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Chapter 17

Lipid Peroxidation and Antioxidants in Arterial Hypertension

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http://dx.doi.org/10.5772/50346

1. Introduction

This chapter aims at giving a critical overview of the major oxidant and antioxidant changes in arterial hypertension, summarizing the experimental and clinical evidence about the involvement of oxidative stress in the pathophysiology of hypertension, either as a cause or a consequence of this disease. This review also provides a description of the biomarkers commonly used to evaluate lipid peroxidation and antioxidant defenses in experimental and human hypertension. Finally, we review the strategies (antioxidants, antihypertensive drugs) known to prevent or ameliorate oxidative damage, both in animal models of hypertension and hypertensive patients.

2. Pathophysiological role of oxidative stress in arterial hypertension

2.1. ROS sources and oxidative pathways involved in the pathogenesis of hypertension

In aerobic organisms, the beneficial effects of oxygen come with the price of reactive oxygen species (ROS) formation. These highly bioactive and short-lived molecules can interact with lipids, proteins and nucleic acids, causing severe molecular damage. However, living organisms have evolved specific mechanisms to adapt to the coexistence of ROS. In physiological conditions, there is a delicate balance between oxidants and antioxidants that not only protects our cells from the detrimental effects of reactive oxygen species (ROS), but also allows the existence of redox signaling processes that regulate cellular and organ functions. However, the disruption of redox homeostasis, leading to persistent high levels of ROS, is potentially pathological [1, 2]. Besides ROS, another group of molecules collectively designated as reactive nitrogen species (RNS) also exerts important functions in diverse physiological and pathological redox signaling processes. The excess of RNS is often termed nitrosative stress [3, 4].

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ROS can be classified into two main categories: free radicals [e.g. superoxide ($O_2^{\cdot-}$), hydroxyl ($HO^{\cdot}$), peroxyl ($ROO^{\cdot}$)], which are highly reactive species due to the presence of one or more unpaired electrons, and non-radical oxidants [e.g. singlet oxygen ($^1O_2$) hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl)] that have generally more specific reactivity and higher stability [3, 5, 6]. RNS include nitric oxide ($^{\cdot}NO$) and nitrogen dioxide radicals ($^{\cdot}NO_2$ and also non radicals such as nitrous acid (HNO_2), peroxynitrite ($ONOO^-$), peroxynitrous acid (ONOOH) and alkyl peroxynitrites (ROONO) [3]. Among biological ROS and RNS, $O_2^{\cdot-}$, H_2O_2, $^{\cdot}NO$ and $ONOO^-$ appear to be especially relevant in neuronal, renal and vascular control of blood pressure [3, 7 ,8] (Table 1). Major sources of ROS (and also RNS) within these systems include, but are not limited to, NADPH oxidases, xanthine oxidase, mitochondrial respiratory chain enzymes, $^{\cdot}NO$ synthases and myeloperoxidase [3, 8, 9].

	Free radicals	Non radical oxidants
ROS	$O_2^{\cdot-}$ $HO^{\cdot}$ $ROO^{\cdot}$	H_2O_2 HClO
RNS	$^{\cdot}NO$	$ONOO^-$

Table 1. Reactive oxidant species involved in cardiovascular and renal physiology or pathophysiology

NADPH oxidases (Nox) are enzyme complexes that catalyze the reduction of molecular oxygen using NADPH as an electron donor. Generally, the product of the electron transfer reaction is $O_2^{\cdot-}$ but H_2O_2 is also rapidly formed from dismutation of Nox-derived $O_2^{\cdot-}$ due to the presence of superoxide dismutase (SOD) in the cells or by spontaneous reaction. Nox-derived ROS have been shown to play a role in host defense and also in diverse signaling processes [10]. The Nox family comprises seven members (Nox1-5 and Duox1-2) with distinct tissue distribution and functions [10, 11]. So far, only Nox1, Nox2 and Nox4 have been shown to play relevant roles in hypertension pathophysiology [5, 8, 10]. These isoforms are localized in major sites of blood pressure control. For example, Nox1, Nox2 and Nox4 are expressed in the central nervous system where they appear to regulate sympathetic nerve activity [8]. Nox2 and Nox4 participate in the regulation of renal functions and contribute to end-organ damage associated with hypertension [8, 12]. In the vasculature, Nox1 controls smooth muscle cell growth and migration, Nox2 contributes to endothelial dysfunction and Nox4 controls vascular smooth muscle cell differentiation and improves endothelial-dependent vasodilatation [8, 13, 14].

Xanthine oxidoreductase has two inter-convertible forms, xanthine dehydrogenase (XDH) and xanthine oxidase (XO), that participate in purines metabolism catalyzing the conversion of hypoxanthine to xanthine and xanthine to uric acid [15, 16]. XDH preferentially uses NAD^+ as an electron acceptor while the oxidase reduces molecular oxygen in a reaction that generates $O_2^{\cdot-}$ and H_2O_2 [15, 16]. The XO form predominates in oxidative stress conditions and may contribute to endothelium dysfunction due to its localization in the luminal surface of vascular endothelium [16, 17]. Besides the production of ROS by XO, both XDH and XO generate uric acid which possesses antioxidant properties, such as scavenging of $ONOO^-$ and $HO^{\cdot}$, prevention of oxidative inactivation of endothelium enzymes and stabilization of Vitamin C

[18-22]. On the other hand, uric acid may also have prooxidant and proinflammatory effects [23, 24]. Indeed, high systemic levels of uric acid are associated with increased cardiovascular disease and poor outcome but it is not clear whether these effects reflect deleterious actions of uric acid or the oxidative damage caused by XO-derived ROS [23, 25].

Mitochondrial respiratory chain enzymes are primary intracellular sources of ROS. More than 90% of the total oxygen consumed by aerobic organisms is utilized by mitochondrial oxidases which produce ATP in a process coupled to the reduction of cellular oxygen to water [26]. About 1-4% of the oxygen used in these reactions is converted to $O_2^{\cdot-}$ and H_2O_2 which can be largely detrimental to mitochondrial functions if not adequately detoxified [26-28]. ROS levels in the mitochondria are regulated by the respiratory rate and manganese SOD [29]. Hypertensive animals have increased mitochondrial ROS production in the vessels, kidney and CNS [30-32].

$^{\cdot}NO$ synthases (NOS) constitute a family of enzyme isoforms (neuronal NOS, nNOS; inducible NOS, iNOS; endothelial NOS, eNOS) that produce $^{\cdot}NO$ in a reaction that converts L-arginine to L-citrulline [28]. However, in conditions of limited bioavailability of the cofactor tetrahydrobiopterin, or the substrate L-arginine, these enzymes become unstable and reduce molecular oxygen to $O_2^{\cdot-}$ instead of $^{\cdot}NO$ production (uncoupled NOS) [28, 29]. NOS uncoupling is more often described for eNOS and is triggered by oxidative/nitrosative stress [28, 33]. Numerous experimental studies have shown that arterial hypertension is associated with eNOS dysregulation and endothelial dysfunction [34, 35].

Myeloperoxidase (MPO) is a heme protein secreted by activated neutrophiles and monocytes in inflammatory conditions and produces several oxidizing molecules that can affect lipids and proteins [28, 36]. MPO uses H_2O_2 to produce ROS such as HOCl, chloramines, tyrosyl radicals and nitrogen dioxides [36, 37]. Although MPO-derived ROS have a primary role in microbial killing, they also cause tissue damage in the heart, vessels, kidney and brain and appear to contribute to endothelial dysfunction [37, 40]. Figure 1 illustrates the major sources of ROS and/or RNS generation.

Of all the putative oxidative pathways involved in the pathogenesis of hypertension, the impairment of endothelial-dependent vasorelaxation by $O_2^{\cdot-}$ is by far the most studied [41-44]. In conditions of increased $O_2^{\cdot-}$ bioavailability, this ROS rapidly inactivates endothelial-derived $^{\cdot}NO$ leading to endothelial dysfunction [41]. In addition, $O_2^{\cdot-}$ may also modulate vascular tone by increasing intracellular Ca^{2+} concentration in vascular smooth muscle cells and endothelial cells [45]. The imbalance between $O_2^{\cdot-}$ and $^{\cdot}NO$ also affects the renal function, leading to enhanced sodium reabsorption and increased $ONOO^-$ formation, which contributes to tissue damage [12, 46]. In the CNS, elevated $O_2^{\cdot-}$ generation also appears to contribute to hypertension by reducing the cardiovascular depressor actions of $^{\cdot}NO$ in the rostral ventrolateral medulla [47]. In recent years H_2O_2 has also emerged as a pivotal molecule in the pathophysiology of arterial hypertension [48-50]. Of note, H_2O_2 seems to be even more harmful than $O_2^{\cdot-}$ due to its higher life span and diffusibility within and between cells [7, 51]. Furthermore, the conversion of $O_2^{\cdot-}$ to H_2O_2 appears to be favored in cardiovascular diseases since the expression and activity of SOD is enhanced by

inflammatory cytokines in hypertension or in response to the pressor peptide, angiotensin II [7]. Several prohypertensive effects have been described for H_2O_2, such as increased vasoconstriction, vascular hypertrophy and hyperplasia, decreased diuresis and natriuresis and also increased spinal sympathetic outflow [7, 50, 52-58]. Increasing evidence has also shown that H_2O_2 amplifies oxidative stress by stimulating ROS generation by NADPH oxidases, XO and eNOS [7, 51]. In addition, H_2O_2 also appears to enhance the activation of the intrarenal renin-angiotensin system, a major regulator of blood pressure and renal function [49]. Altogether, these effects propagate H_2O_2 generation and prolong the redox pathologic signaling involved in blood pressure dysregulation. The oxidative mechanisms contributing to hypertension are summarized in Table 2.

