that aimed to determine the benefit/risk ratio of antiplatelet therapy (aspirin/clopidogrel) for prevention of new (cerebral) vessel occlusions vs. induction of new bleeding events in patients with previous ischemic hemorrhage. Antiplatelet treatment in the RESTART trial did not change the cumulative incidence of all major hemorrhagic or occlusive vascular events ($P = 1.0$). There was an unexpected 50 % *reduction* of recurrent symptomatic spontaneous intracerebral hemorrhages in patients on antiplatelet treatment vs. those without. Unfortunately, the primary endpoint (fatal or nonfatal morphologically proven recurrent symptomatic intracerebral hemorrhage) was not significantly reduced ($P = 0.060$). Possibly, the study was underpowered, as also discussed by the authors. In addition, only one out of 12 eligible patients was included into the study. Main reasons were the unwell situation of the patients (28 %) and unwillingness of the doctors to accept a randomization procedure for treatment (26 %). Overall, this resulted in a probably too small sample size despite prolongation of the study duration to 4 years and might also have caused a selection bias. The randomization (treatment) was started after an avoidance period of >30 days in the vast majority (74 %) of patients, the median of all patients being 76 days. This could also have contributed to a selection bias since previous studies (IST, CAST) have shown that apparently all beneficial preventive effects of aspirin treatment on recurrent strokes in nonselected stroke patients were seen within the first 3–5 weeks after the acute event. This was also the time when most recurrent vascular events occurred [77].

Stroke subtypes. The "ischemic stroke" populations in clinical trials were quite different. Lacunar strokes, representing about one third or more of total ischemic strokes, were excluded from participation in the COMPASS trial but amounted to more than half (53 %) of patients in the MATCH trial. The SALT and THALES trials excluded atrial fibrillation and cardioembolic strokes, respectively, while the UK-TIA trial and the ESPS-2 trial did not and the European Atrial Fibrillation trial by definition used only atrial fibrillation patients. Given these differences in the composition of the study groups and the low efficacy of aspirin in prevention of lacunar strokes [44] this could

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result in study population-dependent results as the reasons for the efficacy of stroke prevention are also determined by the stroke subtype.

A review of 10 early randomized, placebo-controlled trials (9/10) of aspirin in 6,171 patients with previous stroke or TIA has shown that long-term regular aspirin reduced the total risk of combined vascular events (stroke, myocardial infarction, vascular death) by 13 %. There was a large interindividual variability and no clear dose dependency [79]. Regarding an annual risk of strokes in this population of 15–20 %, an absolute RR by 1–2 % is low and much less than the protection from myocardial infarctions. This indicates that the vast majority of ischemic strokes even in high-risk populations is not prevented by aspirin prophylaxis [79, 80]. One reason might be platelet activation and aggregation by nonaspirin-sensitive mechanisms [81], another, and more reliable, the heterogenous pathogenesis of ischemic strokes.

For these reasons, the importance of the stroke subtype for the clinical efficacy of antiplatelet treatment is obvious but not always sufficiently appreciated in clinical trials. This issue was studied in more detail in a large prospective cohort study. Patients were classified by cause and subtype of stroke. Mortality was measured 4 weeks after the initial ischemic episode. Of the 1,457 patients included, 650 (45 %) were using aspirin (median dose 75 mg; range 75 to 300 mg) prior to the stroke. Prior use of aspirin was associated with lower 4-week mortality (14 % vs. 20 %; $P < 0.01$). These beneficial effects were seen in patients with atherosclerotic stroke (15 % vs. 21 %; $P < 0.05$) and patients with cardioembolic stroke (21 % vs. 34 %; $P < 0.05$), but not among patients with lacunar strokes (10 % vs. 11 %; $P = 0.8$). These data suggested that prior use of low-dose aspirin is associated with a small but significant reduction in stroke mortality and that lacunar stroke with its different and complex etiology [3–5] is less affected by aspirin than atherothrombotic or cardioembolic strokes [82]. These data agree with the negative findings in patients with lacunar stroke in the SP3P study. In this trial, addition of clopidogrel to aspirin in 3,020 patients with recent symptomatic lacunar stroke did not reduce the risk of recurrent stroke during an observation period of 3.4 years but significantly increased the risk of bleeding [83].

In one study on stroke patients the risk of recurrent stroke was similar between patients who had presented with lacunar and nonlacunar strokes. Recurrent strokes in patients presenting with lacunar stroke were typically nonlacunar. These findings suggest that the pathophysiology of these strokes is related to the stenosis rather than small vessel disease. The authors suggested that patients presenting with lacunar strokes should be included in trials investigating secondary prevention for symptomatic intracranial stenosis [5]. Nevertheless, a different pathophysiology of lacunar stroke vs. others and consequently a different role of platelets exists [3].

One metaanalysis of six trials on primary and secondary stroke prevention has essentially confirmed this [84]. Another recent metaanalysis of 16 randomized trials on secondary prevention of lacunar strokes by treatment with antiplatelet agents has confirmed a lack of benefit from clopidogel and aspirin therapy in lacunar stroke patients [44]. Nevertheless, many strokes might be of the mixed type and there might be

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some effect by antiplatelet treatment due to prevention of secondary platelet activation because of local cerebral ischemia.

4.1.2.5 Aspirin and other drugs

Several attempts have been undertaken to increase the efficacy of antiplatelet treatment for prevention of recurrent stroke. Antiplatelet alternatives to aspirin are clopidogrel or ticagrelor, alone or in combination, or the combination of aspirin with dipyridamole. Of interest is also cilostazol, a vasodilator with endothelium-protective properties and a weak inhibitor of platelet aggregation which, interestingly, does not prolong bleeding time [85]. This pharmacological profile could result in a synergistic effect with aspirin on platelet aggregation and improvement of endothelial dysfunctions in vascular pathologies. Finally, in patients with atrial fibrillation, oral anticoagulants/NOACs, alone or in combination with antiplatelet drugs, have increasingly become the treatment of choice.

Clopidogrel and ticagrelor. ADP antagonists, such as clopidogrel and ticagrelor, are alternatives to aspirin in stroke prevention. In five trials among 29,357 patients who had (recent) ischemic stroke, clopidogrel was slightly more effective than aspirin in reducing recurrent vascular events within 12 months. Pairwise metaanalysis showed a significant RR in the occurrence of major cardiovascular and cerebrovascular events (OR: 0.72; 95 % CI: 0.53–0.97) and recurrent ischemic stroke (OR: 0.72; 95 % CI: 0.55–0.94) in patients who received clopidogrel versus aspirin. There was also a lower risk of bleeding for clopidogrel (OR: 0.57; 95 % CI: 0.45–0.74) but no difference in overall mortality [86]. These results are similar to an earlier metaanalysis where thienopyridines reduced the odds of a vascular event by 9 % (OR: 0.91; 95 % CI: 0.84–0.98; $2P = 0.01$). Thienopyridines produced significantly less gastrointestinal hemorrhage and upper gastrointestinal upset (indigestion/nausea/vomiting) than did aspirin [87].

In contrast, a retrospective nationwide cohort trial in Taiwan compared aspirin with clopidogrel for major adverse cardiovascular event reduction within 1 year after ischemic stroke and found that clopidogrel conferred a higher risk of recurrent stroke and myocardial infarctions than aspirin [88]. However, the population were ethnic Chinese with frequent (50–65 %) CYP2C19 loss-of-function genotypes [89]. This might result in reduced bioactivation and antiplatelet efficacy of clopidogrel [90].

Dual antiplatelet therapy. Of interest is the combined use of both agents because of possible synergism between these differentially and independently from each other acting drugs. However, the question is whether any expected efficacy benefit can be obtained at an acceptable risk of bleeding events.

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The POINT trial was a randomized study in a total of 881 patients with minor ischemic stroke or high-risk TIA. Patients were treated with either clopidogrel plus aspirin (at a dose of 50 to 325 mg per day) or the same range of doses of aspirin alone. The primary efficacy outcome was the risk of a composite of ischemic stroke, myocardial infarction or cardiovascular death at 90 days.

The trial was halted after 84 % of the anticipated number of patients had been enrolled because the data and safety monitoring board had determined that the combination of clopidogrel and aspirin was associated with both a lower risk of major ischemic events and a higher risk of major hemorrhage than aspirin alone at 90 days. Major ischemic events occurred in 5.0 % of patients receiving aspirin plus clopidogrel and in 6.5 % of patients receiving aspirin plus placebo (HR: 0.75; 95 % CI: 0.59–0.95; $P = 0.02$), with most events occurring during the first week after the index event. Major hemorrhage occurred in 0.9 % of patients receiving clopidogrel plus aspirin and in 0.4 % of patients receiving aspirin plus placebo (HR: 2.32; 95 % CI: 1.10–4.87; $P = 0.02$).

The conclusion was that treatment of patients with minor ischemic stroke or high-risk TIA with combined clopidogrel and aspirin had a lower risk of major ischemic events but a higher risk of major hemorrhage at 3 months as compared to aspirin alone. The benefits of clopidogrel–aspirin occur predominantly within the first 21 days and outweighs the low but ongoing risk of major hemorrhage.

When compared with the results of the CHANCE trial [91], using a similar treatment approach with clopidogrel–aspirin (see below) but showing no increase in major hemorrhages, the POINT data suggest that limiting clopidogrel–aspirin use to 21 days may maximize the benefits and reduce the risk after high-risk TIA or minor ischemic stroke [92].

The POINT and CHANCE trials indicated a higher vascular protection with DAPT, that is, standard dose (75 mg/day) clopidogrel with aspirin at the price of excess bleeding. This probably reflects the higher potency of DAPT as opposed to aspirin alone on platelet function and circulating platelet activation markers than low-dose aspirin alone [21, 29, 93]. Whether this translates into clinical benefit, however, is uncertain and possibly also dependent on the subtype of stroke. DAPT with aspirin and clopidogrel was superior to aspirin alone in early treatment of noncardioembolic stroke [94]. In patients with recent lacunar stroke, DAPT with aspirin and clopidogrel was effective in patients with symptomatic carotid stenosis and reduced mortality [95, 96]. The recent "Clopidogrel in High-risk patients with Acute Non-disabling Cerebrovascular Events" (CHANCE) trial [91] showed that DAPT with aspirin plus clopidogrel versus aspirin alone reduced the recurrence of strokes (ischemic and hemorrhagic) in 5,170 patients within 1 year from 14.0 % to 10.6 % (HR: 0.78; 95 % CI: 0.65–0.93; $P = 0.006$) at an unchanged number of moderate to severe bleeding events (0.3 % vs. 0.4 %; $P = 0.44$) [91].

In the "Acute stroke or transient ischemic attack treated with aspirin or ticagrelor and patient outcomes" (SOCRATES) trial, 13,199 patients with a nonsevere ischemic stroke (not cardioembolic) or TIA were treated. Patients were randomly assigned within 24 hours after symptom onset to receive either ticagrelor (180 mg loading dose on day 1 followed by 90 mg twice daily for days 2 through 90 combined with aspirin) or aspirin alone (300 mg on day 1 followed by 100 mg daily for days 2 through 90). The primary endpoint was the time to the occurrence of stroke, myocardial infarction or death within 90 days.

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During the 90 days of treatment, a primary endpoint event occurred in 6.7 % of patients treated with ticagrelor and in 7.5 % of patients treated with aspirin (HR: 0.89; 95 % CI: 0.78–1.01; $P = 0.07$). Ischemic stroke occurred in 5.8 % of patients treated with ticagrelor and in 6.7 % of patients treated with aspirin (HR: 0.87; 95 % CI: 0.76–1.00). Major bleeding occurred in 0.5 % of patients treated with ticagrelor and in 0.6 % of patients treated with aspirin; intracranial hemorrhage occurred in 0.2 % and 0.3 %, and fatal bleeding occurred in 0.1 % and 0.1 %, respectively.

The conclusion was that in patients with acute ischemic stroke or TIA, ticagrelor was not superior to aspirin [97].

However, a follow-up analysis indicated some beneficial effects of ticagrelor alone versus aspirin alone in a prespecified subgroup of SOCRATES patients with ipsilateral atherosclerotic stenosis: 6.7 % of patients of this subgroup on ticagrelor versus 9.6 % of patients on aspirin had a recurrent vascular event (stroke, myocardial infarction or death) within 3 months (HR: 0.68; 95 % CI: 0.53–0.88; $P = 0.003$). There were no differences in major and minor bleeding events [98].

To test the relevance of this finding, the "Acute stroke or transient ischemic attack treated with ticagrelor and aspirin for prevention of stroke and death" (THALES) trial was conducted and showed a superiority of the combined treatment versus aspirin alone at 30 days in primary outcome (5.5 % vs. 6.6 %; HR: 0.83; 95 % CI: 0.71–0.96) and stroke alone (HR: 0.81; 95 % CI: 0.69–0.95), but not death. There was a marked increase in severe bleeding events in the aspirin alone group (0.5 % vs. 0.1 %; HR: 3.99; 95 % CI: 1.74–9.14), mainly driven by increased intracranial bleeding [99]. However, there were also several questions, in particular with respect to the exclusion of patients receiving reperfusion therapy, being inevitable because of the 24-h inclusion window. There was also a much higher loading dose of aspirin compared with the CHANCE and POINT trials, possibly contributing to increased bleeding [100].

No significant additional therapeutic benefit but a 3-fold increase in life-threatening bleeding events was seen after combined treatment with aspirin (75 mg/day) plus clopidogrel versus clopidogrel alone in the MATCH trial in 7,599 high-risk patients with recent ischemic stroke or TIA, 53 % being of the lacunar subtype [101]. Two further clopidogrel (plus aspirin) secondary prevention trials had looked at the efficacy of DAPT in specific subtypes of ischemic stroke. The "Atrial Fibrillation Clopidogrel Trial With Irbesartan for Prevention of Vascular Events" (ACTIVE) trial studied patients with atrial fibrillation who could not use vitamin K antagonists. During an observation period of 3.6 years, DAPT reduced the risk of major vascular events, including stroke (2.4 % vs. 3.3 % per year; HR: 0.72; 95 % CI: 0.62–0.83; $P < 0.001$), but significantly increased the risk of major hemorrhages (2.0 % vs. 1.3 % per year; HR: 1.57; 95 % CI: 1.29–1.92; $P < 0.001$) [102]. The "Secondary Prevention of Small Subcortical Strokes" (SPS3) in patients with recent symptomatic lacunar stroke found no reduction of recurrent strokes during a follow-up of 3.4 years but also a significant increase in major bleeding events and overall mortality [83]. Taken together, comedication of clopidogrel with aspirin in most of the unselected trials did not improve

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efficacy in nonselected ischemic strokes or TIA but rather increased bleeding, and this increased bleeding appeared to be independent of the stroke subtype.

Despite positive results for the total population in the ticagrelor versus clopidogrel – both together with aspirin – PLATO trial, there were no differences regarding the rate of strokes but rather more hemorrhagic strokes with ticagrelor than with clopidogrel (0.2 % vs. 0.1 %; $P = 0.10$). No beneficial effects with respect to stroke prevention were obtained [103].

Prasugrel, a third-generation thienopyridine, given on top of aspirin did not reduce the rate of strokes in the TRITON-TIMI-38 trial [104] but increased bleeding [105]. For this reason, prasugrel is contraindicated in patients with a history of TIA or stroke, having a higher risk of stroke (thrombotic stroke and intracranial hemorrhage).

A recent metaanalysis assessed the efficacy and safety of P2Y12 receptor inhibitor plus aspirin versus aspirin alone in patients treated within 24 h after acute noncardioembolic ischemic stroke or TIA. The primary efficacy endpoint was recurrent stroke and the primary safety endpoint was severe bleeding. A total of five randomized trials with 21,808 individuals were identified. P2Y12 receptor inhibitor plus aspirin compared with aspirin was associated with a lower risk of recurrent stroke (HR: 0.75; 95 % CI: 0.68–0.83). Ticagrelor plus aspirin compared with aspirin was associated with an increased risk of severe bleeding (HR: 3.98; 95 % CI: 1.74–9.10) and intracranial hemorrhage (HR: 3.32; 95 % CI: 1.33–8.25), whereas clopidogrel plus aspirin was associated with a similar hemorrhagic risk as aspirin. The conclusion was that P2Y12 receptor inhibitor plus aspirin vs. aspirin alone given within 24 h after acute noncardioembolic ischemic stroke or TIA reduces the risk of subsequent stroke. However, the risk of severe bleeding, including intracranial hemorrhage, was higher with ticagrelor plus aspirin vs. aspirin alone [106]. Another metaanalysis came to similar results and additionally found that discontinuation of DAPT within 21 days, and possibly even as early as 10 days after starting, is likely to maximize the benefit and minimize harms. Any benefit beyond 21 days is extremely unlikely [107].

What is the role of DAPT after high-risk TIA or minor stroke? Specifically, does DAPT with a combination of aspirin and clopidogrel lead to a greater reduction in recurrent stroke and death over the use of aspirin alone when given in the first 24 hours after a high-risk TIA or minor ischemic stroke? One expert panel recommended to initiate DAPT within 24 hours of the onset of symptoms and to continue it for 10–21 days, although current practice is typically to use a single drug [108]. A recent network analysis including 22,098 patients and five randomized trials also came to the conclusion that short-term DAPT with aspirin and either ticagrelor or clopidogrel is effective after minor ischemic stroke or TIA [109].

Dipyridamole. Dipyridamole is a vasodilator and weak inhibitor of platelet aggregation. Both actions are probably due to accumulation of cyclic GMP (cGMP) after inhibition of the cGMP-specific phosphodiesterase V and subsequent activation of the

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NO/cGMP pathway [110]. Because of this mode of action, dipyridamole should synergize with aspirin with respect to inhibition of platelet aggregation.

The "European Stroke Prevention Study-2" (ESPS-2) was the first study to compare aspirin and dipyridamole alone and in combination with each substance alone in a placebo-controlled randomized trial. Dipyridamole was applied in a new extended-release formulation which allowed much higher total doses of dipyridamol because of the slow release of the active compound [111].

A total of 6,602 nonselected ischemic stroke/TIA patients (including patients with atrial fibrillation!) were treated with aspirin (25 mg twice daily), extended-release dipyridamole (200 mg twice daily), the combination of both or placebo over 2 years in a double-blind, randomized manner. Primary endpoints were stroke and death, secondary endpoints were TIA and other vascular events.

Compared to placebo, the incidences of new strokes and vascular events were significantly reduced by aspirin alone (18 %), dipyridamole alone (16 %) and the combination of the two (37 %). All of these changes were highly significant. There was no significant reduction of mortality, myocardial infarction alone or other vascular events alone. Side effects were bleeding (aspirin groups), gastrointestinal intolerance and headache (dipyridamole groups).

The conclusion was that aspirin and dipyridamole at the dose and formulation used are equieffective in the secondary prevention of stroke and TIA. The combination of aspirin plus extended-release dipyridamole acts additively and is significantly more effective than either treatment alone [111].

This study was the first to report a therapeutic benefit (as well as an increased bleeding tendency) for aspirin at the extremely low dose of 25 mg twice daily in secondary prevention of stroke. Similar results were reported for extended-release dipyridamole alone as well as an additive effect for the combined use. These were important findings and the therapeutic consequences for clinical reality were discussed intensively [112]. The ESPS-2 trial differed from earlier dipyridamole/aspirin trials because of the relatively high dose of (extended-release) dipyridamole (400 mg vs. 75 mg in older trials), resulting in a dipyridamole/aspirin ratio of 8:1. This ratio of dipyridamole/aspirin was previously reported to have an additive effect on human platelet aggregation in healthy volunteers [113]. No previous study had shown before any clinical efficacy of the extremely low dose of aspirin (25 mg twice daily) – the Dutch-TIA trial with 30 mg aspirin/day had no placebo arm. The Danish "Very low-dose Aspirin Study" was placebo-controlled and found a dose-dependent inhibition of platelet function by aspirin (50–100 mg/day) in patients with carotid endarterectomy. This was, however, not accompanied by an improved clinical outcome [53] and atherosclerotic vessel disease is probably the most sensitive type to low-dose aspirin treatment, as already shown in ACE [52]. The metaanalyses of the ATTC also have shown a reduced antiplatelet effect of aspirin doses less than 75 mg/day [80]. Thus, a clinically suboptimal aspirin dose cannot be excluded. No changes in the incidence of myocardial infarctions were seen in the ESPS-2 trial [114], although the number of patients with CVD (35 %) and peripheral arterial occlusive disease (22 %) was substantial. On the

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other hand, significantly more patients in the two dipyridamole groups (29 %) finished the study prematurely than in the aspirin or placebo groups (22 %), mainly because there were more gastrointestinal events and headaches. The incidence of orthostatic hypotension, a possible problem of dipyridamole in the elderly [115], was not reported.

Another large clinical trial following ESPS-2 in secondary prevention of stroke was the "European/Australasian Stroke Prevention in Reversible Ischaemia Trial" (ESPRIT) study. The study was addressed to reconfirm the ESPS-2 data in a similar, randomized study population but with an open design.

Aspirin (30–325 mg/day, median dose: 75 mg/day) was given alone (1,376 patients) or in combination with 200 mg twice daily dipyridamole (1,363 patients), mostly (83 %) in an extended-release formulation, to patients with previous stroke of arterial origin or TIA within the last 6 months. Important exclusion criteria were atrial fibrillation and/or a cardiac source for emboli as well as high-degree carotid stenosis requiring surgery or major bleeding complications. Primary endpoint was the composite of vascular death, nonfatal myocardial infarction and stroke as well as severe bleeding. The mean follow up was 3.5 years.

At least one primary outcome event was obtained in 13 % of patients in the combined treatment group and in 16 % of patients in the aspirin alone group (OR: 0.80; 95 % CI: 0.66–0.98) in the ITT analysis. This was equivalent to a reduction of the absolute vascular risk by 1 % per year. There were no differences between the two treatment groups in cerebral or cardiac events as single endpoints and no differences in the occurrence of severe bleeding events. However, more than one third of patients (34 %) in the combined group stopped medication prematurely because of side effects, mainly (26 %) headache, while only 13 % of the aspirin group interrupted treatment, mostly because of medical reasons.

The conclusion was that this study – combined with the results of previous trials (metaanalyses)– provides sufficient evidence to prefer the combination of aspirin plus extended-release dipyridamole over aspirin alone as antithrombotic treatment for secondary prevention after cerebral ischemia of arterial origin [116].

These data are interesting, however, the study had several limitations [112, 117]. Although the endpoint adjudication process was blinded, the design was open and the benefit of combined treatment was small with wide interindividual variations. One third of patients discontinued treatment because of side effects of dipyridamole, mainly (26 %) because of headache (similar to ESPS-2). This is an issue of concern because of possible problems with patient adherence to combined long-term treatment in real life. Interestingly, dipyridamole-induced headache has been suggested as a predictor of its clinical efficacy in both the ESPS-2 and ProFESS studies [118]. It was also surprising and remained finally unexplained by the authors why the dipyridamole-related almost 3-fold (34 % vs. 13 %) higher dropout rate during treatment did not result in any significant differences between the "ITT" and "on-treatment" analyses of the study [117] – one would have expected that interruption of an effective treatment should result in worse clinical outcome.

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Another randomized trial on secondary prevention of stroke was the "Prevention Regimen for Effectively avoiding Second Strokes" (PRoFESS), comparing aspirin/extended-release dipyridamole with clopidogrel (2008). The primary outcome was first recurrence of stroke. The study was designed as a noninferiority trial [119]. The trial did not meet the predefined criteria for noninferiority and, therefore, was inconclusive. No subtype specification of strokes was done. The rates of recurrent stroke with aspirin plus extended-release dipyridamole amounted to 9.0 % vs. 8.8 % with clopidogrel. However, there were more major hemorrhagic events (HR: 1.15; 95 % CI: 1.00–1.32) and hemorrhagic strokes (HR: 1.42; 95 % CI: 1.11–1.83) in the dipyridamole/aspirin group: 4.1 % vs. 3.6 % in the clopidogrel-treated patients [119]. Interestingly, the PRoFESS trial included fewer patients of Caucasian descent; many participants came from Asia, with a possibly different genetic background for intracerebral hemorrhage [15].

According to a Cochrane analysis of 29 studies on dipyridamole for preventing stroke and other vascular events in patients with vascular disease, a small additional benefit of dipyridamole was only shown for cerebral ischemias [120]. Thus, the therapeutic value of dipyridamole in stroke prevention is limited. Triple antiplatelet therapy (aspirin, clopidogrel, dipyridamole) was not better than DAPT due to increased bleeding [121].

Cilostazol. Cilostazol is a vasodilator which also inhibits platelet function. Both actions are mediated by accumulation of cyclic AMP (cAMP) after inhibition of phosphodiesterase III and subsequent activation of the cAMP/PKA signaling pathway in platelets and the endothelium. Cilostazol also enhances some actions of adenosine [85]. This pharmacological profile could result in a synergistic action with aspirin on platelet aggregation and improvement of endothelial dysfunctions in vascular pathologies.

Available data confirm a stroke-preventive potential of cilostazol that is comparable to or even better than that of aspirin as well as a low bleeding risk specifically in (East) Asian populations [16, 122]. It has also been suggested that cilostazol retards the progression of atherosclerosis [123]. A metaanalysis of 12 placebo-controlled randomized trials in secondary prevention in atherothrombosis involving 5,674 patients reported a marked decrease of cerebrovascular events by cilostazol as the major effect (OR: 0.58; 95 % CI: 0.43–0.78; $P < 0.001$) with no increase of bleeding [124]. Similar results were obtained in a recent Cochrane analysis. There was also a reduced risk of total vascular events including strokes by cilostazol. Most notably was the substantial *reduction* of hemorrhagic strokes (0.53 % vs. 2.01 %; OR: 0.26; 95 % CI: 0.13–0.55) [125]. Another metaanalysis confirmed the therapeutic advantages of cilostazol vs. aspirin in secondary stroke prevention: There was a significant reduction of hemorrhages, including cerebral bleeding events, by 73 % and a 28 % reduction in the composite endpoint of stroke, myocardial infarction and vascular death [126] by cilostazol compared to

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aspirin. Combined use of cilostazol (200 mg/day) plus aspirin (100 mg/day) in 165 patients with ischemic stroke because of stenosis in the responsible intracranial artery (noncardioembolic strokes) yielded slight improvements in stroke and silent brain infarcts versus aspirin alone during a 2-year observation period in the randomized "Cilostazol-Aspirin Therapy Against Recurrent Stroke with Intracranial Artery Stenosis" (CATHARSIS) trial [124]. A double-blind randomized trial in East Asian patients with ischemic stroke at high risk of cerebral hemorrhage (history of previous intracerebral hemorrhage or microbleeds), the "Prevention of cardiovascular events in Asian patients with ischemic stroke at high risk of cerebral hemorrhage" (PICASSO) trial, has shown that cilostazol was noninferior to aspirin (100 mg/day) for the prevention of cardiovascular events, including stroke, but did not reduce the risk of hemorrhagic stroke [127].

These data are very interesting, specifically for patients with stroke as a consequence of large artery diseases (atherosclerosis), and, hopefully, will be followed by larger prospective, randomized trials in the near future in non-Asian populations. These are known for their (genetically defined?) increased risk of strokes and cerebral bleeding events [14, 15] but also frequent (50–65 %) CYP2C19 defective genotypes [89]. This might retard cilostazol metabolism (inactivation) in vivo [128] and thereby increase its pharmacodynamic potency [85].

Taken together, cilostazol appears effective for long-term secondary stroke prevention without increasing the hemorrhage risk. More trials in Western countries should assess its antithrombotic properties in stroke prevention as well as its effects on cognitive decline and functional outcome, particularly in lacunar stroke and other presentations of small vessel diseases [129].

Coumarin-type anticoagulants. An alternative therapeutic option in stroke prevention to antiplatelet agents is the use of oral anticoagulants. Warfarin-type compounds inhibit generation of vitamin K-dependent synthesis of zymogens of clotting factors, most notably (pro)thrombin, but have no direct antiplatelet effects. They reduce the risk of stroke and cardiovascular events in patients with nonvalvular chronic or paroxysmal atrial fibrillation and cardiogenic embolism and are significantly more potent than aspirin in this group of patients. According to a Cochrane analysis, dose-adjusted warfarin reduced stroke and other vascular events in patients with nonvalvular atrial fibrillation by about one third, being significantly more effective than antiplatelet drugs [130]. Another metaanalysis compared adjusted-dose warfarin (six trials, 2,900 participants) and antiplatelet agents (eight trials, 4,876 participants). Compared with the control group, anticoagulants reduced stroke by 64 % (95 % CI: 49–74 %) and antiplatelet drugs by 22 % (95 % CI: 6–35 %) [131]. The absolute increases in major extracranial hemorrhages were small (≤0.3 % per year) [131]. This result was mainly driven by the "Stroke prevention in atrial fibrillation" (SPAF) study. This study showed an overall treatment effect of 44 % compared with placebo with no apparent

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increase in clinically relevant bleeding events. However, a post hoc analysis of these data indicated that aspirin reduced noncardioembolic strokes much more efficiently (reduction 100 %) than those of cardioembolic origin (reduction 31 %) ($P = 0.01$) [132, 133].

The incidence of recurrent strokes in patients with atrial fibrillation is reduced from 12 % to 11 % per year by aspirin, that is, by 1 % by aspirin alone, while oral anticoagulants of the coumarin type reduce the risk in this patient population by 4 %, dependent on the accuracy of dosing, that is, the "time in therapeutic range" of the patient (INR: 2–3). According to a Cochrane analysis, this is equivalent to a reduction of stroke incidence by two thirds (OR: 0.36; 95 % CI: 0.22–0.58). However, the incidence of severe extracranial bleeding is increased 4-fold (OR: 4.32; 95 % CI: 1.55–12.10) [134]. Aspirin is significantly less effective but also causes fewer bleeding events [135]. Thus, coumarin-type anticoagulants are superior to aspirin or other antiplatelet drugs in patients with atrial fibrillation but the question remains whether this can be obtained at an acceptable risk of bleeding events. The prospective randomized "Birmingham Atrial Fibrillation Treatment of the Aged" (BAFTA) has also confirmed the superiority of anticoagulation by warfarin (INR: 2–3) versus aspirin (75 mg/day) in a population-based study in elderly patients with nonvalvular atrial fibrillation [136].

BAFTA was a prospective, randomized but open trial which was intended to answer the question whether a stronger anticoagulation with an oral anticoagulant (warfarin) in the elderly is superior to aspirin or bears an unacceptable high risk of bleeding, considering possible polypharmacy, compliance problems and multimorbidity of elderly individuals.

A total of 973 elderly patients (>75 years of age, average 82 years) with atrial fibrillation and treated by general practitioners was included. They were randomized to receive either warfarin (INR: 2–3) or aspirin (75 mg/day). The mean follow-up period was 2.7 years. Primary endpoints were fatal or disabling stroke (ischemic or hemorrhagic), other intracranial hemorrhages or clinically relevant arterial embolism. The adherence to warfarin was 67 % and to aspirin it was 76 % at the end of the study. The most frequent reason for noncompliance was change to alternative medication, which was permitted according to the study protocol.

The primary endpoint was reached by 24 patients (21 strokes) in the warfarin group but in twice as much, 48 patients (44 strokes), in the aspirin group. This was equivalent to an annual absolute risk of 1.8 % in the warfarin group but 3.8 % in the aspirin group, equivalent to an absolute RR by 2 % (O: 0.48; 95 % CI: 0.28–0.80; $P = 0.003$). There were no differences in the risk of extracranial bleeding events, the annual risk being 1.4 % in the warfarin and 1.6 % in the aspirin group (O: 0.87; 95 % CI: 0.43–0.73).

The conclusion was that well-controlled treatment with oral anticoagulants in elderly patients with atrial fibrillation is superior to aspirin as long as there are no contraindications and the patient accepts the additional efforts related to oral anticoagulant treatment [136].

This study was the first to show superiority of warfarin vs. aspirin in stroke protection in patients with atrial fibrillation at no increased risk of bleeding events. However, warfarin is not the first choice of treatment in other risk groups of stroke, including noncardioembolic stroke and symptomatic intracranial arterial stenosis. The reason is the high risk of severe bleeding events. Two studies, the "Warfarin-Aspirin Recur-

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rent Stroke Study" (WARSS) [137] and the "Warfarin Aspirin Symptomatic Intracranial disease" (WASID) study [138], have addressed this issue in stroke patients.

The WARSS study included a total of 2,206 nonselected patients (mean age 63 years, 41 % women) who had suffered a noncardioembolic ischemic stroke within the previous 30 days. Patients were randomized to aspirin (325 mg/day) or warfarin (INR: 1.4–2.8) in a double-blind manner. Primary endpoint was recurrent ischemic stroke or death from any cause within 2 years.

The primary endpoint was reached by 17.8 % of patients in the warfarin group and by 16.0 % of patients in the aspirin group. This was not significantly different ($P = 0.25$). Major hemorrhages occurred at a rate of 2.2 % per year in the warfarin group but only at 1.5 % per year in the aspirin group ($P = 0.10$). The corresponding values for minor hemorrhages were 12.9 % for aspirin but 20.8 % for warfarin ($P < 0.001$).

The conclusion was that both warfarin and aspirin are reasonable therapeutic alternatives in the prevention of recurrent ischemic stroke. However, warfarin is associated with an increased risk of bleeding [137].

The WASID study was another prospective trial in patients with transient ischemic attacks or nondisabling stroke. The main inclusion criterion was angiographically verified 50–99 % stenosis of a major intracranial artery. Atrial fibrillation was an exclusion criterion. Patients were randomized to warfarin (INR: 2–3) or aspirin (1,300 mg/day) in a double-blind fashion. The primary endpoint was ischemic stroke, cerebral hemorrhage or death.

After 569 patients had undergone randomization, enrollment was stopped prematurely because of safety concerns in the warfarin group. After a mean follow-up of 1.8 years, there were significantly more severe adverse events including deaths in the warfarin group (9.7 %) as compared to aspirin (4.3 %) ($P = 0.02$). The numbers for major hemorrhages in the warfarin group were 8.3 % vs. 3.2 % ($P = 0.01$), and for myocardial infarction or sudden death these numbers were 7.3 % vs. 2.9 % ($P = 0.02$). The rate of death from vascular sources was 5.9 % vs. 3.2 % ($P = 0.16$) and the rate of death from nonvascular sources was 3.8 % vs. 1.1 % ($P = 0.05$). There was no difference in efficacy. A primary endpoint occurred in 22.1 % of patients in the aspirin group and in 21.8 % of patients in the warfarin group ($P = 0.83$).

The conclusion was that warfarin was associated with significantly higher rates of adverse events but produced no benefit above aspirin. Aspirin should be used in preference of warfarin for patients with intracranial arterial stenoses [138].

As a consequence of these studies, warfarin was replaced as first-line treatment for stroke prevention by antiplatelet drugs, except in patients with atrial fibrillation [139].

Triple vs. double therapy in atrial fibrillation patients with coronary heart disease. Triple therapy, that is, the combination of DAPT with an anticoagulant, is guideline recommendation in patients with atrial fibrillation who need an acute coronary intervention. The "What is the Optimal Antiplatelet and Anticoagulant Therapy in Patients with Oral Anti-Coagulation and Coronary Stenting?" (WOEST) study investigated whether one antiplatelet agent alone (clopidogrel) in combination with warfarin is sufficient to reduce the rate of thrombotic events in patients with atrial fibrillation undergoing PCI and perhaps yields lower rates of bleeding than in the triple treatment group [140].

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A total of 753 patients with atrial fibrillation (70 %) and/or ACS (25 %) who underwent coronary stenting were randomized to triple therapy with oral anticoagulant plus clopidogrel (75 mg/day) and aspirin (75 mg/day) or only clopidogrel without aspirin for at least 1 month after insertion of the drug-eluting stent. Follow-up was 1 year, primary endpoint was the occurrence of all bleeding events and secondary endpoint was a combined vascular endpoint including target vessel revascularization.

The incidence of bleeding events was markedly lower in the double therapy than in the triple therapy group (HR: 0.36; 95 % CI: 0.26–0.50). There were more cardiovascular events (stroke, myocardial infarction) in the aspirin-free group and an increased death rate ($P = 0.027$).

The conclusion was that combined treatment of these patient populations with oral anticoagulants with only one antiplatelet compound (no aspirin) yields the same clinical benefit but causes less bleeding [140].

Despite some criticisms [141] regarding the relatively low number of patients of whom only 70 % had atrial fibrillation and 25 % had ACS, the low periprocedural INR of 2.0 which was kept unaltered throughout the 1-year follow-up period and the fact that the larger number of bleeding events in the triple group was entirely driven by minor bleeding events, three subsequent registry studies were going into a similar direction [142]. These data suggest that inhibition of thrombin formation by antithrombotics in combination with one single antiplatelet drug such as clopidogrel, leaving out aspirin, might be an alternative approach to triple treatment. However, there was a tendency for an increased rate of cardiovascular endpoints, including death, leading to the suggestion that removal of aspirin might be associated with a reduced antiplatelet effect. This issue has been studied intensively in several randomized trials in patients receiving oral anticoagulation who underwent stenting or presented with ACS. Bleeding was the primary endpoint. Efficacy, here prevention of stent thrombosis or myocardial infarction, was less with dual (omission of aspirin) than triple therapy. This suggests that omission of aspirin from triple therapy in patients receiving oral anticoagulation should not be recommended until it is sure that omitting aspirin does not cause harm (Fig. 4.1.2-5) [143].

New oral anticoagulants. The pharmacological spectrum of oral anticoagulants has been widened with the introduction of new, direct acting oral anticoagulants (NOACs), i. e., antithrombins (dabigatran) and inhibitors of factor Xa (rivaroxaban, apixaban, edoxaban). All of these compounds demonstrated superior or at least equal efficacy in comparison vitamin K antagonists in managing thrombotic risks, specifically ischemic stroke in patients with atrial fibrillation [144]. This included both increased efficacy and reduced risk of intracranial [145] and extracranial bleeding events [144, 146–148]. The reduced incidence of strokes was largely due to a reduced number of hemorrhagic strokes as well as a reduced rate of severe extracranial bleeding events. This resulted in the clinical approval of NOACs. Currently, NOACs are about to replace coumarin-type anticoagulants in stroke prevention in patients with atrial fibrillation [149]. For other stroke isoforms the situation is less clear. Available data suggest that NOAC-

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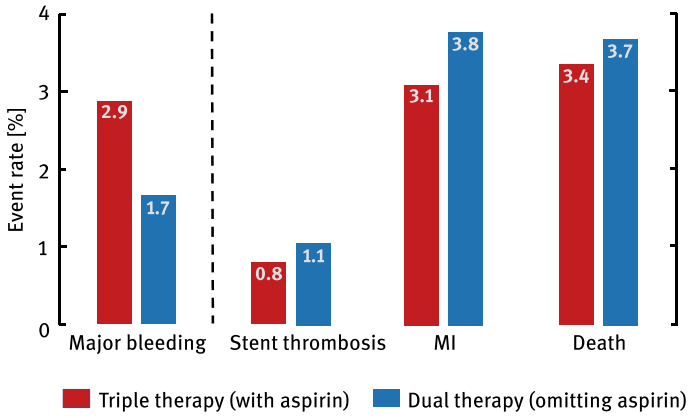

Figure 4.1.2-5: Rates of major clinical endpoints in four trials (pooled) comparing aspirin omission (dual therapy) versus aspirin administration (triple therapy) in patients receiving oral anticoagulation who present with ACS or undergo PCI [143].

based dual therapy, that is, rivaroxaban and clopidogrel, appears to provide an optimal risk/benefit ratio for the majority of these patients. Adding aspirin to this primary choice for up to 4 weeks in patients at especially high ischemic risk would likely reduce atherothrombotic events [150].

Although vitamin K antagonists have a relatively narrow safety margin, they can be kept within the therapeutic range by appropriate INR monitoring. This is not possible with NOACs because of the absence of a clinically useful surrogate parameter of their clinical efficacy. There are also large variations in plasma levels associated with the short half-life which do not exist for warfarin-type compounds, which are "forgiving drugs" – one forgotten intake is not associated with an increased risk of stroke. Whether the risk/benefit ratio is also improved in other forms of ischemic stroke and whether all NOACs are the same needs also to be determined.

There are no sufficient studies so far on the effects of these agents on stroke caused by atherosclerosis of the major intracranial arteries [151], the subtype of ischemic stroke that is the domain of antiplatelet treatment. Therefore, until more data are available, there should be no undifferentiated use of NOACs, also because of missing monitoring, the reversible mode of action with half-lives in the range of several hours – that of warfarin is about 2 days – and actually little experience with antidotes. In addition there is a tendency to more (gastrointestinal) bleeding events with NOACs although with large interindividual variability between the different trials [152].

4.1.2.6 Actual situation

General aspects. In addition to treatment of hypertension and lipid-lowering therapy, if appropriate, antiplatelet therapy with aspirin is efficient in stroke prevention,

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specifically in populations at high risk. However, current prophylaxis by antiplatelet treatment only prevents <20 % of (ischemic) cerebral infarctions and even might cause (hemorrhagic) strokes by itself. One clinical problem is the complex etiology of (ischemic) strokes, containing subgroups that are less sensitive to antiplatelet treatment than others. The multimorbidity of the (elderly) stroke patient, including frequent polypharmacy, compliance problems and bleeding risk, is of additional concern.

Primary prevention. Aspirin is not recommended for primary cardiovascular prevention, including prevention of a first stroke as outlined in detail in Section 4.1.1.

Secondary prevention. The situation with secondary prevention is more complex [153]. The 2021 AHA/ASA guidelines for secondary prevention of noncardioembolic ischemic stroke and TIA generally recommends (in addition to management of risk factors) antiplatelet/antithrombotic agents in the absence of contraindications and also now groups recommendations by etiological stroke subtypes [154]. In this context an actual metaanalysis of 57 RCTs has shown that aspirin (≤150 mg/day), clopidogrel and ticagrelor appear to be among the best choices to prevent non-cardioembolic ischemic strokes. Only aspirin at >150 mg/day significantly reduces all-cause mortality (OR: 0.86; 95% CI: 0.76–0.97) but also increases the risk of hemorrhagic events [155]. Comedication of dipyridamole to aspirin has been shown to be statistically slightly more effective than aspirin alone. However, the clinical significance of this action appears to be small and is reduced by a high rate of noncompliance and adverse effects of dipyridamole (headache). In addition, there is no (additional) beneficial effect of the combined treatment on mortality [154]. Data of the recent "Triple Antiplatelets for Reducing Dependency After Ischaemic Stroke" (TARDIS) trial, studying triple antiplatelet therapy (aspirin, clopidogrel and dipyridamole) vs. clopidogrel alone or combined aspirin and dipyridamole, have shown that stronger antiplatelet treatment did not reduce the incidence and severity of recurrent stroke or TIA, but did significantly increase the risk of major bleeding [156]. DAPT with aspirin plus clopidogrel for secondary stroke prevention is not generally recommended. Additional vascular effects by compounds such as cilostazol might be useful and open the door for a more disease-oriented treatment.

Anticoagulants, i. e., NOACs, rather than oral anticoagulants of the coumarin type at medium INR (2–3) have shown promising results in the COMPASS trial (Section 4.1.1) in particular in the subgroup of patients with stable peripheral or carotid artery disease [157]. Interestingly, the total vascular benefit in the combined aspirin plus rivaroxaban group was mainly driven by a significant reduction in strokes, 0.9 % in the combined treatment group, as opposed to 1.6 % in the aspirin alone group ($P < 0.001$), and not by a reduced rate of myocardial infarctions (Section 4.1.3). However, in the stroke subgroup lacunar and hemorrhagic strokes were an exclusion criterion as well as recent strokes within 1 month before the trial was started. This is interesting at the

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background of a recent review of ischemic stroke trials showing most of the beneficial effects of aspirin on prevention of recurrent strokes within the first 2 weeks after the acute event (Fig. 4.2.1-4) [77].

NOACs are currently under intense study in patients with cardioembolic stroke of undetermined source (ESUS). In the PIONEER AF-PCI trial, two rivaroxaban-based treatment regimens significantly reduced bleeding complications compared to conventional triple therapy without increasing embolic or ischemic complications following PCI. Dual therapy with rivaroxaban and clopidogrel appeared to provide an optimal risk/benefit ratio. In the RE-DUAL PCI trial, dual therapy with dabigatran also reduced bleeding complications compared to conventional triple therapy. The Phase III NAVIGATE ESUS study, evaluating the efficacy and safety of rivaroxaban in comparison to aspirin in patients with a recent embolic stroke of undetermined source (ESUS), was stopped early because of futility. This decision was made as the trial showed comparable efficacy between rivaroxaban and the standard of care, aspirin. In addition, an increase in major bleeding was observed in the rivaroxaban arm: 1.8 %, as opposed to 0.7 % per year in the aspirin group (HR: 2.7; 95 % CI: 1.68–4.39; $P < 0.001$) [158]. Similar negative results – increased bleeding – were obtained in trials on the combined use of antithrombotics with antiplatelet agents.

Summary

Acute cerebrovascular ischemia, clinically appearing as TIA or ischemic stroke, is caused by atherothrombosis, cardiac embolism or small vessel disease (lacunar infarction). Among the subtypes of ischemic strokes, strokes of vascular (atherothrombotic) origin are particularly sensitive to antiplatelet/aspirin treatment. Aspirin appears to be less effective in lacunar stroke. Stroke prevention in patients with atrial fibrillation as a major reason for cardioembolic stroke in the elderly is the domain of oral anticoagulants, that is, direct acting oral anticoagulants (NOACs), as a first-line treatment. NOACs are about to replace coumarins because of the much lower risk of intracerebral bleeding.

The USPSTF (2022) does only recommend low-dose aspirin for adults aged 40 to 59 years with an estimated 10 % or greater 10-year risk for a first heart attack or stroke in the abscence of increased risk of bleeding (level C). In contrast, aspirin is recommended for early management of patients with acute noncardioembolic stroke. Clopidogrel is an alternative as well as combined aspirin/extended-release dipyridamole which appears to be slightly more effective than aspirin alone (all IA level of recommendation in the 2021 ESC guidelines) [159]. Combined aspirin/clopidogrel and ticagrelor appears to be more effective in large artery strokes and minor strokes or TIA. However, there is also an increased risk for bleeding. There might be a role for cilostazol alone or in combination with antiplatelet agents in (East) Asian populations that deserves further studies in Western societies.

Overall, a relative reduction of ischemic strokes by 10–15 % at the cost of an about 5 % increase of bleeding events (cerebral and gastrointestinal) by aspirin is low and less than has to be expected for effective thrombosis prevention in the cerebral circulation. One reason for this is the complex stroke etiology and the mix of subtypes, including lacunar strokes, with a different etiology, in many clinical trials. However, beneficial effects in cardiovascular prevention and perhaps also positive effects on prevention of (colorectal) cancer could be added, since both events are more frequently observed in the elderly (Section 4.3.1), although this has been disputed by the

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USPSTF edition 2021. Increased bleeding is always a problem and it is a principal question whether further inhibition of clotting beyond a critical limit is really useful or just dangerous because of too many severe bleeding events, including gastrointestinal bleeding events by NOACs. In any case, a more subtype-specific treatment of stroke and TIA is highly desirable and there is no reason to postulate that "one size (of drug selection) fits all" (etiological forms) of ischemic stroke is a suitable strategy [153].

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4.1.3 Peripheral arterial occlusive disease

4.1.3.1 General aspects

Etiology. Peripheral arterial occlusive disease (PAD) is a debilitating atherosclerotic syndrome with stenoses and occlusions in peripheral arteries, predominantly (90 %) of the lower limbs. The clinical symptoms range from intermittent claudication during

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exercise (Fontaine stage II) to severe peripheral limb ischemia with pain at rest (stage III) and, finally, ulcers and gangrene (stage IV), eventually requiring limb amputation. Intermittent claudication is the most frequent form of PAD with ischemic pain during walking as the first clinical symptom. This ischemic pain occurs when blood flow is insufficient to meet the metabolic demands of leg muscles in ambulatory patients.

PAD is a systemic, polyvascular disease that reflects an aggressive type of atherosclerosis and thrombosis. Consequently, PAD is associated with a considerably increased cardiovascular morbidity and mortality [1, 2]. Therefore, the health problems of PAD patients do not result solely in a reduced walking distance and ambulatory pain – although these might be the first clinical symptoms – but also in the coexisting coronary and cerebrovascular morbidities and the associated increased vascular thrombotic risk and mortality [3, 4].

Epidemiology. Currently, more than 200 million people worldwide suffer from PAD. The incidence is increasing [5] and may become even higher with increasing life expectancy [6, 7]. Longer life is also associated with a longer exposure to vascular risk factors, such as smoking, a sedative lifestyle and comorbidities, most notably diabetes. The disease is frequently underdiagnosed, mainly because about only one half of individuals with PAD are symptomatic [8, 9]. PAD is also underestimated in its prognostic value for other thrombotic complications of generalized atherosclerosis, i. e., myocardial infarction, stroke and sudden cardiac death. According to a population-based study in Germany, about twice as many elderly patients with PAD also had a manifestation of cerebrovascular or cardiovascular disease than individuals without PAD [10], a finding also seen in other countries [11].

Pathophysiology. The pathophysiological reasons of chronic underperfusion of lower extremities are much more complex than underperfusion of cerebral or coronary vessels. There are multiple abnormalities of endothelial and platelet functions as well as of plasmatic coagulation and fibrinolysis. Procoagulatory and proinflammatory factors synergize and this on the background of a pathological altered endothelial function [12].

PAD is associated with platelet hyperreactivity as also seen with other manifestations of generalized atherosclerosis but also with hypercoagulopathy. HTPR is frequently observed not only in response to platelet-stimulatory agonists such as ADP [13], but also upon local shear stress in stenotic areas with nonlaminar blood flow [14]. Platelet hyperreactivity in PAD is functionally reflected by enhanced platelet secretion (serotonin, growth factors, CD40L), thrombin formation [15], expression of adhesion molecules at the platelet surface (P-selectin) [16] and CD40L [12], elevated plasma β-thromboglobulin and shortened platelet survival [13]. In addition to their fundamental role in arterial thrombus formation, platelets are also a primary source of inflammatory mediators [12] and act as a trigger for activation of white cells and

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formation of NETs. Inflammation stimulates local thrombosis and vice versa. Consequently, beneficial actions of antiplatelet therapy should include both inhibition of platelet-dependent thrombus formation and inhibition of platelet-triggered inflammatory reactions via white cell effects. The complexity of pathogenetic factors, including those that are platelet-independent, will limit therapeutic approaches that only affect one pathophysiological variable of the disease, such as disturbed platelet function. Effective antithrombotic treatment of PAD should also involve inhibition of thrombin generation and action because the outstanding role of thrombin as prothrombotic and platelet-activating factor [1].

4.1.3.2 Thrombotic risk and mode of aspirin action

PAD and thromboxane. The antiplatelet actions of low-dose aspirin are determined by its effect on platelet-dependent thromboxane formation (Section 2.3.1). Elevated plasma thromboxane levels and enhanced urinary excretion of the thromboxane metabolite 11-dehydro-TXB_2 (11-DH-TXB_2) are generally found in PAD [13, 17]. On average, urinary excretion of 11-DH-TXB_2 is about twice as much in PAD patients as compared to age- and sex-matched controls and becomes largely normalized by low-dose aspirin treatment. This suggests platelets as the major site of formation [18]. Interestingly, enhanced 11-DH-TXB_2 excretion in PAD patients was seen only in association with coexisting cardiovascular risk factors, such as diabetes, hypercholesterolemia or hypertension. These patients and those without risk factors had a comparable severity of PAD (intermittent claudication) with comparably low levels of the ABI (0.60–0.63). This suggested that PAD per se is not a trigger of platelet activation in vivo and that the increased rate of thromboxane biosynthesis rather reflects the influence of coexisting disorders (Fig. 4.1.3-1) [18].

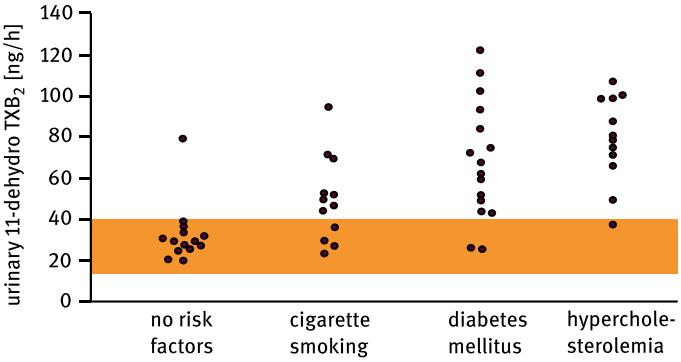

Figure 4.1.3-1: Urinary excretion of 11-DH-TXB_2 in subgroups of patients with claudicatio intermittens (Fontaine stage II) [18].

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This finding agrees with a population-based study in Finland, showing that PAD (claudication) was more frequent in persons with coronary heart disease or diabetes. After adjusting for symptoms and signs of coronary heart disease, claudication had no independent effect on mortality in men [19]. Thus, enhanced circulating thromboxane levels may represent a common link between atherosclerotic cardiovascular disease and the thrombotic complications of PAD. Aspirin reduces the platelet thromboxane-related elevated vascular risk [20] and is, therefore, likely to be more potent in patients with coexisting atherosclerotic disorders than in those without. Since the probability of thrombotic occlusions of all arteries, including those of the limb, increases with the progression and severity of any coexisting atherosclerotic vascular disease, antiplatelet/antithrombotic agents are probably more effective at more advanced stages of PAD because of their action on these coexisting disorders [21].

Coagulation and fibrinolysis. Besides disturbed platelet functions, there are other hemostatic abnormalities that contribute to the atherothrombotic risk in PAD. For example, blood viscosity and plasma fibrinogen levels are elevated, possibly in relation to enhanced activity of "tissue factor" [22] and increased thrombin formation [15]. In addition, there is disturbed fibrinolysis [16]. A "circulating plasma constituent" was found to directly stimulate platelet function in PAD patients via the GPIIb/IIIa receptor [23], as did fibrinogen [14], and this in an aspirin-insensitive manner. These findings confirm an inflammatory, thrombin-associated component of platelet hyperreactivity which appears to be more pronounced in peripheral arteries than in other vascular beds. Possibly, the large mass of ischemic muscles of the leg might be more prone to generation and release of inflammatory mediators from platelets, white cells and the endothelium than the relatively "small" heart or brain, and this in particular during reperfusion after successful spontaneous or medical lysis of preformed vessel narrowing or obstruction by thrombi.

Endothelial dysfunction. In addition to abnormalities in platelet and white cell function, coagulation and fibrinolysis in patients with PAD, there is also evidence for severe endothelial dysfunction. This has been most convincingly demonstrated in older studies on patients with type 2 diabetes, who according to the Framingham database have a 3–5-fold increased risk of developing symptomatic PAD [24]. In these patients, there was also a markedly increased generation of reactive oxygen species by the vasculature. Antioxidative defense was reduced, resulting in the formation of AGE proteins in the vessel wall which further enhance endothelial dysfunction [25–27].

Aspirin and platelet functions. Aspirin is the most intensively studied antiplatelet drug in PAD. Several studies indicated that platelets in patients with PAD are relatively "aspirin-resistant" [23, 28–31] and might be more sensitive against inhibition

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by other types of antiplatelet agents, such as clopidogrel or other ADP antagonists [14, 15, 32, 33]. For example, aspirin-treated platelets of PAD patients exhibited unchanged spontaneous or serotonin-induced platelet aggregation [34] and were also not inhibited after stimulation by ADP in vitro. These data underline the need for more effective antithrombotic treatment [35]. Selective platelet inhibition by low-dose aspirin may be less effective in PAD patients than in patients with other manifestations of atherothrombosis. For a long time there was no convincing evidence that aspirin is beneficial for treatment of claudication or rather for treatment of the associated increased cardiovascular risk [7]. There was also no consensus whether mono- or dual antiplatelet treatment should be preferred [36] or any other vasoactive or antithrombotic/antiinflammatory compound [22]. This has changed after the COMPACT and VOYAGER studies, respectively, and aspirin became an important part of dual antiplatelet or combined antiplatelet/antithrombotic treatment of PAD in patients at elevated cardiovascular risk (see below).

4.1.3.3 Clinical trials: primary prevention

General aspects. Prior to VOYAGER and COMPASS, there were only very few if any studies that addressed the symptoms and severity of PAD, such as progression to critical limb ischemia or surgical interventions, as a primary or even only target of therapeutic vascular prevention. Consequently, there was little evidence that antiplatelet medications, such as aspirin, will protect from claudication or its atherothrombotic complications in otherwise healthy individuals.

