Modulation of Oxidant and Antioxidant Enzyme Expression and Function in Vascular Cells

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Abstract—Pathological conditions that predispose to cardiovascular events, such as hypertension, hypercholesterolemia, and diabetes, are associated with oxidative stress. These observations and further data derived from a plethora of investigations provided accumulating evidence that oxidative stress is decisively involved in the pathogenesis of endothelial dysfunction and atherosclerosis. Several enzymes expressed in vascular tissue contribute to production and efficient degradation of reactive oxygen species, and enhanced activity of oxidant enzymes and/or reduced activity of antioxidant enzymes may cause oxidative stress. Various agonists, pathological conditions, and therapeutic interventions lead to modulated expression and function of oxidant and antioxidant enzymes, including NAD(P)H oxidase, endothelial nitric oxide synthase, xanthine oxidase, myeloperoxidase, superoxide dismutases, catalase, thioredoxin reductase, and glutathione peroxidase. Data from numerous studies underline the importance of dysregulated oxidant and antioxidant enzymes for the development and progression of atherosclerotic disease in animal models and humans. Specific pharmacological modulation of key enzymes involved in the propagation of oxidative stress rather than using direct antioxidants may be an approach to reduce oxygen radical load in the vasculature and subsequent disease progression in humans. This review focuses on the modulation of expression and activity of major oxidant and oxidant enzymes expressed in vascular cells. (Hypertension. 2004;44:381-386.)

Key Words: free radicals ■ oxidative stress ■ enzymes ■ gene regulation ■ atherosclerosis

The family of reactive oxygen species (ROS) includes highly bioactive, short-living molecules that are derived from reduction of molecular oxygen. Multiple enzyme systems use different substrates as sources of electrons to produce a variety of ROS, including superoxide, hydroxyl radical, hydrogen peroxide, peroxynitrite, hypochlorous acid, and lipid radicals. Several enzymes are expressed in vascular tissue that contribute to production as well as to degradation of ROS (Figure 1). Under physiological conditions, ROS formation and elimination are delicately balanced in the vascular wall. However, enhanced activity of oxidant enzymes and/or reduced activity of antioxidant enzymes lead to oxidative stress.

Oxidant Enzyme Systems

NAD(P)H Oxidase
A major source of ROS in vascular cells is the NAD(P)H oxidase, which consists of the membrane subunits gp91phox and p22phox and the cytosolic subunits p67phox, p47phox, and the small GTPase rac1. The subunits assemble on activation and form the functional enzyme, which after electron transfer to molecular oxygen produces superoxide radicals. In contrast to endothelial cells (EC) and adventitial cells, gp91phox is expressed only at low levels in vascular smooth muscle cells (VSMC) and is (functionally) replaced by the homologue nox1. VSMC from resistance arteries, however, express a functionally active gp91phox subunit. Regulation of the expression levels and translocation of the different NAD(P)H oxidase subunits leads to changes in activity of the entire enzyme system.

Xanthine Oxidase
Xanthine oxidase catalyzes the oxidation of xanthine and hypoxanthine during purine metabolism. This enzyme reduces molecular oxygen, leading to the formation of both superoxide and hydrogen peroxide. Xanthine oxidase is capable of producing large amounts of ROS under pathological conditions. The enzyme is not only expressed in vascular cells but also circulates in the plasma and binds to endothelial cell extracellular matrix.

Myeloperoxidase
Myeloperoxidase is a hemoprotein expressed in neutrophils and monocytes, which is secreted during activation of these cells and localizes in and around endothelial cells after leukocyte degranulation. Myeloperoxidase uses hydrogen peroxide to produce hypochlorous acid in a 2-step reaction involving the redox reaction intermediate compound I. The enzyme also exerts classic peroxidase activity and oxidizes a variety of organic substrates to reactive oxygen intermediates.
The superoxide dismutases (SOD) are a major cellular defense system against superoxide in all vascular cells. Three different isoforms of SOD have been identified: the mitochondrial manganese-containing SOD (MnSOD, SOD-2), the cytosolic copper/zinc-containing SOD (CuZnSOD, SOD-1), and the extracellular SOD (ecSOD, SOD-3), which is also a copper/zinc-containing enzyme that is mainly produced and secreted by VSMC and binds to glycosaminoglycans in the vascular extracellular matrix on the endothelial cell surface. EcSOD plays an important role in the regulation of the oxidant status in the vascular interstitium.