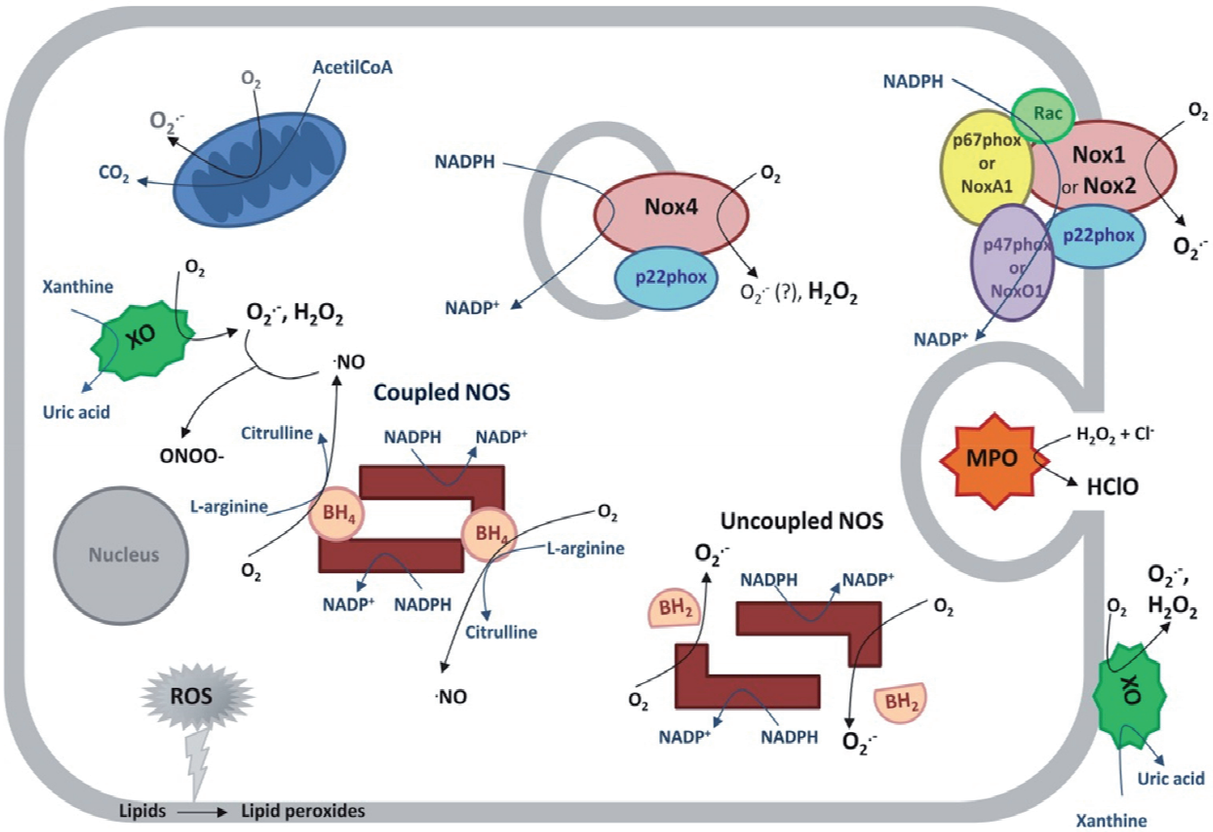


Figure 1. Sources of ROS and/or RNS generation - In normal cells, 1–2% of electrons carried by the mitochondrial electron transport chain leak from this pathway and pass directly to oxygen generating superoxide radical ($O_2^{\cdot-}$) which can be a source of other ROS. $O_2^{\cdot-}$ can also be formed by xanthine oxidase (XO) which catalyzes the oxidation of hypoxanthine and xanthine. All NOX enzymes utilize NADPH as an electron donor and catalyze transfer of electrons to molecular oxygen to generate $O_2^{\cdot-}$ and/or H_2O_2. Nitric Oxide synthases (NOS) generate ·NO and L-citrulline from arginine and O_2. Under pathologic conditions of oxidative stress, or when tetrahydrobiopterin (BH_4) or L-arginine are deficient, NOS enzymes become structurally unstable (uncoupled NOS) resulting in production of $O_2^{\cdot-}$ rather than ·NO. Activated monocytes also secrete a heme enzyme, myeloperoxidase(MPO), that uses H_2O_2 as a substrate to generate products that can oxidize lipids and proteins. One of these oxidants is hypochlorous acid (HOCI) which plays a critical role in host defenses against invading bacteria, viruses, and tumor cells but may also injure normal tissue. Within cell membranes, ROS can trigger lipid peroxidation, a self-propagating chain-reaction that can result in significant tissue damage.

Affected organ	Oxidative stress consequences	Major ROS and RNS involved
Vasculature	Impaired endothelium-dependent vasodilation	$O_2^{\cdot-}$, $^{\cdot}NO$, $ONOO^-$
	Increased vasoconstriction	$O_2^{\cdot-}$, H_2O_2
	Increased hypertrophy and hyperplasia	$O_2^{\cdot-}$, H_2O_2
Kidney	Decreased blood flow	$O_2^{\cdot-}$, H_2O_2
	Increased salt reabsorption	$O_2^{\cdot-}$, H_2O_2
	Tissue damage	$HO^{\cdot}$, HClO, $ONOO^-$
Brain/Spinal cord	Increased sympathetic efferent activity	$O_2^{\cdot-}$, H_2O_2

Table 2. Putative oxidative pathways leading to arterial hypertension

2.2. Evidence for redox changes in experimental and human hypertension

In the last two decades several studies have consistently observed increased oxidative stress in experimental and human arterial hypertension. Studies in diverse experimental models of hypertension have demonstrated raised prooxidant activity and ROS levels, altered antioxidant defenses and increased ROS-mediated damage, both at peripheral and central sites of cardiovascular regulation [8, 33, 59]. In human hypertensive patients there is also evidence of redox dysfunction. $O_2^{\cdot-}$ release from peripheral polymorphonuclear leucocytes is higher in hypertensive patients than in normotensive subjects [60]. Plasma H_2O_2 production is also raised in hypertensive patients. Furthermore, among still normotensive subjects, those with a family history of hypertension have a higher H_2O_2 production [61, 62]. An elevation of several oxidative stress byproducts, such as malondialdehyde, 8-isoprostanes, 8-oxo-2′-deoxyguanosine, oxidized low density lipoproteins, carbonyl groups and nitrotyrosine has also been observed in plasma or serum, urine or blood cells of hypertensive patients [63-66]. Furthermore, both enzymatic and non-enzymatic antioxidant defenses appear to be significantly reduced in human hypertension [65, 67]. Alterations of redox biomarkers in human hypertension are summarized in Table 3.

	Biomarker	**Evaluated in:**	**Alteration in hypertensive patients**	**References**
ROS/RNS	$O_2^{\cdot-}$	Peripheral PMN	↑	[60]
	H_2O_2	Plasma	↑	[61, 62]
		Lymphocytes	↑	[68]
	NOx	Plasma	↓	[69]
		Urine	↓	[70]

	Biomarker	Evaluated in:	Alteration in hypertensive patients	References
Prooxidant enzymes	NADPH oxidase activity	Mononuclear cells	↑	[71]
	p22phox (Nox subunit) mRNA and protein expression	Mononuclear cells	↑	[71]
Oxidative or nitrosative stress byproducts	Malondialdehyde (MDA)/Thiobarbituric acid reactive substances (TBARS)	Plasma	↑	[67, 72]
		Erythrocytes	↑	[64, 73, 74]
		Mononuclear cells and whole blood	↑	[65]
	F2-Isoprostane (or 8-isoprostane or 8-epi-PGF2α)	Plasma	↑	[63, 66, 74]
		Urine	↑	[63, 74, 75]
	8-Oxo-2′-deoxyguanosine		↑	[65]
			↑	[76, 77]
	Carbonyl groups	Serum	↑	[64]
	Oxidized low density lipoproteins	Plasma	↑	[63, 78]
	3-Nitrotyrosine	Plasma	↑	[66, 79]
Redox status	GSSG/GSH	Mononuclear cells and whole blood	↑	[65]
	GSH/GSSG	Erythrocytes	↓	[74]
Antioxidants	GSH	Mononuclear cells and whole blood	↓	[65]
		Erytrocytes	↓	[64]
	Uric acid	Plasma	↑	[79]
		Serum	↑	[80]
	Vitamin C (ascorbic acid)	Plasma	↓	[67]
		Serum	↓	[81]
	Vitamin E (α-Tocopherol,	Erytrhocytes	↓	[67]
	Total antioxidant status (TAS)	Plasma	↓	[63, 82]
	Ferric reducing activity of plasma (FRAP)	Plasma	↓	[74, 83]

	Biomarker	Evaluated in:	Alteration in hypertensive patients	References
	SOD activity	Erythrocytes	↓	[64, 74]
		Whole blood and mononuclear cells	↓	[65]
	Catalase activity	Erythrocytes	↑ or ↓	[64, 74]
		Whole blood and mononuclear cells	↓	[65]
	Glutathione peroxidase activity	Erythrocytes	↓	[64, 74]
		Whole blood and mononuclear cells	↓	[65]
	Glutathione-S-transferase activity	Erythrocytes	↑	[64]

NOx- nitrites and nitrates; PMN – Polymorphonuclear leucocytes; GSH – reduced glutathione; GSSG- oxidized glutathione;

Table 3. Altered oxidative/nitrosative stress biomarkers in human arterial hypertension

2.3. Oxidative stress as a cause for arterial hypertension

Whether oxidant imbalance is a cause or a consequence of high blood pressure remains a debatable question. The hypothesis that oxidative stress contributes to arterial hypertension is supported by several lines of evidence: (1) the induction of oxidative stress by the administration of lead or the glutathione synthesis inhibitor, buthionine sulfoximine, or the SOD inhibitor, sodium diethyldithiocarbamate, increases blood pressure in rats [48, 84]; (2) the infusion of H_2O_2 into the renal medulla leads to hypertension [48]; the treatment of hypertensive animals with antioxidants or inhibitors of ROS production prevents or attenuates hypertension [50, 85-87]; (3) the manipulation of genes related to ROS generation or elimination can alter blood pressure [88, 89]; (4) the *in vitro* exposure of cells and tissues to exogenous oxidants reproduces events involved in the pathophysiology of hypertension [43]; (5) systemic and tissue redox dysfunction appears to precede the blood pressure elevation [90].

