Oxidative Stress at the Vascular Wall. Mechanistic and Pharmacological Aspects

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During the process of energy production in aerobic respiration, vascular cells produce reactive oxygen species (ROS). A growing body of evidence indicates that oxidative stress refers to a condition in which cells are subjected to excessive levels of ROS. Overall vascular function is dependent upon a fine balance of oxidant and antioxidant mechanisms, which determine endothelial functions. Considerable experimental and clinical data indicate that intracellular oxidant milieu is also involved in several redox-sensitive cellular signaling pathways such as ion transport systems, protein phosphorylation, and gene expression and thus also plays important roles as modulator of vascular cell functions such as cell growth, apoptosis, migration, angiogenesis and cell adhesion. Overproduction of ROS under pathophysiologic conditions is integral in the development of cardiovascular diseases. This fact has raised an intensive search of new pharmacological approaches to improve vascular hemostasis and particularly those intended to decrease oxidative stress or augment the antioxidant defense mechanisms.

Key Words: Oxidative stress, Vascular functions, Endothelial cells, Vascular smooth muscle cells, Pharmacology.

Introduction

The endothelium is now recognized not only as a physical barrier between blood and vascular wall but also as an important and strategically located organ with multiple endocrine and paracrine functions. Additionally, it is able to sense changes in hemodynamic forces and blood-borne signals and responds by releasing vasoactive substances. Under physiological conditions, the vascular endothelium acts as an inhibitory regulator of vascular contraction, leukocyte adhesion, vascular smooth muscle cell growth and platelet aggregation through the production of an array of biologically active molecules (1,2).

Growing evidence indicates that oxidative stress refers to a condition in which cells are subjected to excessive levels of molecular oxygen or its chemical derivatives called reactive oxygen species (ROS) (3,4). Overproduction of ROS under pathophysiologic conditions is integral in the development of cardiovascular diseases. Therefore, it is important to highlight the term overproduction, because considerable data indicate that the intracellular oxidant milieu is also involved in cellular signaling and thus also plays important roles as modulator of vascular cell functions (5–7). This increased oxidative stress impairs endothelial functions, now considered as a serious causative factor of vascular dysfunction, and plays an important role in the pathophysiology of several vascular diseases including atherosclerosis, diabetes mellitus, neuronal disorders, and ischemia-reperfusion injury (8,9).

Furthermore, overall vascular function is dependent upon the balance of oxidant and antioxidant mechanisms, which determines endothelial function.

In the present review we summarize the main sources of ROS at vascular wall, the interacting capacity of ROS with some redox-sensitive processess involved in physio- and pathophysiological processess as well as the main action

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of ROS on endothelial and vascular smoth muscle cells. Finally, some pharmacological approaches to circumvent the ROS effects at vascular wall are also discussed.

Vascular Oxidant Stress

Vascular cells produce energy by reducing molecular oxygen to water during aerobic respiration. During this process, reactive species are generated such as superoxide anion ($O_2^{-}\cdot$) hydrogen peroxide (H_2O_2), peroxynitrite ($OONO^-$), hypochlorous acid (HOCl) and the hydroxyl radical ($OH^{\bullet}$), among others (10,11). Under homeostatic conditions, these molecules play regulatory roles in cellular function (Figure 1), and antioxidant defenses are critical to modulate their steady state balance, which is now recognized as a key mechanism for the maintenance of vascular health (12).

An important consequence of vascular oxidant stress, with dramatic results on vascular homeostasis, is the impaired nitric oxide bioavailability produced by its inactivation by superoxide. Superoxide anion rapidly reacts with nitric oxide and eliminates its biological activity (13).

Sources of ROS

In vascular wall, several enzymatic systems produce $O_2^{-}\cdot$ and its derivatives in the vasculature, including NAD(P)H oxidases, xanthine oxidase (XO), nitric oxide synthases (NOS), and myeloperoxidase (MPO).

NAD(P)H oxidase

Compelling evidence suggests that NAD(P)H oxidases, also known as NOX enzymes, constitute the main enzymatic source of endothelial and vascular $O_2^{-}\cdot$. Nox proteins represent the catalytic subunits of these enzymes and vary in terms of their mode of activation and need for cofactor activation (14). Nox1 protein levels are quite low in vascular cells but can be induced by stimuli such as PDGF and angiotensin II (Ang II) (14). Nox2, previously known as $gp91^{phox}$, is expressed in endothelial and adventitial cells of large vessels and in the vascular smooth muscle cells of smaller vessels (15–17). Nox4 is constitutively expressed and active in vascular smooth muscle cells (VSMC) and endothelial cells (EC) (18,19). All Nox enzymes require $p22^{phox}$, which serves as a docking protein for other subunits and stabilizes the Nox proteins (19).

