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REVIEW SERIES

Reactive oxygen species and vascular biology: implications in human hypertension

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Increased vascular production of reactive oxygen species (ROS; termed oxidative stress) has been implicated in various chronic diseases, including hypertension. Oxidative stress is both a cause and a consequence of hypertension. Although oxidative injury may not be the sole etiology, it amplifies blood pressure elevation in the presence of other pro-hypertensive factors. Oxidative stress is a multisystem phenomenon in hypertension and involves the heart, kidneys, nervous system, vessels and possibly the immune system. Compelling experimental and clinical evidence indicates the importance of the vasculature in the pathophysiology of hypertension and as such much emphasis has been placed on the (patho)biology of ROS in the vascular system. A major source for cardiovascular, renal and neural ROS is a family of non-phagocytic nicotinamide adenine dinucleotide phosphate (NADPH) oxidases (Nox), including the prototypic Nox2 homolog-based NADPH oxidase, as well as other Noxes, such as Nox1 and Nox4. Nox-derived ROS is important in regulating endothelial function and vascular tone. Oxidative stress is implicated in endothelial dysfunction, inflammation, hypertrophy, apoptosis, migration, fibrosis, angiogenesis and rarefaction, important processes involved in vascular remodeling in hypertension. Despite a plethora of data implicating oxidative stress as a causative factor in experimental hypertension, findings in human hypertension are less conclusive. This review highlights the importance of ROS in vascular biology and focuses on the potential role of oxidative stress in human hypertension.
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INTRODUCTION

Hypertension is a leading cause of morbidity and mortality globally.[1] The exact etiology is elusive, with only about 5% of hypertensive patients having a known cause. However, it is evident that blood pressure elevation is due to complex interactions involving multiple organ systems (heart, kidney, brain, vessels), between many genes, physiological systems (cardiovascular, renal, neural, immune) and environmental stimuli. At the molecular level, numerous factors have been implicated in the pathophysiology of hypertension including activation of the renin–angiotensin–aldosterone system, inflammation, aberrant G protein-coupled receptor signaling and endothelial dysfunction.[2–4] Common to these is oxidative stress due, in large part, to excess production of vascular reactive oxygen species (ROS), to decreased nitric oxide bioavailability and to decreased antioxidant capacity.[5]

ROS, originally considered to cause cell damage, are now recognized to be key signaling molecules that mediate diverse biological responses such as induction of host defense genes, activation of transcription factors, phosphorylation of kinases and mobilization of ion transport systems.[6–8] In the vascular system ROS has a physiological role in controlling endothelial function and vascular tone and a pathophysiological role in inflammation, hypertrophy, proliferation, apoptosis, migration, fibrosis, angiogenesis and rarefaction, important in vascular remodeling and endothelial dysfunction associated with hypertension.[9–11]

The relationship between free radicals and hypertension was suggested in the early 1960s,[12] but it was some 40 years later that this association was investigated in greater detail when it was demonstrated that angiotensin II (Ang II)-mediated hypertension in rats increases vascular superoxide production via membrane NAD(P)H oxidase activation.[13] Almost all experimental models of hypertension display some form of oxidative excess.[14–20] As inhibition of ROS-generating enzymes, anti-oxidants and ROS scavengers reduce blood pressure, whereas pro-oxidants increase blood pressure, it has been suggested that ROS are causally associated with hypertension, at least in animal models.

Despite the plethora of data supporting a role for oxidative stress in experimental hypertension, the evidence in human hypertension is weak.[21–23] It is still unclear whether oxidative stress causes hypertension in humans and only a few small clinical studies showed a blood pressure-lowering effect of anti-oxidants,[24–26] with most large anti-oxidant clinical trials failing to demonstrate any cardiovascular benefit and blood pressure lowering.[27–29] Nevertheless, what is evident is that oxidative stress has a critical role in the molecular mechanisms

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associated with cardiovascular and renal injury in hypertension and that hypertension itself can contribute to oxidative stress. A greater understanding of the (patho)biology of ROS may lead to new mechanistic insights and novel diagnostics and treatments for hypertension. ROS production in vessels, as well as other organs, including the heart, kidneys and brain, likely participate in blood pressure regulation.[30–32] This review will focus on ROS, the vascular system and hypertension, specifically relating to the clinical significance.

