Reactive oxygen species and vascular remodelling in hypertension: Still alive

Shaoping Xu MD PhD, Rhian M Touyz MD PhD

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Reactive oxygen species (ROS) are reactive derivatives of O_2 metabolism, including superoxide anion, hydrogen peroxide, hydroxyl radical and nitric oxide. All types of vascular cells produce ROS, primarily via cell membrane-associated NAD(P)H oxidase. Cardiovascular diseases, such as hypertension, are associated with increased ROS formation (oxidative stress). Oxidative excess in the vasculature reduces levels of the vasodilator nitric oxide, causes tissue injury, promotes protein oxidation and DNA damage, and induces proinflammatory responses. ROS are also important intracellular signalling molecules that regulate vascular function by modulating vascular cell contraction/dilation, migration, growth/apoptosis, and extracellular matrix protein turnover, which contribute to vascular remodelling. Interventions to decrease ROS bioavailability regress remodelling and reduce blood pressure in experimental hypertension. Such strategies may have therapeutic potential in cardiovascular diseases.

Key Words: *Endothelium; Free radicals; Inflammation; Redox signalling; Smooth muscle cells; Vessels*

Les espèces oxygénées radicalaires et le remodelage vasculaire en cas d'hypertension : Toujours d'intérêt

Les espèces oxygénées radicalaires (EOR) sont des dérivés radicalaires du métabolisme de l'oxygène, y compris l'anion de superoxyde, le peroxyde d'hydrogène, l'hydroxyle et le monoxyde d'azote. Tous les types de cellules vasculaires produisent des EOR, surtout par l'oxydase NAD(P)H associée aux membranes cellulaires. Les maladies cardiovasculaires, comme l'hypertension, sont reliées à une formation accrue d'EOR (stress oxydatif). L'excédent oxydatif dans le système vasculaire réduit les taux de monoxyde d'azote vasodilatateur, provoque des lésions tissulaires, favorise l'oxydation protéique et les dommages à l'ADN et induit des réponses pro-inflammatoires. Les EOR sont également d'importantes molécules de signalisation intracellulaire, qui régularisent la fonction vasculaire en modulant la contraction et la dilatation, la migration la croissance et l'apoptose des cellules vasculaires, ainsi que le renouvellement des porines extracellulaires, qui contribuent au remodelage vasculaire. Les interventions en vue de réduire la biodisponibilité de l'EOR font régresser le remodelage et réduisent la tension artérielle dans les cas d'hypertension expérimentale. Ces stratégies peuvent avoir un potentiel thérapeutique en présence de maladies cardiovasculaires.

Reactive oxygen species (ROS) are ubiquitous reactive derivatives of O_2 metabolism found in all biological systems. ROS were traditionally regarded as byproducts of aerobic metabolism. However, ROS have recently been recognized to act as signalling molecules in vascular cells and to play a role in cellular events associated with vascular remodelling in cardiovascular diseases (1). ROS function as important intracellular and intercellular second messengers to modulate many downstream signalling molecules, such as protein tyrosine phosphatases (PTPs), protein tyrosine kinases, transcription factors, mitogen-activated protein kinases (MAPKs) and ion channels. Induction of these signalling cascades leads to vascular smooth muscle cell (VSMC) growth and migration, expression of proinflammatory mediators and modification of the extracellular matrix (ECM). In addition, ROS increase intracellular free Ca^{2+} concentrations, a major determinant of vascular reactivity. ROS influence signalling molecules by altering the intracellular reduction-oxidation (redox) state and by oxidative modification of proteins. In physiological conditions, these events are highly regulated and play an important role in maintaining vascular function and integrity. Under pathological conditions, dysregulation of ROS, due to enhanced production and/or reduced antioxidant potential, contributes to increased bioavailability of free radicals and consequently to oxidative stress, which results in vascular dysfunction and remodelling through oxidative damage.

