Reactive Oxygen Species in the Vasculature
Molecular and Cellular Mechanisms
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Abstract—Accumulating evidence indicates that reactive oxygen species (ROS) play major roles in the initiation and progression of cardiovascular dysfunction associated with diseases such as hyperlipidemia, diabetes mellitus, hypertension, ischemic heart disease, and chronic heart failure. ROS produced by migrating inflammatory cells as well as vascular cells (endothelial cells, vascular smooth muscle cells, and adventitial fibroblasts) have distinct functional effects on each cell type. These include cell growth, apoptosis, migration, inflammatory gene expression, and matrix regulation. ROS, by regulating vascular cell function, can play a central role in normal vascular physiology, and can contribute substantially to the development of vascular disease. (Hypertension. 2003;42:1075-1081.)

Key Words: antioxidants ■ vascular disease ■ muscle, smooth, vascular ■ endothelium ■ free radicals ■ macrophages ■ oxidative stress

Accumulating evidence indicates that oxidative stress plays a major role in the initiation and progression of cardiovascular dysfunction associated with diseases such as hyperlipidemia, diabetes mellitus, hypertension, ischemic heart disease, and chronic heart failure. Oxidative stress is a state in which excess reactive oxygen species (ROS) overwhelm endogenous antioxidant systems. ROS have distinct functional effects on each cell type in the vasculature and can play both physiological and pathophysiological roles. In this review, we will focus on vascular endothelial cells (ECs) and smooth muscle cells (VSMCs), because the effects of ROS on adventitial fibroblasts were reviewed recently.1 We will also briefly discuss the clinical implications of oxidative stress.

Reactive Oxygen Species
One of the most important ROS in the vasculature is superoxide (O$_2^{-}$), which is formed by the univalent reduction of oxygen.2 This reaction is mediated by several enzyme systems including NAD(P)H oxidases and xanthine oxidase ( XO). Although O$_2^{-}$ can exert effects on vascular function, it is also pivotal in generating other reactive species. Reaction of O$_2^{-}$ with NO generates peroxynitrite, a potentially deleterious ROS. Dismutation of O$_2^{-}$ by superoxide dismutase (SOD) produces the more stable ROS, hydrogen peroxide (H$_2$O$_2$), which is then converted enzymatically into H$_2$O by catalase and glutathione peroxidase (GPx). H$_2$O$_2$ can also react with reduced transition metals to be converted to the highly reactive hydroxyl radical (OH), or it can be metabolized by myeloperoxidase (MPO) to form hypochlorous acid (HOCI). Virtually all types of vascular cells produce O$_2^{-}$ and H$_2$O$_2$.3

Enzymatic Superoxide Production
Multiple enzymatic systems produce O$_2^{-}$ and its derivatives in the vasculature, including NAD(P)H oxidases, XO, nitric oxide synthases (NOS), and MPO. The relative importance of each of these proteins appears to vary with the physiological state of the vasculature.

NAD(P)H oxidases consist of multiple subunits: the electron transfer moieties (gp91phox, nox1 or nox4), p22phox, and regulatory subunits (p47phox, p67phox, and rac1). The expression pattern of these subunits varies among vascular cells.4 What makes the NAD(P)H oxidases so important in vascular function is their responsiveness to a variety of agonists, such as angiotensin (Ang) II.5 Enzyme activation occurs over the short term by stimulation of specific intracellular signals6 and over the long term by upregulation of the enzyme subunits.7,8 Even low Ang II concentrations (0.1 nmol/L) increase NAD(P)H oxidase-derived ROS, suggesting that this enzyme system is important physiologically.5

In certain circumstances, NOS can generate O$_2^{-}$ in addition to NO. NOS utilizes L-arginine as a substrate to synthesize NO in a tetrahydrobiopterin (H$_4$B)-dependent manner. If the concentration of L-arginine or H$_4$B is low, or if H$_4$B is oxidized, NOS becomes uncoupled and generates significant amounts of O$_2^{-}$9. This occurs in hypertension, where activation of NAD(P)H oxidases leads to oxidation of H$_4$B and production of large amounts of O$_2^{-}$ from endothelial NOS.10