VASCULAR GENERATION OF ROS

ROS are produced as intermediates in reduction–oxidation (redox) reactions leading from O_2 to H_2O.[33,34] The sequential univalent reduction of O_2 is: $O_2 \xrightarrow{e^-} \cdot O_2^- \xrightarrow{e^-} H_2O_2 \xrightarrow{e^-} OH\cdot \xrightarrow{e^-} H_2O + O_2$. Of the ROS generated in vascular cells, $\bullet O_2^-$, and H_2O_2 appear to be particularly important. In biological systems, $\bullet O_2^-$ is short-lived owing to its rapid reduction to H_2O_2 by superoxide dismutase (SOD), of which three isoforms have been characterized in mammals: copper/zinc SOD (SOD1), mitochondrial SOD (SOD2) and extracellular SOD (SOD3).[35,36] The major vascular SOD is extracellular SOD. The charge on the superoxide anion makes it difficult to cross the cellular membranes, except possibly through ion channels. H_2O_2 has a longer lifespan than $\bullet O_2^-$, is relatively stable and is easily diffusible within and between cells. The distinct chemical properties between $\bullet O_2^-$ and H_2O_2 and their different sites of distribution suggest that different species of ROS activate diverse signaling pathways, which lead to divergent, and potentially opposing, biological responses.

All vascular cell types produce ROS, including endothelial, smooth muscle, adventitial fibroblasts and perivascular adipocytes, and can be formed by many enzymes, including xanthine oxidoreductase, uncoupled nitric oxide synthase, mitochondrial respiratory enzymes and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase[37–44] (Figure 1). Of these mitochondrial enzymes and NADPH oxidase seem to be particularly important in hypertension.

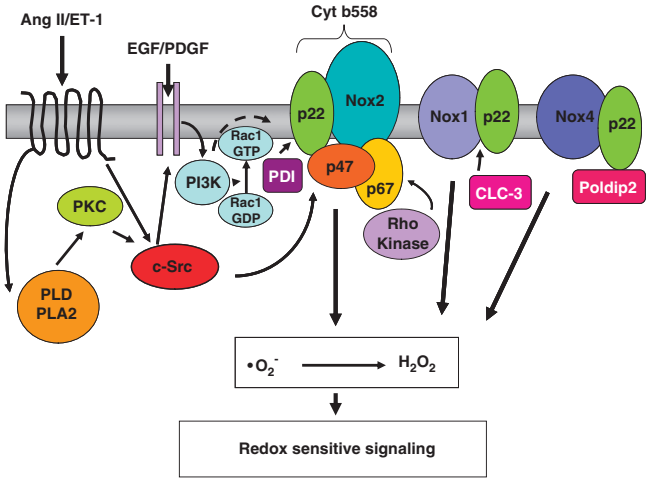

Figure 1 Regulation of Noxes in vascular cells. Activation of NADPH oxidase involves multiple subunits and many signaling pathways involving c-Src $p21^{Ras}$, protein kinase C (PKC), phospholipase D (PLD), phospholipase A_2 (PLA_2) and Rho kinase. All Noxes, except Nox5, appear to have an obligatory need for p22phox. Nox2 requires p47phox and p67phox for its activity. Oxidase activation involves Rac translocation, phosphorylation of p47phox and its translocation, possibly with p67phox, and p47phox association with cytochrome b558. Induction of Nox mRNA expression is observed in response to many stimuli including vasoactive agents (angiotensin II (Ang II), endothelin 1 (ET-1)), growth factors (epidermal growth factor (EGF), platelet-derived growth factor (PDGF)), amongst others. Recently identified Nox regulators include ClC-3, Poldip2 and protein disulfide isomerase (PDI). PDI associates with p22phox to regulate Nox2. Poldip2 associates with p22phox to activate Nox4, leading to regulation of focal adhesion turnover and vascular smooth muscle cell migration. Nox1 is regulated by the chloride/proton exchanger ClC-3. Cyt, cytochrome; GDP, guanosine diphosphate; GTP, guanosine triphosphate; PI3K, phosphoinositide 3-kinase.

Mitochondrial production of ROS

More than 95% of O_2 consumed by cells is reduced by four electrons to yield two molecules of H_2O via mitochondrial electron transport chain complexes (I–IV), with 1–2% of the electron flow leaking to O_2 to form $\bullet O_2^-$ under normoxic conditions.[45] Normally this $\bullet O_2^-$ is rapidly scavenged by antioxidant enzymes, including mitochondrial manganese SOD and glutathione peroxidise. Damaged or dysfunctional mitochondria overgenerate $\bullet O_2^-$ creating a state of redox imbalance and consequent oxidative stress. Intramitochondrial $\bullet O_2^-$ production triggers damaging reactions through production of H_2O_2, leading to altered adenosine triphosphate synthesis, cellular Ca^{2+} dysregulation and induction of mitochondrial permeability transition, all of which predispose to cell death.[46] Ang II and endothelin-1 stimulate mitochondrial ROS generation in endothelial and vascular smooth muscle cells and in rat aorta *in vivo*.[47–51] Mechanisms whereby these vasoactive agents induce such actions are unclear but could involve opening of mitochondrial potassium channels ($mitoK_{ATP}$)[52] and mitochondrial permeability transition.[53] Ang II-induced Nox activation has also been shown to induce mitochondrial ROS formation.[54]