Among ROS, attention has focused on the highly reactive free radical superoxide anion ($\bullet O_2^-$) and the more stable hydrogen peroxide (H_2O_2). ROS are formed as intermediates in redox processes leading from O_2 to H_2O. The univalent reduction of O_2, in the presence of a free electron, yields $\bullet O_2^-$, which is cell membrane-impermeable (Figure 1). In physiological conditions, the favoured reaction of $\bullet O_2^-$ is dismutation by superoxide dismutase (SOD) yielding H_2O_2, which is scavenged by catalase and glutathione peroxide to produce H_2O. In the vasculature, all types of cells, including endothelial cells (ECs), VSMCs and fibroblasts, produce $\bullet O_2^-$ and H_2O_2 to varying degrees (2). When generated in excess, $\bullet O_2^-$ reacts with nitric oxide (NO) to produce peroxynitrite, a potentially deleterious oxidant, leading to decreased NO bioavailability. H_2O_2 is lipid-soluble and crosses cell membranes. A mismatch between ROS formation and the defense ability of antioxidants results in increased bioavailability of ROS, leading to a state of oxidative stress (3).

Through their cell-damaging effects, ROS have been implicated to play a role in vascular injury associated with cardiovascular diseases, such as hypertension, atherosclerosis, restenosis and diabetic vascular complications (1-3). In hypertension, small arteries undergo structural remodelling due, in large part, to increased cell growth, cell migration, ECM deposition and inflammation (4). All of these processes are influenced to varying degrees by ROS (2) (Figure 1).

Kidney Research Centre, Ottawa Health Research Institute, University of Ottawa, Ottawa, Ontario
Correspondence: Rhian M Touyz, Canada Research Chair in Hypertension, Kidney Research Centre, Health Research Institute/University of Ottawa, 451 Smyth Road, Ottawa, Ontario K1H 8M5. Telephone 613-562-5800 ext 8241, fax 613-562-5487, e-mail rtouyz@uottawa.ca
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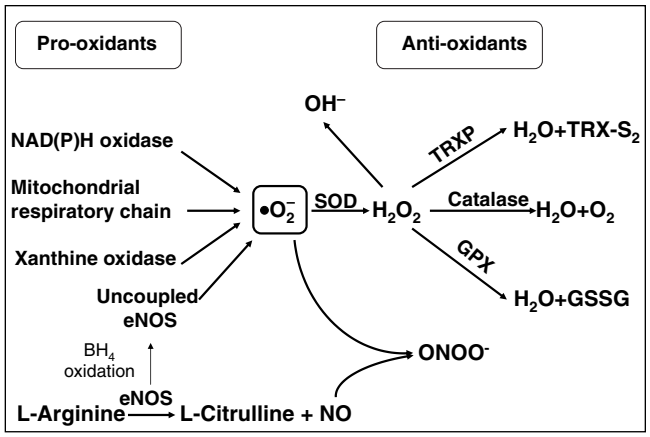

Figure 1) *Possible role of reactive oxygen species in vascular remodelling and implications in the pathogenesis of hypertension. Angiotensin II-stimulated NAD(P)H oxidase in vascular cells results in the univalent reduction of O_2 in the presence of a free electron to yield superoxide anion ($\bullet O_2^-$), which in turn is dismutated to hydrogen peroxide (H_2O_2). In the presence of nitric oxide (NO), peroxynitrite ($ONOO^-$) can be formed. Increased intracellular levels of reactive oxygen species contribute to vascular inflammation, growth, altered contraction and dilation (vascular tone), and endothelial dysfunction. These events in turn lead to vascular remodelling, arterial narrowing, increased peripheral resistance and consequently to increased blood pressure. BH_4 Tetrahydrobiopterin; eNOS Endothelial nitric oxide synthase; GPX Glutathione peroxidase; GSSG Oxidized glutathione; $\bullet OH^-$ Hydroxyl radical; SOD Superoxide dismutase; TRXP Thioredoxin peroxidase; TRX-S_2 Thioredoxin disulphide*

