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Rhian M. Touyz

*Hypertension*. 2004;44:248-252; originally published online July 19, 2004;

doi: 10.1161/01.HYP.0000138070.47616.9d

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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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## Reactive Oxygen Species, Vascular Oxidative Stress, and Redox Signaling in Hypertension What Is the Clinical Significance?

Rhian M. Touyz

**Abstract**—Metabolism of oxygen by cells generates potentially deleterious reactive oxygen species (ROS). Under normal conditions the rate and magnitude of oxidant formation is balanced by the rate of oxidant elimination. However, an imbalance between prooxidants and antioxidants results in oxidative stress, which is the pathogenic outcome of oxidant overproduction that overwhelms the cellular antioxidant capacity. The kidney and vasculature are rich sources of NADPH oxidase–derived ROS, which under pathological conditions play an important role in renal dysfunction and vascular damage. Strong experimental evidence indicates that increased oxidative stress and associated oxidative damage are mediators of renovascular injury in cardiovascular pathologies. Increased production of superoxide anion and hydrogen peroxide, reduced nitric oxide synthesis, and decreased bioavailability of antioxidants have been demonstrated in experimental and human hypertension. These findings have evoked considerable interest because of the possibilities that therapies targeted against free radicals by decreasing ROS generation or by increasing nitric oxide availability and antioxidants may be useful in minimizing vascular injury and renal dysfunction and thereby prevent or regress hypertensive end-organ damage. This article highlights current developments in the field of ROS and hypertension, focusing specifically on the role of oxidative stress in hypertension-associated vascular damage. In addition, recent clinical trials investigating cardiovascular benefits of antioxidants are discussed, and some explanations for the rather disappointing results from these studies are addressed. Finally, important avenues for future research in the field of ROS, oxidative stress, and redox signaling in hypertension are considered. (*Hypertension*. 2004; 44:248-252.)

**Key Words:** free radicals ■ antioxidants ■ resistance ■ arteries ■ hypertension, essential

Compelling experimental evidence indicates that reactive oxygen species (ROS) play an important pathophysiological role in the development of hypertension. This is due, in large part, to  $\cdot\text{O}_2^-$  excess (oxidative stress) and decreased NO bioavailability in the vasculature and kidneys and to ROS-mediated cardiovascular remodeling.<sup>1-3</sup> In human hypertension, biomarkers of systemic oxidative stress are elevated.<sup>4</sup> Treatment with superoxide dismutase (SOD) mimetics or antioxidants improves vascular and renal function, regresses vascular remodeling, and reduces blood pressure (BP).<sup>5,6</sup> Mouse models deficient in ROS-generating enzymes have lower BP compared with wild-type counterparts, and Angiotensin II (Ang II) infusion fails to induce hypertension in these mice.<sup>7</sup> Furthermore, experimental models with compromised antioxidant capacity develop hypertension.<sup>8</sup> In cultured vascular smooth muscle cells (VSMCs) and isolated arteries from hypertensive rats and humans, ROS production is enhanced, redox-dependent signaling is amplified, and antioxidant bioactivity is reduced.<sup>9</sup> Accordingly, evidence at multiple levels supports a role for oxidative stress in the

pathogenesis of hypertension. This article highlights recent developments relating to vascular ROS in hypertension and consider the significance of oxidative stress in clinical hypertension. ROS in other organ systems, such as the heart, nervous system, and kidneys, have also been implicated in the pathophysiology of hypertension. In particular, increased renal  $\cdot\text{O}_2^-$  production is associated with NO bioinactivation, which influences afferent arteriolar tone, tubuloglomerular feedback responses, and sodium reabsorption, important in long-term BP regulation.<sup>10</sup> These aspects are examined in recent excellent reviews<sup>11,12</sup> and will not be discussed further here.

### ROS in Vascular Damage in Hypertension

Vascular ROS are produced in endothelial, adventitial, and VSMCs and derived primarily from NAD(P)H oxidase, a multisubunit enzyme catalyzing  $\cdot\text{O}_2^-$  production by the 1 electron reduction of oxygen using NAD(P)H as the electron donor:  $2\text{O}_2 + \text{NAD(P)H} \rightarrow 2\text{O}_2^- + \text{NAD(P)} + \text{H}^+$ .<sup>13</sup> Vascular NAD(P)H oxidase comprises at least 4 components: cell

Received May 10, 2004; first decision May 25, 2004; revision accepted June 25, 2004.