2.4. Oxidative stress as a consequence of arterial hypertension

Arterial hypertension is associated with oscillatory shear stress and vascular stretch caused by increased vascular pressure. These mechanical forces are known to induce oxidative stress and vascular damage [91]. Furthermore, there is evidence that lowering blood pressure *per se* causes reduction of oxidative stress and improvement in endothelial function [92]. Several antihypertensive drugs with distinct mechanisms of action have been shown to decrease oxidant biomarkers in experimental and human hypertension [93-95]. However, there is limited evidence supporting the use of antioxidants to lower blood pressure in human hypertensive patients [5, 92]. Nevertheless, the failure of these studies does not

exclude a role for oxidative stress in human essential hypertension but instead suggests that the antioxidant supplementation approach was not the appropriate therapeutic strategy [96].

3. Biomarkers of redox status in arterial hypertension

The evaluation of redox status may provide valuable information about the pathogenesis and progression of arterial hypertension and related cardiovascular and renal diseases. However, the short lifetime of ROS turns their assessment in animal models and humans a significant challenge, leading to a growing interest in the development and validation of oxidative stress biomarkers. Traditional approaches to evaluate oxidant status have frequently relied on indirect measurements of ROS bioavailability (e.g. evaluation of prooxidant and antioxidant activity, oxidized products from ROS and the GSH/GSSG ratio) as indicators of normal biological processes, pathogenic processes, or pharmacologic responses to therapeutic intervention [9, 96-99].

A biomarker of oxidative stress is classically defined as a biological molecule whose chemical structure has been modified by ROS and that can be used to reliably assess oxidative stress status in animal models and humans [100]. The ideal biomarker of oxidative stress depends on its ability to contribute to an early indication of disease severity and/or its progression, as well as to evaluate therapy efficacy. The measurement of redox status biomarkers may also help to clarify the pathophysiologic mechanisms mediating oxidative injury and may allow the prediction of disease. Ideally, biomarkers of oxidative damage for human studies would be evaluated in specimens that can be collected relatively easily, such as blood or urine. However, to serve these purposes, an ideal biomarker of oxidative damage should fulfill several conditions, such as: a) being a stable product, not susceptible to artifactual induction, oxidation, or loss during sample handling, processing, analysis, and storage; b) having a well-established relationship with the generation of ROS and/or progression of disease; c) allowing direct assessment in a target tissue or being able to generate a valid substitute that quantitatively reflects the oxidative modification of the target tissue; d) being present at concentrations high enough to be a significant detectable product; e) showing high specificity for the reactive species in question and free of erroneous factors from dietary intake; f) being noninvasive; g) being measurable by a specific, sensitive, reproducible and inexpensive assay; h) being measurable across populations; i) being present in concentrations that do not vary widely in the same persons under the same conditions at different times [97].

3.1. Systemic and tissue antioxidant defenses

ROS are involved in many biological processes including cell growth, differentiation, apoptosis, immunity and defense against micro-organisms [1, 101, 102]. Low or moderate concentrations of ROS are beneficial for living organisms. However, high concentrations of ROS can cause direct damage of macromolecules such as DNA, proteins, carbohydrates, and lipids, or disrupt redox signaling and control pathways, leading to a myriad of human

diseases [103]. ROS bioavailability is determined by the balance between their production by prooxidant enzymes and their clearance by various antioxidant compounds and enzymes [1]. As defined by Halliwell and Gutteridge, an antioxidant is any substance that, at low concentration, is able to significantly delay or inhibit the oxidation of an oxidizable substrate [104]. Biological antioxidant defenses have evolved to match the diversity of prooxidants and several enzymatic and non-enzymatic molecules exist in cells and body fluids to control ROS levels within the physiological range [105]. The coordinated action of antioxidants results in the interception and deactivation of the damaging species. For example, the radical chain events initiated by free radicals can be terminated by the interaction of radicals with different non-enzymatic antioxidants [e.g. GSH, ascorbic acid, uric acid, α-tocopherol, etc] or prevented by specialized enzymatic defenses such as SOD, catalase and glutathione peroxidase (GPx) [105, 106]. The reduction of antioxidants bioavailability disrupts redox homeostasis leaving organisms more vulnerable to oxidative damage. Therefore, antioxidants may be useful biomarkers for risk stratification and disease prognostication.

3.2. Enzymatic antioxidants defenses

All eukaryotic cells possess powerful antioxidant enzymes which are responsible for neutralizing ROS.The first line of defense against ROS is achieved by SOD which is active in catalyzing the detoxification of $O_2^{\cdot-}$. This radical can be readily converted into H_2O_2 by SOD enzymes present in the cytosol and organelles (Cu,Zn-SOD or SOD-1), mitochondria (Mn-SOD or SOD-2) and extracellular fluids (EC-SOD or SOD-3) [36, 107, 108]. H_2O_2 generated in this reaction can be further decomposed to water and oxygen. This is achieved primarily by catalase in the peroxisomes and also by GPx enzymes in the cytosol and mitochondria [107, 108]. GPx are selenium-containing enzymes whose activity is dependent on GSH availability [108]. Besides neutralizing H_2O_2, GPx also degrades lipid hydroperoxides to lipid alcohols [36]. These reactions lead to the oxidation of GSH to GSSG. Catalase and GPx are differentially required for the clearance of high-levels or low-levels of H_2O_2, respectively [36]. Figure 2 illustrates major antioxidant enzymatic pathways.

In addition to these key antioxidant enzymatic defenses, there are other specialized enzymes with direct and/or indirect antioxidant functions. Glutathione reductase (GR) is responsible for the replenishment of GSH from GSSG disulphide. Glutathione-S-transferase catalyzes the conjugation of GSH with reactive electrophiles and is also involved in the detoxification of some carbonyl-, peroxide- and epoxide-containing metabolites produced within the cell in oxidative stress conditions [109]. Peroxiredoxins are selenium-independent enzymes that decompose H_2O_2, organic hydroperoxides and peroxynitrite [110]. Thioredoxin (Trx) and glutaredoxin (Grx) systems include several enzymes that regulate the thiol-disulphide state of proteins and influence their structure and function [110]. Trx isoforms reduce disulphide bonds in proteins, especially in peroxiredoxins and Trx reductase regenerates the oxidized Trx. Grx protects proteins SH-groups from irreversible oxidation by catalyzing S-glutathionylation and restores functionally active thiols through catalysis of deglutathionylation [110]. Grx enzymes are functionally coupled to GR which reduces the GSSG produced in the deglutathionylation reaction [110].

