The contemporary notion that oxidative stress contributes to vascular wall pathology dates back some 25 years, when chemical modification of LDL was found to permit macrophage foam cell formation, and subsequent data indicated that vascular cells promoted LDL lipid oxidation (e.g., LDL oxidation) to produce a similarly modified LDL. It is now clear, however, that oxidative stress in the vascular wall involves much more than the oxidation of LDL lipids. Risk factors for atherosclerosis are associated with an increased arterial wall flux of reactive oxygen species that not only may oxidize biological targets (i.e., lipids), but also directly produce phenotypic changes in vascular cells such as inducing smooth muscle cell proliferation, adhesion molecule expression, and premature senescence. Many of these cellular responses have been implicated in both the development and the clinical manifestations of atherosclerosis.

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In the cellular environment, the most common reactive oxygen species produced is superoxide ($O_2^-$) because it is the product of a single electron added to oxygen. Cells and tissues contain abundant superoxide dismutase that converts superoxide to hydrogen peroxide (H$_2$O$_2$), a species that has garnered considerable interest as an endogenous signaling molecule. A landmark study by Sundaresan and colleagues demonstrated a requirement for intracellular H$_2$O$_2$ generation in the mitogenic effects of platelet-derived growth factor on smooth muscle. Subsequent investigation points to a host of phenotypic responses that involve intracellular reactive oxygen species as signaling molecules (reviewed by Chen et al). As a result of this body of work, one can construct a paradigm (Figure 1) relating cellular responses to the phenotype of the vascular wall. In this model, the presence or absence of vascular disease represents a balance between tissue responses typically categorized as either normal or related to injury. With regard to the former, a normal vasculature is characterized by laminar flow, endothelial elaboration of nitric oxide (NO), and the resultant vasodilation, inhibition of platelet adhesion, low matrix turnover, and smooth muscle cells historically described as “quiescent.” In contrast, vascular disease is characterized by an injury response that involves mediators such as angiotensin to induce production of cellular reactive oxygen species that, in part, mediate vasoconstriction, matrix turnover, and a “proliferative” smooth muscle cell phenotype. According to this paradigm, one may propose that vascular disease represents a dysregulation of the injury response. Because the cellular generation of reactive oxygen species is a critical part of this response, understanding the mechanism(s) of injury-related reactive oxygen species production has become a subject of considerable interest.

Among the cellular sources of reactive oxygen species associated with injury, NADPH oxidase is perhaps the best characterized. This multicomponent enzyme consists of cytosolic accessory proteins (Rac, p47phox, p67phox) that, on stimulation, associate with the membrane catalytic subunits (Nox, p22phox) to facilitate superoxide generation (Figure 2). For many years, this enzyme was thought to exist as a single isoform only in phagocytes and its function restricted to antimicrobial action. This notion changed drastically with the discovery of multiple Nox isoforms that now represent an enzyme family containing at least 5 members. The phagocyte enzyme is now known as Nox2, and several members of this Nox family exist in vascular cells (Figure 2), with many appearing to be upregulated in the setting of atherosclerosis and arterial injury. Consistent with these observations, the activity of this enzyme family has been implicated in atherosclerosis, hypertension, and the response to arterial injury.

Although there is considerable enthusiasm for the involvement of NADPH oxidases in the pathogenesis of vascular disease, much of the supportive evidence is circumstantial. Although it is known that the neutrophil oxidase (Nox2) requires p22phox, p47phox, and Rac1 for full activity, our knowledge concerning the contribution of these proteins to other Nox isoform activity is not yet clear. As a consequence, little information exists on the individual Nox family members with regard to their contribution to vascular pathology. Two important articles in this issue of Circulation have broken new ground and have begun to bridge this gap in knowledge with regard to the Nox1 isoform of NADPH oxidase. Dikalova and colleagues used a “gain-of-function” strategy to overexpress Nox1 and determine its implications for vascular pathology. In particular, they used elegant genetic techniques to specifically overexpress Nox1 in smooth muscle cells where this isoform has been implicated in the response to angiotensin II. They found that overexpression of Nox1 in smooth muscle produced a basal increase in vascular reactive oxygen species production without any overt vascular pathology. In response to angiotensin II...
infusion, however, animals with smooth muscle cell Nox1 overexpression demonstrated greatly exaggerated hypertension and medial arterial hypertrophy as compared with animals with normal Nox1 levels. These data are consistent with published observations that angiotensin II–induced reactive oxygen species production induces smooth muscle cell hypertrophy and contributes to the angiotensin II–induced vasomotor response.20 The work by Dikalova and colleagues specifically identifies smooth muscle cell Nox1 as a plausible source of angiotensin II–induced reactive oxygen species production and supports the notion that Nox1 contributes to angiotensin II–mediated vascular pathology. Given the broad efficacy of angiotensin-converting enzyme inhibitors to ameliorate vascular disease,21 the data by Dikalova and coworkers would support speculation that Nox1 may contribute to atherosclerosis in general.