From the CIHR Multidisciplinary Research Group on Hypertension, Clinical Research Institute of Montreal, University of Montreal, Quebec, Canada. Correspondence to Rhian M. Touyz, MD, PhD, Clinical Research Institute of Montreal, 110 Pine Ave W, Montreal, H2W 1R7. E-mail [touyzr@ircm.qc.ca](mailto:touyzr@ircm.qc.ca)

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DOI: 10.1161/01.HYP.0000138070.47616.9d

membrane-associated p22phox and gp91phox (or gp91phox [nox2] homologues, nox1 and nox4), and cytosolic subunits, p47phox and p67phox.<sup>13,14</sup> Vascular NAD(P)H oxidase is regulated by humoral (cytokines, growth factors, and vasoactive agents) and physical factors (stretch, pulsatile strain, and shear stress).<sup>13</sup> Physiologically, ROS are produced in a controlled manner at low concentrations and function as signaling molecules<sup>14</sup> to maintain vascular integrity by regulating endothelial function and vascular contraction-relaxation. Under pathological conditions, increased ROS bioactivity leads to endothelial dysfunction, increased contractility, VSMC growth, monocyte invasion, lipid peroxidation, inflammation, and increased deposition of extracellular matrix proteins, important factors in hypertensive vascular damage.<sup>15,16</sup>

Impaired endothelium-mediated vasodilation in hypertension has been linked to decreased NO bioavailability. This may be secondary to decreased NO synthesis or to increased NO degradation because of its interaction with  $\cdot\text{O}_2^-$  to form ONOO<sup>-</sup>.<sup>17</sup> Vasomotor tone is also modulated through direct ROS effects on  $[\text{Ca}^{2+}]_i$ .<sup>18,19</sup> Whereas VSMC  $\cdot\text{O}_2^-$  is associated primarily with vasoconstriction, endothelial  $\text{H}_2\text{O}_2$  has been described as an endothelium-derived relaxing factor.<sup>20</sup>

Molecular processes underlying ROS-induced vascular changes involve activation of redox-sensitive signaling pathways. Superoxide anion and  $\text{H}_2\text{O}_2$  stimulate mitogen-activated protein kinases, tyrosine kinases, and transcription factors (NF $\kappa$ B, AP-1, and HIF-1) and inactivate protein tyrosine phosphatases.<sup>14,21</sup> ROS also increase  $[\text{Ca}^{2+}]_i$  and upregulate protooncogene and proinflammatory gene expression.<sup>14</sup> These processes occur through oxidative modification of proteins by altering important amino acid residues, by inducing protein dimerization, and by interacting with metal complexes such as Fe-S moieties.<sup>14</sup> Changes in the intracellular redox state through thioredoxin and glutathione systems may also influence signaling events.<sup>14,21</sup>

### Oxidative Stress in Experimental Hypertension

Vascular oxidative stress has been demonstrated in spontaneous (genetic) and experimental hypertension. Spontaneously hypertensive rats (SHR) and stroke-prone SHR, genetic models that develop hypertension spontaneously, exhibit increased NAD(P)H driven  $\cdot\text{O}_2^-$  generation in resistance (mesenteric) and conduit (aortic) vessels.<sup>5,8,22,23</sup> This is associated with NAD(P)H oxidase subunit overexpression and enhanced oxidase activity.<sup>3,6,22,24</sup> Several polymorphisms in the promoter region of the p22<sup>phox</sup> gene have been identified in SHR.<sup>25</sup> This has clinical relevance because an association between a p22<sup>phox</sup> gene polymorphism and NAD(P)H oxidase-mediated  $\cdot\text{O}_2^-$  production in the vascular wall of patients with hypertension and atherosclerosis has been described.<sup>26</sup> Increased expression of p47phox has been reported in the renal vasculature, macula densa, and distal nephron from young SHR, suggesting that renal NAD(P)H oxidase upregulation precedes development of hypertension.<sup>3,27</sup> Diminished NO bioavailability as a consequence of enhanced vascular  $\cdot\text{O}_2^-$  generation and downregulation of the thioredoxin system may also contribute to oxidative stress in SHR

and stroke-prone SHR.<sup>1,8</sup> Treatment with antioxidant vitamins, NAD(P)H oxidase inhibitors, SOD mimetics, and  $\text{BH}_4$  and Ang II type-1 ( $\text{AT}_1$ ) receptor blockers decrease vascular  $\cdot\text{O}_2^-$  production and attenuate development of hypertension in these models.<sup>5,22,23</sup> Taken together, these findings suggest that oxidative stress in genetic hypertension involves enhanced NAD(P)H oxidase activity and dysfunctional endothelial nitric oxide synthase (uncoupled NOS) and is regulated, in part, by  $\text{AT}_1$  receptors.