Although endothelial and vascular oxidases appear to be constantly active, generating low levels of ROS, they are regulated by humoral factors as demonstrated for cytokines, growth factors, and vasoactive agents as well as by physical factors including stretch, pulsatile strain, and shear stress (20). Interestingly, hydrogen peroxide and lipid peroxides can stimulate activity of the NAD(P)H oxidases in vascular smooth muscle cells, leading to a feed-forward increase in ROS production in vascular wall (21,22).

The protagonic role of this enzymatic system in cardiovascular diseases has been evidenced by several reports showing that increased levels of p22phox, p47phox, p67phox and Nox subunits are present in both human atherosclerotic coronary arteries (23) and diabetic vessels (24), in association with increased superoxide production. This suggests that upregulated gene expression and/or post-transcriptional increases in protein levels are important in mediating increased NAD(P)H oxidase activity in human vascular disease. For example, angiotensin II increases NAD(P)H oxidase activity by transcriptional upregulation of subunit expression (25). However, it is clear that the

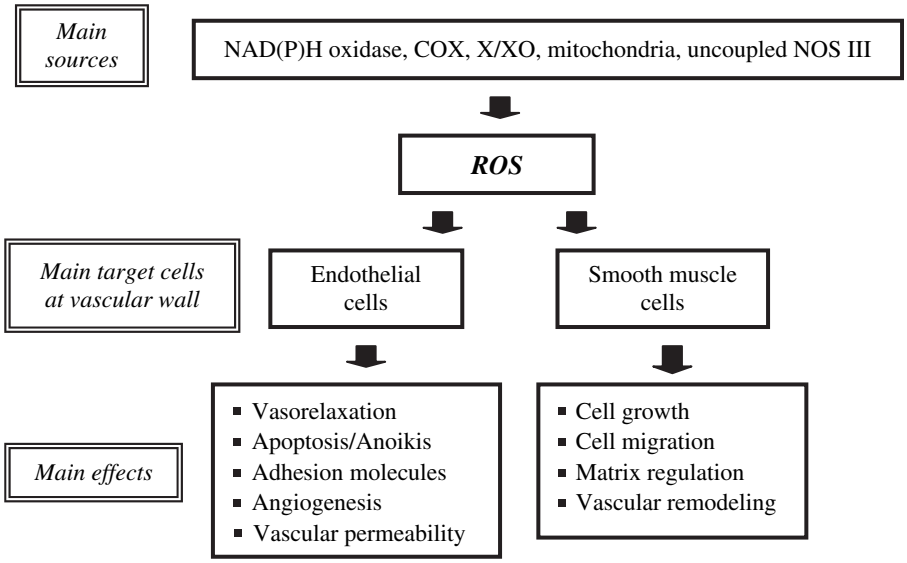

Figure 1. Schematic depiction of main sources of ROS at vascular wall. Once produced, ROS reach both endothelial and smooth vascular cells where they are involved in several cell functions relevant to vascular homeostasis.

cytosolic regulatory proteins p47phox, p67phox and the small g protein Rac-1 also play an important part in regulating NAD(P)H oxidase activity in cardiovascular disease states by acute activation of the enzyme complex, i.e., by phosphorylation and translocation of p47phox (26).

COX

Cyclooxygenase is another source of $O_2^{-}\cdot$ production, particularly in cerebral circulation (27). PGH synthase and lipoxygenase are able to co-oxidize substances such as NAD(P)H (28).

X/XO

Another source of vascular ROS is the xanthine oxidoreductase enzyme system. The xanthine dehydrogenase (XDH) activity present in vascular endothelium is readily converted into xanthine oxidase (XO) by processes including thiol oxidation and/or proteolysis (29), and the the ratio of XO to XDH in the cell is therefore critical to determine the amount of ROS produced by these enzymes. Xanthine oxidase metabolizes hypoxanthine, xanthine, and NADH to form $O_2^{-}\cdot$ and H_2O_2 and appears to be an important source of ROS production in ischemia/reperfusion (30) and hypercholesterolemia (31). Thus, xanthine oxidase has the potential to be an important source of ROS production under certain pathophysiological conditions.

A controversial point has been raised about the expression of XDH in vascular wall, because different immunohistochemical studies have failed to demonstrate the presence of XDH in endothelial cells or other cardiovascular tissues (32). It has been suggested that XO in endothelial cells originates from other organs and that the enzyme is probably taken up via heparin-binding sites (33,34).