Xanthine oxidoreductase is ubiquitous and appears in two interconvertible, yet functionally distinct, forms: xanthine dehydrogenase and XO.11 XO metabolizes hypoxanthine, xanthine, and NADH to form O$_2^{-}$ and H$_2$O$_2$. XO-generated
ROS have been implicated in various clinicopathologic entities, including ischemia/reperfusion injury, hypercholesterolemia and endothelial dysfunction in chronic heart failure.11,12 Recently, the role of MPO in vascular pathology has been highlighted. MPO is abundant in phagocytes and catalyzes H2 O2 to produce HOCl and other oxidizing species.13 It also utilizes NO to generate reactive nitrogen species, thereby reducing NO bioactivity and increasing oxidative stress.14,15

**Effects of ROS on Vascular Cells**

**Endothelial Cells**

Many functions of the endothelium are affected by ROS. The most well known is endothelium-dependent vasorelaxation, which is impaired by a loss of NO bioactivity in the vessel wall.16 ROS also cause EC apoptosis, increase monocyte adhesion, and play a role in angiogenesis (Figure).

**Impaired Endothelium-Dependent Vasorelaxation**

In animal models, endothelial dysfunction occurs in association with increased ROS in numerous disease conditions,17 due to inactivation of NO by O2-. Importantly, SOD16 and probucol (a lipid-lowering drug with antioxidant potential)18 improve endothelium-dependent vasorelaxation in hypercholesterolemic animals. Endothelial dysfunction induced by Ang II infusion,19 deoxycorticosterone acetate (DOCA)-salt,20 or heart failure21 is reversed by SOD. Moreover, heterozygous deletion of GPx leads to impaired endothelium-dependent vasorelaxation.22 Because GPx is responsible for removal of peroxides, these data suggest that ROS other than O2- also contribute to control of vasomotor function.

**Apoptosis/Anoikis**

Endothelial injury or exposure to O2- and H2O2 induces apoptosis (programmed cell death) of ECs, which leads to EC loss and results in atherogenesis and a procoagulative state.23 Importantly, EC apoptosis stimulated by oxidized LDL, Ang II, high glucose, and TNF-α is inhibited by SOD, catalase, NAC, and antioxidant vitamins.23 These data strongly suggest that ROS regulate apoptotic mechanisms induced by a variety of stimuli. Another type of programmed cell death, anoikis, results from detachment of ECs from extracellular matrix. This process is also associated with increased intracellular ROS, probably from mitochondria, and is inhibited by NAC and diphenylene iodonium (DPI), a inhibitor of flavin-containing enzymes such as NAD(P)H oxidases.24

**Expression of Adhesion Molecules**

The endothelium normally presents an inert inflammatory surface. However, many proinflammatory stimuli induce the expression of adhesion molecules on ECs, leading to monocyte adhesion and ultimately atherosclerotic lesion formation. Expression of several adhesion molecules, including vascular cell adhesion molecule-1 (VCAM-1) and intracellular adhesion molecule-1 (ICAM-1), is ROS-dependent. Interleukin-1β and TNF-α-induced VCAM-1 gene expression is suppressed by the antioxidants pyrrolidine dithiocarbamate (PDTC) and NAC.25 Furthermore, NO, which, because of its ability to scavenge radicals, can act as an antioxidant and reduces VCAM-1 expression stimulated by TNF-α, possibly by inhibiting the formation of peroxyl-fatty acids.26 Induction of VCAM-1 and ICAM-1 expression by oscillatory shear stress is also inhibited by NAC.27 Taken together, these observations suggest that ROS promote adhesion of inflammatory cells.

**Angiogenesis**

Angiogenesis is important not only for physiological processes such as embryonic development and wound repair but also for pathological processes such as cancer, diabetic retinopathy, and atherosclerosis. EC migration, proliferation, and tube formation are essential events in the process of angiogenesis. ROS may be directly involved in all these mechanisms, as H2O2 has been shown to induce proliferation and migration of ECs and to mediate lymphocyte-activated tubulogenesis.28 ROS also act as mediators of angiogenic
growth factors, such as vascular endothelial growth factor (VEGF). It has been reported that NAD(P)H oxidases regulate not only the induction of VEGF expression but also VEGF-induced angiogenesis.

