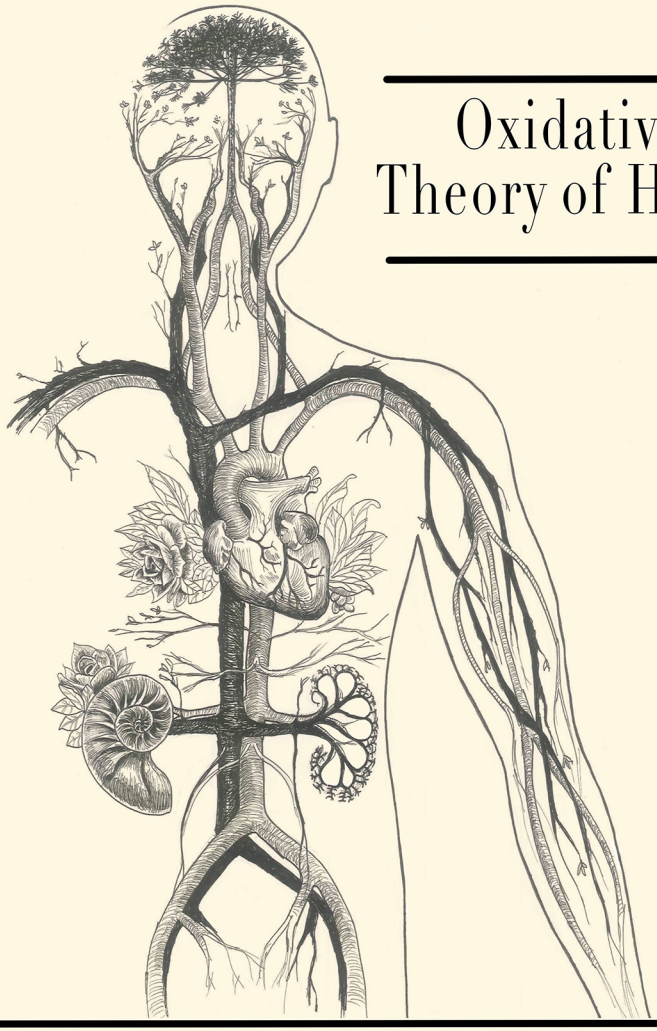

Oxidative Stress Theory of Hypertension

Edited by
Ramón Rodrigo

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PREFACE

Hypertension is a major independent and progressive risk factor for cardiovascular disease. It still remains a leading cause of morbidity and mortality worldwide and the biggest contributor to global mortality accounting for around 10.4 million deaths annually, according to recent data provided by the World Health Organization. Unfortunately, being asymptomatic, about one-third of patients are unaware of having this condition, remaining untreated and thus increasing the risk of stroke and heart disease. These characteristics have contributed to this pathology being referred to as a silent killer. When this disease remains chronically untreated, it gives rise to the development of complications often referred as target-organ damage—mainly kidney, heart, and brain— damage that is often not reversible. Consequently, the causes of death in hypertensive patients include heart failure, end-stage chronic renal disease, or stroke.

Most causes of hypertension are not identifiable, being a multifactorial development and referred to as primary or essential hypertension, accounting for more than 90% of cases. The rest of hypertensive patients have identifiable factors that cause secondary hypertension, with a known direct cause such as kidney disease or endocrine derangements, among others. The pathophysiological mechanism of hypertension has not been well elucidated; however, reactive oxygen species appears to be involved in both classes of hypertension, having a causal role in this pathology. Indeed, reactive oxygen species are mediators of hormones causing vasoconstriction in the physiological conditions; however, when the antioxidant defense system is overwhelmed by the production of these species, oxidative stress occurs and participates in triggering pathophysiological cascades. Consequently, a functional impairment ensues in the vascular wall leading to the development of endothelial dysfunction, followed by structural changes including inflammation, atherosclerosis, or fibrosis, among others. It is noteworthy that oxidative stress is involved in each one of the stages of this pathological sequence of events. Thus, it is reasonable to consider the therapeutic role of antioxidants in reducing the functional and structural oxidative damage caused by increased reactive oxygen species. However, human studies have shown inconsistent results when using antioxidants as therapy for hypertension. Despite this, most of the antihypertensive drugs currently in use have antioxidant properties. Therefore, it is reasonable to

propose that natural exogenous antioxidant supplements are candidates to be tested, based on their ability to contribute in a synergistic effect with antihypertensive drugs, especially when the latter are recognized to evoke a suboptimal therapeutic response in hypertensive patients. Unfortunately, the treatment of hypertension is aimed at lowering blood pressure rather than against the causal agent. Indeed, it is known that despite the numerous drugs available as pharmacological resources for the treatment of hypertension, and the need to indicate multidrug regimens, treatment-resistant hypertension is present with a prevalence between 11% and 21% of cases and has more than doubled during the last 25 years. Furthermore, with recent findings demonstrating a decreased incidence of cardiovascular events when applying strict goals for systolic blood pressure, usually in the range of 120 and 140 mmHg, it is reasonable to assume that future clinical interest will remain centered in blood pressure lowering drugs.

The inconsistency between human studies and experimental protocols in showing the association of hypertension and oxidative stress biomarkers indicates that studies are still lacking a complete knowledge of the pathophysiology behind oxidative stress-dependent hypertension. The aim of this book is to present available data related to the pathogenic mechanisms accounting for the role of oxidative stress in the induction, development, and maintenance of human arterial hypertension. In addition, the contribution of antioxidants as therapeutic agents to treat or prevent hypertension is also discussed as part of the paradigm presented here.

In order to reach our objective, the following chapters are presented:

1. **Role of the endothelium in vascular homeostasis**. Endothelial cells play a key role in the response of the vascular wall to local and systemic stimulus. Thus inflammation, hemostasis, angiogenesis, and vascular tone are modulated by mediators of these cells. Among these functions, the modulation of vasomotor tone is particularly sensitive to the intracellular redox balance occurring in endothelial and vascular smooth muscle cells. This balance, in turn, determines the predominance between vasoconstriction vs. vasodilation, mainly increasing blood pressure through cell signaling effects of ROS and oxidative stress.

2. **Involvement of ROS in blood pressure modulation**. The generation of oxidative stress induces blood pressure effects not solely due to the direct biological actions of ROS on the vascular wall, but also at the level of central nervous system (rostral ventrolateral medulla) and juxtaglomerular apparatus. In turn, renin

release leads to angiotensin II production, thereby inducing NADPH activation, an event also resulting from the effects of other hormones such as endothelin-1 and urotensin II, among others, all of which should exert actions responsive to antioxidants; however, clinical data remains controversial and new studies are needed. The involvement of peptides belonging to the non-canonical renin-angiotensin system, such as angiotensin-(1–7), angiotensin-(1–9), AT2R and Mas receptors, and the enzymes that participate in those reactions, remain to be determined.

3. **Role of oxidative stress in essential hypertension**. It is of interest to note that myofibrils contraction will occur whenever ROS increases within the vascular smooth muscle cells, including the above-mentioned receptor pathway, but also any other ROS source. The best characterized ROS source in the vascular wall is NADPH oxidase, but several enzymes may also contribute in this process (xanthine oxidase, uncoupled eNOS, iNOS, and mitochondrion, among others). Together with the direct ROS-induced vasoconstriction, the reduction in NO bioavailability and downregulation of prostacyclin synthase are mechanisms able to account for the production of imbalance between vasodilators and vasoconstrictors in favor of the latter.

4. **Oxidative stress and secondary hypertension**. Although most cases of hypertension are essential, identifiable causes account for 5%–10% of cases. The various etiologies include renal and endocrine origin. The mechanisms include the contribution of the vasomotor areas of the central nervous system and the renin-angiotensin-aldosterone axis, among others, with oxidative stress involved in all of them. Increased reactive oxygen species is associated to a wide spectrum of effects on the vascular wall, ranging from endothelial dysfunction, to intima-media thickness, and other vascular events leading to increased blood pressure.

5. **Antioxidants and therapy of hypertension**: l**ooking forward.** The relationship between oxidative stress and blood pressure modulation results from the vascular vasoconstrictor response to reactive oxygen species, but also to a decreased bioavailability of vasodilator mediators. Therefore, from a therapeutic point of view of hypertension, it is relevant to counteract the occurrence of oxidative stress. Indeed, most of antihypertensive drugs currently in use have antioxidant

properties. In addition, it is of interest that refractory hypertension has been increasing despite new drugs added to the treatment of patients. Other exogenous naturally occurring antioxidants, such as antioxidant vitamins or polyphenols, need to be studied in clinical trials to explore the possibility of reducing refractory hypertension in potentiating the antioxidant defense system with safe and easily available compounds.

The aim of this book is to provide updated research advances consistent with the association of oxidative stress and human hypertension, presenting a therapeutic target of a relevant health problem with a higher mortality despite the use of a great deal of resources. Particularly notorious is the need to use three or more antihypertensive drugs against resistant hypertension. However, further research of antioxidants as potential adjunct antihypertensive agents is still needed.

About the Editor

Dr. Ramón Rodrigo, Master's in Medical Sciences, is Full Professor at the Faculty of Medicine, University of Chile, Institute of Biomedical Sciences, Molecular and Clinical Pharmacology Program. As a researcher in the field of oxidative stress he has published five books as editor, 40 book chapters, and about 250 articles in journals, having more than 8500 citations. As an academic of medical sciences, he is a member of the Board of two Doctorate and one Master Programs of the University of Chile, he has been coeditor of a textbook, and directed numerous Master and Doctorate theses.

CHAPTER 1

ENDOTHELIUM AND VASCULAR HOMEOSTASIS

LUCÍA DEL VALLE[1], RAÚL CASTILLO[1], RAMÓN RODRIGO*[2]

[1] School of Medicine, Faculty of Medicine, University of Chile, Santiago, Chile

[2] Molecular and Clinical Pharmacology Program, Institute of Biomedical Sciences, Faculty of Medicine, University of Chile, Santiago, Chile

***Corresponding author:**
Dr. Ramón Rodrigo
Full Professor
Molecular and Clinical Pharmacology Program
Institute of Biomedical Sciences
Faculty of Medicine, University of Chile
Avda. Independencia 1027, CP 8380453
Email: rrodrigo@med.uchile.cl
Phone: +56229786126

Abstract

Endothelial function, its interactions, and its alteration and malfunction, are responsible for the development of several vascular diseases. The NO pathway, endothelium-derived hyperpolarization, and several biological signals such as angiotensin II, acetylcholine, endothelins, or eicosanoids are part of the many endothelial-dependent processes in arterial function. Reactive oxygen species (ROS) are a family of highly reactive molecules which play a crucial role in a large number of biological processes and systems. ROS react with several key components to vascular function. While many of these interactions are well understood and documented, such as the NO pathway, other processes have been more difficult to develop into a coherent narrative regarding their effects, such as ROS interactions with endothelium-derived hyperpolarization. This chapter aims to develop a

comprehensive overview on how oxidative stress and reactive oxygen species interact with the endothelium and vascular homeostasis, becoming a key pathophysiological component in the development and sustainment of hypertension.

Abbreviations

ACh	Acetylcholine
ADMA	Asymmetric dimethylarginine
Ang II	Angiotensin II
BH2	Dihydropterin
BH4	Tetrahydrobiopterin
Cav-1	Caveolin-1
COX	Cyclooxygenase
ECM	Extracellular matrix
EDHF	Endothelium-derived hyperpolarizing factor
eNOS	Endothelial isoform of nitric oxide synthase
EP	Prostaglandin E2 receptor
ET-1	Endothelin 1
ETA	Endothelin-1 A receptors
ETB	Endothelin-1 B receptors
H2O2	Hydrogen peroxide
iNOS	Inducible nitric oxide synthase
IL-1B	Interleukin-1β
MAPK	Mitogen activated protein kinase
MMP	Matrix metalloproteinases
mPGES-1	Microsomal prostaglandin E synthase-1
NADPH	Reduced nicotinamide adenine dinucleotide
NE	Norepinephrine
NO	Nitric oxide
NOS	Nitric oxide synthase
Nox	NADPH oxidases
O2•−	Superoxide anion
ONOO-	Peroxynitrite
PGE2	Prostaglandin E2
PGH	Prostaglandin H
PGI2	Prostaglandin I2 (Prostacyclin)
ROS	Reactive oxygen species
SNA	Sympathetic nervous system activity
TRP	Transient Receptor Potential
TRPV4	TRP vanilloid type 4

TxA2	Thromboxane A2
VSMC	Vascular smooth muscle cells
XDH	Xanthine dehydrogenase
XO	Xanthine oxidase

1. Vascular structure and determinants of blood pressure

Blood pressure is determined as the product of cardiac output and vascular resistance. When it comes to primary hypertension, the main pathophysiological mechanism involved in increased blood pressure is an increase in vascular resistance. According to the Poiseuille equation, the resistance to blood flow is inversely proportional to the fourth power of the vessel radius. Thus, a decrease in the lumen diameter of an artery increases the resistance proportionally more, raising arterial blood pressure. Therefore, changes in the luminal diameter of vessels are the main determinants of blood pressure.

Vascular tone and wall thickness are the main determinants of arterial luminal diameter. A brief description of each component of the vascular structure is discussed below.

1.1 Endothelium

The endothelium is a single layer of cells located at the intima, the innermost layer of a vessel. It is a complete organ which participates in the vascular system regulation. The endothelium controls vascular function in response to multiple stimuli, including hormones, neurotransmitters, and vasoactive inputs. The vascular regulation is performed through various vasoactive factors, which primarily control the muscle layer of the vessel. Its function is not limited to the vascular tone though—it also has multiple roles in the organism having an effect on platelet aggregation, inflammation, and coagulation homeostasis.

The endothelium-derived factors that participate in vascular regulation can be either vasodilators or vasoconstrictors. Among vasodilators we can find nitric oxide (NO), prostaglandin I2 (PGI2, prostacyclin), and endothelium-derived hyperpolarizing factor (EDHF). On the other hand, vasoconstrictive factors include thromboxane A2 (TxA2) and endothelin 1 (ET-1). Disturbances in these factors can lead to an increase in vasoconstriction, due to either reduced availability of vasodilators, or to an excess of vasoconstrictors, leading to pathologies such as arterial hypertension.

1.2 Smooth muscle cells

As previously mentioned, the main determinants of blood pressure are vascular tone and wall thickness. Both mechanisms depend on the vascular smooth muscle cells (VSMC) to produce an effect. VSMC can modify the luminal diameter through short-term regulation of the vascular tone, mainly determined by endothelial activity. VSMC also regulate wall thickness, which directly affects the luminal diameter. This can produce long-lasting effects in blood pressure. The structural remodeling happening in the layer of VSMC is determined by a plethora of cell signals, some being endothelium-mediated, cardiovascular hormones-mediated, or physical stimuli. There are several cell pathways involved in proliferation and hypertrophy, but Ras/mitogen activated protein kinase (MAPK) pathway signaling has been demonstrated to be an important pathway for this process. Oxidative stress has been demonstrated to cause cell proliferation and muscle layer hypertrophy, mainly through activation of the MAPK pathway. It is currently being discussed which receptors and activators of the MAPK pathway are the ones involved. It has been proposed that reactive oxygen species interact with proto-oncogene tyrosine-protein kinase Src (c-Src), a protein from tyrosine kinase receptors, which may change the reactivity of the receptors to different signals [1]. Some of these include the insulin-like growth factor receptor, the epidermal growth factor receptor, and the platelet-derived growth factor receptor. MAPK signaling contributes to enhanced cell survival signals, cell division, and expression of Gqα/PLCβ1 proteins, resulting in VSMC hypertrophy [2].

1.3 Adventitia

The adventitia layer of the vascular wall is a connective tissue layer mainly composed of fibroblasts and collagen. Most of the adventitia is extracellular matrix, and the main protein in the extracellular matrix is collagen. Changes in adventitia thickness and composition lead to changes in vascular homeostasis, mainly elasticity, which has been shown to decrease in arterial walls of the elderly.

Increased reactive oxygen species production by adventitial fibroblast NADPH oxidase (Nox) has been shown to cause vascular remodeling, increasing wall thickness, and reducing vascular elasticity. Nonetheless, it has been proposed that the importance of adventitia in vascular homeostasis is contributing to oxidative stress in the vessel wall, distributing ROS to other components of the arteries. This has been demonstrated by Ang II stimulation. Additionally, Ang II in the adventitia causes the release of

vasoactive hormones such as growth factors and ET-1, which may further regulate vascular structure and function via autocrine or paracrine signaling mechanisms [3].

2. Endothelium-dependent vasoregulators

2.1 Nitric oxide

In 1984, Furchgott and Zawadzki described that the endothelium-dependent vasodilation was preceded by an increase in cyclic guanosine monophosphate (cGMP), proposing an endothelium-derived relaxing factor as responsible for this increase. That factor is in fact, nitric oxide [4].

Nitric oxide can cause vasodilation either by soluble guanylyl cyclase in a dependent or independent manner. Within the VSMC, NO binds and activates the soluble guanylyl cyclase enzyme, which converts guanosine triphosphate into cyclic guanosine monophosphate. The latter activates cGMP-dependent protein kinase, leading to lower cytosolic Ca^{2+} concentrations and subsequent vasodilation. NO also stimulates the endoplasmic reticulum calcium ATPase, which also reduces the intracellular Ca^{2+} concentration and produces vasodilation. NO is known to have pleiotropic effects besides the aforementioned vasodilation. Among them we find a decrease in inflammation, vascular cell proliferation, platelet adhesion, and tissue factor inhibition. NO is mainly produced by the endothelial isoform (eNOS) of nitric oxide synthase (NOS). There are other isoforms, such as neuronal and inducible NOS. In hypertension, there is an alteration in the expression of these enzymes, but also a compensatory upregulation of neuronal isoform [5, 6].

2.1.1 Nitric oxide synthase reaction

NOS catalyzes the reaction of molecular oxygen with the amino acid substrate L-arginine to produce L-citrulline and NO [7]. L-arginine transport is impaired both in hypertensive and normotensive subjects with a genetic background of essential hypertension [8]. Furthermore, L-arginine supplementation improves endothelial dysfunction in hypertension [9].

Asymmetric dimethylarginine (ADMA) is an endogenous competitive inhibitor of NOS [10, 11] and also inhibits cationic amino acid transporters that supply intracellular NOS with L-arginine from the plasma. ADMA can be increased by reduced nicotinamide adenine dinucleotide phosphate oxidase (NADPH oxidase) activity [12] and oxidative stress [13, 14]. Oxidative stress-dependent increases in circulating ADMA could lead to

eNOS uncoupling [15], vasoconstriction [16], and a subsequent marked increase in blood pressure [15, 17, 18]. In fact, patients with hypertension show significantly higher plasma concentrations of ADMA and a reduced plasma ratio of L-arginine/ADMA [19].

Tetrahydrobiopterin (BH4) is an essential cofactor for the catalytic activity of all three NOS isoforms, as it increases L-arginine binding and stabilizes the active dimeric form of the enzyme [20–22]. Dihydrofolate reductase catalyzes the regeneration of BH4 from its oxidized form, dihydropterin (BH2) [23, 24]. Dihydrofolate reductase expression can be downregulated by endothelial NADPH oxidase-derived H_2O_2, resulting in BH4 deficiency and thus uncoupling of eNOS [25]. Uncoupled eNOS produces superoxide, leading to the oxidation of BH4 to BH2, which leads to further eNOS uncoupling, resulting in a positive feedback mechanism that perpetuates oxidative stress [19].

2.1.2 Nitric oxide synthase interaction with caveolae, caveolins, and oxidative stress

Under basal conditions, eNOS is inactive, due to its union with membrane invaginations called caveolae [26]. Oxidized low-density lipoproteins (LDL), regarded as a representative parameter of oxidative stress, causes depletion of caveolae cholesterol in cultured endothelium via the scavenger receptor CD36, leading to eNOS redistribution away from the plasma membrane and diminished capacity to activate the enzyme [27]. Due to the provision of cholesterol esters by high-density lipoproteins (HDL), this molecule maintains caveolae cholesterol content, retains eNOS in the domain, and thereby preserves the capacity of eNOS activation [28].

Caveolins are the main coat proteins of caveolae. Studies in endothelial cells demonstrate that eNOS has the capacity to directly interact with caveolin-1 (Cav-1) or caveolin-3 and that this interaction results in inhibition of NO production [29]. Furthermore, in vivo experiments support the role of caveolin-1 (Cav-1) as a negative regulator of eNOS [30]. In fact, Cav-1 deletion prevents hypertensive vascular remodeling induced by angiotensin II [31], while the presence of caveolae with Cav-1 expression increased significantly in the aortas of rats with pulmonary hypertension [32]. It has been shown that Interleukin-1β (IL-1B) induces the upregulation of Cav-1 [33], while NADPH oxidase-derived ROS are involved in human neutrophil IL-1β secretion [34]. Thus, we propose that oxidative stress-dependent production of IL-1β could be involved in Cav-1 induced hypertension.

2.2 Norepinephrine

To this date, several studies assessing either indirect or direct markers of sympathetic function have provided compelling evidence that in early stages of hypertension or in young hypertensive subjects, the sympathetic nervous system is upregulated. Norepinephrine (NE) is a powerful vasoconstrictor agent released by the sympathetic nervous system on adrenergic receptors of the vascular smooth muscle. The activation of α1-adrenergic receptors by NE can lead to VSMC proliferation via the MAPK pathway. On the other hand, oxidative stress can mediate a central activation of the sympathetic nervous system by overexpression of iNOS, resulting in increased blood pressure [35].

2.3 Angiotensin II

Angiotensin II (Ang II) is a potent vasoactive peptide that can be formed in several vascular beds, as long as they contain angiotensin I converting enzyme. When Ang II production increases above normal levels, it can lead to vascular remodeling and endothelial dysfunction in association with increased blood pressure levels. Also, despite the commonly described context of enhanced sympathetic nervous system activation in hypertension, it has been described that chronically elevated plasma levels of Ang II lead to a salt-sensitive form of hypertension that is associated with a differential pattern of peripheral sympathetic outflow. This phenomenon is called the "Ang II-salt sympathetic signature," being characterized by a transient reduction in sympathetic nervous system activity (SNA) to the kidneys, no change in SNA to skeletal muscle, and a delayed activation of SNA to the splanchnic circulation. Thus, SNA is differentially regulated in Ang II-salt rats, the splanchnic vascular bed being the primary target of the sympathetic nervous system in this model of hypertension. [36].

On the other hand, in terms of a possible relationship between oxidative stress and Ang II dependent hypertension, a wealth of evidence has emerged implicating Ang II-induced ROS generation in the pathogenesis of hypertension, NADPH oxidase being possibly the predominant source of derived ROS production in the brain [37]. Also, reactive oxygen species may play a role by impairing sympathetic vasoregulation in the skeletal muscle in the context of this type of hypertension. In fact, chronically elevated Ang II increases muscle ROS, which disrupts the normal NO-dependent attenuation of sympathetic vasoconstriction [38]. On the other hand, prostaglandin E2 (PGE2) acting on endothelin-1 receptors contributes

to excessive ROS levels derived from NADPH oxidase and mitochondria in response to Ang II [39, 40].