Impaired activity and/or decreased expression of mitochondrial electron transport chain complexes I, III and IV have been implicated in vascular aging and cardiovascular disease,[55] and an association between mitochondrial dysfunction and blood pressure has been reported in human and experimental hypertension.[56–58] Ang II-sensitive hypertension is also linked to mitochondrial-derived oxidative stress, as AT_1 receptor blockade attenuates H_2O_2 production[59] and mitochondrial dysfunction in SHR, and in mice, Ang II infusion is associated with decreased expression of cardiac mitochondrial electron transport genes.[60] In deoxycorticosterone acetate–salt hypertension, mitochondrial-derived ROS, via endothelin-1/endothelin A receptors, has an important role in oxidative vascular damage.[61,62] In humans, mitochondrial heritability for systolic blood pressure is about 5% and mitochondrial effects may account for 35% of hypertensive pedigrees.[63,64] In African Americans with hypertension-associated end-stage renal disease, mitochondrial–DNA mutations in the kidneys have been identified.[65]

Nox family NAD(P)H oxidase-derived ROS

NAD(P)H oxidases were originally considered as enzymes expressed only in phagocytic cells involved in host defense and innate immunity. Recent evidence indicates that there is a family of NAD(P)H oxidases, based on the discovery of gp91phox homologs. The new homologs, along with gp91phox are now designated the Nox family of NAD(P)H oxidases[66–68] and are key sources of ROS in the vasculature. The prototypical phagocytic NAD(P)H oxidase comprises five subunits: p47phox ('phox' stands for phagocyte oxidase), p67phox, p40phox, p22phox and the catalytic subunit gp91phox (also termed Nox2).[69,70] In unstimulated cells p47phox, p67phox and p40phox, exist in the cytosol, whereas p22phox and gp91phox are in the membrane, where they occur as a heterodimeric flavoprotein, (cytochrome b558). On stimulation p47phox is phosphorylated and the cytosolic subunits

form a complex that translocates to the membrane, where it associates with cytochrome b558 to assemble the active oxidase, which transfers electrons from the substrate to O_2 forming $\bullet O_2^-$.[71,72] Activation also requires participation of Rac 2 (or Rac 1) and Rap 1A. In vascular cells, NADPH oxidase is constitutively active.

The mammalian Nox family comprises seven members, characterized primarily by the catalytic subunit that they utilize. These include Nox1, Nox2 (formerly gp91phox), Nox3, Nox4, Nox5, Duox1 and Duox2.[73–75] All Noxes are transmembrane proteins that have conserved structural properties and that transport electrons across biological membranes to reduce O_2 to $\bullet O_2^-$. Nox1, Nox2, Nox4 and Nox5 have been identified in vascular tissue. Characterization of Noxes as they pertain to vascular biology and hypertension has recently been reviewed.[76,77] In vessels, in addition to vascular cells possessing functional Noxes, resident macrophages, neutrophils and platelets express NAD(P)H oxidase, particularly in pathological states. Accordingly these cells can also contribute to vascular oxidative stress in disease.

Nox1, found mainly in colon epithelial cells, is also expressed in other cell types, including endothelial and vascular smooth muscle cells.[78,79] Nox1 requires p22phox, p47phox and p67phox for its activity. It is regulated by the redox chaperone protein disulfide isomerase in vascular smooth muscle cells[80] and has been implicated in vascular smooth muscle cell migration, proliferation and extracellular matrix production, effects mediated by cofilin.[81]

Nox2 is the catalytic subunit of the respiratory burst oxidase in phagocytes, but is also expressed in vascular, cardiac, renal and neural cells.[82,83] Human Nox2 is a highly glycosylated protein that runs with an apparent molecular mass of ~70 to 90 kDa on SDS-polyacrylamide gel electrophoresis. Nox2 is unstable without p22phox and requires the cytosolic subunits for its full activation. In neutrophils Nox2 localizes to intracellular and plasma membranes and in vascular smooth muscle cells it also localizes with the cytoskeleton. The *Nox2* gene, located on the X chromosome, is inducible and is highly regulated by Ang II and stretch and is upregulated in experimental hypertension.[84–86]