**Vascular Smooth Muscle Cells**

Many functions of VSMCs also depend on the production of ROS (Figure). Perhaps the most well-studied of these processes is cell growth, but ROS are also involved in migration as well as expression of inflammatory mediators and matrix components. In addition, ROS have been implicated in VSMC contraction.

**VSMC Growth**

Synthetic VSMCs, whose phenotype has been altered to support growth, are found in cardiovascular diseases such as hypertension, atherosclerosis, and restenosis after balloon angioplasty. ROS production is intimately involved in many of the processes leading to both hypertrophic and proliferative VSMC growth.

It has been known for many years that the vasoactive peptide Ang II can induce VSMC hypertrophy. This peptide was used to provide one of the first demonstrations that ROS are involved in the hypertrophic response. Ang II–induced VSMC hypertrophy is inhibited by catalase and p22phox antisense, thus implicating NAD(P)H oxidase-derived ROS in the growth response.

ROS also mediate the full proliferative response to agonists such as PDGF and thrombin. \( \text{H}_2\text{O}_2 \) itself induces VSMC proliferation, although this effect is critically dependent on the concentration of \( \text{H}_2\text{O}_2 \) to which cells are exposed (see below). Furthermore, VSMC proliferation by PDGF or thrombin requires \( \text{H}_2\text{O}_2 \) generation, as it is inhibited by catalase, NAC, or DPI. Endogenously produced \( \text{H}_2\text{O}_2 \) may also be important in modulating survival and proliferation of VSMCs, because overexpression of catalase inhibits basal smooth muscle proliferation while increasing the rate of apoptosis.

Although the effects of ROS described above are positive for VSMC growth, ROS induce VSMC apoptosis and differentiation as well. Li et al. showed that exposure of VSMCs to glucose/glucose oxidase or diethylmaleate induces apoptosis through the formation of hydroxyl radicals, whereas Su et al. reported that endogenous ROS can increase VSMC maturation and differentiation. These apparent paradoxical effects may be explained by the identity and amount of ROS to which VSMCs are exposed. High concentrations of \( \text{H}_2\text{O}_2 \) (500 \( \mu \text{mol/L} \) to 1 \( \text{mmol/L} \)) induce apoptosis, whereas moderate concentrations (100 \( \mu \text{mol/L} \)) cause cell cycle arrest in G1.

**Migration**

VSMC migration is considered to be one of the major components of vascular pathogenesis. Although the precise molecular mechanisms of VSMC migration are unclear, a role for ROS has clearly been demonstrated. Sundaresan et al. showed that PDGF-induced VSMC chemotaxis is inhibited by catalase overexpression. This has recently been confirmed by Weber et al., who demonstrated that VSMC migration stimulated by PDGF is inhibited by NAC, DPI, ebselen, and dominant-negative Rac, suggesting that \( \text{O}_2^- \) production through the NAD(P)H oxidase is critical for agonist-stimulated VSMC migration. It will be of great interest to identify the cascade of signaling molecules that are responsible for ROS-dependent VSMC migration.

**Matrix Regulation**

Degradation and reorganization of the extracellular matrix by matrix metalloproteinases (MMPs) are prominent events in physiological and pathological vascular remodeling. Recent studies have revealed that activity of MMPs can be modulated by ROS. Rajagopal et al. demonstrated that pro-MMP-2 and pro–MMP-9 secreted from human VSMCs are activated by ROS. Insight into the mechanism by which ROS regulate MMP activity was provided by Fu et al., who found MPO-derived HOCl activates MMP-7 by oxygenation of the cysteine residue, which is a mechanism distinct from the well-known proteolytic cleavage of MMP proenzyme. Gene expression of MMPs can also be regulated by ROS. In VSMCs exposed to mechanical stretch, MMP-2 mRNA is increased in an NAD(P)H oxidase-derived, ROS-sensitive manner. Thus, ROS modulate matrix remodeling at multiple levels.