Mitochondria

The contribution of mitochondria to the production of ROS in vascular wall is less understood, although significant contributions have been made in the last 5 years (35).

Recent evidence suggests that increased mitochondrial $O_2^{-}\cdot$ generation in endothelial cells is particularly prominent in some pathological settings. Hyperglycemia induces mitochondrial $O_2^{-}\cdot$ production, which is involved in the pathogenesis of diabetic complications (36). Similarly, the adipokine leptin also induces mitochondrial $O_2^{-}\cdot$ production by increasing fatty acid oxidation (37). In hypoxia-reoxygenation and ischemia-reperfusion, mitochondrial-derived $O_2^{-}\cdot$ radicals are increased, where the enhanced $O_2^{-}\cdot$ is at least partially responsible for a rise in endothelial permeability (38).

Dysfunctional or Uncoupled Endothelial Nitric Oxide Synthase (NOS III)

NOS III is a complex homodimeric oxidoreductase that shuttles electrons from the reductase domain to the oxidase domain that contains the heme active site. Under some conditions, NOS generates superoxide rather than NO (39), a phenomenon that is known as NOS uncoupling, meaning that electrons flowing from the NOS III reductase domain to the oxygenase domain are diverted to molecular oxygen rather than to L-arginine. One of NOS cofactors, tetrahydrobiopterin (BH_4), appears to have a key role in regulating NOS function by ''coupling'' the reduction of molecular O_2 to L-arginine oxidation. Exogenous BH_4 partially restores NOS III-dependent NO production and reduces NOS uncoupling in hypertension (40), hypercholesterolemia (41), and smokers (42). Thus, BH_4 availability is a crucial factor in the balance between NO and $O_2^{-}\cdot$ generation by NOS III.

Interaction of ROS with Redox-sensitive Processes in Vascular Wall

The production of ROS in and around vascular endothelium has a marked influence in a variety of redox-sensitive processes, which in turn influences cellular phenotype (Figure 2).

Ion Transport Systems

Although the functional links beteween ROS production and ion transport systems are not yet well understood, some evidence suggests that they are becoming increasingly apparent. At present, many calcium channels are known to be affected by ROS (43). Calcium-regulated potassium channels appear to mediate the vasodilation induced by H_2O_2. In fact, elevated levels of H_2O_2 have been shown to cause calcium-dependent release of NO from the endothelium (44) and potassium channel-dependent relaxation of vascular smooth muscle cell (VSMC) (45).

Increases in intracellular Ca^{2+} have been also detected in response to H_2O_2 treatment of VSMCs (46). Endothelial cells treated with hypoxanthine and hypoxanthine oxidase and H_2O_2 showed a transient release of Ca^{2+} from intracellular stores (47).

Hyperpolarization in vascular smooth muscle cells due to opening of potassium channels plays a central role in several mechanisms of vasodilation. Effects of ROS on hyperpolarization mechanisms of dilation involving potassium channels, although not fully understood, are very important because hyperpolarization-mediated dilation often compensates for the absence of other dilator mechanisms (48).

Oxygen sensing and reactivity to changes in the concentration of oxygen is a fundamental property of cellular physiology. Thus, vascular ion channels are potentially controlled by multiple redox-linked mechanisms, and this is likely to be responsible for the diversity of observations that have been made. The role of mitochondria-induced ROS signal has been raised as attractive models in oxygen-sensing mechanisms (49).