**Glutathione Peroxidase**

Reduced glutathione plays a major role in the regulation of the intracellular redox state of vascular cells by providing reducing equivalents for many biochemical pathways. Glutathione peroxidase (GPX) is a selenium-containing antioxidant enzyme that effectively reduces hydrogen peroxide and lipid peroxides to water and lipid alcohols, respectively, and in turn oxidizes glutathione to glutathione disulfide. In the absence of adequate GPX activity or glutathione levels, hydrogen peroxide and lipid peroxides are not detoxified and may be converted to hydroxyl radicals and lipid peroxyl radicals, respectively, by transition metals (eg, Fe²⁺). The GPX/glutathione system is thought to be a major defense in low-level oxidative stress.

**Catalase**

Catalase is an intracellular antioxidant enzyme that is mainly located in cellular peroxisomes and to some extent in the cytosol, which catalyzes the reaction of hydrogen peroxide to water and molecular oxygen in a 2-step reaction involving Compound I. By removing hydrogen peroxide, it indirectly detoxifies superoxide radicals, which are turned into hydrogen peroxide by SOD. The enzyme also has peroxidase activity and reacts with organic peroxides and hydrogen donors to water and organic alcohols. Catalase is very effective in high-level oxidative stress and protects cells from hydrogen peroxide produced within the cell. The enzyme is especially important in the case of limited glutathione content or reduced GPX activity and plays a significant role in the development of tolerance to oxidative stress in the adaptive response of cells.

**Thioredoxin Reductase**

Thioredoxin reductase is an antioxidant enzyme that participates in thiol-dependent cellular reductive processes. The enzyme regenerates reduced thioredoxin, which serves as reducing equivalent, and may also directly reduce lipid hydroperoxides. In addition, thioredoxin and glutaredoxin, which catalyzes glutathione-dependent disulfide reduction, are reductants for plasma GPX, allowing the latter enzyme to reduce hydroperoxides in surroundings with low levels of glutathione. Moreover, thioredoxin was shown to enhance MnSOD expression. The thioredoxin system may effectively regenerate proteins that were inactivated by oxidative stress.

**Regulation of Oxidant and Antioxidant Enzyme Expression and Activity**

There is a tight regulation of production and elimination of ROS in the vasculature. However, several agonists and pathological conditions may lead to dysregulated expression and activity levels of the oxidant and antioxidant enzyme systems.
systems; in contrast, certain interventions and drugs may decrease oxidative stress.

Angiotensin II

Angiotensin II (Ang II) is a potent stimulus for the production of ROS in vascular cells. One of the main actions in this context is the activation of the NAD(P)H oxidase. Ang II enhances NAD(P)H oxidase activity within minutes of stimulation because of changes in the assembly of the enzyme subunits, but longer incubation periods for hours markedly increase enzyme activity, mainly as a result of upregulated subunit expression, including nox1 and rac1 in VSMC, p22phox, gp91phox, p67phox, and p47phox in EC and VSMC of resistance vessels, and p67phox in fibroblasts. Activation and membrane translocation of rac1 seem to be particularly important for the activation of the enzyme complex. Ang II stimulation causes enhanced expression, GTPase activity, and membrane translocation of rac1 in VSMC. In animals made hypertensive by Ang II infusion, vascular superoxide production, NAD(P)H oxidase activity, and expression levels of the p22phox, gp91phox, p67phox, and nox1 subunits were enhanced. Further experiments with SOD injection or genetic disruption of p47phox demonstrated the importance of NAD(P)H oxidase-mediated superoxide production for Ang II–induced hypertension. In other models of hypertension, an increased NAD(P)H oxidase activity was found, which was either associated with increased Ang II levels (2-kidney–1-clip rats) or could be normalized by treatment with an AT1 receptor antagonist (spontaneously hypertensive rats). Ang II infusion has also been shown to upregulate vascular eNOS expression, which was accompanied with uncoupling of the enzyme, reduced NO production, and enhanced superoxide release. In a genetic model of endogenous Ang II overproduction, enhanced activity of vascular and renal xanthine oxidase was found. These studies indicate that besides NAD(P)H oxidase, other enzymes contribute to Ang II–induced oxidative stress in the vasculature, as well. Probably as an adaptive negative feedback mechanism, Ang II increases vascular expression of ecSOD in vitro and in vivo. Finally, modulation of AT1 receptor expression levels by various agonists influences Ang II–induced ROS production in vascular cells.