Among the few prospective randomized primary prevention trials on aspirin and PAD is a follow-up subgroup analysis of the Physicians' Health Study (Section 4.1.1) [37].s

The study population was a subgroup of the US-PHS in about 20,000 apparently healthy US physicians aged 40–84 years. Participating doctors were treated for 5 years with aspirin (325 mg each other day) or placebo in a double-blind, randomized design. Previous peripheral arterial surgery or preexisting claudication at baseline were exclusion criteria. Primary endpoint of the substudy was vascular surgery at lower limbs at 4 years or later after randomization.

Within a total observation period of 4 years, 56 study participants had to undergo peripheral arterial surgery, 20 of them in the aspirin group and 36 in the placebo group. This was equivalent to a 46 % relative RR by aspirin (RR: 0.54; 95 % CI: 0.30–0.95; $P = 0.03$). No difference was seen in the incidence of claudication or the number of individuals that developed self-reported new claudication: 112 in the aspirin group and 109 in the placebo group ($P = 0.92$). Interestingly, from the nine new claudicants who had to undergo peripheral arterial surgery, one was in the aspirin but eight were in the placebo group ($P = 0.03$).

The conclusion was that long-term administration of aspirin will not protect from atherogenesis, that is, not retard the development or progression of atherosclerosis (in the limbs) in its early stages. However, aspirin will be beneficial in the more advanced stages of PAD when thrombosis within the narrowed vessels plays a critical role for clinical symptoms and may require surgical treatment [21]).

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In this study, the severity of the (developing) disease, by definition, was lower, the individuals were much younger than the typical PAD patients, the number of smokers was lower and the duration of the disease was shorter. In addition, PAD complications were not a primary study endpoint but were calculated from a post hoc analysis of the data. Despite these limitations, the data suggest that administration of aspirin might be effective in prevention of thrombotic vessel occlusion of peripheral arteries but not in preventing the occurrence and progression of atherosclerosis [7]. These findings and conclusions are similar to prevention of myocardial infarction (Section 4.1.1) and stroke (Section 4.1.2).

The AAAT trial. More information on primary prevention in asymptomatic PAD patients was expected from the "Aspirin for Asymptomatic Atherosclerosis Trialists" (AAAT) trial. The main inclusion criterion was a reduced ABI that was determined in a general screening program. ABI was considered as an early, noninvasive marker for atherosclerotic alterations in the vessel wall with predictive value for later cardiovascular thrombotic events [38]

AAAT was a randomized, placebo-controlled prospective double-blind study in apparently healthy Scottish individuals, aged 50–70 years at entry. Participants had to be free from known cardiovascular diseases or treatment with antiplatelet or anticoagulant drugs. The main inclusion criterion was an ABI of <0.95. Primary endpoint was the occurrence of ACS, stroke or revascularization. Secondary endpoints were TIA or claudication. The total observation period was 8.2 years.

A total of 250 persons who fulfilled the inclusion criteria were randomized and received either enteric-coated aspirin (100 mg/day) or placebo. The study was terminated individually if the participant needed antiplatelet/antithrombotic long-term treatment due to reaching a vascular endpoint.

The mean age of the participants at the beginning of the study was 52 years, and about 70 % were female. The mean ABI at the beginning of the study was 0.86, the mean total cholesterol was 238 mg/dl and the mean blood pressure was 148/84 mmHg. There were 13.7 primary events per 1,000 patient-years in the aspirin group and 13.3 primary events per 1,000 participants in the group receiving placebo. Among the events were 14 and 12 peripheral revascularizations, respectively. There were 22.8 secondary events per 1,000 patients-years in the aspirin as opposed to 22.9 secondary events in the placebo group, including 32 individuals in each group with intermittent claudication. None of these differences were significant and the mortality was also unchanged. There was a tendency to more bleeding events and ulcers in the aspirin group which, however, was also not significant.

The conclusion was that prophylactic aspirin in individuals without symptomatic cardiovascular disease or asymptomatic PAD is useless [39].

This was the first large randomized prospective and placebo-controlled trial in individuals with low-degree asymptomatic PAD and is, therefore, of particular interest for the evaluation of prophylactic aspirin in primary prevention. Of interest is also the study concept – early identification of individuals with possibly elevated cardiovascular risk by determination of an easy accessible and highly specific functional parameter of reduced limb perfusion. ABI can be measured by each GP without particular equipment.

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By combination with conventional laboratory risk markers it could help to estimate the benefit/risk ratio in primary prevention. The German "Epidemiological trial on ankle brachial index" (getABI) on 6,880 nonselected persons with subclinical or manifest PAD had already shown that an ABI below 0.90 is associated with a linear increase of cardiovascular mortality [40, 41]. Similar results, that is, a significant increase of the cardiovascular risk at an ABI of ≤0.85, had previously also been reported by others [42]. In this context, the AAAT study has shown that this correlation does apparently not exist for individuals with lower-degree PAD (ABI ≤ 0.95).

Possible explanations for these negative findings in the AAAT trial are a too low number of events and the low ABI index as inclusion criterion. In addition, the compliance rate amounted to only 60 %. Possibly, the number of participants was also too low for a statistically safe conclusion. An Italian metaanalysis on antiplatelet agents, including 29 randomized trials in patients with claudication and/or an ABI of ≤0.99 (!), reported a significant reduction of cardiovascular adverse events using a combined vascular endpoint (OR: 0.779; 95 % CI: 0.639–0.950; $P = 0.014$) in the thienopyridine-treated patients vs. control and a tendency for aspirin (OR: 0.847; 95 % CI: 0.653–1.097; $P = 0.084$) that, however, did not reach significance [43]. The calculations of case numbers in the AAAT study were based upon an ambitioned estimated reduction of vascular events by aspirin by 25 % (!). The real RR was much smaller. This is not totally unexpected: According to the metaanalysis data of the Antiplatelet Trialists, the reduction of vascular events in primary prevention was only 12 % [44]. In the AAAT study, there was an *increase* by 3 %, however with a 95 % CI between +27 % and –16 %. This is well within the AAAT limits [45].

4.1.3.4 Clinical trials: secondary prevention

General aspects. The complex pathogenesis of PAD also defines the treatment goals of the disease. The first is relief of ischemic symptoms, in particular leg pain and prevention or at least retardation of progression of the disease to critical arterial stenosis and, finally, critical limb ischemia. One option is percutaneous transluminal angioplasty (PTA). PTA requires appropriate antithrombotic treatment to prevent reocclusions and other vascular events whose incidence is markedly increased during the first weeks after the intervention [46]. There are several attempts to reach these goals: treatment or avoidance of risk factors (diabetes, cigarette smoking and immobility) and administration of antiplatelet/antithrombotic drugs [2, 7, 47].

The metaanalysis of the Antithrombotic Trialists' Collaboration on secondary prevention (including randomized studies published until 1997) [48] contained 42 studies on 9,214 patients with PAD. There was a 23 % reduction of serious vascular events in PAD patients as compared to a 22 % reduction in the total cardiovascular risk population. This might have been expected because of the frequent association of PAD with other manifestations of atherosclerosis. The real benefit of aspirin on PAD-specific endpoints cannot be deducted from these data.

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Prospective trials. There are few prospective trials on aspirin treatment in PAD with PAD-related complications as a primary study endpoint. The prospective, placebo-controlled double-blind CLIPS trial in 366 PAD patients with low-grade PAD (Fontaine I/II) studied aspirin (100 mg/day) vs. vitamins E and C for two years. Neither treatment was associated with any significant change in adverse vascular events. Inclusion of this trial in a metaanalysis of other randomized trials of antiplatelet therapy in PAD made the overall results highly significant ($P < 0.001$) and suggested that low-dose aspirin could reduce the incidence of vascular events by 26 % [49].

In addition to these studies, mostly involving patients at medium to moderate stages of PAD (claudicants), another prospective trial on the efficacy of aspirin in PAD was performed in patients at end stages of the disease, i. e., diabetic patients with active limb gangrene or recent amputation.

A total of 231 patients were treated with aspirin (650 mg/day) and dipyridamole or placebo for 5 years. Primary endpoints were death from atherosclerotic vascular disease plus amputation of the opposite extremity for gangrene.

In comparison to controls there were no differences to treated patients in vascular deaths (22 % and 19 %) or amputation of the opposite extremity (20 % vs. 24 %). There was no change in the incidence of myocardial infarctions but a 50 % reduction in cerebrovascular events (stroke or TIA; 8 % vs. 19 %), which, however, was not significant.

The conclusion was that antiplatelet agents have no effect on primary vascular endpoints but might affect the incidence of strokes, which, however, was only a secondary endpoint in the study [50].

It is questioned whether any (conservative) treatment at this final stage of the atherosclerotic disease can still improve the symptoms or even retard the progression of the disease. However, the studies in claudicants do suggest that (high-dose) aspirin can reduce the incidence of thrombotic vessel occlusions in leg arteries, although it probably cannot retard the atherosclerotic process. This would be in line with the primary prevention data in the Physicians' Health Study (see above) [21].

4.1.3.5 Clinical trials: peripheral transluminal angioplasty

A remarkable Austrian prospective, although small and nonrandomized study tried to predict the clinical outcome of aspirin-treated PAD patients subjected to elective PTA from aspirin-induced inhibition of platelet aggregation [51].

A total of 100 patients (30 female, 70 male) with intermittent claudication were subjected to elective PTA. Clinical outcome (new vessel occlusion after successful intervention) and platelet function were monitored for 12 months in a prospective, compliance-controlled manner. All patients were treated with 100 mg aspirin/day. Whole blood aggregometry was used to compare inhibition of collagen and ADP-induced platelet aggregation by aspirin with the clinical outcome.

All patients showed complete inhibition of arachidonic acid-induced, that is, thromboxane-dependent, platelet aggregation by aspirin (which was an inclusion criterion) at all study time

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points. Late reocclusions (eighth to 52nd week) at the site of PTA occurred exclusively in male patients, for whom in vitro aggregometry failed to prove sufficient inhibition by collagen and ADP. In these patients, the risk of recurrent vessel occlusion was increased by 87 %.

The conclusion was that only 40 % of male patients showed the expected effect of aspirin on in vitro platelet aggregation after ADP and collagen and that whole blood aggregometry was capable of predicting patients at elevated risk of reocclusion following PTA (Fig. 4.1.3-2) [51].

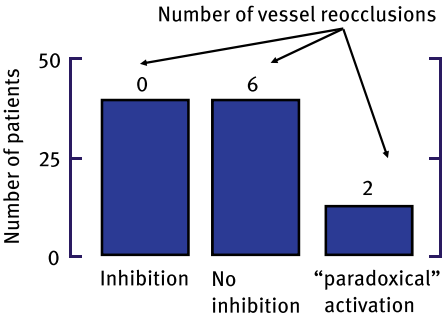

Figure 4.1.3-2: Predictive value of aspirin-induced inhibition of ADP-induced platelet aggregation (whole blood aggregometry) for the incidence of reocclusions in 96 patients after successful PTA and treatment with 100 mg/day aspirin. Numbers on top mark the number of reocclusions in dependency on the aspirin efficacy (for further explanations see text) [51].

This appears to be the only clinical study which tried to correlate a PAD-specific vascular outcome – reocclusion of PTA-treated vessels – with the antiplatelet action of aspirin as a prognostic surrogate parameter. The results clearly indicate that insufficient inhibition of platelet aggregation by aspirin was associated with an increased risk of vessel reocclusion. One major difference between this and most other studies on antiplatelet agents in PAD patients was the use of whole blood aggregometry. This technique also considers red cells, white cells and other plasma constituents as modifying factors for platelet function and antiplatelet actions of aspirin [52–54]. This may better reflect the complex situation of platelet activation in PAD patients in vivo than measurement of platelet function in any conventional ex vivo assays using platelet-rich plasma. More randomized trials in sufficiently sized studies are necessary to establish the relationship between reduced platelet sensitivity to aspirin and reduced therapeutic benefit in PAD patients subjected to PTA. These should also include further thrombosis-relevant parameters and might be most interesting at the background of the recent COMPASS and VOYAGER trial data on PAD patients (see below) [55–57].

A Cochrane analysis of 14 randomized trials in patients with symptomatic PAD reported a 60 % reduction of recurrent obstructions of the dilated artery segment with aspirin (50–330 mg/day) alone or combined with dipyridamole as compared to placebo at 12 months. At 6 months after endovascular treatment, a positive (nonsignificant) effect on patency was also seen with 50–100 mg/day aspirin. It was concluded

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that aspirin at daily doses of 50–300 mg, starting prior to femoropopliteal endovascular interventions, appears to be effective and safe. No positive effect was seen with warfarin-type anticoagulants [58]. A more recent Cochrane analysis of 16 trials with 5,683 PAD patients has confirmed these positive findings for aspirin and other antiplatelet drugs as compared to placebo or no treatment (OR: 0.42; 95 % CI: 0.22–0.83; $P = 0.01$). This effect was not seen for venous grafts, but was solely observed for all time points in prosthetic grafts, including the final time point of 12 months (OR: 0.19; 95 % CI: 0.10–0.36; $P < 0.00001$). There was no evidence of differences in side effects (including bleeding or infections), amputation, cardiovascular events or mortality between the treatment groups [59].

4.1.3.6 Aspirin and other drugs

Aspirin will probably not modify the claudication per se since the progression of the disease is mainly determined by aspirin-independent coexisting disorders. Accordingly, treatment with antihypertensives, antidiabetics and lipid-lowering drugs is frequent and generally recommended in PAD patients with the appropriate risk [2, 60].

Clopidogrel and other ADP antagonists. The currently best studied antiplatelet alternative to aspirin in long-term prophylaxis of ischemic events with proven benefit in PAD patients is clopidogrel. In the CAPRIE trial [61], 6,452 patients, about one third of the total study population, had PAD as a qualifying entry event. All patients were randomized to 325 mg/day aspirin or 75 mg/day clopidogrel and followed up for about 2 years.

In the subgroup with PAD as qualifying disease, clopidogrel (as compared with aspirin) caused a 24 % relative RR in the combined vascular endpoint ischemic stroke/myocardial infarction/vascular death. However, the 95 % CI was wide (9–36 %), and the group included not only patients with claudication but also those with endovascular treatment [61].

The conclusion was that clopidogrel reduced the risk of vascular events in PAD patients, being slightly more effective than aspirin in a post hoc analysis [62].

In five further comparative studies with aspirin and other antiplatelet agents, including clopidogrel, antiplatelet medication was always better than placebo or no treatment. There were no differences in efficacy or safety in patients with symptomatic PAD after monotreatment with clopidogrel or ticagrelor. In a double-blind, event-driven, randomized trial on 13,885 patients with symptomatic PAD, during a medium follow-up of 30 months, the primary efficacy endpoint (combined cardiovascular events) occurred in 10.8 % of patients receiving ticagrelor and in 10.6 % of patients receiving clopidogrel, acute limb ischemia occurred in 1.7 % of the patients in both groups and major bleeding occurred in 1.6 % of patients. Thus, ticagrelor was not superior to clopidogrel for the reduction of cardiovascular events in PAD patients [63]. In other words,

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the selection of antiplatelet drugs in PAD patients should be done with respect to the individual cardiovascular risk factor profile of the respective patients.

Warfarin-type oral anticoagulants. Older studies with warfarin-type oral anticoagulants suggested that these compounds were of limited value in PAD [64]. This was recently confirmed in the randomized but open "Warfarin Antiplatelet vascular evaluation" (WAVE) trial on more than 2,100 PAD patients (claudication intermittens). The study compared warfarin (INR: 2.0–3.0) with aspirin (81–325 mg/day) alone or in combination with the anticoagulant over 3 years. There was no reduced rate of myocardial infarctions, stroke or cardiovascular death but a significant increase in moderate and life-threatening bleeding events in the combined treatment group: 4.0 % vs. 1.2 % (O: 3.41; 95 % CI: 1.84–6.35; $P < 0.001$). The conclusion was that the combination of warfarin with antiplatelet treatment has no additional benefit but significantly increases severe bleeding and, therefore, is not to be recommended [65].

In one multicenter, randomized but open trial on 2,690 patients who had undergone infrainguinal grafting, treatment over 21 months with oral anticoagulation (INR: 3.0–4.5) was found to be superior to aspirin to prevent infrainguinal vein graft occlusion and to lower the rate of ischemic events, while aspirin (80 mg/day) was found to be more effective in the prevention of nonvenous graft occlusion and caused less bleeding [66]. Whether the benefits of oral anticoagulants outweigh the risk of severe and life-threatening bleeding at this high INR requires more prospective randomized trials but might be out of time after the introduction of NOACs.

New oral anticoagulants. Interesting new data on the effects of the NOAC rivaroxaban and aspirin, alone and in combination, in PAD patients were obtained in a (prespecified) subgroup analysis of the COMPASS trial (Section 4.1.1) [67].

The COMPASS trial contained a subgroup of 7,440 patients with PAD (stages II–IV). After a 30-day run-in, eligible patients were randomly assigned to receive rivaroxaban (2.5 mg twice daily) plus aspirin (100 mg/day), rivaroxaban (5 mg twice daily) or aspirin (100 mg/day). The primary outcome was a composite of cardiovascular death, myocardial infarction or stroke. PAD outcome was a secondary endpoint and included major adverse limb events including major amputation or a new pulse deficit leading to an intervention. The median duration of treatment was 21 months. This was shorter than originally planned. The decision for premature stop was made by the safety-monitoring board because of favorable outcomes in one subgroup of the whole study.

The combination of rivaroxaban plus aspirin compared with aspirin alone reduced the composite vascular endpoint in this subgroup to 5 % vs. 7 % (HR: 0.72; 95 % CI: 0.57–0.90; $P = 0.0047$) and major adverse limb events including major amputation to 1 % vs. 2 % (HR: 0.54; 95 % CI: 0.35–0.82; $P = 0.0037$). Rivaroxaban (5 mg twice daily) compared with aspirin alone did not significantly reduce the composite endpoint (6 % vs. 7 %; HR: 0.86; 95 % CI: 0.69–1.08; $P = 0.19$), but reduced major adverse limb events including major amputation to 40 [2 %] vs. 60 [2 %] (HR: 0.67; 95 % CI: 0.45–1.00; $P = 0.05$). The combined use of rivaroxaban plus aspirin increased

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major bleeding as compared with the aspirin alone group (77 of 2,492 [3 %] vs. 48 of 2,504 [2 %] total participants; HR: 1.61; 95 % CI: 1.12–2.31; $P = 0.0089$), which was mainly gastrointestinal. Major bleeding occurred in 79 of 2,474 (3 %) patients with rivaroxaban (5 mg twice daily) and in 48 of 2,504 (2 %) patients in the aspirin alone group (HR: 1.68; 95 % CI: 1.17–2.40; $P = 0.0043$). All-cause mortality was unchanged.

The conclusion was that low-dose rivaroxaban taken twice a day plus aspirin once a day reduced major adverse cardiovascular and limb events when compared with aspirin alone. Although major bleeding was increased, fatal or critical organ bleeding was not. This combination therapy represents an important advance in the management of PAD patients. Rivaroxaban alone did not significantly reduce major adverse cardiovascular events compared with aspirin alone, but reduced major adverse limb events and increased major bleeding [55, 57].

These data are promising and suggest inhibition of thrombin generation together with direct antiplatelet effects as a useful new and effective approach in treatment of PAD-related limb events [68]. Importantly, high-dose rivaroxaban alone reduced "major adverse limb events" (MALE) including major amputations more than did aspirin alone ($P = 0.05$). Another subgroup analysis of PAD patients of the COMPASS trial showed that the combination of rivaroxaban plus aspirin reduced the incidence of major adverse limb events by 43 % ($P = 0.01$), total vascular amputations by 58 % ($P = 0.01$) and all peripheral vascular outcomes by 24 % ($P = 0.02$) [68]. Beneficial effects on critical limb ischemia were also seen after vorapaxar, a thrombin receptor (PAR-1) antagonist, in the subgroup of PAD patients in the TRAP-2-TIMI 50 trial [69].

The combination of low-dose rivaroxaban (2.5 mg twice daily) to attenuate thrombin generation and aspirin (100 mg once daily) to reduce platelet activation has been licensed for secondary prevention in patients with CAD or PAD. The 18 % mortality reduction with this dual inhibition is a unique finding that has not been demonstrated with other intensified antithrombotic regimens and represents a paradigm shift for secondary prevention. Successful translation of the results of the COMPASS trial into clinical practice depends on identifying high-risk patients who will benefit most. Such patients include those with polyvascular disease or symptomatic PAD and those with other high-risk features such as diabetes and renal impairment [1].

Similar results were obtained in the "Vascular outcomes study of aspirin along with rivaroxaban in endovascular or surgical limb revascularization for peripheral artery disease" (VOYAGER-PAD) in patients with symptomatic PAD undergoing revascularization. Rivaroxaban plus aspirin reduced the risk of adverse cardiovascular and limb events with an early benefit from limb ischemia regardless of clopidogrel use. The data suggest the combined treatment with rivaroxaban plus aspirin after lower extremity revascularization regardless of concomitant clopidogrel, with a short course (<days) associated with less bleeding [56].

Dipyridamole. Whether the efficacy of aspirin in treatment of PAD can be increased by comedication of dipyridamole is unproven. According to a Cochrane analysis involving 29 trials with more than 23,000 participants, dipyridamole did not reduce the in-

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cidence of vascular death in the presence or absence of antiplatelet treatment. A modestly reduced risk of vascular events was seen with dipyridamole only in patients presenting with cerebral ischemia but not with other manifestations of atherothrombosis, including PAD (HR:0.88; 95 % CI: 0.81–0.95) [70].

Cilostazol. Cilostazol is an orally active inhibitor of phosphodiesterase III (PDE III) and an inhibitor of adenosine uptake. The compound resembles dipyridamole in several aspects, including its synergism with adenosine [71]. Cilostazol has been shown in four randomized, controlled trials to improve the function of ischemic legs, possibly by retarding the progression of atherosclerosis [47]. The compound appears not to negatively interfere with platelet inhibitors, including aspirin, and does not prolong bleeding time [72]. Cilostazol is approved for treatment of PAD (claudication) in several countries and can be applied in combination with aspirin. Whether cilostazol also has beneficial effects on clinical outcome, that is, vascular mortality and other cardiovascular events, in PAD patients is unknown [73].

4.1.3.7 Actual situation

In clinical practice, the optimum conservative treatment of PAD is still a matter of discussion as also seen from the multitude of agents that are recommended (by the manufacturers) for this purpose. There is, however, no doubt that nonmedical measures, including smoking cessation and exercise training in claudicants (evidence levels IA!), are first-line recommendations as is an appropriate diet and, if indicated, weight loss. In addition to treatment of basal diseases (diabetes, hypertension and hypercholesterolemia) by appropriate drugs, antiplatelet drugs such as aspirin in combination with an antithrombin/FXa inhibitor appear to be the treatment of first choice in PAD. Moreover, dual antiplatelet therapy may be superior to aspirin or other antiplatelet monotherapy [36]. In general, the efficacy of aspirin monotherapy is low, regarding PAD-related perfusion problems, including the progression of limb atherosclerosis from claudication to critical limb ischemia. However, it adds to the protection from other cardiovascular thrombotic events (myocardial infarction, stroke) by its antiplatelet effects. Oral anticoagulants such as warfarin increase the bleeding risk and are of no therapeutic benefit. NOACs in combination with aspirin are a promising new development for treatment of PAD. An interesting alternative development is cilostazol, a compound which mainly acts on vascular endothelium but also has tissue-protective, antiapoptotic and platelet-inhibitory properties.

Summary

Platelet hyperreactivity is part of the overall thrombotic/inflammatory syndrome in PAD patients. Further abnormalities of the hemostatic system include enhanced coagulation, disturbed fibrinolysis and endothelial dysfunction. Probably, because of this complexity, aspirin is less effective

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as sole antiplatelet/antithrombotic drug in PAD patients on PAD events than in patients suffering from other forms of generalized atherosclerosis. HTPR ("resistance") against aspirin is frequently observed.

PAD patients are at a 2–4-fold elevated risk of acute thromboembolism in the coronary and cerebral circulations. These are the life-threatening events in PAD patients rather than critical limb ischemia or amputation. According to the 2021 guidelines of the ESC, all patients with symptomatic PAD should be considered for treatment with low-dose aspirin or other antiplatelet drugs to reduce cardiovascular morbidity and mortality (evidence level IC) [74]. Dual antiplatelet treatment appears to be particularly useful for prevention of complications after revascularization [36].

Exciting new data came from the COMPASS and VOYAGER trials, studying the NOAC (factor Xa inhibitor) rivaroxaban. Most notably for PAD, there was a significant protective effect of rivaroxaban on major adverse limb events (HR for amputations: 0.54) as opposed to aspirin alone although at the price of increased severe bleeding events. Further studies with NOACs are underway. The combined use of FXa inhibitors (rivaroxaban) plus aspirin might become the treatment option of choice for PAD patients.

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4.1.4 Venous thromboembolism

4.1.4.1 General aspects

Etiology. Venous thromboembolism (VTE), clinically presenting as deep vein thrombosis (DVT) with pulmonary embolism (PE) as the major complication, is a dangerous

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and potentially fatal complication of acute surgical interventions. This "provoked" VTE is due to an iatrogenic activation of the clotting system by the operative procedures. Rather unpredictable is spontaneous, unprovoked VTE, frequently due to genetic predisposition in persons with inborn anomalies in clotting factors such as factor V-Leiden and/or other medical conditions (birth control pills, hormonal replacement therapy) and chronic medical illnesses (cancer!) and (guideline-conform) stop of secondary thrombosis prophylaxis to prevent recurrent VTE.

The thrombotic risk is also increased by certain comedications and environmental factors. At high risk of VTE are individuals who undergo operations with large injuries of soft tissue, such as hip or knee arthroplasties. These patients are frequently used to study the efficacy of antithrombotic measures for prevention of VTE. Another group of patients at elevated risk for VTE are bed-ridden persons and individuals immobilized by sitting in an airplane during long-distance flights (≥10 h). Both conditions will facilitate venous stasis in the absence of sufficient washout of locally accumulating clotting factors.

Epidemiology. The overall incidence of symptomatic VTE after elective surgical interventions amounts to about 1 %, including about one third of PE, and is increased to about 2 % at older age (>70 years). The vascular risk is dependent on the kind of surgery and amounts to 2–3 % in high-risk procedures, such as hip or knee arthroplasties [1]. The annual risk of recurrent VTE in persons without modifiable risk factors after stop of guideline-conform treatment with oral anticoagulants (warfarin, NOACs) amounts to 6–10 % per year, with the highest risk during the first 1–2 months after stopping anticoagulant use [2, 3]. All these numbers indicate that prevention of VTE and/or PE by appropriate measures is a clinically highly relevant issue.

Pathophysiology. The Virchow triad of venous thrombogenesis mentions blood "constituents" but not explicitly platelets as relevant pathogenetic factors. However, there are numerous and well-established interactions between platelets and white cells, facilitated by local inflammatory conditions, for example varicosis with local stasis and disturbed endothelial functions [4]. All of these have thrombin formation and action as a common final denominator. Activation of the plasmatic clotting system starts with the availability of "tissue factor" from different sources, in case of veins preferentially from circulating microparticles [5]. This is followed by the generation of the procoagulatory factors Xa (FXa) and IIa (thrombin) [4, 6, 7]. Thrombin and fibrin formation is also associated with the activation of platelets and white cells. Stimulation of fibrin formation, inhibition of fibrinolysis and a number of other inflammatory events are important contributing factors to wound healing and scar formation and might be affected by inhibition of thrombin formation and action.

During these processes, the endothelium remains morphologically intact. The venous thrombus initially adheres with a red fibrin- and red cell-rich layer at the en-

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dothelium. This is followed by luminal apposition of white platelet- and white cell-rich layers with irregular platelet clumps inside the thrombus [4]. The developing adherent thrombus will then grow towards the lumen with a luminally increasing proportion of platelets [8].

Platelets enhance the prothrombotic/proinflammatory changes and facilitate luminal thrombus growth. They stimulate leukocyte accumulation at the adherent thrombus, which grows in the direction of the blood stream, as well as fibrin formation and the formation of NETs [8, 9]. Negatively charged phospholipids at the surface of activated platelets potentiate these reactions by activation of the tenase and prothrombinase complexes [10]. In animal studies, platelet depletion inhibits these processes and largely prevents venous thrombus formation while depletion of neutrophils does not modify thrombogenesis [11]. This confirms platelets as an important pathophysiologic determinant of VTE [12]. In addition, experimental studies in a mouse model of VTE with uninjured endothelium clearly suggest that platelet-dependent thromboxane formation is another key event for both thrombin action and thrombus formation. Consequently, prevention of VTE via inhibition of platelet-dependent thromboxane (and thrombin) formation by aspirin appears to be a useful antithrombotic strategy [13] in addition to antithrombotic agents without direct effects on platelet functions (Fig. 4.1.4-1).

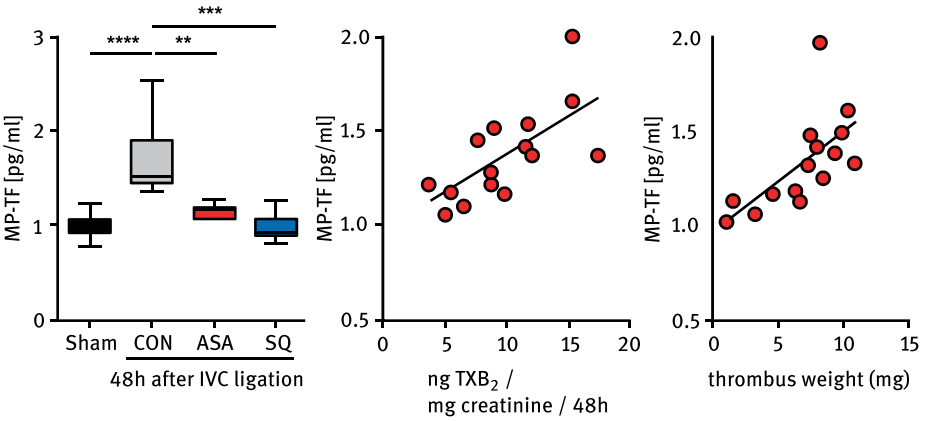

Figure 4.1.4-1: Circulating microparticle-bound tissue factor (MP-TF) is elevated in mice with partial (80 %) inferior vena cava ligation. This effect is prevented by pretreatment of mice with oral aspirin (ASA) (3 mg/kg) or the thromboxane (TX) receptor blocker SQ 29,548 (SQ). Note the linear correlation between MP-TF plasma levels and the TX metabolite (TXB_2) excretion and thrombus weight at 48 h after vessel ligation. Asterisks (*) denote significant changes in TF levels [13].

Inside the clot, thrombin formation is the key event for maintaining long-lasting (auto)activation of the clotting system [14] as well as for stimulation of clot-related platelet functions. Consequently, thrombi are not only the result but also – more im-

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portantly – the source of long-lasting thrombus-associated release of procoagulatory, platelet/white cell- stimulating and proinflammatory mediators.

4.1.4.2 Thrombotic risk and modes of aspirin action

Thrombotic risk. Similarly to experimental studies, clinical data also suggest a relationship between platelet reactivity and VTE [15]. Atherosclerosis is a risk factor not only for arterial but also for spontaneous VTE [16], while patients with idiopathic VTE exhibit a higher risk for arterial thromboembolism [17, 18]. Studies in surgical intensive care unit patients have shown an aspirin-sensitive contribution of platelets to (venous) thrombogenesis, clot strength and fibrin formation [19]. These and other data indicate a pathophysiological connection between arterial and venous thromboembolism. For these reasons, it has to be expected that antiplatelet agents, such as aspirin, may be an option not only to prevent (retard) arterial but also venous thrombus formation.

Mode of aspirin action. Aspirin has multiple targets to modify venous thrombus formation. These include antiplatelet effects via inhibition of COX-1-dependent thromboxane formation but also inhibition of thrombin generation and antiinflammatory effects (Section 2.3.1). There are multiple interactions between zymogens of clotting factors and platelets that interact with thrombin formation by aspirin at antiplatelet doses [20–24]. Inhibition of platelet activation by aspirin also causes inhibition of thrombogenic and proinflammatory platelet–white cell interactions and NET formation [9, 13]. Recent work has identified a "high-mobility group box 1 protein" (HMGB-1) from platelets that acts as mediator of NET formation and is considered a master regulator of the prothrombotic cascade in veins [25]. HMGB-1 also has specific binding sites for salicylates. Salicylate additionally inhibits HMGB-1-induced expression of COX-2 and proinflammatory cytokines at medium (100 μM) concentrations [26]. These findings, together with the generation of proinflammatory dioxolanes by thrombin-stimulated platelets that can be blocked by aspirin in antiplatelet doses (Fig. 2.3.2-2) [27], are important new experimental findings in favor of an antiinflammatory/antithrombotic action of low-dose aspirin in prevention of VTE.

4.1.4.3 Clinical trials – primary prevention

General aspects. The multifactorial pathogenesis of VTE requires a multimodal, individualized treatment strategy. This involves early mobilization, stockings and other physical measures. Pharmacologically, inhibition of thrombin formation is the primary target of prevention (and treatment) of VTE. Conventional standard drugs used to be low-molecular weight heparins (LMWHs) and pentasaccharide (fondaparinux) as well as coumarin-type oral anticoagulants (warfarin) [7]. Currently, direct acting oral anticoagulants (NOACs), that is, inhibitors of thrombin (dabigatran) [28] or factor Xa

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(e. g., rivaroxaban) [29], are attractive alternatives to coumarin-type anticoagulants, balancing the risk of appearance of a VTE versus the risk of producing major bleeding by drug treatment, for example in joint arthroplasty – there are very similar figures of 1–2 % for each of these compounds. This supports the concept of an individualized treatment strategy for an optimal benefit/risk ratio [30].

Antiplatelet drugs such as aspirin were not considered as primary option for prevention of VTE for a long time. According to an early metaanalysis of the Antiplatelet Trialists' Collaboration, aspirin reduced the risk of VTE and symptomatic PE in patients with orthopedic surgery significantly, by 30 % and 52 %, respectively [31], but was less effective than heparins or coumarins [32]. The evaluation of these metaanalyses has to consider the heterogeneity of the included trials with respect to duration and aspirin dosing, comorbidities and cotreatments. Nevertheless, the bleeding risk was less than that after coumarin treatment. This resulted in an improved benefit/risk ratio and, finally, in a restart of prophylactic aspirin use in prevention of VTE, frequently as part of a multimodal antithrombotic approach [33].

Observational trials. There are no clear data from early observational trials comparing aspirin with LMWH or coumarins as preventives of VTE in surgical conditions. Reasons for this are different evaluation criteria (symptomatic vs. asymptomatic VTE or PE) and heterogeneity in patient populations, comedications and drug treatment protocols. There were also different definitions of kind, localization and severity of bleeding events – the key safety endpoint of all studies [34, 35]. Nevertheless, most retrospective observational trials appeared to show a clinical net benefit for aspirin, in particular as part of a multimodal approach, that is, together with other preventive measures.

Sharrock and colleagues [36] analyzed the effects of different anticoagulation protocols on VTE prophylaxis with respect to efficacy in patients with total hip or knee arthroplasty. According to 20 single trials published between 1998 and 2007, including more than 15,000 patients, there was an about twice as high 3-month mortality after standard anticoagulation as compared with a multimodal approach with aspirin as antithrombotic agent [36]. Limitations of this review were a high percentage (50 %) of nonrandomized trials, heterogeneity of the treatment groups with different comorbidities and absent detailed information about bleeding events.

Another large, retrospective cohort study on patients with knee arthroplasty in 307 US hospitals came to similar conclusions.

A total of 93,840 patients undergoing primary knee arthroplasty received guideline-directed treatment with warfarin (51,923), LMWH/Fondaparinux (37,198) or (multimodal) aspirin (4,719). Efficacy endpoints were VTE/ PE. Safety endpoints were surgical site bleeding events and infections and mortality over 2 years.

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During this observation period, the lowest rates of VTE occurred in the aspirin group (2.3 %) as compared to warfarin (4.0 %) ($P < 0.01$) or LMWH/fondaparinux (3.1 %) (nonsignificant). Surgical site bleeding initially tended to be higher with the injectable agents and warfarin ($P < 0.01$), but the adjusted analysis found no differences. There were no differences in the adjusted risks of bleeding, infection or mortality.

The conclusion was that aspirin when used in conjunction with other clinical care protocols may be an effective preventive for VTE in certain patients undergoing knee arthroplasty [37].

In addition to the general problems of observational trials (Section 4.1), further limitations of this study were the retrospective design and the fact that patients in the aspirin group had a significantly lower baseline risk for VTE ($P < 0.01$). In addition, the authors critically commented that only 5 % of their study patients were treated with aspirin, as opposed to 40 % with injectable drugs (heparins) and 55 % with coumarin-type anticoagulants [37].

Another metaanalysis including 30,270 patients who received aspirin or warfarin as thromboprophylaxis after total joint arthroplasty indicated that aspirin, even in patients at elevated risk of VTE, was as effective as warfarin but safer with respect to bleeding events [38]. A recent retrospective single-institution study on 35,860 patients undergoing total joint arthroplasty was focused on safety – severe bleeding events. There were less than half (0.5 %) major bleeding events in patients treated with aspirin compared to patients treated with anticoagulants (1.2 %) [39].

According to these and other recent reviews on prevention of VTE with aspirin following joint surgery [40–42] no significant difference in effectiveness of VTE prevention was found between aspirin, LMWH and warfarin. NOACs were more effective, but increased bleeding. However, the quality of many studies was low. There was a substantial heterogeneity between them [43] as well as possible selection bias of patients with a tendency to give patients at lower risk aspirin and others "more potent" anticoagulants. This might result in an overestimation of the benefits of aspirin. Another tissue of concern are the different and sometimes even opposite guideline recommendations by the AAOS and the ACCP in the United States [44]. The randomized "Comparative effectiveness of pulmonary embolism prevention after hip and knee replacement" (PEPPER) trial might find an answer. The study was started in 2016 and will compare open-label aspirin (162 mg enteric-coated/day – no loading dose!) with warfarin and rivaroxaban (10 mg) in 20,000 patients for 6 months. Primary efficacy endpoint are specific joint functions and patient well-being, primary safety endpoints are all-cause mortality plus VTE (PE and DVT). The study is estimated to be completed by February 2023.

Randomized trials. A first randomized, controlled trial on aspirin versus warfarin in primary prevention of VTE in patients undergoing hip surgery after hip fractures was published in 1989. The study reported a significant reduction of the combined endpoint DVT/PE for the aspirin group (10.6 %) and the warfarin group (9.2 %) com-

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pared with the placebo group (30.2 %) ($P < 0.001$) at a comparable risk of bleeding [45].

The by far largest prospective, randomized, placebo-controlled trial on primary prevention of VTE in surgical patients was the multicenter "Pulmonary Embolism Prevention" (PEP) study [46].

Aim of the study was the prevention of postsurgical PE and DVT with low-dose aspirin in patients with elective hip or knee arthroplasty or proximal femur or femoral neck fracture.

A total of 13,356 patients with hip fractures and 4,088 patients with elective joint arthroplasty were included, randomized and treated with aspirin (160 mg/day) or placebo. Further thromboprophylactic measures such as LMWH were allowed. In addition, physical treatment (stockings/early mobilization) was also possible, according to the standard treatment protocols of the participating clinics. Already existing treatment with aspirin or anticoagulants (fractionated or unfractionated heparin) was no exclusion criterion. Patients on chronic aspirin because of arterial thrombotic risk were excluded. Efficacy endpoint was symptomatic VTE, PE or death within an observation period of 35 days after surgery.

In the patients with hip fractures, aspirin significantly reduced the proportion of symptomatic VTE from 1.5 % to 1.0 % ($P = 0.03$) and the proportion of PE from 1.2 % to 0.7 % ($P = 0.002$). This corresponded to a relative RR by 29 % and 43 %, respectively. The proportion of fatal PE was reduced from 0.6 to 0.3 %. In heparin-treated patients (44 %), aspirin reduced the proportion of VTE from 2.3 % to 1.4 %, as opposed to 1.7 % and 2.6 % in the aspirin and placebo groups without heparins. There was a small but significant increase in periprocedural bleeding events requiring transfusion in the aspirin group (2.9 % vs. 2.4 %; $P = 0.04$) but no change in total mortality.

The risk of VTE in the subgroup with elective joint surgery was small, amounting to 1.4 % with placebo and 1.1 % with aspirin (HR: 0.81; 95 % CI: 0.47–1.42). In both groups together there was a reduction in the risk of symptomatic VTE (VTE plus PE) by 34 %.

By consideration of earlier metaanalyses, the conclusion was that aspirin treatment reduced the risk of VTE and PE by at least one third. Aspirin might be considered as an option for routine use as a preventive of VTE in patients at elevated thrombotic risk (Fig. 4.1.4-2) [46].

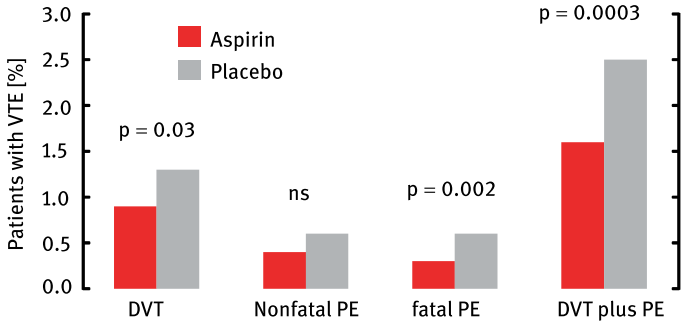

Figure 4.1.4-2: Incidence of pulmonary embolism (PE) and deep vein thrombosis (DVT) in 13,356 patients subsequent to surgical interventions (hip surgery, elective hip and knee arthroplasty). Patients were treated with aspirin (160 mg/day) plus heparin or placebo plus heparin [46].

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This study caused a number of controversial discussions [31] and was not considered in the guidelines for prevention of VTE by the "American College of Chest Physicians" (ACCP) 2008 and the American College of Physicians (ACP) of 2011 [47–50]. Important points of concern among others were changes in and unclear definitions of primary endpoints for VTE and bleeding events during the study. In addition, over 40 % of patients received concurrent LMWH or unfractionated heparin and the proportion of nonfatal PE under aspirin was unchanged. The combination of data from hip fracture patients with those of patients with elective joint surgery was also questioned – the last without significant antithrombotic actions of aspirin [31, 51].

Slightly different, but essentially comparable results were obtained in a pooled analysis of 14 prospective randomized trials with more than 33,000 patients with hip and knee surgery, including those from the PEP trial. The hypothesis was that aspirin will cause fewer operative site bleeding events without increasing the incidence of thromboembolic events. The frequency of clinically relevant, symptomatic VTE and PE in aspirin-treated patients was not different from that of patients treated with warfarin or heparins (LMWH, fondaparinux). However, the RR of periprocedural bleeding events at the operation site was 4.9-fold (warfarin), 6.4-fold (LMWH) and 4.2-fold (fondaparinux) higher than that after aspirin treatment. The author concluded that these data support the use of aspirin for prophylaxis of VTE after major orthopedic surgery [48].

A metaanalysis including 13 randomized clinical trials has compared the clinical outcome of more than 20,000 patients subjected to hip or knee surgery after treatment with aspirin, anticoagulants or placebo. Aspirin was associated with a nonsignificantly reduced VTE risk as compared with other strategies (RR: 0.87; $P = 0.43$) but was significantly superior to placebo (RR: 0.65; 95 % CI: 0.47–0.89; $P = 0.008$). There were no significant differences between the groups in mortality or any bleeding (Table 4.1.4-1) [52].

The first available prospective randomized study of aspirin vs. LMWH in patients after hip arthroplasty was the Canadian "Extended Prophylaxis Comparing Low Molecular Weight Heparin to Aspirin in Total Hip Arthroplasty" (EPCAT) study. The study showed that extended prophylaxis for 28 days with aspirin to patients subjected to unilateral total hip arthroplasty was noninferior to and as safe as dalteparin for the prevention of VTE, suggesting that aspirin may be considered a reasonable alternative to LMWH for extended thromboprophylaxis after hip replacement [53]. This study was also the first to document a noninferiority of aspirin vs. LMWH (Dalteparin) in a combined therapeutic approach.

Another prospective and randomized although small trial on aspirin (100 mg/day), a NOAC (rivaroxaban) and LMWH in prevention of postsurgical VTE in 324 patients with osteoarthritis and knee replacement showed the lowest incidence of DVT (2.9 %) for rivaroxaban compared to heparin (12.4 %) and aspirin (16.4 %). There were no differences in overall outcome between aspirin and LMWH. In contrast, there was increased postoperative blood loss and more wound complications with rivaroxa-

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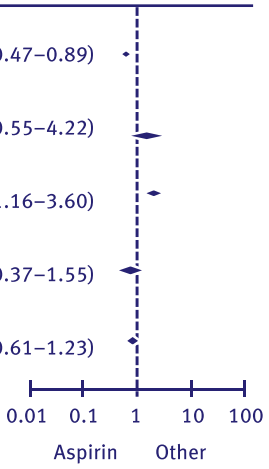
Table 4.1.4-1: Effects of aspirin vs. other treatment or placebo on prevention of VTE in 13 randomized controlled trials on hip or knee arthroplasty (numbers indicate the number of patients, numbers in brackets indicate the number of events) [52].

Study or subgroup	Aspirin total	Other total	Weight	HR mean (95 % CI)	Risk ratio
Aspirin vs placebo					
Number (events)	6,869 (150)	6,871 (239)	36.9 %	0.65 (0.47–0.89)	
Aspirin vs rivaroxaban					
Number (events)	1,877 (39)	1,879 (26)	18.7 %	1.52 (0.55–4.22)	
Aspirin vs warfarin					
Number (events)	66 (27)	65 (13)	8.5 %	2.05 (1.16–3.60)	
Aspirin vs heparin					
Number (events)	1,013 (72)	1,532 (86)	35.9 %	0.76 (0.37–1.55)	
Total					
Number (events)	9,825 (280)	10,347 (364)	100.0 %	0.87 (0.61–1.23)	



ban. The conclusion was that clinicians using rivaroxaban for anticoagulant therapy should closely monitor the hemoglobin level and wound healing [54].

All drugs that inhibit thrombin formation (FXa inhibitors) or action (dabigatran) bear not only a mechanism-based risk of bleeding but also a risk for disturbed wound healing because of disturbed coagulation. Thrombin, the major procoagulatory factor, is not only important for coagulation, i. e., thrombotic vessel occlusion, but also for thrombus-associated tissue growth and wound healing processes. Experimental thrombi in vitro generate and release thrombin and factor Xa over many hours. This process is markedly reduced by inhibitors of thrombin and factor Xa [14]. It is currently under discussion whether NOACs (rivaroxaban) bear a clinically relevant enhanced risk of wound healing complications [55–57].

The noninferiority of aspirin versus the factor Xa inhibitor rivaroxaban in preventing VTE after total hip or knee arthroplasty was confirmed in the "Extended venous thromboembolism prophylaxis comparing rivaroxaban with aspirin following total hip and knee arthroplasty" II (EPCAT-II) trial [58].

EPCAT-II was the first large-sized double-blind, randomized, prospective trial to compare aspirin with direct oral anticoagulants (NOACs) as preventives of VTE in patients after total hip or knee surgery beyond hospital discharge.

Eligible patients were undergoing total hip or knee arthroplasty. All patients received oral rivaroxaban (10 mg) once daily, starting at the day of surgery until postoperative day 5 (included). Patients were then randomly assigned to continue rivaroxaban at the same dose or to switch to

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aspirin (81 mg daily) for an additional 9 days after total knee arthroplasty or for 30 days after total hip arthroplasty. Patients were followed for 90 days for symptomatic VTE (the primary effectiveness outcome) and bleeding complications, including major or clinically relevant nonmajor bleeding (the primary safety outcome).

A total of 3,424 patients (1,804 undergoing total hip arthroplasty and 1,620 undergoing total knee arthroplasty) were enrolled in the trial. VTE occurred in 11 of 1,707 patients (0.64 %) in the aspirin group and in 12 of 1,717 patients (0.70 %) in the rivaroxaban group (difference, 0.06 percentage points; 95 % CI: 0.55–0.66; $P < 0.001$ for noninferiority and $P = 0.84$ for superiority). Major bleeding complications occurred in eight patients (0.47 %) in the aspirin group and in five patients (0.29 %) in the rivaroxaban group (difference, 0.18 percentage points; 95 % CI: −0.65 to 0.29; $P = 0.42$). Clinically important bleeding occurred in 22 patients (1.29 %) in the aspirin group and in 17 (0.99 %) in the rivaroxaban group ($P = 0.43$).

The conclusion was that among patients who received 5 days of rivaroxaban prophylaxis after total hip or total knee arthroplasty, extended prophylaxis with aspirin was not significantly different from that with rivaroxaban in the prevention of symptomatic VTE (Table 4.1.4-2).

Table 4.1.4-2: Primary safety outcomes in the EPCAT-II trial in patients undergoing hip or knee arthroplasty. Note the very low numbers of symptomatic VTE after surgery with no difference between the aspirin and rivaroxaban groups: 0.64 % vs. 0.70 % [58].

Outcome	Total hip arthroplasty		Total knee arthroplasty	
	Rivaroxaban (*N* = 902)	Aspirin (*N* = 902)	Rivaroxaban (*N* = 815)	Aspirin (*N* = 805)
	n (%)		*n* (%)	
Venous thromboembolism	5 (0.55)	4 (0.44)	7(0.86)	7 (0.87)
Pulmonary embolism	2 (0.22)	2 (0.22)	4(0.49)	3 (0.37)
Proximal deep-vein thrombosis	1 (0.11)	1 (0.11)	3 (0.37)	3 (0.37)
Pulmonary embolism and proximal deep-vein thrombosis	2 (0.22)	1(0.11)	0	1 (0.12)
Major bleeding	3 (0.33)	3 (0.33)	2 (0.25)	5 (0.62)
All bleeding	7 (0.78)	11 (1.22)	10 (1.23)	11 (1.37)

This trial is of considerable importance for primary prevention in patients who undergo surgeries that expose them to a substantial risk of VTE. The hybrid strategy of combining an initial short-term treatment with an inhibition of thrombin formation appears to be logic, also from the point of view that the surgery-induced prothrombotic alterations in the clotting cascade are more likely to be dominant in the first days after injury than thereafter. It should be considered that relatively few patients with previous VTE or cancer or other very high-risk conditions were included. Also, only about 15 % of patients had an additional mechanical thrombosis prophylaxis. Finally, the overall bleeding rates were very low, probably due to the fact that more than 50 % of patients received perioperative tranexamic acid, an antifibrinolytic agent (Section 3.1.2) [59].

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A randomized but open clinical trial studied VTE prevention in adult orthopedic trauma patients admitted to a trauma center (operative extremity fractures or a pelvis or acetabular fracture). Patients were randomized to receive LMWH (30 mg enoxaparin) twice daily (n = 164) or 81 mg aspirin twice daily (n = 165). The primary outcome was a composite endpoint of bleeding complications, deep surgical site infection, DVT, PE and death within 90 days of injury. There was no evidence of superiority between LMWH and aspirin for VTE prevention in these patients. Of the patients of either group, 59–60 % were event-free in the weighted time. There was no statistically significant difference between the two treatment arms with a patient preference for aspirin [60].

DVT and long-distance flights. The LONFLIT-1 and -2 studies have shown that long-distance flights (>10 hours) might cause asymptomatic DVT in 4–6 % of individuals at elevated thrombotic risk. The usefulness of aspirin prevention vs. LMWH was studied in the LONFLIT-3 study [61]:

A total of 300 individuals at elevated thrombotic risk were randomized to aspirin (400 mg/day for 3 days, starting 3 days before the flight), enoxaparin (weight-adapted injection 2–4 hours before flight) or placebo.

A DVT occurred in 4.8 % of individuals in the placebo group, in 3.6 % of individuals in the aspirin group and in no one of the heparin group. DVT was asymptomatic in 60 % of subjects; 85 % of DVTs were observed in passengers in nonaisle seats. Mild gastrointestinal symptoms were reported in 13 % of patients taking aspirin.

The conclusion was that one dose of LMWH is an important option to consider in high-risk subjects during long-haul flights [61].

Thus, aspirin is no replacement for LMWH prophylaxis in this indication.

4.1.4.4 Clinical trials – secondary prevention

Long-term prevention of recurrent VTE or unprovoked VTE because of genetic or environmental predispositions used to be the domain of oral anticoagulants, now with particular focus on the new direct acting NOACs, such as the rivaroxaban-type antithrombins (dabigatran). Two prospective randomized, placebo-controlled trials have studied whether extended treatment with low-dose aspirin after the end of the guideline-directed anticoagulation period (usually 6 months) will have a beneficial effect on prevention of VTE: the "Warfarin and Acetylsalicylic Acid" (WARFASA) study [2] and the "Aspirin to Prevent Recurrent Venous Thromboembolism" (ASPIRE) study [62].

The WARFASA trial included 402 patients with a previous unprovoked VTE. All patients received a guideline-consistent treatment with oral anticoagulants for 6–18 months and were then randomly assigned to receive aspirin (100 mg/day) or placebo for another 2 years in a double-blind manner. The primary efficacy outcome was recurrent VTE, the primary safety outcome major bleeding.

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In comparison with placebo, aspirin reduced the incidence of new VTE from 11.2 % to 6.6 % per year (HR: 0.58; 95 % CI: 0.36–0.93; $P = 0.02$). This was equivalent to a reduction of recurrent VTE after withdrawal of oral anticoagulants by almost 41 %. There was no difference in major bleeding events – one patient per group. The total number of venous and arterial thromboses was not significantly reduced by aspirin (HR: 0.67; 95 % CI: 0.43–1.03; $P = 0.06$).

The conclusion was that aspirin reduced the risk of recurrent VTE in patients with unprovoked VTE who had discontinued anticoagulant treatment with no apparent increase in the risk of major bleeding events [2].

The ASPIRE trial included 822 patients with a previous unprovoked thromboembolism. The patients received anticoagulants (heparin/warfarin) for at least 6 weeks, but mostly over 3 months, and were afterwards assigned to receive either aspirin (100 mg/day) or placebo for another 37 months. Primary endpoint was the occurrence of VTE.

In comparison with placebo, aspirin tended to reduce the incidence of recurrent VTE from 6.5 % to 4.8 % (HR: 0.74; 95 % CI: 0.52–1.05), which was not significant ($P = 0.09$). However, aspirin reduced the rate of a prespecified secondary composite endpoint (VTE, myocardial infarction, stroke, cardiovascular death) from 8.0 % per year to 5.2 % per year (HR: 0.66; 95 % CI: 0.48–0.92), i. e., by 34 %. This was mainly driven by an about 50 % reduction by aspirin of arterial thrombotic events: from 10 to 19 ($P = 0.01$). There were no significant differences in major or clinically relevant bleeding events (0.6 % per year with placebo vs. 1.1 % per year with aspirin; $P = 0.22$) or any other serious adverse event.

The conclusion was that aspirin did not significantly reduce the rate of recurrent VTE but did significantly reduce the rate of major vascular events at no increase in bleeding, suggesting an improved net clinical benefit [62].

Taken together, there was a reduction in venous and arterial thromboembolism by one third ($P = 0.002$). The major effect was seen in the first year (Fig. 4.1.4-3).

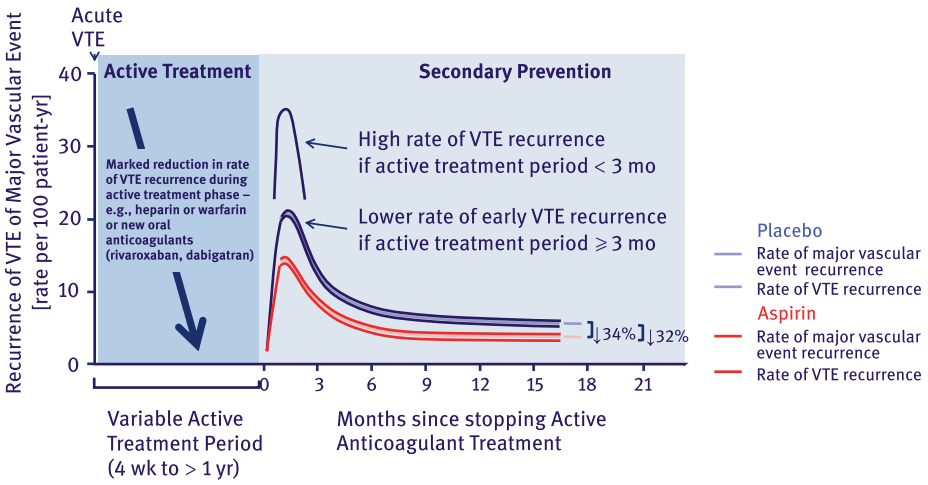

Figure 4.1.4-3: Effect of aspirin on the risk of recurrence of venous thromboembolism (VTE) and major vascular events [33].