Given previous data that NADPH oxidase is required for angiotensin II–induced smooth muscle cell hypertrophy,19 it is notable that overexpression of Nox1 alone only induced mild medial hypertrophy with no hypertension despite a demonstrable increase in the reactive oxygen species signal.18 The authors explain these findings by noting compensatory upregulation of antioxidant enzymes (eg, catalase, manganese superoxide dismutase), that potentially mitigated the influence of reactive oxygen species. One must also consider, however, an alternative interpretation that reactive oxygen species from Nox1 are not sufficient to recapitulate the entire spectrum of the vascular wall angiotensin II response. This view coincides with in vitro data that angiotensin II induces reactive oxygen species–dependent and –independent responses that are both required for smooth muscle cell hypertrophy.20 Under this scenario, reactive oxygen species–mediated signals would best be viewed as either permissive or exerting a modulatory influences on vascular phenotype rather than being strictly required for specific cellular responses. In this regard, the role of reactive oxygen species could be likened to that of NO, a signaling molecule with a largely modulatory role in the vasculature that often counterbalances phenotypic responses associated with reactive oxygen species (Figure 1).

In a classic complement to the “gain-of-function” strategy employed by Dikalova and coworkers, the study by Matsuno and colleagues employs a “loss-of-function” approach. These investigators created mice deficient in Nox1 by disrupting the Nox1 gene. Because the gene is on the X chromosome, it was possible to study male mice (eg, Nox1/H11002/H11002/Y) to determine the effect of Nox1 on the response to angiotensin II. Compared with wild-type mice, Nox1-null mice exhibited attenuation of both the reactive oxygen species flux and the hypertensive response to angiotensin II after 7 days of treatment.23 These findings are in total agreement with the notion that Nox1-derived reactive oxygen species contribute to angiotensin II–induced hypertension.18 The angiotensin II–induced increase in medial hypertrophy was not blunted in Nox1-null mice despite this reduced blood pressure. These data by Matsuno and colleagues suggest that Nox1 is not necessary for angiotensin II–induced medial hypertrophy, a finding that contrasts with the conclusions derived from Nox1 overexpressing mice.18

How can we reconcile the findings from these 2 studies? One possible explanation relates to the different strategies employed: enzyme overexpression versus deletion. The former approach requires considerable care with regard to interpretation. In any study that uses overexpression of a protein, it is possible that the extent of overexpression does not mimic the situation normally observed in nature, with the
potential consequence of introducing nonspecific effects. Focusing on the study of Dikalova and colleagues, it seems that Nox1 overexpression afforded an angiotensin II–induced reactive oxygen species flux far exceeding that observed with angiotensin infusion alone. In this instance, it is possible that an “excess” nonphysiological reactive oxygen species flux produced additional medial hypertrophy. Indeed, it is known that the smooth muscle cell response to reactive oxygen species is highly dose dependent. Another possible reason for the discrepancy between studies is that reactive oxygen species could be responsible for the medial hypertrophy, but any source will suffice. This contention is consistent with observations that compared with normal animals, angiotensin II still produced an increased (albeit blunted) reactive oxygen species flux in Nox1-null mice in association with the upregulation of Nox2 and Nox4. This Nox1-independent reactive oxygen species flux could be sufficient to support the medial hypertrophy. Data from Dikalova and colleagues that tempol (an antioxidant compound) attenuated both the reactive oxygen species signal and medial hypertrophy in response to angiotensin II would tend to support this viewpoint.

Despite their differences, these 2 studies add collectively to our understanding of vascular pathology. For example, both studies demonstrated that Nox1-derived reactive oxygen species have an impact on the hypertensive response to angiotensin II, perhaps through scavenging of NO, a potent vasodilator known to regulate blood pressure. In addition, both studies have effectively separated hemodynamic responses from effects on medial hypertrophy. In saline-treated animals, Dikalova and colleagues found medial hypertrophy with Nox1 overexpression in the absence of hypertension, whereas Matsuno et al demonstrated reduced angiotensin II–induced hypertension in Nox1-null mice with no effect on hypertrophy. Together, these findings are consistent with a previous report that mice deficient in another NADPH oxidase isoform (Nox2) have no defect in angiotensin II–induced hypertension. Data from Dikalova and colleagues that reactive oxygen species flux could be sufficient to support the subsequent phenotypic response(s). In summary, the 2 genetic models of Nox1 manipulation presented here represent an important milestone in research relating oxidative stress to vascular disease. It is clear that NADPH oxidases play a role in vascular pathology and the control of vascular phenotype. We need more models in which the genes for various NADPH oxidase isoforms are either overexpressed or deleted. Only with the advent of these models can we hope to test the precise contribution of NADPH oxidases in vascular pathology. Because injury and inflammation are known to be important for vascular disease, understanding the role of NADPH oxidases in these processes is of paramount importance, and this knowledge may help us develop new strategies for the treatment of cardiovascular disease.

References


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Oxidative Stress and the Vascular Wall: NADPH Oxidases Take Center Stage
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