Growth Factors and Cytokines

Many growth factors are known to influence oxidant and antioxidant enzyme function. Platelet-derived growth factor, epidermal growth factor, transforming growth factor-β, and thrombin lead to increased subunit expression and activity of the NAD(P)H oxidase and to decreased ecSOD expression in VSMC. The proinflammatory cytokines interferon-γ, interleukin-1, and especially tumor necrosis factor-α seem to promote oxidant effects in vascular cells by activating NAD(P)H oxidase and xanthine oxidase, although there are differential regulations of antioxidant enzymes, for example, ecSOD, MnSOD, and GPX, presumably because of compensatory mechanisms. During endotoxemia, lipopolysaccharides induce oxidative stress by enhancing xanthine oxidase and NAD(P)H oxidase expression and activity, and superoxide, hydrogen peroxide, and peroxynitrite formation.

Reactive Oxygen Species

ROS not only are products of oxidant enzymes but also are involved in the regulation of oxidant and antioxidant enzymes. Superoxide enhances the activity of myeloperoxidase and may activate xanthine oxidase in EC. The reaction product of superoxide and NO, peroxynitrite, leads to oxidative destruction of tetrahydrobiopterin, which causes uncoupling of eNOS, as demonstrated in various animal models, including Ang II–induced hypertension, DOCA-salt hypertension, and SHR. However, peroxynitrite may induce thioredoxin reductase expression in EC, possibly as a protective mechanism during oxidative stress. Hydrogen peroxide is capable of activating NAD(P)H oxidase and reducing the activity of CuZnSOD and ecSOD, but may also increase the expression of catalase and eNOS.

Nitric Oxide

NO rapidly reacts with ROS, and the scavenging of ROS as well as the formation of new radicals by these reactions influence cellular function of vascular cells. NO leads to upregulation of ecSOD and MnSOD in VSMC and inhibits xanthine oxidase activity in hypoxia.

Lipids

Hypercholesterolemia is associated with increased oxidative stress, eNOS uncoupling occurs, and NAD(P)H oxidase activity is increased. Low-density lipoprotein (LDL) cholesterol and oxidized LDL upregulate nox1 and gp91phox expression and NAD(P)H oxidase activity. As an adaptive process, increased MnSOD and catalase expression were found.

Hormones

Estrogens act antioxidative in vascular cells. 17β-Estradiol decreases NAD(P)H oxidase activity by downregulation of rac1 expression and activity (VSMC) and gp91phox expression (EC), and enhances eNOS expression and activity in EC. Similar effects were seen with the selective estrogen receptor modulator raloxifene. Furthermore, 17β-estradiol was shown to enhance expression and activity of MnSOD and ecSOD in VSMC, and to induce the thioredoxin system in EC by increasing thioredoxin, glutaredoxin, and thioredoxin reductase expression. In contrast to estrogen, progesterone increases ROS production, decreases the expression of MnSOD and ecSOD, and abolishes the effects of estrogen on SOD expression and ROS production in VSMC (unpublished observations). In humans, progesterone was reported to counteract the beneficial effects of estrogen on endothelial function.