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The INSPIRE trial, another evaluation of both studies together, using standardized evaluation criteria, has confirmed these findings [3]. Aspirin treatment after the end of anticoagulant use reduced the risk of recurrent VTE at 30 months in comparison to placebo by 42 % (HR: 0.58; 95 % CI: 0.40–0.85; $P = 0.005$). The number of severe bleeding events was not different between the two groups: 0.4 % per year for aspirin and 0.5 % per year for placebo.

These interesting findings have reanimated the discussion about the long-term medical prevention of recurrent VTE after completion of an initial treatment period with anticoagulants. This also under consideration of the finding that patients with VTE are also at an elevated risk for cardiovascular arterial events, i. e., myocardial infarctions [17]. In addition, aspirin is inexpensive, does not require monitoring (in contrast to warfarin) and does not accumulate in patients with renal insufficiency (in contrast to dabigatran and rivaroxaban) [33].

4.1.4.5 Aspirin and other drugs – NOACs

After publication of the WARFASA and ASPIRE trials, NOACs became also of interest for secondary prevention of recurrent or unprovoked DVT after the end of guideline-recommended oral anticoagulation. In placebo-controlled trials, the factor Xa inhibitors rivaroxaban (EINSTEIN-EXT) [63], apixaban (AMPLIFY-EXT) [64], edoxaban [65] and dabigatran (RE-SONATE) [66] were more potent than aspirin and equipotent to warfarin (Fig. 4.1.4-4) [31].

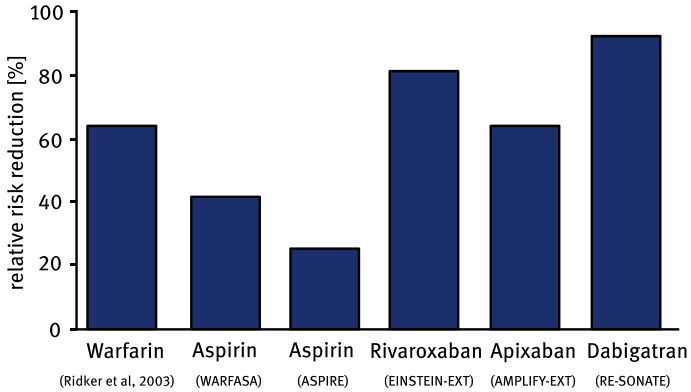

Figure 4.1.4-4: Relative risk reduction of VTE by antithrombotic drugs and aspirin vs. placebo for extended treatment in placebo-controlled secondary prevention trials after the end of guideline-directed anticoagulant treatment. The risk is calculated referring to the placebo effect of the respective trial. For more details see text (after [31]).

The pioneering study that compared directly two doses of rivaroxaban with aspirin was the EINSTEIN CHOICE trial [67].

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A total of 3,396 patients were included in this multicenter, randomized, double-blind trial after the end of a 6–12-month standard anticoagulation treatment (vitamin K antagonist or NOAC) because of symptomatic DVT or PE. This treatment had not to be interrupted for more than 7 days after randomization. Exclusion criteria were (among others) clinically necessary continuation of standard anticoagulants or antiplatelet agents.

The patients received rivaroxaban (10 or 20 mg/day) or enteric-coated aspirin (100 mg/day) for one year. Primary efficacy endpoint was a combination of symptomatic fatal or nonfatal VTE and unexplained death for which PE could not be ruled out. Safety endpoints were major and fatal bleeding events.

The primary efficacy endpoint was reached in 1.5 % of patients on 20 mg rivaroxaban and in 1.2 % of patients on 10 mg rivaroxaban, as opposed to 4.4 % of patients on aspirin (HR for 10 mg rivaroxaban vs. aspirin: 0.26; 95 % CI: 0.14–0.47; $P < 0.001$). The primary safety endpoint was reached in 0.5 % and 0.4 % of patients on 20 and 10 mg/day rivaroxaban, respectively, as opposed to 0.3 % in the aspirin group. The incidence of other clinically relevant bleeding events was 2.7 % and 2.0 % for the two rivaroxaban groups and 1.8 % for aspirin. None of these differences between the treatment groups were significant.

The conclusion was that in patients with VTE in equipoise for continued anticoagulation, rivaroxaban at both doses was more effective and did not result in any significantly higher bleeding risk than aspirin [67].

This study was the first to demonstrate clinical superiority of a NOAC, here rivaroxaban, over aspirin in a head-to-head comparison in long-term prevention of secondary VTE. These results can, however, not be transferred to all patients with an indication of long-term anticoagulant treatment. According to the inclusion criteria of this study, no patients were considered who had stopped anticoagulants more than one week prior to randomization and also no patients with need for antiplatelet therapy or continuation of anticoagulation. In addition, there was a large number of provoked VTEs and the patients of the (later) aspirin group had a much higher rate of anamnestic VTEs *prior* to randomization than the rivaroxaban groups: 8.8 % vs. 1.5 % and 1.1 %. More studies are definitely needed.

These studies should also address the safety issue, after the 10 mg/day dose in the COMPASS trial was found to cause significant more bleeding events than aspirin [68]. In particular gastrointestinal bleeding events appear to be a problem for NOACs [69]. A dose reduction is under discussion. In any case, more data are needed, also on alternative NOACs such as edoxaban or apixaban.

4.1.4.6 Actual situation

In case of primary VTE prevention, the actual guidelines of the ACCP from 2012 contained for the first time a special chapter regarding prevention of VTE in orthopedic surgery. This included aspirin together with anticoagulants as another therapeutic option. The American Academy of Orthopaedic Surgeons (AAOS) had already in 2007 recommended aspirin for the same indication as an alternative to anticoagulants. The guidelines from 2011 now recommend “pharmacological agents” without more detailed classification, which probably include aspirin. Thus, both societies did

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accept aspirin as a pharmacological option for prevention of VTE despite their different definitions of efficacy: only symptomatic or fatal PE (AAOS) but no DVTs vs. all DVTs and PE (ACCP) [51]. European societies, such as the British National Institute of Health and Care Excellence (NICE), have changed their VTE recommendations in 2018 with the major consequence of increased use of aspirin for VTE chemical prophylaxis [70]. The guidelines of the European Society of Anaesthesiology from 2017 do not recommend aspirin as thromboprophylaxis in general surgery. However, they do recommend aspirin for major orthopedic surgery considering that it may be less effective than LMWHs (level 1C) [71]. Thus, the situation is complex and a final answer definitely requires more prospective randomized trials, specifically in primary prevention.

The current discussion about aspirin for secondary unprovoked VTE prevention is mainly focused on the question whether aspirin treatment may be sufficiently effective for long-term protection of VTE or whether this will be the future area of NOACs. Although NOACs – and warfarin – appear to be more potent than aspirin, they also cause more bleeding events and the balance between the two depends on the individual risk of the patient. In any case, it may be a wrong strategy to assume that "one size (of all antithrombotics) fits all (clinical needs)."

Summary

Venous thrombi primarily result from increased thrombin generation and its local accumulation during venous stasis. Platelets not only are the most sensitive targets for thrombin but also markedly enhance thrombin formation as well as growth and stability of the developing thrombus. For these reasons, platelet inhibition is a therapeutically relevant goal also for prevention of venous thrombosis. Excellent new experimental data on the key role of platelets in NET formation and generation of inflammatory mediators (HMGB-1) and their sensitivity against aspirin (salicylates) became recently available and confirm the hypothesis of the fundamental role of platelets also in venous thrombus formation.

Vascular complications of venous thrombosis subsequent to provocation by surgery or other prothrombotic conditions are symptomatic VTE and PE. Prevention of VTE in patients at risk is primarily focused on thrombin inhibitors, mainly LMWHs, coumarins (warfarin) and NOACs. There may also be a role for aspirin, especially in acute, injury-associated thrombosis prevention, as well as after hospital discharge or long-term prophylaxis of recurrent, unprovoked VTE. Several studies have suggested that aspirin in a multimodal approach together with stockings and early mobilization is not inferior to anticoagulants in primary prevention and has the advantage of a low bleeding risk. Similar considerations apply for secondary long-term prevention after the initial anticoagulant treatment is finished.

With an individualized risk assessment and as part of a multimodal approach, possibly as a follow-up after initial antithrombins ("hybrid" strategy), aspirin is safe as thromboprophylactic agent in primary arthroplasty. It is not associated with an increased incidence of symptomatic VTE, PE or death and also not with problems in wound healing. A final assessment of the role of aspirin as compared with NOACs is actually not possible. In secondary prevention, the EINSTEIN-CHOICE study on rivaroxaban versus aspirin has shown for the first time superiority of rivaroxaban over aspirin at comparable bleeding rates.

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Open issues in primary prevention are a more precise determination of the individual risk profile, including bleeding and other peri- and postoperative complications (wound healing, perioperative joint infections). In secondary prevention, open issues include the role of (cardiovascular) comorbidities, elucidation of possible differences between different NOACs and the safety issue of bleeding.

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4.1.5 Pregnancy-induced hypertension (preeclampsia) and preterm delivery

4.1.5.1 General aspects

Etiology. Pregnancy-induced hypertension (PIH) as isolated hypertension (blood pressure ≥ 140/90 mmHg) after the 20th week of gestation or as hypertension with albuminuria (≥0.3 g/24 h) (preeclampsia) is a multisystem disorder of pregnancy, characterized by variable degrees of placental malperfusion. Release of soluble factors from the placenta into the circulation causes maternal vascular endothelial injury, which leads to hypertension and multiorgan injury [1]. PIH is the leading cause of maternal, fetal and perinatal morbidity and mortality. The maternal clinical symptoms of PIH include functional disturbances of vital organs such as the kidney, liver and CNS. They become manifest in the second third of pregnancy although the initiating pathophysiological events occur already shortly after implantation of the cytotrophoblast in the uterus [2, 3]. The reasons for these dysfunctions are different but finally result in disturbed trophoblast growth and differentiation. Clinical symptoms are pathological immune reactions with secondary systemic inflammatory and stress responses in the maternal circulation as well as disturbed blood supply to the fetus. These finally result

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from poor placentation in early pregnancy (weeks 8–18) [4]. Removal of the placenta by delivery or abortion terminates the clinical symptoms.

Pathophysiology. The uterine life of the fetus starts with the invasion of the embryonic cytotrophoblasts into the endometrium. In physiological conditions, these cells migrate into the maternal spiral arteries of the uterus and transform them from small-caliber resistance vessels into large-caliber capacity vessels. This vascular remodeling is the prerequisite for later adequate placental blood perfusion and oxygen supply to the growing fetus. These processes are disturbed in preeclampsia as there is apparently no differentiation of the cytotrophoblast from the epithelial into the endothelial, invasive phenotype [5]. Instead, the small-caliber muscular spiral arteries persist and maintain a pulsatile flow within the intervillous compartment. This causes pulsatile changes of perfusion pressure and oxygen saturation. Results are oxidative stress and disturbed perfusion, followed by systemic endothelial dysfunction including multiple signs of maternal systemic vascular inflammation [4].

Pathophysiological determinants of impaired angiogenesis in preeclampsia are too low activities of angiogenic growth factors, such as "vascular endothelial growth factor" (VEGF) and "placenta-induced growth factor" (PIGF). The reason for that is most likely an enhanced formation and expression of antiangiogenic factors, such as the "soluble fms-like tyrosine kinase-1" (sFlt-1 = soluble fragment of the VEGF receptor), dissolved endoglin (sENG) and others. Antiangiogenic proteins such as sFlt-1 bind to and inactivate growth factors (VEGF, PIGF) and subsequently block their natural function as inductors of angiogenesis in the placenta [3, 6–10]. The result is insufficient placental vascularization with the consequence of increased vascular resistance in the uteroplacental circulation, pulsatile blood flow and oxidative stress. Antiangiogenic and proinflammatory mediators also enter the maternal circulation and cause there a syndrome of diffuse endothelial dysfunction with signs of systemic inflammation (activation of neutrophils, generation and release of reactive oxygen species and inflammatory cytokines) [1]. There is activation of NF-κB signaling pathways and expression of COX-2 in the systemic vasculature of women with preeclampsia [11]. The vasculature becomes more sensitive against vasoconstrictors, such as angiotensin II and, perhaps, thromboxane A_2 (TXA_2). This causes vasoconstriction with subsequent hypertension and formation of microthrombi [2]. Another maternal consequence is a disturbed barrier function of the glomeruli with subsequent albuminuria [3, 4, 12–15]. These undesirable consequences for mother and child are determined by the severity of these alterations and may result in (preterm) eclampsia with intrauterine growth retardation (IUGR), abortion or death [5].

Epidemiology. The overall incidence of PIH amounts to 3–8 % of pregnancies worldwide [1, 5]. Every third case is associated with severe perinatal morbidity and there is a significant (20–25 %) perinatal mortality. The incidence is higher in nulliparae

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or women above the age of 35 years and also increased by genetic predisposition as well as preexisting morbidities, such as hypertension, diabetes, kidney diseases, overweight and coagulopathies [3, 16]. No curative treatment is known. For these reasons, early diagnostics, for example by clinical measures (blood pressure, albuminuria) or, better, by appropriate laboratory biomarkers, is of utmost importance.

4.1.5.2 Vascular risk and modes of aspirin action

Angiogenesis and antiangiogenic factors. A most attractive approach to modify basic pathophysiological processes in preeclampsia would be an interference with the formation and/or action of antiangiogenic mediators, such as Flt-1 [3]. In vitro studies suggest inhibition of hypoxia-induced sFlt-1 production by aspirin in cell cultures of human cytotrophoblasts [17] in a concentration-dependent manner, starting at concentrations of 0.1 mM. This action of aspirin was accompanied by inhibition of sFlt-1-induced trophoblast invasion, leading to the hypothesis that aspirin could prevent preeclampsia by (these) trophoblast-associated actions [18]. Interestingly, aspirin at about the same concentrations has also been shown in vitro to improve trophoblast integration into myometrial microvascular endothelial cells [19]. These concentrations of aspirin are high and not likely to be expected in vivo after administration of antiplatelet doses. However, a recent study has provided evidence for inhibition of soluble antiangiogenic factors (Flt-1, endoglin) and for improved oxygen defense due to elevated superoxide dismutase (SOD) and reduced MDA levels by low-dose oral aspirin (100 mg/day) in preeclamptic women (Fig. 4.1.5-1) [20]. Improved placentation by aspirin in case of trophoblast abnormalities is likely to be involved in the protective action of aspirin [21]. It is currently unknown whether higher doses of aspirin that are recommended for prevention of preeclampsia (≥100 mg) [22] as opposed to the lower dose of 81 mg for cardiocoronary prevention [23] are possibly explained by these additional actions of aspirin on placental/trophoblast tissues.

Alterations in the metabolism of arachidonic acid – reduced vascular prostacyclin (PGI_2) generation and action. Changes in arachidonic acid metabolism associated with platelet hyperreactivity and inflammatory reactions are among the earliest findings regarding the pathology of PIH. An increased generation of arachidonic acid-derived metabolites as well as an altered spectrum of placenta-derived products is typical for pregnancy [24] and has important functional consequences for thrombosis and fetal perfusion. In normal pregnancies, PGI_2 production is upregulated 2–3-fold. This occurs early, during the first weeks of normal gestation, and remains at an elevated level throughout pregnancy until delivery [25]. A largely missing upregulation of this pregnancy-induced PGI_2 formation is one of the first abnormalities of arachidonic acid metabolism in women at risk of developing PIH. It becomes clinically detectable prior to the 20th week of gestation, that is, a long time before the onset of clinical symptoms of the disease (Fig. 4.1.5-2) [26–28], and is maintained at the low level until

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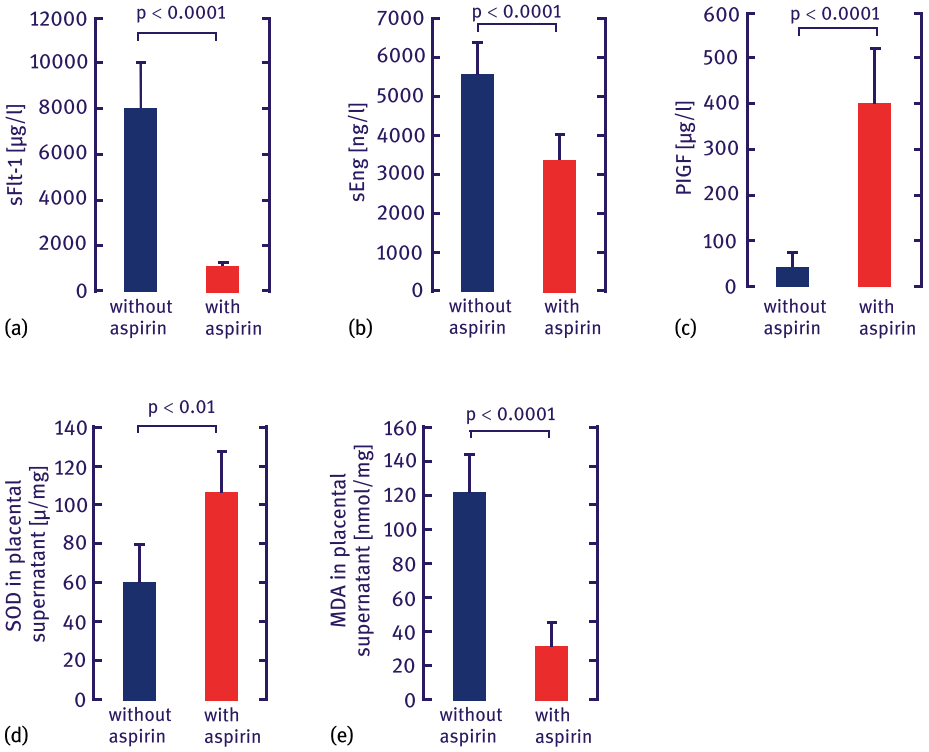

Figure 4.1.5-1: Aspirin treatment (100 mg/day, given from week 17 until delivery) improves oxygen defense and the level of angiogenic activities in women with preeclampsia compared to untreated women. The elevated serum levels of the antiangiogenic factors soluble fms-like tyrosine kinase-1 (sFlt-1) (a) and soluble endoglin (sENG) (b) are reduced by aspirin, while the level of the proangiogenic placental growth factor PlGF (c) is increased. The oxygen defense of placental tissue is improved (d) (elevated superoxide dismutase [SOD] levels) while the enhanced peroxidation status (e) (malondialdehyde [MDA]) is reduced (modified after [20]).

delivery [25, 26, 29–32]. PGI_2 levels return to normal values with the first weeks after delivery [33, 34].

Any insufficient PGI_2 production probably not only promotes thrombotic events within the placental circulation but may also reduce fetoplacental blood flow [35]. Lack of vasodilatory prostaglandins reduces the refractory state of vascular smooth muscle cells against vasoconstrictors, such as angiotensin II and, perhaps, TXA_2, with the clinical consequence of hypertension. Interestingly, endogenous, endothelium-derived PGI_2 appears to be a much more potent "endothelium-dependent relaxant factor" for human umbilical arteries than endogenous nitric oxide [35–37].

Reduced upregulation of PGI_2 biosynthesis is also accompanied by enhanced platelet reactivity and enhanced (largely) platelet-dependent thromboxane production [25, 27, 33]. In addition, the antiplatelet effects of PGI_2 are markedly reduced in

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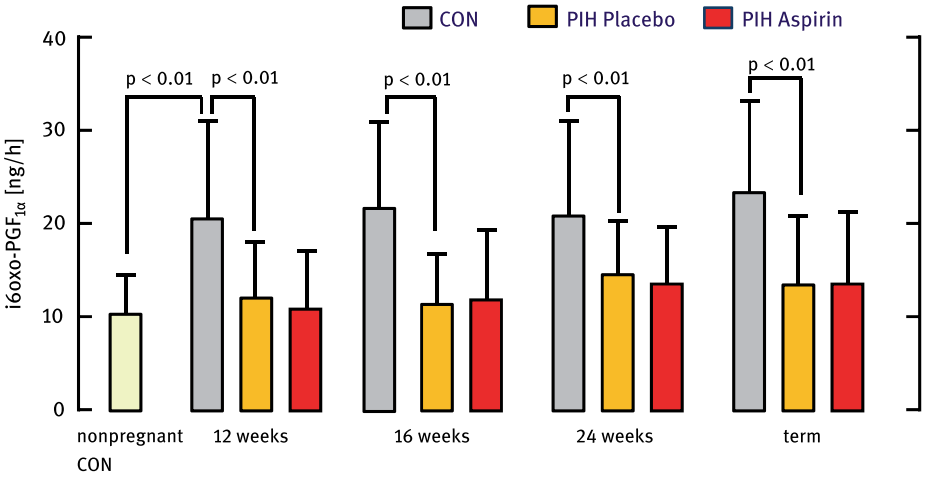

Figure 4.1.5-2: Renal excretion of the immunoreactive PGI_2 metabolite i6-oxo-PGF1α before, during and after gestation in normotensive gravidae (CON) and gravidae at high risk of PIH treated with aspirin (60 mg/day) or placebo. Treatment was started at the 12th week of gestation. Note the significant increase in PGI_2 metabolite excretion at 12 weeks and the significant suppression of PGI_2 metabolite excretion in women at risk of PIH which is unchanged by low-dose aspirin treatment (after data in [25]).

women with PIH as opposed to normal pregnancies [38], possibly because of reduced affinity of the platelet PGI_2 (IP) receptors against this agonist [39]. Unfortunately, there are only very few research data on the regulation and function of COX-1 and COX-2 in PIH [11]. The therapeutic administration of PGI_2 in PIH was not successful, possibly because of its potent blood pressure-lowering activity [40]; in two reported cases it was even fatal [41].

What are the reasons for the missing increase of PGI_2 biosynthesis despite the enhanced needs in pregnancy? The disturbed morphological transformation of the trophoblast into the endothelial phenotype as described above might be one explanation. Alternatively or additionally, endothelial injury by oxidative stress due to pathological persistent pulsatile flow conditions might suppress PGI_2 biosynthesis. Higher oxygen pressure of pulsatile flow allows for increased lipid peroxidation as seen from the enhanced formation of isoprostanes and malondialdehyde (MDA) in the preeclamptic placenta [42] and circulating blood (Fig. 4.1.5-3) [42]. Fatty acid peroxides are specific inhibitors of prostacyclin synthase [43, 44]. They additionally act as potent vasoconstrictors by themselves. Thus, the reduced PGI_2 generation due to inhibition of PGI_2 biosynthesis is possibly caused by enhanced levels of lipid peroxidation products. Lipid peroxide (MDA) and isoprostane formation is principally nonenzymatic in nature and, therefore, not sensitive to inhibition by aspirin (Fig. 4.1.5-3).

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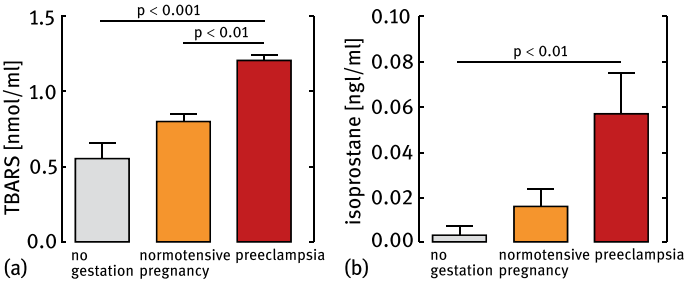

Figure 4.1.5-3: Lipid peroxidation levels as measured by thiobarbituric acid-reactive substances (TBARS) (a) and isoprostanes (b) in plasma of women with preeclampsia as opposed to normotensive pregnant women and nonpregnant women. In preeclamptic women of this study, elevated lipid peroxidation was also associated with elevated levels of antiangiogenic protein (sFtl-1) and the inflammation marker TNFα (not shown) [42].

Alterations in the metabolism of arachidonic acid – generation of thromboxane A_2 (TXA_2). Associated with reduced PGI_2 formation in preeclampsia are platelet hyperreactivity and slightly enhanced thromboxane formation. Platelets [25, 33] and the decidual cells and trophoblasts of the placenta [45] are major sites of thromboxane biosynthesis. Genetic studies additionally suggest increased vascular expression of the thromboxane synthase gene in omental arteries of preeclamptic women, possibly due to reduced DNA methylation [46]. It has been hypothesized that these processes may trigger inflammation in vascular cells, culminating in endothelial dysfunction, hypertension and edema [10].

The group of *Giuseppe Remuzzi* and colleagues from Italy [25] was the first to study in more detail the clinical efficacy of aspirin in women at high risk for PIH treated with low-dose (60 mg/day) aspirin. In their study, aspirin treatment was started at the 12th week of gestation. At these doses, there was an almost complete inhibition of serum thromboxane (>90 %) and also a significant reduction of (mainly platelet-derived) thromboxane metabolite excretion at unchanged levels of PGI_2 metabolite excretion by aspirin treatment (Fig. 4.1.5-4). These data and others [47] confirm TXA_2 inhibition as a relevant pharmacological target for prevention of PIH and aspirin as a useful drug to reach this goal.

Thromboxane and tissue injury. Enhanced platelet-derived TXA_2 formation appears to be a normal event in pregnancy but appears to become additionally increased in women at risk for preeclampsia (Fig. 4.1.5-4). Enhanced thromboxane levels are probably also involved in coagulopathy (thrombocytopenia), inflammation and vasoconstriction. Aspirin reduced these elevated levels markedly at unchanged excretion of a PGI_2 metabolite [25]. The increased circulating thromboxane levels in the maternal circulation return to normal shortly after delivery [33]. Enhanced, aspirin-sensitive thromboxane (metabolite) excretion and reduced free platelet count, related to in-

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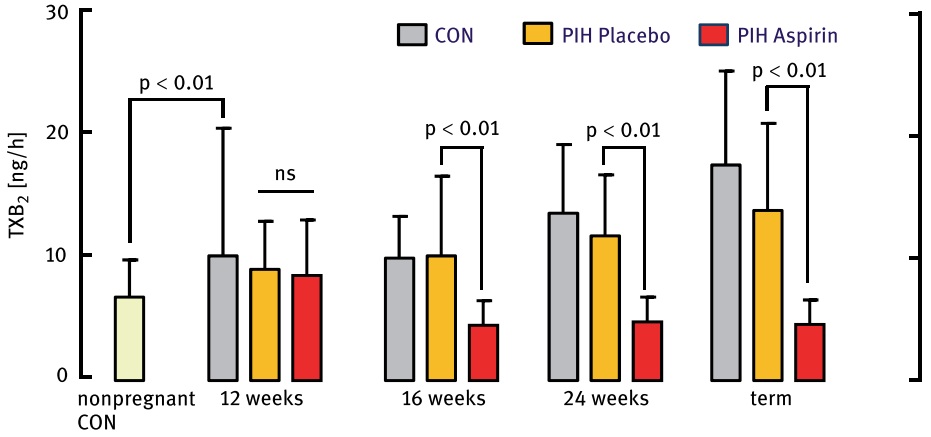

Figure 4.1.5-4: Renal excretion of immunoreactive TXB_2 ($iTXB_2$) before, during and after gestation in normotensive gravidae (CON) and gravidae at high risk of PIH treated with aspirin (60 mg/day) or placebo. Treatment was started at the 12th week of gestation. Note the significantly elevated TX excretion at 12 weeks with a further increase during pregnancy and the marked inhibition by aspirin (after data from [25]).

creased platelet consumption in PIH [48], were correlated with the increase in blood pressure and elevated plasma levels of lactate dehydrogenase (LDH), an index protein of tissue injury [47]. For these reasons, long-lasting inhibition of thromboxane formation and/or action might be a useful treatment tool for women at risk for PIH. Aspirin became the candidate of choice, based upon the assumption that the detrimental systemic alterations in preeclampsia are largely thromboxane-related. Low-dose aspirin (81 mg/day) "resistance" (HTPR) of platelets of women at high risk for preeclampsia was also observed. This "resistance" could be largely overcome by increasing the aspirin dose [49], as also seen in some platelet studies in stroke patients (Section 4.1.2) and patients undergoing cardiac bypass surgery (Section 4.1.1). Aspirin "resistance" in women at risk for PIH might be associated with the abnormal synthesis of nonaspirin-sensitive lipids, including isoprostanes and sphingolipids, by placental tissue [24]. This might also affect the numerous paracrine actions of platelets on white cells and the endothelium (Fig. 2.3.2-5) [50]. Prevention of enhanced uteroplacental/platelet thromboxane formation will be more effective to prevent later systemic abnormalities of the disease when started early, i. e., prior to the completion of placentation [51, 52].

Aspirin rapidly passes the placenta and enters the fetal circulation, approaching pharmacologically active levels in the umbilical circulation [53, 54]. There is no evidence for any clinically relevant fetal toxicity of aspirin (Section 3.1.2) and also no enhanced bleeding risk if the treatment is terminated 1–2 weeks prior to delivery. There is also no evidence for any clinically relevant constriction of the ductus arteriosus Botalli by aspirin (Section 3.1.2) [55].

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In this context, it should be noted that measurement of thromboxane (metabolite) excretion is a useful tool to assess thromboxane/lipid peroxidation-associated abnormalities in women at risk for preeclampsia. Serum thromboxane levels can be used as an index of aspirin's efficacy to block platelet COX-1 mediated thromboxane formation but provide no information about thromboxane formation in vivo.

In contrast to plasma or urinary thromboxane (metabolite) levels, serum thromboxane has no predictive value for determination of the thrombotic risk in high-risk pregnancies [25, 56]. Serum thromboxane is largely unchanged in normal pregnancies as well as in preeclampsia and reduced by aspirin in both conditions to a similar extent [33, 34]. This is not surprising, since serum thromboxane is only a marker of the (platelet) thromboxane forming *capacity* and not of the actual *levels* of biosynthesis triggered by platelet-stimulating factors or the placenta. These numbers can be determined by measuring the excretion of thromboxane (metabolites), for example in urine. However, measurement of serum thromboxane is a useful compliance control for adherence to aspirin treatment (see below).

Lipoxins. LXs are antiinflammatory arachidonic acid-derived lipid mediators, generated by intercellular interactions of acetylated COX-2 with other lipoxygenases (ATL or LXA_4) (Section 2.2.1). LXs have been reported to reduce neutrophil activation and their adhesion to endothelial cells induced by inflammatory mediators, circulating in plasma from preeclamptic women [15]. LXA_4 was also found to prevent antiphospholipid antibody toxicity for trophoblast migration and its interaction with endothelial cells [57]. Whether there is a relation of preeclampsia to a deficiency in endogenous LXA_4 is under discussion [15, 58]. Plasma levels of LXA_4 are reduced in women at high risk for preeclampsia. Treatment with aspirin resulted in an increase in ATL and IL-10 levels and reduced the IL-8 plasma concentration. These data suggest a potential antiinflammatory role of aspirin through the ATL pathway in pregnant women at risk for preeclampsia [59]. More research on this most interesting new aspect of aspirin action in preeclampsia is definitely needed.

4.1.5.3 Clinical trials

General aspects. There is no curative treatment of PIH except delivery, and no drugs have been shown to influence disease progression [1]. Effective prevention of the disease by modifying the early disturbances in trophoblast pathology and angiogenesis is difficult since the pathophysiological background of these events is complex and any intervention, if possible, may only be effective in (very) early stages of pregnancy. These are time points before clinical signs have fully developed. Several options are under discussion: oral calcium, LMWHs, metformin and others. However, aspirin is the only preventive drug for preeclampsia that is supported by strong evidence [1]. Treatment with aspirin is focused on disease-related alterations in arachidonic acid metabolism, specifically the inhibition of TXA_2 production and its mul-

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tiple consequences for thrombotic and inflammatory conditions of the mother and fetus.

A piece of history. After a brief report in 1978 [60], *Crandon* and *Isherwood* [61] were the first to show in a clinical trial that nulliparae who took aspirin (plus dipyridamole) more than once each other week during pregnancy were at a significantly lower risk of recurrent PIH than those who had not taken these medications [61]. The first randomized but open trial by *Beaufils* and colleagues studied 102 women in early pregnancy at high risk of preeclampsia. The authors confirmed that combined treatment of aspirin/dipyridamole prevented recurrent PIH in some patients [62]. In this study, women received aspirin (150 mg/day) plus dipyridamole (300 mg/day) from the 3rd month of gestation until delivery. Preeclampsia and severe IUGR occurred in a significant percentage of untreated but in none of the aspirin/dipyridamole-treated women. This suggested that regular low-dose aspirin/dipyridamole might protect from preeclampsia in women at elevated risk for the disease. This finding stimulated several larger prospective, randomized, double-blind trials using aspirin as a single medication, however with controversial results.

The first large, placebo-controlled, double-blind, prospective, randomized trial testing low-dose aspirin as a preventive measure of PIH and preeclampsia in pregnant women at elevated risk for PIH was published by *Henk C. S. Wallenburg* and colleagues from Rotterdam (the Netherlands) [63].

Pregnant nulliparous, healthy women, normotensive at the 26th week of gestation, were subjected to an angiotensin II sensitivity test in order to detect abnormal vasoconstriction as an index parameter for pathological vascular reactivity. From a total of 207 women, 46 were found to react with enhanced pressure responses. These patients at risk were enrolled into the study at the 28th week of gestation and treated with aspirin (60 mg/day) or placebo until delivery. This aspirin dose reduced thromboxane generation by platelets (as determined by measuring MDA levels) on average by 89 %.

One intrauterine death (asphyxia?) and two cases of slight hypertension occurred in the aspirin group, but 12 cases of a usually severe preeclampsia occurred in the placebo group. No adverse effects of aspirin treatment on either mother or child were observed. Specifically, there were no hemorrhages or any evidence for constriction of the ductus arteriosus Botalli.

The conclusion was that in these individuals at high risk for PIH, here identified by a pathological angiotensin II vasoconstrictor test, treatment with low-dose aspirin was a useful protective measure [63].

This trial set the stage for all further studies on low-dose aspirin as a single drug to prevent PIH/preeclampsia. Interestingly, MDA, an enzymatic byproduct of thromboxane synthase but mainly a nonenzymatic breakdown product of lipid peroxidation, was taken by Wallenburg as surrogate parameter for thromboxane formation. In fact, MDA, like isoprostanes, is a useful global marker of (enhanced) lipid peroxidation inside the placental circulation which is a well-known feature of preeclampsia (Fig. 4.1.5-3) [42].

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Early placebo-controlled trials and metaanalyses. These positive results stimulated several large, randomized, placebo-controlled, double-blind studies. These studies, done in the following 10–20 years, yielded different results and, in most cases, could not confirm the impressive beneficial effects of the early studies. Among them was the probably worldwide largest prospective randomized, placebo-controlled trial on the efficacy of aspirin in prevention of preeclampsia, the "Collaborative Low-dose Aspirin Study in Pregnancy" (CLASP) [64, 65].

A total of 9,364 women were randomly assigned to film-coated aspirin (60 mg/day) or a matching placebo until delivery. Women were eligible if they were between the 12th and 32nd (!) weeks of gestation and were at "sufficient" preexisting risk of preeclampsia or IUGR. The majority of patients (74 %) was included for prophylaxis of preeclampsia, because of an enhanced risk for the disease including history of preeclampsia or IUGR in a previous pregnancy, chronic hypertension or renal diseases. Further risk factors were maternal age, family history or multiparous pregnancies. In total, 62 % were enrolled at the 20th week of gestation or earlier. Main study endpoints were proteinuric preeclampsia, duration of pregnancy, birth weight and stillbirth and neonatal death ascribed to preeclampsia or IUGR.

Overall, 6.7 % of women allocated to the aspirin group developed proteinuric preeclampsia, compared to 7.6 % of those allocated to the placebo group. This 12 % relative RR by aspirin was not significant. There was no significant effect of aspirin on the incidence of IUGR, stillbirth or neonatal death. Aspirin significantly reduced the rate of preterm delivery in comparison with placebo (19.7 % vs. 22.2 %; $2P = 0.003$), and there was a significant trend ($P = 0.004$) towards progressively greater reductions in proteinuric preeclampsia, the more preterm the delivery occurred. Aspirin treatment was not associated with a significant increase in placental hemorrhages or bleeding events during preparation for epidural anesthesia. However, there was a slight increase in the number of blood transfusions after delivery. Aspirin was generally safe for the fetus and newborn infant with no evidence of an increased likelihood of bleeding.

The main conclusion was that these findings do not support routine prophylactic or therapeutic administration of antiplatelet drugs such as aspirin in pregnancy to all women at increased risk of preeclampsia or IUGR. However, there is also no evidence for an aspirin-related risk to mother or child when given during pregnancy, even in high-risk women [64, 65].

CLASP was one out of eight larger placebo-controlled, randomized studies published between 1993 and 1998. The daily aspirin doses in all of these studies were low (50–60 mg/day). This dosing was based on the hypothesis that low-dose aspirin is sufficient for inhibition of platelet-dependent thromboxane formation without major effects on vascular prostacyclin biosynthesis [25, 66]. The start of treatment was also highly variable – between the 12th and the 32nd week. Cochrane library data (1985–2017) now indicate that aspirin treatment should be started before the 16th week of gestation and at a dose of 100 mg/day or more (see below) [22].

4.1.5.4 Clinical trials – reasons for data variability

General aspects – the PARIS trial. The different outcomes with aspirin in prevention of PIH in clinical trials were somehow surprising and suggested additional variables from the patients' side as determinants for the study outcome rather than a variability

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in the pharmacodynamic action of aspirin. It is possible that the less impressive results of the large CLASP study as compared with the several smaller ones might have been due to a too heterogeneous patient selection, which diluted any beneficial effect by inclusion of (relatively) more patients with a low risk profile [67–69]. On the other hand, small-sized studies with negative results might not have been published at all, possibly causing a publication bias. Three variables with particular relevance to clinical outcome and treatment efficacy are: (i) patient population and individual risk profile, specifically concomitant diseases; (ii) beginning (and end) of treatment; and (iii) selection of dose and control of adherence of the patient to regular drug intake (compliance!). The frequently cited "Perinatal Antiplatelet Review of International Studies" (PARIS), a retrospective metaanalysis of primary prevention of preeclampsia by antiplatelet treatment [70], is a nice example to demonstrate the complexity of this issue.

The PARIS study was a metaanalysis on aspirin prophylaxis of preeclampsia, based upon individual patient data from 32,217 women and their 32,819 babies. Data were obtained from 31 randomized preeclampsia primary prevention trials. The prespecified main outcomes included: preeclampsia, death in utero or death of the baby before discharge from the hospital, preterm birth at less than 34 weeks of gestation and IUGR. Only randomized studies of antiplatelet agents (mostly aspirin) vs. placebo or no treatment were included.

For women assigned to receive antiplatelet agents, the RR of developing preeclampsia was reduced by 10 % (HR: 0.90; 95 % CI: 0.84–0.97), that of delivering before 34 weeks by 10 % (HR: 0.90; 95 % CI: 0.83–0.98) and that of having a pregnancy with a serious adverse outcome by 10 % (HR: 0.90; 95 % CI: 0.85–0.96). There was no effect on other parameters, including death of fetus or baby, IUGR or bleeding events. The outcome was similar in several subgroups studied, including those with late start of treatment, different dosing and preexisting medical conditions.

The conclusion was that administration of antiplatelet agents during pregnancy causes moderate but consistent reductions in the risk of developing preeclampsia, preterm delivery (before 34 weeks' gestation) and pregnancy with serious adverse outcomes [70].

This metaanalysis is important, in particular with respect to safety aspects of long-term aspirin administration during pregnancy (Section 3.1.2). However, it also suffers from the principal problems of all metaanalyses, that is, the mix of (single) patient data from different studies with different study designs, entry and exclusion criteria, duration, aspirin doses and definitions of clinical outcome (Table 4-2). Only data from 31 trials (out of 115 trials that were considered by the authors as potentially eligible!) were included. This is about one quarter from the total available information. The aspirin doses varied between 50 and 150 mg/day. There was a 16 % better outcome at doses of ≥75 mg as compared to doses of <75 mg, which, however, was not significant. A preplanned ≥150 mg aspirin subgroup analysis was not conducted because of too small numbers of patients. Randomization and start of treatment at optimum time points, prior to the 20th week of gestation, was only done in about half of participating women. Women with preexisting medical conditions (i. e., renal disease, diabetes, hypertension), that is, a possibly different etiology of preeclampsia, as op-

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posed to the "idiopathic" form of the disease, were also included. Finally, more than 37 % of data extracted from the 31 studies came from *one* (principally negative) trial (CLASP) (see above). This indicates a rather mixed dataset which might have underestimated the real benefits of aspirin prophylaxis because of paying too little attention to the individual risk profile.

Individual risk profile. The clinical symptoms of preeclampsia are influenced by the individual risk profile and might be further aggravated by external risk factors and preexisting medical conditions [71]. Thus, improved diagnostics, identifying patients with risk factors early, before the appearance of clinical symptoms, might be a useful approach to increase the success rate of treatment and was applied in the ASPRE trial (see below).

Beginning and end of treatment. Because the pathogenesis of preeclampsia is related to early abnormalities of the uteroplacental circulation, treatment is probably most effective when started before placentation is completed [72], that is, prior to the 16th week of gestation [51, 73]. This was confirmed by a metaanalysis of the effects of aspirin and the start and doses of aspirin treatment on the incidence of preterm preeclampsia (Table 4.1.5-1) [22].

Table 4.1.5-1: Effects of aspirin at daily doses of <100 or ≥100 mg on prevention of preterm preeclampsia. Treatment was started before or after the 16th week of gestation. The metaanalysis included 16 studies and a total of 18,907 participants (modified after [22]).

Start of treatment	Aspirin-dose	Number of patients (events / no events)		HR (95 % CI)
		Treatment	No treatment	
≤16. week	<100 mg	60/1805	100/1794	0.59 (0.29–1.19)
>16. week	<100 mg	146/4122	144/4134	1.00 (0.80–1.25)
≤16. week	≥100 mg	17/1145	57/1114	0.33 (0.19–0.57)
>16. week	≥100 mg	27/276	31/278	0.88 (0.54–1.43)
total		**271/9456**	**362/9451**	**0.62 (0.45–0.87)**

Late start of treatment, for example between the 13th and 26th (mean 18th–22nd) weeks of gestation [74], or even until the 32nd week of gestation in the CLASP trial, will be less effective though not fully ineffective [75]. Treatment should be stopped after the 34th week of gestation because of a questionable benefit and the avoidance of perinatal bleeding events (Section 3.1.2).

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Aspirin dosing. Most of the studies which failed to show convincing beneficial effects of aspirin on prevention of preeclampsia were performed with 60 mg aspirin/day or less. These doses were chosen in order not to (further) reduce the impaired vascular PGI_2 production. However, these doses may not always be sufficient for clinically relevant inhibition of thromboxane formation, specifically in situations of increased platelet reactivity (aspirin "resistance") in pregnant women. Higher doses (100–150 mg/day) of aspirin were frequently more effective than lower ones (50–80 mg/day) in an older metaanalysis [76]. In one recent prospective cohort study, 29 % of women had a lack of platelet inhibition by 81 mg/day aspirin, which was in most cases overcome with doubling the dose [49, 77]. Cochrane data of women at risk of developing preeclampsia indicated a reduction of preeclampsia by 65 % (RR: 0.35; 95 % CI: 0.24–0.52) in the subgroup of women treated with higher doses of aspirin (>75 mg/day) as compared to an only 15 % RR for all patients [78]. An updated version of this metaanalysis of all randomized trials comparing antiplatelet agents with either placebo or no antiplatelet agent confirmed the beneficial effects of low-dose aspirin (50–150 mg/day) for reduction of pregnancies with adverse outcome – 20 fewer events per 1,000 women treated [79]. Another systematic review and metaanalysis of randomized controlled trials that evaluated the prophylactic effect of aspirin on prevention of preeclampsia showed significant effects only for aspirin doses of ≥100 mg/day and start of treatment prior to the 16th week of gestation (Table 4.1.5-1 [22]; Table 4.1.5-1). Taken together, these data suggest that aspirin doses of 100–150 mg/day might be considered optimal, and that is also what most guidelines recommend today.

Circadian variations. Another explanation of variable treatment results with antiplatelet agents is the insufficient consideration of possible circadian variations in drug efficacy. *Ramón C. Hermida* and coworkers from Vigo (Spain) were the first to detect in a placebo-controlled randomized trial in pregnant women at elevated risk for preeclampsia that the (weak) blood pressure-lowering action of aspirin (100 mg/day) showed a clear circadian variation. The blood pressure-lowering effect was most prominent when aspirin was given at least 8 hours or later after awakening but apparently absent when given in the morning at awakening time (Fig. 4.1.5-5) [80]. These findings were confirmed by the authors in a later similarly designed trial with the same aspirin dose, starting on average at 13.5 weeks of gestation. Aspirin ingested at bedtime, but not upon awakening, significantly decreased blood pressure and reduced the incidence of preeclampsia, gestational hypertension, preterm delivery and IUGR [81]. These chronobiological aspects have probably not been considered in most of the aspirin trials in preeclampsia – but were considered in the ASPRE trial – with a positive outcome in favor of aspirin (see below) [82].

Compliance. Insufficient patient compliance is another possible explanation for treatment failure in prevention of gestation-related diseases. Specifically, pregnant

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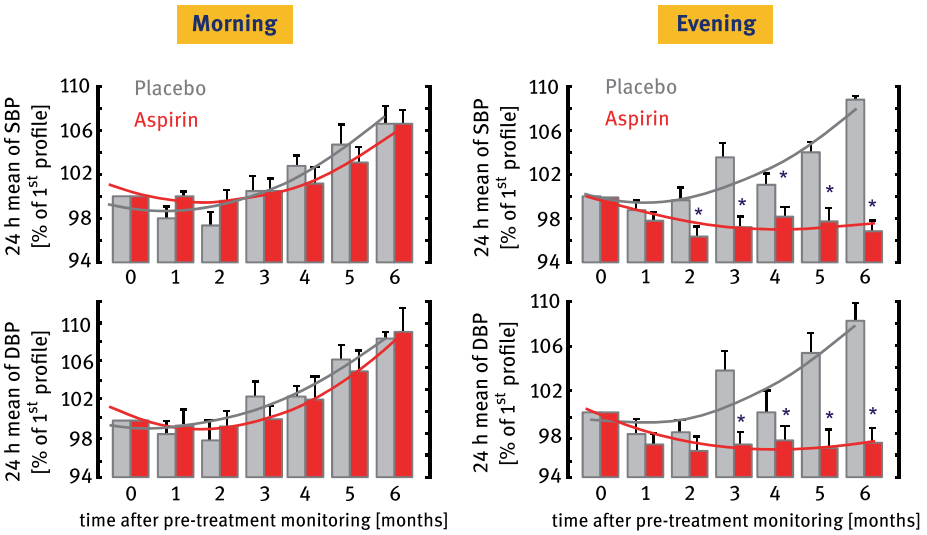

Figure 4.1.5-5: Time-dependent alterations in arterial systolic (SBP) and diastolic (DBP) blood pressures in pregnant women at risk for preeclampsia and their modification by low-dose aspirin given in the morning or in the evening. Asterisks (*) denote significant changes compared with placebo [80].

women may not wish to take any drugs because of possible injury to the fetus. The compliance rates according to tablet counts were only 57 % in the "Jamaica Low-dose Aspirin Study Project" (JLASP) [83] and 42 % in the "Barbados Low-dose Aspirin Study in Pregnancy" (BLASP) [84]. The assessment of compliance is also dependent on the method used, for example determination by personal pill count might differ from more objective methods, such as thromboxane determination.

An impressive example for the (in)validity of pill count as an estimate for women's compliance of aspirin use in prevention of preeclampsia was published by Hauth et al. (1995). Patient compliance in this study was 94 % according to tablet count but only 79 % according to serum thromboxane (TXB_2) determination, an objective parameter for aspirin intake in terms of efficacy.

Interestingly, an at least 2-fold reduction in thromboxane levels was seen in 33 % of patients in the placebo group. Obviously, these patients had taken aspirin or aspirin-containing medications for other reasons. When all pregnancies included in the study were divided into those with at least 2-fold reduction in serum thromboxane levels and those without – independently of randomization to the placebo or aspirin group – there was a clear correlation between inhibition of thromboxane formation and clinical outcome, i. e., rates of preeclampsia, IUGR and preterm delivery [85, 86].

4.1.5.5 Actual situation

The ASPRE trial. The most recent and probably quite influential study on aspirin prophylaxis of preeclampsia was the "Aspirin for evidence-based PREeclampsia prevention" (ASPRE) trial [82, 87].

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ASPRE was a multicenter, double-blind, randomized and placebo-controlled trial to study the efficacy of early aspirin on the prevention of preterm eclampsia, requiring termination prior to the 37th gestational week in women at high risk.

In total, 1,776 women with singleton pregnancies and high risk for preterm eclampsia were randomized to receive one coated aspirin tablet (150 mg/day) *at night* or a matching placebo. Treatment was started during the 11th–13th week of gestation and lasted until the 36th week of gestation. Primary endpoint was delivery with preeclampsia before 37 weeks of gestation (preterm eclampsia). Secondary outcomes were adverse events to the mother, fetus or newborn.

All women underwent an intensive screening program in the 11th–13th week of gestation. This program included the measurement of biomarkers (PlGF and others), biophysical markers (mean arterial blood pressure, pulsatile index of umbilical artery by Doppler ultrasound) and clinical parameters (personal health status, preceding or accompanying diseases, medications). A risk score was defined with an estimated detection rate of preterm eclampsia of 75 % accepting about 10 % wrong positive results. Compliance was controlled by pill counting [87].

Only 13 out of the 798 participants of the aspirin group (1.6 %) but 35 out of the 822 participants in the placebo group (4.3 %) developed preterm preeclampsia. This was equivalent to a >60 % reduction of the primary endpoint by aspirin (OR: 0.38; 95 % CI: 0.20–0.74; $P = 0.004$). The reported compliance was >85 % in 80 % of patients. There were no significant between group differences in the incidence of serious adverse events. However, the trial was not powered for these secondary outcomes. Aspirin did not reduce the incidence of term preeclampsia.

The conclusion was that treatment with low-dose aspirin in women at high risk for preterm eclampsia reduced the incidence of the disease as compared with placebo (Fig. 4.1.5-6) [82].

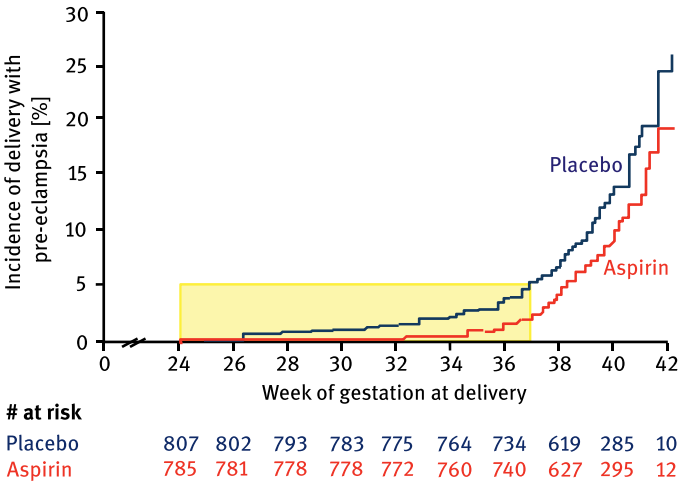

Figure 4.1.5-6: Incidence of delivery with preeclampsia in women at high risk for preterm eclampsia according to combined multimarker screening. The yellow area highlights the rate of preeclampsia before the 37th week of gestation [82].

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In the well-defined high-risk population of ASPRE, the incidence of preterm eclampsia after aspirin treatment was reduced highly significantly to half of that with placebo. This study was one of the first to use screening by combined multimarkers for early identification of women at risk and also considered possibly circadian variations in drug efficacy [80].

In a follow-up subanalysis of the ASPRE study, the effect of aspirin on length of stay in the neonatal intensive care unit was determined. Overall, the mean length of stay was longer in the placebo than in the aspirin group: 2.06 versus 0.66 days; a reduction of 1.4 days, corresponding to a reduction of hospitalization of 68 % (95 % CI: 20–86 %). This reduction was mainly due to a decrease in the rate of births at <32 weeks' gestation, mainly because of prevention of early preeclampsia [88].

The recommendations of the USPSTF from 2014 for treatment of women at high risk for preeclampsia had already reduced the rates of recurrent preeclampsia among women with a history of preeclampsia by 30 % within 2 years (HR: 0.70; 95 % CI: 0.452–0.95) [89]. A most recent evidence report on aspirin use to prevent preeclampsia and related morbidity and mortality reports an incidence of 4–30 % in women at increased risk of PIH in a metaanalysis of 18 trials. Aspirin significantly reduced the risk of preeclampsia (HR: 0.85; 95 % CI: 0.66–0.96), the risk of preterm birth (RR: 0.80; 95 % CI: 0.67–0.95) and the risk of IUGR (HR: 0.82; 95 % CI: 0.68–0.99). The absolute RR for preeclampsia by aspirin amounted to 1–6 % across larger trials (including >300 participants) [90]. With the exception of a Swedish registry study (see below) [91], there was no association between aspirin use and postpartum hemorrhage (HR: 1.03; 95 % CI: 0.94–1.12) and other bleeding or long-term harms.

A recent large, population-based registry study from Sweden investigated the risk of hemorrhagic complications in pregnant versus nonpregnant women.

Aspirin use was registered in 1.8 % of the 313,624 women giving birth between 2013 and 2017 according to the Swedish Pregnancy register.

Pregnant aspirin users had a higher risk of intrapartum bleeding (2.9 % vs. 1.5 % in nonusers; OR: 1.63; 95 % CI: 1.30–2.05) and postpartum hemorrhages (10.2 % vs. 7.8 %; OR: 1.23; 95 % CI: 1.08–1.39) [91]. There was also an elevated risk of neonatal intracranial hemorrhage (0.07 % vs. 0.01 %; OR: 9.66; 95 % CI: 1.88–49.48).

The conclusion was that aspirin use during pregnancy is associated with increased postpartum bleeding and postpartum hematoma. When offering aspirin, these risks need to be weighed against the potential benefits recommended [91].

This register study is interesting, because of both its large size and the clinical outcome, although the data are somehow different to other trials and metaanalyses. The recommended daily aspirin dose for prevention in Sweden is 75 mg. Cessation of aspirin only occurred at the 36th week of pregnancy. This is close to term delivery and might have been too late to avoid aspirin-related perinatal bleeding events.

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Risk calculation. The positive results with aspirin in ASPRE were obtained in a study with identification of women at high risk for eclampsia by risk markers. Earlier studies have indicated that screening at the 11th–13th week of gestation will only identify less than 40 % of cases of term preeclampsia [92]. Currently the selection of suitable biochemical, biophysical and clinical parameters is under discussion. Attractive candidates are biomarkers for angiogenic imbalances, such as soluble antiangiogenic proteins (sFlt-1, endoglin/PIGF) [93], inflammation markers such as TNFα [15] and others [7, 94–96]. A useful tool might also be the sFlt-1/PIGF ratio with a reportedly 100 % (!) specificity for early-onset preeclampsia [95]. A combined setting might help to cover the multifactorial pathogenetic background of the disease better than just one (group of) parameter(s) alone [87, 97]. The pattern of biomarkers differs between different high-risk groups for preeclampsia (diabetes, hypertension, previous preeclampsia), suggesting that multiple pathogenic pathways might be involved in clinical preeclampsia and the inclusion of clinical criteria (Doppler ultrasound of the umbilical artery, blood pressure, anamnestic risk factors, etc.) will also be helpful.

Summary

Preeclampsia – pregnancy induced hypertension (PIH) with proteinuria – is a multisystem disorder of pregnancy with different etiologies. PIH is a leading cause of fetal and maternal morbidity and mortality. The pathophysiology of the disease is likely to be determined by impaired implantation of the trophoblast inside the uterus, causing a number of follow-up reactions. These include disturbed angiogenesis in the fetal circulation, possibly via enhanced formation of antiangiogenic proteins, enhanced oxidative stress inside the placental tissues and subsequent systemic inflammation with endothelial injury in the maternal circulation. No curative treatment of the disease is available except delivery and no drugs have been shown so far to influence the progression of the disease.