**Inflammatory Gene Expression**

It has been recognized that atherosclerosis is an inflammatory disease in which various cytokines play a significant role in the progression of vascular lesions. One of the major mechanisms by which cytokine gene expression is increased is the activation of nuclear factor-\( \kappa \)-B (NF-\( \kappa \)-B). NF-\( \kappa \)-B is a ROS-sensitive transcription factor and has a central role in the expression of proinflammatory genes, including monocyte chemotactic protein-1 (MCP-1) and interleukin-6. In VSMCs, Ang II and TNF-\( \alpha \) were shown to induce these genes through the activation of NF-\( \kappa \)-B in a ROS-dependent manner.

**Contraction**

As discussed above, it is well established that \( \text{O}_2^- \) regulates vasomotor tone through the inactivation of NO. However, the direct effect of ROS on VSMC is controversial. \( \text{H}_2\text{O}_2 \) induces vasorelaxation of pulmonary, coronary, and mesenteric arteries. Contrast, ROS generated by XO are vasoconstrictive in aorta, and ROS-induced contraction is augmented in spontaneously hypertensive rats (SHR). Furthermore, contraction of aorta to Ang II is inhibited by catalase. These apparent discrepancies may be due to the vascular bed studied or the concentration of ROS generated in the particular system. Further work is necessary to fully elucidate the effects of ROS on contraction.

**Clinical Implications**

**Atherosclerosis**

As discussed above, ROS limit the bioavailability of NO and induce inflammatory gene expression, cell growth/apoptosis, migration, and matrix reorganization, all of which are central mechanisms for initiation and progression of atherosclerosis. NAD(P)H oxidases, XO, and MPO have been implicated as potential sources of ROS in this disease.
Atherosclerotic lesions in human coronary arteries show intense expression of gp91phox in the vulnerable shoulder region of the plaque, coincident with macrophage localization. In addition, nox4 is upregulated during the atheroma phase of lesion formation but is reduced in advanced lesions. The functional significance of these enzyme systems was confirmed by animal experiments. Genetic deletion of p47phox in ApoE knockout mice (ApoE/−/−, a model of atherosclerosis), results in a reduction of lesion area in the descending aorta compared with that in ApoE/−/− mice. This study indicates that NAD(P)H oxidase–derived ROS generation has a requisite role in atherosclerotic lesion formation.

Less is known about the role of XO and MPO in atherosclerosis. In hypercholesterolemic rabbit aorta and human atherosclerotic coronary artery, a role for XO-derived ROS in impaired endothelium-dependent vasorelaxation was reported. Furthermore, XO is present at high concentrations in atherosclerotic plaques. In human subjects, there is a remarkable association between blood MPO levels and risk of atherosclerosis, suggesting a protective role for MPO in the progression of atherosclerosis. Further investigations are needed to define the cause-effect relation between XO, MPO, and atherosclerosis.

One of the defining features of atherosclerosis is the episodic nature of lesion development. Hemodynamic influences are proposed to be responsible for the discontinuous nature of plaque formation because lesions tend to form in regions of disturbed flow. Whereas introduction of laminar shear stress presents a transient prooxidant signal that is quickly corrected by upregulation of antioxidant enzymes, oscillatory shear stress activates both NAD(P)H oxidases and XO in the absence of a compensatory upregulation of SOD. Based on the known effects of ROS on endothelial function (see above), these data suggest that ROS produced by disturbed flow may be one mechanism by which these areas are predisposed to lesion formation.

Hypertension

A number of studies have suggested that oxidative stress is deeply involved in the pathogenesis of hypertension. These effects are mediated by inactivation of NO by O$_2^-$ in the vasculature and the kidney, and by H$_2$O$_2$-induced vessel remodeling. Vaziri et al showed that induction of oxidative stress by glutathione depletion causes severe hypertension in normal rats. Furthermore, Ang II–induced O$_2^-$ production and hypertension are markedly blunted in mice lacking the p47phox subunit of NAD(P)H oxidase. Renovascular hypertension (RVH), an Ang II–dependent form of hypertension, is accompanied by increased oxidative stress in animal models and human subjects. The cell-permeable SOD mimetic tempol lowers blood pressure in the 1-kidney, 1-clip model of RVH. Oxidative stress plays a role in low renin hypertension as well. In the DOCA-salt hypertension model, vascular production of O$_2^-$ is increased in association with upregulation of p22phox and long-term treatment with tempol lowers blood pressure. Finally, in the SHR, blood pressure can be lowered with PEG-SOD, tempol, or an antioxidant-rich diet.