Glucose and Insulin

Data from animal and human studies show that diabetes mellitus is associated with increased oxidative stress. In cultured EC and VSMC, NAD(P)H oxidase activity and superoxide production are enhanced by high glucose levels involving protein kinase C activation, and an upregulation of
p22phox expression was found in isolated arteries stimulated with high glucose. In experimental models of diabetes, such as the streptozotocin-treated rat (model of type 1 diabetes), high glucose levels are associated with enhanced vascular NAD(P)H oxidase activity and superoxide production, increased expression of gp91phox and p22phox, protein kinase C activation, reduced glutathione levels, enhanced xanthine oxidase activity, dysfunctional eNOS, and impaired endothelial function. In blood vessels from patients with type 2 diabetes, an upregulation of p22phox, p47phox, and p67phox, and an enhanced NAD(P)H oxidase activity were found. In genetically diabetic rats (model of type 2 diabetes), a similar activation of the NAD(P)H oxidase was demonstrated. Taken together, metabolic changes associated with diabetes mellitus lead to a strong activation of several oxidant enzyme systems in the vasculature.

Drugs

Antioxidants such as vitamin C, vitamin E, probucol, tiron, or N-acetylcysteine directly scavenge and inactivate ROS rather than interfering with expression and function of oxidant and antioxidant enzymes.

Allopurinol and its active metabolite oxypurinol are direct inhibitors of xanthine oxidase and suppress xanthine oxidase-mediated ROS production in vascular cells. Oxypurinol decreased ROS production and lowered blood pressure levels in diabetic animals and SHR. In addition, allopurinol and oxypurinol were shown to reduce oxidative stress and to improve endothelial function in diabetic, hyperuricemic, and hypercholesterolemic patients.

HMG-CoA reductase inhibitors (statins) block not only cholesterol synthesis but also isoprenoid metabolism, which is needed for posttranslational modifications of numerous regulatory proteins, such as small GTP-binding proteins. Statins lead to upregulation of eNOS expression and activity in EC, and to increased expression and activity of catalase in VSMC. Otherwise, statins inhibit ROS production by reduction of NAD(P)H oxidase activity, decreased expression of nox1 in VSMC and gp91phox, p22phox, and p47phox in EC, and inhibition of activity and geranylgeranylation-dependent membrane translocation of rac1 GTPase. Taken together, the described effects of statins result in decreased vascular ROS production, increased NO bioactivity, and improvement of endothelial function.

Peroxisome proliferator-activated receptors (PPAR) are ligand-activated transcription factors, which have been shown to mediate anti-inflammatory actions in vascular cells. Activators of PPARα (lipid-lowering fibrate derivatives) and PPARγ (antidiabetic thiazolidinediones) reduce the expression of p22phox and p47phox, decrease NAD(P)H oxidase activity and ROS production, and increase CuZnSOD and catalase expression and NO release in EC. Moreover, PPARα agonists diminish ROS production in Ang II-infused rats and decrease p22phox expression and improve endothelial function in diabetic rats. These data show that PPAR agonists may exert favorable effects on the oxidant status of vascular cells.

Angiotensin-converting enzyme inhibitors and AT1 receptor antagonists are inhibitors of the renin-angiotensin system that do not act as direct antioxidants. However, these drugs inhibit the actions of Ang II and thus block the activation of oxidant enzymes and redox-sensitive genes by Ang II (see Angiotensin II section of this article). In many cell culture and animal studies, their antioxidant potential was demonstrated, associated with improvement of endothelial function and inhibition of atherosclerotic lesion formation in the absence of blood pressure reduction. Antioxidant effects and improvement of vascular function were confirmed in human studies.

Other antihypertensive drugs, namely β-blockers and calcium channel antagonists, have been shown to exert antioxidant properties as well. Third-generation β-blockers like carvedilol and nebivolol may enhance NO release in EC, increase vascular glutathione content, inhibit ROS formation, and reduce lipid peroxidation. Several calcium channel blockers were shown to inhibit oxidation of LDL and other lipids in animals and humans. For example, nifedipine increases NO bioavailability by decreased ROS formation in EC and enhances MnSOD expression in VSMC.