GENERATION OF ROS IN THE VASCULATURE

Potential sources of vascular $\bullet O_2^-$ generation include NAD(P)H oxidase, uncoupled nitric oxide synthase (NOS), xanthine oxidase and mitochondria. Among them, NAD(P)H oxidase appears to be the major source in vascular cells. Vascular NAD(P)H oxidases that are low-output, slow-release enzymes differ from phagocytic NAD(P)H oxidases in their structural and biochemical characteristics (5) (Table 1). Phagocytic NAD(P)H oxidases comprise a plasma membrane-spanning cytochrome b558 composed of a large catalytic subunit, gp91^{phox} (nox2), and a small subunit, p22^{phox}, together with cytosolic regulatory subunits p47^{phox}, p67^{phox}, p40^{phox} and the small GTPase rac (6). Phagocytic NAD(P)H oxidase uses intracellular NADPH and transfers electrons across the membrane to extracellular O_2 (6). However, ROS generation in vascular cells appear to be intracellular. gp91^{phox} (nox2), p22^{phox}, p47^{phox} and p67^{phox} have been identified in ECs, adventitial fibroblasts (5) and VSMCs from small human resistance arteries (7). Studies on VSMCs from aortas and other large arteries demonstrate that p22^{phox}, p47^{phox} and rac are expressed, whereas nox2 and p67^{phox} are either absent or present in very low concentrations. Instead, the nox2 homologues, nox1 and nox4, appear to be the major catalytic subunits in these cells (8).

On cell stimulation, p47^{phox} becomes phosphorylated and the cytosolic subunits form a complex, which then migrates to the membrane, where it associates with cytochrome b558 to assemble the active oxidase, which transfers electrons from the substrate to O_2, leading to $\bullet O_2^-$ generation (6). In vascular cells, nox4 expression is abundant and may play an important role in constitutive $\bullet O_2^-$ production in nonproliferating cells.

TABLE 1
Differences between phagocytic and vascular NAD(P)H oxidase

Characteristic	Phagocytic oxidase	Vascular oxidase
Name	NAD(P)H oxidase	nox enzymes
Activity	Basal state inactive	Constitutively active
Primary function	Host:defense reactions	Signal transduction
Mode of activity	Inducible	Inducible
Nox isoform	gp91^{phox} (nox2)	gp91^{phox}/nox1/ nox4/nox5
Pattern of $\bullet O_2^-$ release	Burst-like	Slow and sustained
Concentration released	High	Low (1% to10% of phagocytic)
Site of $\bullet O_2^-$ release	Extracellular	Intracellular
Substrate	NAD(P)H	NAD(P)H/NADH
Small guanine nucleotide-binding protein	rac2	rac1

On stimulation, nox1 is upregulated and may be important in vascular pathology (9). Recently, nox organizer 1 (NOXO1) and nox activator 1, homologues of p47^{phox} and p67^{phox}, respectively, have been cloned from colon epithelial cells (10). Similar to p47^{phox}, NOXO1 binds to p22^{phox}, which is required for nox1-dependent activity. NOXO1 is prelocalized at membranes together with nox1 and p22^{phox} in unstimulated cells (10). However, the functional significance of these homologues in the vasculature remains unclear.

Endothelial nitric oxide synthase (eNOS) is a calcium-dependent flavoenzyme that generates NO in a process involving oxidation of the amino acid L-arginine by the reduction of molecular O_2. All three NOSs, eNOS, neuronal NOS and inducible NOS can also generate $\bullet O_2^-$ in conditions of substrate (arginine) or cofactor (tetrahydrobiopterin [BH_4]) deficiency (11). These findings have led to the concept of 'NOS uncoupling', where the activity of the enzymes for NO production is decreased in association with an increase in NOS-dependent $\bullet O_2^-$ formation. BH_4 itself is highly susceptible to oxidative degradation, and the initial oxidative loss of BH_4 in response to increased ROS production by NAD(P)H oxidases has been shown to amplify oxidative stress through the resulting loss of NO production and increased NOS-dependent $\bullet O_2^-$ generation (8,11). In spontaneously hypertensive rats (SHR), in spite of the increased expression and activity of NOS, $\bullet O_2^-$ is elevated and NO production is reduced. In deoxycorticosterone acetate-salt hypertensive mice, BH_4, and NO are improved or restored by treatment with BH_4, eNOS gene deletion, apocynin, or p47^{phox} gene deletion, suggesting a role for NAD(P)H oxidase in NOS uncoupling (8).