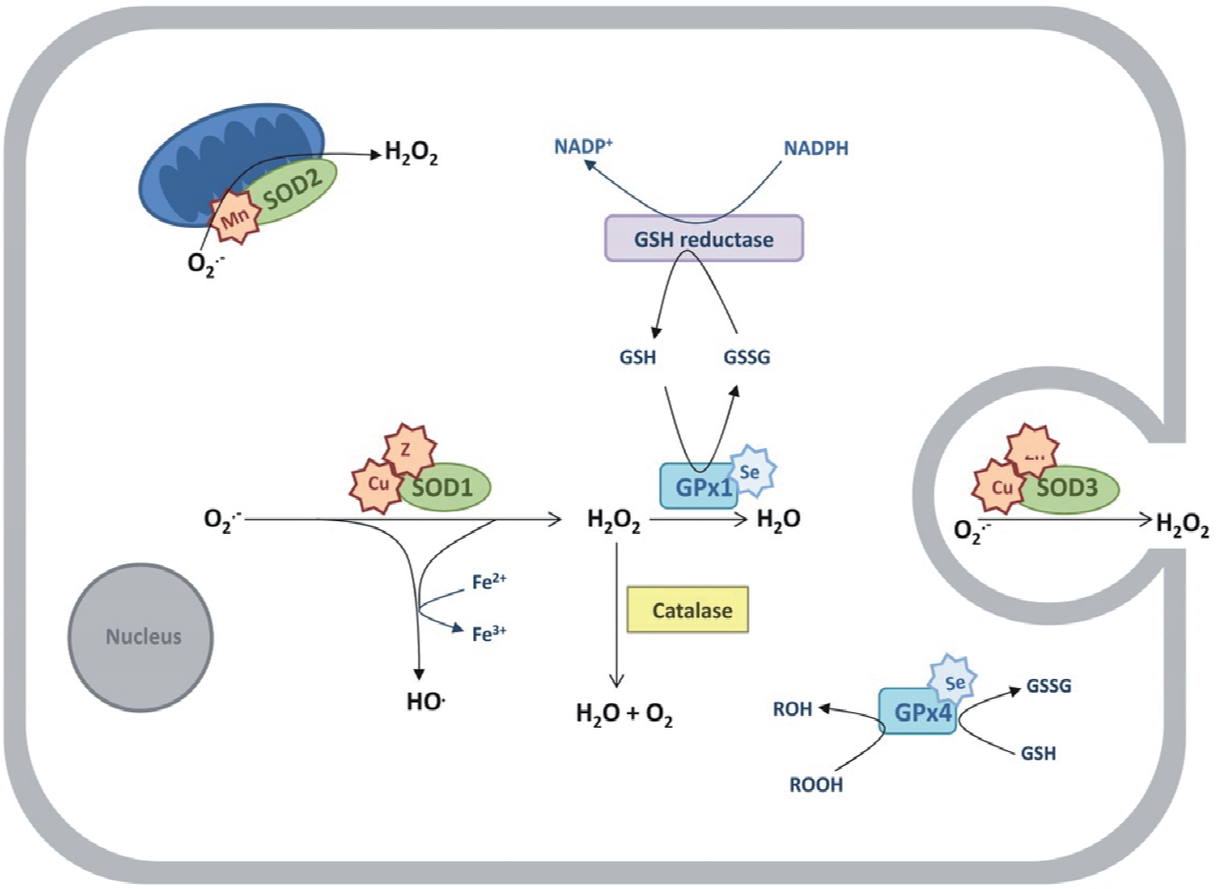


Figure 2. Major antioxidant enzyme defenses

Hypertensive patients have reduced activity and decreased content of antioxidant enzymes, including SOD, GPx, and catalase [43]. However, several studies have also described an adaptive increase in antioxidant enzyme activities in some experimental models of hypertension [50, 111, 112]. The uncoordinated activity of antioxidant enzymes may aggravate oxidative stress. For example, the increased dismutation of $O_2^{\cdot-}$ by SOD significantly increases H_2O_2 concentration, and may lead to deleterious consequences for the tissue in the absence of compensation of catalase and GPx activities [113]. Examples of altered antioxidant defenses in human and experimental hypertension are shown in Table 3 and Table 4, respectively.

Biomarker	Evaluated in:	Alteration	Hypertension Model	Reference
SOD	Brain	↓ expression and activity of Mn-SOD	Spontaneously hypertensive rats (SHR)	[114]
		↓ Cu, Zn-SOD activity	Stroke prone spontaneously hypertensive rats (SHRSP)	[115]
		↓ expression and activity of SOD1 and SOD2	SHR	[116]

Biomarker	Evaluated in:	Alteration	Hypertension Model	Reference
	Kidney	↓ expression of EC-SOD	SHR	[117]
		↑ SOD activity	Angiotensin II (Ang II) induced hypertension	[49]
		↓ expression of SOD1 and SOD3	SHR	[118]
	Arteries	↑ SOD activity	Hypertension induced by renin-angiotensin system (RAS) activation	[50]
		↓ SOD activity	SHR	[119, 120]
		↑ expression and activity of Cu, Zn-SOD and Mn-SOD	SHR	[121]
Catalase	Brain	↓ Catalase expression and activity	SHR	[116]
		↓ Catalase activity	Renovascular hypertensive rat; SHR	[122, 123]
	Kidney	↑ Catalase activity	SHR; Ang II-induced hypertension;	[49, 122]
		↓ Catalase expression	SHR	[124]
		↑ Catalase expression	SHR	[125]
	Arteries	↑ Catalase activity	Hypertension induced by RAS activation	[50]
		↓ Catalase activity	SHR	[120]
GPx	Brain	↓ GPx activity	SHR	[122]
	Kidney	↑ GPx activity	Ang II-induced hypertension	[49]
		↓ GPx expression	SHR	[124, 125]
		↓ GPx activity	SHR	[122]
	Arteries	↑ GPx activity	Hypertension induced by RAS activation	[50]
		↓ GPx activity	SHR	[120]
		↓ GPx expression	Salt- sensitive hypertension (Ovariectomized female rats)	[126]

Table 4. Alterations in major antioxidant enzyme defenses in the brain, kidney and arteries in experimental models of hypertension

3.3. Non-enzymatic antioxidants defenses

Non-enzymatic antioxidants such as GSH, ascorbic acid (vitamin C) and α-tocopherol (vitamin E) play an excellent role in protecting the cells from oxidative damage [4]. GSH has a potent electron-donating capacity that renders GSH both a potent antioxidant *per se* and a conventional cofactor for enzymatic reactions that require readily available electron pairs. In physiological conditions, GSH is present inside the cells mainly in its reduced form and less than 10 percent of total GSH exists in the oxidized form, GSSG [127]. Therefore, intracellular GSH status can be used as a sensitive marker of the cell health and resistance to toxicity. Furthermore, it has been demonstrated that GSH depletion can lead to cell apoptosis [128]. The measurements of GSH and GSSG have been considered useful indicators of the status of oxidative stress [4, 129]. Vitamins E and C are among the major dietary antioxidants. The vitamins have received considerable attention in clinical trials of primary and secondary prevention of cardiovascular diseases (CVD) and cancer. Vitamin E is found in lipoproteins, cell membranes and extracellular fluids. It terminates lipid peroxidation processes and converts $O_2^{\cdot-}$ and $HO^{\cdot}$ to less reactive forms [130]. Vitamin C, a water soluble antioxidant, is found in high concentrations in the adrenal and pituitary glands, liver, brain, spleen and pancreas. It is hydrophilic and can directly scavenge ROS and lipid hydroperoxides. Vitamin C can also restore oxidized vitamin E and can spare selenium [131]. Carotenoids, such as β-carotene are lipid soluble antioxidants that function as efficient scavengers of $^1O_2$ but may also quench $ROO^{\cdot}$ radicals [108]. Uric acid is a highly abundant aqueous antioxidant, considered to be the main contributor for the antioxidant capacity in the plasma [96, 132]. It has the ability to quenche $HO^{\cdot}$ and $ONOO^-$ and may prevent lipid peroxidation [21, 132]. The scavenging of $ONOO^-$ by uric acid is significantly increased in the presence of Vitamin C and cysteine which regenerate the urate radical formed in these reactions. Uric acid also acts as a chelator of iron in extracellular fluids [16]. However, once inside the cells, uric acid appears to exert prooxidant effects. It is not clear whether the correlation between the raised plasma levels of uric acid and cardiovascular risk are due to increased ROS generation by XO or to the prooxidative effects of uric acid itself. Some authors speculate that the increased concentrations of urate might be an adaptive mechanism that confers protection from oxidative damage [132]. It is likely that uric acid effects have different consequences depending on the surrounding microenvironment [21]. Bilirrubin, the end-product of heme catabolism, also appears to function as a chain-breaking antioxidant [133]. Low circulating bilirrubin levels are considered a risk factor for cardiovascular diseases [134]. Plasma albumin, the predominant plasma protein, is also an antioxidant due to its sulfhydryl groups and is able to scavenge MPO-derived chlorinated reactive species and $ROO^{\cdot}$ radicals [108, 135].

The combined antioxidant activities of aqueous- and lipid-antioxidants, including GSH, vitamins, uric acid, bilirrubin, albumin, etc, can be evaluated in the plasma and serum by several assays that measure the ability of the antioxidants present in the sample to inhibit the oxidation of the cation radical $ABTS^+$ [2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid] (Total Antioxidant Status assay), to reduce a ferric-tripyridyltriazine complex (Ferric Reducing Ability of Plasma, FRAP assay) or to trap free radicals (Oxygen Radical Absorbance Capacity, ORAC assay; Total radical Trapping Parameter, TRAP) [50, 136-138].

The measurement of the overall antioxidant capacity may be more representative of the *in vivo* balance between oxidizing and antioxidant compounds than the evaluation of individual antioxidants [139]. Nevertheless, these assays have also some limitations. First, they correlate poorly with each other as the various antioxidants react differently in each assay. Second, in biological fluids, uric acid appears to account for more than 50% of the total antioxidant activity measured in most assays [108]. However, the putative protective effect of uric acid is debatable [140, 141].

Under conditions of high ROS levels it is expected a decrease of non-enzymatic antioxidants defenses in plasma, since the need for neutralization ROS species implies a higher consumption of endogenous antioxidants. For example, decreased levels of antioxidant vitamins C and E have been demonstrated in newly diagnosed untreated hypertensive patients compared with normotensive control subjects [142-144].

3.4. Systemic, urinary and tissue markers of lipid peroxidation

Measuring oxidative stress in biological systems is complex and requires accurate quantification of ROS or damaged biomolecules. One method to quantify oxidative stress is to measure lipid peroxidation. Lipids that contain unsaturated fatty acids with more than one double bond are particularly susceptible to the action of free radicals. The peroxidation of lipids disrupts biological membranes and is thereby highly deleterious to its structure and function [145]. A large number of by-products are formed during this process and can be measured by different assays. Common biomarkers of lipid peroxidation damage include hydroperoxides, which are primary products generated in the initial stages of lipid peroxidation, and secondary products formed at later lipid peroxidation stages, such as malondialdehyde (MDA) or F2-isoprostanes (Table 5) [146, 147] .The lag time required for the exponential generation of lipid peroxidation products can also be used to evaluate the susceptibility of lipid molecules to free radical damage. Therefore, lipids with higher resistance to oxidative stress exhibit longer lag times than those which are easily attacked by free radicals [147].