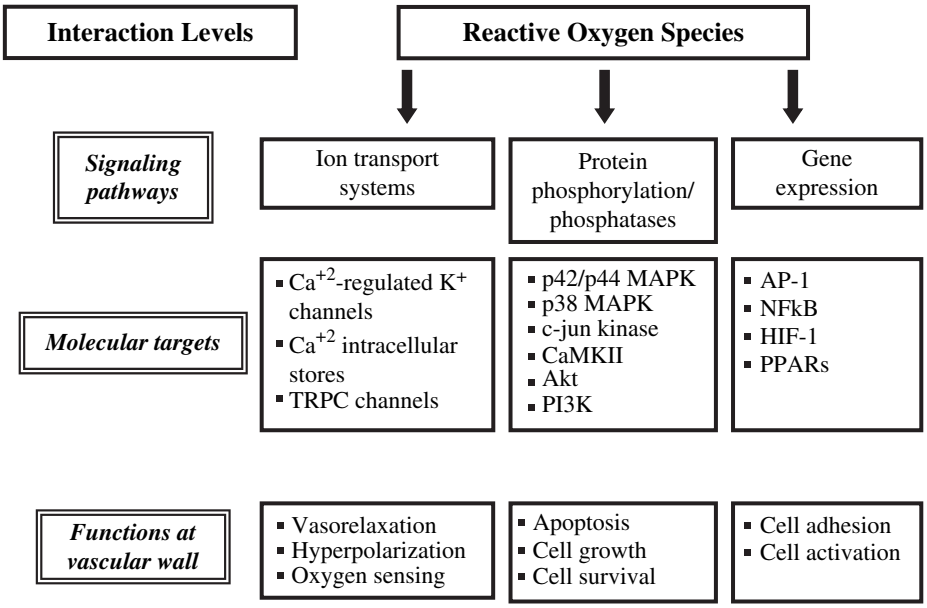

Figure 2. At vascular wall, ROS interact with redox sensitive-signaling pathways through a myriad of target proteins, which in turn influence cellular phenotype.

Protein Phosphorylation

Reversible protein phosphorylation is a key biochemical event in cell-signaling pathways. It is now well established that redox processes markedly influence the balance of the activities between various mitogen-activated protein kinases (MAPK) in vessel wall, such as p42/p44 MAPK, stress-activated or c-Jun N-terminal kinase, and the p38 MAPK-associated pathways (50,51). Of particular interest, H_2O_2-mediated protein phosphorylation is involved in wall vessel physiology. Hydrogen peroxide upregulates endothelial nitric oxide synthase expression via a calcium/calmodulin-dependent protein kinase II (CaMKII)-mediated mechanism (52). On the other hand, H_2O_2 has also been shown to inhibit phosphatases, probably by the direct oxidation of cysteine in the active site of these enzymes (53,54). There is evidence that oxidant mechanisms may induce an increment in autophosphorylation of receptor-linked tyrosine kinases that activate MAPK pathways (55), and H_2O_2 has been observed to stimulate tyrosine phosphorylation of the epidermal growth factor receptor in VSM cells (56), whereas nitrosation of a thiol on $p21^{ras}$ stimulates the activation of p42/p44 MAPK (57). A phosphorylation mediated by cGMP-dependent protein kinase has also been demonstrated to activate the p42/p44 MAPK pathway in VSM (58). Thus, ROS have multiple ways of interacting with processes linked to the various protein phosphorylation-phosphatases systems.

Akt kinase, which lies downstream of phosphoinositide 3-kinase (PI 3-kinase), is another key redox-sensitive element in the control of several processes at the vascular wall (59–62). As recently demonstrated, Akt is involved in a redox-sensitive pathway in endothelial cells during the expression of the NAD(P)H oxidase subunit p22phox (63). Similar to p38 MAPK, both exogenous H_2O_2 and Ang II activate Akt in SMCs. (64) and the VEGF-induced growth and survival in EC (65–67).

Several of the beneficial effects of red wine polyphenols have also been ascribed to the redox-sensitive activation of the PI3-kinase/Akt pathway in endothelial cells (68,69).

Gene Expression

ROS may modify several functions in vascular wall, particularly in EC and VSMC, by modulating gene and protein expression through the regulation of some redox-sensitive transcription factors, such as nuclear factor-κB, the activator protein-1, HIF-1 and the peroxisome proliferator-activated receptor (PPAR) family of transcriptional activators activating several transcriptions.

AP-1

AP-1 is activated by prooxidant stimulus in both ECs and SMCs by agents such as H_2O_2, oxLDL and the lipid peroxidation product 4-hydroxy-2-nonenal (70–72). Furthermore, regulation of the vascular inflammatory genes MCP-1 and ICAM-1 by H_2O_2 is mediated by AP-1 binding elements in the promoters of these genes (73,74). The redox-mediated AP-1 transcriptional pathway also plays an important role in Ang II-induced ET-1 gene expression (75), as well as in the Il-8 induction by hyperglycemia (76). Interestingly, the cardiovascular hormone atrial natriuretic peptide (ANP) exerts anti-inflammatory effects on tumor necrosis factor-alpha-activated endothelial cells by

inducing mitogen-activated protein kinase phosphatase-1 (MKP-1) through the activation of the transcription factor AP-1 (77).

