Editorial

Nox Response to Injury

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Szöcs and colleagues, in this issue of *Arteriosclerosis, Thrombosis and Vascular Biology*, have added to our understanding of the role of NAD(P)H oxidase and reactive oxygen species in vascular injury. The study was undertaken by using the rat carotid artery balloon injury model, a well-characterized model of neointimal formation. In this model, vascular balloon injury leads to medial smooth muscle cell proliferation and migration across the internal elastic lamina to form the neointima. Adventitial myofibroblasts may also migrate across the media and into the neointima within the first week after balloon injury. The processes of proliferation and migration of smooth muscle cells and fibroblasts in vitro are critically dependent on the production of reactive oxygen species. Moreover, several reports suggest that vascular production of reactive oxygen species increases rapidly after balloon injury, and that treatment with nonspecific antioxidant regimens can inhibit experimental neointimal formation. Although the relevance of the rat balloon injury model to human post-angioplasty restenosis is questionable, the well-defined kinetics of neointimal formation in this model provide a unique opportunity to explore the cellular and enzymatic sources of reactive oxygen species during vascular cell proliferation and migration in response to injury in vivo.

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NAD(P)H Oxidases in the Vasculature: Structural and Functional Considerations

Recent studies indicate that nonphagocytic NAD(P)H oxidases are major sources of reactive oxygen species in the vascular wall. Like the phagocytic respiratory burst NADPH oxidase, non-phagocytic NAD(P)H oxidases reduce molecular oxygen to generate superoxide, which is in turn converted to hydrogen peroxide. However, unlike the phagocytic NADPH oxidase, the NAD(P)H oxidases present in blood vessels are constitutively active, producing relatively low levels of reactive oxygen species under basal conditions and generating higher levels of oxidants in response to growth factors and cytokines. These attributes are consistent with a role for nonphagocytic NAD(P)H oxidases in cellular signaling.

Both the phagocytic and nonphagocytic oxidases are multimeric enzymes composed of plasma membrane associated–proteins as well as cytosolic factors. In the phagocytic NADPH oxidase, the plasma membrane–associated proteins gp91phox and p22phox compose the flavocytochrome b55 complex, which forms the catalytic subunit of the oxidase. Although fibroblasts are known to possess gp91phox, this subunit is not highly expressed in smooth muscle cells, suggesting that other subunits must be present in the latter cells to form catalytically active NAD(P)H oxidase. Recently, two smooth muscle cell homologs of gp91phox, termed nox1 and nox4, were identified by this same group of investigators. The investigators found that antisense nox1 mRNA inhibited smooth muscle cell superoxide production and growth, whereas overexpression of nox1 in fibroblasts increased superoxide production and proliferation. Moreover, in smooth muscle cells, treatment with the growth-promoting agonist angiotensin II upregulated the expression of nox1 while downregulating that of nox4, suggesting that the expression of these proteins may be positively and negatively related, respectively, to smooth muscle cell growth. Considering the many differences between smooth muscle cells grown in culture versus those resident within the arterial wall, however, it remained to be determined whether nox expression is similarly altered during cell growth coincident with production of superoxide in vivo.

Localization of Superoxide Production and Nox Expression in Balloon-Injured Vessels

In the present study, Szöcs et al examined the vascular distribution of superoxide in the balloon-injured carotid artery using the fluorescent probe dihydroethidium in conjunction with laser confocal microscopy. Early after balloon injury, increases in superoxide were detected that temporally and spatially colocalized with activated smooth muscle cells and fibroblasts. Late (7 days or more) after balloon injury, marked increases in superoxide were observed throughout the vessel wall, coinciding with maximal neointimal formation. The neointimal cells were virtually all of vascular origin, and predominately smooth muscle cells, as has been described previously.