3.5. F2-isoprostanes

F2-isoprostanes are prostaglandin F2α isomers primarily produced by free radical-catalyzed peroxidation of the polyunsaturated fatty acid (PUFA), arachidonic acid [97]. Although there is also evidence of F2-isoprostane formation by the action of cyclooxygenase, it is currently assumed that systemic and urinary F2-isoprostanes are mostly derived from free radical-induced lipid peroxidation, independently of cyclooxygenase enzymatic activity. Therefore, F2-isoprostanes have been regarded as reliable biomarkers of oxidative stress. Furthermore, F2-isoprostanes have been shown to exert potent vasoconstrictor effects on animal and human vessels, suggesting a pathogenic role in cardiovascular diseases and have been extensively used as markers of lipid peroxidation in human diseases [74, 75,148]. Their high stability and presence in measurable concentrations in many biological tissues and fluids, under physiological and pathological conditions, has also allowed the establishment of reference intervals and the comparison or monitoring of disease states

[97,149,150]. Urine specimens are particularly suited for F2-isoprostanes measurements. First, the ex vivo formation of F2 isoprostanes is minimized in these samples due to the low urinary lipid content, avoiding the need for time-sensitive sample processing [97, 149, 151, 152]. Second, they provide a noninvasive route for systemic oxidative stress evaluation. Although they can also be locally produced in the kidney, many studies have demonstrated that urinary F2-isoprostanes are mainly derived from free F2-isoprostanes filtered from the circulation [97, 149, 151, 152]. Only hydrolyzed isoprostanes are excreted into the urine whereas blood plasma samples contain both free and esterified isoprostanes. Since plasma samples have considerable amounts of arachidonic acid, the addition of preservatives, such as butylated hydroxytoluene (BHT) and indomethacin, and the storage at -80°C, are recommended to avoid degradation and/or ex vivo formation of F2-isoprostanes [97.

3.6. TBARS

The free radical attack to PUFAs in cellular membranes leads to the disruption of cell structure and function. MDA, one of the end products of these oxidative reactions, can be detected in several biological fluids and tissues and is therefore used as a biomarker of lipid peroxidation and oxidative stress [153]. High MDA levels indicate a high rate of lipid peroxidation [154]. The reaction of MDA with 2-thiobarbituric acid (TBA) is frequently used to estimate oxidative stress [155]. MDA reacts with TBA under conditions of high temperature and acidity generating 2-thiobarbituric acid reactive substances (TBARS) that can be measured either spectrophotometrically or spectrofluorometrically. However, these products can also be formed by sample autooxidation under assay conditions or by cross-reactivity with non-MDA substrates such as bile pigments, proteins, carbohydrates and other aldehydes. Therefore, TBARS measurements often originate doubts due to their limited specificity as markers of lipid peroxidation [156]. Nevertheless, undesirable autooxidation and non-MDA substrates reactivity can be minimized by adding BHT during sample preparation. Plasma TBARS measurements have been reported to correlate with some clinical features of cardiovascular disease, preeclampsia, ischemia/reperfusion, chronic kidney disease and cerebrovascular disorders [157-160]. Since the TBARS assay may overestimate MDA, other methods can be used to evaluate lipid peroxidation products, such as the lipid hydroperoxide (LPO) test. The principle of the LPO test is that in the presence of hemoglobin, lipid hydroperoxides are reduced to hydroxyl derivates with the equimolar production of a methylene blue product, which can be quantified spectrophotometrically [161].

3.7. HNE

The aldehyde 4-hydroxy-2-nonenal (4-HNE) is one of the most cytotoxic products of free radical attack on ω6-PUFA, namely arachidonic and linoleic acids, being able to react with diverse biological molecules such as proteins, peptides, phospholipids and nucleic acids. It also acts as an important mediator of oxidant-induced signaling, cellular proliferation and apoptosis [97, 162]. 4-HNE can be detected in plasma and several biological tissues under physiological conditions but its generation is significantly raised in pathological states associated with oxidative stress [97, 162-164]. Renovascular hypertensive rats showed

increased 4-HNE deposition in the intima of injured mesenteric arteries, suggesting the presence of free radical injury and cytotoxicity induced by 4-HNE [163]. A wide diversity of effects have been demonstrated for 4-HNE depending on its concentration. Concentrations below 0.1 microM are within the physiological range and appear to induce chemotaxis and stimulation of guanylate cyclase and phospholipase C [165]. 4-HNE concentrations between 1-20 microM inhibit DNA and protein synthesis and stimulate phospholipase A2. Higher concentrations (100 microM and above) are cytotoxic and genotoxic leading to cell death [165]. Thus, 4-HNE represents a broad indicator of lipid peroxidation.

3.8. Early stage of lipid peroxidation products

Lipid hydroperoxides are the primary products of lipid peroxidation and can further react to form secondary products including aldehydes such as MDA and 4-HNE [166,167]. Therefore, lipid hydroperoxides may be used to evaluate initial stage or acute lipid peroxidation while MDA and 4-HNE appear to be more representative of chronic oxidative stress. Recent reports described that 13-hydroperoxyoctadecadienoic acid (13-HPODE), a precursor to 3-hydroxyoctadecadienoic acid (13-HODE) is able to react with proteins forming adducts by covalently binding to specific amino acid residues. The Hexanoyl-Lysine (HEL) adduct results from the oxidative modification of ω6-PUFAs such as linoleic acid, the predominant PUFA in the human diet, and arachidonic acid [168]. HEL may be another useful biomarker for detecting and quantifying the earlier stages of lipid peroxidation. Monoclonal antibodies and ELISA kits have been developped, and HEL can be detected in oxidatively modified LDL, in human atherosclerotic lesions, human urine and serum. It has been also reported that HEL is formed in rat muscle during exercise and that its formation is inhibited by antioxidants such as flavonoids [169].

The lipid peroxidation biomarkers most commonly evaluated in hypertensive patients or experimental hypertension are shown in Table 3 and Table 5, respectively.

Lipid peroxidation biomarker	Measured in:	Alteration	Experimental model of hypertension	References
MDA	Plasma	↑	SHR	[170]
	Aorta	↑	Salt-loaded SHR	[94]
TBARS	Plasma	↑	Hypertension induced by RAS activation	[50]
	Plasma	↑	Ang II-induced hypertension	[171]
	Plasma, Heart	↑	Mineralocorticoid-induced hypertension	[172]
	Urine	↑	Salt-sensitive hypertension	[173]
	Aorta, Left Ventricle	↑	Ang II-induced hypertension	[174]

Lipid peroxidation biomarker	Measured in:	Alteration	Experimental model of hypertension	References
F2-**Isoprostanes**	Plasma	↑	Salt-sensitive hypertension	[175]
	Plasma	↑	Glucocorticoid-induced hypertension	[87]
	Urine	↑	SHR	[170, 176]
4-HNE	Mesenteric arteries	↑	Renovascular hypertension	[163]
	Aorta	↑	SHRSP	[177]
4-HNE adducts	Blood	↑	SHR	[178]

Table 5. Lipid peroxidation biomarkers in experimental hypertension

3.9. Other prooxidant biomarkers

Besides antioxidants and lipid peroxidation parameters, there are other important indexes of oxidant status. These include the expression and activity of prooxidant enzymes, ROS concentration, byproducts formed by ROS/RNS interaction with DNA (8-hydroxy-2-deoxyguanosine) or proteins (3-nitrotyrosine, carbonyl groups) and redox-sensitive transcription factors such as nuclear factor kappa B (NF-KB). Major sources of cellular ROS include Nox enzymes, mitochondrial electron transport enzymes, uncoupled NOS, XO and MPO. Table 6 summarizes several prooxidant biomarkers evaluated in experimental models of hypertension.

Biomarkers of prooxidant status	Evaluated in:	Alteration	Hypertension model	Reference
Mitochondrial electron transport chain enzymes/ mitochondrial ROS production	Brain	Oxidative impairment of mitochondrial enzymes	SHR	[30]
	Kidney	↑ mitochondrial ROS production	SHR, Mineralocorticoid hypertension	[179, 180]
NADPH oxidase family enzymes (or NOXs)	Brain	↑ NADPH oxidase activity	Salt-loaded SHRSP	[181]
	Kidney	↑ Nox4 expression, ↑ NADPH oxidase activity	Ang II–induced hypertension	[49]
	Arteries	**↑ expression of NAD(P)H oxidase subunits (p67(phox) and gp91(phox)**	Ang II-induced hypertension	[182]
		↑ Nox1 and Nox4 expression	SHRSP	[183]

Biomarkers of prooxidant status	Evaluated in:	Alteration	Hypertension model	Reference
		↑ NADPH oxidase activity	Ang II-induced hypertension; Hypertension induced by RAS activation	[50, 184-186]l
eNOS	Arteries	Uncoupled eNOS/ ↑ eNOS-derived ROS	SHR	[34]
	Arteries	↑ eNOS expression/↓ eNOS activity	SHR	[35]
XO	Arteries	↑XO activity	SHR	[187]
	Arteries	↑XO expression	SHR	[188]
H_2O_2	Kidney, Blood /urine	↑ renal production/↑ production in plasma /↑ urinary excretion	Ang II-induced hypertension	[49]
MPO	Arteries	↑ MPO activity	SHRSP	[189]
	Kidney, Heart, Brain	↑ MPO activity	Renovascular hypertension	[190]
GSH/GSSG	Kidney	↓ ratio	SHR	[191]
	Plasma, Heart, kidney	↓ ratio	Salt-sensitive hypertension	[192]
3-nitrotyrosine	Kidney	↑ expression	SHR	[193]
Protein carbonyl groups	Arteries, Heart, Kidney	↑ expression	SHR	[194]
	Kidney	↑ expression	SHR	[195]
8-Hydroxy-2-deoxyguanosine (8-OH-dG)	Arteries, Heart, Kidney,	↑ expression	SHR	[194].
NF-κB	Kidney	↑ activation	SHR; Ang II-induced hypertension	[49, 196]
	Arteries	↑ activation	Ang II-induced hypertension	[197]

Table 6. Other Prooxidant status biomarkers in experimental hypertension

4. Prophylactic and therapeutic strategies to reduce oxidative damage in arterial hypertension

A plethora of studies has demonstrated that hypertension is associated with an imbalance between oxidants and antioxidants that leads to altered cell signaling and oxidative damage.