NF-κB

NF-κB was the first eukaryotic transcription factor shown to respond directly to oxidative stress. A huge amount of experimental data supports the activation of the transcription factor NF-κB as a key redox-sensitive event associated with vascular dysfunction (78). In ECs, NF-κB is a prime target for ROS, and its activation by cytokines, hypercholesterolemia, ischemia-reperfusion, advanced glycation end-products, and the renin-angiotensin system has been linked to increased expression adhesion molecules such as E-selectin, ICAM-1, V-CAM-1 (79,80). Interestingly, vascular endothelial growth factor-mediated induction of manganese superoxide dismutase also occurs through redox-dependent regulation of NF-κB (81). In VSMC, NF-κB activation is reported to be essential for proliferation (82). On the other hand, NF-κB-mediated transcription has also been pointed out as an important mechanism in cell survival (83–85).

HIF-1

ROS can also modulate gene expression through the redox-sensitive activation of the transcription factor HIF-1 (hypoxia-inducible factor-1), which may increase the expression of genes involved in angiogenesis, energy metabolism, cell proliferation, and vascular remodeling (86). Recent studies have begun to delineate the ways in which ROS may regulate gene transcription through HIF-1. The redox regulation of HIF-1 activity appears to be mediated largely through ROS-dependent changes in HIF-1α stability as well as by posttranslational regulation of HIF-1 activity. However, the precise mechanisms through which the latter regulation occurs remain unclear, with evidence for regulation through activation of the phosphatidylinositol 3-kinase/Akt pathway or through a thiol-sensitive mechanism (87).

Peroxisome Proliferator-Activated Receptors

PPAR activators were shown to inhibit the activation of inflammatory response genes by negatively interfering with the NF-κB, STAT and AP-1 signaling pathways in cells of the vascular wall (88–90). Several studies have demonstrated that PPARs may be viewed as redox-sensitive transcription factors in the vasculature by their ability to be selectively activated by oxidatively modified fatty acids (91–94).

Effects of ROS at Vascular Wall

Endothelial Cells

Vasorelaxation. The role of ROS in vasorelaxation has become evident in at least two ways: first, via interactions with NO and second, through the direct effects of H_2O_2. A large body of evidence from both experimental animals and human subjects suggests that oxidative inactivation of NO plays an important role in various pathologic conditions. It is well established that endothelium-derived NO undergoes a very rapid reaction with $O_2^{-}\cdot$ (95) thereby reducing NO bioavailability. Increased superoxide production by vascular NAD(P)H oxidases and xanthine oxidase is responsible for NO deficit observed in several models of vascular disease, including hypercholesterolemia (96), atherosclerosis (97,98), hypertension (99–101), and heart failure (102). Reactive oxygen species also diminish NO bioavailability in humans (103). Local SOD activity at the vessell wall is therefore a very important element in regulating the $NO/O_2^{-}\cdot$ balance.

Compelling evidence indicates that H_2O_2 released from the endothelium (after conversion from $O_2^{-}\cdot$) may account for EDHF activity in murine and human mesenteric arteries and human coronary arterioles, where it is involved in flow-induced dilatation (104). Endothelial CuZnSOD plays a pivotal role in converting $O_2^{-}\cdot$ (generated probably mainly by NO synthase) to H_2O_2, to the extent that it was proposed to act as an EDHF synthase.

Finally, interaction of advanced glycation end products with their receptor in endothelial cells triggers the generation of ROS, where NAD(P)H oxidase plays a central role (105). This situation, in addition to AGEs capabilities to reduce NOS III expression by increasing the rate of NOS III mRNA degradation (106), renders a reduced nitric oxide bioavailability at vascular wall.

Apoptosis/Anoikis

A large body of evidence indicates that endothelial exposure to ROS induces apoptosis (programmed cell death), which leads to EC loss and results in atherogenesis and a procoagulative state (107). Importantly, EC apoptosis stimulated by oxidized LDL, Ang II, high glucose, and TNF-α are inhibited by SOD, catalase, NAC, and antioxidant vitamins (107). These data strongly suggest that ROS regulate apoptotic mechanisms induced by a variety of stimuli. Very interestingly, low doses of ROS may also act as signaling molecules and they exert anti-apoptotic functions in endothelial cells via upregulation of the redox-regulator Trx-1 (108).

ROS seem to also be involved in a form of apoptosis called anoikis (109) that results from detachment of ECs from extracellular matrix. Anoikis is induced by the loss of cell−matrix interactions, but its exact mechanisms and pathophysiologic role in cardiovascular disease are not fully understood. This process is also associated with increased intracellular ROS probably from mitochondria and is inhibited by NAC and diphenylene iodonium (DPI), an inhibitor of flavin-containing enzymes such as NAD(P)H oxidases. (110). Eicosapentaenoic acid, a polyunsaturated

fatty acid contained in fish oil, was shown to protect endothelial cells from anoikis (111), which may contribute to the antiatherogenic and cardioprotective effects of fish oil.