Vascular oxidative stress has also been demonstrated in experimentally-induced hypertension, such as Ang II-mediated hypertension, Dahl salt-sensitive hypertension, lead-induced hypertension, obesity-associated hypertension, mineralocorticoid hypertension, and aldosterone-provoked hypertension.<sup>28–30</sup> Activation of vascular NAD(P)H oxidase and xanthine oxidase and endothelial nitric oxide synthase uncoupling<sup>13,14,17,31,32</sup> have been implicated in amplified  $\cdot\text{O}_2^-$  generation in experimental hypertension. Inhibition of ROS generation with apocynin (NAD(P)H oxidase inhibitor) or allopurinol (xanthine oxidase inhibitor) and radical scavenging with antioxidants or SOD mimetics decrease BP and prevent development of hypertension in most hypertensive models.<sup>5,6,22,23,27</sup> These beneficial effects have been attributed to normalization of endothelial function, regression of vascular remodeling, reduced vascular inflammation, and improved renal function.

### Oxidative Stress in Human Hypertension

Clinical studies demonstrated increased ROS production in patients with essential hypertension, renovascular hypertension, malignant hypertension, and preeclampsia.<sup>33–35</sup> 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.<sup>4,36</sup> Accumulation of ROS by-products from oxidized genomic and mitochondrial DNA have also been demonstrated in hypertensive individuals.<sup>4</sup> Polymorphonuclear leukocytes and platelets, rich  $\cdot\text{O}_2^-$  sources, also participate in vascular oxidative stress and inflammation in hypertensive patients.<sup>37,38</sup> In never-treated mild-to-moderate hypertension, lipid peroxidation and oxidative stress are not increased,<sup>39</sup> suggesting that ROS may not be critical in the early stages of human hypertension, but could be more important in severe hypertension. Decreased antioxidant activity (SOD, catalase) and reduced levels of ROS scavengers (vitamin E, glutathione) may contribute to oxidative stress.<sup>4,36</sup> Activation of the renin-angiotensin system has been proposed as a mediator of NAD(P)H oxidase activation and ROS production.<sup>1,13–15</sup> In fact, some of the therapeutic BP-lowering actions of  $\text{AT}_1$  receptor blockers and angiotensin-converting enzyme (ACE) inhibitors have been attributed to NAD(P)H oxidase inhibition and decreased ROS production.<sup>40,41</sup> It has also been suggested that p22phox polymorphisms may be important in altered NAD(P)H oxidase-generated  $\cdot\text{O}_2^-$  production in human cardiovascular disease. In particular, the -930(A/G) polymorphism in the p22(phox) promoter may be a novel genetic marker associated with hypertension.<sup>26</sup> However, recent data suggest that homozygous individuals with the T allele of the C242T *CYBA* polymorphism may have reduced

vascular oxidative stress compared with wild-type carriers and heterozygous individuals.<sup>42</sup> Hence, the exact significance of p22phox polymorphisms in clinical hypertension awaits clarification.

### **Modulating ROS Bioavailability: Therapeutic Role in Human Hypertension**

Based on experimental evidence of the importance of oxidative stress in vascular damage, there has been great interest in developing strategies that target ROS in the treatment of hypertension and other cardiovascular diseases. Therapeutic approaches that have been considered include mechanisms to increase antioxidant bioavailability or to reduce ROS generation by decreasing activity of  $\cdot\text{O}_2^-$ -generating enzymes.<sup>43</sup> Gene therapy targeting oxidant systems are also being developed, but their use in clinical hypertension remains unclear.

The potential of antioxidants in treating conditions associated with oxidative stress is supported by experimental investigations, observational findings, small clinical studies, and epidemiological data.<sup>1,44,45</sup> However, findings are inconsistent and clinical trial data are inconclusive. To date, at least 7 large trials have been published regarding antioxidant vitamin effects on risks of cardiovascular disease: the Cambridge Heart Antioxidant Study (CHAOS; 2002 patients); Alpha Tocopherol, Beta-Carotene cancer prevention study (ATBC; 27 271 males); Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico (GISSI)-Prevenzione trial (3658 patients); Heart Outcomes Prevention Evaluation (HOPE) study (2545 subjects); Medical Research Council/British Heart Foundation (MRC/BHF) heart protection study (20 536 adults); Primary Prevention Project (PPP; 4495 patients); and the Antioxidant Supplementation in Atherosclerosis Prevention (ASAP) study (520 subjects) have recently been reviewed.<sup>46,47</sup> Except for the ASAP study, which demonstrated that 6-year supplementation of daily vitamin E and slow-release vitamin C reduced progression of carotid atherosclerosis, the other studies failed to demonstrate significant beneficial effects of antioxidants on BP or on cardiovascular end points. Thus, overall results of clinical trials are disappointing given the consistent and promising findings from experimental investigations, clinical observations, and epidemiological data. However, many of these trials enrolled patients with significant cardiovascular disease, and the choice of antioxidant vitamins used may not have been ideal, as discussed below.