Leading clinical symptoms of the disease are those of a generalized endothelial dysfunction, clinically presenting with hypertension, proteinuria and general signs of inflammation. These symptoms are associated with a pathology of arachidonic acid metabolism inside the fetoplacental unit. The increasing prostacyclin formation with progression of pregnancy is largely abolished prior to occurrence of symptoms while the pregnancy-related increase in thromboxane generation tends to be further enhanced. Platelet hyperreactivity, increased platelet-dependent thromboxane formation and oxidative stress will cause prothrombotic and inflammatory reactions prior to the clinical onset of the disease.

Prevention of enhanced (platelet) thromboxane formation is the rationale for prophylactic use of low-dose aspirin (about 150 mg/day). A moderate but significant 10–15 % improvement of clinical outcome, including prevention of preeclampsia, prolongation of gestation and prevention of IUGR and increased perinatal mortality, is well established by several metaanalyses in women at increased risk. Whether improved diagnostic procedures in addition to blood pressure measurements or testing for proteinuria might further increase the clinical efficacy of aspirin by allowing earlier identification and treatment of women at risk remains to be shown.

Aspirin treatment should be started early (before the 16th week of gestation) and finished at the 34th week because metaanalyses have suggested a small postpartum bleeding risk. There is no evidence for significant side effects of aspirin at low doses on clinical outcome of mother or fetus (premature closure of the ductus arteriosus, pulmonary hypertension) or fetal development (miscarriages, malformations) (Section 3.1.3).

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4.1.6 Aspirin "high on treatment platelet reactivity" (HTPR, aspirin resistance)

4.1.6.1 General aspects

Variability in drug responses. Considerable heterogeneity exists in the way individuals respond to drugs in terms of both efficacy and safety. Antiplatelet drugs, such as aspirin, ADP-P2Y12 antagonists, GPIIb/IIIa blockers and other compounds which are used for prevention of thrombotic events are no exception from the rule. They are effective in a certain proportion of patients but are ineffective in others [1]. The clinical consequences of this variability for prevention of vascular thrombotic events are well documented in the metaanalyses of the AATC: There is an overall about 15–20 % relative protection in unselected individuals taking aspirin for secondary prevention of

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thromboembolic events [2] but with a wide disease-dependent variation in efficacy between 0 % and 50 % [3, 4]. The phenomenon of reduced efficacy of antiplatelet agents was named "High on treatment platelet reactivity" (HTPR) or – in the case of aspirin – aspirin "resistance."

A piece of history. Anecdotally, the first report on platelet aspirin "HTPR," as a failure of the compound to inhibit platelet function, was seen – but not commented on – in a paper which first described the inhibition of prostaglandin (thromboxane) biosynthesis by aspirin in human platelets. In this study, aspirin largely prevented thrombin-induced prostaglandin (thromboxane) production but not the thrombin-induced platelet serotonin secretion [5]. This "HTPR" can now be explained by the use of thrombin, a strong platelet-stimulating agent, that does not require "support" of the platelet COX/thromboxane pathway for a full platelet response. This finding of a separation of inhibition of platelet COX-1, that is, inhibition of platelet-dependent thromboxane formation, from inhibition of platelet aggregation/secretion is more than 50 years old, but still not generally appreciated. Moreover, it is now known that there are multiple drug- and disease-related reasons for insufficient inhibition of platelet function by aspirin. In the meantime, HTPR, originally named aspirin "resistance" by Helgason et al. [6], became a frequent observation – and an issue of concern – in clinical thrombosis prevention trials. The large interindividual variability of the antiplatelet effect of aspirin in clinical trials on thrombosis prevention additionally suggested that there will be probably many more cases of disease-related drug treatment failures than an insufficient pharmacological action of the drug. Thus, clinical treatment failure and a failure of aspirin to work pharmacologically are *no* synonyms but, unfortunately, are frequently mixed up in an inappropriate way, which has caused much confusion [7, 8].

4.1.6.2 Definition and types of aspirin – HTPR

"HTPR" against antithrombotic drugs is not restricted to aspirin. "Resistance" is an established term also for the efficacy of other antiplatelet and antithrombotic drugs. "Resistance" (HTPR) to the antiplatelet effects of clopidogrel appears in 20–30 % of patients. In many cases this can be explained by insufficient generation of the active metabolite due to genetically fixed insufficient enzymatic hepatic bioactivation [9]. For example, 50–65 % of the East Asian population carry defective CYP2C19 genotypes with reduced bioactivation of clopidogrel [10]. Similarly, "resistance" to coumarin-type anticoagulants such as warfarin is due to a genetically fixed mutation of one key enzyme of vitamin K metabolism [11]. In the case of aspirin, th situation is more complex. In addition, there is neither an uniform, clinically accepted definition of aspirin "resistance" (HTPR) nor any generally accepted procedure for its determination.

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Drug-related forms of aspirin HTPR. Aspirin HTPR can be defined in pharmacological terms as the inability of the drug to hit its molecular target, that is, platelet COX-1 [12]. Clinical HTPR to aspirin can be caused by multiple mechanisms and only very few of them are causally related to a pharmacodynamic failure of aspirin action [12, 13]. *Artur-Aron Weber* and colleagues from Düsseldorf (Germany) [14] have proposed a typological approach to classify several forms of aspirin HTPR in pharmacological terms. This was done by comparing the potency of aspirin to inhibit platelet-dependent thromboxane formation with thromboxane-dependent platelet aggregation under well-defined in vitro conditions. This allows for a separation of three different types of pharmacological HTPR. This assay has also been successfully used for classification of the clinical syndrome of aspirin "resistance" [15].

In the Weber assay, aspirin "resistance" was measured by simultaneous determination of inhibition of collagen-induced thromboxane formation and inhibition of platelet aggregation by aspirin in vitro. A low dose (1 µg/ml) of collagen was chosen, which under the conditions of this assay required release of endogenous arachidonic acid for a full aggregation response. In case of incomplete or missing inhibition of platelet aggregation after oral aspirin ex vivo, three different reaction profiles could be separated according to the alterations in thromboxane formation and platelet aggregation after in vitro addition of aspirin:

Type I (pharmacokinetic) resistance: No inhibition of platelet aggregation after oral treatment in vivo but inhibition of aggregation and thromboxane formation after treatment with aspirin in vitro. This suggests that aspirin does work in these platelets as expected but was not bioavailable in sufficient amounts in vivo at the site where it was needed – the COX-1 channel of platelets. Most likely explanations are missing compliance (!) or competition with other drugs for salicylate binding inside the hydrophobic channel of COX-1 (NSAIDs).

Type II (pharmacodynamic) resistance: No inhibition of platelet aggregation after oral treatment in vivo but partial inhibition of platelet aggregation and thromboxane formation after addition of aspirin in vitro which can be (partially) antagonized by increasing the aspirin dose. This "true" pharmacological resistance suggests a pharmacodynamic failure of aspirin to act, for example because of reduced sensitivity of the platelet COX-1, different gene polymorphisms or residual platelet activity due to enhanced expression of the platelet GPIIb/IIIa receptors. To this type belong also disease-induced forms of HTPR, for example provision of prostaglandin endoperoxides for platelet thromboxane synthase via an upregulated COX-2 from inflammatory cells (macrophages and others).

Type III (pseudo)resistance: No inhibition of platelet aggregation after oral treatment in vivo and no inhibition of platelet aggregation in vitro despite complete inhibition of thromboxane synthesis after addition of aspirin. Possible reasons are platelet activation by nonthromboxane-dependent pathways, for example many platelet agonists (thrombin, high-dose collagen, thromboxane) or isoprostanes (Fig. 4.1.6-1) [14].

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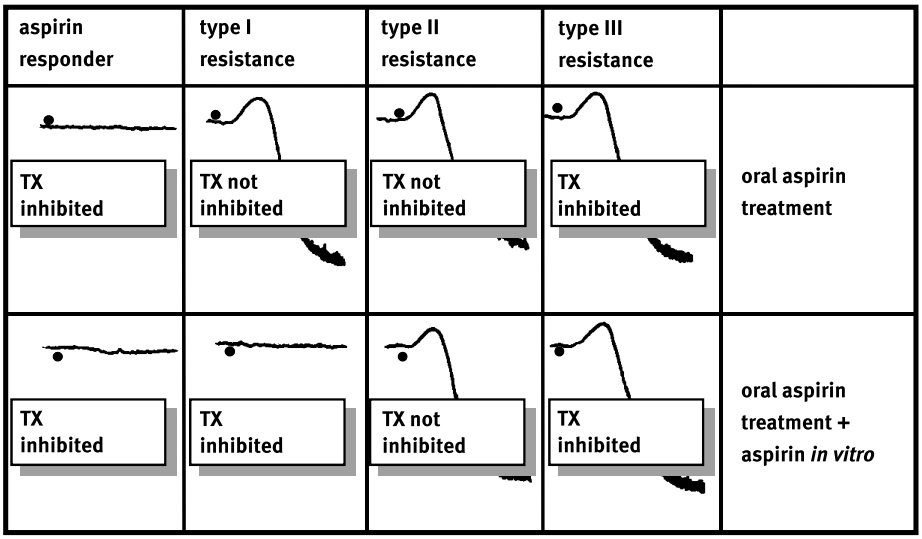

Figure 4.1.6-1: Typology of aspirin resistance (HTPR) according to Weber et al. [14]. For further explanations see text.

Pharmacological HTPR vs. treatment failure. The pharmacological failure of aspirin to act is due to its inability to sufficiently inhibit platelet COX-1, that is, incomplete (<95 %) inhibition of COX-1-mediated thromboxane formation by regular aspirin administration in antiplatelet doses (75–325 mg/day) [16]. This pure pharmacodynamic HTPR is rare and accounts for only 1 % or even less of treated individuals [17–19]. In contrast, clinical treatment failure of aspirin, that is, thrombotic vessel occlusion despite efficient inhibition of platelet-dependent thromboxane formation, is much more common. It is detectable in up to 50 % of patients, dependent on the method of determination, definition of normal values and clinical conditions of the patient [20–31]. Independently of the relevance of this finding for clinical outcome, this also means that pharmacological inhibition of platelet-dependent thromboxane formation by aspirin cannot be directly translated into its clinical efficacy as antiplatelet/antithrombotic drug. Multiple mechanisms contribute to a poor clinical outcome of "aspirin-resistant" patients in different clinical conditions [8, 32–34]. In addition, the intensity of antiplatelet effects of aspirin is not likely to correlate with its clinical efficacy if thrombotic events are not primarily aspirin-sensitive, for example lacunar vs. large cerebral artery atherosclerotic stroke [35–37]. On this background, it is not surprising that the term "aspirin resistance" was originally introduced to describe the variable and dose-dependent antiplatelet effects of aspirin in prevention of ischemic stroke (Section 4.1.2) [20].

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4.1.6.3 Detection of aspirin HTPR

There are principally two mechanism-based methods to detect "resistance" or HTPR of platelets to aspirin – the measurement of one or more parameters of platelet function ex vivo or the determination of (platelet-dependent) thromboxane formation. Both approaches have advantages and disadvantages, the most important disadvantage for both being (i) the absence of *one* generally accepted technology of measurement; (ii) the absence of generally accepted "normal" values to allow for standardization and comparisons between different laboratories; and (iii) the (still) poorly defined predictive value of HTPR for clinical outcome, that is, the transfer of laboratory data into medical reality of the clinics [38].

Measurement of platelet function. Determination of platelet functions *ex vivo* or *in vitro* provides direct information about platelet reactivity in response to a long list of well-defined platelet agonists and also generates easily understandable readouts. Here, Gustav Born's photometric assay of light transmission aggregometry of platelets in citrated platelet-rich plasma is still a most popular technology to study platelet aggregation and to detect aspirin HTPR. However, any study of blood platelets *ex vivo* or *in vitro* is done under conditions which differ fundamentally from the *in vivo* situation. *In vivo*, circulating platelets permanently interact with blood components and the vessel wall and are under continuous exposure of blood-borne, platelet-active factors [39]. In all *ex vivo* assays, platelets are removed from their natural environment, and in most of them they are studied under static conditions in a small reaction vial. Several platelet-active factors from blood are labile. They are already inactivated when the assay starts (NO, prostacyclin, thromboxane) or not present for other reasons (endothelial ADPase). Mechanical stimulation of platelets by blood sampling procedures, centrifugation or pipetting (shear stress) might result in uncontrolled *ex vivo* activation prior to addition of the platelet-stimulating agents. There are no red cells in platelet-rich plasma, which is used for many *in vitro* platelet function assays. Red cells markedly increase platelet reactivity [40, 41] and will reduce the antiplatelet actions of aspirin [42]. Finally, measurement of platelet function in platelet-rich plasma does not consider possible effects of aspirin on platelet–white cell interactions that may influence the platelet-dependent hemostatic process and are important sources of inflammatory, prothrombotic mediators (cytokines). Thus, any analysis of platelet aspirin sensitivity that is solely based on measures of platelet inhibition *in vitro* after stimulation by one particular agonist will not capture the full antithrombotic potential of antiplatelet treatment by antiplatelet drugs [38, 39, 43].

All of the numerous platelet function tests have limitations. Immediately after blood sampling, platelets first have to be "paralyzed" by withdrawal of external Ca^{++} or addition of thrombin inhibitors to avoid spontaneous aggregation. This is essential for later stimulation by a selected agonist of choice. Most importantly, different platelet function assays do not measure the same platelet-derived signal. Conse-

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quently, they may provide different results even in the same patient [43–48], and platelets that are "resistant" in one assay are not necessarily "resistant" in another [49].

Platelets not only form aggregates but also generate and release several TXA_2-sensitive and -insensitive proinflammatory/prothrombotic storage products (Fig. 2.3.1-2). Hence, platelet activation and secretion support platelet-mediated prothrombotic conditions, partially via interaction with other cells in the vicinity, most notably monocytes/macrophages and polymorphonuclear leukocytes [50]. Mediators include P-selectin, soluble CD40L and other inflammatory, immunogenic and growth-promoting factors (Fig. 2.3.2-5) [50–54], the platelet-derived lipid mediator S1P being of particular interest as an inflammatory and mitogenic compound [55]. The significance of modulation by aspirin of these complex interactions between platelet-derived mediators, thromboxane and other cells, the so-called "heterotypic" platelet functions [56], will be considerable and highly relevant to the clinical outcome [53]. None of these paracrine platelet functions can be determined by measuring platelet aggregate formation in conventional *in vitro* assays. Consequently, there is apparently no predictive value of aspirin HTPR determined by aggregometry for clinical outcome in patients with stable atherosclerotic CVD (ASCVD) (see below) [57, 58]. Current evidence does also not suggest routine diagnostic utility for aspirin HTPR in individuals after PCI [59] except some high-risk patients [31].

Thromboxane formation. A different option to determine aspirin HTPR is the measurement of aspirin action on platelet-dependent thromboxane (TXA_2) formation, either as the stable hydrolysis product TXB_2 or as one of its multiple metabolites in urine. Serum TXB_2 levels correspond to the capacity of thromboxane biosynthesis. They are a very useful surrogate parameter for the pharmacodynamic efficacy of aspirin and a valid compliance control. Inhibition of serum thromboxane should amount to at least 95 % of capacity [16]. This is equivalent to thromboxane levels of about 25 ng/ml [60]. Less inhibition is considered clinically ineffective because of the nonlinear correlation between inhibition of thromboxane formation and inhibition of thromboxane-dependent platelet functions [16, 48]. However, serum thromboxane has no natural correlate in vivo and provides no information about the platelet reactivity status in vivo. Platelet-derived thromboxane levels, for example in bleeding time blood, amount to only about 1 % of the thromboxane-forming capacity of platelets in blood serum (Section 3.1.2).

Another approach to estimate the aspirin action on thromboxane levels in the cardiovascular system in vivo is measurement of thromboxane metabolite excretion, such as 11-DH-TXB_2, in urine [61]. A significant, aspirin-sensitive elevation of plasma and urinary 11-DH-TXB_2, associated with platelet activation in unstable angina, severe atherosclerosis, PAD and PE is well known (Table 4.1.6-1) [61–69]. This confirms the

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Table 4.1.6-1: Urinary levels of 11-dehydro-TXB_2 (11-DH-TXB_2) before and after aspirin treatment in selected studies. *Serum TX was also measured and completely inhibited. **Acute coronary syndrome. ***Escalating aspirin doses between 325 and 2,600 mg/day. Cr: creatinin [63–70].

n	11-DH-TXB_2 in urine		% Reduction	Reference
	before Aspirin	after Aspirin		
5	273 ± 65 pg/mg cr	9–13 pg/mg cr	95–97*,***	FitzGerald et al., 1983 [70]
24	75 ± 13 (SEM) ng/mmol cr	17 ± 3 ng/mmol cr	77*	Montalescot et al., 1994 [63]
24	815 ± 183 (SEM) ng/g cr	266 ± 114 ng/g cr	67	Uyama et al., 1994 [66]
64	ca. 450 pg/mg cr	ca. 160 pg/mg cr	72	Cipollone et al., 2000 [64]
16–71	1,386 (176–3844, range) ng/g cr	783 (149–7,415) ng/g cr	44	Bruno et al., 2002 [65]
24	180 ± 142 (SD) ng/mmol cr	40 ± 23 (SD) ng/mmol cr	75	Gonzalez-Conejero et al., 2005 [67]
267	7,082 ± 12,813 (SD) pg/mg cr	1,354 ± 886 (± SD) pg/mg cr	81**	Matsuura et al. 2012 [69]
54	3,665 ± 2,465 (SD) pg/mg cr	996 ± 845 (SD) pg/mg cr	73	Lopez et al., 2014 [68]

usefulness of this parameter for the prediction of thromboembolic risk but not really the platelet reactivity status.

11-DH-TXB_2 is the most frequently determined metabolite of TXA_2 but only one out of about 20 degradation products of $TXA(B)_2$ in urine [71]. The conversion rate of TXB_2 into this metabolite is about 7 % [72]. The proportional conversion of TXB_2 into 11-DH-TXB_2 can be considerably changed by environmental factors. The conversion rate in smokers is twice as high as in nonsmokers [73]. A varying proportion of 11-DH-TXB_2 in urine is probably not platelet COX-1-derived, but derived from prostaglandin endoperoxides from other sources, such as monocytes/macrophages [74] and/or vascular cells with COX-2 upregulation (see below) [75]. Accordingly, there is a high variation in both published 11-DH-TXB_2 levels in urine and their reduction by aspirin treatment (Table 4.1.6-1). Until now, there is some empiric [76, 77] but no generally accepted definition of a threshold or a normal range of urinary 11-DH-TXB_2 excretion. A prospective cohort study on the predictive value of urinary 11-DH-TXB_2 for clinical outcome (mortality) in aspirin-treated patients with stable CAD has suggested a cut-off point of 1.6 ng/mg creatine for the prediction of mortality over 5 years [78]. However, the multiple variables mentioned above as well as the fact that about 30 % of total body thromboxane formation as measured from urinary excretion of 11-DH-TXB_2 – in contrast to serum TXB_2 – is not aspirin-sensitive (Table 4.1.6-1) indicate that urinary 11-DH-

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TXB_2 is no reliable biomarker of aspirin-sensitive alterations in platelet reactivity but rather a general index of oxidative stress and/or the severity and inflammatory state of atherosclerotic alterations of the vessel wall. This hypothesis is confirmed by studies in cholesterol-fed COX-1/ApoE knockout mice. These experiments showed that non-platelet sources of COX-1 and TXA_2 that are inaccessible to standard doses of aspirin may contribute to the development of atherosclerosis in these animals [79].

4.1.6.4 Mechanisms of aspirin HTPR

General aspects. There are two principally different reasons to explain reduced or absent inhibition of platelet functions by aspirin: drug-related and disease-related. The former are mostly due to pharmacokinetic and, rather random, pharmacodynamic reasons. The vast majority of clinical aspirin HTPR is disease-related, in most cases due to stimulation of platelet functions (not solely aggregation!) by aspirin-insensitive mechanisms. Typical examples are strong platelet stimuli, such as thrombin, that do not require thromboxane formation for a full platelet aggregation/secretion response as well as COX-2 upregulation in inflammatory cells or the vessel wall which will provide prostaglandin endoperoxide precursors to the (platelet) thromboxane synthase. An overview of drug-related and disease-related mechanisms of aspirin HTPR is shown in Table 4.1.6-2.

Table 4.1.6-2: Drug- and disease-related mechanisms of aspirin HTPR ("aspirin resistance").

Drug-related (drug failure)
Pharmacokinetics
Insufficient bioavailability of nonmetabolized, active aspirin in blood
Interactions of binding inside the COX-channel with NSAIDs and other lipophilic agents
Overexpression of multidrug resistance protein 4 (MRP4)
Pharmacodynamics
Impaired sensitivity of platelet COX-1
Gene polymorphism(s)
Increased platelet expression of GP IIb/IIIa,
changes in platelet proteomics
Disease-related (treatment failure)
Provision of thromboxane precursors (PG-endoperoxides) by COX-2 from non-platelet sources (monocytes/macrophages, vascular smooth muscle cells, endothelial cells)
Aspirin-insensitive mechanisms of platelet activation and secretion (Thrombin, ADP, shear stress, isoprostanes)
Increased platelet turnover rate with a higher proportion of immature, more reactive platelets
Increased protein glycation (diabetes)

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Insufficient bioavailability of active, nonmetabolized aspirin in blood. A possibly too low bioavailability of low-dose aspirin (75 mg/day) was repeatedly described for enteric-coated, retarded-release preparations. These were less bioequivalent to a plain preparation of the same dose and had an overall 20 % probability of treatment failure as seen from an insufficient reduction of serum thromboxane levels [80]. Another trial found an up to 49 % apparent HTPR for a 325-mg enteric-coated single-dose aspirin preparation but not for the same dose of plain aspirin. This was explained by a delayed and reduced drug absorption in the small intestine [81], that is, a type I pharmacokinetic HTPR (Fig. 4.1.6-1) [14]. Prolonged exposition to aspirin esterases in the gut and/or increased aspirin esterase activity in the blood might result in a reduced intravascular bioavailability of uncleaved aspirin [82]. Interestingly, there is no evidence that aspirin induces (plasma) aspirin esterase activity [83]. A 3-fold higher incidence of aspirin HTPR (insufficient inhibition of serum thromboxane) was recently reported for an enteric-coated preparation but not standard plain aspirin at the same dose in diabetics. The authors explained this by insufficient aspirin absorption [84]. Further mechanisms, in particular shortened platelet survival with an enhanced proportion of reactive immature and aspirin-hyperreactive platelets in diabetics, might also be contributing factors (Section 4.1.1).

The probably most frequent reason for reduced bioavailability of aspirin as an explanation for aspirin treatment failure is missing compliance [85, 86]. Insufficient adherence to aspirin treatment amounts to up to 40–50 % in (controlled!) long-term cardiovascular prevention trials and might be even higher in real life.

Negative interactions with NSAIDs and other lipophilic agents with aspirin binding inside the COX channel. Another pharmacokinetic reason for insufficient antiplatelet activity of aspirin are negative interactions of NSAIDs [87–92] and other lipophilic analgesics, such as dipyrone (metamizole) [90, 93], with initial aspirin binding in the hydrophobic substrate channel of COX-1. Binding of one of these competitor compounds will prevent the initial (reversible) binding of aspirin via the salicylate chain and the subsequent (irreversible) acetylation of $serine_{529/530}$. (Fig. 2.2.1-4). NSAIDs and dipyrone are highly lipophilic and have an about three orders of magnitude (Section 2.2.1) higher affinity to these primary binding sites inside the COX-1 channel than aspirin and salicylate. In the presence of these compounds, such as ibuprofen (half-lives in blood 2–4 h), no aspirin can bind and will become degraded in the blood by esterases (Section 2.2.1) [88–92].

This interaction is clinically relevant. A follow-up substudy to the US-PHS trial has found that participants who had taken NSAIDs (not specified) at a rate of more than 60 tablets per year lost their aspirin-related protection from myocardial infarction. This effect was similar to a poor drug adherence to aspirin (Fig. 4.1.6-2). This indicates that insufficiently sustained blockade of platelet COX-1 either by low patient compliance or displacement of aspirin by more lipophilic agents even at regular in-

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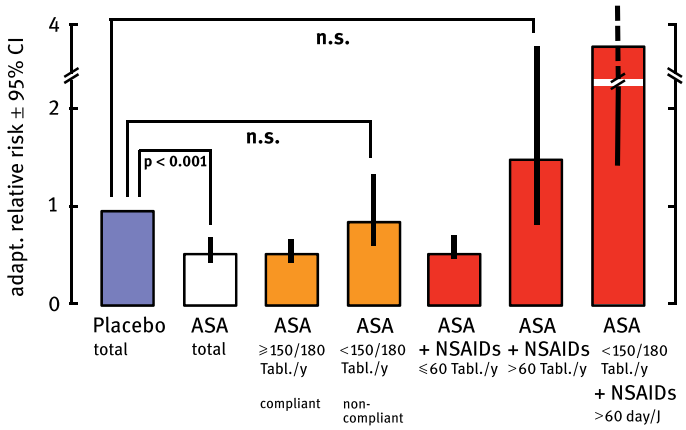

Figure 4.1.6-2: Correlation between aspirin (ASA) compliance, use of NSAIDs and risk of a first myocardial infarction in the US Physicians' Health Study (US-PHS). Note that both insufficient compliance and simultaneous (repeated) intake of NSAIDs (not specified) antagonize the cardioprotective action of aspirin. Tabl./y = tablets per year (modified after [95]).

take might cause aspirin "resistance." This negative interaction was also seen in another study on aspirin "resistance" in ACS patients. The group of patients exhibiting aspirin HTPR (10 %) with an associated worse clinical outcome also received NSAIDs as a comedication 3 times more frequently ($P < 0.01$) [94].

Fig. 4.1.6-3 is an overview of the negative interactions of different NSAIDs and paracetamol (acetaminophen with aspirin in vitro. It is evident that all of the compounds inhibit the arachidonic acid-induced platelet aggregation but behave differently when studied in the presence of aspirin, suggesting different reaction sites of NSAIDs and paracetamol vs. aspirin inside the COX-1 channel.

This type of interaction is not seen with selective COX-2 inhibitors [96] which cannot enter the COX-1 channel and also not for diclofenac [90, 91, 97], a compound with a significant COX-2-inhibitory potential. In contrast, dipyrone (metamizole), a nonantiinflammatory analgesic, is also a potent inhibitor of the antiplatelet effects of aspirin [98]. According to a nationwide observational study in Germany, this interaction between aspirin and metamizole is possibly also clinically relevant. Cotreatment with metamizole for control of chronic pain in patients with a previous cardiovascular event and subsequent secondary prevention with aspirin resulted in excess mortality [99], which was partially driven by myocardial infarctions and strokes.

These findings confirm iatrogenic, pharmacokinetic interactions of aspirin with other lipophilic agents, such as NSAIDs, that are able to displace or to prevent aspirin (salicylate) binding to its binding site(s) inside the hydrophobic COX-1 channel (Section 2.2.1) [100]. Today warning labels are placed by many health authorities worldwide for ibuprofen-type compounds for patients who need to take aspirin regularly for cardiovascular protection.

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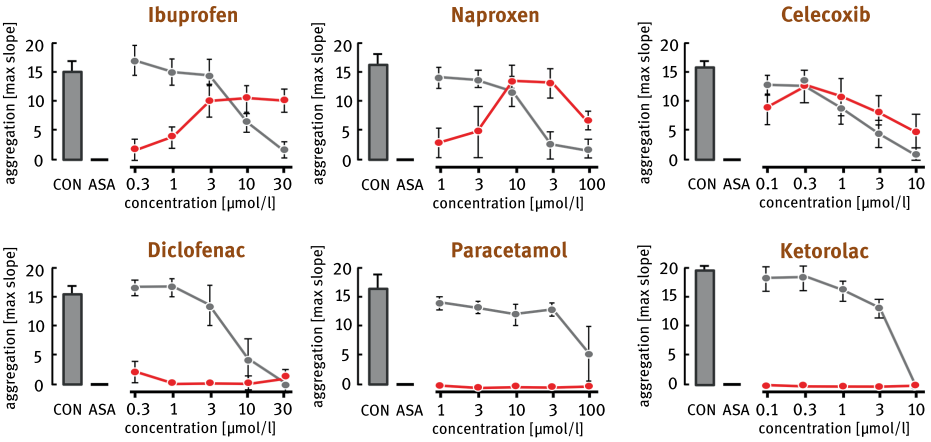

Figure 4.1.6-3: Antiplatelet effects of selected NSAIDs as compared to acetaminophen (paracetamol) in vitro – modification of aspirin-induced inhibition of platelet aggregation. Platelet stimulation with arachidonic acid in vitro causes platelet aggregation (gray columns), which is completely prevented in the presence of 30 µM aspirin (ASA). Gray lines demonstrate concentration-dependent inhibition of platelet aggregation for different NSAIDs and acetaminophen (paracetamol) in the absence of aspirin, red lines demonstrate aggregation if aspirin and NSAIDs were added simultaneously, shortly (5 min) before stimulation by arachidonic acid. Ibuprofen, naproxen and celecoxib prevent the antiplatelet effect of aspirin, while diclofenac, paracetamol (acetaminophen) and ketorolac do not although they are also capable of inhibition of platelet aggregation by themselves [91].

Overexpression of multidrug resistance protein 4 (MRP4). MRP4 is an efflux transporter protein that is involved in platelet aggregation and thrombus formation [101]. Aspirin is a substrate for MRP4 and can be extruded from platelets through this transportation pathway [102]. Regular aspirin intake at antiplatelet doses (100 mg/day) upregulates MRP4 at the mRNA and protein levels in megakaryocytes and platelets, respectively [103, 104]. This may result in reduced antiplatelet effects of aspirin, that is, HTPR, as seen from increased aggregation and serum thromboxane levels in patients after several weeks of aspirin treatment [105]. In patients under chronic aspirin treatment, platelets that present high MRP4 levels have an increase of residual platelet reactivity, which is due in part to incomplete COX-1 inhibition, and in part to COX-1-independent mechanisms (Fig. 4.1.6-4). Conversely, pharmacological inhibition of MRP4, for example by cotreatment with statins that are also substrates for MRP4, enhances the antiplatelet effects of aspirin and might overcome aspirin HTPR in certain clinical settings.

Impaired sensitivity of platelet cyclooxygenases. Platelets of patients undergoing CABG become largely resistant to conventional doses of oral aspirin, that is, 100 mg/day, within a few days after the surgical intervention [106]. This HTPR can be

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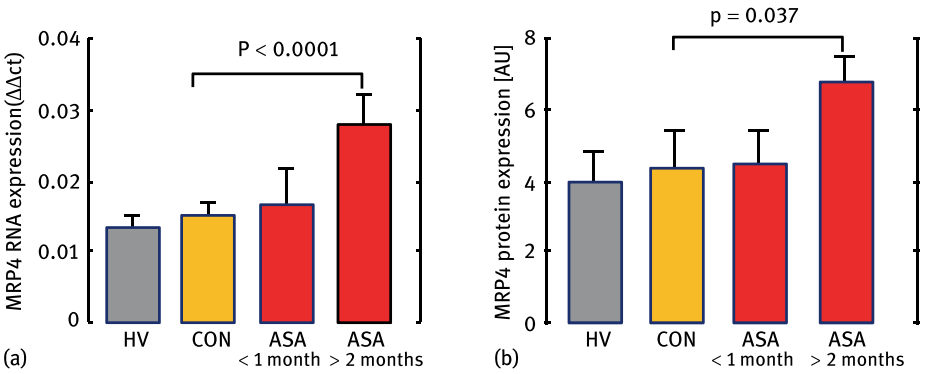

Figure 4.1.6-4: MRP4 RNA (a) and protein expression (b) in platelets from healthy volunteers (HV), aspirin-free patients (CON) and patients under chronic aspirin (ASA) (100 mg/day) for <1 month or >2 months (modified after data in [104]).

overcome in vitro by increasing the aspirin concentration [107] and is accompanied by enhanced expression of an immunoreactive COX-2 protein in platelets (Fig. 4.1.6-5). Interestingly, this immunoreactive COX-2 found in CABG patients was insensitive to inhibition by coxibs [107, 108]. The explanation was a new splice variant of COX-2 mRNA (COX-2a) that was about 200-fold upregulated in CABG patients. This was due to a shift in the reading frame of the enzyme [107, 109]. The resulting COX-2a protein was 16-fold upregulated in platelets of these patients [107, 110] but was enzymatically inactive.

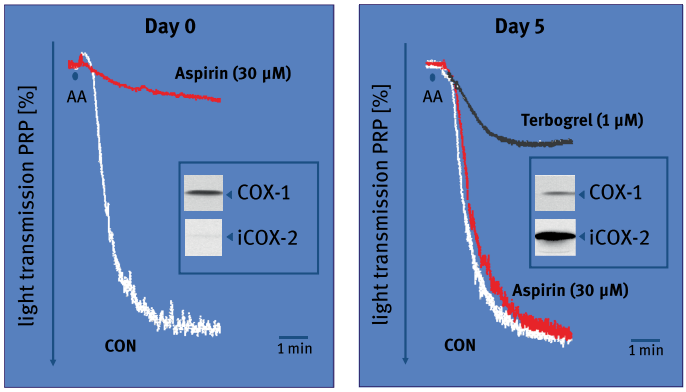

Figure 4.1.6-5: Expression of COX isoforms after coronary artery bypass grafting (CABG) and arachidonic acid (AA)-induced platelet aggregation before (day 0) and after (day 5) CABG in one patient. Different effects of aspirin and terbogrel (combined thromboxane synthase inhibitor and receptor antagonist) are observed in vitro. The aspirin HTPR at day 5 is associated with enhanced expression of an immunoreactive COX-2 (i-COX-2, probably COX-2a). No change is seen in COX-1 protein expression (modified after data in [107]).

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Thus, a reduced sensitivity of COX-1 against aspirin is the most likely explanation for HTPR to aspirin in these conditions. In patients with chronic ischemic heart disease a defective suppression of platelet COX-1 activity by aspirin was also described in terms of enhanced variability of serum TXB_2 generation which was not sensitive to COX-2 inhibition [108]. HTPR under these conditions might be explained by gene polymorphisms in functionally relevant target enzymes for aspirin, most importantly platelet COX-1 [44]. In addition, aspirin exposure may also alter a set of platelet mRNA that at baseline correlate with platelet function [111]. Another reason are (post)translational modifications of the COX-1 enzyme, for example after coronary bypass surgery [107].

Gene polymorphisms, platelet proteomics and enhanced expression of GPIIb/IIIa. A common platelet-based denominator of the variability of antiplatelet effects of antiplatelet agents is the proteomic signature. Antiplatelet drugs, including aspirin, differ markedly in their effects on functional protein clusters in platelets, suggesting that individuals might differ in their "proteome barcode" [112]. Experimental studies have shown that aspirin modulates protein release from the platelet secretome, regardless of the agonist [113] but distinct between individuals who were "aspirin-resistant" and those who were not [114].

Several prothrombotic gene polymorphisms relevant to the antiplatelet effects of aspirin have been described [115]. These include modifications of COX-1, such as the A842G/C50T gene polymorphism [116–118]. Genotypic alterations in the expression of the platelet GPIIIa receptor [119] have also been found. Recently, a marked increase in platelet GPIIIa expression has been found in "aspirin-resistant" individuals. Although this appears to be a rare event, it is a very interesting finding and one of the few examples of "true" pharmacodynamic HTPR to aspirin [120]. Another genetic variant of interest is the thrombin PAR-4 receptor. Signaling differences by the PAR4-120 variant have been shown to result in the enhancement of both G_q and G_{13} activation and an increase in thrombus formation resulting in a potential HTPR to traditional antiplatelet therapies targeting COX-1 and the P2Y12 receptor [121].

Provision of thromboxane precursors (prostaglandin endoperoxides) by nonplatelet sources with COX-2 upregulation. In the inflammatory conditions of advanced-stage atherosclerosis, nonplatelet sources of PG-EPs, the immediate precursors of TXA_2, become increasingly relevant to thromboxane production by the platelet thromboxane synthase. Potential sources for thromboxane precursors inside the circulation are vascular cells with COX-2 upregulation, such as endothelial cells [122], secretory vascular smooth muscle cells (Fig. 2.2.1-5) [123] and monocytes/macrophages [74]. This allows for transcellular precursor (prostaglandin endoperoxide) exchange and for thromboxane formation by the platelet's thromboxane synthase, which is not inhibited by aspirin (Fig. 4.1.6-6). Thus, enhanced provision of endoperoxides by COX-2 from nucle-

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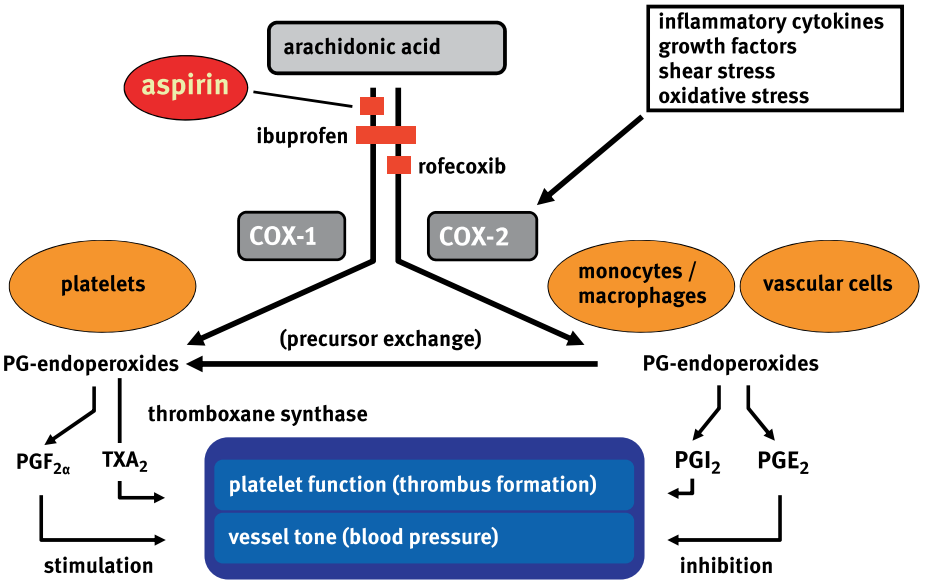

Figure 4.1.6-6: Arachidonic acid metabolism via COX-1 and COX-2 in the cardiovascular system in vivo. Shown are the modes of action of aspirin, ibuprofen (nonselective COX-1/COX-2 inhibitor) and rofecoxib (selective COX-2 inhibitor). Note the provision of prostaglandin (PG) endoperoxides to the (uninhibited!) platelet thromboxane synthase via an upregulated COX-2 from vascular cells (endothelium, smooth muscle cells) and monocytes/macrophages (for further explanations see text).

ated cells might clinically present as aspirin HTPR because of the lower sensitivity of COX-2 in nucleated cells to inhibition by aspirin in vivo.

This COX-2-mediated and platelet COX-1-independent thromboxane synthesis in platelets is largely aspirin-resistant. It causes increased urinary excretion of thromboxane (metabolites) but is not detected by measuring serum thromboxane levels. The only partial inhibition of renal 11-DH-TXB_2 excretion by aspirin by a maximum of 60–70 % in most studies at doses around 100 mg/day (Table 4.1.6-1), frequently associated with increased, aspirin-insensitive isoprostane release [64, 124], rather appears to indicate the activity and progression of the systemic atherosclerotic process. This incomplete inhibition of TX metabolite excretion by aspirin at low antiplatelet doses is not a pharmacological failure of the drug to act but rather suggests a transient and weak inhibition of COX-2 by aspirin in vivo, combined with platelet activation by nonaspirin-sensitive mediators (see below). Accordingly, even near complete inhibition of (platelet-derived) serum thromboxane by aspirin is not paralleled by complete inhibition of 11-DH-TXB_2 excretion (Table 4.1.6-1).

Aspirin-insensitive mechanisms of platelet aggregation and secretion. In contrast to in vitro testing, platelets in vivo will regularly become activated by multiple stim-

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uli that usually act in concert. Many of them are "aspirin-resistant" because they do not require activation of the platelet thromboxane pathway to become effective. Most important is thrombin, especially in situations of massive acute thrombin formation, such as acute ischemic syndromes [125]. Platelet activation by not only ADP [32, 126], higher concentrations of collagen and adrenaline [127], but also emotional stress [128, 129] and shear stress [130, 131], is largely aspirin-resistant. Aspirin does also not inhibit platelet stimulation by endogenous TXA_2 from whatever source. If these stimuli become critical to platelet activation, aspirin will not inhibit platelet aggregation/secretion despite sufficient inhibition of platelet-dependent thromboxane biosynthesis.

Similar considerations apply to isoprostanes, arachidonic acid metabolites that are formed by nonenzymatic, free radical catalyzed reactions [132]. Isoprostanes cause platelet activation possibly via the thromboxane (TP) receptor [133]. They are increasingly formed in vivo when COX activation and oxidant stress coincide, for example in stable and unstable angina [64] or type 2 diabetes [134]. Consequently, platelet activation by isoprostanes is not sensitive to aspirin [64]. Oxidative stress with its multiple effects on platelets, inflammation and endothelial function is considered a major contributor to HTPR in (type 2) diabetics [135].

Increased platelet turnover rate – immature platelets. Circulating human platelets express small amounts of immunoreactive COX-2 protein in addition to COX-1 [136]. The likely source of COX-2 are immature, reticulated platelets [137]. They are released from the bone marrow via the lung into the circulation and appear there as early as 4–6 hours after aspirin ingestion [138]. Diminished antiplatelet effects of aspirin and increased aspirin HTPR have been described in these immature platelets, possibly because of increased reactivity and uninhibited COX- activity (Section 2.3.1) [139, 140].

Increased platelet turnover, for example in myeloproliferative diseases and diabetes or platelet destruction by artificial surfaces, such as extracorporeal circulation [106], will result in an increased proportion of new, partially immature, reactive and aspirin-naïve platelets. These platelets might functionally antagonize the inhibition of COX-1-dependent thromboxane formation of aspirin-treated platelets as soon as they exceed a certain percentage (>5 %) of total circulating platelets. This and a "carry-over" of COX-2 from the bone marrow by immature platelets [141] possibly contribute to the reduced antiplatelet effects of aspirin in some type 2 diabetics and patients with essential thrombocytopenia [76, 137, 142–144]. This HTPR can possibly be overcome by shortening the aspirin treatment interval from once to twice daily (Fig. 4.1.1-6). Aspirin three times daily had no stronger effects but caused more gastrointestinal discomfort during a 2-week treatment period [145]. This twice daily approach of aspirin use in patients with enhanced platelet turnover was considered a new strategy to overcome aspirin HTPR in these patients [146]. One randomized double-blind trial confirming this concept in patients with essential thrombocythemia is available [145], those in other patients at elevated cardiovascular risk should follow (Section 4.1.1).

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Increased protein glycation. Several studies have shown insufficient inhibition of platelet function in diabetics by aspirin. In addition to shortened platelet survival, increased oxidative stress and increased turnover rates, hyperglycemia has been suggested as another variable to explain HTPR in diabetics with poor metabolic control. There is a competition in glycation between glucose and aspirin which might be overcome by increasing the aspirin dose [147]. Clinically, glycated albumin levels in type 2 diabetics have been reported not only to interact with antiplatelet treatment but also to be associated with a doubling of risk of recurrent stroke [148].

4.1.6.5 Clinical trials

General aspects. The importance of TXA_2 for vascular atherothrombotic events is underlined by studies demonstrating that enhanced urinary excretion of thromboxane metabolites in aspirin-treated individuals is associated with an increased risk of myocardial infarction or vascular death [33, 69, 149, 150]. This clinical outcome is clearly the most important information that is to be expected from all laboratory measurements of aspirin HTPR. Dependent on the method of measurement and the definition used, approximately 5–45 % of cardiovascular and 5–65 % of stroke patients are considered clinically "aspirin-resistant" [22, 24–27, 151, 152]. As outlined above, these numbers, elaborated by different techniques and protocols in highly variable clinical conditions, are hard to compare. More importantly, they are not equivalent to a pharmacological failure of aspirin to act, i. e., a true pharmacodynamic aspirin HTPR. In most cases, it are disease-related risk factors that cause a platelet hyperreactive state which is not necessarily aspirin-sensitive.

The dose issue. The "Aspirin-induced Platelet Effect Study" (ASPECT) by Gurbel and colleagues is one of the few prospective randomized trials designed to study aspirin HTPR in terms of different biomarkers and its modification by different doses in patients with coronary heart disease [46].

A total of 125 stable outpatients with coronary heart disease were randomized in a double-blind, double crossover investigation to receive aspirin at 81 mg, 162 mg and 325 mg daily for 4 weeks, each over a 12-week period. Platelet aggregation was determined by different contemporary assays and thromboxane metabolite excretion was measured in urine. The aim was to determine the degree of platelet responsiveness to aspirin, to compare the different techniques and to evaluate the relation of aspirin doses to platelet inhibition. "HTPR" was defined according to a standard protocol.

At any single dose, HTPR to aspirin was lowest – 0–6 % – in the overall group when arachidonic acid was used as the stimulus. It was increased to 1–27 % when other methods were used, the figures seen with PFA-100 being the highest (Table 4.1.6-3). Platelet responses to aspirin, as measured by collagen-, ADP- and PFA-100-induced light transmission, were dose-related (81 mg/day vs. 162 mg/day; $P \leq 0.05$) and there was also a dose-related inhibition of 11-DH-TXB_2 excretion.

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The inhibition became stronger with repeated dosing in all assays. No carry-over effects were observed.

The conclusion was that the assessment of aspirin HTPR is highly assay-dependent, arachidonic acid stimulation being the most sensitive stimulus. The dose-dependent effects despite nearly complete inhibition of arachidonic acid-induced aggregation suggest additional antiplatelet effects of aspirin that are COX-1-independent [46].

Table 4.1.6-3: Effects of assay and doses on measurement of aspirin HTPR in 125 patients with stable coronary heart disease. Abbreviations: LTA: light transmission aggregometry; TEG: thromboelastogram (for further explanations see text) (modified after [46]).

Technology for determination of aspirin resistance	Number of aspirin resistant patients according to definition					
	81 mg	162 mg	325 mg	1 dose	2 doses	3 doses
LTA-AA	2	1	0	2	1	0
LTA-Collagen	12	2	1	14	1	0
LTA-ADP	19	11	10	27	7	3
TEG-AA	5	3	5	11	2	0
PFA-100	32	14	21	42	15	5
urinary 11-DH-TXB_2	31	22	14	42	16	5
VerifyNow	7	4	4	13	2	0

This study and similar results from others in "aspirin-resistant" individuals [153] have resulted in considering arachidonic acid-induced platelet aggregation as the most specific assay for measuring platelet reactivity to aspirin. This is correct. However, pure arachidonic acid-induced platelet aggregation is not observed in vivo. Here, arachidonic acid release is simply an accompanying amplification step of platelet stimulation and not an independent acting factor by its own. Similar to measurement of thromboxane formation in serum, platelet aggregation, induced by added high-dose arachidonic acid is an in vitro artifact, but useful for analyzing the efficacy of aspirin to inhibit platelet COX-1-dependent thromboxane formation.

Aspirin HTPR and long-term cardiovascular outcome. A metaanalysis of 20 clinical studies including about 3,000 patients taking aspirin for secondary cardiovascular prevention reported laboratory aspirin HTPR in about 28 % of patients. This was associated with a 4-fold increased risk of adverse cardiovascular events. The conclusion was that patients biochemically identified as having laboratory aspirin HTPR are more likely to also have clinical HTPR to aspirin and a significantly higher risk of recurrent cardiovascular events as opposed to patients who are identified as (laboratory) aspirin-sensitive [152].

The possible predictive value of inhibition of platelet function by aspirin was analyzed in the prospective observational "Antiplatelet drug HTPRs and ischemic events

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study” (ADRIE) trial [57] in a total of 771 patients with symptomatic atherosclerotic disease. A total of 16 % of these patients suffered a major cardiovascular event during the observation period of 3 years. No differences in clinical outcome were seen between patients with and without HTPR (Fig. 4.1.6-7). Similar results were reported in the “Aspirin nonresponsiveness and clopidogrel in clinical endpoint trial” (ASCET) [58]. There were no differences in the clinical endpoint between patients with and without HTPR to either aspirin or clopidogrel. HTPR according to platelet function testing appeared not to have any predictive value for future major cardiovascular events in patients with stable angina [57, 58]. This confirms the suggestion that monitoring antiplatelet therapy should be considered for investigational purposes only [12].

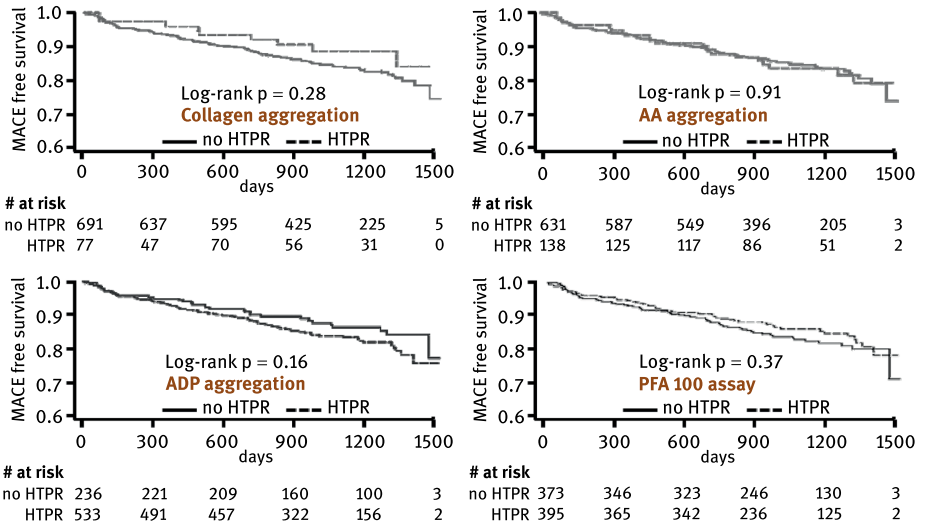

Figure 4.1.6-7: Major adverse cardiovascular event (MACE)-free survival curves for patients with and without “high on treatment platelet reactivity” (HTPR) evaluated in aggregation-based assays using collagen, arachidonic acid (AA), ADP and collagen/epinephrine as stimulating agents. Data were obtained from the ADRIE trial [57].

Alternatively, determination of thromboxane metabolite excretion was considered to allow a more useful vascular risk estimation since it considers more parameters than just platelet reactivity to aspirin. In 2002, Eikelboom and colleagues were the first to present data from a subgroup of patients of the HOPE trial, which demonstrated a worse clinical outcome in aspirin-treated patients with higher thromboxane metabolite excretion [33].

The HOPE study was designed to compare the ACE inhibitor ramipril and vitamin E with placebo for secondary prevention in high-risk cardiac patients. A total of 5,529 patients were enrolled and a retrospective subgroup analysis was done when the main study was finished, using a nested

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case-control design. All of the patients provided baseline urine specimens. Aspirin treatment was started at least 6 months before study entry. Thus, all (compliant) patients were on aspirin during the study and there was no urinary sample available from nonaspirin-treated patients, that is, no untreated control group.

During the 5-year follow-up, 488 of these patients suffered a heart attack, stroke or fatal vascular event. The urinary 11-DH-TXB_2 level of these patients was compared with 488 matched controls of the same study who did not suffer an event. It was found that with increasing urinary 11-DH-TXB_2 concentrations, there was an increasing risk for cardiovascular events. The difference was significant between the highest quartile of urinary 11-DH-TXB_2 excretion and the lowest one. The median urinary 11-DH-TXB_2 level was 22.8 ng/mmol creatinine in the myocardial infarction cases and 20.3 ng/mmol in the controls ($P = 0.001$), the median in cases of cardiovascular death was 24.0 ng/mmol creatinine in cases and 19.9 in controls ($P < 0.001$). No differences were seen with stroke.

The conclusion was that (i) high 11-DH-TXB_2 levels in urine of aspirin-treated patients are indicative of resistance to aspirin and (ii) these patients are at elevated risk for myocardial infarction and cardiac death [33].

This study was the first to show a statistical relationship between aspirin "HTPR" – here defined as insufficient inhibition of systemic thromboxane production – and cardiovascular risk. The study is used occasionally as an argument that aspirin "HTPR" of platelets might have deleterious consequences for the patient. This might be, but has not been studied in this trial. The pharmacological "HTPR" of the patients' platelets, for example by measuring serum TXB_2, was not determined. It is well known that a significant but variable proportion of excreted 11-DH-TXB_2, in most studies about one third at antiplatelet doses (Table 4.1.6-1), is not platelet COX-1-derived, and most likely made from prostaglandin endoperoxides of nucleated cells, that is, from nonplatelet sources. Finally, by comparison with other studies (Table 4.1.6-3), the differences between "resistant" and control patients suffering myocardial infarctions – 20.8 vs. 22.8 ng/mmol creatinine – were significant ($P = 0.001$) but small. In contrast to the complete HOPE study population, this subgroup also varied significantly in the cardiovascular risk profile – the infarct patients bore a markedly higher vascular risk. There was also no nonaspirin control group. The most likely explanation for the worse outcome of patients with elevated 11-DH-TXB_2 excretion might be their more advanced stage of atherosclerosis with an expectable poor prognosis.

Aspirin, HTPR and acute interventions. In an observational study, Chen and colleagues [154] reported that cardiac patients undergoing elective PCIs and combined treatment with aspirin and clopidogrel were more likely to have periprocedural myonecroses if their platelets were "aspirin-resistant" according to the VerifyNow-platelet assay. In a later prospective nonrandomized trial, these authors also found a higher incidence of atherothrombotic vascular events in aspirin-treated (80–325 mg/day) patients with stable CAD if they were "resistant" according to the VerifyNow-platelet assay [155]. In contrast, no increased appearance of myonecrosis in low-risk, "aspirin-resistant" patients undergoing elective PCI was reported by Buch and col-

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leagues [156] and also no difference was reported in a case-control study on cardiac patients with a history of myocardial infarction [157]. Both studies also used the VerifyNow technique.

Valles and colleagues reported an increase of myonecroses in "aspirin-resistant" patients with acute STEMI which they explained by thromboxane-dependent and -independent mechanisms. There was a lower in vitro sensitivity of platelet COX-1 to aspirin at the time of the acute event, which completely disappeared within the following 24–48 h [158]. Interestingly, the HTPR against aspirin-induced inhibition of thromboxane formation could be overcome by cotreatment with atorvastatin [159]. Since statins are known inhibitors of platelet function and thromboxane formation [160], their comedication might significantly improve the antiplatelet actions of aspirin in acute myocardial infarction. It is possible that the aspirin HTPR in the acute phase is caused by nonaspirin-sensitive platelet stimulation, for example by thrombin (Section 4.1.1), generated by a fresh platelet–fibrin clot, specifically in STEMI, the first 2–4 hours of ACS. At this time, platelets are hyperaggregable to ADP (Fig. 4.1.1-3) [161]. This might contribute to a refractory state of these platelets against inhibition by oral ADP antagonists, both from the thienopyridine type and ticagrelor, as seen from the FABOLUS-PRO trial [162] and the PRIVATE ATLANTIC platelet substudy [163]. This HTPR could be corrected by GPIIb/IIIa blockers, indicating that it is platelet-specific [162].

There are also mixed data with aspirin "HTPR" in DAPT of acute coronary interventions. The large-scale prospective registry study of the ADAPT-DES trial in patients undergoing PCI found no significant association between HTPR and ischemic events, including death and stent thrombosis [164], while another large-scale observational registry trial, the ISAR-ASPI registry, reported the opposite result, i. e., HTPR was associated with higher mortality and/or stent thrombosis [34]. Both studies differed in several methodological aspects, including methods of determination of platelet function. Thus, the clinical relevance of lower than usual inhibition of platelet function by aspirin as a determinant of cardiovascular risk is unclear but in all likelihood has little or nothing to do with a failure of aspirin to block platelet-dependent thromboxane formation.