Adhesion Molecules

At present, the expression of several adhesion molecules including vascular cell adhesion molecule-1 (VCAM-1) and intracellular adhesion molecule-1 (ICAM-1) is widely accepted to be a ROS-dependent process. (112). On the other hand, endogenous synthesis of nitric oxide is responsible for inhibition of cytokine-induced expression of intracellular adhesion molecule-1, vascular cell adhesion molecule-1, and endothelial leukocyte adhesion molecule-1 (113–115), rendering both monocytes and neutrophils very limited in their ability to migrate into the vascular wall. Because ROS markedly reduced NO bioavailability at vascular wall, it is expected that adhesion molecule gene expression is suppressed by the antioxidants, as extensively demonstrated (116). Additional factors that can modulate the magnitude and/or nature of the vascular inflammatory responses as the CD40/CD40 ligand signaling system seem to also be related to oxidative stress-mediated mechanism that require the activation of NAD(P)H oxidase (117).

Angiogenesis

Angiogenesis, a process of new blood vessel growth, is present in some vascular pathological entities such as diabetic retinopathy and atherosclerosis. EC migration, proliferation, and tube formation are essential events in the process of angiogenesis. Recent reports suggest that ROS play an important role in angiogenesis; however, its underlying molecular mechanisms remain unknown (118). VEGF induces angiogenesis by stimulating EC proliferation and migration primarily through the receptor tyrosine kinase VEGF receptor2 (Flk1/KDR). ROS derived from NAD(P)H oxidase are critically important for VEGF signaling *in vitro* and angiogenesis *in vivo*, as recently demonstrated (119). Furthermore, Ang II, a major stimulus for vascular NAD(P)H oxidase, also plays an important role in angiogenesis (120).

Endothelial Barrier Dysfunction

There is substantive evidence that oxidant stress increases vascular endothelial permeability. This notion is further supported by *in vitro* and *in vivo* studies in which direct treatment ROS or ROS generating systems increased transendothelial permeability (121). ROS cause intercellular gap formation, cell shape change, and actin filament reorganization (122). These morphological features implicate impaired cell-cell adhesion and consequently, impaired intercellular junctions, as a primary determinant of increased paracellular permeability.

At present, there is limited information regarding the effects of oxidant stress on the function and organization of adherens and tight junctional proteins.

Increased extravasated FITC-albumin has not been associated with altered distribution of ZO-1 as detected by immunogold localization in rat lungs perfused with H_2O_2 (123). However, others have reported that *in vitro* H_2O_2 treatment of human and bovine endothelial cells resulted in the redistribution of occludin and dissociation from ZO-1 (124,125).

H_2O_2 treatment of endothelial cells promotes cadherin internalization as a possible mechanism explaining reduced cadherin expression (126). We have reported that exposure of endothelial cell monolayers to advanced glycation endproducts, which are able to induce a marked oxidative stress in endothelial cells, induced decreases in the levels of VE-cadherin, β-catenin and γ-catenin, resulting in increased monolayer permeability and cell migration. These effects were completely reversed by antioxidants such as NAC and PDTC (127).

Vascular Smooth Muscle Cells

One of the most extensively studied VSMC functions altered by ROS is cell growth, although they are also involved in cell migration as well as in VSMC contraction, expression of inflammatory mediators and matrix components.

Vascular Smooth Cell Growth and Migration

ROS production is intimately involved in many of the processes leading to both hypertrophic and proliferative VSMC growth.

Ang II can induce VSMC hypertrophy by a redox-sensitive mechanism. Ang II-induced VSMC hypertrophy is inhibited by catalase and p22phox antisense (128,129), thus implicating NAD(P)H oxidase-derived ROS in the growth response. Furthermore, VSMC proliferation by PDGF or thrombin requires H_2O_2 generation, as it is inhibited by catalase, NAC, or DPI (130,131).

VSMC migration is considered to be one of the major components of vascular pathogenesis. Although the precise molecular mechanisms of VSMC migration are not fully understood, a role for ROS has clearly been demonstrated. PDGF-induced VSMC chemotaxis is inhibited by catalase overexpression (130). In additon, VSMC migration stimulated by PDGF is inhibited by NAC, DPI, ebselen, and dominant-negative Rac, suggesting that $O_2^{-}\cdot$ production through the NAD(P)H oxidase is critical for agonist-stimulated VSMC migration (132).