2.4 Acetylcholine

Acetylcholine (ACh) is an endothelium-dependent vasodilator released after its generation following mitochondrial production of acetyl-CoA. Once liberated, ACh triggers calcium release from the internal store of endothelial cells, nitric oxide production, and thus, artery relaxation. The nitric oxide depletion caused by ROS, discussed before, worsens the ACh-mediated vasodilation response [41]. To this date, it has been widely described that endothelium-dependent vasodilation to ACh is reduced in the forearm of essential hypertensive patients [42]. Thus, it is fair to hypothesize that the increase of availability of ACh, resulting from the effect of antiacetylcholinesterases, may prevent autonomic imbalance and reduce inflammation, yielding beneficial effects for cardiovascular disorders in hypertension, which is exactly what happens with the administration of Donepezil in spontaneously hypertensive rats [43].

2.5 Endothelium-derived hyperpolarizing factor

In addition to other vasodilating factors, endothelial cells relax the vascular muscle layer through hyperpolarization of the smooth muscle cells. The mechanism through which the endothelium produces hyperpolarization in the smooth muscle cells varies between vascular types and species, but there are two well-documented mechanisms: diffusible factors and contact-mediated pathways. It seems that in normal conditions, the endothelium-derived hyperpolarizing factor (EDHF) system serves as a vasodilator reserve. In the case of NO pathway dysfunction or downregulation, upregulation of EDHF has been observed. The interplay between NO and EDHF is complex and not properly understood. It is estimated that, in normal conditions, the NO pathway inhibits EDHF activity through various mechanisms, such as inhibition of cytochrome P-450 enzyme activity [44], inhibitory effect on cation channels [45], and reduced permeability of gap junctions [45, 46].

Hydrogen peroxide (H_2O_2) is known to induce vascular relaxation through EDHF activation [47, 48]. Therefore, H_2O_2 behaves both as an inhibitor of the NO vasodilator system, and as a vasodilator through the EDHF system. A reduction of EDHF function has been seen in prolonged hypertension, mainly through endothelial dysfunction [49]. The precise mechanisms in which this takes place have not been fully described.

Myoendothelial gap junctions are responsible for the electrical propagation of hyperpolarization from endothelial cells to smooth muscle cells, but it seems to have little impact in the reduction of EDHF function [50].

2.5.1 Endothelium-derived hyperpolarizing factor and oxidative stress

As discussed before, EDHF's activity is probably impaired in hypertension, although there are studies which argue that EDHF function is the same in normotensive and hypertensive subjects, and mainly acts as a backup vasodilator to compensate for decreased NO pathway function. We think this might be the case in physiological conditions and early stages of hypertension pathophysiological development. This has been observed by an initial upregulation of EDHF system activity in early hypertension, and then impairment in later stages of the disease [51].

2.5.2 Endothelium-derived hyperpolarizing factor and hypertension

Vascular cells produce a number of different ion channels (SKCa, IKCa, KIR, KATP, Kv, TRPs, CaCCs), and alterations in their expression have been documented in hypertension [52]. A study argues that the impaired EDHF function observed in animal models of hypertension is caused by alterations of function and expression of the aforementioned channels, thus suggesting that the alterations in ion channels could become therapeutic targets for the prevention and reversal of EDHF dysfunction and endothelial dysfunction that comes with hypertension [50].

2.5.3 Transient receptor potential

Transient receptor potential (TRP) channels are non-selective cation channels, and they play a role in hyperpolarization generation in the endothelium, regulating vascular function [53–55]. Specifically, TRP vanilloid type 4 (TRPV4) may be involved in this function [55, 56]. TRPV4 is downregulated in spontaneously hypertensive rats, and it seems to be a consequence of hypertension, due to the preserved number and function that prehypertensive patients show [56]. Also, TRPV4 knockout mice presented a decrease in EDHF function [57]. Some TRP channels seem to be regulated through direct binding of ROS. Regulation of TRPM2 is distinctive and involves direct binding of oxidant-derived second messengers [58]; however, its role in EDHF function is unclear.

2.6 Endothelin 1

Endothelin 1 (ET-1) is widely known for being a potent vasoconstrictor peptide. Its continuous release from endothelial cells can be inhibited by NO [59, 60]. In return, ET-1 has a strong inhibitory effect on NO-mediated vasodilation [61], but it also reduces β-adrenergic receptor-dependent relaxation [62], being a potent antivasodilatory factor. At elevated concentrations, ET-1 can promote inflammation and vascular smooth muscle cell proliferation [63, 64].

ET-1 effects depend on the activation of ETA and ETB receptors [65, 66]. ETB participates in ET-1 clearance and in the release of endothelial prostacyclin and NO with subsequent vasodilation. ETB inhibition increases circulating ET-1 levels and blood pressure in healthy subjects. In contrast, inhibition of ETA receptors causes coronary dilation, increased coronary blood flow, and decreased coronary resistance [66, 67]. The production of ET-1 is regulated at a genetic level [68], being upregulated by inflammatory factors (such as transforming growth factor beta, tumor necrosis factor alpha, interleukins, insulin, and Ang II), and downregulated by NO, PGI2, hypoxia, and shear stress [68–70].

We can find an example of ET-1 upregulation in the vascular wall of salt-dependent models of hypertension [71, 72]. Furthermore, an increase of ET-1 plasma levels is also induced by acute mental and physical stress in human adults and adolescents, which correlates with stress-induced increases in blood pressure in prehypertensive young adults with verified family histories of cardiovascular disease, being the stress-induced release of ET-1 probably involved in the acute stress-induced pressor response [73, 74]. Reactive oxygen species contribute to this ET-1 induced pressor response to acute stress. In fact, the increase in reactive oxygen species occurs downstream of ET-1 receptor activation [75]. Also, the increased vascular oxidative stress in animal models of hypertension is associated with activation of the ET (endothelin) system via ET receptors [76], this oxidative stress being independent of NADPH oxidase and probably mediated by the mitochondria [77]. Nevertheless, it has been shown that ET-1 augments vascular superoxide production at least in part via an ET(A)/NADPH oxidase pathway in low-renin mineralocorticoid hypertension [71].

2.7 Eicosanoids

Prostacyclin (PGI2) and TxA2 are eicosanoids produced by oxidation of arachidonic acid catalyzed by cyclooxygenase (COX) enzymes [78]. These

compounds have opposite effects on vascular tone, with prostacyclin being responsible for vasodilation, while TxA2 produces vasoconstriction. Thus, the balance between the two contributes to the homeostatic regulation of normal blood pressure by the subsequent modification of the vascular resistance [79]. The disturbance of this balance can lead to hypertension, with increased levels of TxA2 and at the same time, reduced levels of prostacyclin. In fact, prostacyclin knockout mice present increased rates of hypertension, increased fibrosis, vascular injury, and kidney infarction [80], while transgenic overexpressing PGIs mice are protected against hypoxic pulmonary hypertension [81]. Also, elevations in blood pressure and cardiac hypertrophy of hypertensive rat models can be attenuated when the TxA2 receptor is knocked out [82]. Now, in the clinical context, hypertensive men exhibit an increased TxA2 to prostacyclin ratio when compared to normotensive subjects. Impaired prostacyclin biosynthesis in hypertensive patients could account for the typical increased vascular resistance and complications of the hypertensive state [83].

The TxA2 to prostacyclin ratio is also increased during preeclampsia, being partially responsible for hypertension, increased vascular reactivity, and increased platelet aggregation associated with the disease [84]. Oxidative stress is considered a major molecular determinant of preeclampsia [85]. There is, in fact, a relationship between oxidative stress and increased TxA2 in preeclampsia. Glutathione peroxidase—an enzyme that converts lipid hydroperoxides to less reactive alcohols—may be deficient in this disease. Consequently, lipid hydroperoxides result increased, thus inhibiting PGI2 synthase enzyme activity while prostaglandin H synthase, the cyclooxygenase component is stimulated. Therefore, increased TxA2 to prostacyclin ratio accounts for vasospasm and exacerbation of placental ischemia, increased cell damage, and increased lipid peroxidation, enhancing the oxidative stress. Patients with this disease show higher total plasma vitamin E levels [86] when compared to normotensive pregnancies, possibly reflecting enhanced antioxidant defense in response to oxidative stress in women with preeclampsia.

During inflammatory conditions, the production of eicosanoids—particularly PGE2—is enhanced by the action of the inducible COX-2. Microsomal prostaglandin E synthase-1 (mPGES-1), a COX-2 downstream enzyme, controls both baseline and inducible PGE2 production [87]. PGE2 modulates vascular tone through four PGE2 receptor (EP) subtypes (EP1–4). EP1 and EP3 receptors mediate excitatory and contractile effects, whereas EP2 and EP4 receptors mediate inhibitory and vasodilator effects [40]. Increased PGE2 production and microsomal prostaglandin E synthase-1 (mPGES-1) expression are observed in vessels from several models of

hypertension and in peripheral cells from hypertensive patients. Furthermore, mPGES-1-derived PGE2 acting on EP1/EP3 receptors and activating JNK/ERK1/2 pathways contributes to the excessive ROS levels derived from NADPH oxidase and mitochondria in response to Ang II [39, 40]. A summary of all these factors is presented in Table 1-1.

3. Role of reactive oxygen species in vascular homeostasis

The situation most frequently discussed when talking about reactive oxygen species and the endothelium is endothelial dysfunction through oxidative stress, meaning the loss of regulation of ROS mechanisms. Nevertheless, it is widely known that physiological ROS production plays important roles in various cellular processes, such as gene expression, proliferation, and in the case of the endothelium, even vasodilation through EDHF system activation [47, 48, 88].

The loss of regulation of ROS systems, also known as oxidative stress, causes pathophysiological phenomena including ischemia reperfusion damage, atherogenesis, and chronic inflammation, among many others. Specifically, it generates endothelial dysfunction [88, 89].

This is evident in hypertension, as there is an established impairment of antioxidant activity. Antioxidant enzymes (superoxide dismutase, catalase and glutathione peroxidase) are significantly decreased in prehypertension and in stage I and stage II hypertension groups when compared to control individuals [90].

3.1 Reactive oxygen species sources in the vasculature and their interactions

3.1.1 NADPH oxidases

NADPH oxidases (Nox) are the major ROS source in the endothelium [91]. They can be upregulated by various signals, such as Ang II, vascular endothelial growth factor, and platelet-derived growth factor, among others [92]. Endothelial-specific Nox-2 overexpression in mice increases Ang II-induced hypertension, vascular oxidative stress, endothelial dysfunction, vascular fibrosis, and left ventricular diastolic dysfunction [93, 94]. It can be said that in pathophysiological conditions, Nox activity translates into worse hypertension outcomes, and it has been suggested to become a pharmacological target [91]. But there are also a number of studies pointing to better endothelial function and outcomes when Nox activity is increased in the endothelium. Overexpression of Nox4 produces enhanced endothelium-

derived hyperpolarization, but not altered nitric oxide bioactivity, and decreased blood pressure compared to wild mice [95]. It is worth noting that Nox4-overexpression mice only had this alteration and did not have hypertension. It was suggested that perhaps Nox4 was “the good Nox” homologue [96]. Proinflammatory mediators that induce Nox1 or Nox2 appear to downregulate Nox4 expression [97]. Nox4 is the only vascular Nox homologue that directly produces hydrogen peroxide (H2O2), unlike the other homologues that primarily produce superoxide anion (O_2•−), and thus do not scavenge NO nor produce peroxynitrite (ONOO-) [98]. It was recently concluded that Nox4 protects from chronic hemodynamic overload-induced cardiac remodeling, by demonstrating that Nox4 knockout mice had worse pressure overload-induced cardiac remodeling and dysfunction when compared to wild-type mice [99]. However, the available data do not allow concluding that Nox4 upregulated expression can lead to better hypertension control, since most of the studies were not performed using a hypertension animal model. Therefore, it could be said that Nox4 indeed plays an important role in preserving normal endothelial function, but its role in oxidative stress conditions or once endothelial dysfunction is already established remains unclear.

Nox5 is the latest identified NADPH oxidase, and unlike the other subtypes, it does not require any NADPH oxidase subunit to function, being also capable of producing ROS at lower Ca2+ levels. Another feature that makes Nox5 different is that it is not present in mice like the other Noxes, so it is especially hard to experiment with. In vascular cells, Nox5 isoforms Nox5α and Nox5β are its major ROS sources, being activated by thrombin, platelet-derived growth factor, Ang II, and ET-1.

Nox5 has been implicated in cell proliferation, angiogenesis, and migration. In animal models, Nox5-derived ROS participate in VSMC proliferation and migration in atherogenesis. Moreover, Nox5 expression is increased in several cardiovascular diseases, among which we find hypertension. In fact, Nox5 expression in mice is associated with renal function impairment and higher blood pressure [100].

3.1.2 Xanthine oxidase

Xanthine oxidase (XO) is a hepatic and vascular endothelium enzyme that catalyzes the oxidation of hypoxanthine and xanthine to form superoxide ($•O_2^-$), leading also to the production of uric acid, NO, and ROS, thus having a relevant role in oxidative stress. Furthermore, this enzyme is also upregulated by NADPH oxidase activation, being a possible mechanism of an oxidative stress vicious circle [101]. Xanthine oxidase

exists in two different forms: xanthine dehydrogenase (XDH) and XO. The cellular increase of the XO to XDH ratio would be a critical step in some point of the development of endothelial dysfunction and hypertension, among other cardiovascular diseases. In fact, the enzyme's activity is increased both in hypertensive patients and in patients with Ang II-associated coronary disease, while spontaneously hypertensive rats show higher levels of the endothelial form of XO and increased ROS production, leading to increased vasoconstriction [102, 103].

It has been demonstrated that the enzyme inhibitor allopurinol can improve cardiac hypertrophy in spontaneously hypertensive rats but has a minimal impact on blood pressure. Thus, in terms of the specific role of this enzyme in the pathogenesis of hypertension, XO would be participating in the end organ damage rather than in the development of the disease itself [104]. For example, both endothelial XO and plasma XO activity are increased in human atherosclerotic plaques, suggesting that XO-derived superoxide contributes to the development of hypertension-induced atherosclerosis [105–107]. Therefore, plasmatic uric acid has been proposed as a potential oxidative stress biomarker [107, 108]. On the other hand, while experimental models of hypertension exhibit increased XO activity in the kidney, long-term inhibition of XO with allopurinol reduced renal XO activity without lowering blood pressure, supporting again that XO would not necessary participate in the development of the disease itself but in the end organ damage produced by hypertension [109].

3.2 Reactive oxygen species and notch signaling

In the endothelium, notch signaling regulates endothelial functions through influence in other pathways responsible for angiogenesis, inflammation, and apoptosis. The notch pathway has been associated with endothelial dysfunction [110].

ROS have demonstrated to regulate angiogenesis through notch signaling; even noting notch effects on cell proliferation, migration, and adhesion are mediated by ROS, and Nox4-dependent phosphorylation of vascular endothelial growth factor receptor [111, 112]. Moreover, the notch pathway controls Nox4 activity and ROS production [111].

4. Chronic vascular remodeling

The process of chronic vascular remodeling in hypertension requires the phenotypic change of VSMC from a contractile to a proliferative and synthetic phenotype, thus leading to vascular hypertrophy and with that, to

a thicker muscle layer, marked stiffness, and finally to diminished distensibility. Changes in the oxidative status can lead to several changes at a molecular level, such as cytoskeletal assembling, altered relationship between growth and apoptosis, cell senescence, and VSMC redistribution [100].

During the process of vascular remodeling of hypertension, the extracellular matrix (ECM) undergoes degradation to facilitate the proliferation and migration of VSMC. Different proteases participate in this process, among which matrix metalloproteinases (MMP) are found. These are divided into subtypes according to their specific substrates; standing out among them is the MMP-2, being part of the gelatinase subtype. The latter have been described as being present in physiological conditions in most cells. However, certain stimuli—such as reactive oxygen and nitrogen species—can increase their expression, while mechanical stress induced by hypertension can increase their activity. On the other hand, Ang II is also capable of increasing both the expression and activity of MMP-2.

MMP-2 participates in the entire process of vascular dysfunction and remodeling in hypertension, from the acute to the chronic phase, for example through degradation of type IV collagen and laminin in the basement membrane of VSMC, allowing its detachment and migration to the vascular lumen or its rearrangement in the muscular layer. In addition, MMP-2 induces an interaction between VSMC and ECM that ends in persistent migration associated with a phenotypic change of VSMC, from the contractile phenotype to a synthetic one. [113]

5. Clinical findings and significance

As oxidative stress participates in the hypertension pathogenesis, several authors have hypothesized that either a decrease of ROS production or an increase of the antioxidant scavenging of ROS could, by reducing oxidative damage, lead to an improvement of vascular dysfunction and thus to lower blood pressure in the hypertensive patient.

Vitamins C and E have been proposed as a therapeutic strategy to decrease oxidative stress, increase NO bioavailability, and reduce blood pressure. The oral administration of vitamin C in patients with hypertension resulted in improved endothelium-dependent vasodilatory function, higher blood antioxidant capacity, and reduced blood pressure [114]. Yet, clinical trials using antioxidants have had disappointing results regarding sustained decrease in hypertension. It has been suggested that such outcomes can be explained by timing of delivery and insufficient dosage. [101]

Other antioxidant treatment approaches have been proposed, including XO inhibitors such as allopurinol, and NADPH inhibiting compounds. Future research will elucidate the feasibility of using these drugs as hypertension treatment.

Conclusions

The relationships between vasodilator and vasoconstrictor mechanisms exposed in this chapter, and how they interact between one another is of great complexity (Figure 1-1). The redox imbalance occurring at cellular level causes effects involving pathophysiological processes in proliferation, migration, ECM status, and inflammation, among others. All these processes are dependent on the time of development of increased blood pressure. Loss of regulation of ROS production can lead to eNOS uncoupling due to BH4 oxidation, thus impairing endothelium-dependent vasodilation and other endothelial functions, thereby configuring endothelial dysfunction. In physiological conditions, ROS produced by Nox4 cause vasodilation through EHDF activation, and upregulate the Notch1 pathway, which leads to eNOS activation, anti-inflammatory and antiapoptotic effects, and, in term, maintains correct endothelial function. However, this capacity can be overwhelmed, and the maintenance of oxidative stress can give rise to other effects leading to increased VSMC proliferation and increased ECM deposition, resulting in increased stiffness of the vascular wall. The latter could also be damaged by the occurrence of a ROS-induced atherogenesis process. Consequently, the antihypertensive effects of antioxidants could be effective during the first stages of increased blood pressure development, but could be lost in the chronic state due to established damage and pathological remodeling of the vascular wall. This view could contribute to account for the controversial results of available clinical data.

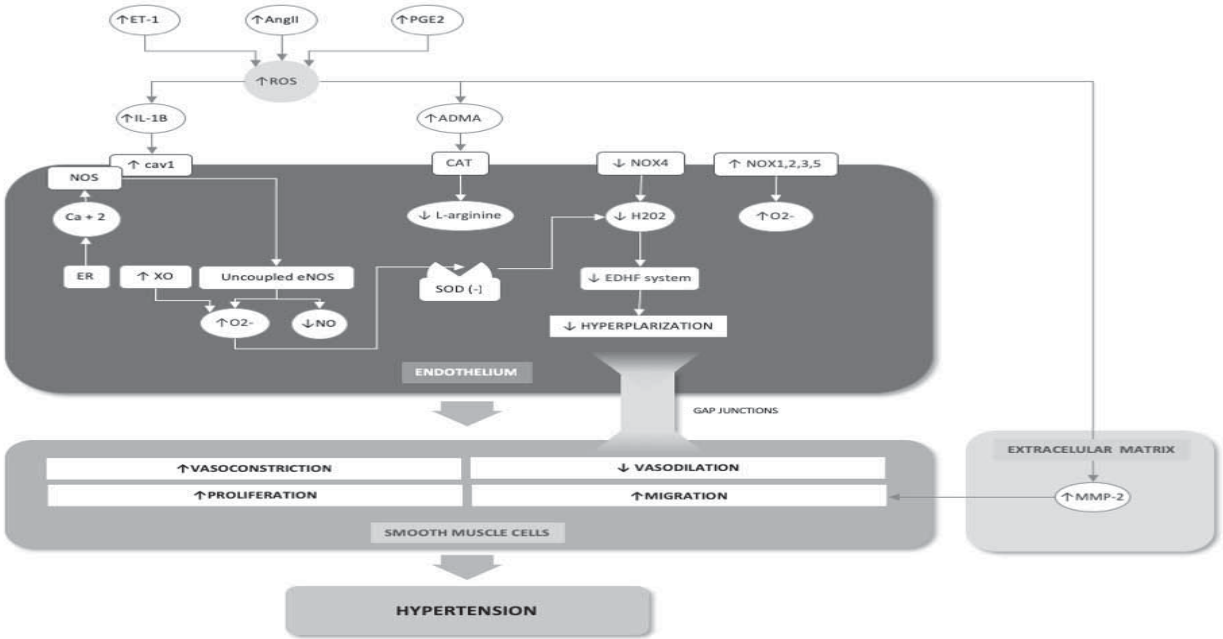

Figure 1-1: Role of reactive oxygen species in vascular homeostatis

Figure 1-1: Role of reactive oxygen species in vascular homeostatis

Higher levels of ET-1, Ang II, and PGE2 leads to increased ROS. ROS increases levels of ADMA and IL-1B. IL-1B increases Cav-1. Cav-1 interaction with NOS prevents it activation. ADMA inhibits catalase, which leads to lower levels of L-arginine and thus lower NO-mediated vasodilation. Increased ADMA uncouples eNOS, while oxidative stress oxidizes BH4 to BH2, which also uncouples eNOS. This uncoupled eNOS produces ROS (possible oxidative stress perpetuation mechanism).
XO is upregulated by NADPH oxidase activation, being a possible mechanism of oxidative stress perpetuation.
NADPH oxidase 4 produces H2O2 directly, in contrast to other homologues of Nox that produce •O2−, and then SOD protein converts them to H2O2. H2O2 produces vasodilation through the EDHF system. When O2•− is present, especially in chronic conditions, it leads to oxidative stress and endothelial dysfunction.
MMP-2 induces extracellular matrix degradation allowing proliferation and migration of smooth muscle cells.