Electron leakage from the mitochondrial electron transport chain constitutively produces $\bullet O_2^-$, usually rapidly degraded by manganese SOD. However, under some pathological conditions, such as hypoxia/reoxygenation, mitochondria may be a significant source of $\bullet O_2^-$ in a ceramide-dependent fashion (12,13). In deoxycorticosterone acetate-induced hypertension, a model of endothelin (ET)-dependent hypertension, although NAD(P)H oxidase and xanthine oxidase activities are increased, only mitochondrial generation of ROS was normalized by ET_A receptor antagonist, indicating that mitochondria may play a role in ET-1-driven oxidative stress (14).

Xanthine oxidase requires reduction of molecular O_2 to catalyze oxidation of hypoxanthine to xanthine and xanthine to urate, thereby generating $\bullet O_2^-$. The possible contribution of xanthine oxidase to ROS elevation in hypertension has been assessed using specific inhibitors. Such treatments normalize ROS formation in microvessels from rats fed a high-salt diet, and increase endothelial-dependent relaxation in arteries from SHR and rats overexpressing renin and angiotensinogen genes, suggesting that xanthine oxidase is another potential source of elevated ROS generation in hypertension that could impair vascular function and structure (8).

ROS IN HUMAN AND EXPERIMENTAL HYPERTENSION

Clinical studies demonstrated increased ROS production in patients with essential hypertension, renovascular hypertension, malignant hypertension and preeclampsia (15). In general, these findings are based on increased levels of plasma thiobarbituric acid reactive substances and 8-epi-isoprostanes, biomarkers of lipid peroxidation and oxidative stress. Studies in cultured VSMCs derived from resistance arteries of hypertensive patients revealed enhanced formation of ROS (16). Patients with essential hypertension have decreased levels of antioxidant glutathione, and activity of SOD is reduced (17).

Increased levels/activity of vascular NAD(P)H oxidase has been implicated as the primary source of excess $\bullet O_2^-$ in essential hypertension (5). Activation of the renin-angiotensin system has been proposed as a major stimulator of NAD(P)H oxidase activation and ROS production in human hypertension (5). Because of this interaction between renin-angiotensin II and $\bullet O_2^-$-generating systems, it is not surprising that some of the therapeutic blood pressure-lowering actions of renin-angiotensin converting enzyme inhibitors and renin-angiotensin II type I receptor blockers may be mediated by inhibiting NAD(P)H oxidase activity and reducing ROS production.

Polymorphisms in the $p22^{phox}$ gene have been suggested to play a role in altered NAD(P)H oxidase-generated $\bullet O_2^-$ production in human cardiovascular disease (18). In particular, the $-930^{A/G}$ polymorphism in the $p22^{phox}$ promoter may be a novel genetic marker associated with hypertension (18). A single nucleotide polymorphism in the $p22^{phox}$ gene has been linked to altered arterial compliance (18). However, to confirm that these polymorphisms are indeed markers for hypertension, studies in large populations are necessary.

Although clinical studies provide compelling evidence that oxidative stress is important in the pathophysiology of hypertension, not all human hypertension is redox-dependent. In never-treated, mild-to-moderate hypertension, lipid peroxidation is not increased (19). In some studies, renin-angiotensin II type I receptor blockade did not improve endothelial function and $\bullet O_2^-$ production was unaltered in hypertensive subjects (20). Furthermore, many large clinical trials on antioxidants failed to demonstrate beneficial therapeutic effects on blood pressure and cardiovascular outcomes (21-23). Reasons for these discrepancies probably relate to the heterogeneous nature of hypertension and to the complexities of redox biology in the cardiovascular system.