4.1.6.6 Actual situation

According to current knowledge, true pharmacodynamic aspirin "HTPR" is a very random event affecting about 1 % of patients [17–19, 32, 46, 81]. Any laboratory HTPR does not reflect adequately the clinical reality of aspirin-related treatment failures. There are clinical data suggesting a relationship between clinical outcome, i. e., possible treatment failure in case of pharmacological aspirin HTPR [154, 155, 158]. However, large prospective randomized trials are still missing and it is entirely possible that the vast majority of treatment failures with aspirin is due to disturbed platelet sensitivity (hyperreactivity), platelet stimulation by aspirin-insensitive factors (thrombin, ADP,

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high shear stress) or low compliance. Specifically, a clear distinction should be made between poor responders on antiplatelet treatment, that is, potentially "resistant" patients, and those with a high residual platelet reactivity [44].

The general recommendation to date is that regarding the uncertainties of the transfer of measurements of platelet function in vitro into clinical reality, the absence of standardized procedures of measurement, the assay-related differences in results and the absence of proven, effective alternatives, patients should not be routinely tested for possible aspirin HTPR. Serum thromboxane levels serve as a useful predictor of the pharmacological potency of aspirin to block platelet COX-1, and that is the information which is really needed to prove that aspirin does what it is supposed to do: inhibit platelet COX-1-dependent thromboxane formation.

Summary

The responsiveness of platelets to antiplatelet drugs is known to be variable. In the case of clopidogrel this affects about 20–30 % of patients, has well-defined pharmacokinetic reasons and is associated with poor clinical efficacy. In contrast, aspirin HTPR, defined pharmacologically as an insufficient (<95 % of capacity) inhibition of serum thromboxane formation at standard aspirin doses (75–325 mg/day), is rare and occurs in about 1 % of patients. Clinical treatment failures are much more frequent but in most cases due to medical conditions of the patient rather than due to a failure of aspirin to act.

There are two frequently used laboratory methods to test platelet sensitivity to aspirin: measurement of platelet function (ex vivo) or measurement of inhibition of thromboxane formation, for example in terms of serum TXB_2 or thromboxane metabolite (11-DH-TXB_2) excretion in urine. Both methods have limitations and measure different signals. Specifically, there is a wide variation in 11-DH-TXB_2 excretion and no definition of a normal range, while assays of platelet function have a low predictability and may give different results according to the particular technique and protocols used. Urinary 11-DH-TXB_2 levels might be a surrogate for the overall severity of systemic atherosclerosis and, therefore, a risk predictor for acute vascular atherothrombotic events.

The important issue of a possibly causal relationship between insufficient antiplatelet effects of aspirin and clinical outcome is still a matter of discussion. The about 20–45 % "aspirin-resistant" patients in clinical trials rather confirm the well-known platelet hyperreactivity (HTPR) in relation to the severity of atherosclerosis or acute platelet stimulation by aspirin-insensitive stimuli (thrombin, ADP) in acute prothrombotic situations, i. e., PCI and ACS. There is no alternative to aspirin with respect to its mode of action and no reason to test routinely for a however defined aspirin HTPR.

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4.2 Pain, fever and inflammatory diseases

The therapeutic use of aspirin as an antipyretic analgesic is still the domain of its practical use as an OTC medicine in self-medication of headache or other forms of painful conditions, including treatment of feverish discomfort (Section 4.2.1).

In contrast, aspirin is no longer the drug of choice for treatment of local inflammatory pain, because of the availability of more potent antiinflammatory analgesics. However, some of the patients who suffer from (chronic) inflammatory pain are also at elevated risk for atherothrombotic events and might need regular aspirin intake for cardiovascular prevention. Here, the combined use of aspirin with NSAIDs or coxibs might result in negative drug interactions with the antiplatelet effects of aspirin (Section 4.1.6). Attractive new indications for aspirin are SIRS with the complications of

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sepsis and ARDS as well as possible immunomodulatory effects in patients with HIV. In these cases, there might be a clinically relevant role for platelets as starters or amplifiers of inflammatory/immunological reactions and a role for aspirin as an adjunctive treatment (Section 4.2.2). A most exciting new issue is the question whether the approved clinical treatment of "flu-like" conditions in adults with aspirin might also be extended to another actual flu-like clinical condition – COVID-19 (Section 4.2.2).

Kawasaki's syndrome is an inflammatory disease in children where aspirin is still used as a standard medication at high doses together with immunoglobulin in order to prevent vascular complications of the disease. Here, both antiinflammatory and antiplatelet effects of the compound might contribute to its clinical efficacy in preventing immune vasculitis, coronary aneurysms and myocardial infarctions in affected children at enhanced risk (Section 4.2.3).

4.2.1 Analgesia and antipyresis

4.2.1.1 General aspects

A piece of history. When aspirin was introduced as a medicine at the beginning of the twentieth century, there was a widespread need for a reliable analgesic/antipyretic agent that was better tolerated than the available products, including (sodium) salicylate and should be at least as effective or even more potent than the natural compound. On this background, scientists at Bayer extended the succesful strategy of acetylation of natural compounds to reach this goal to salicylic acid (Section 1.1.2). The product, acetylated salicylic acid was thought to be an inactive prodrug of the active metabolite salicylate. There was no free salicylate inside the stomach lumen, avoiding any physical contact with the stomach mucosa because absorption of intact acetylsalicylic acid and the release of the salicylate metabolite started only in the alkaline conditions of the upper intestine [1]. It should be added that nothing was known at the time about the pharmacokinetics and mode of action of aspirin, except that it was considered not to be a "poison for the heart" [1].

This view of aspirin being only the prodrug of the active metabolite salicylate has changed fundamentally after the multiple and complex pharmacological actions of aspirin were stepwise elucidated. A first milestone was the detection by Sir John Vane that aspirin inhibited prostaglandin biosynthesis, suggesting that this might explain its antipyretic and antiinflammatory actions (Fig. 1.1.3-2) [2]. This *in vitro* assay made it unlikely that salicylate was the (sole) active component and aspirin just an inactive precursor. Subsequent discoveries have further extended the knowledge about the pharmacological profile of aspirin as an antiinflammatory analgesic. Further possible modes of analgesic actions as well as interactions with other central and peripheral pain mediator systems were detected and are outlined in detail in Section 2.3.2.

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4.2.1.2 Pain, fever and mode of analgesic/antipyretic aspirin action

Pain and mode of analgesic actions of aspirin. Pain can result from many reasons and is mediated by both peripheral and central mechanisms of pain perception. Both involve prostaglandins. A contribution of other pain-mediating systems is likely. There are multiple synergisms between the different pain-controlling signaling pathways which are practically used in (fixed) drug combinations to enhance the analgesic efficacy, for example for treatment of headache.

Both the analgesic and antipyretic actions of aspirin are dose-dependent. Aspirin-sensitive synthesis of prostaglandins, the key mediators for pain receptor sensitization (allodynia/hyperalgesia) after tissue injury, also contributes to pain transmission and perception in peripheral nerves and the CNS (Fig. 2.3.2-7) [3]. Functional magnetic resonance imaging (fMRI) has identified distinct areas in the human brain that become activated during acute mechanical pain and are involved in the analgesic action of COX-2 inhibition by parecoxib as well as of aspirin [4]. A more recent fMRI study has confirmed a decreased activation of the anterior cingulate (ACC) and secondary somatosensory cortex (SII) by aspirin in response to trigemino-nociceptive stimulation [5]. It is likely that aspirin in addition to (peripheral and central) inhibition of prostaglandin biosynthesis also interacts with other mediator systems of pain control and pain perception, such as endocannabinoids (anandamide) and serotonin. Unfortunately, and in contrast to basic research on the pathomechanisms of inflammation and immune reactions, there is little clinical mechanism-focused research on the pharmacological mechanisms of pain relief by aspirin and other nonopioid analgesics.

Determination of analgesic efficacy – the placebo effect. Pain and its relief by drugs is a highly subjective experience [6]. Therefore, the patient's self-report, for example by using a visual analog scale, provides a most valid measure for the individual intensity of pain sensitization. In many studies using this method, an at least 50 % reduction of pain intensity over 6 hours is considered clinically significant. In addition, all mechanistic as well as clinical studies on pain have to consider the high placebo rate, underlining the significance of the subjective pain perception and the requirement of an adequate placebo control to determine the analgesic efficacy of drugs. The placebo effect amounts to about 50 % of the drug effect [7]. This is evident from a large metaanalysis of 198 studies on pain relief in osteoarthritis, comparing placebo (193 studies) (RR: 0.51) with no treatment (14 studies) (RR: 0.03) versus standard conservative/surgical procedures [8]. According to these findings, aspirin is an effective analgesic for acute pain of moderate to severe intensity. Higher doses are more effective, but are associated with increased adverse events, including drowsiness and gastric irritation. The pain relief achieved with aspirin is very similar to that seen with paracetamol. There is a significant benefit (estimated in terms of patients reporting at least 50 % pain relief) with aspirin as compared to placebo. The analgesic effect is dose-

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Table 4.2.1-1: Total pain relief (TOTPAR) by aspirin in placebo-controlled trials. Results refer to the number of participants with ≥50 % pain relief over 4–6 hours. Note the dose dependency of the analgesic effect as shown by the decreasing number of patients needed to treat (NNT) with increasing aspirin doses [9].

aspirin dose (mg)	#of trials	# of participants	# of patients [%] with at least 50 % pain relief		TOTPAR relative benefit (98 % CI)	NNT (95 % CI)
			aspirin	placebo		
500	2	213	33	26	1.3 (0.8–2.0)	Not calculated
600 / 650	60	4,630	39	15	2.5 (2.2–2.7)	4.2 (3.9–4.8)
900 / 1000	6	618	41	14	2.7 (2.0–3.7)	3.8 (3.0–5.1)
1200	3	249	61	23	2.9 (2.0–4.2)	2.7 (2.0–3.8)

dependent and becomes significant at doses of 600 mg and more without significant differences between the causes of pain (Table 4.2.1-1) [9].

Pain models. About two thirds of experimental studies on pain have used dental pain, mostly the extraction of the third molar (wisdom tooth), as a pain model. It might well be that findings on this postsurgical pain differ from inflammatory, prostaglandin-mediated pain or the pain of headache, including tension-type headache (TTH) and migraine. After an original report, demonstrating remarkable differences in the analgesic potency of aspirin in different situations of clinical (gynecological/obstetric) pain [6], many follow-up studies have addressed the issue of possible influences of the pain model on the intensity of the analgesic effect of aspirin and other analgesics. According to current knowledge, the pain models used (postoperative, episiotomy, dental pain, etc.), the kind of measurement and the duration of the observation period have no effect on the magnitude of analgesia by aspirin. A metaanalysis has added further evidence to this. The investigators calculated the "under the pain relief versus time curve" equivalent to at least 50 % maximum pain relief over 6 h in dental and postsurgical pain. No major difference was obtained for the two analgesics studied: aspirin (600/650 mg) and paracetamol (acetaminophen) (600/650 mg). This type of studies has limitations, for example by using a pain calculation model rather than an individual pain response. However, a dose dependency of analgesic actions of aspirin, acetaminophen (paracetamol) and ibuprofen was also seen in a systematic review of randomized double-blind trials in acute pain [10]. A recent metaanalysis of 13 randomized trials on the analgesic potency of aspirin (single dose) in randomized trials for treatment of perineal pain in the early postpartum period has confirmed meaningful pain relief for 4–8 hours after aspirin administration (300–1,200 mg) for twice as many women compared to women on placebo treatment (RR: 2.03; 95 % CI: 1.69–2.42) [11].

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Adverse effects. A Cochrane analysis on the analgesic efficacy of aspirin for treatment of acute postoperative pain in adults analyzed 68 randomized, placebo-controlled trials with aspirin at doses between 300 and 1,200 mg. The efficacy parameter was total pain relief over 4–6 hours in participants achieving at least 50 % pain relief.

The NNT was four at a dose of 1,000 mg. A total of 12 % of patients on aspirin and 10 % of patients on placebo reported adverse effects, most frequently drowsiness and gastric irritation. The NNH was 44, i. e., 10-fold higher than the NNT (Table 4.2.1-2) [12]. Importantly, bleeding was not an issue or noteworthy side effect of single or short-term aspirin use at these doses.

Table 4.2.1-2: Relative risk (HR) of adverse effects to aspirin in analgesic doses in placebo-controlled trials as documented by the number needed to harm (NNH). nc: not calculated because the HR was <1 [12].

Dose		Number of trials	Patients with adverse effects with		Relative risk (98 % CI)	NNH (95 % CI)
			aspirin	placebo		
All doses	Total adverse effects	60	313/2,619	261/2,660	1.3 (0.0–1.5)	nc
Aspirin 600/650 mg	Total adverse effects	53	257/1,976	229/2,088	1.2 (1.03–1.4)	44 (23–345)
	Dizziness	30	41/1,429	27/1,557	1.6 (0.9–2.6)	nc
	Drowsiness	33	103/1,542	56/1,672	1.9 (1.4–2.5)	28 (19–52)
	Gastric irritation	11	20/546	6/562	2.5 (1.2–5.1)	38 (22–174)
	Headache	29	34/1,237	56/1,363	0.7 (0.4–1.02)	nc
	Nausea	34	54/1,563	68/1,683	0.8 (0.6–1.2)	nc
	Vomitting	21	12/835	18/927	0.7 (0.4–1.6)	nc

The conclusion was that aspirin is an effective, single-dose analgesic also in postoperative pain. The analgesic effect is dose-dependent and the potency is comparable with that of acetaminophen [9, 12]. However, there are large differences between different trials and not all reviews, including a summary of 10 Cochrane reviews, came to the same conclusion by comparing nonprescription oral analgesics with respect to potency and side effects for treatment of acute pain [13].

Aspirin formulations. The analgesic efficacy of aspirin at a standard single dose of 0.5–1.0 g will be stronger for pharmacokinetic reasons if the compound is given in a predissolved or water-soluble formulation [14]. This results in a more rapid increase in plasma levels (Section 2.1.1) and a more rapid onset and initially higher efficacy of the compound on pain relief (Fig. 4.2.1-1) [15, 16].

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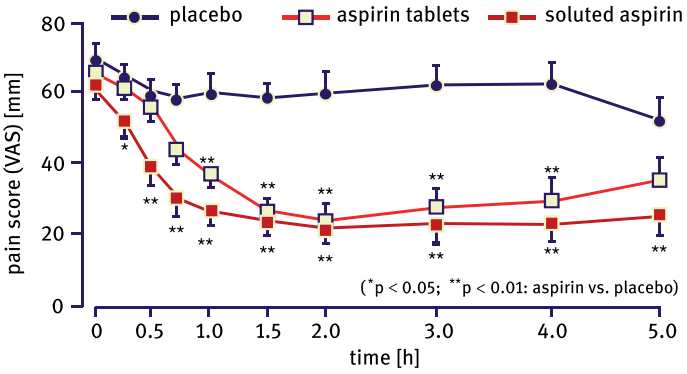

Figure 4.2.1-1: Analgesic potency of aspirin tablets and dissolved aspirin (1.2 g each) in comparison to placebo as determined by individual pain sensitization (postsurgical pain after tooth extraction) (visual analog scale [VAS]). Note the more rapid start of analgesia with soluted aspirin, an initially stronger action of soluted vs. undissolved aspirin at 0.3–1 h and a potency similar to standard aspirin tablets at 2–5 h (modified after [14]).

Soluble aspirin was also found to be more potent than solid acetaminophen in postsurgical pain [17]. Commercial effervescent formulations allow to obtain the same peak plasma level, peak concentrations and half-life as plain aspirin tablets. The time to reach peak plasma levels is considerably shortened to about 30 min instead of 1 h for the plain preparation [18]. Similar benefits are obtained with a mouth-dispersible formulation [19]. The most recent development is a new micronized, fast disintegrating aspirin formulation, allowing not only fast absorption but also significantly higher peak plasma levels of the unmetabolized compound (Fig. 2.1.1). This is associated also with faster and more intense pain relief as compared to the standard plain formulation (Fig. 4.2.1-2) [20].

Another possibility to obtain effective plasma levels even faster is the application of injectable aspirin water-soluble lysine salt (LASAG) (Section 2.1.1). This is of particular advantage in migraine attacks where nausea and vomiting frequently occur.

Fever and mode of antipyretic actions of aspirin. Fever associated with upper respiratory tract infections is of suspected viral origin and therefore mainly subject to symptomatic treatment of the unpleasant symptoms: fever, fatigue, headache and others. Mechanistically, aspirin interferes with endogenous pyrogens and ameliorates subsequent cytokine generation and action as well as amplification of these effects by prostaglandins. This also includes reduction of the upregulated core temperature (Section 2.3.2).

Aspirin does not interact with the physiological temperature control. Therefore, there is no change in normal body temperature by aspirin intake. A significant part of the antipyretic action of aspirin is mediated by salicylate (Fig. 2.3.2-13) [21], due to

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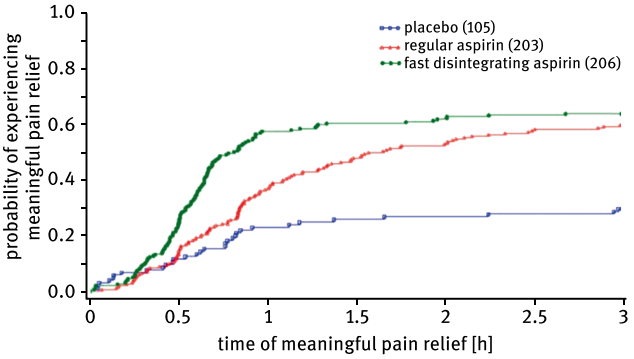

Figure 4.2.1-2: Time to meaningful (according to the patients feeling) pain relief after 500 mg oral aspirin in different galenic preparations given to subjects with postsurgical pain (tooth extraction). The study was randomized, double-blind with the numbers of participants in brackets. Note the faster onset and higher peak level of fast disintegrating aspirin [20].

uncoupling of oxidative phosphorylation as seen from increased oxygen uptake [22], and is associated with sweating, i. e., increased heat loss.

4.2.1.3 Clinical trials

Flu and other feverish diseases. Aspirin as a household remedy is frequently used for treatment of influenza-like symptoms (headache, frontal and maxillary sinus sensitivity to percussion, sore throat, achiness and feverish discomfort). These symptoms typically last for 3–5 days. They are not life threatening but markedly reduce the well-being. The maintenance or restoration of normal daily activity by reducing fever and influenza-like symptoms is the treatment goal and patients frequently use OTC antipyretics for this purpose. Among them, aspirin, ibuprofen and acetaminophen are the most commonly employed drugs.

A prospective, placebo-controlled randomized double-blind trial has compared the antipyretic potency of aspirin with that of acetaminophen in adults. The patients suffered from an acute, noncomplicated infection of the upper airways which was likely to be of viral origin. Patients were treated with single doses of aspirin (500 or 1,000 mg), acetaminophen (500 or 1,000 mg) or placebo. Body temperature was measured in regular intervals; feverish discomfort was evaluated on an interview basis. The total observation period was 6 h.

The average body temperature before treatment was 38.8 °C and remained essentially unchanged over the observation period of 6 h in the placebo group. Both aspirin and acetaminophen reduced the temperature to about 38.0 °C and 37.5 °C after single doses of 500 mg and 1 g, respectively. The antipyretic effect started 30 min after dosing and lasted for at least 6 h. The maximum effect was obtained 2.5–3 h after drug administration. Both compounds were about equipotent, also with respect to improvement of feverish discomfort, headache and achiness. There was no significant difference in side effects between aspirin, acetaminophen and placebo.

The conclusion was that aspirin and acetaminophen are equipotent antipyretics. The action is dose-dependent and there are no differences in side effects (Fig. 4.2.1-3) [23].

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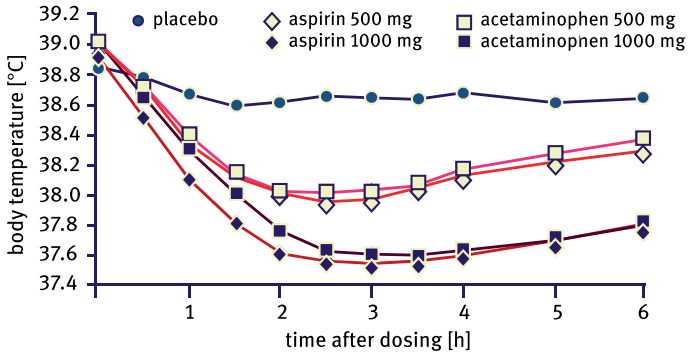

Figure 4.2.1-3: Time course of orally measured body temperature in volunteers with acute uncomplicated febrile upper respiratory tract infection of suspected viral origin. Patients were treated with single-dose aspirin, acetaminophen or placebo. Data are the mean of 78–79 persons per treatment group (modified after [23]).

Interestingly, in vivo and in vitro data suggest that (high-dose) aspirin and salicylate but not traditional NSAIDs will also inhibit the replication of rhinoviruses by interaction with their replication machinery in host cells (Section 2.3.2) [24, 25]. There is at least one double-blind clinical trial in patients suffering from viral infections, suggesting faster recovery and improvements of symptoms after high-dose (3.26 g/day) aspirin as opposed to the virostatic amantadine. However, this high aspirin dose was associated with a number of side effects [26]. Another double-blind trial suggested a markedly improved immune response to influenza vaccine (antibody titer) by simultaneous aspirin administration [27]. Although this has not been studied more systematically, any antiviral action of aspirin will add to the antipyretic action of the compound and is highly welcome. In the absence of any approved medicine for treatment of COVID-19, there is currently some speculation about a possible use of aspirin [28], a drug with well-established antiinflammatory/antithrombotic properties and approval for treatment of "flu-like symptoms" in adults. Because of its tight relation to inflammatory conditions, this discussion is outlined in more detail in Section 4.2.2.

Headache. One of the most frequently occurring forms of pain is primary headache. Two forms exist: TTH and migraine. Treatment in either case is rather single-dose or (repeated) short-term during attacks, frequently by self-medication. Patients are young or middle-aged, frequently female and usually otherwise healthy. They need particular attention for gastric tolerance of drugs, because in situations such as acute migraine attacks, patients may experience nausea or vomiting. Thus, rapid onset of action and less irritation or even bypass of the stomach are desirable.

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Migraine. Aspirin is well established for treatment of acute migraine attacks [29–35]. Diener and colleagues have reviewed the evidence for aspirin as a drug of first choice in treatment of acute migraine attacks in a number of randomized double-blind studies [18]. Overall, these studies showed not only significant beneficial effects of aspirin but also an increased efficacy and improved tolerability by a buffered effervescent preparation. This combination was found to be at least as effective as the combination of aspirin with metoclopramide [36], in all but one [33] study. Similar results were reported for the combination of lysine aspirin plus metoclopramide vs. ergotamine plus caffeine in relieving migraine attacks [37]. Aspirin (plus metoclopramide) was equieffective with triptanes (sumatriptan, zolmitriptan and others) (Table 4.2.1-3) [36, 38, 39]. A metaanalysis of three randomized placebo-controlled trials of effervescent aspirin (1 g) vs. sumatriptan (50 mg) or placebo showed equipotency of aspirin with suma-

Table 4.2.1-3: Prospective double-blind randomized trials with aspirin in migraine: a comparison of three different aspirin formulations vs. other analgesic monotherapy or placebo [19, 29–33, 45, 46]. Abbreviations: G: galenics: p: plain; e: effervescent; i: injectable (lysine salt); PLA: placebo; ERG: ergotamine; SUM: sumatriptan; IBU: ibuprofen.

type of study	G	#	clinical endpoint	outcome	reference
double-blind, parallel; aspirin 1000 mg vs. PLA	p	485	% of patients with two-step improvement on a four-step scale after 2 h	pASA ≫ PLA	[31]
double-blind, parallel; aspirin 900 mg vs. PLA	p	101	% of patients with two-step improvement on four-step scale after 2 h	pASA ≫ PLA	[19]
double-blind, parallel; iLAS (=500 mg aspirin) vs. PLA.	i	40	mean pain reduction on a ten-point VAS	iASA ≫ PLA	[29]
double-blind, cross-over; iLAS 1000 mg vs. ERG 0.5 mg sc.	i	56	pain reduction on a ten-point VAS	iASA = ERG	[30]
double-blind, parallel; iLAS 1000 mg vs SUM 6 mg sc. vs. parenteral PLA	i	279	% of patients with two-step improvement on a four-step scale after 2 h	SUM > iLAS ≫ PLA	[33]
double-blind, parallel; eASA 1000 mg vs. ePLA	e	343	% of patients with two-step improvement on a four-step scale after 2 h	eASA ≫ PLA	[47]
double blind, cross-over; eASA 1000 mg vs SUM 50 mg vs IBU 400 mg vs. PLA	e	312	% of patients with two-step improvement on a four-step scale after 2 h	eASA = SUM = IBU ≫ PLA	[32]
double-blind, parallel; eASA 1000 mg vs. SUM 50 mg vs PLA	e	433	% of patients with complete remission of nausea, photo- and phonophobia after 2 h; % of patients with headache relief	eASA = SUM ≫ PLA	[46]

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triptan, both being significantly more effective than placebo ($P < 0.001$). However, aspirin caused fewer side effects than sumatriptan. This resulted in the recommendation of (effervescent) aspirin as first choice for treatment of migraine attacks and to use a triptan in case of no response [40, 41]. In another Cochrane analysis, 1,000 mg aspirin was shown to be effective for acute migraine headaches, similar to 50 or 100 mg sumatriptan. Addition of 10 mg metoclopramide improves relief of nausea and vomiting. Adverse events were mainly mild and transient, and were slightly more common with aspirin than placebo, but less common than with sumatriptan 100 mg [42]. Aspirin along with ibuprofen and acetaminophen (for nonincapacitating attacks) became level A recommendation for acute treatment of migraine in adults according to the 2015 edition of the American Headache Society [43]. A Cochrane review of six studies with 900 or 1,000 mg acetylsalicylic acid found a significant superiority over placebo for the endpoint of being pain-free after 2 hours in the treatment of migraine attacks (RR: 2.08; 95 % CI: 1.7–2.6).

Of particular interest with respect to both efficacy and side effects is the use of aspirin in mixed combinations, most frequently with paracetamol and caffeine. A first efficacy metaanalysis versus placebo confirmed the superiority of the combination over placebo – as expected – but also showed a greater number of side effects [44].

A recent metaanalysis of randomized, blinded, placebo-controlled trials comparing fixed-dose combinations of aspirin, paracetamol and caffeine (APC) determined the rate ratio (RR) associated with APC versus placebo in treatment of acute migraine attacks. Seven studies with 3,306 participants (2,147 treated with APC and 1,159 treated with placebo) were included. The primary efficacy outcome was being pain-free at 2 h, the recommended primary outcome for the treatment of acute migraine attacks by the International Headache Society.

APC was superior to placebo (19.6 % vs. 9.0 %; RR: 2.2; 95 % CI: 1.4–3.3). For the coprimary efficacy outcome, pain relief at 2 h, APC was superior to placebo (54.3 % vs. 31.2 %; RR: 1.7; 95 % CI: 1.6–1.9). Adverse events were more frequent in the APC group than in the placebo group (10.9 % vs. 7.8 %; RR: 1.7; 95 % CI: 1.3–2.2).

The conclusion was that APC is superior to placebo in the treatment of acute migraine attacks. Efficacy, measured by the pain-free response and pain relief at 2 h, was clinically relevant [44].

The totality of evidence suggests that aspirin at doses from 900 to 1,300 milligrams taken at the onset of symptoms is an effective and safe treatment option for acute migraine headache. In addition, daily aspirin in doses from 81 to 325 mg may be an effective and safe treatment for the prevention of recurrent migraine headaches. The relatively favorable side effect profile of aspirin and its extremely low costs compared with other prescription drug therapies including triptanes may provide additional options for primary healthcare providers treating acute as well as recurrent migraine-type headaches [47].

Tension-type headache. TTH, also known as “normal” or “ordinary” headache, is the most frequent form of headache and probably the best pain “model” in real life. It is a

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"featureless" disease, characterized by nothing but pain in the head. The pathophysiology is unknown but most likely complex. TTH may be episodic or chronic, when occurring at more than 15 days a month. The lifetime prevalence of TTH amounts to 79 % with 3 % suffering from the chronic form [48]. Psychical stress may be involved, as well as musculoskeletal functional or structural abnormalities, such as tension in the head and neck regions. Treatment is usually by OTC self-medication. In contrast to migraine, triptanes do not work in TTH, pointing to a different pathophysiology of the cause of headache.

In a double-blind, placebo-controlled trial, 500 or 1,000 mg aspirin was compared with 500 or 1,000 mg acetaminophen, in a total of 572 compliant individuals. These persons suffered from episodic TTH (not migraine). Treatment was by single doses and the primary endpoint was subjective pain relief (total or worthwhile) after 2 h. Additionally, individual severity of pain was measured by a visual analog scale. Compliance was also controlled.

Aspirin at 1,000 mg had a 76 % responder rate and aspirin at 500 mg had a 70 % responder rate. The responder rates with acetaminophen were 71 % at 1,000 mg and only 64 % at 500 mg. With the exception of 500 mg acetaminophen, all treatments were significantly more effective than placebo with a 54 % responder rate. Outcome was not affected by headache intensity at baseline. Adverse events were reported by 13–19 % of subjects and were mild or moderate. No safety concerns arose.

The conclusion was that 1,000 mg aspirin in moderate to severe headache is significantly more potent than placebo. Aspirin at 500 mg and acetaminophen at 1,000 mg are also effective, but to a lower extent, while 500 mg acetaminophen is ineffective. As expected, there was a high placebo rate [49].

Similar results were obtained in other trials, leading to the conclusion that aspirin could also be considered as first-line treatment in episodic TTH [50]. A recent Cochrane analysis on the use of single-dose aspirin (500 or 1,000 mg) for treatment of episodic tension-time headache came to a slightly more restrictive conclusions: Single-dose aspirin between 500 and 1,000 mg provided some benefit in terms of less frequent use of rescue medications and more participants were satisfied with treatment compared with placebo in adults with frequent episodic TTH who had an acute headache of moderate or severe intensity. There was no difference between a single dose of aspirin and placebo with respect to the number of people experiencing adverse events. However, according to the authors, the amount and quality of the evidence of available studies was very limited for the comparisons between aspirin and placebo and the authors were very uncertain about the results which should be interpreted with caution [51].

4.2.1.4 Aspirin and other drugs

Aspirin, ibuprofen and acetaminophen are the most frequently used antipyretic analgesics. The potency appears to be similar. Aspirin has the advantage to be available in several galenic forms, eventually resulting in faster absorption and faster onset of the analgesic action. It will be interesting to see whether the new fast disintegrating

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formulation of micronized aspirin will do better than the conventional ones. There is no evidence for habit-forming conditions, also after comedication of caffeine or other compounds, such as paracetamol or metoclopramide. The addition of caffeine (≥100 mg) to a standard dose of commonly used analgesics provides a small but clinically relevant improvement of the analgesic efficacy [52]. In addition, coadministration of ascorbic acid has been shown to protect the human stomach from aspirin-induced mucosal injury, possibly by its antioxidant properties [53]. Drowsiness and stomach irritation are possible side effects of short-term use but not bleeding events [9].

4.2.1.5 Actual situation

Aspirin, for example in an effervescent or the new fast disintegrating formulation, is an effective and widely used OTC medication for TTH. The compound is also effective in migraine and other forms of postsurgical or inflammatory pain. The recommended single analgesic dose is 1 g. A metaanalysis of nine clinical trials with this single dose of aspirin in typical OTC medications including TTH showed that 6.3 % of patients on aspirin and 3.9 % of patients on placebo showed adverse effects. Only 3.1 % of patients on aspirin and 2.0 % of patients on placebo reported drug-related gastrointestinal adverse effects [54]. In case of aspirin intolerance or inefficacy, acetaminophen and ibuprofen are alternatives. However, ibuprofen might pharmacologically interact with aspirin and antagonize its antiplatelet effects if regular intake of aspirin is necessary for cardiovascular prevention (Section 4.1.1).

Overall, from a pharmacologist's point of view, the quality and clinical efficacy of nonopioid analgesics are unsatisfactory. There is a definite need to develop new classes of analgesics that more specifically interact with the individual pain perception and transmission pathways, allowing for a more targeted treatment than is currently possible. Attractive new targets are endocannabinoids (anandamide) and other lipid mediators. Unfortunately, available clinical analgesic trials, specifically those on treatment of TTH, are not only frequently of poor quality [9, 51] but also include heterogenous patient populations and use crude global outcomes, such as visual analog scale measures. Although the determination of pain severity and its modification by drugs at an individual basis is important, more mechanism-oriented basic research is highly desirable [55].

Summary

Aspirin is an effective antipyretic analgesic for treatment of several forms of acute pain of moderate to medium severity. Drowsiness and stomach irritation but not bleeding are the most frequent side effects.

For treatment of postsurgical pain, migraine and TTH, aspirin is an established medication with few side effects and low costs. The recommended single dose is 0.5–1 g and the efficacy can further be increased by using effervescent preparations, the new micronized fast-release tablet or parenteral application of water-soluble aspirin salts. For treatment of headache, combined admin-

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istration with paracetamol or caffeine might increase the efficacy while comedication of metoclopramide in migraine attacks might help against nausea and vomiting.

Aspirin is also an effective antipyretic for symptomatic treatment of fever and flu-like conditions. Doses around 1 g in adults start to reduce the elevated body temperature at about 0.5 h after intake with maximum effects at 1–3 h. This is associated with an improved overall feeling. Health authorities have made restrictions regarding aspirin use in (small) children because of the risk of Reye's syndrome. This issue and its actual clinical and research background are outlined and critically discussed in Section 3.3.3.

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4.2.2 Inflammatory diseases and viral infections

4.2.2.1 General aspects

A piece of history. Aspirin was originally introduced into the clinics for treatment of chronic inflammatory pain, associated for example with rheumatoid arthritis and osteoarthritis. Aspirin was introduced to replace salicylate in these indications because

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of tolerance problems with salicylate at the high doses of several grams that had to be used by the patients over days and weeks [1]. Aspirin was better palatable, more potent and caused less gastric discomfort.

The antiinflammatory potency of aspirin was originally thought to be entirely due to its salicylate metabolite [2]. Salicylates, specifically the salicylate ester salicin, are natural products and are considered to be the active antiinflammatory, antipyretic and analgesic ingredient of plant extracts, prepared from willow bark and other natural sources (Section 1.1.1). The antiphlogistic/analgesic efficacy of these preparations has been convincingly demonstrated in placebo-controlled randomized trials [3, 4]. A detailed study on the pharmacology of salicylate, salicylate analogs and derivatives prepared from willow bark is available [5].

It is currently under discussion whether salicylates alone and/or other constituents of these extracts account for the pharmacological efficacy of willow bark. Peak plasma levels of salicylate after oral intake of an extract of willow bark in an analgesic dosage – here equivalent to 240 mg of salicin – were only 1.2 µg/ml. The total systemic bioavailability of salicylate in plasma corresponded to the amount that was found after oral intake of 87 mg of standard aspirin. This strongly suggests that additional factors or nonsalicylate constituents of the willow bark extract contribute to the clinical efficacy of the preparation [6, 7].

A new discovery was the detection of antiplatelet/antithrombotic actions of aspirin which were not shared with salicylic acid and other natural sources of salicylates at comparable doses. The "aspirin-like" NSAIDs rather increased the vascular thrombotic risk despite (reversible) inhibition of platelet thromboxane formation. These findings, accompanied by an improved understanding of the pathophysiological background of inflammation and immune reactions, have also extended our current understanding of aspirin's complex pharmacological mode of antiinflammatory/immunomodulatory actions. Platelet-initiated immunothrombotic effects including thrombin formation and NETosis and their inhibition by aspirin are currently central research tools in sepsis and ARDS [8, 9]. Clearly, at high doses of several grams of aspirin per day, salicylate will additionally exert antiinflammatory effects by its intrinsic physicochemical properties and will accumulate inside (mitochondrial) membranes, uncouple oxidative phosphorylation and trigger multiple follow-up actions on cellular energy metabolism and signal transduction (Fig. 2.2.3-2).

Modes of aspirin action. The major mechanistic cellular mode for these widespread effects of aspirin is the acetylation of target proteins, most notably COX-1/COX-2 and eNOS. This inhibits many platelet functions, including inhibition of generation of platelet-derived inflammatory and immunothrombotic mediators (Section 2.3.1). Beyond thromboxane A_2, these include HMGB-1 [10], S1P [11] and a number of other chemicals (Fig. 2.3.2-4). Another COX product is ATL, an antiinflammatory and inflammation-resolving compound that is formed by acetylated COX-2 in coop-

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eration with white cell lipoxygenases (Section 2.3.1) [12]. In this context, platelets are increasingly considered as important trigger cells that combine thrombotic with proinflammatory and immune reactions [13–15].

Aspirin and salicylates also inhibit virus infections [16]. Unique was here aspirin's mode of action: inhibition of virus replication in the host by modulation of signaling pathways that are required for viral propagation instead of attacking viral genetic structures with their high mutation rates [17]. These actions of salicylate, like the metabolic effects, required higher local concentrations than required for COX inhibition. They became of renewed pharmacological interest with the increasing knowledge about the complex mechanisms of the molecular aspects of inflammation and immune reactions. For these reasons, aspirin is currently investigated as a possible adjunct for treatment of COVID-19 [18] and other viral affections via the respiratory tract.

This section discusses the role of aspirin in systemic inflammatory/immune reactions, rheumatoid arthritis and osteoarthritis as the most common forms of chronic inflammatory and painful diseases. Another topic are acute inflammatory/thrombotic conditions such as SIRS, sepsis and ARDS. Finally, viral infections and their ability to produce a "thrombotic storm" in human immunodeficiency virus (HIV) and COVID-19 infections are discussed, including a hypothesis about the possible role of aspirin in treatment of COVID-19 [18].

4.2.2.2 Rheumatoid arthritis – pathophysiology, mode of aspirin action and clinical trials

Pathophysiology. Chronic rheumatoid arthritis is a multisystem disorder, caused by a pathologic (auto)immune reaction. This results in a chronic systemic inflammation with NF-κB-induced generation of monocyte/macrophage-derived cytokines in the rheumatoid synovium as a key feature [19]. There is an accelerated development of atherosclerosis – from early atheroma formation until thrombus development – all being associated with the chronic systemic (immune) inflammation process [20]. For these reasons, the disease is not only associated with chronic inflammatory pain but also with a significantly shortened life expectancy, mainly due to a by 30–50 % increased risk of atherothrombotic events, in particular of myocardial infarctions [21, 22]. Proinflammatory cytokines are key players in the pathogenesis of both rheumatoid arthritis and atherosclerosis. They cause endothelial dysfunction and oxidative stress [20]. Accordingly, reduced time-averaged disease activity by appropriate drug treatment might reduce the incidence of cardiovascular events in these patients [23].

Patients with rheumatoid arthritis have a higher risk of acute atherothrombotic vascular events than patients with nonrheumatic arthritic diseases or otherwise healthy individuals. In this context it is interesting to note that patients suffering from rheumatoid arthritis at a time, when except aspirin no other pain relieving agent was available, suffered significantly fewer fatal myocardial in-

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farctions and hypertensive heart diseases than age-matched nonrheumatics [24]. In these patients aspirin was probably used regularly at high doses for several months or even years.

Another early study in a small group of patients reported a 30–50 % reduction (not significant!) of cardiovascular ischemic events in patients suffering from rheumatoid arthritis as compared to appropriately matched controls after long-term aspirin treatment for on average 10 years between 1950 and 1975 [25].

These data should not be overinterpreted. However, they are quite remarkable at the background of the rather opposite effects of NSAIDs or even coxibs on the vascular thrombotic risk and the negative interactions with aspirin (see below).

Modes of aspirin action. As expected, prostaglandins are in focus as mediators of pain and inflammation also in rheumatoid arthritis. One role unique to prostaglandins is amplification of cytokine signaling and the resulting long-lasting immune inflammation [26]. The COX-2 protein is markedly upregulated in the synovia of patients with rheumatoid arthritis. This upregulation is probably genetically controlled and a biochemical correlate of disease severity [27]. Inhibition of COXs, including thromboxane-generating COX-1, and the conversion of COX-2 into a 15-lipoxygenase, both by target-specific acetylation, are clinically relevant antiinflammatory actions of aspirin. In addition, salicylate at high local levels (>1 mM) in the inflamed synovia might also contribute to the overall therapeutic efficacy [28]. Its accumulation within the cell membranes, specifically at the acidic pH of inflamed tissue, could further add to the overall antiinflammatory action by nonselective inhibition of kinases, i. e., target enzyme phosphorylation, due to depletion of ATP after uncoupling of oxidative phosphorylation (Section 2.3.2). In vitro studies suggest that salicylate at millimolar concentrations inhibits proliferation and induces apoptosis in human rheumatoid synovial cells [28]. Interestingly, salicylate did not reduce biosynthesis of hyaluronic acid, the most significant compound for maintenance of functional integrity of articular cartilage [29, 30].

Recent experimental studies on endogenous mediators of inflammation and immune reactions have identified new mediators (alarmins, endokines) that are released into the extracellular space after tissue injury and synovial inflammation, respectively, and stimulate inflammatory and immune reactions. They are also surrogates of tissue injury in degenerative joint diseases such as rheumatoid arthritis and osteoarthritis. A new member of this class is HMGB-1 [31]. HMGB-1 can recruit immune cells to inflamed synovia, initiating the adaptive immune response and perpetuating disease [31]. Salicylic acid binds to HMGB-1 in submillimolar concentrations (100 μM) and inhibits its chemotactic actions on leukocytes and on the expression of inflammatory cytokines as well as of COX-2 [32].

The clinical significance of these findings for therapeutic actions of aspirin in treatment of inflammatory joint diseases is currently unknown. However, platelet-derived HMGB-1 (disulfide) is not only a platelet storage product but also a central mediator of platelet-mediated thrombotic/inflammatory processes in high-risk atherothrombotic [10] and VTE patients [33]. In these situations, interactions between aspirin-sensitive

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platelet activation and secretion, NET formation and thromboxane biosynthesis have been established in animal studies [34, 35] and might also be relevant for humans [10]. These fresh insights into the natural history of the disease and the detection of new inflammatory mediators might considerably widen our current view about the use of aspirin as an antiinflammatory/antithrombotic drug.

There are very few historical studies on direct effects of aspirin on inflammatory/degenerative processes inside joints and synovial fluid, respectively. Early studies with high-dose aspirin (4.2 g/day) have shown effective antiinflammatory concentrations of salicylates in the synovial fluid [36]. As had to be expected, salicylate levels in the synovial fluid of patients undergoing knee surgery who received aspirin at antiplatelet doses of 100 mg/day did not result in measurable levels of aspirin and in only low and nonantiinflammatory concentrations of salicylate (ca. 1 µg/ml) [37]. Higher doses were not studied. However, treatment of rheumatoid arthritis is clearly no indication for aspirin anymore.

Clinical trials. After the introduction of NSAIDs for symptomatic treatment of inflammatory pain and the more causally acting "disease-modifying antirheumatic drugs" (DMARDs), such as methotrexate, leflunomide and others, these have replaced high-dose aspirin as an antiinflammatory drug in treatment of chronic inflammatory joint diseases. In addition, observational studies on treatment of patients with rheumatoid arthritis with methotrexate suggest not only beneficial effects of methotrexate on the inflammatory process and its progression but also a reduction of the increased cardiovascular mortality [38, 39]. These antiinflammatory actions are possibly modified in a clinically relevant manner by an interaction of methotrexate with salicylates via adenosine (Section 2.3.2) [40, 41]. Other actions of methotrexate, in particular inhibition of histone deacetylase [42] – aspirin stimulates histone acetylation [43] – might also contribute to this. Elevated methotrexate plasma levels due to reduced methotrexate clearance by aspirin cotreatment might cause toxic effects of methotrexate, which become clinically relevant at high salicylate plasma levels (ca. 70 µg/ml [350 µM] and more) [44–46]. This is equivalent to the intake of several grams of aspirin (Fig. 2.1.1-5) which are not used anymore for treatment of rheumatic diseasesand should definitely be avoided in combination with methotrexate [47].

A significant proportion of patients with rheumatoid arthritis receive aspirin by prescription for cardiocoronary prevention, together with NSAIDs or coxibs for pain relief [48]. This might result in negative drug interactions, specifically inhibition of the COX-1-mediated antiplatelet effects of aspirin (Section 4.1.6) [49] and the COX-2-dependent generation of antiplatelet, vasodilatory prostaglandins, such as PGI_2 and PGE_2 (see below) [50].

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4.2.2.3 Osteoarthritis – pathophysiology, mode of aspirin action and clinical trials

Pathophysiology. In contrast to rheumatoid arthritis, osteoarthritis is not a systemic inflammatory disease but rather a local, degenerative disorder of the joints and joint cartilage, respectively, with a reactive inflammatory and painful component. Injury is localized preferentially in joints that are under high "workload," such as the knees and the hip, and becomes evident after mechanical stress. In contrast to rheumatoid arthritis, osteoarthritis is not associated with increased cardiovascular morbidity or a shortened life span [51]. Pain is the main devastating symptom and arises from different areas of the affected joint: bone (periostitis, subchondrial microfractures and ischemia), synovia (synovitis), stimulation of nerve endings with neuronal inflammation and release of inflammatory mediators as well as periarticular muscle spasms. The local levels of IL-1β and TNFα in the synovial fluid are elevated. COX-2 protein and prostaglandin biosynthesis [52] are also upregulated in the synovia and stimulated by mechanical workload, although to a lower extent than in rheumatoid arthritis. No such changes are seen in patients with traumatic knee injury [27]. Inflammatory pain might induce expression of COX-2 in the spinal cord and cause neuropathic pain. Therefore, the intensity of the reactive, inflammatory reaction not only determines the intensity of pain but might also influence the progression of the degenerative processes in the joint cartilage. Because osteoarthritis is an erosive disease of the cartilage without a chance for *restitutio ad integrum*, but only retardation of progression, the treatment is mainly symptomatic and focused on pain relief and improved or at least maintained mobility.

Modes of aspirin action. These pathophysiological features of osteoarthritis suggest a chronic, self-maintaining and progressive inflammatory condition. Enhanced prostaglandin production, mainly PGE_2, is an accompanying phenomenon which amplifies vascular inflammation and pain and modulates inflammatory white cell activities (Section 2.3.2). Inhibition/modulation of COX activity is, therefore, the major pharmacological strategy. There is no primary indication for aspirin because of the low potency to inhibit the upregulated COX-2 in vivo. Whether the recently detected inhibition of platelet-derived endokine release (HMGB-1) and its proinflammatory actions will have an impact on clinical aspirin use [31, 32] is unknown.

Clinical trials. NSAIDs, presumably those that are available as OTC drugs, such as naproxen, ibuprofen and diclofenac, are the drugs of choice for symptomatic treatment of osteoarthritis. They act more strongly and longer because of their long-lasting inhibition of COX-2 as well as their higher lipophilicity, i. e., improved tissue penetration and accumulation (Fig. 4.2.2-1) [53].

A metaanalysis of 23 randomized placebo-controlled trials indicated that NSAIDs are useful for single or short-term use but not for longer lasting, daily administration.

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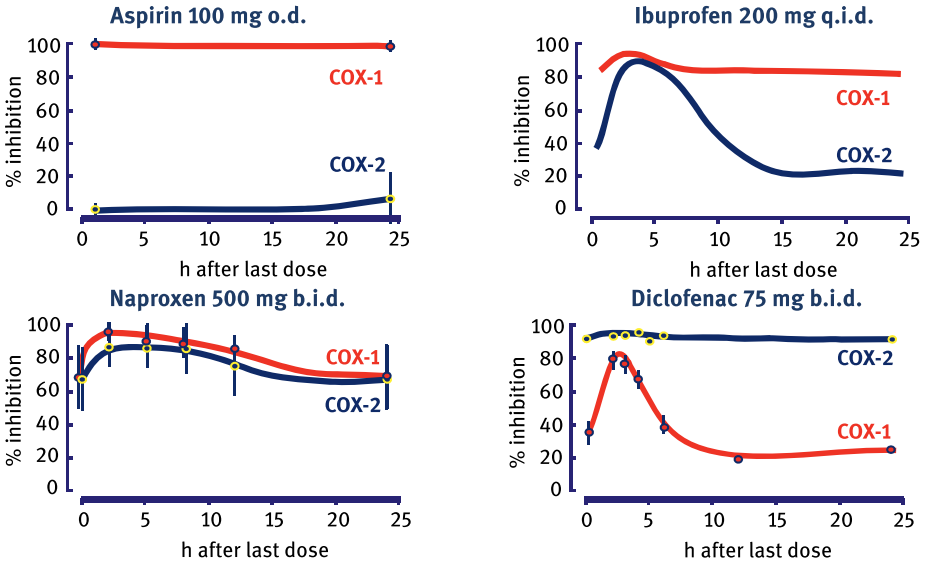

Figure 4.2.2-1: Recovery of COX-1 and COX-2 inhibition after cessation of 4–7 days of continuous daily treatment with aspirin, ibuprofen, naproxen and diclofenac at the doses indicated. Abbreviatiobs; o.d.: once daily; b.i.d.: twice daily; q.i.d.: four times daily (modified after [54] and Grosser personal communication).

The reasons are side effects in particular in the cardiovascular system, the kidneys (water retention) and the gastrointestinal tract [55–57]. Of particular concern are negative interactions with aspirin, in particular in patients who require cardiovascular protection (Section 4.1.1) (see below).

4.2.2.4 Systemic inflammatory response syndrome (SIRS). Pathophysiology, mode of aspirin action and clinical trials. Sepsis and acute respiratory distress syndrome (ARDS)

SIRS is a more or less uncontrolled systemic inflammatory and procoagulant reaction, where platelets play a key role as trigger of interaction with leukocytes and endothelial cells with subsequent modulation of host defense and immune responses [58, 59]. SIRS can result from severe injuries, severe bacterial or viral infections or other systemic (shock) reactions, such as sepsis, the last commonly associated with ARDS. The ultimate negative result will be terminal organ failure due to dysregulated or insufficient body defense mechanisms and death [60]. There is no specific and effective pharmacological treatment available [59, 61] and several options for symptomatic therapy exist.

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Pathophysiology. Most relevant pathophysiological factors for the inflammatory-thrombotic complex in sepsis and ARDS are disturbances of the clotting system with activated platelets as triggers. Platelets generate and release a number of inflammatory mediators (Section 2.3.2), induce the formation of platelet/leukocyte aggregates and NETs and interact with endothelial cells and white cells. Platelets also shed microparticles with significant tissue factor-related procoagulant activity, resulting in enhanced thrombin formation [62, 63]. In addition, platelets are also important players in adaptive immune reactions [64]. As a consequence, accumulated, activated platelets initiate a number of secondary prothrombotic events, such as thrombotic microangiopathy and disseminated intravascular coagulation (DIC), that are aspirin-sensitive [9].

Modes of aspirin action. Several modes of action have been postulated by which aspirin could modulate the pathology of SIRS, sepsis and ARDS: inhibition of COXs, antagonism of proinflammatory NF-κB-mediated pathways, enhanced production of lipoxins and enhanced production of endothelial NO [59], which in turn will inhibit oxidative stress by activation of heme oxygenase-1.

Administration of endotoxin (lipopolysaccharide [LPS]) is one experimental method to mimic the septic condition and SIRS in human. Injection of LPS in healthy volunteers results in upregulation of both COX-1 and COX-2 expression. Aspirin at an antiplatelet dose (81 mg/day for 10 days prior to LPS injection) reduced the COX-1-mediated generation of thromboxane and prostacyclin but not the "flu-like" symptoms and the pyrexial responses to LPS. These were prevented by ibuprofen and celecoxib. These data suggested that both COX enzymes are induced by LPS in white cells and contribute to the prostaglandin response to LPS in humans with a dominating role for COX-2 in mediating the constitutional responses to LPS, while aspirin actions are largely platelet-mediated [65].

Another preclinical trial with LPS has shown that aspirin inhibits LPS-induced pulmonary neutrophilic inflammation, possibly due to inhibition of platelet-dependent thromboxane formation [66]. Thus, clinical benefits from aspirin might be expected from its antiplatelet effects, mainly via inhibition of COX-1. Animal studies have shown that aspirin can prevent ARDS by decreasing neutrophil activation and recruitment in the lung, TNFα expression in pulmonary intravascular macrophages, plasma TXB_2 levels and platelet aggregation in the lungs [67–69]. Aspirin administration in a murine model of ARDS (acid-induced acute lung injury) was associated with improved oxygenation, diminished lung edema and inflammation and increased survival [70]. In addition, there might be an increased formation of antiinflammatory and inflammation-resolving "aspirin-triggered" lipoxin A_4 (ATL) and activation of heme oxygenase-1 which stimulates NO formation via the acetylated COX-2 (Fig. 2.3.2-8) [59, 71]. It is, however, questioned whether aspirin at low antiplatelet doses of 100 mg/day will also be able to prevent the activation of the NF-κB pathway,

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which requires higher local concentrations for most of the actions noted above (for more details see Section 2.3.2).

Taken together, these data suggest that aspirin in sepsis tackles the immunothrombotic cascade upstream of NETs, possibly by an antiplatelet action, and reduces their deleterious consequences for sepsis-induced organ failure [9]. For these reasons, inhibition of deleterious platelet functions by aspirin might represent a most useful tool to attenuate SIRS, ARDS and sepsis and to improve clinical outcome [59, 72–74].

Clinical trials. Several observational trials have provided salutary effects of early aspirin and other antiplatelet agents in critically ill patients [73, 75–80], among them a multicenter clinical study based upon prospective data from the VALID cohort [76].

VALID investigated the effect of aspirin pretreatment on the incidence of ARDS and early clinical outcome in a total of 1,149 critically ill patients including patients with sepsis.

Overall, 368 patients (32 %) developed an ARDS. According to the result of a sophisticated statistical multivariant analysis, aspirin pretreatment (intake of 81–325 mg/day) significantly reduced the incidence of ARDS within the first 4 days of hospitalization (OR: 0.66; 95 % CI: 0.46–0.95; $P = 0.023$). There was a nonsignificant trend (RR: 0.70) to a reduced in-hospital mortality.

The conclusion was that aspirin pretreatment may reduce the incidence of ARDS in critically ill patients and those with sepsis. Prospective randomized trials are needed to further substantiate these findings [76].

A retrospective trial was conducted in a total of 1,802,034 nationwide hospital admissions in the USA in patients with a primary diagnosis of sepsis. A total of 10.86 % participants was on aspirin. Their in-hospital mortality (7.26 %) was lower than in patients without aspirin (10.12 %; $P < 0.001$). The aspirin cohort also had a lower length of in-hospital stay (6.08 versus 7.38 days; $P < 0.001$). The conclusion was that aspirin use was associated with improved survival in patients presenting with sepsis [81].

Similar findings were obtained in a subgroup analysis of seven cohort studies on aspirin from a large metaanalysis on antiplatelet drugs in sepsis. Aspirin markedly reduced hospital mortality in these patients (OR: 0.60; 95 % CI: 0.53–0.68; $P < 0.05$) (Fig. 4.2.2-2) [8]. Another metaanalysis assessed the association between aspirin use prior to ARDS onset and ARDS incidence in 6,764 at-risk patients. The primary outcome was risk of ARDS, and the secondary outcome was the hospital mortality of at-risk patients. Compared to nonaspirin use, prior aspirin use was linked with a significantly lower incidence of ARDS (OR: 0.78; 95 % CI: 0.64–0.96; $P = 0.018$) but had no effect on in-hospital mortality ($P = 0.204$) [82].