By examining the kinetics of expression of subunits of the NAD(P)H oxidase, the authors were able to gain insight into the mechanisms of the enhanced superoxide production. Expression of nox1, gp91phox, and p22phox was elevated within 3 days after balloon denudation, suggesting that NAD(P)H oxidase is rapidly induced after vascular injury in vivo. Increased expression of nox1 and gp91phox implicate both smooth muscle cells and fibroblasts in this process. The subsequent localization of g91phox in the neointima late after balloon injury is an interesting observation. Because gp91phox is preferentially expressed in fibroblasts, one interpretation is that adventitial myofibroblasts have migrated across the internal elastic lamina, as was reported recently by another investigative team. On the other hand, De Leon et al did not observe migration of adventitial myofibroblasts into the neointima after balloon injury. Thus, it is conceivable that a subpopulation of neointimal smooth muscle cells is capable of expressing gp91phox after balloon injury.

Although indices of cell proliferation were not examined in the present study, in an earlier study, the proliferation index decreased rapidly in the late phase after balloon injury and was only slightly elevated after 2 weeks. Thus, although increased vascular superoxide production occurring early after balloon injury likely stimu-
lates cell proliferation, the proliferation process abates despite the continued production of superoxide. Precisely how this occurs is not known. Perhaps an analogy can be drawn, however, to a study by Li and colleagues,17 who reported that the capacity of superoxide to induce proliferation of smooth muscle cells in vitro was dose- and duration-dependent: a single application of a low dose of superoxide stimulated proliferation, whereas higher doses or repeated application induced cytotoxicity.

Late Upregulation of Nox4 After Balloon Injury
One very interesting aspect of the current study is that nox4 expression did not become upregulated until late after balloon injury, coinciding with a marked reduction in the rate of smooth muscle cell proliferation.2 The causes and consequences of this late upregulation of nox4 expression remain to be determined. However, Geiszt et al13 recently reported that overexpression of the same subunit in fibroblasts reduced the rate of proliferation and resulted in an alteration in cell phenotype. Interestingly, the phenotype of neointimal smooth muscle cells in balloon-injured vessels also differs from that of prototypical medial smooth muscle cells.19 Proliferation of neointimal cells reportedly peaks earlier in the post-injury phase,2 before the upregulation of nox4 was detected. Thus, the upregulation of nox4 is more likely consequent to, rather than causal of, growth inhibition. Nevertheless, it is conceivable that overexpression of nox4 in the arterial wall in vivo could attenuate neointimal proliferation after balloon injury.

Another intriguing consideration is that the upregulation of nox4 expression could be pertinent to apoptosis of neointimal cells, which has been observed 7 to 14 days after balloon injury.20,21 Notably, Li et al22 recently reported that hydrogen peroxide can activate NAD(P)H oxidase to induce smooth muscle cell apoptosis, a phenomenon which seems to be enhanced in the rat aorta 14 days after balloon injury.19 This finding suggests that oxidants themselves might be contributing to activation of NAD(P)H oxidase after balloon injury. Whether NAD(P)H oxidase-derived reactive oxygen species contribute to apoptosis of neointimal cells in the late phase after balloon injury, and whether enhanced expression of nox4 plays a role in this process remain to be determined. Because the accumulation of neointimal cells reflects the balance between cell proliferation and apoptotic cell loss, however, these findings suggest that administering antioxidants after balloon injury could conceivably limit vascular remodeling. Dual effects of oxidants to stimulate both neointimal proliferation and apoptosis may help explain experimental and clinical observations that the efficacy of antioxidant therapy to ameliorate post-angioplasty restenosis can explain experimental and clinical observations that the efficacy of oxidants to stimulate both neointimal proliferation and apoptosis may help explain experimental and clinical observations that the efficacy of antioxidant therapy to ameliorate post-angioplasty restenosis can

Conclusions
In summary, Szócs et al1 have provided new insight into the regulation of NAD(P)H oxidase expression after vascular injury. The induction of nox1, gp91phox, and p22phox early after balloon injury is consistent with a role for smooth muscle cell and fibroblast NAD(P)H oxidase in redox-regulation of neointimal proliferation, whereas the late upregulation of nox4 coincides with cessation of cell growth and could play a role in induction of apoptosis. Demonstration of the nox response to injury indicates that expression of nox1 and nox4 in the vascular wall is temporary and spatially regulated in vivo. The factors responsible for modulation of nox1 and nox4 expression after vascular injury and the precise contribution of these novel NAD(P)H oxidase subunits to neointimal formation and remodeling remain to be determined.

References
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