Nox4 is found in vascular cells, fibroblasts and osteoclasts and is abundantly expressed in the kidney.[87,88] In vascular smooth muscle cells, Nox4 and p22phox co-localize with vinculin in focal adhesions and has been implicated in cell migration, proliferation, tube formation, angiogenesis and cell differentiation.[87,88] Nox4 has also been found in the endoplasmic reticulum, mitochondria and nucleus of vascular cells.[89,90] Nox4 produces mainly H_2O_2, whereas Nox1 generates mostly $\bullet O_2^-$ that is subsequently converted to H_2O_2. The difference in the species generated may contribute to Nox-specific actions in cell signaling. Nox4 does not seem to require p47phox, p67phox, p40phox or Rac for its activation, although Nox R1, a Nox4-binding protein was recently identified, which may be important.[91]

Nox5 is a Ca^{2+}-dependent homologue, found in testes and lymphoid tissue, but also in vascular cells.[92,93] Although all Noxes are present in rodents and man, the mouse and rat genome does not contain the Nox5 gene.[94] Unlike other vascular Noxes, Nox5 possesses an amino-terminal calmodulin-like domain with four binding sites for Ca^{2+} (EF hands) and does not require p22phox or other subunits for its activation. Nox5 is directly regulated by intracellular Ca^{2+} ($(Ca^{2+})_i$), the binding of which induces a conformational change leading to enhanced ROS generation.[95] The functional significance of vascular Nox5 is unknown, although it has been implicated in endothelial cell proliferation, angiogenesis and migration, in platelet-derived growth factor-induced proliferation of vascular smooth muscle cells and in oxidative damage in atherosclerosis.[96,97] Vascular Nox5 is activated by thrombin, platelet-derived growth factor, Ang II and endothelin-1.[93,98]

VASCULAR NOX DISTRIBUTION AND REGULATION

Nox distribution

Endothelial cells, vascular smooth muscle cells and adventitial fibroblasts possess multiple Nox isoforms.[73,74] In pathological conditions associated with vascular injury, such as hypertension, macrophages and leukocytes, themselves rich in NADPH oxidase, invade the vessel and become resident cells in the vascular media. Endothelial cells express mRNA and protein for Nox2, Nox4 and associated regulatory proteins, namely, p22phox, p47phox and p67phox.[99] Nox2 is the major source of ROS in endothelial cells under basal conditions and in pathological conditions Nox1 and Nox4 may be upregulated.[100] Nox2, Nox4 and Nox5 seem to localize primarily in the perinuclear area associated with membranes on the endoplasmic reticulum and nucleus although Nox2 is also found in the plasma membrane within cholesterol-enriched domains, which may serve as signaling platforms for ROS generation in vascular disease.[101] Vascular smooth muscle cells possess Nox2 (in human resistance arteries) and Nox4, which are major sources of ROS. Nox1, present in low concentrations in basal states, is upregulated in disease. Adventitial fibroblasts possess Nox2 and Nox4 and perivascular adipose tissue expresses Nox4.[102,103]

Nox regulation

Regulation of Noxes is complex and involves many NADPH oxidase subunits and multiple signaling pathways. All Noxes, except Nox5, seem to have an obligatory need for p22phox.[104] Whereas Nox2 requires p47phox and p67phox for its activity, Nox1 may interact with homologs of p47phox (NAD(P)H oxidase organizer 1) and p67phox (NAD(P)H oxidase activator 1).[105,106] Oxidase activation involves Rac translocation, phosphorylation of p47phox and its translocation, possibly with p67phox, and p47phox association with cytochrome b558. Nox2 and Nox4 are constitutively active. However, induction of Nox mRNA expression is observed in response to physical stimuli, (shear stress, pressure), growth factors (platelet-derived growth factor, epidermal growth factor and transforming growth factor β), cytokines (tumor necrosis factor-α, interleukin-1 and platelet aggregation factor), mechanical forces (cyclic stretch, laminar and oscillatory shear stress), metabolic factors (hyperglycemia, hyperinsulinemia, free fatty acids, advanced glycation end products and G protein-coupled receptor agonists (serotonin, thrombin, bradykinin, endothelin and Ang II).[107–109] Nox enzymes are also regulated by ClC-3, Poldip2 and protein disulfide isomerase. Poldip2 associates with p22phox to activate Nox4, leading to regulation of focal adhesion turnover and vascular smooth muscle cell migration, thus linking ROS production and cytoskeletal remodeling.[110] Ang II an important and potent regulator of cardiovascular NAD(P)H oxidase, activates NAD(P)H oxidase via AT1 receptors through stimulation of signaling pathways involving c-Src $p21^{Ras}$, protein kinase C, phospholipase D and phospholipase A_2[111–113] (Figure 1).