Table 1-1: Systems and effects

Endothelial vasoregulatory system	Effect on physiological conditions	Effect on oxidative stress or pathophysiological conditions	References
Endothelium-dependent vasoregulator systems			
NO pathway	Production of NO with subsequent vasodilation.	• Increased ADMA uncouples eNOS. • Oxidative stress oxidizes BH4 to BH2, which also uncouples eNOS. • The uncoupled eNOS produces ROS which increases intracellular ADMA levels (possible oxidative stress perpetuation mechanism). • Oxidative stress depletes caveolae, leading to eNOS redistribution away from the plasma membrane and diminished capacity to activate the enzyme.	[15, 16, 27]
Endothelium-derived hyperpolarization	The EDHF system serves as a vasodilation reserve. In the case of an NO pathway dysfunction or downregulation, EDHF is upregulated.	• Hydrogen peroxide (H2O2) induces vasodilation through EDHF. • In hypertension there is an initial upregulation of EDHF activity, but an impairment on prolonged hypertension. • Thus, prolonged exposure to ROS causes EDHF and endothelium dysfunction.	[47, 48, 51]

Endothelium-dependent vasoregulators molecules			
Endothelin 1	ET-1 is a potent vasoconstrictor peptide, released from endothelium by a constitutive pathway, which is inhibited by NO.	• The increase in reactive oxygen species occurs downstream of ET-1 receptor activation. • Reactive oxygen species contribute to an ET-1 induced pressor response to acute stress. • Oxidative stress in hypertension animal models is associated to ET receptors activation.	[59, 60, 75–77]
Eicosanoids	PGI2 and TxA2: prostacyclin induces vasodilation while TxA2 produces vasoconstriction.	Oxidative stress participates in preeclampsia, in which there is: • higher plasma vitamin E levels, reflecting enhanced antioxidant defense in response to oxidative stress; • increased TxA2 to PGI2 ratio; • TxA2 analogue prestimulation increases newborn vessels' reaction to $ONOO^-$ induced muscle contraction.	[85, 86]
	PGE2 is known to increase platelet response to their agonists.	• Models of hypertension vessels and peripheral cells from hypertensive patients show increased PGE2 production and mPGES-1 expression. • mPGES-1 derived PGE2 contributes to the excessive ROS levels derived from NADPH oxidase and mitochondria in response to Ang II.	[39, 40]

Angiotensin II	Angiotensin II is a potent vasoactive peptide produced by angiotensin-converting enzymes in several tissues.	• Ang II-induced ROS generation participates in hypertension pathogenesis. • NADPH oxidase is the major source of Ang II-derived ROS production in the brain. • Chronically elevated Ang II increases muscle ROS, which disrupts NO-dependent attenuation of sympathetic vasoconstriction. • PGE2 acting on ET-1 receptors contributes to excessive ROS levels derived from NADPH oxidase and mitochondria in response to Ang II.	[36, 38–40]
Acetylcholine	Acetylcholine (ACh) is an endothelium-dependent vasodilator.	• Endothelium-dependent vasodilatation to acetylcholine is reduced in the forearm of essential hypertensive patients. • Nitric oxide depletion caused by ROS leads to a worsening of the ACh vasodilation response.	[41, 42]
Endothelium-related ROS sources			
NADPH oxidase	NADPH (Nox) enzymes are a major ROS source in the endothelium. They are upregulated by Ang II, VEGF, PDGF, etc. There are several Nox homologues.	• Nox1 and Nox2 are induced by proinflammatory mediators. • Nox1 and Nox2 produce the superoxide anion, which is one of the most reactive ROS, and contributes the most to oxidative stress and nitric oxide depletion.	[91–94, 97–99]

	Nox4 overexpression is linked with better EDH function. Nox4 produces H2O2, unlike the other homologues that primarily produce the superoxide anion (O2•−).	• Proinflammatory mediators reduce Nox4 expression. • Nox4 knockout mice have worse endothelial and cardiac dysfunction.	
Xanthine oxidase	Xanthine oxidase (XO) is a hepatic and vascular endothelium enzyme that catalyzes the oxidation of hypoxanthine and xanthine to form superoxide (O_2•−), leading also to the production of uric acid, NO, and ROS.	• Xanthine oxidase produces uric acid, NO, and ROS • XO is upregulated by NADPH oxidase activation, being a possible mechanism of oxidative stress perpetuation. • Hypertensive rats show higher levels of the endothelial form of XO and increased ROS production, leading to increased vasoconstriction. • XO activity is increased both in hypertensive patients and in patients with Ang II-associated coronary disease. • XO and plasma XO activity are increased in human atherosclerotic plaques. • The enzyme inhibitor allopurinol can reduce renal XO activity and cardiac hypertrophy in hypertension animal models, but have a minimal impact on blood pressure. • Thus, XO would be participating in the end organ damage rather than in the development of the disease itself.	[101–107, 109]

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CHAPTER 2

REACTIVE OXYGEN SPECIES AND BLOOD PRESSURE MODULATION

NICOLÁS BUSTOS[1], RAMÓN RODRIGO*[2]

[1] School of Medicine, Faculty of Medicine, University of Chile, Santiago, Chile
[2] Molecular and Clinical Pharmacology Program, Institute of Biomedical Sciences, Faculty of Medicine, University of Chile, Santiago, Chile
***Corresponding author:**
Dr. Ramón Rodrigo
Full Professor
Molecular and Clinical Pharmacology Program
Institute of Biomedical Sciences
Faculty of Medicine, University of Chile
Avda. Independencia 1027, CP 8380453
Email: rrodrigo@med.uchile.cl

Abstract

In the last few decades, reactive oxygen species (ROS) have been widely considered as one of the fundamental mechanisms responsible for the development of hypertension. ROS have an important role in the homeostasis of the cardiovascular system and they are a known common factor in all the mechanisms regulating blood pressure in physiological as well as pathological conditions that lead to cardiovascular diseases such as hypertension. The aim of this chapter is to provide an updated overview of the role of ROS at the different levels of blood pressure modulation—vascular, neural and renal systems—in order to further understand how an impairment at some levels of the mechanism are determinant in the pathophysiology and progression of hypertension. Finally, antioxidant therapy on clinical trials and future perspectives will be discussed.

Abbreviations

AA	Arachidonic acid
ACE	Angiotensin I converting enzyme
Ang II	Angiotensin II
AT1	Type 1 angiotensin II receptor
BH4	Tetrahydrobiopterin
CACNA1I	Gene encoding low-voltage-activated T-type Ca2+ isoform channels
CNS	Central nervous system
COX	Cyclooxygenase
CVO	Circumventricular organs
DNA	Deoxyribonucleic acid
EDCF	Endothelium-derived contracting factor
EGFR	Epidermal growth factor receptor
eNOS	Endothelial isoform of nitric oxide synthase
ET-1	Endothelin 1
ETC	Electron transport chain
HB-EGF	Heparin-binding EGF-like growth factor
H2O2	Hydrogen peroxide
HOCl	Hypochlorous acid
JGA	Juxtaglomerular apparatus
MAPK	Mitogen activated protein kinase
NADH	Reduced nicotinamide adenine dinucleotide
NADPH	Reduced nicotinamide adenine dinucleotide phosphate
NFκB	Nuclear factor kappa B
Nox	NADPH oxidases
O2•−	Superoxide radical anion
ONOO-	Peroxynitrite
PDGF	Platelet-derived growth factor
PGI2	Prostaglandin I2 (prostacyclin)
PI3K	Phosphatidylinositol 3 kinase
PKC	Protein kinase C
PLA2	Phospholipase A2
PLC	Phospholipase C
PLD	Phospholipase D
PTP	Protein tyrosine phosphatases
PVN	Paraventricular nucleus
RAAS	Renin-angiotensin-aldosterone system
ROS	Reactive oxygen species
RVLM	Rostral ventrolateral medulla

SOD	Superoxide dismutase
SFO	Subfornical organ
TGF	Tubuloglomerular feedback
TNF-α	Tumor necrosis factor alpha
TRPM2	Transient receptor potential melastatin 2
U-II	Urotensin II
UTR	Urotensin II receptor
VSMC	Vascular smooth muscle cells
XO	Xanthine oxidase

1. Reactive oxygen species

ROS are compounds with high reactivity that are generated in cells from an incomplete mitochondrial reduction of molecular oxygen, through enzymatic reactions with intracellular oxidases such as NADPH oxidase, xanthine oxidase, mitochondrial respiratory enzymes, uncoupled eNOS, cyclooxygenases, lipoxygenases, or cytochrome 450 reductases, among other systems [1–3]. The ROS group are intermediates in reduction-oxidation (redox) processes and include unstable free radicals such as the superoxide anion (O_2•−), and non-free-radicals such as hydrogen peroxide (H2O2) [2]. Physiologically, ROS generation is regulated by endogenous cellular antioxidants; the equilibrium defines a redox balance that modulates vascular homeostasis [4, 5]. This regulation includes enzymatic degradation of the free radicals by antioxidant enzymes, such as superoxide dismutase (SOD), catalase, glutathione peroxidase, and a nonenzymatic degradation by glutathione, vitamins, urate, and ubiquinone [2]. Therefore, cellular ROS homeostasis depends on a dynamic balance between production and degradation of free radicals [2, 3].

When ROS generation exceeds this protective capacity, a state of oxidative stress is established. Oxidative stress promotes the alteration of protein synthesis and gene expression, affecting transcription factors, kinases and other cell functional compounds [2, 3]. The different chemical properties of individual ROS molecules have important implications in cellular redox signaling, determining different functions in the cell and homeostasis [6]. ROS participate in maintaining cardiac and vascular integrity in healthy conditions, and play a crucial role in the pathophysiology of several clinical conditions including hypertension [4, 7, 8].

2. Determinants of blood pressure and ROS

A variety of ROS molecules have different effects on cellular function and molecular events that control blood pressure [6]. In this chapter, the role of ROS in the physiological modulation will be discussed as well as in the progression to hypertension along the three main elements that determine blood pressure: vascular modulation, autonomic nervous system, and renal regulation. Mechanisms that contribute to hypertension are complex and involve many pathological processes. In this context, oxidative stress due to NADPH oxidase-derived ROS generation, reduced nitric oxide bioavailability, and insufficient antioxidant capacity, is a common factor of the different pathological mechanisms [9, 10]. Supporting this idea, it has been described that the antihypertensive effect of drugs such as angiotensin I converting enzyme (ACE) inhibitors, angiotensin II receptor antagonist, and calcium channel blockers is in part due to reduction of oxidative stress on vascular tissues [3].

3. Role of ROS on vascular and endothelial regulation

Vasomotor tone depends on a delicate balance between vasoconstrictor and vasodilator components, and under physiological conditions a low concentration of intracellular ROS is essential for the normal redox signaling that maintains vascular function and integrity. Oxidative stress alters this balance, leading to endothelial dysfunction, inflammation, and vascular remodeling [3, 11].

3.1 Sources of ROS in the vasculature

ROS are not only produced in the vessel endothelium but also in vascular smooth muscle cells (VSMC) and adventitial cells [12]. The most important source of ROS in the cardiovascular system involved in blood pressure modulation and hypertension pathophysiology is NADPH oxidase [3, 13–14]. Other enzymes also contribute to ROS generation in vessels such as xanthine oxidase, uncoupled endothelial NO synthase (eNOS), cyclooxygenases (COX), lipoxygenases, cytochrome P450 oxidases and mitochondrial electron transport chain [12, 15–18].

3.1.1 NADPH oxidase

Among many ROS-generating enzymes in the cardiovascular system, NADPH oxidase (Nox) represents one of the major sources of $O_2^{\bullet -}$ and it

plays a key role in the pathophysiology of hypertension [10]. This enzyme catalyzes the reduction of O2 to O_2•− by transferring a single electron from NADPH or NADH [14]. In order to form a functional enzyme, it requires the assembly of five subunits: the cytosolic compounds (p47 phox, p67 phox, and p40 phox) and membrane-bound subunits (gp91 phox, the catalytic subunit, and p22 phox, a membrane regulatory protein) [12, 19]. Seven Nox isoforms have been identified (Nox1–5, DUOX 1 and 2) of which Nox1, 2, 4, and 5 are present in arteries [19–21].

In the vasculature, many effectors can activate NADPH oxidase and thus enhance ROS production and oxidative stress. Depending on the amount of ROS produced in the vascular system, the effect of Nox can be either beneficial or detrimental [6]. One of the major stimuli, which has been widely studied, is angiotensin II (Ang II) which induces NADPH oxidase upregulation by binding to its type 1 receptor (AT1R) [10, 22].

Mice deficient in Nox isoforms or Nox subunits (Nox1, Nox2, Nox4, gp91hox and p47phox knockout mice) have lower basal blood pressure compared with wild-type mice and show lower O_2•− production, and less vascular hypertrophy and endothelial dysfunction than wild-type mice [10–12, 23–29]. Similarly, hypertensive mice that were treated with Nox inhibitors such as apocynin, diphenyleneiodonium, or antioxidant that reduce ROS bioavailability, showed normalized blood pressure and enhanced vascular function [30–32]. All these experiments support the key role of NADPH oxidase in blood pressure modulation.

Other factors that increase Nox expression and activity are endothelin-1, urotensin II, thrombin [33], TNF alpha [34, 35], platelet-derived growth factor (PDGF) [21, 26, 36–39]. Mechanical stimulation on the vascular wall, which is increased during hypertension, is also related with increased Nox-derived ROS [12]. In women with preeclampsia there has been identified autoantibodies against AT1R, that mediate NADPH oxidase activation and oxidative stress leading to upregulation of nuclear factor kappaB (NFκB) and thus contributing to the inflammatory response [40, 41].

Therefore, NADPH oxidase activation is a common factor of the different effectors involved in vasculature regulation and function, including important vasoconstrictors such as Ang II and endothelin I. These factors influence Nox regulation differently according to the isoform, location, and organ where it is expressed.

Thus, the Nox-specific inhibitor represents a novel approach to the treatment of hypertension-associated oxidative damage. Apocynin has been found to blunt the development of hypertension and endothelial dysfunction in already hypertensive rats by oral administration [42]. However, apocynin is nonspecific, lacks selectivity, and has multiple offtarget side effects [11,

43]. In this field, although several NADPH oxidase inhibiting compounds are under investigation, they have not been studied in humans. Nox5 is the most recently discovered Nox isoform and recent studies have shown a particularly important role of this isoform in blood pressure regulation. Unlike Nox1–4, Nox5 does not require p22phox nor cytosolic Nox subunits for its activation [44–46]. Instead, Nox is directly activated by intracellular Ca^{2+} concentration, which binds to an EF-hand Ca^{2+} binding domain, changing its structure to generate O_2•−. In human vascular cells, Nox5 as well as other Nox isoforms, is activated by Ang II, endothelin 1, TNF-α, and PDGF-generating redox signaling [46, 47]. By ROS generation and redox signaling, Nox5 regulates kinases, phosphatases, and transcription factors involved in physiological cellular processes in the cardiovascular, renal, and reproductive system [10, 48], and they are also a key regulator of calcium influx. In VSMC, when ROS levels are increased, the oxidation of calcium channels in the cell membrane or intracellular compartment such as the endoplasmic reticulum leads to a dysregulation of calcium influx, increasing the intracellular free Ca^{2+}. In turn, increased Ca^{2+} is related to ROS production, higher nitrotyrosine levels, and hyperphosphorylation of procontratile signaling molecules such as myosin light chain kinase. Altogether, these effects lead to vasoconstriction [10, 48, 49]. Also, increased Ca^{2+} influences redox-sensitive signaling molecules, inflammation, growth, proliferation, fibrosis, remodeling, and endothelial function. [50–53]. Therefore, Nox5 is proposed to be a cross-talking point between calcium and ROS [49]. Recent genome wide association studies identified Nox5 as a new gene associated with hypertension and systolic blood pressure [54]. Moreover, Nox5 has been recently crystallized, providing new opportunities to develop molecules or drugs specifically targeted to Nox5 [45]. Although much research still needs to be done, the clinical utility of Nox-specific inhibitors is promising [6, 11].

3.1.2 Uncoupled eNOS

In conditions characterized by a deficiency of the substrate arginine or the cofactor tetrahydrobiopterin (BH4), the physiological activity of the enzyme eNOS is switched from NO production to O_2•− generation [55]. In an oxidant environment, O_2•− produced by uncoupled NOS as well as by NADPH oxidase, bio inactivates NO leading to the generation of peroxynitrite (ONOO-), a highly prooxidant molecule, decreasing NO bioavailability and impairing endothelium-dependent vasodilatation [3, 55, 57]. ONOO- can react by an electrophilic aromatic substitution with the tyrosine domain of proteins, thus generating nitrotyrosine, and changing

protein structure and function. ONOO- further contributes to eNOS uncoupling by the oxidation of BH4, enhancing ROS production and leading to oxidative stress [3]. This topic was more extensively discussed in Chapter 1.

3.1.3 Xanthine oxidase

Xanthine oxidase (XO) is a hepatic enzyme that catalyzes the degradation of purines and conversion of hypoxanthine to xanthine and xanthine to uric acid. As a result of the purine degradation pathway, XO oxidizes NADH to form O_2•− and H2O2 [6]. This system is also present in vascular endothelium, generating O_2•− that rapidly reacts with NO to form ONOO- [6, 58]. XO activity has been associated with oxidative damage in biomolecules in patients with hypertension [59]. However, studies have shown that XO may have a function in end organ damage in hypertension rather than an important pathological causal role [60].

3.1.4 Mitochondria

The mitochondrial electron transport chain is a physiologically significant generator of ROS in vascular cells, as a small proportion of electrons escape from the chain at complex I or III to generate superoxide [61, 62]. O_2•− is converted by manganese superoxide dismutase inside the mitochondria to H2O2, which is able to diffuse out of the organelle contributing to oxidative stress. [63]. Mitochondrial ROS mediate essential vascular signaling pathways, for example stabilizing HIF1alpha and mediating adaptation in hypoxic conditions [64, 65]. Mitochondrial ROS also modulates the function and cellular metabolic balance of mitochondria [66]. Changes in mitochondrial redox status may lead to detrimental effects in the vascular wall, relating to endothelial dysfunction by impairing acetylcholine-dependent vasodilatation [67].

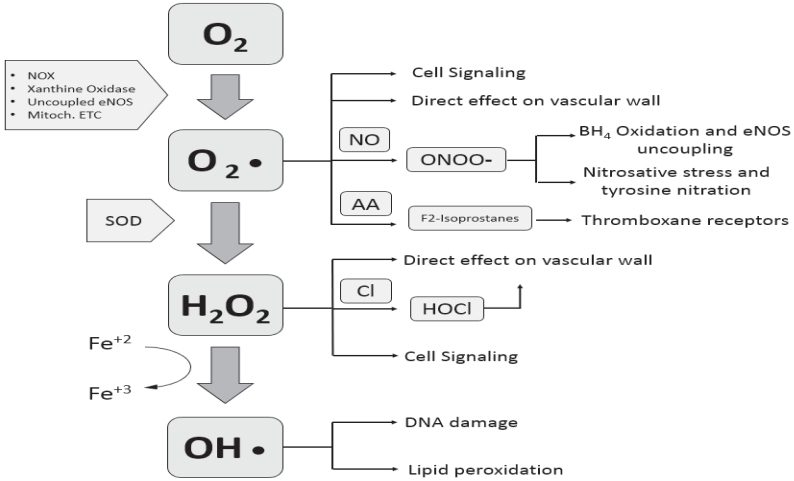

Figure 2-1: Important reactive oxygen species that affect the vascular wall

Schematic overview of the interaction between some of the most relevant ROS that are involved in blood pressure modulation. Superoxide anion (O_2•−) is produced by different sources from molecular oxygen (O2). Superoxide is a highly reactive molecule that can directly affect the vascular wall as well as participate in redox signaling processes or to react with other molecules such as NO to form ONOO-, or AA to form F2-isoprostanes. Peroxynitrite contributes to nitrosative stress, tyrosine nitration of proteins, and eNOS uncoupling, further contributing to oxidative stress. ROS, via BH4 oxidation can, in turn, also enhance eNOS uncoupling.

Superoxide can be converted to hydrogen peroxide by superoxide dismutases. H2O2 can be eliminated enzymatically by glutathione peroxidase, catalase, or thioredoxin peroxidase to form H2O and O2. On the other hand, H2O2 can also undergo, via Fenton reaction in the presence of Fe^{2+}, a spontaneous conversion to hydroxyl radical, an extremely reactive molecule that produces DNA damage and lipid peroxidation. The enzyme myeloperoxidase catalyzes the reaction between H2O2 and Cl in order to produce HOCl; this highly reactive agent is able to inactivate biomolecules including L-arginine and important eNOS substrate, thus further contributing to eNOS uncoupling and ROS production.

Nox, NADPH oxidase; eNOS endothelial nitric oxide synthase; Mitoch ETC, mitochondrial electron transport chain; NO, nitric oxide; ONOO-, Peroxynitrite anion; O_2•−, superoxide anion; SOD, superoxide dismutase; OH•, hydroxyl radical; H2O2, hydrogen peroxide; HOCl, hypochlorous acid; BH_4, tetrahydrobiopterin; AA, arachidonic acid.

Adapted from [68], with modifications.

3.2 Role of vascular and endothelial components

3.2.1 NO

Nitric oxide (NO) plays a key role in the regulation of vascular tone, as it is involved in the maintenance of the integrity of vascular endothelium through different processes such as VSCM proliferation and migration, inhibition of leukocytes, regulating endothelial adhesion, and platelet aggregation [69]. Compared with Ang II, NO has an antagonistic effect on the vascular tone, cell growth, and renal sodium excretion. Moreover, NO decreases ACE synthesis and AT1 receptor expression [3]. In transgenic mice that overexpress Nox1 in VSMC, ROS production increases under the stimulation of Ang II producing eNOS uncoupling and decreasing NO bioavailability, thus impairing vasodilator capacity [70].

3.2.2 Angiotensin II

Angiotensin II (Ang II) is one of the major components in blood pressure regulation. The concept of this peptide has changed from being a simple vasoconstrictor to a complex pleiotropic factor involved in vascular hypertrophy, fibrosis, inflammation, and aging [71]. It has become clear that many of the physiological effects of Ang II on the vasculature, kidney, and heart are in part mediated by ROS such as O_2•− and H2O2. [14, 72].

When Ang II production is increased above physiological levels, it induces vascular remodeling and endothelial dysfunction, which is associated with increased blood pressure level and ROS [3, 73]. This increase is associated with the upregulation of superoxide dismutase activity, possibly in order to compensate for the augmented level of ROS. As long as this compensation is efficient, ROS levels may be normal; however, when ROS production becomes overwhelming and compensatory mechanisms are not sufficient, oxidative stress is able to result in the stimulation of pathological mechanisms [3].

The role of NADPH oxidase in Ang II vascular effect has been widely studied. In animal models of Ang II-induced hypertension, the expression of NADPH oxidase subunits, and generation of ROS are increased [25]. This increase is prevented by the administration of a NADPH oxidase inhibitor [25, 32]. Moreover, in experimental models lacking specific Nox isoforms or Nox subunits, Ang II infusion failed to induce hypertension and endothelial dysfunction [10, 12, 23, 25–27, 29]. On the other hand, in mice that overexpress p22phox in VSMC, vascular hypertrophy and vasopressor effects induced by Ang II are exacerbated compared to control ones [74], thus supporting the idea that effects of Ang II are mediated by oxidative

stress and NADPH oxidase-derived O_2•− [4, 29]. In conditions where Ang II is increased due to activation of the renin-angiotensin-aldosterone system (RAAS), for example; hypertension, diabetes, or atherosclerosis, the activation of Nox by Ang II is augmented [75, 76]. It is believed that Ang II increases expression as well as catalytic activity of Nox and thus increases ROS production [73, 77]. AP-1 and c-Jun activation are critical for induction of Nox1, Nox4, p67phox, p47phox, and p22phox in human aortic VSMC [73, 78].

Together with the canonical Ang II pathway, other peptides, enzymes and receptors have given rise to more complex effects of these systems.

3.2.3 Endothelin-1

Endothelin generated by the endothelium is an important vasoconstrictor. Its type A receptor mediates contraction and is involved in the activation of NADPH oxidase, xanthine oxidase, lipoxygenases, and mitochondrial respiratory chain enzymes [10, 79, 80].

3.2.4 Urotensin II

This hormone is a potent vasoconstrictor in the cardiovascular system that activates NADPH oxidase; however, its effects depend on the vascular bed. It could even function as a vasodilator agent in some isolated vessels [81].

3.2.5 Acetylcholine

In vessels, acetylcholine enhances NO production which diffuses to VSMC, inducing relaxation. Acetylcholine-mediated vasodilatation depends on NO bioavailability which is modulated by ROS levels [82].