Oxidative Stress and Atherosclerosis

Oxidative stress may lead to many cellular events, such as inactivation of NO, oxidative modifications of DNA and proteins, lipid oxidation, enhanced mitogenicity and apoptosis of vascular cells, and increased expression and activation of redox-sensitive genes, such as the receptor for oxidized LDL, adhesion molecules, proinflammatory cytokines, regulators of cell cycle progression, and matrix metalloproteinases (Figure 2). Several cardiovascular risk factors are associated with oxidative stress and dysregulated expression and activity of oxidant and antioxidant enzymes, including arterial hypertension, hypercholesterolemia, diabetes mellitus, and cigarette smoking, and the described effects of ROS and impaired NO bioactivity in vascular cells contribute to the development and progression of atherosclerosis at all stages of the disease (Figure 2).

Increased levels of ROS were demonstrated in all layers of the diseased arterial wall and within the atherosclerotic plaque. Many studies demonstrated an upregulation of several vascular NAD(P)H oxidase subunits and increased enzyme activity in atherosclerotic animals and humans and after vascular injury. Genetic disruption of p47phox strikingly inhibited atherosclerotic lesion formation in mice. Besides dysregulated NAD(P)H oxidase, increased xanthine oxidase activity and reduced ecSOD activity were found in coronary arteries and the plasma of patients with coronary artery disease. Taken together, data from numerous studies underscore the notion that modulation of oxidant and antioxidant enzymes, leading to oxidative stress, plays an important role in the pathogenesis of atherosclerotic disease. Recently, low levels of GPX activity and elevated levels of myeloperoxidase were shown to be an independent risk factor for cardiovascular events in patients with coronary artery disease and in patients presenting with chest pain.

Perspectives

From the available data, it may be assumed that reduction of oxidative stress and restoration of NO bioactivity are important mechanisms for the improvement of endothelial dysfunction, the treatment of more advanced stages of atherosclero-
s, and for the treatment of restenosis after vascular injury. Small clinical trials with antioxidants like vitamins E and C demonstrated improvements of endothelial function and surrogate parameters of atherosclerosis. However, recent large clinical trials with antioxidants like vitamins E and C did not show therapeutic benefits concerning cardiovascular events and mortality. The lack of effect of these substances on hard end points may be explained by pharmacokinetic and pharmacodynamic properties. In contrast to local treatment (eg, intraarterial infusion), antioxidant vitamins may be (partly) inactivated after oral treatment before they reach the vasculature, thus producing only modest increases of their plasma and tissue concentrations. Vitamin E concentrates predominantly in cellular lipid bilayers and lipoproteins, and may therefore not interfere appropriately with ROS accumulating in the cytoplasm or the interstitial space. This vitamin does not react as rapidly with superoxide as does NO, thus leading to incomplete prevention of NO inactivation. Importantly, the antioxidant vitamins E and C may act pro-oxidant themselves by being converted into tocopheroxyl and ascorbyl radicals, respectively, which may enhance lipid peroxidation. In addition, antioxidants may be more beneficial in primary prevention rather than in patients with advanced atherosclerotic disease. However, there is evidence that some drugs that do not act as direct antioxidants, for example, statins and AT1 receptor antagonists, exert their therapeutic benefits at least in part through antioxidant actions. This may have several implications. First, testing diseased individuals for markers of oxidative stress could be used for risk stratification. Treatment with potent antioxidants may potentially be beneficial in this group of patients. Second, given the fact that dysregulated oxidant and antioxidant enzyme expression and function is found in patients with cardiovascular risk factors and manifested atherosclerotic disease (Figure 2), specific pharmacological modulation of key enzymes, such as inhibition of the vascular NAD(P)H oxidase, may be an effective approach to reduce vascular oxidative stress and subsequent disease progression in humans that is potentially more powerful than the use of systemic antioxidants. These important issues should be clarified in future studies.

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