VASCULAR ANTIOXIDANT SYSTEMS

Enzymatic and non-enzymatic antioxidant systems protect against injurious oxidative stress. Major enzymatic antioxidants found in vascular tissue include SOD, catalase, glutathione peroxidases, thioredoxin and peroxiredoxin.[114,115] Non-enzymatic antioxidants include ascorbate, tocopherols, glutathione, bilirubin and uric acid and scavenge OH and other free radicals.[116] Extracellular SOD, the major vascular SOD, is produced and secreted by vascular smooth muscle cells and binds to glycosaminoglycans in the vascular

extracellular matrix and regulates oxidant status in the vascular interstitium.[114,115]

Decreased antioxidant capacity promotes cellular oxidative stress and has been implicated in cardiovascular and renal oxidative damage associated with hypertension.[114] Activity of SOD, catalase and glutathione (GSH) peroxidase is lower and the glutathione disulfide (GSSG)/GSH is higher in plasma and circulating cells from hypertensive patients than normotensive subjects.[117] In mice deficient in extracellular SOD and in rats in which GSH synthesis is inhibited, blood pressure is significantly elevated, demonstrating that reduced antioxidant capacity is associated with elevated blood pressure.[118] Failure to upregulate antioxidant genes and reduced antioxidant capacity are also associated with age-accelerated atherosclerosis.[119]

ROS AND VASCULAR BIOLOGY IN HYPERTENSION

Molecular processes underlying ROS-induced vascular injury involve activation of redox-sensitive signaling pathways. Superoxide anion and H_2O_2 stimulate mitogen-activated protein kinases, tyrosine kinases and transcription factors (nuclear factor-κB, activator protein-1 and hypoxia-inducible factor-1) and inactivate protein tyrosine phosphatases.[120,121] ROS also increase $(Ca^{2+})_i$ and upregulate protooncogene and proinflammatory gene expression and activity[122] (Figure 2). Virtually all processes involved in the inflammatory response involve ROS. Such phenomena occur through oxidative modification of proteins by altering key amino acid residues, by inducing protein dimerization, and by interacting with metal complexes such as Fe–S moieties.[123]

ROS have been implicated in the regulation of vascular tone by modulating vasodilation directly (H_2O_2 may have vasodilator actions) or indirectly by decreasing nitric oxide bioavailability through quenching by $\bullet O_2^-$ to form $ONOO^-$.[124–128] ROS, through the regulation of hypoxia-inducible factor-1, are also important in O_2 sensing,[125] which is essential for maintaining normal O_2 homeostasis. In pathological conditions ROS are involved in inflammation, endothelial dysfunction, cell proliferation, migration and activation, extracellular matrix deposition, fibrosis, angiogenesis and vascular remodeling[129–131] (Figure 3).

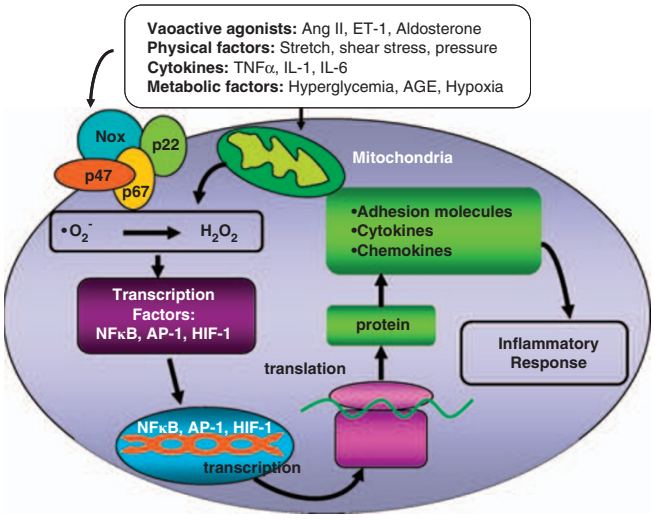

Figure 2 Induction of cellular inflammation by reactive oxygen species (ROS). Generation of ROS in vascular cells by nicotinamide adenine dinucleotide phosphate (NADPH) oxidase or mitochondria, leads to redox signaling that activates transcription factors important in inflammation. AGE, advanced glycation end product; Ang II, angiotensin II; AP-1, activator protein-1; ET-1, endothelin-1; HIF-1; hypoxia-inducible factor-1; IL, interleukin; NF-κB, nuclear factor-κB; TNFα, transforming growth factor α.