Matrix Regulation

Degradation and reorganization of the extracellular matrix by matrix metalloproteinases (MMPs) are key events in

vascular remodeling. Both pro-MMP-2 and pro-MMP-9 secreted from human VSMCs are activated by ROS (133). Interestingly, Ang II induces MMP-2 in a p47phox-dependent manner. It is therefore reasonable to think that the renin-angiotensin system may contribute to plaque destabilization via ROS-dependent induction of MMP-2 (134). Mechanical stretch, a hallmark of arterial hypertension, enhances mRNA expression and proenzyme release of matrix metalloproteinase-2 (MMP-2) via NAD(P)H oxidase-derived reactive oxygen species (135).

Present Facts and Future Hopes

Several pharmacological approaches have been used to improve vascular hemostasis and to decrease oxidative stress. These include treatment modalities that augment the antioxidant defense mechanisms, such as increment of NO production and inhibition of ROS-generating enzymes.

NAD(P)H Oxidase Inhibition

NAD(P)H oxidase is the major source of $O_2^{-}\cdot$ in vascular tissue, and therefore represents an attractive target for pharmacological interventions. However, there is a lack of effective inhibitors targeting the NAD(P)H oxidase system. Diphenyleneiodonium, which has been extensively used as NAD(P)H oxidase inhibitor, can also inhibit several NAD(P)-dependent enzymes such as glucose 6-phosphate dehydrogenase, glyceraldehyde 3-phosphate dehydrogenase, and lactate dehydrogenase (136).

Statins, the amazing pharmacological agents with a broad field of cardiovascular actions, also have inhibitory actions on $O_2^{-}\cdot$ production from NAD(P)H oxidase (137–139) by a mechanism linked to prenylation-dependent Rac translocation and NAD(P)H oxidase inhibiton (140).

A very attractive approach has been recently reported based on the disruption of the active NAD(P)H oxidase complex by means of a chimeric peptide designed to cross cell membranes and then inhibit $p47^{phox}$ association with $gp91^{phox}$ (141), rendering a no-functional complex.

Apocynin, a methoxy-substituted catechol used by Peruvian Indians as an anti-inflammatory agent, is an attractive naturally occurring vascular NAD(P)H oxidase inhibitor. It acts by blocking the assembly of $p47^{phox}$ into the membrane complex (142). *In vivo* administration of apocynin to deoxycorticosterone-acetate-salt hypertensive rats produced a decreased in both vascular $O_2^{-}\cdot$ production and blood pressure (143).

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 (144,145).

Inhibition of the Renin-Angiotensin System

Compelling evidences suggest that Ang II is also an important inducer of $O_2^{-}\cdot$ production in vascular wall through inreased NAD(P)H oxidase activity (146,147) and therefore ACE inhibition and Ang-II receptor antagonisms play key roles in reducing levels of oxidative stress. The beneficial effects of ACE inhibition are far beyond blood pressure-lowering activity and reduced oxidant stress at vascular wall represent another important pharmacological action of ACE inhibitors (148). AT1 receptor antagonists also have marked protective effects at vascular wall, in addition to lowering blood pressure, exerted by the downregulation of NAD(P)H oxidase expression (149,150).

Beta-Blockers

Nebivolol and carvedilol, third-generation β-blockers, in additon to increasing NO synthase (NOS) III activation (151), have antioxidant effects (152,153) that result in substantial increments in NO bioavailability.

Xanthine Oxidase Inhibition

Allopurinol has been shown to improve endothelial function through the reduction of oxidate-stress in type II diabetes, chronic heart failure and cigarette smokers (154–156). A Phase II–III prospective, randomized and double-blind clinical trial (OPT-CHF) is now in progress in 400 CHF patients to test the efficacy of oxypurinol, the active metabolite of allopurinol.

Vitamins and Dietary Antioxidants

Increasing evidence coming from epidemiological studies suggests that a greater intake of antioxidant vitamins such as vitamin E, vitamin C and β-carotene are associated with a reduced risk of cardiovascular disease (157). Vitamin C or vitamin E enhanced NOS activity and attenuated NAD(P)H oxidase activity in rat aorta. In addition, vitamin C treatment resulted in lower oxidation of BH_4 (158). Oxidation of BH_4 by superoxide is a critical element in rendering unfunctional NOS IIII (159).

Despite strong evidence demonstrating antioxidant effects of vitamins C and E in animals and acutely in man, prospective randomized clinical trials have produced contrasting results (160,161). Several explanations may explain the lack of observed benefit in these randomized trials such as the oxidant stress status of the participants and dose and combination of vitamins administered (162). Recently, the beneficial effects of polyphenols, particularly from red wine, have been pointed out based on its antioxidant properties and enhancing nitric oxide synthesis and release by endothelial cells (163).

