Oxidative stress plays an important role in the pathophysiology of vascular diseases. Reactive oxygen species, especially superoxide anion and hydrogen peroxide, are important signalling molecules in cardiovascular cells. Enhanced superoxide production increases nitric oxide inactivation and leads to an accumulation of peroxynitrites and hydrogen peroxide. Reactive oxygen species participate in growth, apoptosis and migration of vascular smooth muscle cells, in the modulation of endothelial function, including endothelium-dependent relaxation and expression of proinflammatory phenotype, and in the modification of the extracellular matrix. All these events play important roles in vascular diseases such as hypertension, suggesting that the sources of reactive oxygen species and the signalling pathways that they modify may represent important therapeutic targets. Potential sources of vascular superoxide production include NADPH-dependent oxidases, xanthine oxidases, lipoxygenases, mitochondrial oxidases and nitric oxide synthases. Studies performed during the last decade have shown that NADPH oxidase is the most important source of superoxide anion in phagocytic and vascular cells. Evidence from experimental animal and human studies suggests a significant role of NADPH oxidase activation in the vascular remodelling and endothelial dysfunction found in cardiovascular diseases.

Oxidative stress is defined as the imbalanced redox state in which pro-oxidants overwhelm antioxidant capacity, resulting in increased production of reactive oxygen species (ROS). ROS have been implicated in the pathogenesis of virtually every stage of vascular lesion formation in atherosclerosis (Madamanchi et al. 2005; Mueller et al. 2005). Increasing evidence supports a role for oxidative stress in the pathogenesis of experimental and essential hypertension (Touyz, 2004). It has been extensively described that ROS contribute to blood pressure elevation by influencing vascular function and structure (Zalba et al. 2001b; Dröge, 2002).

Production and metabolism of reactive oxygen species

Traditionally, macrophages have been assumed to be the source of most of the ROS in the vessel wall, and there is no doubt that these cells play an important role in vessel pathology (Cathcart, 2004). However, it has become clear that virtually all cells in the vessel wall (endothelial, smooth muscle and adventitial cells) produce ROS, in varying amounts and in response to diverse stimuli, which can then act in an autocrine or paracrine fashion to modulate cellular function (Griendling et al. 2000).

ROS play a physiological role in the vessel wall and participate as second messengers in endothelium-dependent function, in smooth muscle cell and endothelial cell growth and survival, and in remodelling of the vessel wall. Each of these responses, when uncontrolled, contributes to vascular diseases (Griendling & Harrison, 1999; Irani, 2000; Taniyama & Griendling, 2003). The major vascular ROS is superoxide anion (\(\cdot O_2^-\)), which inactivates nitric oxide (NO), the main vascular relaxing factor, thus impairing relaxation (Kojda & Harrison, 1999; Cai & Harrison, 2000). Dismutation of \(\cdot O_2^-\) by superoxide dismutase (SOD) produces hydrogen peroxide (\(H_2O_2\)), a more stable ROS, which, in turn, is converted to water by catalase and glutathione peroxidase. \(H_2O_2\) and other peroxides appear to be important in the regulation of growth-related signalling in vascular smooth muscle cells (VSMCs) and inflammatory responses in vascular lesions (Li et al. 1997; Irani, 2000). High levels of \(\cdot O_2^-\), the consequent accumulation of \(H_2O_2\) and diminished
NO bioavailability play a critical role in the modulation of vascular remodelling. Finally, the reaction product between ·O$_2^-$ and NO, peroxynitrite, constitutes a strong oxidant molecule, which is able to oxidize proteins, lipids and nucleic acids, causing cell damage (Beckman & Koppenol, 1996). These pathological processes are associated with hypertension because they contribute to the narrowing of the arterial lumen and consequently to increased peripheral resistance and blood pressure (Fig. 1).

**NADPH oxidase systems**

Not only are there different cellular sources of ROS, but also cells use different enzymes to produce ROS in different circumstances. In addition to mitochondrial sources of ROS, ·O$_2^-$ can be derived from xanthine oxidase, NADPH oxidases, cyclooxygenases, lipoxygenases and uncoupled nitric oxide synthase. On the basis of experimental and clinical studies it has been proposed that NADPH oxidase is the predominant ·O$_2^-$-producing enzyme in the context of oxidative stress in cardiovascular diseases (Mueller et al. 2005).