Oxidative stress has been convincingly demonstrated in various models of genetic and experimental hypertension. SHR and stroke-prone SHR exhibit increased NAD(P)H oxidase-driven $\bullet O_2^-$ generation in mesenteric and aortic arteries (8,15). Increased activation of vascular NAD(P)H oxidase, xanthine oxidase and uncoupling of eNOS have been implicated in enhanced $\bullet O_2^-$ generation in experimental hypertension, such as renin-angiotensin II-induced hypertension, Dahl salt-sensitive hypertension and lead-induced hypertension. Inhibition of ROS generation with apocynin and scavenging of free radicals with antioxidants or SOD mimetics decreases blood pressure and prevents development of hypertension in most experimental models (2).

ROLE OF ROS IN VASCULAR REMODELLING

ROS regulate vascular function by modulating cell growth, apoptosis/anoikis, migration, inflammation, secretion and ECM protein production. During vascular damage in hypertension, when oxidative stress is increased, redox-sensitive growth processes may lead to accelerated proliferation and hypertrophy, further contributing to vascular injury and remodelling (Figure 1). Rao and Berk (24) showed that VSMC DNA synthesis and cell number increased by increasing intracellular ROS. Agonists, such as renin-angiotensin II (5), platelet-derived growth factor (PDGF) (25) and thrombin (26), stimulate VSMC proliferation and migration by induction of intracellular ROS generation, whereas application of $p22^{phox}$ antisense, catalase or diphenyleneiodinium (DPI), which inhibit NAD(P)H oxidase activity, reduced effects by agonists. However, a recent study (27) showed that moderate concentrations of exogenous H_2O_2 (0.1 mM) cause cell cycle arrest, and high concentrations induce apoptosis in vascular VSMCs, or multiphase cell cycle arrest in fibroblasts. The differential responses of vascular cells to ROS may be related to different species generated to varying concentrations, and to compartmentalization of ROS. Differential signalling by ROS, due to concentration or time dependency, may also contribute to variable responses (28).

Redox-sensitive inflammatory processes, including expression of proinflammatory molecules, such as monocyte chemoattractant protein 1 and interleukin (IL)-6, expression of adhesion molecules, including vascular cell adhesion molecule-1 and intracellular adhesion molecule-1, further contribute to vascular remodelling in hypertension (2). In cultured ECs and VSMCs, renin-angiotensin II upregulates expression of vascular cell adhesion molecule-1, intracellular adhesion molecule-1, E-selectin, and proinflammatory cytokines/chemokines, IL-6 and IL-8. These effects are mediated via redox-sensitive processes (29).

ROS also influence vascular remodelling by increasing deposition of ECM proteins, such as collagen, and by modulating matrix metalloproteinases (MMPs), which degrade collagen and other ECM proteins. In cultured ECs, lysophosphatidylcholine, a major component of oxidized low-density lipoprotein, induced generation of ROS and secretion of MMP-2 (30). Inhibition of NAD(P)H oxidase attenuated the effects of lysophosphatidylcholine (30).

SIGNALLING PATHWAYS OF ROS IN REGULATING VASCULAR REMODELLING

ROS influence vascular function by modulating various redox-sensitive signalling proteins, such as MAPKs (31). MAPKs, including extracellular signal-regulated kinase (ERK)-1/2, ERK5, p38 MAPK and *c-jun n*-terminal kinase (JNK), are serine/threonine kinases involved in a number of intracellular

signalling pathways that regulate vascular cell growth, apoptosis, contraction, migration and inflammation. H_2O_2 also influences cell growth by inhibiting nuclear protein transport through ERK1/2-dependent pathways (32). Potential mechanisms for ROS-mediated activation of MAPKs may be via oxidant-induced inactivation of PTPs (33). In VSMC, renin-angiotensin II stimulates ERK1/2 and p38 MAPK activation, whereas transfection of VSMCs with nox1 antisense significantly inhibits p38 MAPK phosphorylation (9). In ECs, increased intracellular ROS activated JNK activity, whereas antioxidant *n*-acetyl cysteine or diphenyleneiodonium inhibited the rise in JNK activity.