3.2.6 Prostaglandins

PGI2 acts as a vasodilator, relaxing the VSMC. Some isoforms may participate in vascular dysfunction under ROS increase. Peroxynitrite-derived free radicals downregulate the activity of the enzyme prostaglandin endoperoxide H synthase-1 (PGHS-1), decreasing PGI2 synthesis and impairing its role on vascular relaxation [83].

3.2.7 Homocysteine

Plasma homocysteine levels are associated with elevated blood pressure [84]. Its elevation impairs NO-induced vasodilation, increases oxidative stress, and stimulates the proliferation of VSMC, therefore contributing to blood pressure elevation [85]. Recent studies suggest that homocysteine-induced endothelial dysfunction is associated with endoplasmic reticulum oxidative stress [86].

3.2.8 Hydrogen peroxide

Calcium-activated potassium channels can be activated by H2O2 in several vascular beds such as cerebral, coronary, and mesenteric vasculature [87–89] Hence, it has been proposed as a potential endothelial-derived hyperpolarizing factor, playing a physiological role in vascular tone, and promoting vasodilation [12]. Nox4 mainly produces H2O2 and has beneficial as well as detrimental effects depending on the stimulation and cell type [90]. In blood vessels, Nox2 generates both O_2•− and H2O2 directly, which affect both NO bioavailability and contractility [91].

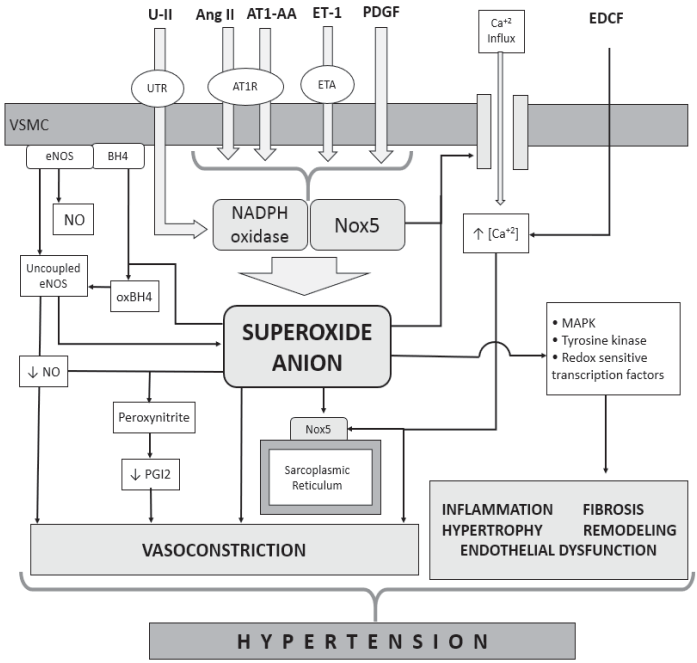

Figure 2-2: Role of NADPH oxidase-derived reactive oxygen species in hypertension

The role of Ang II and other vasoconstrictors in hypertension is, in part, mediated by ROS as their intracellular pathways involve the activation of NADPH oxidase. Superoxide anion produced by Nox, participate in many intracellular processes that lead to a decrease of vasodilator compounds such as NO and PGI2, and activation of molecular redox signaling, transcription factors, and kinases cascades involved in vascular changes, inflammation, and remodeling. Nox 5 activation leads to an important ROS production and calcium influx, related to vasoconstriction. This is a schematic overview of the interrelation between all these pathways to further understand the involvement of ROS in blood pressure modulation and pathophysiology of hypertension.

VSMC, vascular smooth muscle cells; Ang II, angiotensin II; Nox5, NADPH oxidase isoform 5; NO, nitric oxide; eNOS, endothelial nitric oxide synthase; BH4, tetrahydrobiopterin; oxBH4, oxidized tetrahydrobiopterin; PGI_2, prostaglandins I2; Ca^{2+}, free calcium; AT1R, angiotensin II type 1 receptor; AT1-AA, autoantibodies to angiotensin II type 1 receptors; U-II, urotensin II; UTR, urotensin II receptor; ET-1, endothelin-1; ETA, type A endothelin-1 receptor; MAPK, mitogen activated protein kinase; PDGF platelet-derived growth factor; EDCF, endothelium-derived contracting factor. Adapted with modifications from [92].

3.3 Intracellular mechanisms underlying ROS effect in the vasculature

3.3.1 Cell signaling

Growing evidence supports that many of the pathways whereby Ang II and other vasoconstrictors such as endothelin exert their effects involve the activation of NADPH oxidase, and thus ROS production [71]. Ang II regulation of NADPH has complex mechanisms that occur at gene, transcriptional and post-transcriptional level [71]. The activation of the Ang II type 1 (AT1) receptor mediates most of the known actions of Ang II [71]. Via AT1R, Ang II stimulates NADPH oxidase to produce O_2•− and H2O2 [73]. Moreover, Nox-derived ROS also regulates expression and activation of AT1 receptors [93]. AT1 receptors promote intracellular signaling pathways that involve the activation of various protein kinases, subunits of NADPH oxidase, growth factor receptor transactivation [94, 95], among others [22]. Effects of AT1 receptor activation are mediated through different signaling pathways involving second messengers and cytosolic proteins such as phospholipase C (PLC), phospholipase A2 (PLA2), phospholipase D (PLD), protein kinase C (PKC), and the activation of mitogen activated protein kinases (MAPK), tyrosine kinases, phosphatidylinositol 3 kinase (PI3K), c-Jun N terminal kinase (JNK)AKT/ protein kinase, proto-oncogene expression, Ca^{2+} influx, inflammation and cell cycle modulation [96–102]. These kinases and other stimuli are believed to activate NADPH oxidase and thus ROS production [22, 73]. PKC phosphorylates the p47phox subunit, promoting the activation of NADPH oxidase complex and is a common pathway to many other constrictor factors that also activate NADPH oxidase [103]. The biological role of ROS is determined by their chemical properties. ROS can be electrically charged or electrically neutral, hydrophilic, or hydrophobic, conditioning the ability to cross the membrane. [71]. Of the ROS produced in vascular cells, O_2•− and H2O2 appear to be the most important. They are involved in the regulation of many signaling molecules that modulate vascular function such as MAPK, receptor / non-receptor tyrosine kinases, protein tyrosine phosphatases (PTP), and redox-sensitive transcription factors [104, 105].

Nox-derived ROS act as signaling molecules in the vasculature, controlling endothelial function and vascular tone under physiological conditions. In oxidative stress conditions, oxidation of different molecules in vessels cells by ROS lead to vasoconstriction and vascular changes that are key in hypertension development.

Oxidation of thiols leads to structural changes in molecules that result in either activation or inactivation of signaling proteins [71]. For example, oxidation of protein tyrosine phosphatases (PTPS) inactivates its function, leading to increased phosphorylation [71, 106]. PKC is also activated by oxidative stress, leading to phosphorylation and activation of many mechanisms involved in VSMC contraction [68].

3.3.2 Cross-talk between redox status and Ca2+

Oxidative stress also enhances calcium signaling and upregulates rho kinase, playing a role in exacerbated vasoconstriction associated with vascular dysfunction [48, 68]. ROS are also involved in Heparin-binding EGF-like growth factor (HB-EGF) shedding through A disintegrin and metalloproteinase (ADAMS), leading to epidermal growing factor receptor (EGFR) transactivation. This is responsible for Ang II-induced ERK1/2 and AKT signaling, activating cascades that result in protein synthesis that contributes to hypertrophy and cell migration [107–109]. The activation of these molecules is involved in cell growth, migration, regulation of proinflammatory genes, and contraction [68, 71]. Therefore, vascular function and tone is highly regulated by oxidative stress signaling. According to many authors, oxidative stress is crucial for the initiation and progression of endothelial dysfunction and vascular disease [68,110,111].

3.3.3 Role of transient receptor potential melastatin 2 (TRPM2) cation channel

As previously explained, either oxidative stress or altered Ca^{2+} cellular homeostasis can lead to vascular damage in hypertension. However, the mechanism underlying these two effects has not been completely elucidated. Transient receptor potential melastatin 2 (TRPM2) is a multifunctional cation channel that plays central roles in the regulation of calcium influx by oxidative stress. TRPM2 functions as a transducer that converts oxidative stress into Ca^{2+} signaling; it is activated by oxidative stress including H2O2. Indeed, it functions as a sensor for oxidative stress [112]. Recently, it was hypothesized that TRPM2 is a point of cross-talk between redox and Ca^{2+} signaling in vascular smooth muscle cells [113]. The activation of TRPM2 by increased reactive oxygen species would lead to an increase in intracellular Ca^{2+}, through Na^{+}/Ca^{2+}exchanger. This mechanism was studied comparing wild-type and hypertensive mice (LinA3). The generation of reactive oxygen species was increased in vascular smooth muscle cells from hypertensive mice, which was associated

to activation of a TRPM2 modulator, and exaggerated responses to angiotensin II; but these effects were attenuated by catalase, TRPM2 inhibitors, and TRPM2 siRNA, thus accounting for a new player in hypertension-associated vascular dysfunction with the contribution of oxidative stress.

4. Role of ROS on neural modulation of blood pressure

The brainstem is the integrative center of blood pressure neural control. There are two important zones for cardiovascular modulation; the cardioinhibitory zone is composed of parasympathetic premotor neurons from the motor nucleus of the vagus and nucleus ambiguus; and on the other hand, the cardio-excitatory area, mainly represented by the rostral ventrolateral medulla (RVLM) that contains sympathetic premotor neurons. Both zones are integrated in the baroreflex, and have a key role in the short-term blood pressure regulation; increased blood pressure activates baroreceptors leading to a higher firing rate to the nucleus tractus solitarii (NTS), that in turn activates the cardioinhibitory zone and inhibits the RVLM through neural pathways, finally reducing sympathetic tone [2, 114].

The sympathetic efferent outflow of the central nervous system (CNS) is essential for the production and maintenance of most forms of experimental models of hypertension. In these models, ROS play key roles in signaling pathways related to sympathetic overactivation, leading to blood pressure elevation.

4.1 ROS in the central nervous system (CNS)

Accumulating evidence supports that ROS have important effects on the central and peripheral neural mechanisms involved in autonomic function, volume homeostasis, and blood pressure regulation, and therefore cardiovascular function. ROS are important signaling molecules along the CNS. Abundant evidence shows that ion channels and ion exchangers in neurons involved in cardiovascular function are modulated by ROS [115]. At a molecular level, ROS increases neuronal excitability and the sympathetic outflow by downregulating Kv4.3 channel protein expression in neurons, in zones such as the RVLM [116], decreasing the delayed rectifier K^+ current and also enhancing the voltage-gated-L-type Ca^{2+} currents [116, 117]. The general consensus is that oxidative stress in the RVLM and NTS promotes an increase in central sympathetic outflow and baroreflex suppression, mediating neurogenic hypertension [2].

The role of the brainstem-ROS in neural regulation of blood pressure under normotensive condition is less explored as most of the experiments require hypertensive animal models. In normotensive rats, Vitamin C, a well-known antioxidant that is capable of crossing the blood-brain barrier, scavenge ROS in CNS and improves baroreflex sensitivity by modulating the parasympathetic component of the baroreflex [118].

As named above two important neural sites in brainstem are the NTS and the RVLM:

(a) Nucleus tractus solitarii (NTS): ROS are produced under normal physiological conditions in the NTS [119] where they have an excitatory role that involves enhancement in calcium influx in NTS neurons that receive vagal afferences [120], thus participating in NTS neuronal excitability.

(b) Rostral ventral lateral medulla (RVLM): Pharmacological studies suggest that brain stem ROS exerts a general excitatory effect on the sympathetic outflow and cardiac baroreflex under normotensive condition, as microinjection of SOD into the RVLM of normotensive pigs decreased basal sympathetic nerve activity, as well as blood pressure and heart rate [121, 122]. In the RVLM, ROS enhances sympathoexcitatory inputs as they increase glutamatergic excitatory inputs and attenuates GABAergic inhibitory input to RLVM [123, 124]. In this way, ROS in RVLM modulate baroreflex sensitivity and sympathetic tone. Recent studies have shown that upregulation of genes that promote antioxidant enzymes selectively in the RVLM attenuate sympathoexcitation and enhance baroreflex sensitivity [125]. Thus, ROS in RVLM plays an important role enhancing the sympathetic outflow.

4.2 Sources of ROS in CNS

The two major sources of ROS within the CNS are NADPH oxidase and mitochondrial electron transport chain (ETC) [2]. In CNS regulation, mechanisms that involve Ang II signaling are particularly important [14].

4.2.1 NADPH Oxidase

There is now convincing evidence that ROS derived from NADPH oxidase promotes nerve traffic along pathways involved in blood pressure modulation [14, 126, 127]. Inhibition of NADPH oxidase using apocynin improved the baroreflex sensibility in different hypertensive animal models, where this reflex is depressed [114, 128, 129]. Similarly, as in VSMC, NADPH oxidase has a key role in Ang II mediated effects in the CNS.

Inhibition of NADPH oxidase by apocynin blocked Ca^{+2} current induced by Ang II infusion, and abolished the effects in blood pressure of Ang II infusion in the paraventricular nucleus (PVN) in the hypothalamus [130].

Therefore NADPH oxidase contributes to the actions of Ang II on excitability of autonomic central nervous system and is an important mechanism underlying Ang II signaling [4,130].

4.2.2 Mitochondria

As neurons have high bioenergetic requirements, they are often associated with enhanced production of ROS by the mitochondrial ETC [4,131] Mitochondrial ETC dysfunction is accompanied by an increase of ROS generation in the RVLM of hypertensive animal models [2]. The interaction between NADPH oxidase and Ang II is also important in mitochondrial ROS derived regulation. By the activation of AT1R, Ang II induces NADPH oxidase to generate ROS which in turn promotes redox-sensitive suppression of the activity and expression of ETC, as well as downregulation of uncoupling protein, thus leading to mitochondrial ROS generation [116]. Interaction of mitochondrial activity with NADPH oxidase and uncontrolled ROS generation play an important role in the development of neurogenic hypertension under pathological conditions [116].

4.3 Angiotensin II and redox signaling in the CNS

Ang II is a key circulating factor that signals central effector systems, which are determinant in cardiovascular balance [14]. Although many brain regions are sensitive to Ang II, its conformation makes it incapable of crossing the blood-brain barrier [14]. It is believed that Ang II uses circumventricular organs (CVO) that are blood-brain barrier deficient, as an entry door to stimulate local production of Ang II in other areas that are protected by the blood-brain barrier [114]. Subfornical organ (SFO) is the most important CVO, as its neurons, rich in AT1 receptors [126,132] are activated by Ang II in the systemic circulation as well as ventricular system of the brain thus acting as an interphase between peripheral blood and CNS Ang II signaling [14,133–135]. SFO has long been recognized as an important cardiovascular control region modulating fluid homeostasis, and autonomic tone. Through stimulation of the Ang II type 1 receptor subtype (AT1), Ang II enhances sympathetic nerve activity, vasopressin release, and drinking behavior [136]. ROS-related effects in the SFO include activation of PVN causing plasma vasopressin increase (and thus water intake),

upregulation of endothelin-1 in cerebral resistance arterioles and mediating effects of AT1 receptor activation by Ang II [11,137].

It is well known that the activation of pathways that involve ROS generation is one of the mechanisms by which Ang II exerts its role [114, 138]. Suppression of ROS production in the SFO by overexpression of SOD prevented the development of hypertension in Ang II-infused mice [137,139,140]. SFO projects to hypothalamic regions such as the paraventricular nucleus (PVN) and brain stem sites such as RVLM, forming the SFO-PVN-RVLM pathway. In this system, Ang II has a key role as a neurotransmitter and there is now convincing evidence that ROS derived from NADPH oxidase promotes nerve traffic along the SFO-PVN-RVLM pathway [126,138,140].

Chronic peripheral Ang II infusion in mice produces hypertension, which is accompanied by superoxide accumulation in the RVLM and an increase of the sympathetic outflow [141]. Furthermore, selective ablation of AT1R in RVLM prevents hypertension development and superoxide accumulation in the RVLM of Ang II-infused mice [141]. In the same animal models, microinjection of losartan in the RVLM reduces ROS accumulation and improves baroreflex activity [142].

Ang II injections in the RVLM of hypertensive rats activated stress-activated protein kinase/Jun N terminal kinase, ERK 1–2, MAPK. This effect was blocked with the inhibition of NADPH oxidase by apocynin and AT1R antagonist [138]. Other studies also show that in the SFO as well as in the RVLM, NADPH oxidase is required for the full vasopressor effect of Ang II [143].

All studies mentioned above support the idea that Ang II-derived ROS play a role in processing Ang II signal. And for its part, ROS in RVLM impairs the baroreflex and chemoreflex sensitivity [2, 114, 142]. In these pathways, similarly than in VSMC, NADPH oxidase has a key role as an intermediate.

4.4 Effect of NO in CNS

NO is well characterized for its physiological and pathological roles in cardiovascular modulation; however, besides its classical peripheral vasodilatory actions, NO is now recognized as a determinant neuromodulator in autonomic regions in the CNS involved in cardiovascular regulation [2]. Recent studies have shown an important role of Neuronal NOS derived NO on basal hemodynamic and blood pressure in healthy humans [144].

Some studies have shown that NO increases the sensitivity of the baroreflex in the nuclei of tract solitarii which inhibits the sympathetic

response thus producing systemic vasodilation, reduction of the heart rate and decreasing of the inotropic system [2]. However NO neural regulation is highly complex and variable, and not yet completely understood. Evidence shows that different NOS isoform are not equally distributed along the CNS and that NO effect depends on the isoform that produces it causing either baroreflex sensitivity modulation in the NTS and sympathoexcitation/sympathoinhibition in the RVLM, with an important role in the sympathetic basal tone. This is also variable under physiological conditions and during hypertension [145]. NOS interacts with Ang II to regulate ROS production in the RVLM and NTS. NO is capable of reducing the local generation of Ang II and ROS derived production that underlies sympathoexcitation and blood pressure increase [146]. Few studies report the role of NOS uncoupling in the generation of brain stem ROS, its implication in neurogenic hypertension still need to be further studied [2].

5. Role of ROS in renal regulation of blood pressure

By modulation of fluid homeostasis, the kidney is a major contributor to long-term blood pressure regulation and hypertension maintenance. Thus, it is very important to understand the renal mechanisms whereby ROS exert its influence. The latter have a role in the control of renal perfusion and sodium regulation, modulating blood pressure in renal medulla, epithelial integrity and backflux of reabsorbed fluids and electrolytes into the tubular lumen [72, 127].

5.1 Role of ROS in tubuloglomerular function; key role of oxidative stress in the macula densa

Tubuloglomerular feedback (TGF) response, the main component of renal autoregulation, plays an important role in preserving the balance between glomerular filtration rate (GFR) and tubular reabsorption rate [147, 148]. TGF helps in the maintenance of sodium and water homeostasis by regulating GFR and tubular flow in a negative feedback according to changes in NaCl delivery to the macula densa. Therefore, in response to increased tubular flow, more NaCl concentration reaches macula densa, inducing the release of adenosine which causes afferent arteriolar vasoconstriction, decreasing GFR [149]. In the macula densa, oxidative stress is mainly determined by the interaction between NO and $O_2^{\bullet -}$. This balance is essential to maintain the TGF responsiveness in physiological conditions as well as in hypertensive animal models [149–152]. While ROS enhances TGF, NO blunts it [72]. ROS induces vasoconstriction via

juxtaglomerular apparatus (JGA) and VSMC [153]. Superoxide enhances Na/K/2Cl cotransporter via protein kinase C (PKC) activation in the medullary thick ascending limb (mTAL) [127], enhancing the vasoconstriction induced by TGF. Medullary ROS also promotes tubular Na^+ reabsorption and decreases natriuresis [154]. On the other hand, NO generated by eNOS in macula densa, inhibits Na/K/2Cl cotransporter in the mTAL and directly inhibits the NHE3 in proximal tubules [127]. Thus, NO synthesized in macula densa is able to suppress TGF, preventing an increase in blood pressure, and decreasing tubular Na^+ reabsorption, which promote natriuresis [155–157].

In normotensive mice, there is an increased NO generation by the macula densa compared to $O_2\bullet-$, thus, NO dominates in the control of TGF response. On the other hand, in Ang II-induced hypertension, $O_2\bullet-$ production by the macula densa is greater than NO, thus TGF response is mainly controlled by superoxide [149]. Thus, sodium reabsorption is increased and natriuresis is decreased.

5.2 NADPH oxidase in renal function

NADPH oxidase is the predominant source of $O_2\bullet-$ in renal tissue, and is distributed widely in the renal vessels, glomeruli, as well as in nephron segments [158–160]. Activation of NADPH oxidase in the medulla promotes vasa recta vasoconstriction, thereby inducing sodium movement to circulation and reducing natriuresis and thus increasing blood pressure [127]. In hypertensive animal models induced by Ang II, both expression and activity of NADPH oxidase (Nox 2 and Nox4) in the macula densa were enhanced [152].

Studies have proposed a cross-talk between central nervous system and peripheral oxidative stress that include inflammatory targets as T lymphocytes activation, among others that stimulate vascular and kidney generation of NADPH oxidases, promoting hypertensive related vascular changes induced by oxidative stress [161].

5.3 Sympathetic nervous system and renal function

Stimulation of the renal sympathetic nerves produces afferent arteriolar vasoconstriction, renin release and increases Na^+ reabsorption [127]. Activation of α1-adrenergic receptors promote ROS generation and constriction of afferent arterioles, reducing renal blood flow. On the other hand, the activation of β1-adrenergic receptors inhibits ROS generation and produces vasodilatation [127]. In animal hypertensive models, renal

denervation blunts the elevation of blood pressure; however, the role of renal denervation to control hypertension in humans still remains controversial [127].

5.4 Superoxide dismutases

Antioxidant enzymes are highly expressed in the kidney, modulating ROS levels and preventing hypertensive tissue injury [162]. The appropriate function of the antioxidant system to reduce or remove ROS is key to limit tissue injury. The principal scavenging system for removal of O_2•− is the SOD family that catalyzes the conversion of O_2•− to H2O2. Hydrogen peroxide has been described as an endothelium-derived hyperpolarizing factor inducing vasorelaxation, also upregulating eNOS [163] and limiting myogenic responses [162, 164]. Prolonged SOD inhibition is related to increased medullary ROS, reduced medullary blood flow and natriuresis leading to hypertension development [162, 165].

5.5 Renin-angiotensin-aldosterone system (RAAS)

Renin release is regulated by systemic as well as intrarenal signals and there is compelling evidence that oxidative stress regulates renin release and expression [162]. In addition, Ang II production inhibits renin secretion in a negative feedback loop involving oxidative stress [162, 166]. Ang II also regulates aldosterone release from the zona glomerulosa by ROS production [162, 167].

Podocytes as well as mesangial cells generate Ang II in response to overproduction of ROS. In turn, Ang II produces ROS [167], stimulating ROS generation in podocytes, and activating mitochondrial ROS generation, thus inducing podocyte autophagy and apoptosis [169,170]. In addition, Ang II enhances NADPH oxidase-derived O_2•− generation in mesangial cells and increases production of extracellular matrix proteins leading to glomerulosclerosis [171].

Summarizing increased renal ROS have been implicated in renal vasoconstriction, renin release, activation of renal afferent nerves, increased contraction, and augmented myogenic responses of afferent arterioles, enhanced tubuloglomerular feedback, dysfunction of glomerular cells and proteinuria [162].