Therefore, extensive research has been conducted in order to identify the ROS involved in blood pressure dysregulation, as well as the major prooxidant enzymes and antioxidant defenses that contribute to the loss of redox homeostasis in cardiovascular and renal systems. Furthermore, studies on experimental models of hypertension recognized several important neurohumoral stimuli responsible for ROS overproduction and also the main targets for ROS-induced dysfunction [8, 43]. Therapeutic interventions to reduce oxidative stress in hypertension have mostly relied on the administration of drugs that increase antioxidant capacity or inhibit ROS generation. In addition, other strategies aimed at reducing the activation of neurohumoral pathways that stimulate ROS production (upstream mediators) or at blocking/repairing the downstream targets affected by ROS have also been tested [196, 198, 199].

4.1. Targeting oxidative stress in experimental hypertension

The pharmacological modulation of ROS bioavailability in animal models of hypertension has been useful to demonstrate a causative role for oxidative stress in the pathophysiology of hypertension [43, 50]. However, the blood pressure lowering efficacy of these strategies appears to differ when comparing distinct experimental models [48, 50, 85, 200, 201]. This is probably because the development of each animal model was based on a particular etiological factor presumably responsible for human hypertension, such as high salt intake, overactivation of the renin-angiotensin system, genetic factors or renal disease. Since these factors may stimulate different redox pathways, the effectiveness of an antioxidant in one model does not necessarily translate to other models or to human essential hypertension which is known to have a multifactorial nature. Another important observation is that treatments with antioxidants or ROS inhibitors are generally more effective in preventing rather than reversing the hypertension [49, 50, 87, 202]. Indeed, there are several studies demonstrating that ROS activate feed-forward mechanisms that amplify the cardiovascular and renal dysfunction [8, 43, 49, 51]. Once triggered, these pathways may be sufficient to sustain the deleterious effects of oxidative stress even after ROS blockade or elimination [49]. *In vivo* drug treatments targeting oxidative stress in experimental models of hypertension are reviewed below and their effects on blood pressure are summarized in Table 7.

4.1.1. Drugs inhibiting ROS production

Apocynin is a methoxy-substituted catechol (4-hydroxy-3-methoxy-acetophenone), originally extracted from the roots of the tradicional medicinal herb *Picrorhiza kurroa* which has anti-inflammatory properties [203]. Several experimental studies have used apocynin for its ability to inhibit Nox enzymes. The mechanism of inhibition involves the blockade of translocation of cytosolic protein subunits to the membrane which is crucial for the activation of Nox1 and Nox2 [204]. Thus, the effect of apocynin is restricted to inducible Nox enzymes that require cytosolic activators and it does not seem to affect constitutively active Nox isoforms and their putative physiological actions [204]. However, to be an effective Nox inhibitor, apocynin has to undergo a peroxidase-mediated oxidation to be converted into the metabolically active

diapocynin [205-207]. The activation of apocynin occurs in the presence of MPO and H_2O_2 [205, 207]. This fact suggests that apocynin may function only in conditions of high inflammatory and prooxidant activity. Apocynin has also been shown to have direct antioxidant properties, being able to scavenge H_2O_2 derived products [205, 207]. However, it can also function as a prooxidant in resting cells [203]. Nevertheless, it is possible that when administered in conditions of enhanced oxidative stress, the protective effect prevails.

Gp91ds-tat is a chimeric peptide that specifically inhibits NADPH oxidase by preventing the assembly of its subunits. It is constituted by a segment of gp91phox (*gp91ds*) important for the interaction of this membrane subunit with the cytosolic subunit, p47phox, and by a *tat* peptide from the HIV virus, which allows the uptake of the peptide into the cell [208, 209]. However, since it is a peptide it may have poor oral bioavailability and may induce sensitization reactions. Furthermore, the *tat* segment may have side effects on cellular signaling and activity [204, 208]. Thus, it is not suitable for long treatments or to clinical use in the treatment of human cardiovascular diseases. Although it was designed to block Nox2, it may also inhibit Nox1 given the substantial degree of homology between the two isoforms [204]. As for apocynin, Nox4 is not likely to be affected by gp91ds-tat since it is constitutively active and does not require the activation of cytosolic subunits [204].

Allopurinol and its metabolite **oxypurinol** are hypoxanthine and xanthine analogs, respectively, that inhibit XO activity [16]. At low concentrations, allopurinol is a competitive inhibitor of XO, while at higher concentrations it behaves as a non-competitive inhibitor [16]. XO rapidly metabolizes allopurinol into oxypurinol, a noncompetitive inhibitor of the enzyme which has a much higher half-life and is therefore responsible for most of the pharmacological effects of allopurinol [16]. In addition, both allopurinol and oxypurinol have intrinsic antioxidant properties, being able to scavenge ROS such as $O_2^{\cdot-}$, $HO^{\cdot}$ and HClO [210-212]. However, these effects appear to require higher doses than those required for XO inhibition [210]. Allopurinol is approved for the treatment of human patients with gout or hyperuricemia, but it has also potential therapeutic application in cardiovascular diseases. Most common adverse effects are nauseas, diarrheas, hypersensitivity reactions and skin rash [16].

4.1.2. Antioxidants

Tempol (4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl) is a membrane-permeable nitroxide that catalyzes the conversion of $O_2^{\cdot-}$ to H_2O_2 thus functioning as a SOD mimetic [213, 214]. Tempol protects the lipids or proteins from oxidative damage and interacts with other antioxidants to promote the reduction of oxidized lipids [214]. The main antihypertensive effect of this drug is related to the reduction of the $O_2^{\cdot-}$ interaction with $^{\cdot}NO$ which improves vasodilation [213, 214]. It also promotes natriuresis by enhancing the vasodilation of renal medullary vessels in a $^{\cdot}NO$ independent manner [214]. Indeed, tempol has been shown to have sympatholytic actions, being able to inhibit afferent, peripheral and central activation of the sympathetic nervous system [214]. These actions are responsible for the rapid fall of blood pressure and heart rate after acute intravenous administration of tempol [214]. Nevertheless, some studies reported that the formation of H_2O_2 by tempol can

counteract its vasodilator, natriuretic and antihypertensive effects in models of hypertension where H_2O_2 plays a more prominent role than $O_2^{\cdot-}$ [48, 50, 53]. The co-administration of catalase in these conditions restores the protective action of tempol [48, 50].

N-acetylcysteine (NAC) is a thiol containing compound. It is the acetylated derivative of the aminoacid L-cysteine and a precursor for reduced glutathione (GSH) [215, 216]. It appears to have direct antioxidant action since its free thiol can interact with the electrophilic groups of ROS [215]. However, this effect does not seem likely to occur in vivo because NAC has poor oral bioavailability being rapidly metabolized into GSH, among other metabolites [216]. Thus, the main protective action of NAC is probably related to its role as a GSH precursor, which then detoxifies reactive species either by enzymatic or non-enzymatic reactions [216]. In humans, NAC is approved as a mucolytic agent because it destroys the disulphide bridges of mucoproteins [215]. It is also used as an antidote for acetaminophen poisoning which dramatically depletes hepatic GSH content causing severe damage [217]. NAC may also have potential therapeutical applications in the treatment of heart diseases [218].

Polyethylene glycol-catalase is the conjugated form of the enzyme catalase with polyethylene glycol (PEG) which enhances the stability in aqueous solution, reduces immunogenicity and decreases sensitivity to proteolysis, thus increasing the circulatory half-life of catalase [219]. PEG also enhances the catalase association with cells [219]. The antioxidant effect of PEG-catalase results from the enzymatic degradation of H_2O_2 to water.

Ebselen (2-phenyl-1,2-benzisoselenazol-3[2H]-one) is a lipid-soluble seleno-organic compound that mimics glutathione peroxidase activity, being able to react with H_2O_2 and organic hydroperoxides including membrane-bound phospholipid and cholesterylester hydroperoxides [220]. It appears to reduce lipid peroxidation initiated by hydroperoxides but not free radicals initiators [221]. In addition, ebselen reacts rapidly with $ONOO^-$. The ebselen selenoxid product yielded in this reaction is regenerated to ebselen by GSH, which allows its reutilization as a defense against $ONOO^-$ [222, 223]. Ebselen also directly inhibits inflammation-related enzymes such as 5-lipoxygenase, $^{\cdot}NO$ synthases, protein kinase C, NADPH oxidase and H^+/K^+-ATPase by reacting with the SH group, leading to the formation of a selenosulphide complex [221]. Some authors have also proposed that the antioxidant and anti-inflammatory actions of ebselen are mediated through interactions with the thioredoxin (Trx) system [220]. Reduced Trx is important for growth and redox regulation by thiol redox control [220]. Ebselen was found to be an excellent substrate for mammalian TrxR and a highly efficient oxidant of reduced Trx. It also seems to function as a Trx peroxidase or peroxiredoxin mimic, thus contributing to the elimination of H_2O_2 and lipid hydroperoxides [220]. Ebselen has been used in clinical trials for the treatment of patients with acute ischemic stroke or delayed neurological deficit after aneurismal subarachnoid hemorrhage [224, 225].