L-Arginine and BH_4 Administration

BH_4 availability is a crucial factor in the balance between NO and $O_2^{-}\cdot$ generation by eNOS. Administration of both L-arginine and BH_4 can markedly improve NO bioavailability in humans. Since the seminal report of Creager et al. in 1992 (164), several studies have confirmed that both acute and chronic Arg administration improve vascular function in hypercholesterolemia (165) in small-vessel disease (166) and in exercising patients with stable angina pectoris (167). In addition to the previously mentioned effects of BH_4, it also augments NO-mediated effects on forearm blood flow in diabetes (168) and hypercholesterolemic patients (169).

SOD Mimetics

In general, studies aimed at reducing oxidative stress by Cu/Zn SOD have yielded negative results mainly due to short half-life in circulation, inability to penetrate the blood–brain barrier and potential antigenicity have limited its access to the appropriate cellular compartments. However, a number of SOD mimetics are available that cross the membrane and have proved more successful in decreasing oxidative stress and improving endothelial function (170,171). Long-term outcome studies in clinical settings using these promising drugs are needed to address its therapeutic potential.

Calcium Channel Blockers

Very promising effects have been reported on the newly developed dihydropyridine-type calcium antagonist azelnidipine, which is able, in addition to its long-lasting hypotensive effect with a little reflex tachycardia, to inhibit Ang II-mediated growth-promoting signaling in vascular smooth muscle cells (172). Additionally, azelnidipine markedly reduced lipid peroxidation (173), as well as TNF-α-induced IL-8 expression in HUVECs by blocking NAD(P)H oxidase-mediated ROS generation and subsequent AP-1 activation (174).

Gene Transfer Strategies

Two main strategies have been mainly focused to control oxidative stress in vascular wall, either by enhancing nitric oxide synthesis or by diminishing superoxide production. The first involves the nitric oxide synthase genes as potential candidates for cardiovascular gene therapy because of the crucial roles of NO in the cardiovascular system. *In vivo* NOS III gene transfer increased NO levels in cerebrospinal fluid and improved vascular relaxations to bradykinin in a dog model of subarachnoid hemorrhage (175). *Ex vivo* NOS III gene transfer to isolated human saphenous veins improved vascular relaxations to a variety of vasoactive agonists (176). Furthermore, NOS III gene transfer to the aortas and carotid arteries improved acetylcholine-induced vasodilation in a rabbit model of type 1 diabetes (177) and restored NO-mediated endothelial function in models of cardiac failure hypertension (178). *In vivo* intraluminal delivery of NOS III gene to the carotid artery of cholesterol-fed rabbits ameliorated the impaired endothelium-dependent vasodilation in response to acetylcholine (179). Regression of atherosclerosis has also been observed within 3 days after NOS I gene transfer, as evidenced by reduction of vascular adhesion molecule expression, T-lymphocyte and monocyte infiltration, and lipid deposition (180). Interestingly, NOS(s) gene transfer inhibited intimal hyperplasia in several models of vascular injury, including balloon-injured (181) venous bypass grafts (182) and rat aortic allografts (183). Furthermore, *ex vivo* NOS III gene transfer using adenoviral vectors inhibited intimal hyperplasia in cultured human saphenous veins (184). Moreover, NOS III gene transfer restored NO-mediated endothelial function in models of cardiac failure (185). Very recently, an elegant strategy to improve nitric oxide synthesis has been afforded by adenovirus-mediated gene transfer of GTP cyclohydrolase I (GTPCH-I) the rate-limiting enzyme for the de novo BH_4 synthesis (186).

The second strategy relies on adenovirus-mediated superoxide dismutase gene transfer to reduce superoxide release from endothelial cells (187). To date, although results have been quite promising, much effort should be expended to improve current vectors as well as *in vivo* gene-delivering technologies to prior routine clinical applications. Finally, and along the same line of reasoning, perivascular gene transfer delivery of an NADPH oxidase inhibitor peptide reduced overall vascular $O_2^{-}\cdot$ and neointima formation (188).

Conclusions

This review summarizes how complex are the physiological and pathophysiological effects of ROS at vascular wall. Many vascular functions are redox-sensitive processess and the basis to vascular health lies at the balance between ROS-producing and ROS-inactivating systems. An extensive array of experimental animal and human data has raised pharmacological and molecular approaches to reduce oxidative stress. Old drugs with novel mechanism of actions and new approaches to old problems harmonize the aim of improving clinical outcomes based on pharmacological approaches for lowering oxidative stress, a process becoming increasingly recognized as critical in the pathophysiology of vascular diseases.

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