The best-characterized NADPH oxidase is that found in phagocytes, where this enzyme is also the major inducible source of ·O$_2^-$ and where it plays a role in immune protection owing to its bactericidal activity (Cathcart, 2004). The phagocytic oxidase consists of a membrane-associated cytochrome that comprises a large subunit, gp91phox (‘phox’ being derived from phagocytic oxidase), and a small one, p22phox. Besides these, there are at least three cytosolic subunits (p47phox, p67phox and p40phox) and a low-molecular-weight G protein (rac2; Cross & Segal, 2004). All the subunits present on phagocytic

![Diagram](image)

**Figure 1. Generation of reactive oxygen species**

Many enzymatic systems have the potential to generate reactive oxygen species. This flow diagram illustrates the functional and structural consequences of reactive oxygen species in arterial hypertension. An enhanced superoxide production, mainly due to NADPH oxidase activation, is involved in endothelial dysfunction and vascular remodelling by decreasing NO bioavailability and increasing production of H$_2$O$_2$ and ONOO$^-$. eNOS, endothelial nitric oxide synthase; SOD, superoxide dismutase; VSMCs, vascular smooth muscle cells; ECs, endothelial cells.
NADPH oxidase have been identified in vascular cells, as well as p47phox, p67phox and gp91phox (nox2) homologues nox1, nox4 and nox5 (Lassegue & Clempus, 2003). However, several studies have confirmed that p22phox is present in all NADPH oxidase systems and that this subunit is essential for the functionality of the enzyme (Ushio-Fukai et al. 1996; Lassegue & Clempus, 2003). Upon cell stimulation, p47phox becomes phosphorylated, and the cytosolic subunits form a complex which migrates to the membrane where it binds to the cytochrome. Then electrons are transferred from the substrate, NADPH, to O$_2$ leading to O$_2^-$ generation (Brandes & Kreuzer, 2005).

Role of reactive oxygen species in vascular physiology and pathophysiology

The intracellular and extracellular production of ROS and the consequent activation of specific signalling pathways coordinate several integrated physiological responses in cardiovascular tissues. Numerous studies have shown that ROS influence cellular processes in vascular remodelling by turning on several intracellular signalling cascades. ROS potently activate extracellular signal regulated kinases (ERKs), mitogen-activated protein kinases (MAPKs) members important in cell growth and differentiation; receptor and non-receptor tyrosin kinases, which have been implicated in cardiovascular remodelling and vascular damage; and protein tyrosin phosphatases and transcription factors, such as NFkB and AP-1, which induce expression of pro-inflammatory genes that play a role in the vascular inflammation associated with hypertension and atherosclerosis (recently reviewed by Touyz & Schiffrin, 2004; Cai, 2005). Nevertheless, in spite of all this evidence, the exact molecular targets of ROS have not been clarified. The physiological processes coordinated by ROS have been analysed in experimental animal models as well as in human disease, showing their important role in the development of hypertensive vascular disease, since they favour structural and functional alterations such as vascular remodelling, augmented deposition of extracellular matrix proteins, inflammatory processes and increased endothelial permeability.

Several studies have established the role of ROS in growth processes that contribute further to vascular injury and remodelling. Angiotensin type 1 (AT1) receptor-mediated production of O$_2^-$ generated by NADPH oxidase is followed by an increased intracellular H$_2$O$_2$, which may participate as a second messenger for the long-term responses of angiotensin II, such as hypertrophy or hyperplasia of VSMCs (Griendling et al. 1994; Ushio-Fukai et al. 1996; Zafari et al. 1998). Angiotension II-induced hypertrophy can be inhibited by the flavoprotein inhibitor DPI (Griendling et al. 1994), by antisense p22phox transfection (Ushio-Fukai et al. 1996) and by catalase overexpression (Zafari et al. 1998), thus supporting the role of vascular NADPH oxidase. Our group reported an enhanced NADPH oxidase-driven -O$_2^-$ production in the aorta of the adult spontaneously hypertensive rat (SHR), which develops vascular wall hypertrophy (Zalba et al. 2000; Fig. 2). A recent study using transgenic mice that overexpress the p22phox subunit in VSMCs demonstrated that increased H$_2$O$_2$ is associated with vascular hypertrophy (Weber et al. 2005). The importance of these studies in experimental animal models is confirmed by studies with human VSMCs showing that enhanced NADPH oxidase-dependent oxidative stress is associated with angiotensin II-induced vascular remodelling in essential hypertension (Touyz & Schiffrin, 2001).

ROS also modulate vascular remodelling by increasing deposition of extracellular matrix proteins. Collagen degradation depends on the activity of enzymes known as metalloproteinases (MMPs; Wainwright, 2004). These MMPs are secreted by macrophages and VSMCs in an inactive form (Galis & Khatri, 2002). ROS activate MMP2 and MMP9, which promote degradation of basement membrane and elastin, respectively, in cultured human smooth muscle cells (Rajagopalan et al. 1996). In particular, peroxynitrite rapidly increases MMP2 activity in the coronary effluent (Wang et al. 2002). Furthermore, increased aldosterone-induced systemic oxidative stress is associated with an increased collagen and fibronectin deposition related to the development of vascular remodelling (Pu et al. 2003). This study demonstrates for the first time that the inflammatory response is associated with vascular remodelling and enhanced oxidative stress, which may be induced by endothelin-1 in this experimental model of hypertension.

Redox-sensitive inflammatory processes also contribute to vascular remodelling in hypertension. Expression of intracellular adhesion molecule-1 (ICAM-1), which participates in the inflammatory process, is increased in the aorta from aldosterone-infused rats (Pu et al. 2003). Moreover, in an experimental model of angiotensin II-enhanced oxidative stress, where the role of NADPH oxidase is clearly demonstrated, induction of ICAM-1 expression is associated with tissue hypertrophy (Liu et al. 2003).