6. Conclusions and future perspectives

ROS participate directly or indirectly at all levels of blood pressure regulation, affecting key mechanisms and promoting cell signaling events leading to vasoconstriction, vascular remodeling, sodium retention, volume overload, and sympathetic outflow. These regulatory systems do not act separately, but they interact and potentiate each other in either physiological or pathological conditions, where ROS and Ang II seem to be important common factors along this complex cross-talking.

Ang II represents the major vasoactive peptide derived by RAAS activation, as it plays a determinant role in blood pressure modulation at the level of different cell types. Many studies have shown the importance of this peptide in hypertension, where NADPH oxidase activation is essential for Ang II to exert its effect. Thus, Ang II function, as well as the effect of many other factors that activate NADPH oxidase is in part mediated by ROS. NADPH oxidase isoforms play different roles according to the location in the organism as in the cell, and recent investigation aim to elucidate these differential functions in order to further understand its participation in hypertension.

Low ROS levels play physiological roles in blood pressure regulation, as they act in redox signaling of many molecules involved in this process. In contrast, oxidative stress leads to pathological vascular changes, including inflammation and hypertension. Moreover, increased ROS production is involved in perpetuating vascular changes in a positive feedback loop.

It is of interest the proposed a cross-talk between central nervous system and peripheral oxidative stress that include inflammatory target as T lymphocytes activation to stimulate vascular and kidney generation of NADPH oxidases, thus promoting hypertensive related vascular changes in which oxidative stress plays a central role.

Considering the key role of NADPH oxidase in the development of hypertension, it should be further studied the effects in humans of compounds that inhibit specific Nox, searching for a more effective way to modulate ROS production. In VSMC, when ROS levels are increased, the oxidation of calcium channels in the cell membrane or intracellular compartment such as ER leads to a dysregulation of calcium influx, increasing the intracellular free Ca^{2+}. In turn, increased cytosolic Ca^{2+} levels are related to ROS production, higher nitrotyrosine levels, and hyperphosphorylation of procontratile signaling molecules such as myosin light chain kinase, and ultimately vasoconstriction.

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CHAPTER 3

THE ROLE OF OXIDATIVE STRESS IN ESSENTIAL HYPERTENSION

CRISTÓBAL ÁVILA[1], RAMÓN RODRIGO*[2]

[1] School of Medicine, Faculty of Medicine, University of Chile, Santiago, Chile
[2] Molecular and Clinical Pharmacology Program, Institute of Biomedical Sciences, Faculty of Medicine, University of Chile, Santiago, Chile
***Corresponding author:**
Dr. Ramón Rodrigo
Full Professor
Molecular and Clinical Pharmacology Program
Institute of Biomedical Sciences
Faculty of Medicine, University of Chile
Avda. Independencia 1027, CP 8380453
Email: rrodrigo@med.uchile.cl

Abstract

Hypertension is a disease of tremendous global impact. Most hypertension cases worldwide are categorized as essential hypertension, a multifactorial morbid entity. Oxidative stress constitutes a unifying mechanism of injury in many types of disease processes, involving both reactive oxygen species generation and the antioxidant defense systems, key factors in the intracellular redox balance of every tissue, particularly of those involved in the pathophysiology of essential hypertension. The reactive oxygen species family comprises many molecules that have divergent effects on cellular function. Subsequently, oxidative stress may explain the alterations evidenced throughout the kidney, the vascular wall, and the sympathetic nervous system, as there is considerable evidence supporting the involvement of oxidative stress in the pathophysiology of essential hypertension. This opens a new, integrated pathway in the clinical management of essential hypertension, supporting the use of antioxidants,

together with antihypertensive drugs, as a novel strategy to counteract the oxidative stress that determines the development of the disease.

Abbreviations

ACE	Angiotensin I converting enzyme
ACE2	Angiotensin I converting enzyme 2
Ang II	Angiotensin II
Ang (1–7)	Angiotensin (1–7)
Ang (1–9)	Angiotensin (1–9)
AT1	Type 1 angiotensin II receptors
AT2	Type 2 angiotensin II receptors
ATP	Adenosine triphosphate
BBB	Blood-brain barrier
BH4	Tetrahydrobiopterin
CNS	Central nervous system
CVO	Circumventricular organs
eNOS	Endothelial nitric oxide synthase
GFR	Glomerular filtration rate
LDL	Low-density lipoproteins
NADPH	Reduced nicotinamide adenine dinucleotide phosphate
NFκB	Nuclear factor kappaB
nNOS	Neuronal nitric oxide synthase
NO	Nitric oxide
NOX	NADPH oxidase
$O_2^{\bullet-}$	Superoxide radical anion
PVN	Paraventricular nucleus
RAAS	Renin-angiotensin-aldosterone system
ROS	Reactive oxygen species
RVLM	Rostral ventrolateral medulla
SFO	Subfornical organ
SNS	Sympathetic nervous system
TGF	Tubuloglomerular feedback
VSMC	Vascular smooth muscle cells
XO	Xanthine oxidase

1. Essential hypertension: a multifactorial disease

Hypertension is a disease of tremendous global prevalence: more than a quarter of the world's adult population—totaling nearly one billion people—had hypertension in 2000. This proportion is expected to increase by nearly 60 percent—to 1.56 billion—by 2025 [1]. Even more so, it is considered to be the most important risk factor for premature cardiovascular disease [2].

Essential hypertension is defined as elevated blood pressure in which secondary causes (e.g., renovascular disease, primary hyperaldosteronism, and obstructive sleep apnea) have been ruled out [3]. This clinical entity accounts for approximately 90% of total hypertension cases [4]. The present chapter focuses on the role of reactive oxygen species and oxidative stress in the pathophysiology of essential hypertension. Secondary hypertension will be thoroughly discussed in Chapter 4.

Essential hypertension is a complex, multifactorial disease, and the understanding of its pathophysiology is continuously evolving and yet incomplete. Nevertheless, reactive oxygen species (ROS), as discussed in the previous chapters, seem to play a major, ubiquitous role as mediators in the pathophysiological processes underlying essential hypertension.

Given that the foundations of oxidative stress theory in essential hypertension points at an excessive production of ROS, together with an impaired ability of the antioxidant systems to neutralize these, it is relevant to describe the sources of their production. ROS are substances generated within the cell from both enzymatic and nonenzymatic sources, capable of reacting with biomolecules in the cell and also activating signaling pathways. Most relevant enzymatic sources in the vascular wall include: NADPH oxidase (NOX), uncoupled endothelial NO synthase (eNOS), and xanthine oxidase (XO), being the mitochondria and the main nonenzymatic source. The following is a brief description of each one.

1.1 NADPH oxidase

NOX is the main source of ROS in the vascular wall and the kidney [5]. It exerts its function by catalyzing the reduction of molecular oxygen using NADPH as an electron donor, thus generating the superoxide ion (O_2•−). NOX is upregulated by mechanical and molecular stimuli, including shear stress, angiotensin II, endothelin 1, and urotensin II. Its activation in the vasculature has been strongly associated with hypertension [6], through a pathway involving the reaction of NOX-derived O_2•− and nitric oxide (NO), which leads to the formation of peroxynitrite and uncoupling of eNOS,

subsequently upregulating the production of growth factors, proteases, and cellular adhesion molecules by different vascular cell types. This leads to structural changes in vessel walls in a process known as vascular remodeling, a long-term modification involved in the development of essential hypertension.

1.2 Uncoupled eNOS

Under physiological conditions, eNOS functions by synthesizing NO, a mediator responsible for vasodilation, a role which it performs through the activation of signaling pathways involving both endothelial cells and vascular smooth muscle cells (VSMC). Nevertheless, L-arginine and tetrahydrobiopterin, the two main cofactors required for nitric oxide synthesis, are susceptible to deficiency or oxidation (e.g., under pathological oxidative stress levels in the cell), either of which could produce uncoupling of the enzyme and result in decreased nitric oxide production and increased eNOS-mediated generation of O_2•−, initiating a vicious circle of oxidative stress [7]. Moreover, intracellular NO concentrations are also decreased due to its reaction with O_2•−, forming peroxynitrite and perpetuating this vicious circle. The former phenomenon is discussed in Section 3 of this chapter, “Oxidative stress and the dysfunctional endothelium.”

1.3 Xanthine oxidase

This enzyme is involved in the last two steps of purine metabolism, catalyzing the reduction of molecular oxygen to O_2•− and/or hydrogen peroxide during the consecutive oxidation reactions of hypoxanthine to xanthine and finally, to uric acid [8]. Murine models of hypertension have shown increased activity levels of this enzyme in the vascular wall [9]. This phenomenon is discussed *in extenso* in Chapter 1 of this book, “Endothelium and Vascular Homeostasis.”

2. Mitochondria

Complex I of the electron transport chain is the main source of mitochondrial ROS. Although under physiological conditions minor concentrations of ROS are produced, Ang II has been shown to be an important inductor of mitochondrial ROS production in hypertension, thus potentially contributing to the pro-oxidative environment found in the vascular wall of hypertensive subjects [10].

Table 3-1: Main sources of reactive oxygen species in the vascular wall

ROS source	Catalyzed reaction	ROS (and other products)	Stimuli
NADPH oxidase (NOX)	NADPH-dependent reduction of molecular oxygen	Superoxide	Angiotensin II, endothelin 1, urotensin II, shear stress
Uncoupled endothelial NO synthase (uncoupled eNOS)	In conditions with decreased or oxidized L-arginine and dihydrobiopterin, uncoupled eNOS does not produce NO, but catalyzes the reduction of molecular oxygen to superoxide instead	Superoxide. Under physiological conditions, NO	L-arginine deficiency or tetrahydrobiopterin oxidation, in the context of oxidative stress
Mitochondria	Electron transport chain. Complex I is the main source of ROS	Superoxide, hydrogen peroxide, hydroxyl anions	A small fraction of oxygen is reduced to superoxide in physiological conditions. Nevertheless, angiotensin II has been shown to induce the production of mitochondrial ROS
Xanthine oxidase (XO)	Oxidation of hypoxanthine and xanthine	Superoxide, hydrogen peroxide, xanthine, uric acid	Controversial. It is believed that endothelial cells can express XO, regulated by redox-sensitive ways, dependent on endothelial NOX [11]

Consistent with previous findings and the theoretical bases of oxidative stress theory in essential hypertension, a strong correlation between blood pressure and oxidative stress-related parameters (such as antioxidant enzymes activity, total plasma antioxidant capacity, and lipid peroxidation biomarkers) has been documented [12–14]. This correlation has been evidenced both in normotensive and hypertensive patients. Regarding the above, the involvement of ROS in the regulation of blood pressure in normotensive subjects is consistent with the idea that ROS are not exclusive to pathological states, but are also involved in a plethora of physiological functions, such as innate immunity, cellular signaling pathways, oxygen homeostasis regulation, and mitogenic response [15]. Moreover, it has been documented that hypertensive subjects present significantly lower antioxidant enzyme activity, lower total plasma antioxidant capacity, and higher lipid peroxidation biomarkers levels, when compared with normotensives subjects. Even more so, no significant differences in plasma renin, endothelin-1, and homocysteine levels between normotensive and hypertensive subjects were found. These findings suggest that the key disturbance in essential hypertension does not reside in increased systemic levels of blood pressure-related neuroendocrine factors; this is despite that the aforementioned correlation between hypertension and oxidative stress could be explained inasmuch as the latter induces cell signaling pathways leading to increased vasoconstriction first, and remodeling of the vascular wall later. The former is consistent with the available data, as ROS have a proven involvement in redox-sensitive regulation of multiple signaling molecules and second messengers [16]. For example, inducing vasoconstriction through the activation of signaling pathways increases intracellular calcium concentration in vascular smooth muscle cells, thereby contributing to the establishment of increased vascular tone [17].

Systemic and extracellular mechanisms that ultimately associate with the development of hypertension have gained increasing attention as pathophysiological factors. Conversely, the intracellular phenomena on which these mechanisms rely have not been given equal relevance, both from a pathophysiological and clinical perspective [18, 19]. Most pharmacological therapies used in the treatment of hypertension target the extracellular mediators of the renin-angiotensin-aldosterone system (such as angiotensin II receptor blockers and angiotensin-converting enzyme inhibitors) [20], sympathetic system (β-blockers) [21–23], and renal-dependent extracellular volume regulation (diuretics) [24–26].

This chapter will focus on the importance of ROS and oxidative stress as the underlying intracellular elements behind the main pathophysiological

factors involved in the development of essential hypertension, namely renal, vascular, and neural alterations.

3. Intrarenal RAAS and the role of macula densa

For decades, the kidney, in its role as the long-term regulator of extracellular fluid volume (and indirectly, of cardiac output and blood pressure), has been studied in the context of hypertension, as an abnormal renal function is a key component in the pathophysiology of this disease. Accordingly, an interdependent relationship has been established between kidney disease and hypertension, as hypertension can be a cause as well as a consequence of kidney disease [27]. ROS are known to be mediators in a variety of mechanisms involved in kidney diseases: glomerular damage in lipoprotein glomerulopathy and other inflammatory glomerular alterations [28], NOX-activation dependent lipid raft clustering in podocyte damage by homocysteine [29], and LDL-induced tubulointerstitial injury [30]. Nevertheless, studies suggest that the key role played by ROS in renal alterations seen in essential hypertension would be in the macula densa. The latter is a group of specialized epithelial cells located in the distal nephron at the end of the thick ascending limb of Henle's loop, which serve as luminal sensors of sodium chloride (NaCl) concentration. When tubular fluid NaCl concentrations increase, ATP release is promoted in these cells, ultimately being broken down to adenosine in the extracellular space, causing afferent arteriole constriction and subsequently diminishing the glomerular filtration rate (GFR) of the nephron [31]. This mechanism is known as tubuloglomerular feedback (TGF), a main factor in the renal regulation of sodium balance, extracellular fluid volume, and blood pressure.

Interestingly, evidence suggests that ROS are involved in the intracellular mechanism of TGF, since the interactions between NO and O_2•− in the macula densa regulate responsiveness of TGF. Studies conducted in murine models indicate that these interactions vary between normotensive and hypertensive animals. Under physiological conditions, NO is produced in greater amounts than O_2•− in the macula densa. In contrast, both NO and O_2•− are increased, with a predominant increase of O_2•− in Ang II-induced hypertension [32]. Specifically, evidence supports the importance of neuronal nitric oxide synthase (nNOS) as a key factor in the function of the macula densa, regulating the tubuloglomerular feedback response, the natriuretic response to acute volume expansion, and the development of salt-sensitive hypertension [33, 34].

The aforementioned mechanism has great relevance, given that the macula densa modulates TGF and local hemodynamic changes in the kidney that relate to modified intraglomerular pressure through the regulation of the release of renin by the juxtaglomerular cells in the afferent arteriole, an enzyme that serves as the starting point of the renin-angiotensin-aldosterone system (RAAS) [35]. RAAS has been traditionally acknowledged as a cornerstone mechanism in blood pressure regulation, especially in the long term, as it triggers a series of effects on the kidney as well as on a systemic level [36]. For many years, RAAS was considered to be exclusively mediated by the angiotensin-converting enzyme (ACE) / angiotensin II (Ang II) / AT_1 receptor axis, also known as the canonical pathway. Nevertheless, this paradigm has evolved and as an alternate, a counterregulatory pathway has been described: the ACE2 / Ang (1–7) / Mas receptor axis. ACE2 partially resembles the structure of ACE, having a 42% identical structure, with both genes sharing a common ancestor. While ACE is ubiquitously expressed in the endothelium, ACE2 is specifically expressed in the endothelium of cardiac and renal vessels, smooth muscle cells of coronary vessels, and the tubular epithelium of the nephron [37]. This enzyme is responsible for the cleavage of C-terminal residues of both Ang I and Ang II, producing Ang (1–9) and Ang (1–7) respectively. Ang (1–9) is capable of inducing antihypertrophic effects on cardiomyocytes mediated by the binding of the AT_2 receptor [38]. Ang (1–7), on the other hand, binds the Mas receptor (a G-protein coupled receptor), opposing the effects of Ang II in various tissues through the inhibition of cell proliferation, migration, and inflammation, all key components in the development of pathological remodeling in the cardiovascular system [39]. Additional evidence suggests that these effects of Ang (1–7) could be partially mediated by AT_2 receptor binding [40].

Furthermore, another remarkable change to the classical RAAS paradigm is the acknowledgment of multiple tissue-specific RAAS systems, particularly the existence of an intrarenal RAAS, a local autocrine/paracrine system in the kidney as opposed to systemic RAAS, the circulation-borne endocrine system. The difference between these two concepts is crucial to the comprehension of the role of kidney alterations in the pathogenesis of essential hypertension: in essential hypertension, intrarenal RAAS (the most important independently functioning tissue RAAS) is upregulated [41], despite the fact that hypertensive patients tend to have similar levels of plasma angiotensin (related to systemic RAAS activation) to normotensives [42]. Moreover, inappropriate activation of the intrarenal RAAS prevents the kidney from maintaining normal Na^+ balance at normal renal perfusion pressures, and is an important cause of hypertension [43–45]. This is

consistent with the fact that distal renin expression is increased in several forms of experimental hypertension, even in conditions where there is substantial plasma renin suppression [46].

Therefore, oxidative stress in the macula densa cells, characterized as an increased concentration of O_2•− prevailing over NO levels, could be related to an enhancement of intrarenal RAAS activation. This determines a marked impairment of sodium excretion and suppression of the pressure-natriuresis relationship as well as reduced renal blood flow and glomerular filtration rate autoregulatory efficiency [47]. These kidney function alterations are involved in the maintenance of high blood pressure in essential hypertension. Subsequently, targeting RAAS-mediated increased ROS production in the macula densa could be a novel pathway in the therapeutic approach to this disease.

4. Oxidative stress and the dysfunctional endothelium

As discussed previously in Chapter 2, a main factor in the regulation of blood pressure is vascular resistance, which is greatly determined by the response of vascular smooth muscle cells (VSMC) to both vasodilator and vasoconstrictor factors. In pathological conditions endothelial dysfunction can be found, which is an impairment to the normal vascular function characterized by reduced vasodilation, a proinflammatory state, and a prothrombotic setting [48]. On a molecular level, endothelial dysfunction is characterized by reduced NO availability, a major vasodilator derived mainly from eNOS, the predominantly present NOS isoform in the endothelium [49]. Known physiological functions of NO include relaxation of vascular smooth muscle, inhibition of platelet aggregation [50], inhibition of endothelial expression of adhesion molecules [51], and prevention of vascular muscle cell proliferation [52]. Consequently, the stated reduction in the availability of NO is crucial to the production of the aforementioned phenomena, which are characteristic of essential hypertension.

This reduction in NO availability can be explained by the susceptibility of eNOS in the context of a state of oxidative stress. Supraphysiological amounts of ROS, mainly NOX-derived O_2•−, exceed the capacity of the antioxidant system to neutralize them, leading to the oxidation of tetrahydrobiopterin (BH_4), a key cofactor in the synthesis of NO. The former causes eNOS uncoupling, shifting its synthetic activity from NO to O_2•−. Although the deficiency of L-arginine (the limiting substrate in NO synthesis) could also produce eNOS uncoupling, in 2003 it was demonstrated that BH_4 is oxidized in hypertensive vessels, leading to eNOS uncoupling, resulting in increased ROS and reduced NO production by the

enzyme. BH_4 supplementation treatment prevented eNOS uncoupling and blunted the increase in blood pressure ciphers observed in a deoxycorticosterone acetate-salt (DOCA-salt) hypertension model [53]. It is known that O_2•− avidly reacts with NO, producing peroxynitrite ($ONOO^-$), a highly unstable and therefore deleterious nitrogen reactive species, thus creating a vicious circle of increasing ROS and decreasing NO availability.

The association of oxidative stress and essential hypertension has been extensively documented through basic and clinical studies, demonstrating that subjects with hypertension produce excessive amounts of ROS [5, 54–57] and show abnormal antioxidant profiles [58]. Moreover, ROS-generating enzyme-knockout mice have lower blood pressure compared to their wild-type counterparts [59]. Cultured vascular smooth muscle cells and isolated arteries from hypertensive rats and humans show enhanced ROS production, amplified redox-dependent signaling, and reduced antioxidant bioactivity [60]. Moreover, classical antihypertensive agents such as β-adrenergic blockers, ACE inhibitors, angiotensin receptor blockers, and calcium channel blockers may be mediated, in part, by decreasing vascular oxidative stress [61, 62]. Furthermore, drugs like statins have a proven antioxidant effect in the endothelium which mediates a great deal of their therapeutic activities [63].

Evidence suggests that NOX is the initial source of ROS leading to tetrahydrobiopterin oxidation [53], implicating the role of Ang II in the uncoupling of eNOS. In addition, Ang II directly increases the production of mitochondrial ROS, as overexpression of mitochondrial thioredoxin 2, or mitochondrial superoxide dismutase attenuates Ang II-induced hypertension, which demonstrates the importance of mitochondrial ROS in Ang II mediated cardiovascular diseases [10].

The effects of Ang II are mediated by signaling pathways whose enablement is dependent on the activation of G-protein coupled receptors AT_1 and AT_2. The AT_2 receptor appears to have antagonic actions to those of AT_1. The latter activates two signaling pathways: a redox-independent Erk1/2 pathway and a redox-dependent involving Rac, c-Src, Akt, or AMPK [64]. The activation of these redox-dependent pathways is mediated by the stimulation of NOX and mitochondrial ROS [65–68]. Moreover, oxidative stress is involved in the activation of NFκB via nuclear translocation [69], inducing the expression of proteins related to inflammation, stress response, fibrosis, and vascular remodeling.

Despite the apparently nonlimiting role of systemic RAAS activation in the pathophysiology of essential hypertension, the current view [70] is that Ang II generation in tissues does occur (in fact, >90% of tissue Ang II is synthesized locally and not taken up from plasma) [71, 72], but depends on

renal renin and largely, if not completely, on hepatic angiotensinogen. Both diffuse into the interstitium, allowing local Ang II generation to take place in that compartment with the help of membrane-bound, ubiquitously present ACE. This Ang II rapidly binds to AT receptors, a process followed by internalization, explaining why tissue Ang II levels are often high and correlate closely with tissue AT receptor density [73]. Aldosterone is solely synthesized in the suprarenal gland. Furthermore, the expression of the AT_1 receptor is a redox-dependent process via mechanisms involving nuclear factor κB (NFκB) [74], thus, in oxidative stress settings with increased ROS levels, AT_1R-mediated pathways may be overstimulated, further contributing to the production of oxidative stress in the vascular wall [10]. The former concepts, together with the enhancement of a systemic-independent, intravascular RAAS could explain a hypertensive vascular phenotype that appears to relate to increased Ang II activity in the endothelium, even in the presence of normotensive-like Ang II plasma levels.

In summary, endothelial dysfunction is a consequence of increased ROS production, decreased NO availability, Ang II dependent pathways, and the expression of several cellular elements. These processes are interrelated and often develop simultaneously, and therefore it has been a challenging task among researchers to isolate their single contributions to the establishment of endothelial dysfunction and vascular remodeling. Consequently, it has been hypothesized that the relationship between endothelial dysfunction and hypertension is not sequential, but rather a cyclical one, in the form of a vicious cycle [75].