Vitamin C (ascorbic acid) is a water soluble antioxidant found in the body as an ascorbate anion. It acts as a free radical scavenger [226]. Although this effect requires higher concentrations than those achieved in the plasma by oral administration, ascorbate appears to concentrate in tissues in much higher levels than those found in the plasma and can act effectively as a ROS scavenger [227]. In addition, it reduces membrane lipid peroxidation

and regenerates Vitamin E [226]. Recent reports also suggest that Vitamin C can suppress NADPH oxidase activity [227].

Vitamin E is a generic term for a group of compounds including tocopherols and tocotrienols. The isoform α-tocopherol appears to be the most abundant in vivo [227]. Vitamin E terminates the propagation of the free radical chain reaction in lipid membranes and inhibits LDL oxidation [226, 227]. Vitamin E can also have non antioxidant actions primarily through the regulation of enzymes involved in signal transduction. Enzymes inhibited by vitamin E include protein kinases C and B, protein tyrosine kinase, lipoxygenases, mitogen activated protein kinases, phospholipase A2 and cyclooxygenase-2. In contrast, vitamin E has stimulatory effects on protein tyrosine phosphatase and diacylglycerol kinase [228]. Both vitamins C and E have been shown to stimulate the activation of NOS activity and increase ·NO synthesis in endothelial cells and thus may contribute to improved endothelial-dependent vasodilation in hypertension [229]. However, although Vitamins C and E are generally considered to be non-toxic, they can undergo oxidation and generate pro-oxidant molecules [226]. Nevertheless, it appears that this is more likely to occur with Vitamin E, especially in the absence of sufficient Vitamin C to regenerate the α-tocopherol radical [227, 230, 231].

Alpha-lipoic acid (1,2-dithiolane-3-pentanoic acid or thioctic acid) has a wide range of effects on cell functions, acting as an antioxidant, a metal chelator and a signaling mediator [232]. Both lipoic acid (LA) and its reduced form dihydrolipoic acid (DHLA), may scavenge $HO\cdot$ and HClO, although neither species is able to neutralize H_2O_2 [232]. DHLA also regenerates Vitamins C and E and does not become a free radical after reacting with these species. Furthermore, LA and DHLA chelate transition metals, thus reducing the metal-catalyzed free radical damage [232]. LA also contributes to improve antioxidant defenses by increasing the intracellular levels of Vitamin C and GSH. Many of LA protective actions have been attributed to its interference in cell signaling processes [232]. For example, LA effect on GSH appears to be mediated by nuclear factor erythroid 2- related factor 2 (Nrf2), an important transcription factor regulating gene transcription through the Antioxidant Response Element. LA was also shown to interact with several kinases and protein phosphatases [232]. Its interaction with components of the insulin signaling cascade also appears to improve glucose disposal in animal models of diabetes and human diabetic patients [232]. In addition, LA improves endothelial ·NO synthesis and endothelial-dependent vasodilation and prevents deleterious modifications of thiol groups in Ca^{2+} channels [232]. It has also important anti-inflammatory effects by inhibiting the activation of NF-KB, a transcription factor that regulates the expression of proinflammatory genes [232].

Pyrrolidine dithiocarbamate (PDTC), a low-molecular weight thiol compound, has the ability to scavenge oxygen radicals and to chelate metals [233, 234]. It may also act as a prooxidant and a thiol group modulator [233]. PDTC has been shown to interfere with the activation of several transcription factors, being a potent inhibitor of NF-KB [233, 234]. PDTC can also activate other signaling pathways, such as the extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK) and the transcription factor Heat Shock Factor (HSF) [233, 235].

5, 6, 7, 8-Tetrahydrobiopterin (BH_4) is a key cofactor of NOS [236, 237]. It is involved in the formation and stabilization of eNOS and iNOS [236, 238]. In the absence of BH_4, NOS can become uncoupled and starts producing $O_2^{\cdot-}$ instead of $^{\cdot}NO$ [33, 237]. Furthermore, BH_4 also possesses direct antioxidant activity, being able to scavenge $O_2^{\cdot-}$ and $HO^{\cdot}$ [239]. The protective effects of BH_4 on the development of hypertension appear to be due an increase in eNOS activity, a reduction in $O_2^{\cdot-}$ production and a decrease in iNOS expression [199].

Drug	Antihypertensive effect	Lack of antihypertensive effect
Apocynin	Prevented/attenuated mineralocorticoid-induced hypertension [86, 240] Prevented/reversed glucocorticoid-induced hypertension [241] Prevented/reversed adrenocorticotropic hormone-induced hypertension [242] Prevented the development of Ang II-induced hypertension in mice [186] Prevented the development of renovascular hypertension [243] Prevented the development of hypertension induced by RAS activation [50] Reduced blood pressure in borderline and spontaneous hypertension [244] Attenuated salt-sensitive hypertension [245] Normalized blood pressure in a model of hypertension induced by disruption of dopamine D_2 receptor [246]	Failed to prevent the hypertension induced by chronic infusion of endothelin-1 [200] Failed to prevent hypertension in transgenic mice overexpressing renin or angiotensinogen [247, 248] Failed to prevent Ang II-induced hypertension in rats [249, 250]
Gp91ds-tat	Attenuated the blood pressure rise induced by Ang II in mice [209]	Failed to attenuate salt-sensitive hypertension [251]
Allopurinol	Attenuated salt-sensitive hypertension [252] Prevented glucocorticoid-induced hypertension [253]	Failed to prevent or attenuate mineralocorticoid-induced hypertension [254] Failed to prevent glucocorticoid-induced hypertension [255] Failed to prevent or attenuate adrenocorticotropic-induced hypertension [242] Failed to prevent the development of hypertension induced by the blockade of nitric oxide synthesis [256] Failed to prevent the progression of hypertension in young SHR [257]
Oxypurinol	Reduced blood pressure in SHR [258]	
Tempol	Attenuated hypertension in SHR [201] Prevented the progression of hypertension in salt-loaded SHRSP [259] Attenuated mineralocorticoid-induced hypertension [260]	Failed to prevent Ang II-induced hypertension [264] Failed to attenuate hypertension induced by inhibition of superoxide dismutase [48]

Drug	Antihypertensive effect	Lack of antihypertensive effect
	Prevented/attenuated glucocorticoid-induced hypertension [87] Attenuated salt-sensitive hypertension [261] Prevented the development of renovascular hypertension [243] Attenuated high-volume hypertension [262] Attenuated hypertension induced by NO inhibition [112] Partially prevented/reversed adrenocorticotropic hormone-induced hypertension [263]	Failed to prevent hypertension induced by RAS activation [50]
NAC	Attenuated hypertension in young SHR [265] Prevented the development of glucocorticoid-induced hypertension [202] Prevented the development of adrenocorticotropic hormone-induced hypertension [266] Markedly reduced salt-sensitive hypertension [267] Prevented/ attenuated hypertension induced by nitric oxide synthesis inhibition [268]	Failed to reduce blood pressure in adult SHR [265] Failed to attenuate glucorticoid-induced hypertension [202] Failed to reverse adrenocorticotropic-induced hypertension [266] Failed to prevent the development of hypertension induced by the blockade of nitric oxide synthesis [256]
PEG-catalase	Prevented the development of hypertension induced by RAS activation [50] Transiently decreased blood pressure in Ang II-hypertensive rats [49] Reduced blood pressure in high-volume hypertension in mice [269]	Lacked a sustained antihypertensive effect in Ang II-induced hypertension [49]
Ebselen	Attenuated the blood pressure rise induced by Ang II in mice overexpressing p22phox in vascular smooth muscle and in littermate control mice [270]	Failed to prevent the development of hypertension induced by the blockade of nitric oxide synthesis [256]
Vitamin C	Prevented the progression of hypertension induced by salt administration in SHRSP and in SHR [229, 271] Attenuated salt-induced hypertension [272, 273]	Failed to prevent adrenocorticotropic hormone-induced hypertension [274]
Vitamin E	Prevented the progression of hypertension induced by salt administration in SHRSP [229] Attenuated hypertension in young SHRSP [275] Attenuated salt-induced hypertension [273]	Failed to prevent adrenocorticotropic hormone-induced hypertension [274]
Lipoic acid	Reduced blood pressure in SHR [276] Prevented fructose-induced hypertension [277] Prevented/attenuated salt-induced hypertension [278] Prevented mineralocorticoid-induced	

Drug	Antihypertensive effect	Lack of antihypertensive effect
	hypertension [279]	
PDTC	Perinatal administration ameliorated hypertension in SHR offsprings [280] Prevented /Reduced hypertension in SHR [32, 196] Attenuated mineralocorticoid-induced hypertension [260]	
BH_4	Suppressed the development of hypertension in SHR [199] Reduced hypertension in SHR [281]	Failed to attenuate hypertension in castrated SHR [281] Failed to prevent the development of adrenocorticotropic hormone in rat [282]

Table 7. Effect of chronic treatment with antioxidants or inhibitors of ROS production on blood pressure