Ouyang and colleagues published a metaanalysis of 10 cohort trials, seven of them with aspirin, on the efficacy of antiplatelet drugs in patients with sepsis. Aspirin effectively reduced in-hospital mortality in patients with sepsis (OR: 0.60; 95 % CI: 0.53–0.68; $P < 0.05$). A subgroup analysis on the timing of antiplatelet drug adminis-

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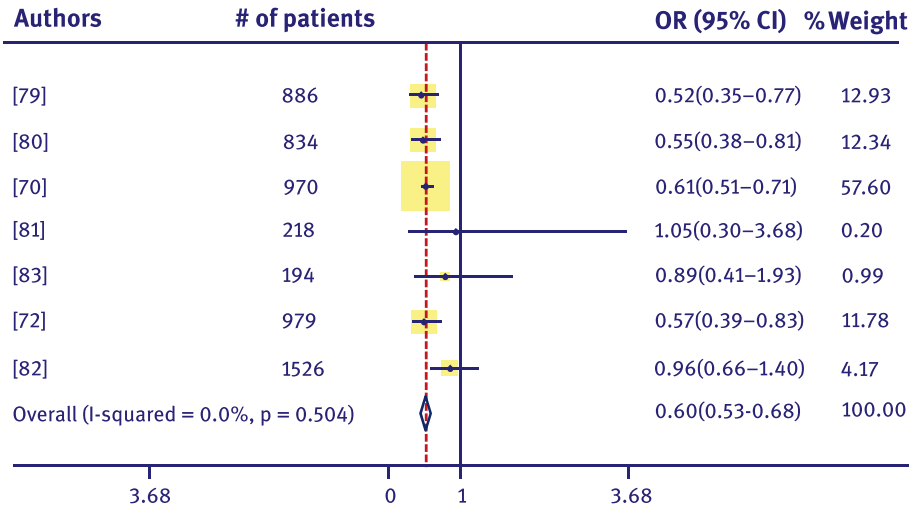

Figure 4.2.2-2: Forest plots showing the effect of aspirin on the mortality rate of at-risk patients with sepsis [8, 73, 75, 83–87].

tration showed that antiplatelet drugs can reduce mortality when administered either before (OR: 0.78; 95 % CI: 0.77–0.80) or after sepsis (OR: 0.59; 95 % CI: 0.52–0.67). Antiplatelet drugs, particularly aspirin, could be used to effectively reduce mortality in patients with sepsis (Fig. 4.2.2-2) [8].

Observational studies cannot replace controlled randomized trials but they present findings under real-world conditions without any particular inclusion and exclusion criteria. Collectively, the vast majority of these studies do show a beneficial effect of adjuvant aspirin at antiplatelet doses, suggesting antiplatelet effects as primary mode of action in sepsis and ARDS [8, 59, 72, 84].

Clinical studies – randomized trials. A randomized, double-blind, placebo-controlled phase II trial was conducted to study whether enteral aspirin (75 mg/day) is safe and effective in improving surrogate outcomes (oxygenation index [OI]) in adult patients with ARDS.

Aspirin or placebo were given for up to 14 days. Unfortunately, the study had to be stopped prematurely due to slow recruitment of patients. At day 7 there was no difference in OI: 54 (aspirin) versus 42 (placebo). Secondary outcomes including safety and other respiratory markers were also not different.

The conclusion was that aspirin was well tolerated but did not improve OI or other physiological outcomes. A larger trial appears not to be feasible using this study design [88].

New information was expected from the "Aspirin to inhibit sepsis" (ANTISEPSIS) trial, a substudy of the ASPREE trial. ANTISEPSIS was a double-blind, placebo-controlled

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study of the effects of aspirin (100 mg/day) on sepsis-related deaths in a population of elderly (>70 years) individuals [80].

ANTISEPSIS was a substudy of ASPREE, a randomized primary prevention trial of low-dose aspirin (100 mg per day) in the Australian cohort of the study. Inclusion criteria were the absence of known cardiovascular diseases, dementia or any disability. A total of 16,703 participants aged 70 years or older at trial entry were enrolled and followed up for a median of 4.6 years (IQR: 3.6–5.6). In total, 8,322 (49.8 %) participants were assigned to receive aspirin and 8,381 (50.2 %) to placebo.

In total, 203 deaths were considered to be associated with sepsis. Univariate analysis showed similar rates of death associated with sepsis in the two study groups (HR for aspirin vs. placebo: 1.08; 95 % CI: 0.82–1.43; $P = 0.57$).

The conclusion was that daily low-dose aspirin treatment did not reduce deaths associated with sepsis in community-dwelling older adults. These findings do not support the use of aspirin as a primary prevention strategy to reduce the burden of sepsis in this population [89].

The whole study is discussed and commented on in detail in Section 4.1.1. At a first view, the results appear not to support the expectations from the numerous observational studies cited above. However, ANTISEPSIS was a primary prevention trial in elderly people at a good health status as seen from the absence of cardiovascular diseases or physical and mental disabilities. The annual mortality rates were only 1.1 % and 1.3 %, respectively, in the two study groups at an average age of the total population of ≥74 years at entry. It should also be noted that the whole ASPREE study was negative with respect to the primary efficacy endpoint. For these reasons, the numerous positive observational studies with aspirin in sepsis and ARDS allow positive expectations for aspirin use as an adjunct to standard care treatment in critically ill patients.

Taken together, the vast majority of observational studies on sepsis and ARDS do suggest salutary actions of aspirin, which are possibly related to its antiplatelet effects, since other antiplatelet agents also appear to work. No such effect is seen with NSAIDs, such as ibuprofen, even at high doses [90]. Any (additional) interaction of aspirin with NF-κB-mediated proinflammatory/prothrombotic signaling pathways is certainly desirable but has not been identified so far in controlled clinical trials. Prospective randomized controlled and appropriately sized clinical trials on septic or ARDS patients are urgently needed. New information is to be expected from the Brazilian "ASpirin for Patients With SEPsis and SeptIc Shock" (ASP-SEPSIS) study, a randomized, placebo-controlled trial of aspirin in patients with sepsis. The study will investigate the efficacy of 1-week treatment with aspirin on clinical outcome and is currently recruiting patients.

4.2.2.5 HIV – pathophysiology, mode of aspirin action and clinical trials

Infections with HIV injure and finally destroy the body-own immune system. This makes the organism highly sensitive to infections and other diseases. Without appro-

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priate treatment, HIV infections can result in an "acquired immunodeficiency syndrome" (AIDS) and death. Specific targets of HIV are $CD4^+$ hematopoietic stem cells.

Pathophysiology. Patients with HIV infections have an increased risk of cardiovascular events, mainly coronary heart disease and myocardial infarctions [91]. This elevated risk is probably related to an enhanced platelet reactivity (HTPR) associated with an overall prothrombotic state due to systemic activation of the immune system. Platelets might act as a trigger and enhancer of further inflammatory, immunogenic and prothrombotic mediator release from platelets and other cells, mainly monocytes/macrophages and the endothelium [13, 14, 59, 92, 93].

Modes of aspirin action. As an antiplatelet and immunomodulating agent, aspirin may modify immune processes indirectly through inhibition of platelet activation and directly through blocking inflammatory pathways in multiple cell types [94]. Studies in healthy volunteers have shown that low-dose aspirin (81 mg daily) initiates production of antiinflammatory ATL [71]. Lipoxins modulate innate immune signaling via inhibition of proinflammatory cytokine production [59] and control innate immunity [95]. Accordingly, low-dose (81 mg/day) aspirin has been shown not only to reduce HTPR in HIV patients under antiretroviral treatment (ART) but also to reduce the elevated number of $CD4^+$ lymphocytes and $CD14^+$ activated monocytes (Fig. 4.2.2-3) [94]. In addition to its immunosuppressive action on macrophages, aspirin also modifies the activity of dendritic cells and all kinds of lymphocytes. These are further potential

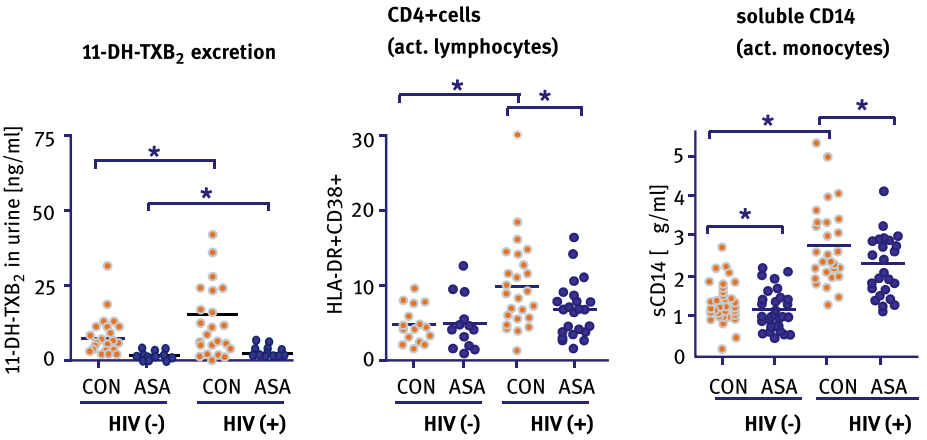

Figure 4.2.2-3: Thromboxane excretion and T-cell and monocyte activation in 44 healthy controls (HIV (–)) and 25 HIV patients (HIV (+)) under ART before and after 1 week of aspirin (ASA) (325 or 81 mg/day) treatment. Elevated plasma P-selectin and GPIIb/IIIa levels and platelet/monocyte coaggregates in HIV (+) individuals were also normalized by aspirin (not shown) (modified after [94]).

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targets to understand the modulation of innate and adaptive immune responses by aspirin in HIV patients [96].

Clinical trials. The morbidity and mortality of patients with HIV infections in developed countries is mainly due to non-AIDS-related events. About 6–15 % of deaths are due to cardiovascular problems, mainly coronary heart disease and myocardial infarction [91]. This proportion might increase with the gradual aging of these patients because of improved ART [97]. In addition to pathological immune reactions, HIV patients also exhibit platelet hyperreactivity (HTPR) with subsequent lymphocyte and monocyte activation. These changes, according to a small, nonrandomized study, can possibly be attenuated by low-dose (81 mg/day) aspirin treatment (Fig. 4.2.2-2) [94]. At the same time, there is normalization of the enhanced thromboxane metabolite excretion in urine (Fig. 4.2.2-2). This suggests that activated platelets contribute to immune activation and inflammation in HIV infection and that low-dose aspirin may be a useful intervention for HIV patients on antiretroviral therapy [94].

Meanwhile, a larger controlled, randomized trial in HIV patients of the same group is available.

A first prospective, randomized double-blind trial was recently conducted in 121 aspirin-naïve HIV patients who had received >48 weeks of ART. Patients were treated with aspirin (100 or 300 mg/day) or placebo over 12 weeks, followed by a 4-week posttreatment observation period. Numerous soluble and cellular surrogate parameters as index biomarkers for the activity of the immune system and endothelial function were measured. Serum thromboxane and urinary thromboxane metabolite excretion were also determined as index parameters for inhibition of COX-1.

There were no significant changes in the soluble biomarkers (CD14, IL-6, CD163, D-dimer) upon aspirin treatment. There were also no changes in T-cell or monocyte activity as well as endothelial function. The mean serum thromboxane level was reduced by >90 % in 49 % and 24 % (!) of the aspirin-treated patients at 300 and 100 mg aspirin. The reductions of urinary thromboxane metabolite excretion were similar in the two treatment groups and amounted to 74 % and 76 %, respectively. The self-reported adherence to the study drug was high: 90 % of participants reported a 100 % adherence.

The conclusion was that inhibition of COX-1-mediated platelet activation by 100 or 300 mg/day aspirin for 12 weeks did not affect HIV-induced activation of the immune system an endothelial function von HIV patients on ART [98].

These results are at variance with a previous small nonrandomized study of the same group in 25 HIV patients who were treated with 81 mg/day aspirin for one week (Fig. 4.2.2-2) [94]. Aspirin reduced serum thromboxane levels by >90 % (95 % had to be obtained for a clinically relevant action) in only 25 % (!) of the aspirin-treated patients of the second HIV study. This is surprising at the background of a self-reported adherence rate of 100 % (!) in 90 % of participants. Alternatively, there might be an HTPR to aspirin ("resistance") which requires higher aspirin dosing and additionally might be aggravated by ART [99, 100]. In any case, aspirin did not work in the majority

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of patients and it is not surprising that the numerous laboratory measurements done in the study were all negative. In addition, the statistically required minimum number of 40 patients per treatment group (according to the authors) was not reached in any of the groups studied. Thus, the data are inconclusive.

The significance of an HTPR against aspirin and ADP antagonists was studied in the "Platelet reactivity in HIV-infected patients on dual antiplatelet therapy for an acute coronary syndrome" (EVERE2ST-HIV) study in HIV patients with ACS [101].

The EVERE2ST-HIV study measured residual platelet aggregation (RPA) ex vivo in 80 ACS patients with HIV and compared it with 160 matched ACS patients without HIV. All patients had an ACS at least 1 month prior to PCI treatment and were on DAPT with aspirin (75–325 mg/day) plus clopidogrel, prasugrel or ticagrelor for at least 30 days prior to platelet function testing. RPA was assessed by conventional platelet function measurements ex vivo. All patients with HIV were on ART.

The proportion of patients with high RPA was elevated in HIV-infected patients. In P2Y12 inhibitor assays, high RPA was found in 23.8 + 2.7 % of HIV patients versus 15.3 + 1.3 % of non-HIV controls ($P = 0.001$). For aspirin, the proportion of RPA amounted to 3.6 + 1.5 % versus 0.4 + 0.1 % ($P = 0.004$). ART with protease inhibitors also increased PRA.

The conclusion was that ACS patients infected with HIV have increased levels of platelet reactivity and a higher prevalence of HTPR to P2Y12 inhibitors and aspirin than non-HIV ACS-patients. Both could contribute to an increased risk of recurrent ischemic events in HIV-infected persons [101].

HIV is known to be associated with elevated risk markers for cardiovascular events, including those for inflammation, coagulation and platelet activation [99]. As pointed out in an editorial to this paper, high ex vivo platelet reactivity in an atmosphere of hypercoagulability and heightened inflammation could present the background for a perfect "thrombotic storm" [102]. Perhaps similar conditions could also exist with other viral infections, such as COVID-19, where antiplatelet treatment with aspirin combined with its anticoagulant and antiinflammatory activities could be an attractive adjunctive treatment option in the absence of other alternatives [103].

4.2.2.6 COVID-19 and flu-like conditions: pathophysiology, modes of aspirin action and clinical trials

General aspects. The acute respiratory syndrome coronavirus-2 (SARS-CoV-2) virus is responsible for the current pandemic that affects millions of people worldwide. Severe COVID-19 is characterized by a hyperinflammatory response with elevated levels of several inflammatory markers, including cRP, cytokines like IL-6 as well as TNFα [104, 105]. In the lung, there is severe endothelial injury, associated with the presence of intracellular virus and widespread thrombosis and microangiopathy [106].

Pathophysiology. Despite intensive thromboprophylactic measures, there is a high incidence of thrombotic complications in COVID-19 patients. Probably, immunothrombosis is the underlying mechanism for this coagulopathy which is triggered by a hy-

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perinflammatory reaction. A hypercoagulable state results from endothelial damage, activation of the complement system and/or platelet hyperactivity. There is NETosis, activation of the coagulation system and a hypofibrinolytic state. Significant crosstalk occurs between the innate/adaptive immune system, endothelium and the coagulation system. D-dimer levels in blood, an index of thrombin activity, have been shown to be a most reliable predictor of disease severity, thrombosis and overall survival. Targeting pathways upstream of coagulation may be a rational approach to prevent the mortality/morbidity due to the COVID-19-associated coagulopathy [107]. This also includes antithrombotics like heparins or aspirin.

Modes of aspirin action. Aspirin is unique as it has several pharmacological properties that are of key importance for fighting viral infections as well as inflammation and thrombosis (Section 2.3.2). Aspirin has the potential to attenuate COVID-19-induced excessive immune activation, cytokine storm, hypercoagulability and multiorgan damage [103, 108] and thus might favorably affect the clinical outcome. Possible sites of action are summarized in Fig. 4.2.2-4 [109]. Importantly, aspirin, in contrast to con-

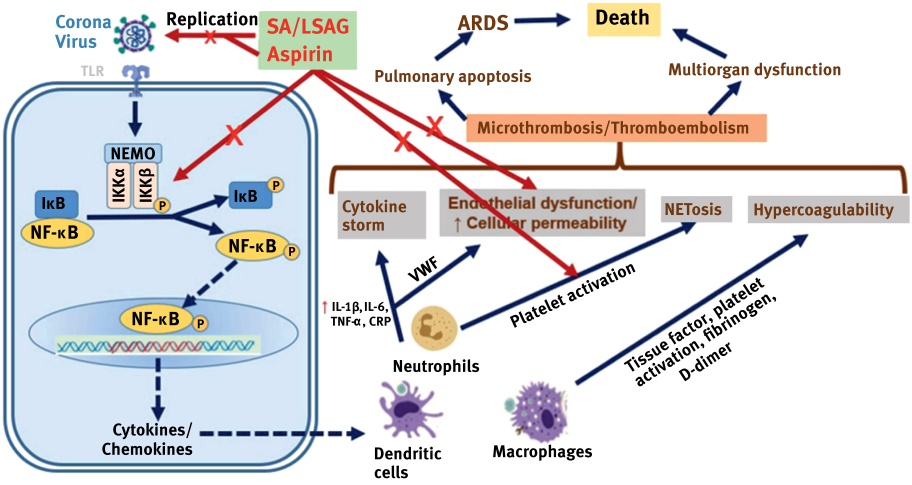

Figure 4.2.2-4: Host cell response to SARS-CoV-2 infection and the role of aspirin in SARS-CoV-2 infection. SARS-CoV-2-induced cytokine storm, endothelial dysfunction, NETosis and hypercoagulability result in microthrombosis/thromboembolism in lungs as well as heart and kidney, leading to multiorgan dysfunction, ARDS and ultimately death in a substantial percentage of patients. Aspirin/salicylate (SA) and LASAG (D,L-lysine acetylsalicylate + glycine) can attenuate viral replication. They inhibit NF-κB activation and the subsequent expression of cytokines and chemokines. In addition, aspirin exerts antiinflammatory and antiplatelet effects and attenuates NETosis, endothelial dysfunction and hypercoagulability. Abbreviations: NF-κB: nuclear factor-κB; IL: interleukin; TNF-α: tumor necrosis factor-α; CRP: C-reactive protein; MCP-1: macrophage chemoattractant protein-1; ICAM-1: intercellular adhesion molecule-1; VCAM-1: vascular cell adhesion molecule-1; NETs: neutrophil extracellular traps; ARDS: acute respiratory distress syndrome [109].

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ventional antiviral agents, does not attack the virus directly, thereby promoting the selection of resistant variants, but indirectly by a host-directed mode of action. This antiviral activity of high-dose aspirin is known since the studies of *Igor Mazur* and colleagues from Münster (Germany) in 2007 [16] and could now be adapted to treatment of COVID-19 (Section 2.3.2) [109].

In this context, targeting pathways upstream of coagulation using novel or repurposed drugs alone or in combination with other antithrombotic agents (heparins) may be a rational approach to reduce the mortality/morbidity due to COVID-19-associated coagulopathy [107], subsequent to pulmonary affection. In order to obtain highly effective concentrations in the lung of COVID patients – the initially most affected organ [106] – "local" application of drugs as an aerosol might be a preferred administration. In patients with obstructive pulmonary diseases (COPD), only 10 % from the dose (250, 500 or 750 mg aspirin twice daily) administered was recovered in urine, as opposed to 70 % after oral intake of the same doses [110]. This suggests significant extravascular accumulation. Of particular interest is aerosolized LASAG (D,L-lysine acetylsalicylate + glycine). Single oral inhalation of up to 750 mg LASAG in healthy volunteers was safe [111]. Aerosolized LASAG was used in a phase II clinical trial for treatment of influenza in hospitalized patients and, reportedly, was effective and well tolerated [112]. Compounds like LASAG, which reduce viral titers, decrease viral protein accumuation and RNA synthesis and impair the formation of virus replication/transcription complexes – all this suggests aerosolized aspirin (LASAG) as an attractive treatment option for viral infections of the upper airways. This also on the background of rapidly changing (retro)viral genomes under the selection pressure of (repeated) vaccinations (Section 2.3.2) [113].

Clinical trials. In an observational cohort study of adult patients with COVID-19, aspirin use at least 7 days before hospitalization or within 24 hours of hospitalization compared to nonaspirin use was associated with lower rates of mechanical ventilation (36 % vs. 48 %) and intensive care unit (ICU) admission (39 % vs. 51 %). In a multivariate analysis, of these data aspirin use remained significantly associated with decreased risk of mechanical ventilation (HR: 0.56; 95 % CI: 0.37–0.85; $P = 0.007$), ICU admission (adjusted HR: 0.57; 95 % CI: 0.38–0.85; $P = 0.005$) and in-hospital mortality (adjusted HR: 0.53; 95 % CI: 0.31–0.90; $P = 0.02$). There were no differences in overt thrombosis or major bleeding between the two groups [114]. This study provided the first clinical evidence supporting aspirin use in patients with COVID-19. Most of these patients were on 81 mg aspirin per day.

In a large study of American veterans with COVID-19, preexisting aspirin prescription was associated with a significant decrease in overall mortality at 14 days (OR: 0.38; 95 % CI: 0.33–0.45) and at 30 days (OR: 0.38; 95 % CI: 0.33–0.45) compared to patients not treated with aspirin [115]. In a retrospective population-based cross-sectional investigation in Israel, aspirin users had a lower rate of COVID-19 (OR: 0.71; 95 % CI:

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0.52–0.99; $P = 0.04$) and a shorter hospitalization (19.8 ± 7.8 vs. 21.9 ± 7.9 days; $P = 0.045$) as compared to nonusers [116].

As reviewed by Tantry and colleagues [109], further observational studies also suggested the benefit of prior aspirin use in reducing the risk of COVID-19 patients and demonstrated a reduced mortality with prior and in-hospital use of aspirin [117, 118]. However, there were also reports of an absence or even an elevated risk of mortality in COVID-19 patients [119, 120]. The reason for the absence of therapeutic (mortality) benefits with aspirin use is not well understood. It might be associated with an HTPR (aspirin "resistance") in COVID-19 patients, due to viral effects on platelet reactivity. Similar HTPRs have also been described for HIV patients under antiretroviral treatment [99, 100].

In a prospective observational study of hospitalized patients with COVID-19, those who were on aspirin therapy had lower urinary 11-DH-TXB_2 levels than patients not on aspirin (3,760 ± 2,295 versus 13,125 ± 11,474 pg/mg creatinine; $P = 0.003$). An inadequate therapeutic aspirin response based on >1,520 pg urinary 11-DH-TXB_2/mg creatinine cut-off was observed in 91 % of patients with COVID-19 on 81 mg daily aspirin and in 50 % of patients with COVID-19 on ≥162 mg daily aspirin [118]. The frequency of thromboinflammation as indicated by >4,200 pg urinary 11-DH-TXB_2/mg creatinine was 81 % in patients with COVID-19 not on aspirin, 55 % in patients on 81 mg aspirin per day and 25 % in patients on ≥162 mg aspirin per day. Moreover, only 17 % of patients had 11-DH-TXB_2 values lower than the cut-off value (<1,520 pg/mg creatinine) for aspirin therapeutic response [121].

For these reasons, in the presence of a highly elevated inflammatory response, endothelial dysfunction and hypercoagulability, low-dose aspirin therapy may not be adequate to produce the strong pharmacodynamic action needed and which translates into improved clinical outcome [109, 122].

The first large prospective randomized but open trial on aspirin as an adjunct to standard of care treatment of patients admitted to hospital with COVID-19 was the "Randomized Evaluation of COVID-19 Therapy" (RECOVERY) trial [123].

RECOVERY was a randomized, prospective open trial comparing several drug treatments in addition to standard care in patients hospitalized with COVID-19. Eligible adults in the aspirin group were randomly allocated to standard care with (7,351) or without (7,541) aspirin (150 mg/day). The observation period was 28 days after randomization. Primary outcome was all-cause mortality, secondary outcomes were time to discharge and progression of the disaese (invasive mechanical ventilation or death).

Overall, 1,222 patients in the aspirin group (17 %) and 1,299 patients in the group at usual care (17 %) died within 28 days (HR: 0.96; 95 % CI: 0.89–1.04; $P = 0.35$). Patients on aspirin had a slightly shorter duration of hospitalization (8 days vs. 9 days) and a higher proportion of them was discharged alive within 28 days (75 % vs. 74 %). Aspirin was associated with a reduction in thrombotic events (4.6 % vs. 1.0 %) and an increase in major bleeding events (1.6 % vs. 1.0 %).

The conclusion was that aspirin in patients hospitalized with COVID-19 did not reduce 28-day mortality or the risk of progression of the disease to invasive mechanical ventilation or death.

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Aspirin was associated with a small increase in the rate of being discharged alive within 28 days [123].

This study is interesting, but has some caveats. A total of 90 % of patients in the aspirin group received at least one dose of the compound, according to the ITT data analysis procedure. This means that 10 % of the patients in the aspirin group did not receive any aspirin while 3 % of the nonaspirin group did. There is no discussion on a per-protocol analysis for more detailed information as, for example, done previously in the ARRIVE study. A 12.5 % mortality reduction was defined as primary endpoint, and the aspirin subgroup was closed as a sufficient number of patients according to these estimations had been recruited. This was day 28 and this period might have been too short. Originally, a total observation period of 6 months was announced for the several kinds of drug treatment, but not for the aspirin group. All patients were on high-dose anticoagulants (heparin, LMWHs). No pharmacodynamic assessment of aspirin's action was performed, such as determination of serum thromboxane, an easily accessible and valid parameter to confirm the antiplatelet efficacy of aspirin. It is, therefore, unknown whether the 150 mg daily aspirin dose was really sufficient to inhibit platelet thromboxane biosynthesis in a situation of hypercoaglability, elevated levels of inflammatory markers, endothelial dysfunction [118, 121] and HTPR to aspirin as mentioned above. Thus, more appropriately sized prospective randomized trials are required to support the results of RECOVERY [109]. The RECOVERY-II trial, including 40,000 participants, is underway and will compare 15 (!) different therapeutic approaches, among them oral aspirin (again 150 mg/day) for their usefulness in COVID-19. According to the investigators, study results are expected in 2032, that is, 10 years from now.

4.2.2.7 Aspirin and other drugs

In addition to aspirin [92, 124], other antiplatelet agents, such as ADP antagonists, also appear to have the property of modulating/inhibiting platelet-mediated inflammatory and immune responses [124, 125]. The clinical relevance of these pleiotropic effects of antiplatelet agents is of considerable interest and requires further studies.

For prevention of COVID-19, mRNA- and vector-based vaccines are available. Both have shown to reduce the severity of infections but do not safely protect from reinfection or viral spread. Questions still remain regarding the benefit/risk ratio. In addition to severe side effects caused by the vaccine itself, although only in a small number of patients according to published data, there is also concern whether mRNA-based vaccines may alter the immunity status. The Pfizer/BionTech vaccine (BNT162b2) was shown in one small study to induce reprogramming of innate immune responses [126]. This important observation, although preliminary, needs to be restudied urgently in a larger number of patients since this might also be relevant for other classes of these antiviral vaccines. Another issue is long-term safety which is completely unknown.

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In 2009 the flu vaccine pandemrix was introduced. Narcolepsia as a severe side effect was detected only months to years later. The risk for vaccinated children was increased 5–14-fold, that for vaccinated adults was increased 2–7-fold. This elevated risk persisted for 2 years after vaccination [127]. Currently, we have less than 2 years of experience with large-scale administration of anticorona vaccines, but there is a significant political pressure to regulate COVID-19 vaccination by law in many countries and to extend vaccination to children aged only 5 years.

New viral mutants are coming up regularly and SARS viruses are no exception from the rule. Although some of the new COVID-19 variants appear to be less dangerous than their predecessor(s), they are possibly also less sensitive to the currently used vaccines. Most notably, none of the administered vaccines is effective for treatment and there is a time-dependent decrease of antiviral protection within a few months. All these are arguments for alternative procedures and improved therapeutic concepts. In the opinion of the author, aspirin would be a great candidate for reasons outlined above (Section 2.3.2) and is on the German market since years for treatment of flu-like conditions. Daily doses up to 2 (elderly) to 3 g are currently recommended for this indication. Oral treatment with paxclovid, a mixed preparation containing the protease inhibitors nirmaltrevir and ritonavir as a "booster" drug component, is currently under discussion. Time will show what the success will be. In any case, treatment will be expensive and probably limited to selected groups of patients. Glucocorticoids might be another option, as well as other medications used for standard of care for patients in the clinics.

4.2.2.8 Actual situation

Aspirin has lost its unique position as the number one agent for treatment of inflammatory pain in patients with rheumatoid arthritis and osteoarthritis. However, it is still of interest as antiinflammatory/immunomodulating agent in systemic inflammatory diseases where (systemic) platelet activation is clinically relevant. Interesting upcoming issues in this context are aspirin in SIRS and sepsis in addition to standard clinical treatment. Under study is also aspirin as possible adjunct in treatment of HIV and COVID-19. Of considerable pharmacological interest for treatment of flu-like conditions and other infections of the upper airways by respiratory viruses is nebulized aspirin (LASAG). A pilot study with an inhaled nanoparticle aspirin preparation resulted in peak plasma levels of 2.9 µg/ml (15 µM) at 2 minutes after inhalation of 100 mg. This was associated with complete inhibition of arachidonic acid-induced platelet aggregation [128]. Similar rapid effects and higher intrapulmonary concentrations are to be expected with aerosolized LASAG [112]. The compound is already on the market in Germany for intravenous application but needs clinical studies for its approval for other indications, including COVID-19.

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Summary

The analgesic/antiinflammatory action of aspirin is primarily due to aspirin itself but can be enhanced by the salicylate metabolite. Nevertheless, aspirin is no longer the drug of choice in patients with rheumatoid arthritis or osteoarthritis. Main reasons are the availability of better tolerated and more potent alternatives, the NSAIDs and coxibs, in addition to disease-modifying agents (DMARDs) such as methotrexate. Most traditional NSAIDs and coxibs increase the cardiovascular risk during long-term treatment. For some of them (ibuprofen), negative interactions with aspirin have been reported, specifically an abolition of the antiplatelet effects of aspirin. This should be considered in clinical practice by drug selection and appropriate timing if the combined use of NSAIDs and aspirin is necessary.

The actually increasing interest in aspirin as an antiinflammatory, immunomodulating agent is mainly due to the new discoveries regarding the central role of platelets in inflammatory and immunological processes. Aspirin inhibits platelet-dependent activation of white cells, NETosis and immunothrombosis. Antiplatelet effects via inhibition of platelet thromboxane formation are involved, as is the anticoagulatory effect via inhibition of thrombin generation. The elucidation of the modification (inhibition) of NF-κB-mediated signaling by salicylates for its clinical efficacy as antiinflammatory agent is also under study.

New potential indications for aspirin as an adjunct of standard antiplatelet/antiinflammatory and antiviral treatment are under study and have provided promising results in numerous observational trials. These include SIRS, sepsis and ARDS. Of increasing interest is the rediscovered antiviral action of salicylates. Whether the unique combination of antiplatelet, antiinflammatory and antiviral actions in the aspirin molecule will become clinically relevant as high-dose treatment for infections via the respiratory tract is under discussion but certainly of interest. This includes application of aerosolized aspirin (LASAG) for viral infections of the upper airways.

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4.2.3 Kawasaki disease

4.2.3.1 General aspects

History and epidemiology. Kawasaki disease (mucocutaneous lymph node syndrome) is a febrile disease of young children – approximately 85 % of patients are below the age of 5 years. The syndrome was originally detected in Japan [1], but later also found in other (East) Asian countries and in Europe. Kawasaki disease is a febrile panvasculitis and a leading cause of acquired heart diseases in small children [2]. In most cases, there are no later complications. However, severe vascular complications may occur in some patients, including thromboembolism, myocardial infarction and aneurysms of the coronary arteries [2, 3]. In this context, Kawasaki disease remains the most common cause of acquired heart disease in children [4].

Etiology and clinics. The etiopathogenesis of Kawasaki disease remains unknown [2]. A genetic background is suggested by the much higher incidence in some (East) Asian countries. There are possibly complex interactions between genetic determinants, bacterial and viral infections and pathological immune reactions [3, 5, 6]. In Japan, there was an impressive decline in mortality from 0.1 % to 0.01 %, mainly due to avoidance and/or appropriate treatment of myocardial infarctions or aneurysm rupture. This underlines the prognostic significance of early diagnosis and adequate treatment.

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The patients present initially with fever, lasting for at least 5 days, combined with signs of acute mucocutaneous inflammation and a pathologic immune reaction. The clinical symptoms include bulbar conjunctiva injection, generalized erythema of skin and mucosae, cervical unilateral lymph node enlargement, palmar erythema and stomatitis [7]. Inflammatory changes in the cardiovascular system suggest a poor prognosis. These include an arteritis of the large arteries, myocarditis and aneurysms of the vessel wall. About 20–25 % of untreated children develop coronary artery aneurysms. Coronary artery aneurysms and myocardial infarctions most commonly occur after the second week of illness. They are paralleled by thrombocytosis and occur at a time-point when fever and mucocutaneous manifestations are subsiding [8]. Approximately half of these abnormalities regresses within the following 5 years.

4.2.3.2 Pathophysiology and mode of aspirin action

Pathophysiology and laboratory findings. The initial feverish phase of the disease is possibly due to infections and is followed by an immune complex vasculitis that occurs when antibodies to the "priming" agent appear in the circulation. Thrombocytosis and activated thrombocytes are consistently found in patients with Kawasaki disease [9] as are immune complexes. These immune complexes also activate and aggregate platelets. This in turn stimulates platelet thromboxane formation and release of platelet-derived vasoactive, proinflammatory mediators. Consequences are elevated levels of plasma thromboxane and platelet activation markers at apparently unchanged levels of PGE_2 [10, 11], suggesting a platelet-related event.

There is no specific diagnostic test for the disease [2]. The laboratory findings are nonspecific and indicative of an immune complex vasculitis. Acute phase proteins and neutrophils are increased. There are elevated plasma levels of inflammatory cytokines, such as TNFα [12], and adhesion molecules, such as ICAM-1 [13]. Somehow indicative of an inflammatory immune reaction is the enhanced generation of cysteinyl leukotrienes during the acute phase of the disease [14] and circulating platelet-activating immune complexes in plasma after the second week of disease [8]. These immune complexes (soluble IL-2 receptors) appear more frequently in those children who later develop coronary abnormalities [15].

Role of platelets. Platelet hyperaggregability in Kawasaki disease is secondary to vasculitis, immune complex–platelet interactions and abnormal blood flow across aneurysmal vessels. Platelets obtained from blood samples of these patients were aggregated and activated with formation of numerous aggregates and heterotypic adhesion to leukocytes and red blood cells. There is an increased proportion of immature platelets and elevated 11-DH-TXB_2, P-selectin and CD40L levels. These changes are much more pronounced in patients with coronary artery lesions. They might be used as biomarkers to indicate the severity of vasculitis, since all of them will promote thrombosis [11] and are aspirin-sensitive.

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Mode of aspirin action. Aspirin significantly reduces the elevated plasma levels of thromboxane, P-selectin and CD40L, probably by inhibition of platelet functions [10]. Consequently, aspirin might also reduce subsequent inflammatory and immunothrombotic reactions (Section 4.2.2) [11, 16]. Initially high-dose and subsequent low, antiplatelet doses of aspirin are used [9]. Interestingly, protein binding of aspirin (salicylate) is significantly lower in children during the acute phase of Kawasaki disease – 73 % vs. 90 %. This results in an on average 2-fold higher level of free salicylate in these patients compared with normoalbuminuric controls [17] and also a significantly higher renal salicylate clearance during the febrile phase [18].

Kawasaki disease and Reye's syndrome. Aspirin has been worldwide banned as an antipyretic analgesic in (small) children suffering from flu-like symptoms because of a suggested elevated risk of Reye's syndrome (Section 3.3.3). In this context, it is interesting to note that it is extremely difficult to find even one single case of Reye's syndrome in children with Kawasaki disease [19] despite the traditionally intense use of high-dose aspirin for initial treatment. There is one case report from Macao [19]. In Japan, up to 200,000 children with Kawasaki disease have been treated with aspirin before 2004 with a recommended initial dose between 30 and 100 mg/kg. Among them, only one case of Reye syndrome has been reported, and this solely in the Japanese literature, being equivalent to a calculated incidence of <0.005 % [20]. This figure is similar to other countries worldwide where only a minority of cases was associated with a (subsequently) reported intake of aspirin (Section 3.3.3). In a recent British guideline for managing Kawasaki disease, the possible risk of Reye's syndrome due to aspirin treatment of (small) children is not even mentioned [2] and, therefore, appears not to be a serious problem. These data do not suggest any important relationship between the (virally induced?) febrile response in Kawasaki disease, Reye syndrome and the use of aspirin, even at the traditionally rather high doses in the early, febrile phase of the disease.

4.2.3.3 Clinical trials

Therapeutic goals. The therapeutic goal of treatment during the acute phase of the illness is to reduce inflammation and immune reactions in an effort to prevent thrombosis and the later occurrence of coronary artery aneurysms [3]. Early recognition and treatment with aspirin and intravenous immunoglobulin have both been shown to be effective. Aspirin was originally given in antiinflammatory doses of up to 100 mg/kg per day in the acute phase of the disease [17, 18] because of a reduced bioavailability of salicylate in the febrile state as a consequence of increased renal clearance; these high doses of aspirin were considered necessary to obtain therapeutic salicylate plasma levels of about 200 µg/ml. In one early trial, this was associated with a nearly complete prevention of coronary artery aneurysms: 3 % vs. 39 % [21].

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Of particular interest was the recent finding of a Chinese research group from Guangzhou (PRC) that the proportion of immature platelets and 11-DH-TXB_2, sP-selectin and sCD40L levels were much more elevated in aspirin-naïve Kawasaki patients with coronary artery lesions than in those without. Aspirin did reduce the elevated concentrations of 11-DH-TXB_2, sCD40L, sP-selectin and immature platelets in the lesion group but not in patients without lesions [11]. The reasons for this are unknown; possibly there is an HTPR or "resistance" to aspirin, as seen with other forms of immunothrombosis, for example HIV and COVID-19 (Section 4.2.2). The number of patients in the study of Pi et al. [11] was small ($n = 44$) and the long-term outcome of these children remained unknown. Two other Chinese groups, from Guangzhou and Taiwan, respectively, were unable to confirm a particular clinical benefit of aspirin as compared to immunoglobulin treatment in retrospective observational trials [22, 23]. Nevertheless, these are important findings that require reinvestigation and confirmation in controlled, appropriately sized, randomized trials.

Combined treatment with immunoglobulins. A metaanalysis on aspirin efficacy in Kawasaki disease indicated that a significant proportion of children still developed coronary artery aneurysms after treatment with aspirin alone. Combined therapy with aspirin and high-dose intravenous γ-immunoglobulin (2 g/kg) given as a single infusion reduced the occurrence of coronary artery aneurysms from 23 % and 17 %, respectively, after 1 month of single aspirin treatment to 9 % and 4 % after 2 months of combined treatment with immunoglobulin [24]. This combination meanwhile became the treatment of first choice [24]. A clinical problem is possible resistance against immunoglobulin.

An older metaanalysis of 1,629 children with acute Kawasaki syndrome in a total of six studies reported no effect of high- (80–120 mg/day) and medium-dose (30–50 mg/day) aspirin combined with intravenous immunoglobulin on the incidence of coronary artery abnormalities and the duration of fever. The conclusion was that 2 g/kg intravenous immunoglobulin combined with at least 30 to 50 mg/kg per day aspirin provides maximum protection against development of coronary abnormalities in children with Kawasaki disease [25]. Another review recommends aspirin as an alternative in patients resistant to immunoglobulin [26], while a more recent metaanalysis recommends low-dose aspirin plus immunoglobulin for the initial treatment [27]. It should be noted that a resistance to immunoglobulin occurs in up to 20 % of cases and that these are the patients at high risk for coronary artery aneurysms in the absence of additional treatment [5, 28]. Randomized, prospective trials are urgently needed to clarify the possible benefits – and risks – of aspirin as an adjunctive to intravenous immunoglobulin.

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4.2.3.4 Aspirin and other drugs

There is no established antiplatelet or antiinflammatory alternative to aspirin yet. However, the optimum dose of aspirin is still under discussion and there might be genetic differences between different (East) Asian populations [29]. Theoretically, antiinflammatory glucocorticoids and TNFα antagonists as well as clopidogrel were suggested for antiplatelet treatment [26]. However, clinical data evaluating the alternative use of glucocorticoids plus γ-globulin have produced confusing results [30, 31]. As 80 % of Kawasaki patients respond to aspirin and γ-globulin and coronary artery aneurysms are most commonly seen in those who fail to respond to γ-globulin, a predictor is needed – although not yet defined – to detect γ-globulin resistance, allowing these patients to be treated with glucocorticoids [2].

There are also different opinions among doctors about the antithrombotic management of patients with Kawasaki disease. A web-based worldwide survey of physicians completed between 2016 and 2017, including 603 physicians from 63 countries, indicated that in patients with normal coronary arteries, 25 % of physicians recommended low-dose aspirin during long-term follow-up (>3 months after diagnosis). In patients with nongiant coronary artery aneurysms, dual antiplatelet treatment (aspirin and clopidogrel) was used by 32 % of physicians, and anticoagulation by 19 %. In patients with giant coronary artery aneurysm, dual antiplatelet was used by 10 % of physicians and anticoagulation by 74 %. Thus, there was significant variation in antithrombotic management of patients with coronary artery aneurysms after Kawasaki disease, with 26 % of physicians not recommending anticoagulation of patients with giant coronary artery aneurysms [32].

4.2.3.5 Actual situation

According to recent metaanalyses, it is still a matter of discussion whether low-dose or high-dose aspirin (in addition to immunoglobulin) should be preferred for initial treatment of Kawasaki disease or even no aspirin at all [27, 33]. Nevertheless, intravenous γ-globulin combined with aspirin appears to be the standard treatment of choice [34]. Immunoglobulin treatment should be started early, preferably within the first 10 days of the illness. Aspirin is still a mainstay of therapy, because of its antiinflammatory and antiplatelet activities [35]. Initially high, antiinflammatory doses were used that were followed by lower, antiplatelet doses. Some clinical data suggest no major differences between high (75–100 mg/kg per day) and low-dose (1–74 mg/kg per day) initial aspirin in combination with immunoglobulin with respect to the duration of fever and the clinical outcome [2, 36]. Rescue therapies for immunoglobulin-resistant patients include corticosteroids as well as infliximab, an antagonist of TNFα. There are limitations for the use of corticosteroids because of a possibly enhanced risk for coronary artery aneurysms [2]. More details about the actual status can be found in the statement of a multidisciplinary writing group of experts of the American Heart Association [37].

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Summary
Kawasaki disease is an acute feverish disease which predominantly affects small children below the age of 5 years. Fever is followed by an immune vasculitis, affecting predominantly (coronary) arteries, associated with a thrombosis tendency. Children with coronary artery abnormalities (aneurysms) are at elevated risk for coronary thrombosis, myocardial infarction and death.

The pathogenesis and etiology of the disease are unknown. Possibly, the disease is initiated by infection, followed by an immune complex vasculitis with the appearance of antibodies in the circulation. These immune complexes cause a prothrombotic state with platelet aggregation, secretion and thromboxane formation as well as generation and release of inflammatory cytokines and expression of adhesion molecules for inflammatory cells at the vascular endothelium.

High-dose intravenous immunoglobulin combined with aspirin is still the treatment of choice. Traditionally, aspirin is initially given in high, antiinflammatory doses of 30–60 mg/kg per day, followed by antiplatelet doses of 3–5 mg/kg per day in later phases of the diseases if there is evidence for coronary abnormalities. Possibly, lower initial doses of aspirin are also effective, since both dose regimes appear to be equipotent with respect to coronary abnormalities [38, 39]. The combined treatment of immunoglobulin with aspirin might reduce the incidence of coronary artery aneurysms, myocardial infarctions and vascular death.

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4.3 Further potential clinical indications

The usefulness of aspirin as a preventive of thrombotic vessel occlusions and treatment of fever, pain and flu-like conditions is established since decades. This covers a broad but not the full spectrum of its pharmacological actions. Other effects, such as the antiinflammatory/antirheumatic effects as well as hypoglycemic activities, are not considered to be of therapeutic value. The major reason is the availability of more effective and better tolerable drug alternatives. The tocolytic action of aspirin was also not considered as clinically valuable; however, it is probably involved in the prevention of preterm deliveries in women at risk for preeclampsia (Section 4.1.5). Similarly, the introduction of aspirin as an adjunct for treatment of severe coagulopathies (DIC), sepsis and ARDS becomes increasingly interesting (Section 4.2.2). The recent exploration of aspirin for prevention of thromboembolic complications in HIV patients under antiretroviral treatment is also an interesting new field of clinical research (Section 4.2.3), as are the current hypotheses about a possible benefit from the antiviral actions of aspirin for treatment of infections of the respiratory tract, including COVID-19 (Section 4.2.2).

An actual example of the potential introduction of aspirin as a drug with presumably significant clinical impact and already more than 100 clinical trials is the chemoprevention of malignant tumors. The numerically most and also most convincing data exist for tumors of the gastrointestinal tract, that is, colorectal carcinomas (CRC) and adenomas, while the efficacy of aspirin as a chemopreventive for other malignancies is rather variable. Nevertheless, a recent large review and metaanalysis of 118 observational studies of cancer survival with and without aspirin in 18 different cancers and a total of about a quarter million patients with cancer suggested an overall 20 % increased survival rate in individuals who took aspirin [1]. However, the bulk of these data was generated from nonrandomized, observational studies, and another recent metaanalysis of 17 CRC cohort studies with 16,654 patients found that postdiagnosis aspirin but not prediagnosis aspirin reduced cancer-specific mortality [2]. With the exception of ASPREE, there are very few large prospective, randomized trials with cancer outcome as a predefined study endpoint. With the possible exception of prostate cancer, it are colorectal tumors (adenomas, carcinomas) which are in focus as possible targets for tumor prevention or treatment by aspirin. Therefore, the discussion of the

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effects of aspirin on prevention of malignancies in men is focused on tumors of the gastrointestinal tract (Section 4.3.1).

Another clinical-experimental field of aspirin research is its use in prevention of neurological diseases with cognitive deficits (dementia). Here, research is not well developed yet. Reasons are the complex pathophysiology of the disease, the problem of early diagnostics in order to prevent or at least retard the progression of the disease and the different etiologies, for example vascular dementia (M. Binswanger) versus nonvascular, neurodegenerative forms of dementia (M. Alzheimer) (Section 4.3.2).

4.3.1 Colorectal tumors (adenomas, carcinomas)

4.3.1.1 General aspects

A piece of history. The first clinical trial on the use of aspirin as a chemopreventive for CRs came from *Gabriel Kune*, Professor of Surgery at the University of Melbourne. (Australia). He compared the incidence of colorectal cancers in individuals who regularly (daily) had taken aspirin with those who did not. Aspirin use resulted in a 40 % reduced risk in individuals who had taken aspirin regularly. Dr. Kune summarized the results of his study as follows:

> ...There was a statistically significant deficit of the use of aspirin and aspirin-containing compounds among cases and these differences remained statistically significant after adjustment for hypertension, heart disease, chronic arthritis, and diet in both males and females. . . . This finding, whatever the mechanism may be, has potential significance in colorectal cancer chemoprevention and merits early confirmation. Aspirin is now widely used in the chemoprophylaxis of cardiovascular disease and may also be useful in a similar way in the prevention of colorectal cancer and perhaps also of other cancers [3].

These data were generated in a retrospective, exploratory case-control study which also noted a significant risk reduction in subjects using NSAIDs other than aspirin. Similar findings were shortly thereafter also obtained in the Boston Collaborative Study [4] and several large prospective epidemiological trials, the "Cancer Prevention Study II" (CPS-II) [5], the "Health professionals follow-up study" (HPFS) in males [6] and the "Nurses Health study" (NHS) in females [7]. They were followed by numerous, mostly observational trials on prevention as well as treatment of solid tumors with aspirin. These studies basically confirmed the data from Kune, have been continued over the years with follow-up analyses in regular intervals and are discussed in more detail below.

Another fresh insight into the clinical benefit/risk ratio of long-term aspirin in prevention of cancer came from a series of reviews and metaanalyses by *Peter M. Rothwell* and colleagues from Oxford (UK) [8–10]. These authors have reused data from earlier cardiovascular prevention trials with aspirin and have focused on the information on

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primary prevention of cancer. These studies had the advantage of a randomized design at the initial phase of the cardiovascular study, followed by a long observation period, currently more than 30 years. These studies confirmed a time-dependently reduced incidence of colorectal cancer by regular aspirin intake for both men and women. Interestingly, aspirin also reduced the risk of distal metastases of preexisting carcinomas, that is, tumor spreading, eventually resulting in a survival benefit in some aspirin treated patients. At the same time, there was a marked and again time-dependent reduction of bleeding events. Tightly related to these studies is also the search for possible modes of action of aspirin as well as the evaluation of suitable biomarkers to identify subgroups of patients who benefit most [11].

Actions of aspirin on nongastrointestinal solid tumors. Chemopreventive actions of aspirin and nonaspirin NSAIDs have been reported for a number of solid tumors. However, the most convincing data, both in experimental settings and clinical studies, were obtained in prevention of malignancies of the gastrointestinal tract [12, 13], CRC and colorectal adenomas being the most intensively studied tumors. According to available trials – the vast majority of them observational – chemoprotective effects of aspirin on cancer incidence and survival of solid tumors outside the gastrointestinal tract (prostate, mamma, bladder, kidney and others) are variable and inconsistent [1, 14–18]. In addition to the colon and rectum, other areas of the gastrointestinal tract are also possible sites for aspirin chemoprotection. This includes carcinomas of the esophagus and stomach [19–26]. The overall incidence rate of CRC and esophageal and gastric cancer in aspirin-taking persons was between 0.70 and 0.75 and the rate of mortality was between 0.55 and 0.70 (conservative estimation) compared to untreated controls [13]. These are remarkable figures and appear to be at least as good as those for cardiovascular protection by aspirin (Table 4.3.1-1).

This section is focused on clinical aspects of prevention and treatment of CRC, including a short summary of aspirin's possible sites of action. A more detailed discussion of the multiple pharmacological mechanisms of chemoprotection can be found in Section 2.3.3 on malignancies.

4.3.1.2 Epidemiology, etiology and pathogenesis

Epidemiology. CRC is the second most prevalent cancer in women and the third most prevalent cancer in men. The incidence is actually 1.84 million new cases and 0.8 million deaths per year worldwide with about equal distribution between sexes (cited after [27]). The tendency appears to be rapidly rising, partially due to an increasing age of the population. However, the incidence is also increasing at younger ages:. In a case-control study of >67,000 US veterans aged 18 to 49 years who underwent screening colonoscopy, the median age of early-onset CRC was 45.3 years and aspirin use was associated with a significantly decreased risk [28]. This prompted the American

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Table 4.3.1-1: Risk ratios for incidence of and mortality from different vascular (myocardial infarction, stroke) and nonvascular (cancer) events during long-term aspirin treatment [13].

Event	Incidence (conservative)	Mortality (conservative)
colorectal cancer	0.70	0.65
oesophageal cancer	0.75	0.55
gastric cancer	0.75	0.70
lung cancer	1.00	0.90
prostate cancer	0.95	0.90
breast cancer	0.95	1.00
myocardial infarction	0.82	0.95
stroke	0.95	1.21
major extracranial bleeding	1.70	–
GI bleeding	–	1.70
peptic ulcer	–	1.70

Society of Cancer to recommend screening colonoscopy in men already at the age of 45 years.

About 70 % of CRC are sporadic tumors, mostly adenocarcinomas, without detectable hereditary background. They probably result from somatic gene mutations during life. Stem cell mutations in the colon become increased at older age. These mutations accumulate over time in the small intestine and colon at a rate of approximately 40 novel mutations per year, despite the large variation in cancer incidence among these tissues [29]. These acquired mutations accumulate steadily if they are not removed in due time by body defense/repair mechanisms [30]. This also explains their preference among individuals at older age (>70 years). The remaining 20–30 % of colorectal malignancies have a hereditary background. For the most part, this includes the "familial adenomatous polyposis coli" (FAP) with hundreds of initially benign polyps in the large intestine. These polyps later become malignant in a significant but highly variable proportion of cases. The other form is the "hereditary nonpolyposis-associated colorectal carcinoma" (HNPCC). The major form is Lynch syndrome as the most frequent inborn predisposition for nonpolyposis CRC with an incidence of 3–5 % and a 75 % risk for becoming malignant [31].

Etiology. The etiology of CRC, like that of other epithelial tumors, is multifactorial and a classical example of multistep carcinogenesis [32]. Reasons are mutations at critical sites of genes that control cell division, apoptosis and DNA repair [33]. Most relevant are defects in the "adenomatous polyposis coli" (APC) gene, a tumor suppressor gene. Mutations in this gene can disturb apoptosis and, therefore, the balance between cell division and programmed cell death (apoptosis). Mutations in the APC gene are particularly frequent and typical for colorectal neoplasias, including FAP – as

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opposed to other nonintestinal solid tumors [34]. In case of simultaneous mutations in other apoptosis-related oncogenes (p-53, k-ras and others), this results in the synthesis of dysfunctional proteins [30, 35]. Further potentially aggravating factors are environmental mutagenic factors, specifically those from the diet (fat, dietary fibers) [36], but also drugs that change the composition of the gut microflora. The gut microbiome is a key determinant for gut homeostasis, host immune activity and intestinal stem cell proliferation/regeneration and might also be involved in aspirin's bioavailability and efficacy [11]. The clinical results are colorectal neoplasias, that is, adenomas or carcinomas. Adenomas are frequent precursor tumors (precancerosis) and proceed to malignancy in about 10 % of cases. The transition time from adenoma to symptomatic cancer is probably at least 5–10 years [37]. This rather long time interval is the reason for (regular) screening for tumors (adenomas) by colonoscopy, allowing removal of adenomas before their transition into malignancy.

Pathophysiology. Defects in the APC gene can result in a premature stop of gene transcription of APC and subsequent incomplete translation into the APC protein. This protein binds in a complex β-catenin, a transcription factor, activated by the Wnt signaling pathway. Functional consequence of a truncated APC protein is the loss of binding sites for β-catenin. This prevents its binding and subsequent phosphorylation and inactivation inside the cytosol. Over 80 % of CRCs have nuclear accumulation of β-catenin, frequently as a consequence of mutations in the APC gene [38]. Instead of binding and degradation, free β-catenin now enters the nucleus and acts there as a coactivator of the transcription factors TCF/LEF. This causes activation of the oncogenic Wnt/β-catenin pathway with subsequent upregulation of several cytokines, chemokines, cell cycle-regulating genes, growth factors and also COX-2. Results are inhibition of apoptosis with subsequent uncontrolled cell division, proliferation and invasion of tumor cells into tissues and induction of tumor angiogenesis and spread, that is, generation of distant metastases as outlined in detail before (Section 2.3.3.3).

4.3.1.3 Modes of aspirin action

General aspects. Aspirin interferes with tumorigenesis, tumor growth and tumor spread at different levels. Central to its proposed cancer-preventive mechanism is the inhibition of COX-1- and COX-2-dependent prostaglandin (PGE_2) and thromboxane (TXA_2) production. Upregulation of COX-2 and increased PGE_2 synthesis in existing tumors is long known to be clinically correlated with the malignancy of the disease (lymph node metastases, tumor size) [39] and the survival rate of patients [40]. A (hypothetical) overview of carcinogenesis (CRC) via the Wnt pathway, the role of prostaglandins and possible sites of action of aspirin are shown in Fig. 4.3.1-1.