Although oxidative stress is regarded as a key element in the development of hypertension, its amelioration may not be experimentally nor clinically effective in the case of already established vascular damage. This may contribute to explain the failure of clinical trials involving antioxidant supplementation in long-term hypertensive patients in contrast to the benefit that the same approach has shown in recently diagnosed hypertensive patients. This concept holds noteworthy relevance regarding the design of therapeutic strategies, as their cost-effectiveness relationship is related to the point of the natural history of the disease in which the intervention is performed.

5. ROS and sympathetic hyperactivation

Along with the renal and vascular alterations in hypertension previously discussed, the sympathetic nervous system (SNS) plays an important role in the pathogenesis of hypertension [76], as the available evidence suggests that central sympathetic outflow is increased in hypertension [77]. SNS activity is not isolated from the previously mentioned elements, as activation of sympathetic pathways in the kidney increases tubular sodium reabsorption, renin release, and renal vascular resistance. This in turn induces a shift of the pressure-natriuresis curve to the right and contributes to chronic elevation of blood pressure [78]. Subjects with essential hypertension show increased sympathetic nerve activity compared with normotensive subjects, both in animal and human studies [79–81].

As Ang II is composed of eight amino acids, it is unable to penetrate the blood-brain barrier (BBB). Nevertheless, and analogically to other pathogenic elements in essential hypertension, oxidative stress and elevated ROS production present themselves as mediators of the action of Ang II in the central nervous system (CNS), through this means it exerts its effects in the CNS and increases central sympathetic outflow. The most accepted hypothesis is that Ang II acts over circumventricular organs (CVO), which lack BBB, acting as a bypass to the CNS. CVOs involved in the regulation of sympathetic outflow include the subfornical organ (SFO), paraventricular nucleus (PVN), and rostral ventrolateral medulla (RVLM). Specific synaptic connections and physiological functions of these organs have been discussed in the previous chapter. It is important to note that in a chronic Ang II infusion murine model of hypertension, AT_1R mRNA expression was reduced in the SFO and increased in the RVLM [82]. Moreover, evidence shows that increased reactive oxygen species, specifically $O_2\bullet-$ in RVLM, contributes to neural mechanisms of hypertension in stroke-prone spontaneously hypertensive rats [83]. Main limitations to the available literature include predominantly animal model-based data with uncertain correlation in human hypertension and the difficulties of intervening pharmacologically the oxidative stress that acts as a foundation of increased central sympathetic outflow in hypertension due to the BBB.

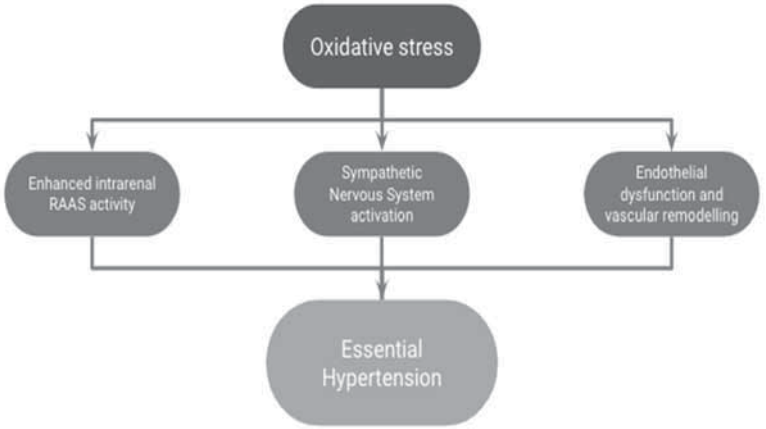

Figure 3-1: A scheme showing the systematization of the pathophysiology of essential hypertension under a paradigm based on the role of oxidative stress.

6. Conclusions

Our understanding of the pathophysiology of essential hypertension is dynamically evolving, as recently described physiological and pathophysiological features of the renin-angiotensin-aldosterone system are expanding beyond the classical paradigm, into the role of novel pathways and local subsystems. However, the available data suggests that oxidative stress is a common phenomenon to all the systems involved in the pathogenic mechanisms of this disease. In conditions of oxidative stress, ROS act as intracellular mediators of the alterations evidenced throughout the renal, vascular, and nervous systems, affecting cellular functions directly through molecular damage and indirectly through redox-dependent signaling pathways. Even more so, local renin-angiotensin-aldosterone system alterations in different tissues appear to be closely related to the occurrence of oxidative stress, as evidence shows that ROS are relevant mediators of the cellular response to the effectors of RAAS, especially of angiotensin II.

Consequently, oxidative stress presents itself as a unifying theory to explain the pathophysiology of essential hypertension, and as a potential therapeutic target for the clinical management of the disease. Evidence regarding interventions to counteract the oxidative stress in the context of hypertension is discussed in Chapter 5.

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CHAPTER 4

OXIDATIVE STRESS AND SECONDARY HYPERTENSION

FLAVIA GIUBERGIA[1], RAMÓN RODRIGO*[2]

[1] School of Medicine, Faculty of Medicine, University of Chile, Santiago, Chile
[2] Molecular and Clinical Pharmacology Program, Institute of Biomedical Sciences, Faculty of Medicine, University of Chile, Santiago, Chile
***Corresponding author:**
Dr. Ramón Rodrigo
Full Professor
Molecular and Clinical Pharmacology Program
Institute of Biomedical Sciences
Faculty of Medicine, University of Chile
Avda. Independencia 1027, CP 8380453
Email: rrodrigo@med.uchile.cl
Phone: +56229786126

Abstract

Although most cases of hypertension are essential, secondary hypertension represents around 5%–10% of them, often generating higher morbimortality in patients. There are various etiologies for secondary hypertension such as renal, renovascular, and endocrine causes, and they all have oxidative stress as a key physiological mechanism in the pathogenesis or maintenance of the disease. The interaction between the renin-angiotensin-aldosterone system, sympathetic nervous system overflow, endothelial dysfunction, and inflammation affects kidney function, endothelial function, and vasomotor areas on the central nervous system. Since the range of action of reactive oxygen species is wide, these species would be a very convenient target to treat in clinical management. The objective of this chapter is to review the role of oxidative stress, and its presence in different forms of secondary hypertension, recognizing redox

imbalance as the common, integrative pathway among the different etiologies.

Keywords: Secondary hypertension, oxidative stress, chronic kidney disease, renovascular hypertension, obstructive sleep apnea, primary aldosteronism.

Abbreviations

ADMA	Asymmetric dimethylarginine
Ang I	Angiotensin I
Ang II	Angiotensin II
AT1-AA	Autoantibodies to type 1 angiotensin II receptor
ATR1	Angiotensin II type 1 receptor
BP	Blood pressure
CB	Carotid body
CIH	Chronic intermittent hypoxia
CKD	Chronic kidney disease
CNS	Central nervous system
CPAP	Continuous positive airway pressure
CTGF	Connective tissue growth factor
DAG	Diacylglycerol
DN	Diabetic nephropathy
DNA	Deoxyribonucleic acid
ECF	Extracellular fluid
eGFR	Estimated glomerular filtration rate
eNOS	Endothelial nitric oxide synthase
GFR	Glomerular filtration rate
GSH	Glutathione
iNOS	Inducible nitric oxide synthase
MDA	Malondialdehyde
MMP-2	Matrix metalloproteinase-2
MR	Mineralocorticoid receptor
mt ROS	Mitochondrial reactive oxygen species
NADPH	Reduced nicotinamide adenine dinucleotide phosphate
NF-κB	Nuclear factor kappaB
NO	Nitric oxide
Nrf2	Nuclear factor erythroid 2–related factor 2
NS	Nitrosative stress
NTS	Nucleus of the solitary tract
OS	Oxidative stress

OSA	Obstructive sleep apnea syndrome
PA	Primary aldosteronism
PlGF	Placental growth factor
PLC	Phospholipase C
RAAS	Renin-angiotensin-aldosterone system
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
rSNA	Renal sympathetic nervous system activity
RVH	Renovascular hypertension
sEng	Soluble endoglin
sFlt-1	soluble fms-like tyrosine kinase-1
SHT	Secondary hypertension
SNS	Sympathetic nervous system
SOD	Superoxide dismutase
TGF-β	Transforming growth factor beta
TNFα	Tumor necrosis factor alpha
VEGF	Vascular endothelial growth factor
VSMC	Vascular smooth muscle cells
XO	Xanthine oxidase

1. Introduction

Hypertension has been classically divided in two types: essential and secondary hypertension (SHT), the latter being the concept used when an identifiable etiology can be found [1], which is the case in around 5% to 10% of hypertensive patients [2]. Refractory hypertension is often due to one of the etiologies of SHT, having a more rapid course, involving higher values of blood pressure (BP), and faster organ damage, implying higher morbidity and mortality rates when it is not adequately treated [3].

Despite appropriate therapy or even efficient removal of the cause, a significant number of these patients do not return to normal BP values [4]. Such residual hypertension indicates that either some patients with secondary hypertension also have concomitant essential hypertension, or that irreversible vascular remodeling has already taken place [5]; the latter can also be a consequence of essential hypertension, which is partly mediated by oxidative stress [6].

There are many etiologies for SHT such as endocrinopathy, kidney disease, renovascular hypertension, preeclampsia, and obstructive sleep apnea syndrome, among others, all of them generated by different pathophysiological pathways but converging on one key mechanism: oxidative stress (OS).

As previously discussed in this book, reactive oxygen species (ROS) are generated within the vascular wall, in endothelial and vascular smooth muscle cells as well as in adventitial fibroblasts. In hypertension, major sources of ROS are xanthine oxidase (XO), uncoupled endothelial NO synthase (eNOS), cyclooxygenase, the mitochondrial respiratory chain and probably the most relevant: nicotinamide adenine dinucleotide phosphate (NADPH) oxidase [7]. Angiotensin II (Ang II), one of the effectors of the renin-angiotensin-aldosterone system (RAAS), represents one of the major vasoactive peptides involved in the regulation and activation of NADPH oxidases, together with cytokines and growth factors. Ang II, via type 1 Ang II receptor (AT1R) upregulates the activity of NADPH oxidase at different levels, stimulating its activation and increasing the expression of its subunits, thereby enhancing vascular ROS generation [8]. It is interesting to note that ROS may regulate AT1R gene expression, thus creating a vicious circle in the affected cells [9].

The same ROS overproduction mediated by Ang II leads to increased sympathetic nervous system (SNS) activation [10] generating autonomic dysfunction, which is also an important mechanism accounting for hypertension development [11]. Sympathetic nerve terminals have been shown to activate T cells in lymph nodes and enhance the expression of homing signals in the vasculature and kidneys. This promotes the migration of inflammatory cells to these organs, which release cytokines that further stimulate the NADPH oxidase in the vasculature and kidneys, finally worsening BP values [12]. In the present chapter, some of the most prevalent etiologies of SHT will be reviewed, showing special interest in the role of OS in the underlying pathophysiology.

2. Chronic kidney disease

Chronic kidney disease (CKD) is not an uncommon cause of secondary hypertension. CKD can result from acute and chronic kidney injury, which consists of a deterioration in the glomerular filtration rate (GFR) (less than 60 mL/min/1.73 m2), often accompanied or preceded by albuminuria (albumin-to-creatinine ratio more than 30 mg of albumin per gram of creatinine). There are various kinds of kidney disease: glomerular disease, tubulointerstitial injury, and obstructive uropathy, and each one of them can be caused by various etiologies.

There is a major correlation between hypertension and CKD, since they play a part in the pathophysiology of one another as well as in the development and evolution. Hypertension is present in >80% of patients with CKD and an estimated GFR (eGFR) <60 ml/min [13]. The prevalence

of hypertension among these patients varies according to the etiology of CKD. In an academic outpatient nephrology clinic in Spain, hypertension was more prevalent among patients with diabetic nephropathy (87%) and polycystic kidney disease (74%), compared with chronic pyelonephritis (63%), glomerulonephritis (54%), hereditary congenital disease (19%), or tubulopathies (5%). [14].

Both essential and secondary hypertension can cause kidney damage leading to nephrosclerosis, and in some instances it may be difficult to determine whether hypertension or renal disease was the initial disorder [15]. It has also been seen that patients with poorly controlled hypertension and CKD have a significantly lower average eGFR when compared to patients with an adequate BP control [16]. It is also important to note that systolic and diastolic blood pressure have been found to be independent risk factors for progression to end-stage kidney disease [17].

Kidneys play multiple roles in hypertension pathophysiology, involving a diminished capacity to excrete sodium, excessive renin secretion in relation to effective circulating volume status, and renal afferent sympathetic nervous system overactivity. Although the prevalence of hypertension among CKD patients increases as eGFR declines, it seems that hypertension development may be more closely related to albuminuria than to eGFR. [18].

Albuminuria triggers inflammation and OS causing significant tissue damage by promoting lipid peroxidation, deoxyribonucleic acid (DNA) damage, and protein modification, as well as mitochondrial dysfunction [19, 20]. This process perpetuates injury upon the glomerulus and further increases ROS production and activation of profibrotic growth factors such as transforming growth factor beta (TGF-β) and connective tissue growth factor (CTGF). This leads to the progression of renal fibrosis and sclerosis, favoring the loss of functional nephrons and the diminution of eGFR, worsening CKD [21]. This whole process is facilitated by enhanced production of vasoactive agents in hypertensive cases, such as Ang II, endothelin-1, and urotensin-2, which have also been linked to higher expression of TGF-β in cultured renal cells and experimental animal models of diabetic nephropathy (DN) [22].

DN is clinically characterized by a progressive increase in glomerular filtration pressure, which leads to albuminuria and subsequent decline in the GFR [23]. DN and other etiologies of glomerular injury run with endothelial dysfunction due to high glucose filtration, OS, and advanced glycation end products which affect the nephron, in addition to the increased RAAS activation altering vasoreactivity and coagulation, finally leading to inflammation [23]. It has been seen that the ROS generated by enhanced

NADPH oxidase 4, mediate extracellular signal-regulated protein kinases 1 and 2 activation, which play a crucial role in high glucose-induced glomerular fibroblast proliferation and activation in vitro [24]. Mitochondrial dysfunction has also been found to be a determinant issue for the development of DN, due to respiratory chain complex alterations, dynamic disorders, and excessive fission, all of them leading to enhanced mitochondrial ROS overproduction. Several therapeutic agents targeting the mitochondria have shown important therapeutic effects in DN [25].

Since there is a very large list of etiologies for CKD, including nephrotoxic drugs and pathological states, the unification of mechanisms that explain the cascade of events that lead to nephron impairment, which directly impacts blood pressure, is yet an unresolved issue Anyway, more research on the field of CKD, associating kidney OS with systemic OS and hypertension must be executed, for a future development of targeted and effective therapeutic agents.

3. Renovascular hypertension (RVH)

Renovascular hypertension (RVH) is a condition in which increased BP is caused by the renal hormonal response to a restricted blood flow reaching the glomeruli due to a diminished luminal diameter of the renal arteries [26].

There are two groups of patients at higher risk for this disorder: the most common scenario (90% of cases) being older arteriosclerotic patients who have a plaque obstructing the renal artery frequently at its origin, often presenting segmental and diffuse intrarenal atherosclerosis or ischemic nephropathy, and patients with fibromuscular dysplasia, where younger white women are the most affected population [27]. Other etiologies worth noting are Takayasu's arteritis, renal arterial aneurysms, aberrant renal arteries, aortic dissection, etc. The estimated prevalence of the disease is 5% or less among the hypertensive population [28].

There are three different models for development of RVH: two kidneys and both arteries stenotic (2K2C), two kidneys and one artery stenotic (2K1C), or the presence of only one kidney and its artery stenotic (1K1C).

The endocrine response to the lack of blood flow reaching the kidney is the release of renin, which promotes the conversion of angiotensin I (Ang I) to Ang II, the latter causing vasoconstriction, aldosterone release from the adrenal glands, and sympathetic nervous system activation [29].

RVH usually behaves as a refractory hypertension, and administration of either an angiotensin-converting enzyme inhibitor or an Ang II receptor blocker can lower the glomerular filtration rate, which may cause acute renal failure (characteristic of bilateral disease or stenosis of a solitary

kidney), worsening BP values [30]. Also, compared with similar BP levels in patients with essential hypertension, RVH patients tend to have higher rates of target-organ injury, and decreased renal function [31].

One key mechanism for maintaining higher BP levels in RVH is chronic activation of the afferent and efferent renal nerves, potentiating SNS overflow [32]. It has been seen that chronic stimulation of renal sympathetic nerve activity is able to increase overall renin secretion and sodium reabsorption, and induce renal vasoconstriction [33].

The excessive amount of Ang II circulating in RVH due to RAAS activation leads to NADPH oxidase activation, which increases local ROS production in nerve cells that regulate cardiovascular function by acting directly on the central nervous system (CNS) and finally generating sympathoexcitation [34]. Activation of sympathetic nervous system has been evidenced in animal studies in association with significantly increased levels of malondialdehyde (MDA), a marker of OS, and reduced glutathione (GSH) levels in the plasma and brain of RVH rats [35].

This phenomenon explains why in rat models of RVH (2K1C) between the third and fourth week after the renal artery clamping, the hypertension has a strong neurogenic component [36, 37]. Also, it has been seen that ganglionic blockade or inhibition of the rostral ventrolateral medulla (an important vasomotor center in the CNS) leads to normalization of blood pressure in 2K-1C rats when compared with a control group [38]. In animal studies a significant increase in mRNA and protein expression of the AT1R receptor has been seen within the hypothalamus on RVH models [36], explaining a way by which the sympathetic overactivation is perpetuated.

There are studies, most of them conducted on animals, demonstrating a role for antioxidant therapy in the management of RVH, [39–43], showing positive results in vascular remodeling and dysfunction, even significantly preventing the rise of BP levels after clipping one renal artery on 2K-1C rats [39]. In the future it would be wise to conduct more extensive studies and clinical trials testing antioxidant therapy in human patients with RVH, especially in early stages, before vascular remodeling has taken place, as well as in patients with known atheromatous disease, who are at a greater risk for developing RVH.

4. Obstructive sleep apnea syndrome (OSA)

This syndrome consists of repeated episodes of complete or partial obstruction of the upper airway during sleep due to increased upper airway collapsibility [44], resulting in chronic intermittent hypoxic and hypercapnic events. It is a very widely spread disease, reaching a prevalence

of 24%–26% in men and 17%–28% in women aged between 30 and 70 years in the United States of America [45]. The prevalence of the disease has increased in developed countries in parallel to the increasing prevalence of obesity. It is estimated that 50% to 60% of people who are obese also have OSA [46]. Since obesity is a well-known risk factor for hypertension development [47] it can be a confounding factor when defining OSA as a cause for SHT, but it has been seen that moderate to severe OSA (apnea-hypopnea index >15/h) is an independent risk factor for hypertension development [48]. After that data was clarified, OSA is recognized as the most common cause of secondary hypertension in adults [49].

It has been suggested that OSA and hypertension can potentiate each other, since acute increases in BP cause a decrease in the tone of the upper airway muscles [50], favoring airway collapse. This phenomenon, together with volume overload and migration of fluids to the upper body during sleep—which can lead to pharyngeal edema—, explains how hypertension can also contribute to OSA [51].

OS and proinflammatory molecules are highly involved in the cardiovascular consequences of OSA: recurrent nocturnal hypoxemia with subsequent reoxygenation produces a phenomenon similar to ischemia reperfusion injury, leading to the release of ROS, inflammatory cytokines, and vasoactive substances, which contribute to endothelial damage [52, 53]. The excess of ROS generates OS in the brainstem and hypothalamic nuclei such as the nucleus of the solitary tract, the primary site for processing the afferent inputs from the carotid body (CB), potentiating an enhanced response to hypoxia at that level [54]. Chronic intermittent hypoxia (CIH) also potentiates the CB chemosensory discharge leading to enhanced carotid chemosensory and ventilatory hypoxic responses [55]. These mechanisms lead to sympathetic overflow, contributing to hypertension development.

It has been seen that CIH increases plasma lipid peroxidation, nitrotyrosine levels, and inducible nitric oxide synthase (iNOS) expression in the CB, the latter being a key factor for its exaggerated response to hypoxemia [56].

High plasma levels of tumor necrosis factor alpha (TNFα) and Interleukin-6 have been found in patients with OSA [57, 58]. It has been seen that ROS and reactive nitrogen species (RNS) contribute to the progression of hypertension in rats exposed to CIH [59]. Also, in patients with moderate to severe OSA, higher levels of myeloperoxidase and oxidized low-density lipoprotein have been found [60], reaffirming the pathogenic role of oxidative stress in the development of hypertension in OSA patients.

It has been seen that the treatment with antioxidants normalized the enhanced CB chemosensory discharge and prevented or reversed the

elevated BP in CIH-treated rats [56]. Also, the treatment with melatonin, a key molecule involved in the sleep–wake cycle and a well-known antioxidant, could ameliorate hypertension in rats with CIH [61].

Nowadays there is a lack of research conducted on humans which tests the effects of antioxidant therapy in OSA, or that compares for example the use of continuous positive airway pressure (CPAP), the election treatment, alone versus CPAP and melatonin. This could also help regulate sleep patterns in these patients by having a more integral approach to the disease.

5. Primary aldosteronism (PA)

For many years, PA was thought to be a rare etiology for hypertension, but that premise has now changed: currently, it is the most common endocrine cause of SHT. PA has a prevalence ranging between 5% and 13% of all patients with hypertension diagnosis [62], even though now it is estimated that the real prevalence of—at least—relatively autonomous aldosterone secretion may be near 30% of hypertensive patients, mostly undiagnosed, since most are asymptomatic [63].

PA consists of an excessive aldosterone production despite having suppressed plasma renin [64]. Aldosterone is normally synthesized from cholesterol in the zona glomerulosa of the adrenal glands, and its production rate physiologically depends on stimulation by Ang II, high serum potassium levels, or the adrenocorticotropic hormone [65]. Together with Ang II, aldosterone is the major effector of the RAAS.

There are various types of PA, the most common being bilateral adrenal cortical hyperplasia (almost 60% of cases), and adrenal cortical aldosterone-secreting adenoma (Conn's adenoma, 35% of cases). Rare causes of PA include primary adrenal hyperplasia (2% of cases), adrenal cortical aldosterone-producing carcinoma (<1% of cases), and adenoma or aldosterone-secreting carcinoma located in an ectopic region (<0.1% of cases) [66].

There is a consensus that the risk profile for organ damage and bad outcomes in patients with PA is higher than age-, sex-, and blood pressure-matched essential hypertensive patients [67], and some studies have shown that cardiovascular complications, like ischemic heart disease, cerebrovascular events, and arrhythmias, are more prevalent in patients with PA than in patients with essential hypertension [68, 69].

It is well known that the hemodynamic role of aldosterone is mediated by genomic actions via activation of the mineralocorticoid receptor (MR) inducing sodium retention and hypokalemia that may be symptomatic, leading to polyuria, polydipsia, paresthesia, and/or muscle weakness [63].

The activation of the MR leads to OS: its infusion on animal models has been associated with upregulation of mRNA of different subunits of NADPH oxidase complex, like the catalytic subunit of NADPH oxidase 2 in heart, vasculature, and kidney tissue [70–72]. Aldosterone was also found to be genotoxic and to produce OS at very low concentrations in renal tubule cells [73].

Besides the genomic effects of aldosterone, it can also exert rapid, nongenomic effects, which enhance the production of ROS via NADPH oxidase [74]. One way this is possible is through activation of phospholipase C which happens after the complex aldosterone-MR is formed, inducing from phosphatidylinositol 4,5-bisphosphate the release of two secondary messengers, such as inositol 1,4,5-trisphosphate and diacylglycerol [75], leading to higher availability of intracellular calcium levels, which induces mitochondria to liberate oxidant radicals [76].