4.2. Antioxidant approaches in human hypertension

Although there is considerable evidence of oxidative stress involvement in the pathophysiology of hypertension, the attempts to demonstrate benefits from antioxidant therapy in human cardiovascular diseases have been very disappointing [5, 96, 283]. Most of the large trials regarding the effects of diet supplementation with Vitamin C, Vitamin E and β-carotene failed to show significant improvements in blood pressure and other cardiovascular endpoints [5, 283]. Furthermore, some of them also led to the conclusion that antioxidant treatment with Vitamin E or β-carotene may even be harmful [283-285]. In contrast, smaller clinical trials have provided some evidence of antioxidant treatment advantages. For example, some studies showed that systemic Vitamin C levels inversely correlates with blood pressure and that Vitamin C supplementation effectively attenuates hypertension [142, 286]. Vitamin E and lipoic acid have also been shown to improve vascular function, though there is not consistent evidence of a blood pressure lowering effect of these agents in human patients [5, 287, 288]. Nevertheless, it has been demonstrated that a high consumption of dietary fruits and vegetables increases plasma antioxidant capacity and reduces blood pressure [289, 290]. Thus, it appears that a diet rich in fruits and vegetables is a better strategy than antioxidant supplementation to improve antioxidant status and cardiovascular health [5]. Overall, the clinical trials with antioxidant supplements have been very unsatisfactory and are in disagreement with the findings obtained in experimental hypertension studies. There are some possible justifications for the disappointing outcomes of these trials. First, the type of the drug used as well as the dose and duration of the therapy might not be adequate [5, 291, 292]. Most trials followed an antioxidant strategy based in the administration of ROS scavengers such as Vitamins C and E. However, these drugs do not neutralize H_2O_2 which has been shown to play a relevant role in the pathophysiology of hypertension and other cardiovascular diseases [5, 7, 48-50]. Furthermore, it is known that human blood and tissues have plenty antioxidants and that several stimuli induce an adaptive increase of enzymatic antioxidant defenses which can

mask the benefits of exogenously administered antioxidants [293]. In addition, the antioxidant doses used in most of the experimental studies have been much higher than those tested in human patients [291]. So, there is the possibility that in humans the antioxidants did not achieve effective concentrations to neutralize ROS. Furthermore, it is not known if orally administered antioxidants can reach the precise sites of increased ROS production as oxidative stress is heterogeneously distributed throughout the organs, tissues and cellular compartments [5, 96, 291]. Indeed, the unspecific scavenging of ROS may even interfere with many important physiological functions in a deleterious manner [29, 96]. Another important limitation of most antioxidants tested is that they can exert themselves prooxidant effects in the absence of a coordinated antioxidant response [5, 96]. For example, Vitamin E needs to be regenerated by Vitamin C otherwise it may cause oxidative damage [231].

There are also drawbacks in clinical trials design. In large trials of antioxidant supplementation, patients have not been recruited accordingly to their redox status [5, 294]. It is unlikely that a beneficial effect of antioxidant therapy would be observed in patients without previous evidence of increased oxidative stress. Another important consideration is that these clinical trials often have heterogeneous populations in terms of the etiology of cardiovascular disease [295]. Indeed, most studies have indiscriminately enrolled any patient at cardiovascular risk [294]. This is in obvious contrast to the homogeneous populations analyzed in experimental studies. Furthermore, some of the patients may be at an advanced stage of disease exhibiting irreversible damage insusceptible to antioxidant interventions [5, 29]. It should also be highlighted that many patients enrolled in these studies were already being treated with drugs such as aspirin, lipid-lowering agents and some antihypertensive drugs which can themselves interfere with oxidant status and mask the effects of additional therapy with antioxidants [5, 92, 296, 297].

So far, most interventions aimed at reducing oxidative stress in human hypertension have relied on antioxidant supplementation. However, it is possible that a strategy based on the inhibition of ROS production is more effective than the antioxidant interventions [5, 96]. The disruption of cardiovascular redox status is most likely triggered by an increase in prooxidant activity rather than a reduction in antioxidant defenses. Indeed, many neurohumoral or ambiental prohypertensive stimuli (angiotensin II, aldosterone, high-salt intake) are known to upregulate the expression and activity of prooxidant enzymes [5, 8, 43]. Nevertheless, there are already some studies that investigated the cardiovascular effects of prooxidant enzyme inhibition. Patients treated with allopurinol showed improvements in vascular function [298, 299]. However, a blood pressure lowering effect of this XO inhibitor has been shown only in newly diagnosed hypertensive adolescents and in hyperuricemic patients with normal renal function [300, 301]. Furthermore, the combination of allopurinol with antihypertensive drugs did not provide additional benefits on blood pressure [299]. This is probably because XO is not a major contributor to the development of hypertension, even though its activity may be increased in pathophysiological conditions [5]. Indeed, compelling evidence indicates that NADPH oxidases are the main contributors to ROS overproduction in cardiovascular and renal diseases [5, 8, 302]. Moreover, Nox-derived ROS

are known to amplify redox dysfunction by inducing the activation of other prooxidant enzymes, such as XO, mitochondrial enzymes and NOS synthases [7, 51]. Since many antihypertensive drugs block upstream activators of Nox enzymes, it is not surprising that the inhibition of XO by allopurinol does not improve blood pressure control in patients already treated with antihypertensive drugs. To date, no Nox inhibitors have been tested in clinical trials although some specific Nox inhibitors have already been developed and patented [96, 296]. Future strategies to demonstrate the benefits of oxidative stress reduction in cardiovascular diseases should include the testing of specific Nox inhibitors in human patients. Moreover, the development of reliable oxidative stress biomarkers for risk stratification and monitoring of therapy is also highly desirable [96, 296]. Table 8 summarizes the possible reasons for the failure of antioxidants in clinical trials.

Limitations related to the drug treatment	Limitations related to the clinical trials design
Inadequate dose or duration of therapy Lack of effect on non-radical oxidants such as H_2O_2 Lack of effect on prooxidant activity Inaccessibility of ROS scavengers to intracellular sites of increased ROS production Some antioxidants may themselves become prooxidants in the absence of a coordinated antioxidant response Unspecific scavenging of ROS may disrupt physiological functions	Lack of previous evidence of increased redox dysfunction in patients analyzed Heterogeneous populations in terms of the etiology of cardiovascular disease Some patients may be at an advanced stage of disease exhibiting irreversible damage Patients treated simultaneously with drugs that interfere with oxidant status (aspirin, lipid lowering agents, antihypertensive drugs) Lack of validated oxidative stress biomarkers for risk stratification and monitoring of therapy

Table 8. Possible reasons for the failure of clinical trials with antioxidants in cardiovascular diseases

4.3. Antihypertensive treatments with direct and indirect antioxidant effects

It is known that first-line antihypertensive drugs such as angiotensin II receptor blockers (ARB) and angiotensin converting enzyme inhibitors (ACEi) can reduce oxidative stress due to their inhibitory effect on angiotensin II, which is a major stimulus for the activation or upregulation of Nox enzymes [5, 296]. ROS such as $O_2^{\cdot-}$ and H_2O_2 are widely recognized as important downstream mediators of Ang II physiological and pathological effects [303]. Nevertheless, some of these antihypertensive drugs also possess antioxidant effects independently of RAS inhibition. For example, captoptil, a thiol-containing ACEi, is a ROS scavenger and a metal chelator [304]. The ARBs candesartan and olmesartan also exhibit antioxidant effects independent of AT_1 receptor blockade or blood pressure control [305-307]. In addition, other agents belonging to the beta-blocker or calcium channel blocker drug classes have also been shown to exert antioxidant effects unrelated to their blood pressure lowering action. The beta-blockers carvedilol and nebivolol appear to possess ROS scavenging properties as well as inhibitory effects on ROS production, such as the inhibition of Nox activation [308, 309]. In addition, nebivolol also increases $^{\cdot}NO$ release from the endothelium, thus attenuating oxidative stress effects on endothelium-dependent vasodilation [309, 310]. The calcium channel blocker lacidipine has also been demonstrated

to have a potent antioxidant activity and to reduce the intracellular production of ROS induced by oxidized LDL [311, 312]. Therefore, even though convincing evidence is lacking regarding a clinical therapeutic effect of antioxidants, there is extensive data showing that currently approved antihypertensive treatments have the ability to modify oxidative stress status.

5. Conclusions

Extensive experimental evidence has shown that unbalanced ROS and/or RNS production can disturb several physiological functions, leading to the genesis and progression of arterial hypertension. Many studies have observed marked alterations in direct and indirect oxidative stress biomarkers, such as lipid peroxidation products, prooxidant enzymes and antioxidant defenses. However, most clinical trials with antioxidants have failed to demonstrate a protective effect on blood pressure and cardiovascular function. This does not necessarily exclude a role for oxidative stress in human cardiovascular diseases but instead suggests that other approaches should be addopted to recover redox homeostasis. The inhibition of Nox enzymes appears to be a promising strategy as these enzymes are major sources of ROS overproduction at cardiovascular and renal sites of blood pressure control. Indeed, several drugs already in use for the treatment of hypertension (e.g. ARBs, ACEi, the β-blocker nebivolol) or dyslipidemia (statins) are known to reduce the activation of Nox enzymes. In addition, there is an urgent need to implement universally validated approaches to evaluate oxidative status in human patients. These should cover a broader range of redox biomarkers and would add valuable information for risk stratification and therapeutic monitoring in human patients.

Acknowledgments

"This work was supported by FEDER funds via COMPETE and by national funds through FCT – Portuguese Foundation for Science and Technology [project grant: PTDC/SAU-TOX/114166/2009]."

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