Pharmacological modes of chemopreventive and, possibly, chemotherapeutic actions of aspirin should be able to explain the following clinical phenomena: (i) Efficacy of antiplatelet doses without a clear dose dependency but with clear evidence that

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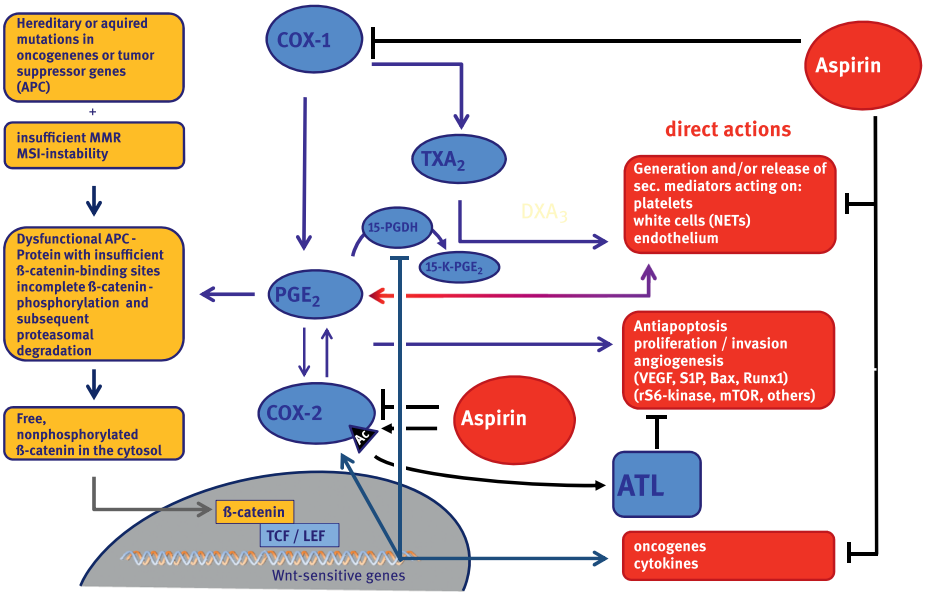

Figure 4.3.1-1: Overview of CRC carcinogenesis. Yellow, the Wnt signaling pathways of CRC pathogenesis; blue, major, CRC-associated changes in prostaglandin formation and metabolism and sites of prostaglandin/thromboxane (COX)-related aspirin actions; red, further potentially aspirin-sensitive targets for direct antioncogenic effects. Gene defects and a dysfunctional APC protein result in free β-catenin accumulation in the cytosol, allowing for its migration into the nucleus, where it acts together with TCF/LEF as a cofactor for induction of Wnt-sensitive genes (yellow). These processes are associated with enhanced COX-2 gene expression and the activation of COX-2/PGE_2-dependent and -independent oncogenic signaling pathways. COX-2/PGE_2 stimulate tumor growth/invasiveness, apoptosis and angiogenesis. PGE_2 accumulates due to downregulated degradation by 15-PGDH. PGE_2/thromboxane production and inflammatory/tumorigenic processes are also stimulated by COX-1 from platelets and (intestinal) epithelial cells. Aspirin inhibits COX-1 and COX-2 and changes the acetylated COX-2 towards a 15-lipoxygenase which generates ATL in cooperation with white cell lipoxygenases (not shown) (blue). Direct inhibitory actions of aspirin/salicylate on multiple, COX-independent targets at higher local concentrations might contribute to these effects (red) (for further explanation see text). Abbreviations: APC: adenomatosis polyposis gene; ATL: aspirin-triggered lipoxin; Bax: proapoptotic gene; COX: cyclooxygenase; PGE_2: prostaglandin E_2; MMR: mismatch repair; MSI: microsatellite instability; S1P: sphingosine-1-phosphate; VEGF: vascular endothelial growth factor; Wnt: oncogene; 15-PGDH: 15-prostaglandin dehydrogenase; TCF/LEF: Wnt-activating cofactors.

nonaspirin antiplatelet agents will have no chemopreventive effects [41, 42]. (ii) Prevention of transformation of healthy, diploid colonic epithelial (stem) cells into proliferating tumor cells with disturbed apoptosis. This on the background that COX(-2) inhibitors have been shown to possess some antitumor actions although healthy colonic epithelial cells do not express COX-2. (iii) Long requirement of treatment with documented clinical effects after regular aspirin intake for about 10 years or more. This is in

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contrast to the cardioprotective effects of aspirin. In the same primary prevention trials which have later been used for studying tumor prevention, optimum efficacy was seen shortly after start of treatment within the first 5 years without further improvements. (iv) Aspirin appears to be preferentially active in gastrointestinal tumors (adenocarcinomas) (colon, stomach, esophagus) but less in nonadenocarcinomas of the gut and several nongastrointestinal carcinomas of other organs (pancreas, bronchi, mamma). However, the data provide mixed information and it should be clarified where these variations come from.

The pharmacological modes of antitumor actions of aspirin have been discussed in detail in Section 2.3.3. For its clinical efficacy, two properties of aspirin appear to be central to its antitumor effects: (i) antiinflammatory/immunomodulating and (ii) antiplatelet actions [11].

Antiplatelet actions. Available clinical data, specifically with respect to the efficacy of low aspirin doses, suggest platelet COX-1 as a relevant target for aspirin-induced chemoprevention. Reasons are the irreversibility of platelet COX-1 acetylation and the survival of the blocked COX-1 protein over the life span of platelets, that is, 8–9 days (Section 2.3.1). In addition, there might be accumulation of cells with "blocked" COX-1 over time. A central role of platelets in tumorigenesis was originally postulated by Gasic and colleagues with respect to the antimetastatic effect of aspirin [43] as well as the significance of platelet-derived factors for angiogenesis and metastatic outgrowth [44, 45]. Platelets are likely sources of elevated circulating thromboxane in patients with FAP and CRC which can be reduced by aspirin intake [46]. Platelets not only form aggregates with themselves but also contain a huge amount of proteins and genetic material, including platelet-specific, tumor-promoting and angiogenic mediators [47]. In addition, platelets can interact with their environment by forming platelet/white cell coaggregates and NETs as well as by priming tumor cells for subsequent metastasis [48]. This plethora of biological activities makes platelets and platelet-derived mediators most attractive candidates as therapeutic targets for cancer prevention. Unfortunately, there is little information about the chemopreventive effects of other antiplatelet agents [41, 42].

Antiinflammatory actions. Aging is associated with increased chronic inflammation, termed inflammaging [11]. Accordingly, experimental and clinical studies also suggest chemopreventive properties for certain NSAIDs, that is, inhibitors of prostaglandin biosynthesis [49]. These compounds are only weak and transient inhibitors of platelet function but do inhibit COXs and prostaglandin formation throughout the body, including tumor cells. PGE_2 is the major metabolite of interest, and COX-2 is the major synthesizing enzyme that is markedly upregulated in CRC, in particular in tumors with poor prognosis [40]. COX-2-derived PGE_2 plays a key role in inflammatory signaling

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and cellular proliferation, survival and growth. Aspirin reduces the incidence of tumors that overexpress COX-2 [50] and changes COX-2 activity towards generation of antiinflammatory ATL [51]. Studies in healthy volunteers have shown that low-dose aspirin significantly inhibits systemic PGE_2 biosynthesis, by 45 % [52], and that intestinal mucosal COX-1 is one source of it [53]. Interestingly, inhibition of PGE_2 biosynthesis by platelet–tumor cell aggregates is, at least in part, platelet-mediated [52]. Aspirin also inhibits prostaglandin synthesis in tumor tissue but does not completely prevent it. Intake of 325 or 650 mg/day aspirin for 2 months resulted in a 50–70 % decrease in PGE_2 production in colonic mucosal samples of patients with a history of colonic cancer. This inhibition was not dose-dependent and disappeared when aspirin was stopped [54]. An about 40 % inhibition of PGE_2 levels in colorectal mucosa was seen after 100 mg/day aspirin for 1 week and correlated with an aspirin-sensitive kinase activity (see above) [53]. These data suggest a therapeutically relevant inhibition of PGE_2 formation by aspirin which, in contrast to inhibition of platelet-dependent thromboxane formation, must not necessarily be complete but is an important variable in tumor prevention.

Taken together, these data confirm a central role of COX-2- and COX-1-derived PGE_2 in tumorigenesis and tumor promotion. Possibly, platelets might act as a kind of trigger in this reaction chain via aspirin-sensitive TXA_2 formation (Fig. 4.3.1-1).

4.3.1.4 Clinical trials – primary prevention

General aspects. Despite the relatively high incidence of CRC in most populations, the developmental period of CRC and consequently the efficacy of chemoprevention are too long to conduct randomized prospective primary trials with CRC as primary clinical endpoint [55]. There might also be ethical concerns for persons who need aspirin prevention for cardiovascular reasons. Therefore, the vast majority of available studies on primary prevention of CRC are epidemiological observational trials. A meta-analysis of methodologically rigorous observational studies showed that data were consistent with those obtained from randomized controlled trials [56]. An important source of information are the initially randomized trials on primary cardiovascular prevention by aspirin. These studies have the advantage of a long observation period, currently (2022) about 30 years and more.

Epidemiological studies. Following the pioneering studies of Gabriel Kune, the Boston Collaborative Study Group conducted another case-control trial which showed that regular intake of NSAIDs (usually aspirin-containing medications) reduced the incidence of CRC by 50 %. This study additionally showed that the risk of CRC appeared to decrease with a longer duration of NSAID (aspirin) intake and to increase after withdrawal. However, none of these trends was significant [4]. Obviously, much larger-sized trials were necessary to identify the chemoprotective actions of aspirin on tumorigenesis.

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A strong impetus in favor of the hypothesis of an anticancerogenic action of aspirin came from *Michael J. Thun* and colleagues from the American Cancer Society [5, 57]. These authors initiated the prospective "Cancer Prevention Study II" (CPS-II), one of the largest epidemiological studies on gastrointestinal neoplasias, including 662,424 adults [57]. The main research question was a possible relationship between aspirin intake and death from colorectal cancer.

The CPS-II study was a prospective cohort trial in both sexes. The medium age at the beginning was 57 years. The participants were asked two questions on aspirin: "How many times in the last month have you used the following [medication]?" and "How long (years) have you used them?" Aspirin was noted together with other medications. Study endpoint was mortality.

The relative mortality from colon cancer among individuals who used aspirin 16 or more times per month for at least 1 year was 0.60 in men (95 % CI: 0.40–0.89) and 0.58 in women (95 % CI: 0.37–0.90), on average 0.58, as compared to persons who did not take aspirin. There was also a trend of a decreasing risk with more frequent and/or prolonged (at least 10 years) aspirin use, again similar in both sexes. Similar results were found with fatal rectum cancer: The combined risk was reduced to 0.66; however, greater reductions were obtained in men. No association was found between the use of acetaminophen and the risk of colon cancer.

The conclusion was that regular aspirin intake at therapeutic doses may reduce the risk of fatal colon cancer. In the paper, published in 1993, there was a similar protective effect also for cancer of the stomach and esophagus. Whether this was due to a direct effect of aspirin, perhaps mediated by the inhibition of prostaglandin biosynthesis, or to other aspirin-sensitive factors, not associated with the prostaglandin system, remained open [5, 58].

The strength of this study was its size and prospective design, establishing dose–response trends with both the frequency and duration of aspirin use in men and women. Its limitations include dependency upon a single brief, self-administered questionnaire, the absence of data on aspirin dosage (as opposed to frequency and duration of use) and reasons for its intake, the possible intake of NSAIDs other than or in addition to aspirin and, particularly, the study's reliance on cancer mortality rates rather than incidence to define the presence of the disease [57]. The CPS-II study, therefore, did also not allow conclusions whether aspirin influenced the development and progression of already existent tumors [59].

The currently last edition of the CPS-II trial was a subgroup analysis, the "Nutrition Cohort Study", established in 1992. This subset contained about 100,000 participants of the CPS-II trial with no history of cancer [60]. This observational analysis has basically confirmed the previous results of the complete cohort, established in 1982, that is, a reduced cancer mortality by regular aspirin intake. However, the data were much less impressive than the previous ones and showed an only 16 % overall lower cancer mortality as opposed to the about 40 % reduction in the original complete study [5, 58] and the 37 % reduction seen after 5 years of randomized aspirin use in a large pooled CRC prevention analysis (see below) [10]. In addition, there was no influence of the duration of daily aspirin intake, that is, more or less than 5 years. In an editorial to this study [61], several critical points of discussion were addressed. These

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included the general problems of nonrandomized trials (Section 4.1), different evaluation criteria regarding definition of malignancies, possible bias because of differences in the composition of the study groups (cigarette smoking!), uncertainties about the real duration of aspirin use by the participants and the finding that the duration of aspirin intake (less or more than 5 years) did not affect cancer mortality. This was at variance with other large CRC prevention trials [10, 62, 63].

There are two more large epidemiological trials on gastrointestinal tumors: the prospective "Health Professional Follow-up Study" (HPFS) in males and the "Nurses' Health Study" (NHS) in female health professionals. The studies have been continued until now and are reevaluated in regular time intervals.

A total of 47,900 male health professionals aged 40–75 years were included into the HPFS study. Participants were asked every second year by a mailed questionnaire on intake of aspirin and other NSAIDs and on history of cancer and other clinically diagnosed medical conditions. Controls were men without regular intake of aspirin.

A decrease in the number of colorectal adenomas was found in a subgroup of 10,521 men subjected to endoscopy for reasons other than bleeding. Regular use of standard aspirin (325 mg) for at least two times a week – more than 92 % of participants took aspirin at least 3 days per week, 51 % even daily – reduced the risk for colorectal cancer as compared to nonusers to 0.68 ($P = 0.008$). A decreased risk was noted for both colon and rectum carcinomas). The inverse association between aspirin use and colorectal cancer became progressively stronger with evidence of more consistent use of aspirin. There was a strong inverse association between aspirin use and advanced (metastatic and fatal) cancer, suggesting that aspirin-related bleeding could further decrease mortality, for example by allowing earlier diagnosis and (surgical) treatment.

The study did not evaluate data on duration of aspirin use. However, supplementary data indicated that a "substantial" proportion of consistent aspirin users had been taking aspirin for at least 10 years.

The conclusion was that regular aspirin use is associated with a reduced risk of (metastatic) colorectal cancer in males [6].

This study additionally showed that regular screening for fecal occult blood loss, possibly combined with colonoscopy, will significantly reduce mortality of the disease. This was confirmed in another randomized controlled trial on 46,000 participants. This study showed a 33 % cumulative decrease in colorectal cancer mortality at 13 years in the group of participants having annual screening for occult blood in stool as compared to those who did not [64].

Follow-up editions of the HPFS/NHS studies have confirmed the previous data but also added some new information. During an 18-year follow-up there was a 21 % reduction of the RR for colorectal cancer (HR: 0.79; 95 % CI: 0.69–0.90) in men who regularly used aspirin at least twice a week. Maximum risk reduction was obtained at doses of more than 14 tablets per week, and at least 6–10 years of continuous use were required. Interestingly, the protective effect disappeared if regular aspirin intake was interrupted for 4 years or more [63]. Thus, a continuous long-term application appears to be required – with accompanying time-dependent risks, in particular of bleeding events [63].

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A similar approach was used for female health professionals in the NHS [7].

This study aimed to determine the effect of standard aspirin (325 mg) on the risk of colorectal cancer in women without previous diagnosis of cancer, familiar adenomatosis coli or ulcerative colitis. A total of 89,446 women were included. The treatment group reported regular aspirin use (two or more standard aspirin tablets) on three consecutive questionnaires at two-year intervals. The rates of colorectal cancer were determined according to the number of the consecutive years of regular aspirin use (defined as two or more standard aspirin tablets per week). The rates were compared with the rates among women who did not take aspirin. All cases of cancer over a period of 12 years were determined. The aim was to define the effect of dose and duration of aspirin treatment on the risk of colorectal cancer.

During the observation period, 331 new cases of colorectal cancer were documented. Regular aspirin intake did not reduce the risk of colorectal cancer as compared with nonusers after four years (OR: 1.05; 95 % CI: 0.78–1.45) or after 5–9 years (OR: 0.84; 95 % CI: 0.55–1.28). There was a slight, nonsignificant risk reduction after aspirin intake for 10–19 years (OR: 0.70; 95 % CI: 0.41–1.20) but a significant reduction after 20 years of consistent use of aspirin (OR: 0.56; 95 % CI: 0.36–0.90).

The conclusion was that regular aspirin use substantially reduces the risk of colorectal cancer in women. Four to six tablets per week appear to be optimal. However, this benefit may not be evident until after at least a decade of regular aspirin consumption [7].

A more recent edition of a subgroup of this study addressed the issue of aspirin dosing and duration of treatment on primary prevention of colorectal adenomas in women. Similar results were obtained.

The adjusted OR for adenoma of regular aspirin users as compared to nonregular users was 0.75 (95 % CI: 0.49–0.80). The risk decreased with increasing aspirin dosing from 0.80 in women who used less than two tablets per week to 0.74 with two to five tablets per week and 0.49 (95 % CI: 0.36–0.65) in those who took more than 14 tablets per week ($P < 0.001$ for trend). Similar dose–response relationships were found among users for ≤5 years and >5 years.

The conclusion was that regular, short-term (≤5 years) aspirin use is inversely associated with the risk for colorectal adenomas but not carcinomas. However, the greatest benefit is obtained at substantially higher doses than those that are used for cardiovascular protection. This requires a more thorough benefit/risk evaluation before aspirin can be recommended for chemoprevention of tumors in the general adult population [65].

A review of publications using the NHS data between 1976 and 2016, that is, 40 years of observation, has identified several environmental factors that increase (red and processed meat, alcohol, smoking, obesity) and decrease (folate, calcium, vitamin D, aspirin, physical activity) the risk of CRC [66]. Among medicines, aspirin appeared to be the only drug that reduced the risk of CRC in primary prevention. The efficacy of aspirin was confirmed in the latest edition of this trial. A suggested benefit of aspirin chemoprotection of CRC necessitates at least 6–10 years of treatment and becomes stronger at 10 years. Remote use and use within the previous 10 years both contribute independently to a decreased risk, though a lower dose may be required for a benefit with longer-term use [67].

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Table 4.3.1-2: Selected epidemiological trials on primary prevention of CRC by aspirin with CRC as primary endpoint. The data reflect the clinical result (OR) at the time of (re)evaluation by comparison with the nonaspirin-treated control groups. Some of these studies are still ongoing and will provide more results in the future. One aspirin standard tablet used in these studies contained 325 mg aspirin [3, 6, 7, 57, 68–70].

Number of participants (acronym)	Frequency of aspirin intake	Study endpoint	OR (±95 % CI)	Reference
715 „User“ 727 „Non-User“	not indicated	CRC	**0.53** (0.40–0.71)	[3]
662,424 persons of both sexes (CPS-II)	≥16 tabl. (325 mg)/ month and >1 year	CRC (mortality)	**0.60** (0.40–0.89) (men) **0.58** (0.37–0.90) (women)	[57] [68]
47,900 men (HPFS)	≥2 times / week	CRC	**0.68** (0.52–0.92)	[6]
89,446 women (NHS)	4–6 tabl. (325 mg)/ week for ≥20years	CRC	**0.56** (0.36–0.90)	[7]
2,279 cases 2,907 controls	>4 tabl. (75 mg)/ week for >1 month	CRC	**0.78** (0.85–0.92)	[69]
1,958 „User“ 7,940 „Non-User“ (Taiwan Study)	50–150 mg/day for at least 3.5 years	CRC	**0.50** (0.28–0.87)	[70]

Table 4.3.1-2 is an overview of results of selected nonrandomized primary prevention trials with CRC as clinical endpoint. All of the available observational studies on CRC chemoprevention by aspirin demonstrate beneficial effects of the compound: On average, long-term regular use will reduce the incidence of and mortality from CRC by about 15–40 %. Similar figures were obtained for prevention from esophageal and stomach cancers but were less consistent for several other solid tumors (Table 4.3.1-1) [13, 56]. Benefits were also found for aspirin with respect to cancer mortality in a recent metaanalysis of 70 published observational studies on aspirin and cancer survival (HR: 0.79; 95 % CI: 0.73–0.84) [1].

Randomized trials. The first randomized, placebo-controlled, prospective studies on aspirin in primary prevention were the cardiovascular prevention trials with thromboembolic vascular events as endpoints. Neither the US-PHS in males nor the WHS in females found any significant change in the incidence of CRC after an initial observation period of 5 or 10 years (RR: 1.03 and RR: 0.97, respectively) [71, 72]. Notably, the treatment was with 325 mg (US-PHS) or 100 mg aspirin (WHS) every *second* day, which might have been less efficient than daily administration [73]. A significant proportion of participants voluntarily moved from the former placebo into the aspirin group for

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the subsequent open part of the trial after the treatment code was opened. The last available evaluation of the WHS trial, now with an observation period of 18 years, has reported a significant decrease in the incidence of CRC, starting after about 12 years of regular intake. Interestingly, there was no change in the incidence of breast cancer in this study, although this cancer occurred five to six times more frequently in this female population [74]. In the WHS study, there was a small increase in gastrointestinal bleeding events (HR: 1.14) and peptic ulcers (HR: 1.17) [74]. Overall, the benefit/risk ratio was considered positive.

The effect of regular aspirin use (more than three tablets a week) on the risk of lethal prostate cancer in the population of the US-PHS was studied in 2009, 33 years after the beginning of the trial. By this time point, 502 men had developed lethal prostate cancer. The incidence was reduced by both current and past aspirin intake (HR: 0.68; 95 % CI: 0.52–0.89; HR: 0.54; 95 % CI: 0.40–0.74) compared to nonusers. Similar data were obtained in a further follow-up in 2015 demonstrating that current and even post-diagnosis regular aspirin intake was associated with a lower risk of lethal prostate cancer [18].

Peter M. Rothwell and colleagues from Oxford (UK) have summarized the randomized cardiovascular prevention trials with daily aspirin intake for long-term effects in a number of excellent reviews [9, 10, 15]. They found a similar reduced incidence of CRC in both women and men, starting at about 3–5 years of regular daily aspirin intake. The beneficial effect of aspirin on the prevention of cardiovascular events within a 5-year observation period was confirmed but also an increased bleeding tendency was found. At longer observation periods (>5 years), there remained only a significantly reduced total risk for cancer while the number of fatal extracerebral bleeds was halved ($P = 0.009$) (Fig. 4.3.1-2).

These data together with those of previous trials and meta-analyses suggested that a major chemopreventive effect of long-term regular aspirin was on cancer [8], specifically of the gastrointestinal tract, and required at least 5 years or more of regular aspirin intake. This is much longer than necessary for cardiovascular protection. These findings agreed well with those from the observational trials discussed before [13].

Lynch syndrome. Lynch syndrome, a genetic defect in MMR genes, is the most frequent inborn form of nonpolyposis predisposition for CRC (HNPCC) with a 75 % risk for later malignancy, as opposed to <3 % in nonhereditary CRC. For these reasons, Lynch syndrome is a particularly interesting model for the chemoprevention of CRC. The prospective, randomized, placebo-controlled "Colorectal Adenoma/Carcinoma Prevention Programme-2" (CAPP-2) trial studied the effect of aspirin (600 mg/day, enteric-coated) on the incidence of CRC in patients with Lynch syndrome: Treatment for 4 years showed no difference vs. placebo (RR: 7.4 % vs. 9.9 %; $P = 0.33$) [75]. However, with longer duration of the study, there was a tendency in favor of aspirin which became significant after 56 months in an "on-treatment" analysis ($P = 0.02$) but not in

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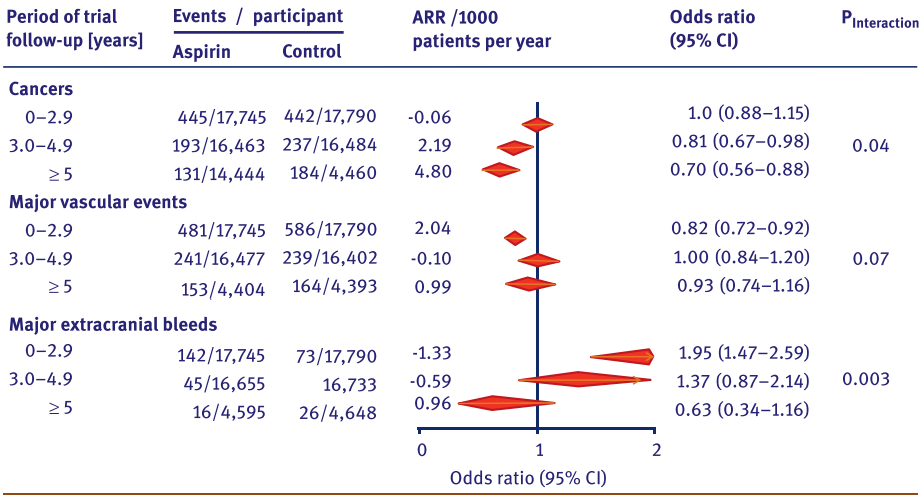

Figure 4.3.1-2: Metaanalysis of the effect of aspirin on the risks of incident cancer, major vascular events and major extracranial bleeding events in six randomized trials of daily low-dose aspirin versus control in primary prevention of vascular events. ARR: absolute risk reduction [10].

the conventional ITT analysis ($P = 0.12$) [76]. In the 10-year follow-up for all Lynch syndrome cancers combined, the ITT analysis did not reach significance but per-protocol analysis showed a significantly reduced overall risk for the aspirin group (HR: 0.63; 95 % CI: 0.43–0.92; $P = 0.018$). Adverse events during the intervention phase between the aspirin and placebo groups were similar, and no significant difference in compliance between intervention groups was observed. The data were interpreted as support of the earlier results for prevention of colorectal cancer with aspirin in Lynch syndrome [77].

4.3.1.5 Clinical trials – secondary prevention

General aspects. In addition to operative tumor resection and conservative measures such as chemotherapy and therapeutic radiation, adjuvant treatment with aspirin might also influence further tumor growth and spread. In fact, several studies have shown that aspirin avoids or at least retards the reoccurrence of tumors (adenomas, carcinomas) and distant tumor metastases in some patients. This led to the search for biomarkers for early detection of recurrent tumors as well as the definition of appropriate risk groups for improved chemoprevention. Overexpression of COX-2 has been early shown to correlate with tumor malignancy [40]. However, COX-2 expression is variable and might be too nonspecific, as are elevated PGE_2 levels in affected tissues and circulating blood [46]. The identification of risk factors and risk groups is currently a major area of cancer research and prevention [11].

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Observational studies. The clinical data with aspirin as an adjunct to conventional chemotherapy are inconsistent and mainly derived from small study groups. One study on 799 eligible patients in stage III CRC reported improved overall 5-year survival rates (HR: 0.48; 95 % CI: 0.23–0.99) and similar data for 843 eligible patients who used COX-2 inhibitors (HR: 0.26; 95 % CI: 0.08–0.81) [78]. Positive data for regular low-dose aspirin as an adjuvant for at least 9 months (95 % of patients took 80 mg/day) on survival rates of CRC patients were found in the "Eindhoven Cancer Registry." Regular intake of aspirin as an adjunct to surgery (91 %) and other therapeutic measures (chemotherapy, radiation) reduced mortality of colon cancer by 35 % (HR: 0.65; 95 % CI: 0.50–0.84; $P = 0.001$). No such effect was seen for rectal cancer and the opposite effect was observed for nonaspirin NSAIDs [79]. A Scottish cohort study also reported a small reduction in total mortality of CRC patients treated with prophylactic-dose aspirin (HR: 0.91; 95 % CI: 0.82–1.00). This effect was only seen in patients taking aspirin prior to diagnosis (HR: 0.86; 95 % CI: 0.76–0.98) [80]. Another case-control study, using essentially the same database, found no effect of aspirin on CRC-specific mortality (OR: 1.06; 95 % CI: 0.92–1.24). However, in these patients, treatment was started only *after* diagnosis of CRC [81]. The aspirin dose in almost every case (>98 %) was 75 mg/day. These negative results agree with another trial where aspirin treatment was started only after diagnosis [80] and support the concept of a chemopreventive rather than therapeutic effect of aspirin on already existing CRC. However, possible bias could probably not be excluded because of the heterogeneity of the study populations and the different study protocols.

For these reasons and, specifically, the probable requirement of long-term treatment, the data from the three large observational trials on CRC (CPS-II, NHS, HPFS), now lasting for more than 30 years, are extremely valuable. Of particular interest are participants who developed CRC. Since there were no sex-related differences, patients of the NHS and HPFS were pooled. This enlarged the number of cases and also allowed a more detailed study of biomarkers and surrogate parameters.

A total of 1,279 individuals from the prospective NHS/HPFS studies who developed a CRC were combined in a prospective cohort trial and followed for 11.8 years. Endpoints were CRC-specific and total mortality.

Patients who started regular aspirin intake only *after* diagnosis of CRC exhibited a significantly reduced mortality which was negatively correlated with the total amount and duration of aspirin intake.

The total mortality over the complete observation period was 35 % in individuals with and 39 % in individuals without aspirin intake. A total of 15 % of aspirin users and 19 % of nonaspirin users died from CRC. The multivariant HR for CRC-specific mortality was 0.71 (95 % CI: 0.65–0.97) in the aspirin users compared with nonusers and the overall mortality was 0.79 (95 % CI: 0.65–0.97). The conclusion was that regular aspirin intake after diagnosis of CRC reduces CRC-specific and overall mortality in CRC patients, presumably in those with COX-2 overexpression [82].

The level of (histochemical) COX-2 expression in colorectal cancer tumor specimens was measured in 636 incident colorectal cancers. A total of 423 (67 %) had moderate or strong COX-2 ex-

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pression. Regular aspirin use conferred a significant reduction in the risk of colorectal cancers that overexpressed COX-2 (HR: 0.64; 95 % CI: 0.52–0.78) but had no influence on tumors with weak or absent COX-2 expression (HR: 0.96; 95 % CI: 0.73–1.26). There was also a higher incidence of cancers in individuals with COX-2 overexpression that could be reduced by aspirin: 56 vs. 37 cases per 100,000 person-years. No such effect was seen in individuals with weak or absent COX-2 expression: 28 vs. 27 cases per 100,000 person-years.

The conclusion was that regular aspirin reduces the risk of CRC in patients with overexpression of COX-2 but not in those with weak or absent expression of COX-2. The risk was also reduced with increased aspirin doses and duration of intake (Fig. 4.3.1-3) [50].

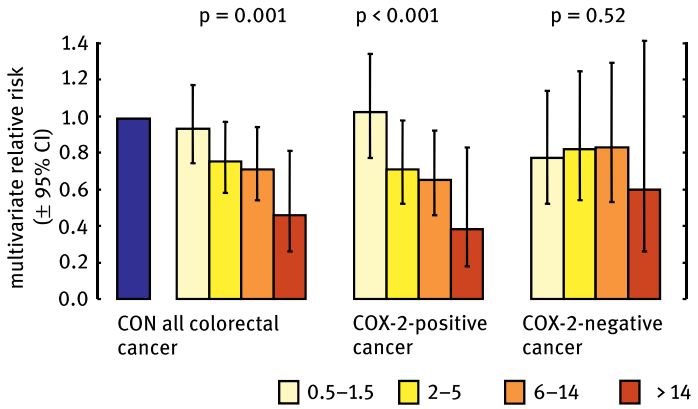

Figure 4.3.1-3: Relative risk of colorectal cancer in relation to COX-2 expression in the tumor tissue and the number of aspirin tablets taken according to combined data from the NHS and HPFS. Aspirin doses were classified according to the number of standard 325-mg aspirin tablets taken per week (after data in [50]).

About two thirds of the CRCs in these studies exhibited moderate to strong COX-2 expression [50]. This confirms the original observation of Desmond Fitzgerald's group of a relationship between COX-2 expression and tumor malignancy [40] and documents an important disease-related role for COX-2-derived products, specifically PGE_2. However, the authors also critically commented that because of the study protocols of the NHS and HPFS studies, it cannot be excluded that any positive aspirin effect already occurred prior to diagnosis with subsequent positive effects on tumor progression [82].

The finding of a correlation between COX-2 expression and tumor malignancy in a relatively large subgroup of patients – 1,226 incident rectal and colon cancers – now allowed for genotyping in order to elucidate genetic alterations which were associated with enhanced COX-2 expression and cancer malignancy. Patients with elevated COX-2 expression combined with two mutations in the PIK3CA gene, a gene which encodes the catalytic subunit of the PI3 kinase [83, 84], were found to be aspirin responders (Fig. 4.3.1-4). Another study confirmed a correlation between beneficial actions of aspirin on cancer-related survival and elevated COX-2 expression but did not see any

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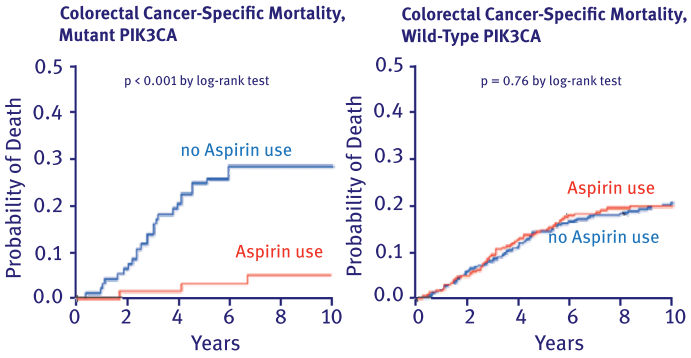

Figure 4.3.1-4: Mortality of participants of the combined NHS/HPFS study with incident CRC during an observation period of 10 years after diagnosis. Aspirin (325 mg once or twice a week) was only effective in individuals with the mutated PIK3CA genotype but not in individuals with the wild-type gene [83].

relation to (nonselected) PIK3A mutations [85]. There were also no correlations of malignancy with mutations in the BRAF protooncogene, encoding a constitutively active B-raf protein that permanently activates oncogenic Wnt signaling. Regular aspirin use (>14 tablets/week) only reduced the cancer risk in patients with the wild-type gene (HR: 0.43; 95 % CI: 0.25–0.75) but not in patients with the mutant [86]. In the same population, it was also found that the antitumor effect of aspirin was only evident in subjects with high expression of 15-PGDH, an enzyme that is downregulated in CRC by β-catenin [87], eventually resulting in maintained, elevated PGE_2 levels and enhancement of its antiapoptotic actions on tumor cells [88].

Another observational study on genetic variations that may confer differential benefit from aspirin or NSAID chemoprevention, involving a total of 8,634 cancer cases, has identified two single-nucleotide polymorphisms (SNPs) that were associated with the risk of CRC and showed a significant interaction with use of aspirin or NSAIDs [89]. These findings, along with others, confirm the existence of genetically defined subgroups in COX-2-overexpressing CRC patients that might particularly benefit from aspirin treatment as well those who are more likely not to. Randomized trials are urgently needed to establish the real benefit for the patient and antiplatelet doses of aspirin.

Randomized trials. The first prospective, randomized, compliance-controlled trial in secondary prevention of CRC with cancer survival as an endpoint showed that aspirin (600 mg twice daily for 2 years) given to patients with invasive colorectal cancer (Dukes B2 and C) shortly after surgery did neither prevent metastasis nor prolong the disease-free interval or survival time [90]. However, the number of patients was small (n = 57). In addition, more advanced chemotherapeutics and surgical measures may not have been available at the time.

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Important information on the role of aspirin in secondary prevention of CRC came from a reevaluation of the large cardiovascular prevention studies with daily aspirin [9]. During the 6.5 years of trial follow-up, 987 participants had a new solid cancer. Allocation to aspirin reduced the risk of cancer with distant metastasis (HR: 0.64; 95 % CI: 0.48–0.84; $P = 0.001$), due mainly to a reduction in proportion of adenocarcinomas that had metastatic versus local disease (OR: 0.52; 95 % CI: 0.35–0.75; $P = 0.0006$). Aspirin reduced the overall risk of fatal adenocarcinoma in the trial populations (HR: 0.65; 95 % CI: 0.53–0.82; $P = 0.0002$), probably due to a reduced number of distant metastases (HR: 0.54; 95 % CI: 0.38–0.77; $P = 0.0007$) but not the risk of other fatal cancers (HR: 1.06; 95 % CI: 0.84–1.32). There was no clear dose dependency [9].

It was also shown that the anticancer effect of aspirin was the major beneficial action of aspirin to explain improved survival (Table 4.3.1-3). These findings agreed well with those from the observational trials as discussed before [13].

Table 4.3.1-3: Effects of daily aspirin on overall and cancer-related mortality in double-blind, placebo-controlled cardiovascular prevention trials. The asterisk (*) indicates not double-blind and not placebo-controlled [9].

Parameter	TPT	UK-TIA	BMDT*	ETDRS	JPAD	SAPAT	POPADAD	AAAT
Patients	5,085	2,435	5,139	3,711	2,539	2,035	1,276	3,350
Median duration of treatment (y)	6.9	4.4	6.0	5.0	4.4	4.2	6.7	8.2
Aspirin dose (mg/d)	75	300/ 1,200	500	650	81/100	75	100	100
OR (95 % CI) for any cancer death	**0.83** (0.62–1.11)	**0.45** (0.25–0.82)	**0.79** (0.55–1.14)	**1.14** (0.56–2.35)	**0.80** (0.47–1.37)	**0.53** (0.25–1.15)	**0.80** (0.47–1.37)	**0.86** (0.63–1.17)
OR (95 % CI) for any death	1.06 (0.87–1.29)	0.90 (0.70–1.14)	0.88 (0.72–1.09)	0.91 (0.77–1.08)	0.88 (0.55–1.40)	0.77 (0.57–1.04)	0.92 (0.68–1.25)	0.94 (0.76–1.17)

Adenoma trials. The vast majority of CRCs develop from colorectal adenomas. Thus, patients with APC are a population at elevated risk for CRCs and adenoma recurrence might be a useful surrogate to determine the efficacy of preventive measures in "secondary" prevention, i. e., adenoma–carcinoma transition or adenoma recurrence after surgical removal. However, this compromise has several limitations. The annual conversion rate of adenomas was found to be 0.25 %. This indicates that an average adenoma-bearing individual is only at a moderate risk of developing colorectal cancer. The annual risk, in addition, is different and varies, dependent on the size and subtype of adenomas, between 3 % and 37 % [91]. Thus, most adenomas do not progress

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to cancer [55]. Finally, the duration of adenoma studies was short, usually less than 5 years, which is possibly too short for the transition of adenomas to carcinomas.

With these limitations in mind, there are currently four randomized, placebo-controlled prospective trials on the effects of aspirin on secondary prevention of colorectal adenomas in high-risk patients.

The "Aspirin Folate Polyp Prevention Study" (AFPPS) included 1,121 patients with a recent history of histological documented colorectal adenoma. The patients were randomized to receive aspirin, 81 mg or 325 mg daily, folic acid (1 mg/day) or a matching placebo. All patients underwent a surveillance colonoscopy 34–40 months after the qualifying examination. A follow-up colonoscopy was performed at least 1 year after randomization. The primary outcome endpoint was the reappearance of colorectal adenomas.

The incidence for this event was 47 % in the placebo group, 38 % in the group given 81 mg/day aspirin and 45 % in the group given 325 mg/day aspirin ($P = 0.04$). The respective risks for advanced lesions in comparison to placebo were 0.59 (81 mg aspirin) and 0.83 (325 mg aspirin). During the treatment period, there were no differences in serious bleeding events between the groups but seven (nonfatal) strokes in the aspirin groups, as opposed to none in the placebo group ($P = 0.06$).

The conclusion was that regular prophylactic use of aspirin had a moderate chemopreventive effect on reoccurrence of colorectal adenomas [92].

Similar positive results were obtained in the "Association pour la Prévention par L'Aspirine du Cancer Colorectal" (APACC) intervention trial, including 272 patients 1 year after polypectomy [93] but could not be confirmed after 4 years [94]. The "UK colorectal adenoma prevention" (UK-CAP) study found a significantly but not drastically reduced adenoma recurrence rate: 23 % after 3 years of aspirin (300 mg/day) vs. 29 % in the placebo (folate) group [95]. A metaanalysis of four randomized, placebo-controlled, double-blind trials on secondary adenoma prevention in a total of 2,698 participants over an average observation period of 33 months showed reappearance of adenomas in 33 % of the aspirin-treated patients (81–325 mg/day) as opposed to 37 % of patients in the placebo group (HR: 0.83; 95 % CI: 0.72–0.96). This was equivalent to a dose-independent reduction in the absolute risk by 6.7 % [96], which was not very impressive.

A particular interesting study was that of Sandler et al. (2003). This appears to be the only published trial on secondary prevention of colorectal neoplasias (adenomas) in patients with surgically removed CRCs.

A total of 517 patients with a previous history of colorectal cancer were included. All patients had curative resection of the primary tumor and colonoscopy with established removal of all polyps. Patients were randomized to enteric-coated aspirin (325 mg/day) or placebo in a double-blind fashion. The patients had at least one colonoscopic evaluation at 13 months (median) after randomization.

Because of significant differences between the treatment groups according to interim results, the study was terminated prematurely. One or more adenomas were found in 17 % of patients in

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the aspirin group and in 27 % of patients in the placebo group ($P = 0.004$). The number of adenomas was lower and the time to detection was longer in the aspirin group. This corresponded to a significant ($P = 0.02$) reduction of the risk for a new adenoma of 0.65 (95 % CI: 0.43–0.94). There were few severe side effects, including one stroke in each group.

The conclusion was that daily aspirin is associated with a significant reduction in the incidence of colorectal adenomas in patients with previous colorectal cancer [97].

The study was the first to show significant protection (in terms of adenoma reappearance) in surgically treated CRC patients, taking 325 mg/day aspirin for at least 1 year. However, despite this significant protective effect of aspirin, adenomas still developed in some patients of the aspirin group. Thus, aspirin cannot be considered as replacement for surveillance colonoscopy [98]. These data were at some variance with the pure adenoma trials of the group reported previously, in particular regarding the aspirin dose [92]. There is possibly a different risk profile in the two types of colonic neoplasias, that is, adenoma and cancer – and a number of different subtypes – that urgently requires further studies.

4.3.1.6 Aspirin and other drugs

NSAIDs and coxibs. Aspirin is the most intensively studied drug for chemoprevention of colorectal cancer. Therapeutic alternatives are other compounds that lower prostaglandin levels, i. e., NSAIDs and coxibs. In addition, sulindac, a prodrug of an active NSAID, was also found to reduce the growth of existing adenomas in patients with FAP. For these high-risk patients, celecoxib was approved by the FDA and the European drug agency EMA according to surrogate data (reduction of intestinal adenomas). However, approval was withdrawn in 2011 after the manufacturer was unable to provide the requested efficacy data for prevention of CRC. No effect was seen with drugs used for symptomatic treatment of osteoarthritic pain (chondroitin sulfate, glucosamine) [99].

There are mixed data with NSAIDs as opposed to the mostly positive results with aspirin [49, 69, 99–102]. A recent case-control study in a cohort extracted from a primary care database identified a total of 15,491 incident cases of CRC and 60,000 randomly selected controls between 2001 and 2014. Nonaspirin NSAID use was associated with a markedly reduced risk of CRC (adjusted OR: 0.67; 95 % CI: 0.63–0.71). The efficacy increased linearly with duration of treatment (P for trend <0.001) and was diminished upon its discontinuation. All individual nonaspirin NSAIDs examined showed a risk which was dependent on the duration of treatment. The concomitant use of PPIs had no impact on the protective effect [99].

No head-to-head comparisons of aspirin and NSAIDs are available. John A. Baron commented on aspirin, NSAIDs and cancer prevention at the background of available trials as follows:

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> . . .Just because aspirin is effective does not mean it necessarily should be used. Aspirin is a real drug, with definite toxicity. As for any preventive intervention, the benefits must be balanced against the risk, particularly when the benefits are delayed whereas the risks are not [61]. . .

There is only one real "aspirin-like drug" bearing an acetylation potential similar to aspirin – APHS (Section 1.1.5). APHS has been shown to inhibit proliferation of COX-2-positive CRC cell lines in vitro [103] but so far has not been studied in vivo.

4.3.1.7 Actual situation

General aspects. There are several explanations regarding the different outcome of aspirin prophylaxis and treatment in patients with CRC as well as other tumors. Clearly, patient-based factors are first-line considerations. This includes the individual genotype, possible comorbidities and the age of the individuals, among others. In any case, the use of biomarkers as well as early diagnostics will be useful.

Primary prevention. If it was true that the chemopreventive effect of aspirin on CRC is due to the same mechanism as the antiplatelet effect but just needs more time to become evident, this could result in reconsideration of the benefit/risk ratio in primary prevention. Although most evidence is from epidemiological trials, there is a huge amount of data regarding long-term use of aspirin, today >30 years, suggesting that regular, long-term aspirin use might protect from CRC [61]. The USPSTF found "adequate evidence" that aspirin use reduces the incidence of CRC in adults after 10 years of use and recommended "low-dose" aspirin for primary cardiovascular and CRC prevention in individuals aged 50–59 years without known bleeding risk and a risk of cardiovascular events of ≥10 % within 10 years with a level of evidence "B" in 2016 [104]. However, the USPSTF removed the rationale for considering low-dose aspirin for prevention of colorectal cancer, possibly because of the data of the ASPREE trial [105].

> ASPREE was a randomized primary prevention trial of low-dose aspirin (100 mg per day) in the elderly. The main exclusion criterion was the presence of cardiovascular diseases, dementia or any physical disability. A total of 19,114 participants aged 70 years and older (US minorities 65 years and older) at trial entry receiving aspirin (9,525) or placebo (9,589) were enrolled and followed up for a median of 4.6 years.
>
> Prior aspirin use was low (11 %). At entry, 19.1 % had a prior diagnosis of cancer; 80.4 % were not known to have cancer. In the aspirin and placebo groups, 981 and 952 cancer events occurred, respectively. There was no significant difference between groups for all incident cancers (HR: 1.04; 95 % CI: 0.95–1.14) but a trend toward increased all-cause mortality was observed (HR: 1.14; 95 % CI: 1.01–1.29) that was driven by cancer death. In addition, aspirin was associated with an increased risk of cancer that had metastasized (HR: 1.19; 95 % CI: 1.00–1.43) or was at stage 4 upon diagnosis.
>
> The conclusion was that in older adults, aspirin treatment had an adverse effect on later stages of cancer evolution. This suggests that in older persons, aspirin may accelerate the progression of cancer and, thus, caution with its use in this group is warranted [105].

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Although a more detailed analysis of the effect of aspirin on cancer incidence in ASPREE did not demonstrate an increase in overall cancer incidence (HR: 1.04; 95 % CI: 0.95–1.14) and CRC incidence (HR: 1.02; 95 % CI: 0.81–1.30) [105], the finding remains that aspirin had no beneficial actions in these elderly persons. Similar results were also obtained in the NHS/HPFS studies. Initiating aspirin use at or after 70 years did not reduce the risk of CRC [107]. Several explanations are possible: The short duration of 4.7 years when the vast majority of participants (89 %) never had used aspirin regularly before enrollment into the study (65–70 years or older). Age might have modified aspirin's chemopreventive effect, for example by age-dependent alterations in DNA methylation and/or different, aspirin-sensitive wild-type BRAF and KRAS genotypes [108].

A model for longitudinal precision chemoprevention in clinical decision making has been recently suggested by *David A. Drew and Andrew T.Chan* (Fig. 4.3.1-5) [11]. It sounds interesting, but will require further studies before realization, specifically regarding the screeening for suitable biomarkers, such as urinary PGE-metabolites as predictors of the individual benefit/risk ratio.

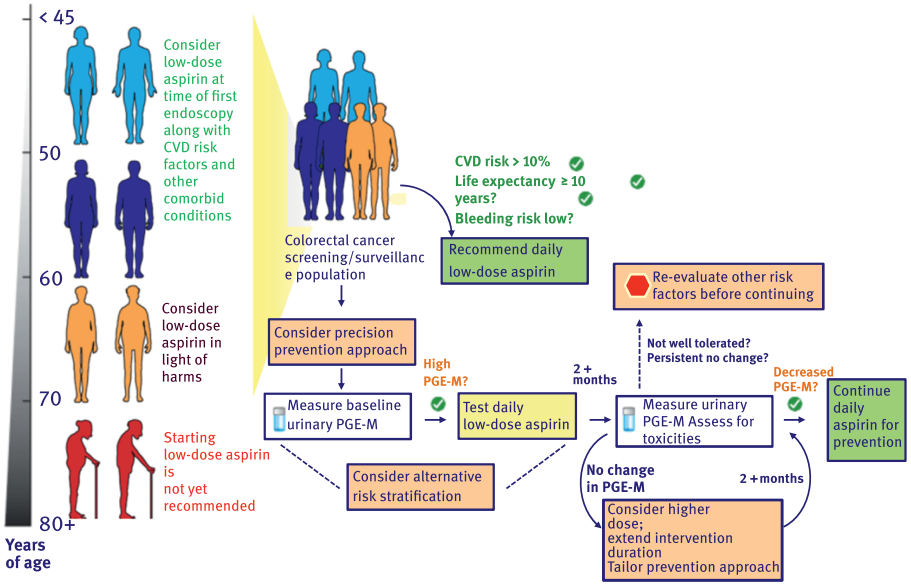

Figure 4.3.1-5: Decision making in chemo- and cardiovascular prevention. For explanation see text [11].

Discussion of the use of aspirin for primary prevention should coincide with an individual's first screening colonoscopy at the age of 45–50 and might result in the recommendation of aspirin directly by considering other factors like bleeding risk and cardiovascular risk. For individuals over

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age 60, CRC risk factors including the presence of adenomas and baseline levels of potentially modifiable biomarkers for CRC risk (PGE-M urinary excretion) could be determined. This could help to tailor individual strategies according to aspirin dose, duration and continuation of use. Precision prevention strategies may be extended to those of advanced age (70 years or older); however, significant consideration of harms is warranted before starting an aspirin prevention regime.

Secondary prevention. There is also an urgent need for further prospective randomized controlled trials in secondary prevention of gastrointestinal neoplasias. The trials should be done under comparable treatment and evaluation criteria in order to clarify the individual benefit/risk ratio. Two large multicenter, double-blind, randomized placebo-controlled phase III trials on secondary prevention of CRC and aspirin are currently underway. One is the "Aspirin for Dukes-C and high risk Dukes-B colorectal cancers" (ASCOLT) trial with 200 mg aspirin per day for 3 years in 2,660 patients [109]. The Add-Aspirin study is a double-blind, placebo-controlled, randomized trial which studies the effect of daily aspirin (100–300 mg) on tumor recurrence and survival after radical cancer therapy in four tumor cohorts: gastroesophageal, colorectal, breast and prostate cancer. After 2 years, there was no evidence of a difference in adherence, acceptance of randomization or toxicity between the different cancer cohorts. Trial recruitment continues to determine whether aspirin could offer a potential low-cost and well-tolerated therapy to improve cancer outcomes. Results are expected in 2025 [110].

Summary

Numerous observational but also randomized trials and metaanalyses suggest that regular long-term intake of aspirin might reduce the risk of CRC by about 15–40 %. This requires regular intake of the compound for at least 5–10 years or more. There is no clear dose dependency. Aspirin at daily doses of about 75–300 mg/day appears to be effective in primary prevention. It might also be considered as an adjunct to standard therapeutic measures in secondary prevention of recurrent gastrointestinal tumors and inhibition of distant metastases. The responder rates are variable. The reasons for this are under study and might be both genetic and epigenetic in nature.

The pharmacological mode of action is unclear. Considering a maximum plasma level of ≤10 µM active acetylsalicylic acid that can be obtained with antiplatelet doses of aspirin, it is most likely that acetylation of COXs (COX-1/COX-2) and antiplatelet actions are involved. Follow-up reactions are inhibition of platelet-dependent thromboxane formation and subsequent secretion of autocrine and paracrine platelet storage products, inhibition of white cell activation and inhibition of COX-derived formation of proinflammatory and mitogenic PGE_2 (for details see Section 2.3.3). The availability of appropriate biomarkers for defining "aspirin-sensitive" patients is desirable.

The use of aspirin for primary prevention is currently under debate. More personalized transfer of the available clinical data into therapeutic guidelines does require more long-term prospective randomized controlled trial and is desirable. Actually (2022) the USPSTF does not recommend aspirin prophylaxis for prevention of colorectal cancer.

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4.3.2 Alzheimer's disease and other neurological disorders

4.3.2.1 General aspects

Inflammatory processes, oxidative stress and associated mitochondrial dysfunction with disturbed energy supply are increasingly identified as relevant factors in the etiology of major neuropsychiatric disorders. This includes schizophrenia, depression and Alzheimer's disease [1]. While it is obvious that the pathogenesis of these diseases is different, inflammatory damage of sensitive neurons, associated with restricted energy supply and/or insufficient removal of metabolic waste, is a common feature that aggravates the disease and facilitates its progression. Most attention in this respect has been focused on Alzheimer's disease, a neurodegenerative disease of the aging brain. In the absence of specific or even causal treatment options, symptomatic treatment with the aim to retard or even prevent the development of the disease is in therapeutic focus. Age-related cognitive decline, not only in Alzheimer's disease, is generally an issue of concern in an aging population.

The pharmacological actions of aspirin in the CNS are well known, for example its effects on specific regions in the CNS as one target of its analgesic and antipyretic activity (Section 2.3.2). These, together with its antithrombotic effects – ischemic disease affects 60–90 % of patients with Alzheimer [2] – also translate into interactions with mediator systems that are relevant for neuropsychiatric disorders, such as endocannabinoids, monoamines (5-HT, noradrenaline), GABA and others [3].

4.3.2.2 Epidemiology, etiology and pathophysiology

Epidemiology. Alzheimer's disease is the most common form of dementia with age as a principal risk factor [2, 3]. Typical for Alzheimer dementia is a progressive loss of memory and higher cognitive functions [2, 4]. Currently about 2 % of the population are affected but the number is likely to increase with increasing life expectancy: More than one third of the people above the age of 85 years suffer from Alzheimer [2]. The disease is diagnosed 2–4 years after the appearance of the first symptoms, usually at the age of about 70. Thus, a delay in onset by 2–3 years, for example by identification of modifiable risk factors and their appropriate prevention or treatment, is clinically most relevant. This would not only improve the quality of life in affected individuals but also save costs for health care providers, hospitalization of the patient clearly being the most unwanted event for both sides. For these reasons, any effective preventive measure is much more desirable than solely the treatment of symptoms, which provides only marginal if any improvement in quality of life.

Etiology and pathophysiology. Age-related accumulation of misfolded proteins with subsequent oxidative and inflammatory damage is typical for the disease. It is a consequence of energy failure and synaptic dysfunction [2]. Brain regions that are involved

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in learning and memory processes become reduced in size as the result of degeneration of synapses and neurons [4] and loss of memory and cognitive functions [5].

Typical of the disease are senile plaques containing aggregated amyloid-β protein and neurofibrillary tangles. Neuronal overexpression of β-amyloid precursor and amyloid-β protein renders the brain more vulnerable to ischemic injury [2, 3]. Importantly, neurofibrillary tangle-containing neurons do not die of apoptosis but rather degenerate. The reason is hyperphosphorylation of tau, the major protein subunit of neurofibrillary tangles [6]. These neurons stimulate local chronic inflammation to remove the cell debris [7]. Biochemically, this includes activation of the complement cascade and generation of chemokines, cytokines and reactive oxygen species [8]. Microglia, a macrophage-like cell population, congregate around amyloid plaques and degenerating neurons and release toxins and inflammatory mediators that in turn promote neurodegeneration [4, 5]. This activation of inflammation-associated signal transduction pathways is not restricted to glial cells but is also seen in neurons and precedes the neurofibrillary pathology, i. e., neurodestruction and (astro)gliosis [9]. Neuroinflammation is a central component of neuronal damage [10] and its prevention a logical target for Alzheimer prevention and treatment by antiinflammatory drugs that inhibit COX activity.

In contrast to many other tissues, COX-2 is expressed constitutively in neurons of the CNS. This neuronal expression of COX-2 is regulated by synaptic activity, suggesting that in the CNS, this COX isoform and its enzymatic products are involved in activity-dependent neuronal plasticity. In contrast, brain microglia, which are crucial to inflammation and subsequent neurodegeneration, do not express high levels of COX-2 after stimulation with inflammatory cytokines or amyloid-β [9]. These findings suggest that selective inhibition of COX-2 may not be an effective treatment strategy for Alzheimer's disease. Nonselective traditional NSAIDs, rather than coxibs, appear to be more useful treatment options [11].

4.3.2.3 Modes of aspirin action

Antiinflammatory actions. Neuroinflammation is a typical and early event in the pathogenesis of Alzheimer's disease. The clinical efficacy of NSAIDs in some clinical trials tends to support the hypothesis that early administration of antiinflammatory drugs might be a useful preventive approach. Aspirin could have similar effects and modify several targets: apolipoprotein E (APOE) isoforms, amyloid-β, neuroinflammation and oxidative stress.

APOE, amyloid-β and PPAR-α. Alzheimer's disease is characterized by build-up of aggregates of amyloid-β. Extracellular deposition of amyloid-β protein as amyloid plaques and vascular amyloid is a typical feature of Alzheimer's disease and probably induced by chronic neuroinflammation. APOE enhances proteolytic breakdown of

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amyloid-β [12]. The isoform APOE-ε4 is less effective and the respective genotype is associated with a significantly increased risk of late-onset sporadic Alzheimer's disease [13]. Treatment with NSAIDs in some studies reduced the incidence of Alzheimer's dementia – not vascular dementia. This effect was most notable in individuals carrying the APOE-ε4 allele. However, neither aspirin nor acetaminophen had any significant effect [14, 15].