More evidence of OS in PA has been found: significant higher levels of urinary excretion of isoprostanes have been described in patients with PA when compared to patients with essential hypertension. Moreover, there have even been studies proving that aldosterone blood levels are accurate to predict OS severity, through serum NADPH oxidase 2 levels, being more specific than the actual BP values [77].

All the mechanisms of damage induced by aldosterone lead to inflammation and fibrosis development on kidneys and vasculature through activation of the immune system and inflammatory mechanisms [78].

In vitro studies have shown that aldosterone activates the nuclear factor erythroid 2–related factor 2 (Nrf2), which is known to be a transcription factor implied in the antioxidant cell response. This factor can be activated by OS as well as by MR activity. It has been observed that this effect on Nrf2, GSH amounts, and target gene levels decrease within 24 hours, even when oxidant levels remained high, so it is probable that an aldosterone-induced Nrf2 adaptive response cannot neutralize oxidative actions of chronically increased aldosterone [79].

In mineralocorticoid excess models, the use of mineralocorticoid receptor antagonists (spironolactone or eplerenone) was associated with significant reduction of the NADPH oxidase activity in rats [80] and with higher bioavailability of nitric oxide in patients, as shown in a small study [81].

It is important that further investigation regarding the role of aldosterone in the generation of OS is directed, especially in humans. It is important also to quantify the contribution of the nongenomic effects of aldosterone in ROS generation and hypertension development, so that the treatment for

states characterized by aldosterone excess can be treated more efficiently in the future using MR blockers in combination with antioxidant therapy.

6. Preeclampsia

Preeclampsia is a severe multisystem syndrome of pregnancy associated with newly onset hypertension, being the leading cause of maternal and fetal morbidity and mortality [82]. Although the cause of this disease remains to be determined, it is associated with the occurrence of oxidative stress and reduction in total antioxidant capacity of plasma [83]. It has been suggested that increased products of oxidative stress might be an underlying mechanism for endothelial dysfunction in preeclampsia [84], thus a correlation between oxidative stress and the initiation and progression of preeclampsia has been proposed [85]. Nevertheless, the development of hypertension in preeclampsia, usually occurring in the third trimester of pregnancy, has a complex multifactorial mechanism that includes several factors indicated below and depicted in Figure. 4-1.

6.1 Increased reactive oxygen species

The contribution of reactive oxygen species is exerted at several levels of blood pressure modulation, mainly by enzymatic mechanisms, as discussed in Chapter 2 of this book. The development of hypertension is due to the impairment of the vasomotor balance between vasoconstriction and vasodilation, both processes being sensitive to redox balance. On one side, the effect of various modulators and vasoconstrictor hormones is mediated by reactive oxygen species. On the other side, vasodilation can result mainly from increased availability of nitric oxide and prostacyclin, among other vasodilator agents [86]. The major vascular source of superoxide anion is the activity of NADPH oxidase [87]. In turn, superoxide anion and other reactive oxygen species act as cell signaling molecules leading to vasoconstriction.

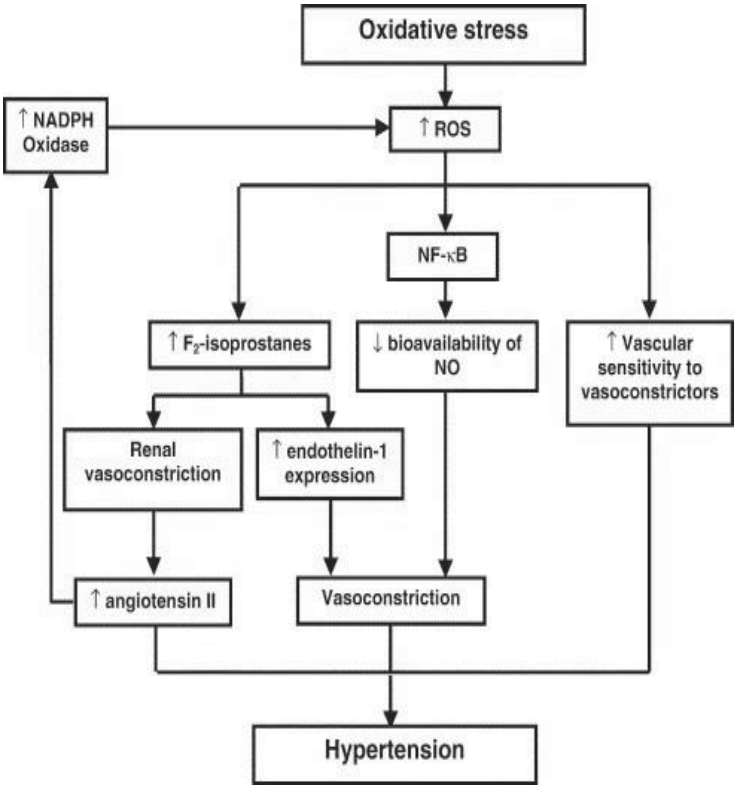

Figure 4-1: Role of oxidative stress in the development of hypertension in preeclampsia. Taken from Rodrigo et al., 2005 [83], with permissions. ROS, reactive oxygen species; NF-κB, nuclear factor kappaB; NO, nitric oxide.

6.2 Decreased nitric oxide bioavailability

The production of nitric oxide via NOSs has a key role in vasodilation of vasculature. Oxidative stress is able to change this response by several mechanisms, such as eNOS uncoupling or downregulation, enhancement of xanthine oxidase, and NADPH oxidase. Particularly relevant is the increased concentration of superoxide, due to its reaction with nitric oxide synthesized by eNOS to form peroxynitrite anion, which destabilizes eNOS to produce further superoxide. In addition, it should be mentioned that superoxide anion contributes to tetrahydrobiopterin oxidation, thus leading to eNOS uncoupling [88, 89].

6.3 Agonistic autoantibodies to type 1 angiotensin II receptor

Once angiotensin II is bound to its type 1 receptor (AT1) in vascular smooth muscle cells, the activation of intracellular cell signaling process leads to protein kinase C pathway activation, thus phosphorylating NADPH p47 subunits leads to the formation of an active complex of this enzyme. Alternatively, this activation could result from the binding of agonistic autoantibodies to the AT1 receptor. These autoantibodies (AT1-AA) were characterized by Wallukat et al. [90]. They do not compete with angiotensin II for the AT1 receptors, but they rather seem to enhance the binding and downstream effects of the hormone. During pregnancy, the production of reactive oxygen species is increased through the effect of AT1-AA, thus causing oxidative-stress-derived hypertension [91]. The role of AT1-AA in the pathophysiology of preeclampsia could be a potential therapeutic target for hypertension. It has been suggested that blocking AT1-AA could serve as a major treatment for the disease and should be further explored [92]. Recently, it was reported that in preeclampsia, AT1-AA signaling stimulates mitochondrial reactive oxygen species production [93].

6.4 Hyperhomocysteinemia

Elevated plasma homocysteine levels have been considered a risk factor for preeclampsia and future risk of cardiovascular diseases [94]. In spite of preeclampsia and hyperhomocysteinemia having been related to the occurrence of oxidative stress, the role of the latter in the pathophysiology of the disease has been poorly studied. With respect to the development of hypertension, hyperhomocysteinemia reduces the nitric oxide bioactivity due to newly formed and stable nitrosothiol [95], among other effects. In fact, elevation of plasma homocysteine levels leads to vascular oxidative stress in preeclamptic women through effects such as decreased activity of antioxidant enzymes, increased proliferation of vascular smooth muscle cells, nitric oxide consumption, and activation of calcium channels [96]. A scheme to account for the involvement of hyperhomocysteinemia in the pathogenesis of hypertension in preeclampsia is depicted in Figure 4-2 [83].

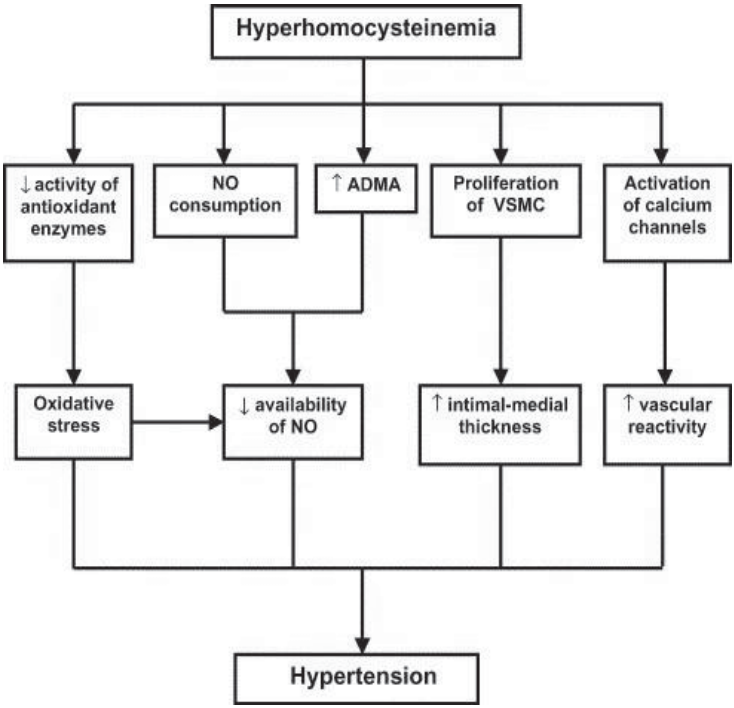

Figure 4-2: Hypothesis to explain the role of hyperhomocysteinemia in the development of oxidative stress-induced hypertension in preeclampsia. Taken from Rodrigo et al., 2005 [83], with permissions. NO, nitric oxide; ADMA, asymmetric dimethylarginine; VSMC, vascular smooth muscle cells.

6.5 Antiangiogenesis-related factors

Some factors modulating angiogenesis are also involved in blood pressure regulation in preeclampsia. Vascular endothelial growth factor (VEGF) is an endothelial mitogen promoting angiogenesis; it is required for the endothelial cell function that induces vasodilation in the vascular wall. It is recognized that VEGF dysfunction exerts a major role in the pathogenesis of pregnancy-induced hypertension [97]. In preeclampsia, some antiangiogenic molecules are released by the placenta to maternal circulation, such as soluble endoglin (sEng) and, particularly, fms-like tyrosine kinase-1 (sFlt-1) in increased amounts before 34 weeks of pregnancy [98]. This occurs together with decreased levels of the

proangiogenic placental growth factor (PlGF). Consequently, preeclamptic women will show a higher sFlt-1/PlGF ratio, a change that becomes significant before gestational week 34 in women destined to develop preeclampsia [99, 100]; however, it has been suggested that the evidence is more limited for the prognosis in women with abnormally high sFlt-1/PlGF ratio [101]. Recently, experimental evidence has revealed that decidual retinoic acid levels may contribute to the pathogenesis of preeclampsia by allowing accumulation at the maternal–fetal interface [102], an interesting finding that needs further study.

Conclusions

OS is present throughout the pathogenesis and maintenance of virtually all etiologies of SHT as seen in Figure 4-3, participating at different levels in the organism. In nephrons of patients with CKD, OS leads to fibrosis and reduction of eGFR, contributing to the latter's fluid accumulation, and increase of plasma renin activating the RAAS. In renovascular hypertension and primary aldosteronism, the elevated aldosterone levels have on their own a detrimental role on heart physiology and worsen plasma OS levels, due to the activation of NADPH oxidases. In RVH there is also an increased Ang II activity, leading to further activation of prooxidant enzymes, producing vasoconstriction and sympathetic nervous system activation, the latter being a key maintenance mechanism for the disease due to its multisystemic effects in vasculature and in kidney tissue. In obstructive sleep apnea there is also a significant neurogenic component, due to OS generated by chronic intermittent hypoxia, generating an effect similar to ischemia reperfusion injury. On the other hand in preeclampsia, blood pressure elevation shows a multifactorial mechanism related to the occurrence of OS and impairment in the profile of the antiangiogenesis-related factors. Overall, we can note the multiple sites where OS has a pathogenic role in the different types of SHT, finally leading to vascular remodeling, thus making prioritary to prevent the damage generated by its chronic existence in order to offer a better prognosis for the affected patients. This can be achieved with systemic antioxidant therapy, targeting several levels in the pathogenesis, and perpetuation of secondary hypertension. Several studies have been conducted trying antioxidant therapy on different SHT models as seen in Table 4-1, but more research is still needed to prove its safety and effectiveness on humans.

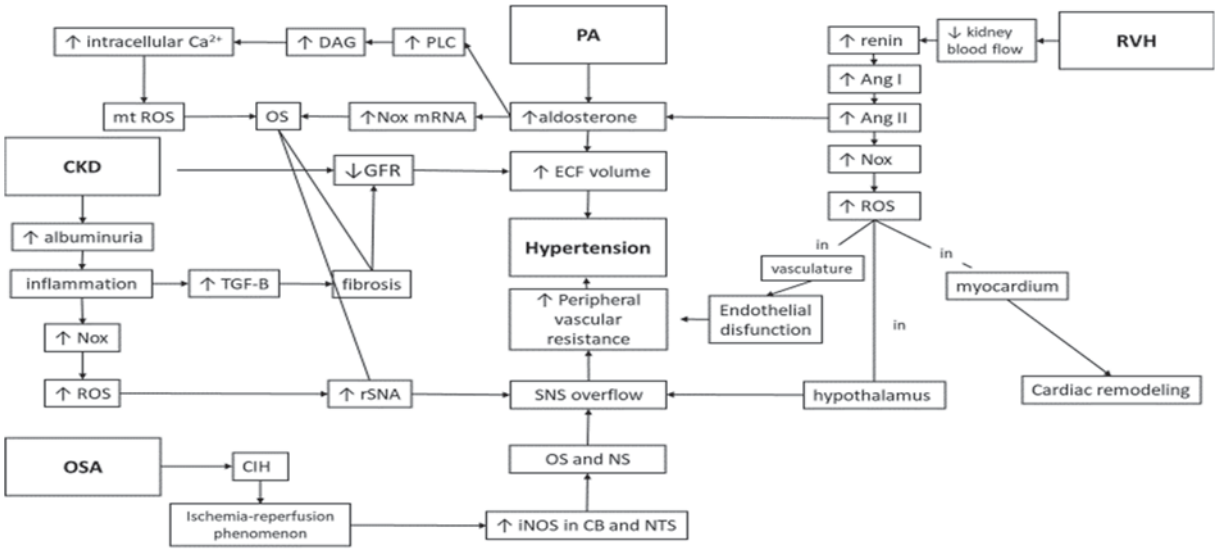

Figure 4-3: Integrated mechanisms for secondary hypertension development and maintenance. PA: primary aldosteronism, RVH: renovascular hypertension, CKD: chronic kidney disease, OSA: obstructive sleep apnea, CIH: chronic intermittent hypoxia, iNOS: inducible nitric oxide synthase, CB: carotid body, NTS: nucleus of the solitary tract, DAG: diacylglycerol, PLC: phospholipase C, ROS: reactive oxygen species, mt ROS: mitochondrial reactive oxygen species NOX: nicotinamide adenine dinucleotide phosphate oxidase, mRNA: messenger ribonucleic acid, OS: oxidative stress, NS: nitrosative stress, SNS: sympathetic nervous system, rSNA: renal sympathetic nervous system activity, TGF-β: transforming growth factor-beta, GFR: glomerular filtration rate, ECF: extracellular fluid, Ang I: angiotensin I, Ang II: angiotensin II.

Table 4-1: Effects of antioxidant therapy on secondary hypertension

Antioxidant	Disease	Model	Results	Reference
Melatonin	CKD 5/6 nephrectomy rat model	Animal model (rats)	Lowered intrarenal angiotensinogen, Ang II and AT1 receptor, lower blood pressure -Lower oxidative stress markers (8-hydroxy-2'-deoxyguanosine) -Higher superoxide dismutase activity -Decreased markers of interstitial fibrosis in the remnant kidneys	[103]
NZ-419 (5-Hydroxy-1-methylimidazoline-2,4-dione)	CKD adenine loaded and 5/6 nephrectomy rat model	Animal model (rats)	-Prevents the progression of chronic kidney disease -Lowers proteinuria	[104]
Curcumin	Adenine induced CKD	Animal model (rats)	-Improved creatinine clearance -Reduced renal morphological damage and histopathological markers of inflammation, fibrosis, and apoptosis -Reduced BP -Reduced albuminuria -Reduced inflammatory cytokines IL-1β, IL-6 and TNF-α, cystatin C, and adiponectin -Increased expression of the antioxidative transcription factor Nrf2	[105]

Allopurinol	CKD	Humans (clinical trial)	-In patients at stage 4 CKD, serum creatinine levels did not decrease significantly and there was no significant increase in GFR -In patients at stage 3 CKD, serum creatinine levels decreased and GFR increased significantly	[106]
Tempol	RVH (2K1C)	Animal model (rats)	-Significantly lower blood pressure -Restoring of endothelial-dependent vascular relaxation -Better endothelium-independent vascular relaxation (using nitroglycerin) -Reduced MDA plasma and mesenteric artery levels in hypertensive groups -Recovered SOD activity on the mesenteric artery -Prevents increases in media thickness of thoracic aorta -Reduces MMP-2 levels in thoracic aortas of 2K1C rats	[39, 42]
Apocynin	RVH (2K1C)	Animal model (rats)	-Significantly lower blood pressure -Restoring of endothelial-dependent vascular relaxation -Better endothelium-independent vascular relaxation (using nitroglycerin) -Reduced MDA plasma and mesenteric artery levels in 2K1C rats	[39, 42]

Vitamin C	RVH (2K1C)	Animal model (rats)	-Lower MAP on 2K1C rats -Higher cardiac baroreflex index (ΔHR/ΔMAP) in control and 2K1C rats -Lowered rSNA activation -Lower AT1 receptor mRNA expression in the clipped kidney -Higher glutathione peroxidase mRNA expression in clipped and nonclipped kidneys	[43]
Allopurinol	OSA	Animal model (rats)	-No difference in systolic blood pressure -Reduced myocardial lipid peroxides -Improves left ventricular contractile function	[107]
Melatonin	OSA	Animal model (rats)	-Significantly lower blood pressure -Lower Nox expression, TNF-α and adhesion molecules levels in thoracic aorta -Increased eNOS and endothelial-dependent relaxation	[61]
Vitamin C	OSA	Humans (clinical trial)	-Increased flow-mediated dilation to a level comparable to that observed in the control group (reduced endothelial dysfunction)	[108]
N-acetylcysteine	OSA	Humans (clinical trial)	-Considerable improvement on slow-wave sleep as sleep time percent and sleep efficiency -Lowering of apnea-hypopnea index, apnea-related arousals, longest apnea episode duration (seconds), oxygen desaturation events per hour and Epworth sleepiness score -Decreased lipid peroxidation -Increase in total reduced glutathione	[109]

N-acetylcysteine	PA	Animal model (previously diabetic rats)	-No difference in blood pressure -Diminished impaired oral glucose tolerance, and decrease in B cell mass - Decreased levels of urinary 8-hydroxy-2-deoxyguanosine (oxidative stress marker)	[110]
Tempol	PA	Cell culture	-Prevents aldosterone-induced DNA damage	[111]
Tempol	PA	Animal model (rats)	-Ameliorated aldosterone-induced podocyte injury and proteinuria -Reduced oxidative stress markers -Attenuated the salt-evoked mineralocorticoid receptor upregulation	[112]

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CHAPTER 5

ANTIOXIDANTS IN HYPERTENSION, LOOKING FORWARD

MARIA-JOSE GOTHE[1], RAÚL CASTILLO[1], RAMÓN RODRIGO[*2]

[1] School of Medicine, Faculty of Medicine, University of Chile, Santiago, Chile
[2] Molecular and Clinical Pharmacology Program, Institute of Biomedical Sciences, Faculty of Medicine, University of Chile, Santiago, Chile
***Corresponding author:**
Dr. Ramón Rodrigo
Full Professor
Molecular and Clinical Pharmacology Program
Institute of Biomedical Sciences
Faculty of Medicine, University of Chile
Avda. Independencia 1027, CP 8380453
Email: rrodrigo@med.uchile.cl

Abstract

The role of reactive oxygen species (ROS) on the pathogenesis of essential hypertension has been extensively documented. ROS are mediators of major physiological vasoconstrictors, increasing intracellular calcium concentrations and reducing the bioavailability of nitric oxide (NO). Recent advances in the understanding of complex redox signaling pathways in the vascular system shed light on the role of oxidative stress in the development of endothelial dysfunction, which has led to growing interest concerning therapeutic possibilities which target ROS in the management of essential hypertension.

This proves to be especially important since essential hypertension constitutes a major risk factor for the development of cardiovascular

disease, the leading cause of morbimortality worldwide; and considering that the current pharmacological approach to treat hypertension is often ineffective, using multiple drug combinations that are not enough to sustain normal blood pressure levels on affected individuals.

In this regard, many approaches have been carried out to treat hypertension including the non-pharmacological dietary approach to treat hypertension (DASH) as well as pharmacological supplementation of antioxidants such as ascorbate, alpha-tocopherol, allopurinol, selenium, N-acetylcysteine, and polyphenols. Despite existing molecular basis and in vitro evidence that supports the use of diverse antioxidants, clinical evidence continues to be controversial. This chapter aims to present novel therapeutic approaches as well as examine the underlying mechanisms, effectiveness, and confounding factors affecting currently available therapy.

Abbreviations

ACEI	Angiotensin-converting enzyme inhibitor
ANG II	Angiotensin II
ARB	Angiotensin receptor blockers
AT1	Angiotensin II receptor type 1
AT2	Angiotensin II receptor type 2
BH4	Tetrahydrobiopterin
CCB	Calcium channel blocker
DASH	Dietary approach to stop hypertension
eNOS	Endothelial nitric oxide synthase
GSH-Px	Glutathione Peroxidase
NAC	N-acetylcysteine
NADPH	Reduced nicotinamide adenine dinucleotide phosphate
NO	Nitric oxide
NOX	NADPH oxidase
OxLDL	Oxidized LDL
PGI2	Prostaglandin I2 (prostacyclin)
ROS	Reactive oxygen species
XO	Xanthine oxidase

1. Introduction

Hypertension remains a leading risk factor for morbidity and mortality worldwide. It is considered the most common cause of cardiovascular disease and the biggest contributor to global mortality, accounting for approximately 9.4 million deaths annually [1]. Although considerable

efforts have been devoted to the study of this disease, underlying mechanisms to explain its development have yet to be elucidated. During the last decades, increasing evidence of the contribution of oxidative stress in the pathological processes accounting for the development of elevated blood pressure has been accumulated [2]. Under normal physiological conditions, reactive oxygen species (ROS) mediate the vasoconstrictor effect of hormones such as angiotensin II, endothelin-1, or urotensin II, among others. In this regard, an enhanced vascular smooth muscle cell response should be expected to occur in any setting favoring increased ROS levels during the steady state [3]. Accordingly, it has been described that patients with elevated blood pressure due to different causes show increased oxidative stress biomarkers levels [4]. Moreover, a direct relationship between plasma 8-isoprostane levels and both systolic and diastolic blood pressure has been reported in hypertensive patients, as well as in normotensive ones [5]. Therefore, it has been suggested that ROS could play an important modulating role in blood pressure control. Consequently, antioxidants have been considered as potential antihypertensive agents, and evidence suggests that antioxidant-rich diets and antioxidant vitamins given as supplements have shown blood pressure lowering properties, particularly in newly diagnosed hypertensive patients without end organ damage [6–7].