The causal relationship between ROS and hypertension probably occurs at the vascular level, at least in part, where oxidative stress promotes endothelial dysfunction, vascular inflammation, increased reactivity and structural remodeling leading to increased peripheral resistance and elevated blood pressure.[132] ROS formation in organs other than the vasculature also contribute to hypertension. Redox signaling in the central nervous system is important in neuronal control of blood pressure. Centrally produced ROS by NAD(P)H oxidase in the hypothalamus and brain stem, nucleus tractus solitarius, subfornical organ, rostral ventrolateral medulla and area postrema are implicated in central control of hypertension, in part through sympathetic outflow.[133] In experimental hypertension, renal Nox activity and ROS generation are increased and antioxidant enzyme activity/expression is reduced.[134] Renal oxidative stress is associated with glomerular damage, proteinuria, sodium and volume retention and nephron loss, all important in the development of hypertension.[134,135]

OXIDATIVE STRESS AND HUMAN HYPERTENSION

Almost all experimental models of hypertension show some form of oxidative excess including genetic forms (spontaneously hypertensive rats, stroke-prone spontaneously hypertensive rats), surgically induced (2K1C, aortic banding), endocrine-induced (Ang II, aldosterone, deoxycorticosterone acetate) and diet-induced hypertension (salt, fat).[21–23,136–138] Sources of ROS in experimental models include Noxes (Nox1, Nox2 and Nox4), xanthine oxidase, uncoupled nitric oxide synthase and mitochondrial oxidases. Mice deficient in ROS-generating enzymes have lower blood pressure compared with wild-type counterparts, and Ang II infusion fails to induce hypertension in these mice.[139,140]

Plasma levels of oxidative markers are increased in patients with essential hypertension, renovascular hypertension, malignant hypertension, salt-sensitive hypertension, cyclosporine-induced hypertension and preeclampsia.[141–143] These findings are based, in general, on increased levels of plasma thiobarbituric acid-reactive substances and 8-epi-isoprostanes, biomarkers of lipid peroxidation and oxidative stress.[144–146] Polymorphonuclear leukocyte- and platelet-derived $\bullet O_2^-$, which also participate in vascular oxidative stress and inflammation, are increased in hypertensive patients.[147]

Hypertensive patients exhibit a significantly higher production of plasma H_2O_2 than normotensive subjects.[148] Additionally, normotensive subjects with a family history of hypertension have greater H_2O_2 production than blood pressure-matched normotensives without a family history of hypertension, suggesting that there may be a genetic component that leads to elevated production of H_2O_2.[149,150] Plasma levels of asymmetric dimethylarginine (endothelial nitric oxide synthase inhibitor) and the lipid peroxidation product of linoleic acid, 13-hydroxyoctadecadienoic acid, a marker of ROS production, were inversely correlated with microvascular endothelial dysfunction and elevated blood pressure in hypertensive patients. To further support a relationship between oxidative stress and blood pressure, Van *et al.*[151] recently showed that myeloperoxidase is positively and independently associated with blood pressure and that this association is strongest in subjects with increased levels of oxidative markers. Phagocytic NADPH oxidase activity is increased in obese subjects, possibly due to hyperleptinemia, and is related to vascular remodeling and preclinical atherosclerosis, risk factors associated with hypertension.[152]