Receptor and nonreceptor tyrosine kinases are also influenced by ROS. Exogenous H_2O_2 induces tyrosine phosphorylation and activation of PDGF receptor and epidermal growth factor receptor (EGFR), probably due to redox-mediated inhibition of dephosphorylation of PDGF receptor and EGFR by inactivation of membrane-associated PTPs (34). Under pathological conditions associated with oxidative stress, such as hypertension, ROS may directly activate cell surface receptors, thereby amplifying the process of $\bullet O_2^-$ generation (2,3,5). The cell survival kinase protein kinase B (Akt), which is a nonreceptor tyrosine kinase, is another redox-sensitive kinase. Both exogenous H_2O_2 and renin-angiotensin II stimulate Akt activation in VSMC (35). Importantly, renin-angiotensin II-induced Akt phosphorylation was inhibited by DPI, overexpression of catalase, and nox1 antisense (9), indicating a role for NAD(P)H oxidase in agonist-induced Akt activation.

The proinflammatory transcription factor, nuclear factor kappa B (NFκB) is regulated in a redox-dependent manner. NFκB regulates the expression of a number of genes involved in immune and inflammatory responses. In cultured ECs, H_2O_2 increases the nuclear translocation of NFκB and binding to target DNA (36), whereas *n*-acetyl cysteine inhibits activation of NFκB by tumour necrosis factor-beta by inhibiting inhibitor kappa B phosphorylation and degradation (37). Both SOD and catalase prevented activation of NFκB by inhibiting renin-angiotensin II-induced inhibitor kappa B degradation (38). These observations indicate that ROS are important for NFκB activation.

In addition to influencing cellular processes associated with growth and inflammation, ROS modulate intracellular free Ca^{2+} concentration, a major determinant of vascular contraction and dilation. Both $\bullet O_2^-$ and H_2O_2 increase intracellular free Ca^{2+} concentration in VSMCs and ECs (39). These processes involve mobilization from reticular stores and activation of Ca^{2+} channels (40,41). These redox-regulated Ca^{2+} processes may be more important in stress responses than in receptor-mediated signalling by growth factors or cytokines, and may play a role in altered vascular contractility in hypertension.

CONCLUSIONS

ROS are produced in the vasculature in a highly regulated manner. $\bullet O_2^-$ and H_2O_2 have important signalling properties, mainly through oxidative modification of proteins and activation of transcription factors that maintain vascular, cardiac and renal function and structure. In hypertension, dysregulation of enzymes such as NAD(P)H oxidase, NOS, xanthine oxidase, mitochondrial enzymes or SOD that generate $\bullet O_2^-$, H_2O_2 and hydroxyl radical or reduced scavenging by antioxidants, results in increased formation of ROS, which has damaging actions on the vasculature. ROS in hypertension contribute to vascular injury by promoting vascular cell growth, ECM protein deposition, activation of MMPs, inflammation, endothelial dysfunction and increased vascular tone. In experimental hypertension oxidative stress is increased and antioxidant levels and activity are decreased. Clinical data suggest that hypertensive patients, especially those with severe hypertension, salt-sensitive hypertension and renovascular hypertension, exhibit oxidative excess. Although inconclusive at present, therapeutic modalities to alter ROS bioavailability by decreasing production and/or by increasing radical scavenging, may regress vascular remodelling, prevent further vascular injury and reduce blood pressure and associated target organ damage in hypertensive patients. Whether it would be preferable, from a clinical viewpoint, to reduce ROS generation rather than increase scavenging of free radicals is unclear at present. However, the potential of SOD mimetics, NAD(P)H oxidase inhibitors and gene transfer strategies to enhance NO and antioxidant bioactivity may provide additional and improved therapeutic options in the management of hypertension and other cardiovascular diseases.

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