The prevalence of treatment-resistant hypertension has increased more than twofold over the past 25 years and for a substantial proportion of US adults, prescription of additional antihypertensive medication is recommended, as more intensive blood pressure lowering algorithms are advised under the 2017 ACC/AHA guidelines [8–9]. Indeed, most people with hypertension require combination therapy to maintain systolic blood pressure under 140 mmHg, in part attributable to treatment-resistant hypertension, defined as blood pressure remaining above the therapeutic goals despite the administration of optimally tolerated doses of three antihypertensive agents from different drug classes, including a diuretic [10]. Nevertheless, antioxidants are still not considered among potential antihypertensive treatments.

The aim of this chapter is to provide a broad picture of some research lines which are currently being pursued, bringing forth clues highlighting the importance of antioxidant therapy in the management of oxidative stress-induced hypertension.

2. Antioxidant effects of antihypertensive drugs

Besides blood pressure reduction achieved through conventional mechanisms (such as inhibition of the renin-angiotensin-aldosterone

system, adrenergic beta receptor and calcium channel antagonism) widespread use of antihypertensive drugs such as ACE inhibitors (ACEI), angiotensin receptor blockers (ARB), dihydropyridine calcium channel blockers and some novel beta blockers have shown to have pleiotropic antioxidant effects that decrease blood pressure in an manner independent of its intended mechanisms. The use of these antihypertensive drugs is linked to a decline in plasma biomarkers of oxidative stress, explained by its direct antioxidant effect as well as the consequential reduction of inflammatory status associated with blood pressure normalization.

Conventional antihypertensive drugs interfere in major pathways involved in immediate blood pressure regulation. Accordingly, several drugs have been developed to stop vasoconstriction mechanisms mediated by the renin-angiotensin system. However, the role of said system in the pathophysiology of hypertension is not limited to smooth muscle cell vasculature contraction, as it is recognized as a major source for free radicals and inflammatory molecules, playing a central role in the decrease of nitric oxide production and bioavailability [5].

2.1 Angiotensin-converting enzyme inhibitors and angiotensin receptor blockers

Several studies conducted on murine models have stated that treatment with ACEI reduces oxidative stress parameters [11–13]. Similarly, a randomized clinical trial study conducted on newly diagnosed hypertensive patients reported significantly lower oxidative stress biomarkers levels in the group treated with enalapril, an ACEI, compared to patients treated with atenolol [14]. Clinical trials on atherosclerotic patients have documented a reduced incidence of hypertensive complications with long-term ACEI treatment [15]. Additionally, further research determined ACEI to be the most effective among antihypertensive drugs since it also provides endothelial protection [16].

Angiotensin II (Ang II) stimulates the activity of endothelial NADPH oxidases with the consequential generation of ROS [17]. Therefore, ARB and ACE inhibitors can effectively reduce NADPH oxidase activity, preventing subsequent oxidative damage. Additionally, an increase in superoxide dismutase activity has been reported for both ARBs and ACEI, as well as increased NO production and bioavailability through the prevention of eNOS uncoupling and enhancement of NO action. In consequence, treatment with both ACEI and ARB results in improved endothelium vasodilation despite their actions upon different targets. ACEI induces NO release by inhibiting bradykinin degradation [18–20], while

ARB, by blocking AT1 receptors, favors the binding of Ang II to AT2 receptors, stimulating the synthesis and release of NO [21, 22]. Moreover, ACEIs containing sulfhydryl groups (-SH) such as captopril, lisinopril, and zofenopril have antioxidant effects on the vascular wall that go even further, exerting a free radical scavenging effect through the thiol residues [23–25].

2.2 Calcium channel blockers

Dihydropyridine calcium channel blockers (CCB) have been widely recognized for their pleiotropic endothelium protecting properties attributed to their antioxidant activity, as they are associated with reduced lipid peroxidation and ROS generation [26–28]. As endothelial cells do not express voltage calcium channels, the reported benefits on endothelial function are likely to obey a different mechanism of action [29, 30]. CCBs are highly lipophilic molecules, as this chemical structure favors electron donation and resonance stabilization, which in turn enables reduction of free radicals [31, 32]. Additionally, eNOS modulation by some CCBs has been reported, enhancing endothelial function through increased NO production [33]. For instance, widespread use of CCBs, such as nifedipine and nicardipine, has been shown to be effective in preventing ROS production from endothelial dysfunction, as well as improving endogenous cellular antioxidant activity in vitro [27]. By protecting endothelial cells from free radicals, CCBs increase NO bioavailability and in turn, improve endothelial function [34, 35]. Recent studies have reported that another CCB, benidipine, is able to protect endothelial cells from oxidized low-density lipoproteins (OxLDL) mediated oxidative damage. In addition, amlodipine prevents endothelial dysfunction in hypertensive patients through its anti-inflammatory and NO producing mechanisms [36–38].

2.3 β-blockers

Chronic treatment with conventional β-adrenoreceptor antagonists, such as atenolol, have shown to reduce blood pressure in hypertensive patients, yet there is no documented activity of NO-dependent vasodilation, contrary to those observed in other classes of antihypertensive drugs [39, 40]. β-adrenergic antagonism, the drug's intended mechanism of action, is by itself relevant to reduce ROS production by blocking the well-known stress inducing catecholaminergic activity [41]. However, new generations of β-blockers, with novel properties and different effectiveness, have increasingly gained interest among the medical and scientific community for their antioxidant properties and favorable effects on endothelial

function, independent of their beta blocker activities. For instance, propranolol inhibits oxidative stress and reduces tissue lipid peroxidation [42]. Similarly, carvedilol can act as a free radical scavenger, reducing lipid peroxidation as well [43].

In addition, some novel β-blockers can interfere on specific regulatory pathways, modulating cellular signaling which results in direct antioxidant effects. For example, nebivolol, a selective β1-blocker, produces NO-dependent vasodilation. This effect was initially attributed to an increased NO synthase expression: inhibition of eNOS uncoupling as well as increased NO generation by promoting eNOS activity [44–47]. However, new experimental evidence suggests that nebivolol's inhibition of NADPH oxidase, with the consequential decrease in ROS production, ameliorates endothelial dysfunction [48–51]. Considering the marked oxidative environment that characterizes this clinical condition, the relevance of nebivolol's effects on NO synthase are questionable, as radicallary species rapidly annul NO activity. Targeting the core pathophysiological process underlying hypertension, such as the inhibition of a major source of vascular ROS, is a promising research that should be further studied.

Similarly, carvedilol, a non-selective β and α1 antagonist, has been shown to enhance endothelial function through antioxidant activity [52, 53]. On this account, treatment with carvedilol in hypertensive patients has proved not only to be useful in the management of high blood pressure, but also has an inhibitory effect on oxidation of low-density lipoprotein [54]. Likewise, another study conducted in healthy individuals reported reduced white blood cell production of ROS when treated with carvedilol. Nevertheless, the exact molecular mechanisms which explain its pleiotropic properties are still not completely elucidated and further investigations are required to conclude with certainty its effects on endothelial dysfunction and hypertension, particularly in the long term [30].

Table 5-1: Antihypertensive effects of antioxidants

Agent	Major antioxidant mechanisms
Angiotensin-converting enzyme inhibitors	Inhibit NADPH oxidase Decrease ROS generation Increase NO bioavailability Increase superoxide dismutase Free radical scavenging
Angiotensin receptor blockers	Inhibit NADPH oxidase Decrease ROS generation Increase NO bioavailability
Novel beta blockers	Increase NO bioavailability Inhibit NADPH oxidase Reduce lipid peroxidation Reduce white blood cell ROS production
Dihydropyridine calcium channel blockers	Decrease ROS generation Inhibit NADPH oxidase Reduce lipid peroxidation

3. Treatment-resistant hypertension

Hypertension is considered a major yet modifiable risk factor for cardiovascular disease. Its asymptomatic clinical presentation leads to patients undergoing extensive periods of time undiagnosed and untreated. Consistently elevated blood pressure increases the risk of cardiovascular complications and has overall detrimental health effects. In consequence, providing patients with early and effective treatment is crucial to limit hypertension's effect on morbimortality. The recent SPRINT trial determined that intensive antihypertensive treatment, targeting < 120mmHg for systolic blood pressure as opposed to conventional < 140mmHg target, reduced the occurrence of acute primary complications such as myocardial infarction, acute coronary syndrome, heart failure, and cardiovascular death in about 25% of patients [55, 56]. Accordingly, a previous meta-analysis had already established a strong correlation between elevated blood pressure and cardiovascular risk [57].

Lifestyle modifications and combined pharmacological treatment with common antihypertensive agents is the current standard of care. Nonetheless,

these drugs may not be suitable for all patients, particularly those with other comorbidities. Additionally, the complex prescription regimes promote patient noncompliance to treatment. Even so, it is estimated that 10%–15% of patients, when prescribed synergistic combinations and maximum dosage, fail to restore blood pressure to acceptable levels [58, 59].

Commonly, the combination consists of a long-action CCB, ACEI or ARB, and a diuretic. It is also considered for treatment-resistant hypertension patients who solely achieved desired blood pressure values with the simultaneous use of ≥ 4 antihypertensive medications including a diuretic [60]. Hence hypertension's increasingly high prevalence, its effect on morbimortality and its massive burden on public health obliges the urgent introduction of new therapeutic approaches [61].

4. Dietary approach to stop hypertension

The dietary approach to stop hypertension (DASH) is an eating pattern that consists of fruits, vegetables, whole grains, and low-fat dairy intake as well as restrictions on sodium and saturated fat content, which resembles the Mediterranean eating pattern [62, 63]. The effect on reducing blood pressure has been extensively studied, is well documented, and has been recommended as an effective nutritional strategy by several US health organizations for the prevention and management of hypertension [64, 65]. Specifically, increasing the antioxidant capacity of the organism by the consumption of flavonoid rich foods such as dark chocolate, black tea, and red wine has shown improved endothelial function [66]. On the other hand, epidemiological studies have linked low antioxidant vitamin dietary consumption with increased risk of cardiovascular disease, which could be explained by their positive effects of endothelial function and vascular contractility [67, 68].

In this regard, the results of a meta-analysis published by the British Journal of Nutrition in 2015 [66] on over 1,900 patients not only confirmed the effects of DASH on blood pressure and cardiometabolic markers, but yielded a significant reduction in the 10-year Framingham risk score for cardiovascular events, suggesting contributions that go beyond its recognized effects lowering blood pressure [66].

However, the underlying mechanism that explains these effects has yet to be elucidated, as it still remains unclear to what extent blood pressure reductions respond to beneficial antioxidant properties of its components, or to the caloric deficit and subsequent weight loss [69–73]. Nevertheless, novel dietary approaches have documented the role of several nutraceuticals such as aged garlic extract, beetroot juice, cocoa flavonoids, lycopene,

resveratrol, pycnogenol, probiotics, etc. with clinically detectable blood pressure lowering effects and meta-analysis or randomized controlled trials level of evidence. The mechanism of action of these novel agents is multiple, exerting its effects on different levels of the complex pathways involved in the pathogenesis of hypertension [74]. In this context, the modulation of nitric oxide (NO) bioavailability is especially relevant considering the high content of inorganic nitrates present in DASH [75, 76].

4.1 Wine

Polyphenols are an exogenous source of antioxidant defenses present in fruit, vegetables, and wine. The beneficial role of polyphenol intake (especially flavonoid and phenolic acid) through rich foods and beverages on cardiovascular and chronic diseases has been documented by numerous epidemiological studies [77, 78]. Many antibacterial, antifungal, antiviral, antineoplastic, hepatoprotective, immunomodulatory, and anti-inflammatory properties that can be attributed to their antioxidant activity have been described [79]. Antioxidant activity is achieved through ROS scavenging, iron chelation, antioxidant enzyme modulation, and enhancement of NO production. [80–83]. Human consumption of polyphenols has shown to increase levels of circulating NO [84], and glutathione; also inhibiting ROS-producing enzymes such as NOX and XO, leading to beneficial effects on endothelial function [6].

In this context, it is interesting to consider that red wine is one of the most abundant sources of polyphenols [85]. The consumption of red wine is linked to decreased all-cause mortality; however, this beverage constitutes a complex mixture of bioactive and bioavailable compounds such as ethanol, resveratrol, flavanoids flavan-3-ols, anthocyanins, and phenolic acids among others. So, the underlying mechanisms that explain its beneficial effects are still unclear and further studies are needed in order to clarify whether its protective effects respond to either a single component or a combination of them. Also, to what extent these are mediated by conventional antioxidant activities, as novel research lines suggest other post-transcriptional mechanisms could be involved [86].

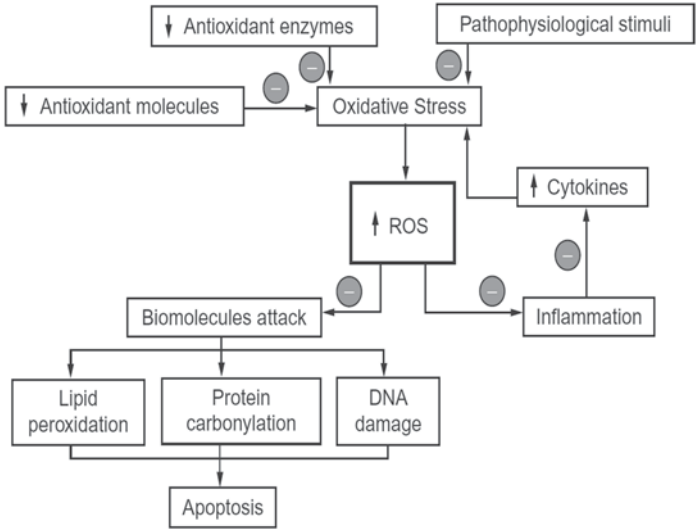

Figure 5-1: Sites of action of polyphenols against biomolecules' oxidative damage. Adapted from Rodrigo et al, 2012 [6]. ⊖: inactivation, decreased expression, or inhibition by polyphenols.

5. Supplements of antioxidant vitamins C and E

5.1 Vitamin C

Vitamin C (ascorbate) is a water-soluble vitamin with potent antioxidant activity in humans, which can be naturally found in numerous fruits and vegetables. Its nature as a reducing agent grants its antioxidant properties, exerting a free radical scavenging role through electron donation. Vitamin C acts as an effective defense mechanism against lipid, protein, and DNA oxidative damage, protecting these molecules' structure and biological function. The effects it exerts as an antihypertensive agent can be explained by the protection of biomolecules from free radical damage. Antioxidant effects on NO generation could in part explain the vasodilatory properties of ascorbate [87–89].

On the vascular wall, vitamin C acts as an enzyme modulator, responsible for the upregulation of eNOS, partly explained by BH4 protection against free radical mediated damage, and downregulation of NADPH oxidases [90, 91]. Additionally, ascorbate seems to have an ET-1 inhibiting effect, preventing both endothelium-dependent and independent vasodilation impairment and IL-6 stimulation mediated by endothelin [92].

The effects of vitamin C on blood pressure has been extensively studied. An inverse relationship between plasma ascorbate levels and blood pressure has been described in both normotensive and hypertensive cohorts, and its supplementation has been associated with reductions in blood pressure levels, as well as improved vascular function in both animal and human models [93–98].

However, several clinical trials on vitamin C antioxidant therapy for hypertension treatment have not reached the expected success, yielding inconsistent findings [99]. To interpret these results it must be taken into consideration that oral administration of vitamin C reaches plasma concentration levels that are way below the established antihypertensive therapeutic threshold [100].

5.2 Vitamin E

Vitamin E (tocopherol) is a lipid soluble chain-breaking antioxidant present in vegetable oils, nuts, seeds, and leafy green vegetables. It provides antioxidant protection in lipid-enriched environments such as membranes, due to its hydrophobic properties [101]. Several studies have documented tocopherol as a cardiovascular disease-protecting agent, improving cardiac performance, decreasing infarct size on acute myocardial infarction, and delaying heart failure progression in both animal and human models. This is concordant with epidemiological data, linking a high dietary vitamin E intake to a reduced incidence of cardiovascular disease [102–104]. Furthermore, antihypertensive properties as well as endothelial function improvement has been documented [105, 106].

Tocopherol can increase PGI2 levels in endothelial cells by exerting an opposing effect on phospholipase A2 and cyclooxygenase 2, thus providing protection against endothelial dysfunction through its vasodilatory properties [107, 108]. Vitamin E can also act as a redox modulator independently of its traditional antioxidant properties through regulation of mitochondrial ROS formation by preventing electron leakage, directly mediating superoxide production and by superoxide scavenging. By doing so, not only is oxidative damage reduced, but also the activity of other redox-sensitive biological modifiers as well as signal transduction pathways being modulated [109, 110].

Paradoxically, clinical trials have not only failed to prove vitamin E benefits on blood pressure and cardiovascular health but have also reported increased mortality rates among high dose supplementation intervention, which poses a dilemma to researchers, as low doses prove ineffectiveness, yet high doses can be detrimental. Moreover, tocopherol tends to concentrate

in lipoproteins, not interacting with cytoplasmic ROS, making the reaction against superoxide and peroxynitrite unlikely. Therefore, vitamin E supplementation in doses over 400 UI is not only ineffective in regulating blood pressure but is also detrimental, increasing mortality rates [111, 112].

5.3 Association of vitamin C and vitamin E

Vitamin C and E association as antihypertensive therapy has been proposed as a promising intervention. Preclinical trials and experimental models have consistently shown that the combination of their individual antioxidant properties acts in a synergic manner, granting beneficial features in blood pressure regulation and endothelial dysfunction. ROS scavenging, NADPH oxidase downregulation, and eNOS upregulation are some of the mechanisms that explain these effects. Furthermore, reduction of the α-tocopheroxyl radical mediated by vitamin C reduces lipid peroxidation activity, reinforcing their beneficial role as antihypertensive agents [90, 100, 113–115].

Nonetheless, clinical trials have been unable to establish the combination as a definite blood pressure lowering intervention among high cardiovascular risk patients due to inconsistent results [110]. These divergent findings could be explained by several factors. It is important to mention the lack of rigorous exclusion criteria in subject selection, to avoid confounding variables. This becomes especially important when analyzing the cohorts followed in larger clinical trials, where a greater proportion of subjects had severe and irreversible cardiovascular disease [116]. Long-term vascular and end organ damage could be responsible for a perpetuated pro-oxidative environment which the aforementioned antioxidant therapy will be unable to counteract. Modifications such as increased expression of NADPH oxidase gp91phox subunit and NOX4 expression on atherosclerotic arteries, as well as AT1 receptor expression (which in turn both stimulates the renin angiotensin system and activates NADPH oxidase in the arterial wall) could explain this major oxidative stress environment within vascular tissue [117, 118].

On the other hand, a study conducted on recently diagnosed mild to moderate essential hypertension patients without evidence of end organ damage, described a significant decrease in blood pressure ciphers in response to an improved antioxidant status following oral administration of vitamin C and E. These findings support the idea of oxidative stress as an essential mechanism in the pathogenesis of hypertension, describing a specific association between oxidative stress status and blood pressure for the first time [119]. Despite these encouraging results, it is important to

emphasize the fact that the positive outcomes of conjunct oral vitamin C and E administration are only seen at the early stages of essential hypertension development, specifically at the endothelial dysfunction phase, which precedes established and irreversible vascular damage. In this context, associated vitamin C and E can be considered as a novel adjunct therapeutic option, counteracting early vascular wall damage mediated by ROS, but with no significant effect on established cardiovascular disease where the chronic deleterious effects of oxidative stress on the vascular wall are practically irreversible [110].

6. Other antioxidants

6.1 Allopurinol

Xanthine oxidase (XO), the enzyme responsible for catalyzing the final steps of purine metabolism to uric acid, is a relevant source of ROS in the vascular endothelium. These ROS have a constricting effect on arteriolar tone, contributing to the pathogenesis of hypertension [120–122]. The reduction in ROS generation that follows the inhibition of XO with agents such as allopurinol has been associated with positive effects on cardiovascular health such as improved endothelial function and blood pressure in preclinical settings [123–125]. Notwithstanding the above, larger randomized controlled trials have failed to replicate these results, suggesting that further research is necessary to elucidate XO inhibition's real benefits [126].

6.2 Selenium

Selenium is a trace element essential to protein structure and enzyme catalytic sites, with a well-documented antioxidant activity. Selenocysteine residues are a crucial constituent of ROS detoxifying selenoenzymes, (glutathione peroxidase, thioredoxin reductase and selenoprotein P) [127]. An adequate selenium dietary intake can prevent the development of several cardiovascular disorders by maintaining full GSH-Px and TR activity [128].

Selenium also displays antioxidant activity by binding to nuclear factor kappa B (NF-κB) essential thiols. This prevents NF-κB union to its nuclear response element in DNA, blocking inflammatory pathways and ROS production mediated by this transcription factor [129–135].

These beneficial antioxidant properties of selenium have been documented by several studies, conducted in both animal models and human clinical trials. Experimental research by Miller et al. in 2001 reported

that low doses of selenium can provide significant protection of human coronary artery endothelium against damage by oxidative stress [127]. Correspondingly, a later study conducted in spontaneously hypertensive rats described how dietary supplementation with selenium was associated with lower levels of cardiac oxidative damage and increased antioxidant expression, as well as reduction in disease severity and mortality [136]. Furthermore, reduced selenium concentration in hypertensive pregnancies has been associated with diminished GSH-Px activity [137]. Given the above, it is reasonable to say that deficiency of selenium might be an underestimated risk factor for the development of high blood pressure that should be further studied due to its potential therapeutic role [138].

6.3 N-acetylcysteine

N-acetylcysteine (NAC) is a well-known sulfhydryl group donor exogenous antioxidant with recognized therapeutic actions. Originally used as a mucolytic agent, this drug can inhibit hypertension-induced pro-oxidative states. It exerts its action by protecting BH4 from oxidation [139], which results in increased levels of NO [140]. Lipid peroxidation inhibition and ROS scavenging properties have also been described [141, 142]. In animal models NAC has shown to improve renal dysfunction and decrease arterial pressure and renal injury in salt-sensitive hypertension [143].

7. Concluding remarks and future perspectives

The role of oxidative stress on the development of essential hypertension has been widely studied and a large body of evidence has documented the several pathways and complex underlying mechanisms of its pathogenesis. Consequently, the idea that antioxidant supplementation could be an effective antihypertensive treatment has been proposed. Basic research, animal models, and preclinical studies have shown promising results, but the outcomes of clinical studies remain controversial, with great discrepancy among different clinical trials.

Essential hypertension is a disease with a complex pathogenesis that is still not fully understood. Even though the effects of ROS on its pathogenesis have been established, no single mechanism can explain this condition, thus oxidative stress constitutes only a contributing factor to its development.

The multicausal nature of essential hypertension, as well as the limitations encountered in clinical trials; especially patient heterogeneity and advanced irreversible disease progression, shed light on the reasons

why studies conducted on human patients have failed to provide successful results.

However, novel trials with interventions based on DASH, wine, and natural dietary antioxidants have shown promising results, setting a precedent to encourage further research, as well as pointing in the direction of successful interventions: a combination of antioxidants, dietary administration, and efficacy on prevention and early-stage disease progression among others.

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