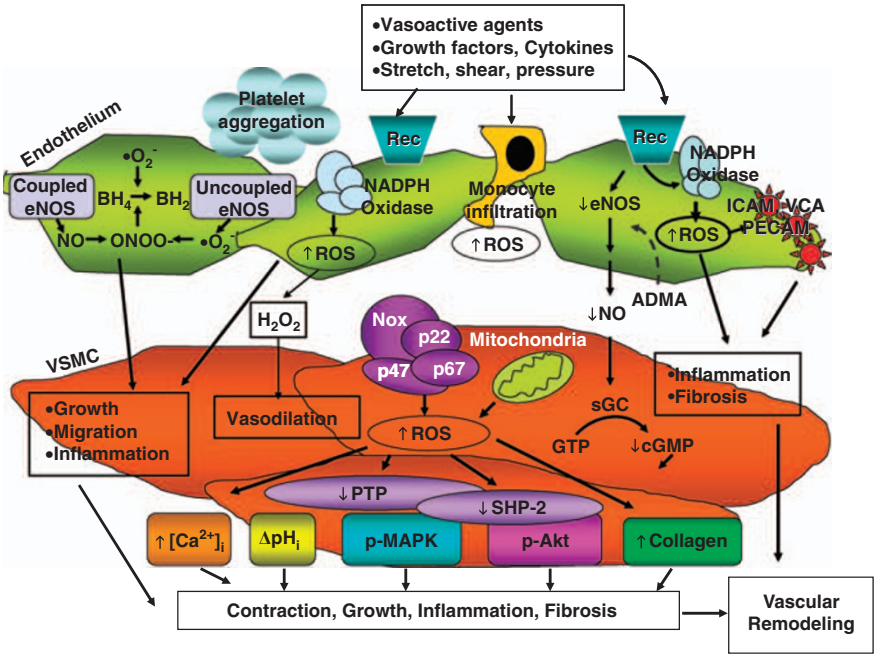

Figure 3 Redox-sensitive mechanisms underlying vascular changes in hypertension. Activation of reactive oxygen species (ROS)-generating enzymes, such as NAD(P)H oxidase, uncoupling of NOS and mitochondrial enzymes in endothelial and vascular smooth muscle cells results in decreased nitric oxide (NO) production and increased generation of $\bullet O_2^-$ and H_2O_2, which in turn influence redox-sensitive signaling molecules including MAPKs, PTPs, ion channels, transcription factors as well as induction of pro-inflammatory adhesion molecules such as platelet/endothelial cell adhesion molecule (PECAM), intercellular adhesion molecules (ICAM) and vascular cell adhesion molecules (VCAM). These processes lead to vascular growth, fibrosis, contraction/dilation, inflammation and platelet aggregation, which underlie vascular damage and structural remodeling in hypertension and other cardiovascular diseases. ADMA, asymmetric *N*(G), *N*(G)-dimethyl-L-arginine, endogenous NOS inhibitor; BH4, tetrahydrobiopterin; cGDP, cyclic guanosine diphosphate; eNOS, endothelial nitric oxide synthase; GTP, guanosine triphosphate; MAPK, mitogen-activated protein kinases; p-, phosphorylated protein; NADPH, nicotinamide adenine dinucleotide phosphate; PTP, protein tyrosine phosphatase; Rec, receptor; sGC, soluble guanylyl cyclase; SHP-2, SH2 domain-containing protein tyrosine phosphatase-2; VSMC, vascular smooth muscle cells.

We showed that ROS production is increased in vascular smooth muscle cells from resistance arteries of hypertensive patients and that this is associated with upregulation of vascular NAD(P)H oxidase.[153,154] The importance of this oxidase in oxidative stress in human cardiovascular disease is supported by studies showing that polymorphisms in NAD(P)H oxidase subunits are associated with increased atherosclerosis and hypertension.[155] In particular, the –930(A/G) polymorphism in the p22(phox) promoter may be a novel genetic marker associated with hypertension.[155,156] p22(phox) −930A/G, A640G and C242T polymorphisms of NADPH oxidase have are also associated with peripheral and central pressures in healthy, normotensive individuals.[52] Polymorphisms −337GA and 565+64CT of xanthine oxidase gene are related to blood pressure and oxidative stress in hypertension, also supporting a role for xanthine oxidase/ROS in hypertension.

Decreased antioxidant capacity also contributes to oxidative stress in patients with hypertension. Hypertensive patients have reduced activity and decreased content of antioxidant enzymes, including SOD, glutathione peroxidase and catalase.[157,158] Decreased levels of antioxidant vitamins A, C and E have been demonstrated in newly diagnosed, untreated hypertensive patients compared with normotensive controls.[158] Moreover, SOD activity has been demonstrated to correlate inversely with blood pressure in patients with hypertension.[158] In patients with white coat hypertension serum protein carbonyl (indicating protein oxidation) was increased and endogenous antioxidant proteins (protein thiol, SOD, glutathione) were decreased compared with normotensive individuals, suggesting a relationship between oxidative stress and hypertension.[159] Antioxidant vitamins reduced blood pressure and arterial stiffness in patients with diabetes or hypertension in small clinical studies,[160] but had no effect in postmenopausal women, in healthy subjects or in pregnant women at risk for hypertension/preeclampsia.[159–162]

THERAPEUTIC POTENTIAL OF ROS MODULATORS IN HYPERTENSION

The potential of antioxidants in treating conditions associated with oxidative stress is supported by experimental investigations, observational findings, small clinical studies and epidemiological data.[161,162] However, findings are inconsistent and clinical trial data are inconclusive.[163–165] In general results of clinical studies investigating cardiovascular effects of antioxidants have been disappointing given the consistent and promising findings from experimental investigations. Reasons for this have been extensively reviewed.[166–169] Harrison and colleagues[170] proposed a new strategy to increase antioxidant capacity without the use of exogenous antioxidants. They suggest that drugs that selectively inhibit multidrug resistant protein 1 would prevent cellular glutathione loss and thereby protect against oxidative damage, endothelial dysfunction and hypertension.[170] Whether such an approach is feasible in hypertensive patients remains to be proven.