Finally, the endothelial cell redox rheostat is primarily regulated by the dynamic interaction between NO and -O$_2^-$. NO inhibits platelet aggregation and adhesion of leucocytes to the endothelium. Increased endothelial permeability results in the extravasation of plasma proteins and the recruitment of inflammatory proteins and cells. Phagocytic cells participate in oxidative stress and inflammation in patients with hypertension (Kristal et al. 1998). In the study by Liu et al. (2003), angiotensin II-enhanced NADPH oxidase activity plays an important role in vascular hypertrophy, induction of ICAM-1 expression and the consequent leucocyte infiltration.
It is important to note that in cardiovascular diseases not only the vascular oxidase but also the phagocytic NADPH oxidase plays an important role in \( \cdot \text{O}_2^- \) production, since monocytes and lymphocytes can infiltrate cardiovascular tissues (Cathcart, 2004). The relevance of this is underlined by findings showing an enhanced phagocytic NADPH oxidase-dependent \( \cdot \text{O}_2^- \) production in hypertensive patients (Fortuño et al. 2004).

Activation and regulation of the NADPH oxidase systems

Several potential stimulating factors of NADPH oxidase systems deserve to be considered in the setting of arterial hypertension. Although vascular NADPH oxidases are constitutive enzymes, humoral factors have been shown to regulate their activity. It has been extensively demonstrated that vasoactive agonists, such as angiotensin II, endothelin 1(ET-1) and tumor necrosis factor-alpha (TNF-α), regulate NADPH oxidase in vascular cells (De Keulenaer et al. 1998; Liu et al. 2003; Pu et al. 2003). Furthermore, our group has recently reported that phagocytic cells from hypertensive patients show a higher response to physiological concentrations of angiotensin II and ET-1 than cells obtained from normotensive subjects, supporting a potential role of these factors in the activation of mononuclear cells in the hypertensive state (Fortuño et al. 2004).

However, mechanical forces, including cyclic stretch and laminar and oscillatory shear stress, stimulate NADPH oxidase activity. In angiotensin II-infused rats, a reduction of blood pressure is associated with normalized aortic \( \cdot \text{O}_2^- \) production (Virdis et al. 2002). In fact, high intraluminal pressure itself elicits increased \( \cdot \text{O}_2^- \) production in rat femoral arterial branches, and this effect is mediated by NADPH oxidase activation (Ungvari et al. 2003). The question of whether mechanical forces may also play a role in regulation of the phagocytic enzymatic system merits investigation. Nevertheless, our findings that \( \cdot \text{O}_2^- \) production is increased in those treated hypertensive patients in whom blood pressure remains raised suggest that mechanical forces may play an important regulatory role in the activation of the phagocytic NADPH oxidase (Fortuño et al. 2004). In this study we showed that there were no differences in the NADPH oxidase activity between treated, although uncontrolled, and not-treated hypertensive patients and, thus, both groups of patients showed elevated \( \cdot \text{O}_2^- \) levels with respect to normotensive subjects and treated hypertensive patients whose blood pressure levels were controled. Nevertheless, humoral factors, which are also modulated by antihypertensive treatment, may also play a role of in the upregulation of NADPH oxidase activity.

Finally, genetic factors might regulate NADPH oxidase-driven \( \cdot \text{O}_2^- \) production in hypertension. A particular interest has been shown in the gene encoding the p22phox subunit, which possesses a significant number of genetic polymorphisms within the promoter and coding sequences, some of which are able to influence gene expression and NADPH oxidase activity (Zalba et al. 2005). Our group reported the existence of five polymorphisms in the promoter region of the p22phox

Figure 2. NADPH oxidase activity and vascular hypertrophy in rat aorta

A, NADH-driven \( \cdot \text{O}_2^- \) production in homogenates from aortic tissue from adult Wistar Kyoto (WKY\(_{30}\)) and spontaneously hypertensive rats (SHR\(_{30}\)). Bars represent means ± s.e.m. of 10 animals in each group.

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gene, which are associated with higher promoter activity in VSMCs of the SHR (Zalba et al. 2001a). More recently, we have characterized the promoter of the human p22phox gene, in which we have discovered a novel variation, the −930A/G polymorphism, which is associated with essential hypertension (Moreno et al. 2003). The relevance of this association has been underlined recently by a study demonstrating the functionality of the −930A/G polymorphism associated with diminished NO bioavailability (San José et al. 2004).

Conclusions

Increased oxidative stress, mainly due to an enhanced NADPH oxidase activity, results in an exaggerated ·O₂⁻ production that favours the accumulation of H₂O₂ and peroxynitrites and that diminishes NO availability. These pathological processes are associated with hypertension, because they contribute to the narrowing of the arterial lumen, and consequently to increased peripheral resistance and blood pressure.

References


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