### **Reasons for Disappointing Outcomes From Antioxidant Prevention Trials**

Overall, data from large prospective randomized clinical trials failed to demonstrate beneficial cardiovascular effects of antioxidants. Potential reasons relate to (1) antioxidants used, (2) patients included in trials, and (3) the trial design itself. With respect to antioxidants, it is possible that agents examined were ineffective and inappropriate and that dosing regimens and duration of therapy were insufficient. For example, vitamins C and E, the most widely examined antioxidants in trials, may have prooxidant properties, with harmful and deleterious interactions. It is also possible that orally administered antioxidants may be inaccessible to the

source of free radicals, particularly if ROS are generated in intracellular compartment and organelles. Furthermore, antioxidant vitamins do not scavenge  $\text{H}_2\text{O}_2$  or HOCl, which may be more important than  $\cdot\text{O}_2^-$  in hypertensive vascular damage. Another factor requiring consideration, is that antioxidants do not inhibit ROS production. Theoretically, agents that reduce oxidant formation should be more efficacious than scavengers in ameliorating oxidative stress. This is based on experimental evidence where it has been shown unambiguously that inhibition of NAD(P)H oxidase-mediated generation of  $\cdot\text{O}_2^-$ , using pharmacological and gene-targeted strategies, leads to regression of vascular remodeling, improved endothelial function, and lowering of BP.<sup>3,6,7,13,24,27</sup> In fact, vascular NAD(P)H oxidase, specifically gp91phox (nox2) homologues, may be novel therapeutic targets for vascular disease.<sup>48</sup>

Regarding individuals included in large trials, most subjects had significant cardiovascular disease, in which case damaging effects of oxidative stress may be irreversible. Another confounding factor is that most of the enrolled subjects were taking aspirin prophylactically. Because aspirin has intrinsic antioxidant properties,<sup>49</sup> additional antioxidant therapy may be ineffective. Moreover, in patients studied in whom negative results were obtained, it was never proven that these individuals had increased oxidative stress. In fact, negative results of clinical trials must be interpreted cautiously in the absence of verification that antioxidant therapy successfully reduces oxidative stress. To date, there are no large clinical trials in which patients were recruited based on evidence of elevated ROS formation. It is possible that in the absence of oxidative stress, antioxidants are ineffective.

Finally, none of the large clinical trials were designed to examine effects of antioxidants specifically on BP. Trials with well-characterized "oxidatively stressed" cohorts investigating appropriate antioxidants (or other ROS modulators) specifically on BP and development of hypertension need to be conducted to definitively test the role of oxidative stress in human hypertension.

### **Current Recommendations and Clinical Implications**

In view of present data, it is suggested that the general population consumes a balanced diet emphasizing antioxidant-rich fruits and vegetables and whole grains. Currently, antioxidant supplementation is not recommended for the prevention or treatment of hypertension. This advice, which is consistent with the guidelines of the American Heart Association<sup>50</sup> and the Canadian Hypertension Society,<sup>51</sup> considers the role of the total diet in influencing disease risk, and is supported by findings from the Dietary Approaches to Stop Hypertension (DASH) and a recent trial from the United Kingdom, which demonstrated that subjects consuming high fruit and vegetable diets had significantly reduced BP.<sup>52</sup>

It has also been suggested that some of the beneficial effects of classical antihypertensive agents such as  $\beta$ -adrenergic blockers (carvedilol), ACE inhibitors, AT<sub>1</sub> receptor antagonists, and Ca<sup>2+</sup> channel blockers may be mediated, in part, by decreasing vascular oxidative stress.<sup>40,41</sup> These effects have been attributed to direct inhibition of

NAD(P)H oxidase activity, as shown for AT<sub>1</sub> receptor blockers, and to intrinsic antioxidant properties of the agents. The potential role of antihypertensive drugs as modulators of vascular oxidative stress is currently an area of active research.

### Where to From Here?

Experimental evidence supporting a role for oxidative stress in vascular injury and hypertension is convincing. What remains unclear is (1) exactly how ROS regulate signaling molecules in the cardiovascular system, (2) what tips the balance to a prooxidant state in hypertension, and (3) whether scavenging free radicals is indeed the best way to decrease ROS bioavailability. Future research should focus on (1) elucidating mechanisms whereby free radicals and the redox state modify signaling proteins in the cardiovascular system, (2) exploring why superoxide-generating systems are upregulated and antioxidant systems are downregulated in hypertension, and (3) determining whether it is preferable to prevent or limit formation of free radicals by targeting the source of ROS rather than by scavenging them once they have been generated. Furthermore, sensitive and specific biomarkers that can be used clinically to assess the oxidant status of patients need to be developed. Finally, clinical trials designed to specifically address the role of oxidative stress in the development of hypertension need to be undertaken. Accordingly, the need to pursue research in the field of ROS, oxidative stress, redox signaling, and hypertension is more important than ever. With a greater insight into the 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 actions of vascular oxygen-free radicals can be reduced and beneficial effects of NO $\cdot$  can be enhanced. Such therapies would be useful in the prevention and management of many disease processes associated with vascular damage, including hypertension.

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