Theoretically, agents that reduce oxidant formation should be more efficacious than non-specific, inefficient antioxidant vitamin scavengers. This is based on experimental evidence, in which it has been demonstrated that inhibition of Nox-mediated $\bullet O_2^-$ generation, using pharmacological and gene-targeted strategies, leads to regression of vascular remodeling, improved endothelial function, and lowering of blood pressure.[171,172] In fact Nox isoforms may be attractive

therapeutic targets for vascular disease.[171–173] New peptide inhibitors that have been developed to specifically target NADPH oxidases include the 18 amino acid peptide gp91ds-tat, which interferes with Nox and subunit assembly, because nine of the amino acids mimic the region of p22phox that interacts with p47phox.[174] PR39, a naturally occurring 39 amino acid proline- and arginine-rich peptide that binds to Src homology domain 3 of p47phox also prevents association between p47phox and Nox, thereby inhibiting oxidase assembly and activation.[175] Specific Nox inhibitors, including GKT136901, which induce allosteric changes, may also be promising candidates to reduce ROS generation.[176] However, the safety and effectiveness of all of these agents require confirmation in humans.

Another interesting approach is targeting glucose-6-phosphate dehydrogenase, which is a source of NADPH, the substrate for NAD(P)H oxidase.[177] Inhibition of glucose-6-phosphate dehydrogenase has been shown to ameliorate development of pulmonary hypertension, possibly through decreased oxidative stress. To date only investigational glucose-6-phosphate dehydrogenase inhibitors are available.

Other pharmacological interventions that reduce oxidative stress include: apocynin, a methoxy catechol that is activated by intracellular peroxidases into active metabolites, which inhibit NADPH oxidase, tetrahydrobiopterin (BH4), polyphenols and flavonoids.[175] Recent studies demonstrated that pycnogenol, a polyphenol and melatonin, protect the vasculature against oxidative damage, independently of blood pressure changes.[178,179]

Some of the beneficial effects of classical antihypertensive agents such as ß-adrenergic blockers, angiotensin-converting enzyme inhibitors, AT_1 receptor antagonists and Ca^{2+} channel blockers may be mediated, in part, by decreasing vascular oxidative stress.[180–184] These effects have been attributed to direct inhibition of NADPH oxidase activity and to intrinsic antioxidant properties of the drugs.

In view of current data and the lack of evidence to prove the benefits from use of antioxidants to prevent cardiovascular disease, antioxidant supplementation is not recommended for the prevention or treatment of hypertension.[185] However, most therapeutic guidelines suggest that the general population consumes a diet emphasizing antioxidant-rich fruits and vegetables and whole grains.[186,187] Another important lifestyle modification that may have cardiovascular protective and blood pressure lowering effects by reducing oxidative stress is exercise. In experimental models of hypertension and in patients with coronary artery disease, exercise reduced vascular NAD(P)H oxidase activity and ROS production, ameliorated vascular injury and reduced blood pressure.[188,189]

CONCLUSIONS

Extensive data confirm the importance of ROS in the physiological control of vascular function, through regulation of endothelial function and vascular tone via tightly controlled redox-sensitive signaling pathways. Uncontrolled production/degradation of ROS results in oxidative stress, which induces cardiovascular, renal and neural damage with associated increase in blood pressure. Although oxidative damage may not be the sole cause of hypertension, it facilitates and augments blood pressure elevation in the presence of other pro-hypertensive factors, such as salt-loading, activation of the renin–angiotensin system and sympathetic hyperactivity. Compelling findings from experimental and animal studies suggest a role for oxidative stress in the pathogenesis of hypertension, possibly through increased activation of Noxes. The exact role of specific Nox isoforms however remains unclear. From a clinical viewpoint current data on the causative role of ROS in hypertension are less conclusive. This may relate to heterogeneity of populations studied, inappropriate or insensitive methodologies to evaluate oxidative state clinically and sub-optimal antioxidant therapies used. Further research in the field of oxidative stress and human hypertension is warranted. In particular, there is an urgent need for the development of sensitive and specific biomarkers to assess the oxidant status of patients. Also needed are clinical trials designed to specifically address the role of oxidative stress in the development of hypertension. With a better understanding of mechanisms regulating ROS metabolism and identification of processes that promote oxidative excess, it should be possible to target therapies more effectively so that detrimental vascular actions of oxygen free radicals can be reduced and beneficial effects of nitric oxide can be enhanced.

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