Formation of the amyloid-activated microglia complex is an early event in the disease [16]. Aspirin (1 mM) has been found in vitro to nearly completely prevent the precipitation of extracellular fibrils from dissolved amyloid-β precursor protein in a cell-free system. If this antiamyloid mechanism also works in vivo, it might result in reduced extracellular deposition of amyloid-β fibrils in brain tissue, thereby retarding the progression of the disease. In this context, it is interesting to note that aspirin was found to decrease hyperphosphorylation of τ [17] and to stimulate apoptosis by this mechanism [6]. Another study was unable to confirm disaggregation of preformed amyloid fibrils by aspirin [18]. In any case, the stimulatory effects of aspirin on lysosomal biogenesis and improved amyloid-β clearance are of considerable interest as possible strategies for drug-induced removal of dysfunctional neurons.

Peroxisome proliferator-activated receptor-α (PPAR-α) has been identified as a strong ligand and novel target of aspirin in the brain. Activation of PPAR-α by aspirin stimulates a series of downstream signaling pathways in the hippocampus that could potentially ameliorate different Alzheimer's disease-related pathologies [19]. Without PPAR-α, aspirin fails to mediate upregulation of neurotrophic factors and plasticity-associated genes in the hippocampus [20]. PPAR-α is also essential for stimulation of lysosomal biogenesis and autophagic clearance of amyloid-β plaques in a mouse model of Alzheimer's disease [21, 22]. Reversal of cognitive deficits is critically dependent on PPAR-α and these actions are seen at low aspirin doses [20].

COX and prostaglandins. In addition to its antiinflammatory actions, the antiplatelet effects of aspirin might be relevant to Alzheimer's disease. Alzheimer patients have a 3–4-fold increased urinary excretion of thromboxane metabolites and of the lipid peroxidation marker 8-iso-PGF2α. The plasma levels of vitamin E, an antioxidant, are reduced and are inversely correlated with the excretion of these metabolites. These data suggest persistent platelet activation and reduced oxygen defense in Alzheimer's disease [23]. Low-dose aspirin (100 mg/day) markedly reduced urinary excretion of a thromboxane metabolite while the urinary excretion of the (nonenzymatically formed) stress marker 8-iso-PGF2α was unchanged [23]. The clinical correlation with cognitive defects of Alzheimer patients with local inflammation is difficult and many questions remain.

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4.3.2.4 Clinical trials

General aspects. Because of the multifactorial pathogenesis of Alzheimer's disease and the lack of any causal treatment, multiple therapeutic options were investigated although none of them yielded really convincing results [2, 24]. Overall, they are focused on retardation of the development of the disease and treatment of symptoms. Since neuroinflammation and disturbed apoptosis are crucial to exaggeration of the disease, antiinflammatory/antiapoptotic approaches might be useful, if they are applied *before* manifest brain injury emerges. Aspirin and NSAIDs are particularly attractive and were subject to most preventive studies [25] although with less convincing results [24].

Epidemiological trials. A number of retrospective and prospective observational trials have studied whether aspirin and/or traditional NSAIDs can prevent or retard the progression of the disease. One of the first trials showing a reduced prevalence of Alzheimer in regular users of aspirin and NSAIDs was the prospective population-based "Baltimore Longitudinal Study of Aging."

The Baltimore Longitudinal Study of Aging examined whether the risk of Alzheimer's disease was reduced among users of aspirin and paracetamol (acetaminophen) as compared to traditional NSAIDs in 1,686 participants. Information was collected by biennial examinations during an observation period of 6 years. The question was whether self-reported medication with these drugs and the duration of use had any relation to the risk of the onset of Alzheimer's disease.

The risk for Alzheimer's disease was inversely correlated with increasing duration of NSAID use. The HR amounted to 0.40 in individuals with more than 2 years of reported use and 0.65 in those with less than 2 years of reported use. The overall risk for aspirin users was reduced to 0.74. This number was not significantly different from controls and no trend for decreasing risk with longer use was found. Paracetamol had no effect at all (Fig. 4.3.2-6).

The conclusion was that regular intake of NSAIDs reduces the risk of Alzheimer's disease. This protective effect is more pronounced with longer use, suggesting that an inflammatory process might be involved [26].

In a comment to this study, it was discussed whether aspirin might have been less effective because a significant number (about 60 %) of Alzheimer patients in this study had vascular dementia. This dementia is frequently related to small-vessel intracranial atherosclerosis (independent of infarction or primary neurodegeneration of classical Alzheimer) [27]. This might also have resulted in an increased proportion of participants taking aspirin at (very) low doses (65–85 mg/day) for cardiovascular prevention. This dose might be too low for COX(-2) inhibition in vivo [26]. In addition, there are no convincing clinical data for improved cognitive abilities by aspirin in patients with primary vascular dementia [28, 29].

Another population-based retrospective study confirmed that long-term use of aspirin and NSAIDs might decrease the risk of developing Alzheimer's disease [30], while no beneficial effect for aspirin was seen in a small Swedish population-based

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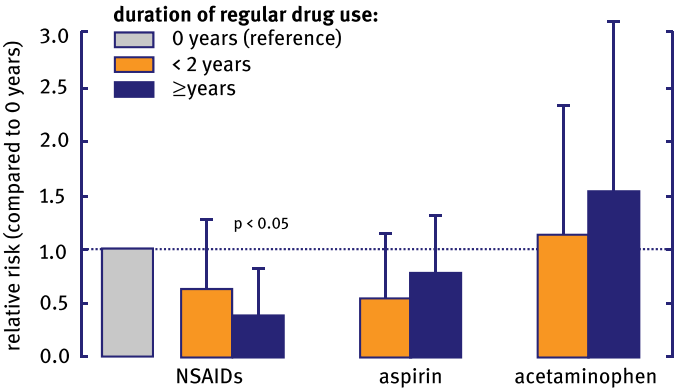

Figure 4.3.2-6: Baltimore Longitudinal Study of Aging. Relative risk of Alzheimer's disease by type and duration of medication use. Medications were NSAIDs, aspirin and acetaminophen (paracetamol). There is a small but significant reduction by traditional NSAIDs taken for at least 2 years, but no significant change by aspirin or acetaminophen [28].

prospective trial [31]. However, no differentiation between aspirin use by prescription or self-medication was made in the Swedish study and no information about strength and dosages was provided. This study is at variance with a retrospective observational trial from Australia [32].

The "Sydney Older Persons Study" was a retrospective case-control study in a total of 647 recruited individuals of 75 years of age or older (average 81 years), where 163 patients had diagnoses of dementia (different categories) and were compared with 373 nondement controls from the same population sample. Aim of the study was the detection of a possible relationship between the used drugs, in particular NSAIDs and aspirin, and the incidence of Alzheimer's disease.

Fifty drugs or drug groups were identified and those with inverse associations were further analyzed. There was an inverse association between intake of NSAIDs and aspirin and the occurrence of Alzheimer. No associations were seen with vascular dementia or any other diagnosis. There was no evidence for a dose dependency for either NSAIDs or aspirin at low (<175 mg/day) and medium doses (>175 mg/day).

Several potential mechanisms for the effects of NSAIDs and aspirin were discussed. Since antiplatelet doses of aspirin were equieffective to higher doses, it was also assumed that the beneficial effects of aspirin might have been due to its antiplatelet activity and were possibly related to inhibition of amyloid-β release from activated platelets.

The conclusion was that the data did not support a high-dose antiinflammatory action of NSAIDs or aspirin in Alzheimer's disease [32].

An Australian study did morphologic examinations of postmortem brain tissue of 12 Alzheimer patients, of whom five were on long-term antiinflammatory medications. There was no reduction in inflammatory microglia or neuropathological changes despite antemortem improved cognitive performance by antiinflammatory drug treatment [33]. However, these findings have been controversially discussed [34] and the number of patients was low. Opposite results were obtained in another population-

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based retrospective cohort trial in an older population (≥80 years at inclusion) in Sweden. Here, users of high-dose aspirin, but not paracetamol, low-dose aspirin (75 mg) or other NSAIDs – even if given occasionally – had a significantly lower prevalence of Alzheimer's disease and better maintained cognitive functions than nonusers [35].

The probably largest currently available epidemiological study on the possible relation between NSAID and aspirin intake and the risk of Alzheimer's disease was the prospective Rotterdam study [29].

The Rotterdam study was a prospective, population-based cohort trial in 6,989 subjects aged ≥55 years (about 80 % of the total cohort was aged <75 years) who were free of dementia at baseline. On average, each participant was followed for about 7 years. Endpoints were death, dementia or the end of the study period. Only medications prescribed by a physician were considered. There was no control for cardiac or other vascular indications for prophylactic aspirin use. Complete information about prescriptions was available in an automated form from pharmacy records. A clinical diagnosis of dementia and its possible reason was done according to standard criteria.

A total of 394 subjects received a diagnosis of dementia during the study. Out of these, 293 had Alzheimer's disease, 56 had vascular dementia and 45 had other types of dementia. Use of NSAIDs at any time was associated with a reduced risk of Alzheimer while no effect was seen with paracetamol. The HR of Alzheimer's disease was 0.95 in subjects with short-term NSAID use (up to 1 month), 0.83 in those with intermediate-term use (1–24 months) and 0.20 (95 % CI: 0.05–0.83) in those with long-term cumulative use (more than 24 months). A total of 2,314 individuals (33 %) were on aspirin or other oral salicylates, almost all of them at antiplatelet doses, i. e., <300 mg/day. There was a nonsignificant risk reduction to 0.76 (95 % CI: 0.49–1.19) in long-term aspirin users: The risk of vascular dementia was not reduced by NSAIDs but significantly increased by aspirin.

The conclusion was that regular long-term use of NSAIDs, that is, for 2 years or more, is associated with a significantly reduced risk for Alzheimer's disease but does not protect from vascular dementia. Paracetamol has no effect on any of these parameters. Prospective randomized primary prevention trials are to be recommended [29].

The more recent "Cardiovascular Health Cognition Study" investigated the association of Alzheimer's disease with the use of NSAIDs in 329 dementia-free individuals ≥65 years of age. This study also confirmed a significant risk reduction by NSAIDs (HR: 0.63; 95 % CI: 0.45–0.88) but did not find a significant effect for aspirin or acetaminophen. Interestingly, this risk reduction was only seen in patients carrying an APOE-ε4 allele (HR: 0.34; 95 % CI: 0.18–0.65). One explanation for the failure of aspirin was that the dose which was taken by these individuals – mostly for cardiocoronary prevention – was possibly too low to provide the same antiinflammatory neuroprotection as other NSAIDs [14], a limitation also discussed in the "Baltimore Longitudinal Study of Aging" trial [26]. No effect of regular aspirin intake and long-term changes (2–6 years) of cognitive functions was seen in another population-based cohort trial. In this study, patients with already existent cognitive defects or cerebral insult were excluded [36].

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The effect of aspirin on cognitive decline in patients with Alzheimer's disease was studied in a Chinese observational trial. Participants (median age 73 years) were separated into three groups: normal cognition (509), mild cognitive impairment (MCI) and Alzheimer's disease (372). Each group contained aspirin users, mostly 81 mg/day (82 %), and nonusers. The Mini-Mental State Examination was used to determine the cognitive outcome.

There were no significant differences in outcome in any of these groups between aspirin users and nonusers. However, in a longitudinal analysis over the up to 14-year observation period, the use of aspirin in the Alzheimer group was associated with slower cognitive decline over time. There were no changes in the other groups.

The conclusion was that there might be an association between aspirin use and slower cognitive decline in Alzheimer patients, which may be dependent on the clinical stage [37].

The multiple problems with observational, nonrandomized studies are well known. Specifically, there is a huge amount of possible bias, here regarding a different etiology of the disease, selection bias for the elderly participants, bias from drug interactions and multimorbidities in the elderly, variable compliance and duration of the studies and more. For these reasons, the results of these investigations should be interpreted with caution.

Randomized trials. The available prospective studies on aspirin and the CNS have mainly been focused on stroke. They showed a protective effect in secondary stroke prevention and also some effect (in addition to a reduced number of myocardial infarctions) in primary prevention of patients at elevated risk. However, these are no genuine Alzheimer patients and there is no evidence that aspirin protects from vascular dementia [28]. The only available prospective randomized "Alzheimer's disease anti-inflammatory prevention trial" (ADAPT) trial on antiinflammatory compounds, comparing celecoxib with naproxen and placebo [38], was negative, also in a more recent follow-up reevaluation [39]. In addition, treatment with these drugs appears to be only effective if it is started before neurological deficits become evident.

The double-blind, placebo-controlled ASPREE trial in the elderly investigated whether regular aspirin increases the healthy life span, defined as survival free of dementia and disability. There were no differences between the aspirin (100 mg/day) and placebo groups with respect to all major efficacy outcomes, including prevention of depression in older people [40], but there was an increased risk of severe bleeding events. The mental disability aspects of this study are discussed in detail in Section 3.1.3. According to a recent Cochrane analysis of randomized trials, there is no evidence for beneficial effects of low-dose aspirin or NSAIDs (naproxen, celcoxib) for prevention of dementia [24]. This conclusion is based on three nonaspirin trials which all had to be terminated prematurely due to adverse effects with the study drugs in other trials. The only aspirin versus placebo trial is ASPREE, which was also terminated prematurely and is discussed above. Because of the limited number of randomized trials and the heterogeneity of the studies which according to the authors of the Cochrane analysis [24] did not even allow to combine data from these studies

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to give summary estimates, it is difficult to make any firm conclusions on aspirin and cognitive deficits at this time.

4.3.2.5 Aspirin and other drugs

Many different classes of drugs have been tested for prevention and treatment of dementia and its behavioral disturbances, including Alzheimer's disease. NSAIDs and aspirin are just one group of them. Others are antioxidants, monoclonal antibodies against amyloid-β, polyphenolic extracts from grape seeds (resveratrol) and many others [2]. These and further drugs and chemicals, including estrogen, selenium, Ginkgo biloba extract, vitamin E and others, were under investigation in several primary and secondary prevention trials [41] with mixed results. One double-blind, placebo-controlled, randomized clinical trial involving 613 patients with mild to moderate Alzheimer's disease has shown some benefit, that is, reduced progression of the disease with α-tocopherol [42]. A metaanalysis of 15 randomized, placebo-controlled trials in patients with dementia showed similar beneficial effects for Ginkgo biloba extract EGb 761 [43]. While these reports are interesting, the multitude of therapeutic approaches also documents the uncertainty about the best pathophysiological target as well as the heterogeneity of cerebral dysfunctions in Alzheimer and the overall morbidity in the elderly.

4.3.2.6 Actual situation

Although some data from observational studies tend to support the use of antiinflammatory agents for prevention of Alzheimer's disease, there are also many negative studies and a definite lack of randomized controlled trials. The lack of benefit in the ASPREE trial indicates that aspirin might not be a useful approach to improve cognitive functions in the elderly but rather causes health problems (bleeding). ASPREE also tells us that prevention of Alzheimer's disease should start early and this strategy could be facilitated by definition of useful risk markers. More prospective randomized, appropriately sized controlled trials in appropriate populations with Alzheimer-related cognitive deficits as primary clinical endpoint are needed. These studies should start early, that is, before the age of 70 years, and should allow an observation period that is long enough to detect clinically meaningful improvements.

Summary

Alzheimer's disease is a neurodegenerative disorder of the brain, associated with progressive loss of memory and cognitive functions. The pathophysiology of the disease is complex and involves multiple inflammatory processes and a disturbed apoptosis of affected neurons, platelet activation, peripheral signs of inflammation and reduced oxidative defense. Aspirin might affect these processes at different levels, including activation of PPAR-α, upregulation of plasticity-associated genes, removal of amyloid-β plaque burden in hippocampal neurons and antiinflammatory actions.

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Available clinical studies are mostly epidemiological observational trials. They provide mixed results for the numerous drugs studied, including aspirin. They need to be confirmed by appropriately sized, prospective randomized trials considering also the variable pathophysiology of the disease, including genetic variations (APOE) and the frequent comorbidities of the elderly.

Currently, there is one randomized prospective, placebo-controlled trial on prevention of cognitive deficits by low-dose aspirin in the elderly (ASPREE). No benefits with respect to primary prevention of mental illnesses were found. There is no drug known so far that has been proved to be effective in Alzheimer prevention. Alzheimer is a disease of the elderly, who might take aspirin for protection from atherothrombotic events, including stroke or vascular dementia (M. Binswanger). On this background, any additional positive effect of any compound on cognitive functions, including aspirin, is clearly desirable.

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Abbreviations

AA	Arachidonic acid
ABI	Ankle/brachial index
ACE	Angiotensin converting enzyme
ACF	Aberrant cryptic foci
ACS	Acute coronary syndrome
ADMA	Asymmetrical dimethyl arginine
ADP	Adenosine diphosphate
AECD	Aspirin-exacerbated cutaneous disease
AERD	Aspirin-exacerbated respiratory disease (“aspirin-sensitive asthma”)
AHA	American Heart Association
AMPK	Adenosine monophosphate-activated protein kinase
APHS	2-(acetoxy-phenyl)hept-2-ynyl sulfide
ARDS	Acute respiratory distress syndrome
ART	Antiretroviral treatment
ATP	Adenosine triphosphate
ATT	Antiplatelet/antithrombotic Trialists
AP-1	Activator protein-1
APC	Adenomatous polyposis coli
ASA	Acetylsalicylic acid (aspirin)
ASCVD	Atherosclerotic coronary vascular disease
ATL	Aspirin-triggered lipoxin
AUC	Area under the curve
Bcl-2	B-cell lymphoma-2
b. i. d.	Two times daily
BiP	Immunoglobulin-binding protein
CABG	Coronary artery bypass graft
CAD	Coronary artery disease
cAMP	Cyclic adenosine monophosphate
cGMP	cyclic guanosine monophosphate
CIN	Chromosomal instability
CD39	5′-Nucleotidase
cEBPβ	CCAT/enhancer-binding protein β
CKD	Chronic kidney disease
CNS	Central nervous system
CoA	Coenzyme A
COX	Cyclooxygenase
CRE	cAMP responsive element
CRP	c-reactive protein
CYP	Cytochrome P450
Cys-LT	Cysteinyl leukotriene
DAPT	Dual antiplatelet treatment
DDAVP	1-Deamino-8-(D)-arginine vasopressin
11-DH-TXB$_2$	11-Dehydro-thromboxane B$_2$
DIC	Disseminated intravascular coagulation
DMARDs	Disease-modifying antirheumatic drugs
DNA	Desoxyribonucleic acid
DNP	2,4-dinitrophenol

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DVT	Deep vein thrombosis
EC	Enteric coated
EC_{50}	50 % effective dose
EDRF	endothelium-derived relaxing factor
EGF	Epidermal growth factor
EMA	European Medicines Agency
ENG	Endoglin
eNOS	Endothelial nitric oxide synthase
EP	(PG) Endoperoxides
ESC	European Society of Cardiology
ESUS	Embolic stroke of undetermined source
FAAH	Fatty acid amide hydrolase
FAP	Familiar adenomatous polyposis coli
FDA	(US) Food and Drug Administration
Flt-1	fms-like tyrosine kinase-1
GA	Gentisic acid
GAPDH	Glycerolaldehyde-3-phosphate dehydrogenase
GFR	Glomerular filtration rate
G6PD	Glucose-6-phosphate dehydrogenase
GI	Gastrointestinal (tract)
GPIIb/IIIa	Glycoprotein IIb/IIIa
GU	Gentisuric acid
HNPCC	Hereditary nonpolyposis-associated colorectal carcinoma
HETE	Hydroxyeicosatetraenoic acid
HIV	Human immunodeficiency virus
HMGB-1	High-mobility group box 1 protein
HNPCC	Hereditary nonpolyposis-associated colorectal carcinoma
HO-1	Heme oxygenase-1
HPLC	High-performance liquid chromatography
HR	Hazard ratio
HSP	Heat shock protein
5-HT	5-Hydroxytryptamine (serotonin)
HTPR	High-on-treatment platelet reactivity
ICAM-1	Intercellular adhesion molecule-1 (CD54)
ICU	Intensive Care Unit
ID_{50}	50 % inhibitory dose
IEM	Inborn error of metabolism
IFN-γ	Interferon γ
IKKβ	Inhibitory kinase β
IL	Interleukin
iNOS	Inducible nitric oxide synthase
INR	International normalized ratio
ITT	Intention-to-treat (analysis)
IUGR	Intrauterine growth retardation
JNK	c-Jun N-terminal kinase
LASAG	D,L-lysine acetylsalicylate-glycine
LDH	Lactate dehydrogenase
LMWH	Low-molecular weight heparin
LOX	Lipoxygenase

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LPS	Lipopolysaccharide
LT	Leukotriene
LUF	Luteinized unruptured follicle
LX	Lipoxin
MACCE	Major adverse cardiac and cerebrovascular events
MACE	Major adverse cardiac events
MCSF	Macrophage colony-stimulating factor
MDA	Malondialdehyde
MMR	Mismatch repair
MRP4	Multidrug resistance protein 4
MSI	Microsatellite instability
mTOR	Mammalian target of rapamycin
NET	Neutrophil extracellular trap
NFAT	Nuclear factor of activated T-cells
NICE	National Institute for Health and Care Excellence
NIH	National Institute of Health
NF-κB	Nuclear factor-κB
NMDA	N-Methyl-D-aspartate (glutamate) receptor
NNH	Number needed to harm
NNT	Number needed to treat
NO	Nitric oxide
NOAC	New oral anticoagulant
NOS	Nitric oxide synthase
NSAID	Nonsteroidal antiinflammatory drug
NSTEMI	Non-ST elevation myocardial infarction
o. d.	Odds ratio
OTC	Over-the-counter
PAF	Platelet-activating factor
PAI-1	Plasminogen activator inhibitor-1
PAD	Peripheral arterial occlusive disease
PAR	Protease-activated receptor
PCI	Percutaneous coronary intervention
PDGF	Platelet-derived growth factor
PE	Pulmonary embolism
PG	Prostaglandin
PG-EP	Prostaglandin endoperoxides
PGHS	Prostaglandin-H synthase
15-PGDH	15-Prostaglandin dehydrogenase
15-PGT	Prostaglandin transporter]
PHA	Phytohemagglutinin acetate
PIGF	Placenta-induced growth factor
PIH	Pregnancy-induced hypertension
PKC	Protein kinase C
PMA	Phorbolmyristate acetate
PMN	Polymorphonuclear cell
PPAR	Peroxisome proliferator-activated receptor
PPI	Proton pump inhibitor
PSGL	P-selectin glycoprotein ligand
PUB	Perforation, ulcer and bleeding

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PTCA	Percutaneous transluminal coronary angioplasty
RCS	rabbit aorta contracting substance
RCT	Randomized controlled trial
RR	Risk reduction
RSK	Ribosomal S6 kinase
Runx-1	Runt-related transcription factor-1
SA	Salicylic acid
SAG	Salicylic acid-acyl-glucuronide
SIRS	Systemic inflammatory response syndrome
SNP	Single nucleotide polymorphism
S1P	Sphingosine-1-phosphate
SPG	Salicylic acid-phenol-glucuronide
STAT	Signal transducer and activator of transcription
STEMI	ST elevation myocardial infarction
SU	Salicyluric acid
SUPG	Salicyluric acid-phenol-glucuronide
TCF/LEF	T-cell factor/lymphoid enhancer family
TGFß	Transforming growth factor ß
t. i. d.	Three times daily
TNF	Tumor necrosis factor
TOTPAR	Total pain relief
tPA	Tissue plasminogen activator
TRAIL	Tumor necrosis factor-related apoptosis-inducing ligand
TTH	Tension-type headache
TGFß	Transforming growth factor ß
TX	Thromboxane
UGT	UDP-glucuronyltransferase
USPSTF	US Preventive Services Task Force
VCAM	Vascular cell adhesion molecule
VEGF	Vascular endothelial growth factor
VTE	Venous thromboembolism
Wnt	Wingless & Int-1 (drosophila gene)

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Acronyms of clinical trials

AASER	"Aspirin for primary prevention of cardiovascular disease and renal disease progression in chronic kidney disease patients" study	Goicoechea et al., 2018	281 [28]
AAAT	Aspirin for Asymptomatic Atherosclerosis Trialists' Trial	Fowkes et al., 2010	450 [38]
ACE	Aspirin and Carotid Endarterecomy	Taylor et al., 1999	419 [52]
ACTIVE	Atrial Fibrillation Clopidogrel Trial with Irbesartan for Prevention of Vascular Events	Connolly et al., 2009	426 [102]
ADAPT	Alzheimer Disease Anti-Inflammatory Prevention Trial	Aisen et al., 2003	608 [38, 39]
ADAPTABLE	Comparable effectiveness of aspirin dosing in cardiovascular disease	Jones et al., 2021	370 [197]
ADRIE	Antiplatelet Drug Resistances and Ischemic Events	Reny et al., 2012	517 [57]
AFPPS	Aspirin/Folate Polyp Prevention Study	Baron et al., 2003	592 [92]
APACC	Association pour la Prévention par l'Aspirine du Cancer Colorectal	Benamouzig et al., 2003	592 [93]
ARES	"Aspirin Regimens in EsSential thrombocythaemia"	Tosetto et al., 2021	142 [82]
ARRIVE	Aspirin to reduce risk of initial vascular events	Gaziano et al., 2018	356 [102]
ASCEND	A study of cardiovascular risk in diabetes	ASCEND group, 2018	361 [137]
ASPECT	Aspirin-induced Platelet Effects	Gurbel et al., 2007	515 [46]
ASPIRE	Aspirin to Prevent Recurrent Venous Thromboembolism	Brighton et al., 2012	471 [62]
ASPRE	Aspirin for evidence-based preeclampsia prevention	Rolnik et al., 2017	493 [82]
ASPREE	"Aspirin in Reducing Events in the Elderly"	McNeil et al., 2018, 2021; Eisen et al., 2021	242 [1]; 352 [89]; 384 [67]; 594 [105]
ATACAS	Aspirin and Tranexamic Acid for Coronary Artery Surgery	Myles et al., 2016	231 [77, 78]
ATLAS-ACS-2-TIMI-51	Rivaroxaban in Patients with a Recent ACS	Mega et al., 2012	385 [275]

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ATT	Antiplatelet/Antithrombotic Trialists	ATT Baigent et al., 2009	345 [56]
BAFTA	Birmingham Atrial Fibrillation Treatment of the Aged Study	Mant et al., 2007	432 [136]
BLASP	Barbados Low-dose Aspirin study in Pregnancy	Rotchell et al., 1998	492 [84]
BLSA	Baltimore Longitudinal Study of Aging	Stewart et al., 1997	605 [26]
BMDS	British Male Doctors' Trial	Peto et al., 1988	349 [77]
	Boston Collaborative Drug Surveillance Group Study	Group Study Investigators	338 [11]
BRAVO	Blockade of the Glycoprotein IIb/IIIa Receptor to Avoid Vascular Occlusion	Arionov et al., 2008	370 [194]
CAPP-2	Colorectal Adenoma/Carcinoma Prevention Programme-2	Burn et al., 2008, 2011, 2020	586 [75–77]
CAPRIE	Clopidogrel vs. Aspirin in Patients at Risk of Ischemic Events	CAPRIE – Steering Committee, 1996	377 [237]
CARS	Coumadin Aspirin Reinfarction Study	CARS Investigators, 1997	384 [264]
CAST	Chinese Acute Stroke Trial	CAST Collaborative Group, 1997	420 [76]
CATHARSIS	Cilostazol-Aspirin Therapy against Recurrent Stroke with Intracranial Stenosis	Uchiyama et al., 2015	431 [124]
CCSG	Canadian Cooperative Study Group	CCS-Group, 1978	417 [67]
CHAMP	Combined Hemotherapy and Mortality Prevention	Fiore et al., 2002	384 [265]
CHANCE	Clopidogrel in High Risk Patients with Acute Non-Disabling Cerebrovascular Events	Wang et al., 2015	425 [91]
CHARISMA	Clopidogrel for High Atherothrombotic Risk, Ischemic Stabilization, Management, and Avoidance	Bhatt et al., 2006	379 [98]
CLASP	Collaborative Low-Dose Aspirin Study in Pregnancy	CLASP Collaborative Group, 1994	488 [64, 65]
CLIPS	Critical Leg Ischemia Prevention Study	Catalano et al., 2007	452 [49]

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COMPASS	Cardiovascular Outcomes for People using Anticoagulation Strategies	Eikelboom et al., 2017; Bonaca et al., 2020	386 [280]; 455 [67]
	Cottbus Reinfarkt-Studie	Hoffmann & Förster, 1987	368 [182–184]
CPP	Collaborative Perinatal Project	Slone et al., 1976	238 [14]
CPS-II	Cancer Prevention Study II	Thun et al., 1991, 1993	582 [5, 58]
CURE	Clopidogrel in Unstable Angina to Prevent Recurrent Ischemic Events	Yusuf, 2001	367 [26]
	Danish very low-Dose Aspirin after carotid endartectomy trial	Boysen et al.	428 [53]
	Dutch TIA-trial	Dutch-TIA Trial Study Group	418 [69]
EAGER	"Effects of Aspirin in Gestation and Reproduction"	Levine et al., 2018	218 [22]
EINSTEIN-CHOICE	Rivaroxaban or Aspirin for Extended Treatment of Venous Thromboembolism	Weitz et al., 2017	474 [67]
EPCAT	Extended Prophylaxis Comparing Low Molecular Weight Heparin to Aspirin in Total Hip Arthroplasty	Anderson et al., 2013	468 [53]
EPCAT-II	Extended venous thromboembolism prophylaxis comparing rivaroxaban with aspirin following total hip and knee arthroplasty	Anderson et al., 2018	469 [58]
ESPRIT	European and Australian Stroke Prevention in Reversible Ischemia Trial	Halkes et al., 2006	429 [116]
ESPS-2	European Stroke Prevention Study-2	Diener et al., 1996	428 [111]
ETDRS	Early Treatment Diabetic Retinopathy Study	ETDRS-Invest., 1992	359 [127]
EVERE2ST-HIV	Platelet reactivity in human immunodeficiency virus infected patients on dual antiplatelet therapy for an acute coronary syndrome	Hauguel-Moreau et al., 2017	555 [101]
GEMINI-ACS-1	Rivaroxaban Versus Acetylsalicylic Acid in Addition to Either Clopidogrel or Ticagrelor Therapy in Participants With Acute Coronary Syndrome	Ohmann et al., 2017	385 [277]
getABI	German Epidemiological Trial on Ankle Brachial Index	Diehm et al., 2006	451 [41]
GLOBAL LEADERS	Global Leaders	Vranckx et al., 2018	380 [252]
HOPE	Heart Outcomes Prevention Evaluation	Eikelboom et al., 2002	517 [33]

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HOSTEXAM	Host-extended antiplatelet monotherapy	Koo et al., 2021	377 [238]
HOT	Hypertension Optimal Treatment	Hansson et al., 1998	279 [44]; 353 [95]
HPFS	Health Professionals Follow-Up Study	Giovannucci et al., 1993	583 [6]
INSPIRE	Aspirin to Prevent Recurrent Venous Thromboembolism	Simes et al., 2014	473 [3]
ISAAC	International Study of Asthma and Allergies in Childhood	Beasley et al., 2008, 2010	325 [96, 97]
ISIS-2	International Study on Infarct Survival-2	ISIS-2 Group, 1998	364 [13]
IST	International Stroke Trial	IST-Collab. Group, 1997	420 [76]
JLASP	Jamaica Low-Dose Aspirin Study Project	Golding, 1998	492 [83]
JPAD	Japanese Primary Prevention of Atherosclerosis with Aspirin for Diabetes	Ogawa et al., 2008	360 [129]
LEDA	renaL disEase progression by aspirin in diabetic pAtients	Violi et al., 2017	278 [36]
LONFLIT-3	Venous thrombosis from air travel	Cesarone et al., 2002	471 [61]
MATCH	Aspirin and Clopidogrel Compared with Clopidogrel	Diener et al., 2004	426 [101]
	Melbourne Colorectal Cancer Study	Kune et al., 1988	575 [3]
NHANES III	Third National Health and Nutrition Examination Survey	Shen et al., 2014	271 [21]
NHS	Nurses' Health Study	Giovannucci et al., 1995; Chan et al., 2007; Lee et al., 2016	584 [7]; 584 [65]; 584 [66]
OASIS-7	Organization to Assess Strategies for Ischemic Syndromes	Mehta et al., 2010	378 [245]
PARIS	Perinatal Antiplatelet Review of International Studies	Askie et al., 2007	488 [70]
PEGASUST TIMI 54	Prevention Regimen for Effectively Avoiding Second Prevention with Ticagrelor of Secondary Thrombotic Events in High-Risk Patients with Prior Acute Coronary Syndrome	Bonaca et al., 2015	379 [250, 251]
PEP	Pulmonary Embolism Prevention Study PEP-Study	PEP Study Group, 2000	467 [46]
PEPPER	Comparative effectiveness of pulmonary embolism prevention after hip and knee replacement	Pellegrini et al., 2019	466 [44]

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PHS	Public Health Service Study	Hurwitz et al., 1987	318 [27]
PICASSO	Prevention of cardiovascular events in Asian patients with ischemic stroke at high risk of cerebral hemorrhage	Kim et al., 2018	431 [127]
PLATO	Platelet Inhibition and Patient Outcomes trial	Wallentin et al., 2009	379 [24]
POINT	Platelet-oriented Inhibition in New TIA and Minor Ischemic Stroke	Johnston et al., 2019	425 [92]
POPADAD	Prevention of Progression of Arterial Disease and Diabetes	Belch et al., 2008	359 [128]
PPP	Primary Prevention Project	de Gaetano et al., 2001	355 [100]
PROFESS	Prevention Regimen for Effectively Avoiding Second Strokes	Sacco et al., 2008	430 [119]
RECOVERY	Randomised Evaluation of COVID-19 Therapy	RECOVERY Cooperative Group, 2022	558 [123]
RESTART	Restart or Stop Antithrombotics Randomized Trial	RESTART group, 2019	421 [78]
	Rotterdam Study	In 't Veld et al., 2001	607 [29]
SALT	Swedish Aspirin Low-dose Trial	SALT Collab. Group, 1991	419 [75]
SAPAT	Swedish Angina Pectoris Aspirin Trial	Juul-Möller et al., 1992	362 [146]
SOCRATES	Acute Stroke or Transient Ischemic Attack Treated with Aspirin or Ticagrelor and Patient Outcomes	Johnston et al., 2017; Amarenco et al., 2017	425 [97, 98]
SPAF	Stroke prevention in atrial fibrillation study	SPAF-Study Group, 1993	431 [133]
SPS3	Second Prevention of small subcortical Strokes	Benavente et al., 2012	426 [83]
	Sydney Older Persons Study	Broe et al., 2000	606 [32]
THALES	Acute Stroke or Transient Ischemic Attack treated with Ticagrelor and Asprin for Prevention of Stroke and Death	Johnston et al., 2020	426 [99]
TIPS-3	The International Polycap Study – 3	Yusuf et al., 2021	390 [307]
TPT	Thrombosis Prevention Trial – Medical Research Council's General Practice Research Network	TPT, 1998	355 [99]
TWILIGHT	Ticagrelor with aspirin or alone in High-Risk patients after coronary intervention	Mehran et al., 2019	381 [253]

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UK-HARP-I	First United Kingdom Heart and Renal Protection Study	Baigent et al., 2005	277 [32]
UK-TIA	UK Transient Ischemia Trial	Frith et al., 1998	417 [68]
US-PHS	US Physicians' Health Study	US-PHS	346 [72–74]
VOYAGER	Vascular outcomes study of ASA along with rivaroxaban in endovascular or surgical limb revascularization for peripheral artery disease	Hiatt et al., 2020	456 [56]
WARFASA	Warfarin and Acetylsalicylic Acid	Becattini et al., 2012	471 [2]
WARSS	Warfarin – Aspirin Recurrent Stroke Study	Mohr et al., 2001	433 [137]
WASH	Warfarin/Aspirin Study in Heart failure	Cleland et al., 2004	279 [48]
WASID	Warfarin – Aspirin Symptomatic Intracranial Disease	Chimowitz et al., 2005	433 [138]
WATCH	Warfarin and Antiplatelet Therapy in Chronic Heart Failure	Massie et al., 2009	279 [49]
WHS	Women's Health Study	Ridker et al., 2005	350 [86]
WOEST	What is the Optimal Antiplatelet and Anticoagulant Therapy in Patients with Oral Anti-Coagulation and Coronary Stenting	Dewilde et al., 2013	433 [140]

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Index

Acetaminophen (Paracetamol)
– Alzheimer 606
– Analgesic actions 537
– Asthma 301, 324
– Clinical trials 534
– Hepatotoxicity 269
– Reye's syndrome 323, 324
Adenosine 170
ACE inhibitors
– Coronary vascular disease 387
– Heart failure 279
– Hypertension 278
Acetylsalicylic acid (ASA) see *aspirin*
Acute coronary syndrome (ACS) see *coronary vascular disease*
Adenomatous polyposis coli (APC) 190
– Clinical trials 591
– Pathophysiology 190
Allodynia 194
Angina pectoris see *coronary vascular disease*
Alzheimer's disease 602
– Clinical trials 605
– COX-2 604
– Epidemiology 602
– Etiology 602
– Modes of aspirin action 603
– NSAIDs 605
– Pathophysiology 602
Anandamide 100
Anticoagulants 431
– Cerebrovascular disease 431
– Coronary vascular disease 383
– General aspects 150
– New Anticoagulants see *NOAC*
– Peripheral arterial disease 455
– Venous thrombosis 471
Antiplatelet drugs 138
Antipyretic analgesics 531, 534
Antitumorigenic effects see *malignancies*
APHS 35, 47, 96, 100, 148
Apixaban see *NOAC*
Arachidonic acid
– Cancer 189
– General aspects 25, 28, 90
– Pain 25
Arthritis see *Osteoarthritis*; see *Rheumatoid arthritis*
Aspirin see also *special items*; see also *"salicylate"*
– Absorption 64, 65
– ACE-inhibitors 387
– Acetylation targets 80
– Adenosine 170
– Alcohol 64
– Analgesic actions 172
– Analogs 47, 100
– Antiinflammatory actions 34, 162
– Antimicrobial actions 170
– Antiplatelet actions 35, 137
– Antipyretic actions 35, 177
– Antitumor actions 41, 185, 576
– Antiviral actions 171, 555
– Bioavailability 63, 66
– Biotransformations 77, 81
– Bleeding risk 35, 227
– Bleeding-time 221
– Chemical properties 52
– Clinical applications 331
– COVID-19 172, 556
– COX-inhibition 29, 89, 255, 263
– Craven studies 37
– Desensitization 300
– Determination 56
– Diabetes 142, 278, 357
– Dioxolanes 95
– Dissolution 63
– Distribution 71
– Dosing 141, 134, 331, 414, 419, 515
– Ductus arteriosus Botalli 240
– Elderly patients 241
– Endocannabinoids 175
– Energy metabolism 24, 119
– Esterases 78
– Excretion 77
– Fecundability 238
– Fertility 237
– Fetal toxicity 238
– Fibrinolysis 150
– Formulations 53, 66, 532
– Gene transcription 106

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– Gene polymorphism 197, 512
– Generics 53
– GI-bleeding 227
– Habituation 216
– Heart failure 279
– Heme oxygenase-1 (HO-1) 169
– Hemostasis 136
– Hepatotoxicity 269
– History 1, 13
– Hyperpyrexie 214
– Hypersensitivity see *"aspirin exacerbated respiratory disease, see "urticarial/angioedema"*
– Inflammation 160, 542
– Inhibition of cyclooxygenases 36, 91
– Inhibition of platelet function 27, 36, 137, 142
– Inhibition of thromboxane formation 27, 137, 139, 142
– Inhibition of kinases 109
– Metabolism 77, 81
– Methotrexate
– Miscarriage 239
– Salicylate-interactions 93, 145
– Modes of action 26, 46, 80, 87, 556
– Mortality 213
– Nitric oxide 106, 148
– NSAIDs 93, 98, 145, 170, 388, 509
– Pharmacokinetics 62
– Plasma levels 57, 71
– Pregnancy 237
– PPI 262, 372
– Renal failure 276
– « Resistance » see *Aspirin "resistance"*
– Reye's syndrome 128, 309
– "Spanish flu" 211
– Serotonin 175, 410
– Sphingosine-1-phosphate (S1P) 163, 193
– Stomach see *GI tract*
– Statins 388
– Synthesis of 13
– Teratogenicity 238
– Thrombin formation 138, 225
– Teratogenicity 238
– Thromboxane 137
– Venous thrombosis 464
– Withdrawal 229, 371
"Aspirin exacerbated respiratory disease" (AERD) 292
– Clinical trials 299
– COX-1 298
– COX-2 298
– Epidemiology 292
– General aspects 293
– Leukotrienes 294
– Modes of aspirin action 297
– Pathophysiology 293
– Prostaglandins 296
– Treatment 300
"Aspirin-induced asthma" see *Aspirin exacerbated respiratory disease, AERD*
Aspirin "Resistance" (HTPR) 500
– Cardiovascular outcome 516
– Causes 503
– Clinical Trials 515, 517
– Definition 501
– Detection 504
– General aspects 500
– Modes of aspirin action 507
– Platelet function 504
– Stroke outcome 413
– Thromboxane 505
"Aspirin-triggered lipoxin (ATL)" 30, 96, 195
– Colorectal carcinomas 586
– General aspects 96
– eNOS stimulation 160
Audiovestibular system see *ototoxicity*

Bleeding 221
– Bleeding risk 223, 227
– Bleeding risk in surgical interventions 228
– Bleeding time 35, 224
– General aspects 221, 227
– GI bleeding 257
– Pregnancy and labour 241
– Treatment 230

Cancer 185, 575; see also *malignancies and special items*
Cardiovascular disease see *coronary vascular disease*
Cerebrovascular disease 409
– Anticoagulants 431
– Cilostazol 430
– Clopidogrel 424
– Dipyridamole 427
– Epidemiology 410
– Etiology 409
– Modes of aspirin action 411

EBSCOhost - printed on 2/11/2023 5:24 AM via . All use subject to https://www.ebsco.com/terms-of-use

– NOAC 434
– Pathophysiology 409
– Platelets 411
– Primary prevention 415
– Secondary prevention 416
– Stroke subtypes 422
– Thromboxane 413
Cilostazol
– Cerebrovascular disease 430
– Mode of action 430
– Peripheral arterial disease 457
Clinical trials, types of 334
Clopidogrel
– Cerebrovascular disease 424
– Coronary vascular disease 377
– Peripheral arterial disease (PAD) 454
– "resistance" 501
Colorectal adenomas see *adenomatosis polyposis coli*
Colorectal cancer 575
– Clinical trials 581
– COX-1 188, 192, 580
– COX-2 189, 193, 580
– Coxibs 593, 608
– Epidemiology 576
– Etiology 577
– General aspects 185, 575
– Lynch syndrome 586
– Modes of aspirin action 191, 578
– NSAIDs 593
– Pathophysiology 187, 578
– Primary prevention 581
– Prostaglandins 185, 580
– Secondary prevention 587
Coronary vascular disease (CVD) 338
– ACE inhibitors 387
– Acute coronary syndrome (ACS) 363, 385
– Anticoagulants 383
– Clopidogrel 377, 379
– Coronary artery bypass surgery (CABG) 373
– Coumarins 383
– Coxibs 388
– Epidemiology 339
– Etiology 339
– General aspects 338
– Modes of aspirin action 340
– NOACs 385
– NSAIDs 388
– Pathophysiology 339
– Platelets 137, 341, 343
– Primary prevention 344, 353 357
– Secondary prevention 363, 367
– Statins 388
– Thromboxane 341
Circadian rhythm 342, 491
COVID-19 171, 555
– Clinical trials 557
– Modes of aspirin action 171, 556
COX
– General aspects 89, 91
– Historical aspects 29
– Inhibition by NSAIDs 91
– Isofoms 89, 510
COX-1
– Antiplatelet actions of aspirin 137, 144
– Colorectal carcinomas 188
– Gastric mucosa 253
– General properties 91
– "Aspirin-induced asthma" (AERD) 298
– Acetylation by aspirin 29, 91, 93
– Interaction with NSAIDs 98
– Platelet functions 137
COX-2
– Acetylation by aspirin 30, 46, 95
– Adenomatosis polyposis coli 190, 591
– Colorectal carcinomas 189
– Gastric mucosa 251
– General aspects 89
– Inflammation 163
– Inhibition by aspirin 29, 166
– Lipoxins 30, 167
– Transcription factors 31
– Tumor promotion 189, 579
COX-3 see *COX-isoforms*
Craven, Lawrence L. 37
Cyclooxygenase(s) see *COX*
Cytochromes 81

Dabigatran see *NOAC*
Deep vein thrombosis see *venous thromboembolism*
Desmopressin (DDAVP) 231
Diabetes
– Antiplatelet action of aspirin 142, 358
– Clinical trials 359
– General aspects 357
– Thrombotic risk 357
Dicarboxylic fatty acids 124, 312

EBSCOhost - printed on 2/11/2023 5:24 AM via . All use subject to https://www.ebsco.com/terms-of-use

Dioxolanes 95, 164
Dipyridamole 456
– Cerebrovascular disease 427
– Mode of action 427
– PAD 456
Dipyrone (Metamizol) 99, 389, 509
Disease-modifying antirheumatic drugs (DMARDs) 546
Dreser, Heinrich 14
Ductus arteriosus Botalli
Duisberg, Carl 13, 20

Edoxaban see *NOAC*
Eichengrün, Arthur 14, 18
Eicosanoids 28
Elwood, Peter C. 40
Endocannabinoids 91, 175
Endothelium 145
– Anticoagulatory factors
– Dysfunction 146, 448
– Fibrinolysis 151, 448
– General aspects 134, 145
– Heme oxygenase-1 169
– Inflammation
– NO-production (eNOS) 148, 169
– Prostacyclin production 146
Energy metabolism 119
– Modes of aspirin action 119
– Modes of salicylate action 121
– Oxidative phosphorylation 24

Fatty acid metabolism 121
Fecundability 238
Ferritin 169
Fertility 237
Fever 177
Flu
– Flu-like conditions and COVID-19 171, 534
– Modes of aspirin action 171, 555
– Spanish flu and aspirin 211
Fever 177
– Aspirin modes of action 177
– General aspects 177
– Salicylates 177
Fibrinolysis 150, 448

Gastrointestinal tract (GI tract)
– Clinical trials 258, 263
– Coxibs 263
– GI bleeding 227, 262, 372
– Helicobacter pylori 258
– Modes of aspirin action 252
– Mucosal injury 64, 249
– NSAIDs 263
– Prostaglandins 251
Gentisic acid 79
Gerhardt, Charles Frédéric 18

Headache see *tension-type headache*; see *migraine*
Hearing disturbances see *ototoxicity*
Heart failure 299
Helicobacter pylori 258
Heme oxygenase-1 169
Hemorrhage see *bleeding*
Hemorrhagic stroke see *cerebrovascular diseases*
Hemostasis
– General aspects 134
– Modes of aspirin action
HIV 552
– Clinical trials 554
– Modes of aspirin action 553
– Pathophysiology
HMGB-1 165, 365, 464, 545
Hoffmann, Felix 14, 18
Hypertension
– Antihypertensive treatment and aspirin 278

Ibuprofen 240, 388
Indomethacin 388
Inflammation 160
– General aspects 160, 502
– HMGB-1 165, 365
– Lipoxins 167
– Modes of aspirin action 162, 553
– Platelets 162, 163
– Prostaglandins 163
– Salicylates 167
Ischemia see *particular organs*
Isoprostanes 483, 514, 604

Kawasaki disease 567
– Clinical trials 569
– Etiology 568
– General aspects 567
– Mode of aspirin action 568
– Pathophysiology 568

EBSCOhost - printed on 2/11/2023 5:24 AM via . All use subject to https://www.ebsco.com/terms-of-use

– Reye-syndrome 322
Kidney
– Analgesic nephropathy 275
– Chronic kidney disease 276
– Clinical trials 275
– General aspects 273
– Mode of aspirin action 274
Kinases
– Inhibition by salicylates 24, 109
– Roles in cancer
– Roles in inflammation
Kolbe, Hermann 10
Kune, Gabriel 41

Leukotrienes 293
– AERD 293
– Ototoxicity 287
Lipoxins 96, 167, 297, 486; see also *aspirin-triggered lipoxin*
– Aspirin-triggered lipoxin (ATL) 30, 95, 168, 195
– Inflammation 167
– Stimulation of NO production 169
Liver 269
– Clinical studies 270
– General aspects 269
– Mode of aspirin actions 121–123, 270
– Reye's syndrome 128
Lyell syndrome 306
Lynch syndrome 586

Malignancies see *cancer*
Majerus, Philip W. 36, 137
Malondialdehyde (MDA) 483, 487
Metamizol see *dipyrone*
Methotrexate 546
Methylsalicylate 52, 211
Migraine 536
– Clinical trials 537
– Modes of aspirin action
MRP4 510
Myocardial infarction see *coronary vascular disease*

New oral anticoagulants see *NOAC*
Nitric oxide (NO)
– Endothelial NO-synthase (eNOS) 108
– Inducible NO-synthase (iNOS) 106
– Stimulation of eNOS by aspirin 148, 169
NOAC see also *particular compounds*
– Cerebrovascular disease 434
– CVD 388
– PAD 455
– Venous thrombosis (VTE) 469, 473
Nonsteroidal antiinflammatory drugs (NSAIDs) see also *particular compounds*)
– Alzheimer's disease
– Antiplatelet effects 99, 508
– Colorectal carcinoma
– COX-1/COX-2-selectivity 98
– CVD 385
– Ductus arteriosus 240
– Osteoarthritis 547
Nuclear factor kB (NFkB) 113, 197

Organ toxicity see also *individual organs*
Osteoarthritis 547
– Clinical trials 547
– Modes of aspirin action 543
– Pathophysiology 547
Ototoxicity 284
– Clinical trials 287
– Modes of aspirin action 285
– Pathophysiology of hearing loss 285
– Prestin 286
– Prostaglandins 287
– Tinnitus 284
Oxidative phosphorylation see *energy metabolism*

Pain 528
– Acetaminophen 530–532
– Endocannabinoids
– General aspects 530
– Headache 535
– Mediators of pain 530
– Migraine 536
– Modes of aspirin action 172, 530
– Placebo effects 530
– Prostaglandins 173
– Serotonin 175
Paracetamol see *acetaminophen*
Peripheral arterial occlusive disease (PAD) 445
– Anticoagulants 455
– Cilostazol 457
– Clinical trials 449
– Clopidogrel 454
– Dipyridamole 456

EBSCOhost - printed on 2/11/2023 5:24 AM via . All use subject to https://www.ebsco.com/terms-of-use

– Endothelial dysfunction 448
– Epidemiology 446
– General aspects 445, 448
– Modes of aspirin action 447
– NOACs 455
– Pathophysiology 446
– Peripheral transluminal angioplasty 452
– Platelet function 448, 452
– Primary prevention 449
– Secondary prevention 451
– Thromboxane 447
Plants and salicylates 105
– Salicylate and plant resistance 105
– Vegetables as sources of salicylates 73
– Willow bark 9
Platelets 137
– ACS 340
– Aspirin-dosing 141
– Bleeding time 35
– Colorectal carcinomas 186
– COX-1 92, 137, 510
– General aspects 36
– inflammation 162, 347
– Mode of aspirin action 36
– Response variability 504; see *Aspirin "resistance"*
– Thrombosis 137
– Thromboxane formation 137, 505
– Tumorigenesis 41
– Turnover rate 141, 514, 524
Polypill 390
PPI 262, 372
Prasugrel 138, 377, 427
Preeclampsia 479
– Aspirin dosing 490
– Clinical trials 486
– Etiology 479
– General aspects 479
– Lipoxins 486
– Modes of aspirin action 481
– Pathophysiology 480
– Prostacyclin 481
– Thromboxane 484
Pregnancy 237
– Aspirin use in pregnancy 237
– Bleeding 241
– Ductus acrteriosus Botalli 240
– Miscarriages 239
– Teratogenicity 238
Pregnancy-induced hypertension see *preeclampsia*
Prestin 286
Prostacyclin see also *prostaglandins*
– ACS 342
– Endothelial cells 146
– Myocardial ischemia 343
– Preeclampsia 481
Prostaglandins
– Asthma 296
– General aspects 28
– Inflammation 27, 163
– Malignancies 185, 191, 580
– Pain mediators 173
– Receptors 28, 147, 173, 177
– Synthase(s) see *COX*
Proton pump inhibitors (PPI) 262, 372

Reye's syndrome 309
– Acetaminophen 323
– Aspirin 310
– Clinical studies 316
– Etiology 313
– History 309
– Laboratory findings 311
– Metabolic actions of salicylate 128, 312
– Morphological findings 312
– Pathogenesis 314
– Salicylate levels 129, 315
Rheumatoid arthritis 544
– Clinical trials 546
– Modes of aspirin action 545
– Pathophysiology 544
Rivaroxaban see *NOACs*
Rothwell, Peter M. 575, 586

Salicin 10, 50
Salicylate (Salicylic acid) 51
– Antiinflammatory actions 11, 543
– Antipyretic actions 177
– Antirheumatic actions 11
– Antiviral actions 171
– Biotransformations 71, 79, 81
– Bleeding 223
– Chemical properties 51
– Determination 57
– Energy metabolism 24, 125

EBSCOhost - printed on 2/11/2023 5:24 AM via . All use subject to https://www.ebsco.com/terms-of-use

– Excretion 83
– Fatty acid metabolism 122
– Gastric mucsal injury 552
– History 10
– Inflammation 162
– Inhibition of kinases 109
– Inhibition of prostaglandin synthesis
– Natural sources of 10, 73
– Ototoxicity 286
– Pharmacokinetics 63, 69, 71
– Plant resistance 105
– Plasma levels 71
– Protonophoric properties 51, 125
– Synthesis of 10
– Toxicity 209
– Transcription factors 108, 113, 197
– Uncoupling of oxidative phosphorylation see *energy metabolism*
Salicylic acid see *salicylate*
Salicyluric acid 79
Sepsis 548
– Clinical trials 550
– General aspects 548
– Modes of aspirin action 549
Serotonin (5-HT) 175
Sphingosine-1-phosphate (S1P) 163, 164, 193
Stevens-Johnson syndrome 307
Stomach see also *GI-tract*
– Aspirin absorption 64
– Bleeding 262, 271
– Helicobacter pylori 258
– "Ion trapping" 65
Stone, Edward 9
Stroke see *Cerebrovascular diseases*
Systemic inflammatory response syndrome (SIRS)
– Clinical trials 550
– General aspects 548
– Modes of aspirin action 549
– Pathophysiology 549
Szczeklik, Andrzej 225, 292

Tension-type headache 537
– Clinical trials 538
– Modes of aspirin action 174, 539
Thrombin
– Bleeding time 224
– CVD
– General aspects 548
– Modes of aspirin action 549
– Inhibition by aspirin 225
– PAD
– Wound healing
Thrombocythemia 141
Thrombosis 222
– Arterial thrombosis 136
– General aspects 336
– Venous thrombosis 136, 461
Thromboxane (TX)
– ACS 342
– Aspirin "resistance" 510
– Bleeding time 224
– Blood (plasma) levels
– General aspects 548
– Metabolites 505
– Modes of aspirin action 549
– Paracrine functions 139
– Peripheral arterial disease (PAD) 447
– Platelet functions 137
– Preeclampsia 484
– Serum levels 143
– Stroke 411
– Urinary excretion 542, 447, 506
Thun, Mihael J. 582
Ticagrelor 138, 424
Tinnitus see *ototoxicity*
Toxicity 207; see also *individual organs*
– Acute toxicity 209
– Children 214
– Chronic toxicity ("salicylism") 214
– Clinical symptoms 211, 214
– General aspects 209
– Laboratory findings 213
– Pathophysiology 211
– Treatment 215
Tranexamic acid 231
Transcription factors 108, 113, 197

Urticaria 306

Vane, Sir John R. 26
Venous Thromboembolism (VTE) 461
– Etiology 462
– General aspects 136, 461
– Long-distance flights 471
– Mode of aspirin action 464
– NOACs 473

EBSCOhost - printed on 2/11/2023 5:24 AM via . All use subject to https://www.ebsco.com/terms-of-use

– Pathophysiology 462
– Platelets 463
– Primary prevention 464
– Secondary prevention 471
– Thrombin 463
Von Heyden, Friedrich 11

Warfarin see *anticoagulants*
Willow bark 54
Wu, Kenneth K. 30, 47

EBSCOhost - printed on 2/11/2023 5:24 AM via . All use subject to https://www.ebsco.com/terms-of-use