When investigating the role of oxidative stress in models of vascular disease, the effect of the intervention on both the enzymes that produce ROS and those responsible for their removal must be considered. For example, Ang II not only increases NAD(P)H oxidase activity but also upregulates eNOS, possibly to compensate for the increased ROS. In situations where this compensatory effect is efficient, ROS levels may appear normal even in the face of prooxidant stresses such as Ang II. In Dahl salt-resistant rats on a low salt diet, Ang II levels are high but O$_2^-$ is actually decreased, perhaps because of the accompanying increase in MnSOD expression. This ability to increase antioxidant defenses may be sufficient to protect the vasculature from low levels of oxidant stress, allowing ROS to function as signaling molecules. However, when ROS production becomes overwhelming, compensatory mechanisms are inadequate and pathophysiological consequences ensue.

Diabetes Mellitus

Cardiovascular complications are the leading cause of morbidity and mortality in patients with diabetes mellitus (DM). Evidence implicating ROS in the development of diabetic vascular dysfunction has been expanding rapidly in recent years. Hyperglycemia and increased free fatty acids in the bloodstream, the chief characteristics of DM, can both lead to leakage of O$_2^-$ from the mitochondrial respiration process and NAD(P)H oxidase activation. Furthermore, saphenous veins and internal mammary arteries from diabetic patients have increased NAD(P)H oxidase activity and uncoupling of eNOS compared with that in matched control subjects. Increased production of ROS, in turn, leads to impaired endothelium-dependent vasorelaxation in models of both type 1 and type 2 DM. The relation between ROS and the vascular complications of DM is discussed more fully in a recent review.

Restenosis After Angioplasty

Restenosis is a frequent complication of balloon angioplasty. In this pathological process, VSMCs undergo apoptosis, proliferation, and migration. Adventitial fibroblasts also participate in lesion formation by differentiating into myofibroblasts and migrating to the neointima. As discussed above, ROS are able to induce these phenotypic changes, suggesting that they may contribute to the pathogenesis of restenosis in vivo.

Superoxide production is increased after balloon injury, especially in medial and neointimal smooth muscle cells and adventitial fibroblasts. ROS appear to be functionally important, because neointima formation is inhibited by antioxidants. Although the oxidase(s) responsible for the production of ROS after balloon injury have not been fully identified, a role for NAD(P)H oxidases has been reported. DPI abolishes O$_2^-$ generation in porcine coronary arteries. Subunits of the enzyme, including p47phox, nox1, nox4, gp91phox, and p22phox, are upregulated after injury.

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Very recently, Jacobson et al. reported that a specific peptide inhibitor for NAD(P)H oxidases inhibited restenosis, suggesting a mechanistic role for these enzymes in restenosis.

Controversy in Clinical Trials

Although numerous basic and animal studies have clearly identified a major role for ROS in the development and progression of cardiovascular diseases, the clinical benefits of antioxidant administration have been disappointing. However, a critical evaluation of the extant clinical trials suggests several reasons why antioxidants are sometimes not effective for primary and secondary prevention of cardiovascular diseases in large-scale clinical trials. First, administration of antioxidants to patients with established lesions does not provide coverage during the establishment of vascular disease, when ROS production is increased (e.g., initiation of the lesion). Second, there may be a difference in the extent of the contribution of specific ROS to various cardiovascular diseases, thereby limiting the effectiveness of specific antioxidants. For example, the antioxidant vitamins do not scavenge H2O2 or HOCl, which may be more important than O2− in the development of atheroma (see above). Finally, the antioxidants tested so far are weak compared with the endogenous antioxidant defense systems, and many times the effectiveness of the antioxidants to reduce ROS was not tested or was tested only against O2−-induced processes. Better antioxidants are necessary to truly test the concept of oxidative stress as a central regulator of human vascular disease. Future clinical trials as well as basic studies must be designed to answer the questions such as these.

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References


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