Editors

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The Oxidative Paradox
Another Piece in the Puzzle
Cam Patterson, Nageswara R. Madamanchi, Marschall S. Runge

Because atherosclerosis is a disease of many risk factors, a complex pathology, and diverse clinical manifestations, the as yet unattained Holy Grail of atherosclerosis research is a unifying pathway to explain its many aspects and provide a single point at which to measure risk or intervene in its clinical course. If vascular biology possesses such a relic, it would have many of the characteristics attributed to oxidative stress. Risk factors for atherosclerosis, such as hypertension and hyperlipidemia, are potent stimuli for the generation of reactive oxygen species (ROS) in experimental systems, and it is likely that cigarette smoking and diabetes mellitus share oxidative histories. At the molecular level, signaling in response to proatherogenic agents, such as α-thrombin, platelet-derived growth factor, and angiotensin II, requires the generation of ROS via oxidase systems. In fact, many of the hallmarks of atherosclerosis, including endothelial dysfunction, smooth muscle cell (SMC) proliferation, and inflammatory recruitment, occur in response to ROS. In this issue of Circulation Research, Schieffer et al contribute to our understanding of these events by demonstrating that angiotensin II–induced cytokine activation via the Janus kinase/signal transducer and activator of transcription (STAT) pathway requires oxidant generation by the NAD(P)H oxidase in SMCs. Their data are consistent with the notion that ROS are central mediators of adverse events in atherosclerosis. The oxidative paradox, then, is the inability of clinical investigators to definitively demonstrate modulation of ROS by the use of antioxidant therapies has any effect on atherogenesis.

Angiotensin II is the best-characterized stimulus for ROS generation in SMCs. The study by Schieffer et al describes the role of ROS as stimuli for inflammatory events that may contribute to plaque instability. Although many of the issues raised in this study have been addressed previously, the nature of the NAD(P)H oxidase that likely generates ROS in SMCs merits attention. The classical NAD(P)H oxidase described in neutrophils consists of several components, p22phox, p67phox, gp91phox, and a rac GTPase, that are recruited to create the oxidative burst. The study by Schieffer et al is the second to implicate a particular component of this oxidase, p47phox, in the oxidase of SMCs. Using electroporated antibodies to target p47phox, they find that this protein (and presumably the oxidase in which it participates) is necessary for ROS generation, STAT activation, and interleukin-6 synthesis elicited by angiotensin II. It should be noted that their data, although compelling, are not conclusive in this regard. Decreased p47phox protein in cells electroporated with an anti-p47phox antibody may indicate decreased de novo p47phox protein synthesis. However, neutralizing-antibody experiments are notoriously difficult to interpret, and alternative explanations include the possibility that p47phox antibody complexes are insoluble or that antibody binding to p47phox elicits its degradation.

p47phox is not the only component of the SMC oxidase shared with the neutrophil oxidase. There is also good evidence that p22phox is present in both. However, equally compelling evidence points to critical differences in the phagocytic and nonphagocytic oxidases. First, these oxidases have important differences in substrate use. The neutrophil oxidase consumes NADPH preferentially, whereas the SMC oxidase favors NADH. Second, the amounts of ROS generated by these oxidases differ by several orders of magnitude and also differ in the rate by which ROS is generated. Third, evidence is mounting that the components of these two oxidases differ structurally. A member of the gp91phox family, NOX1, probably participates in the SMC oxidase in place of gp91phox; and p67phox has been difficult to detect in SMCs, indicating that it may either be dispensable or replaced by another component.

The novel structure of the SMC oxidase (and perhaps other nonphagocytic oxidases) may explain how these oxidases mediate nonmicrobical functions, particularly by generation of ROS at slower rates and lower intracellular concentrations. Perhaps more importantly, it is possible that the function of the SMC oxidase can be regulated independently of oxidase systems in other cell types, a significant point is oxidases in nonvascular cells are essential for other purposes. The present study provides confirmatory evidence that p47phox is a component of the SMC oxidase. Identification and reconstitution of the SMC oxidase, which we anticipate would include additional novel components in comparison with the neutrophil oxidase, will be crucial to our understanding of the atherogenic role of ROS. It should also be emphasized that a component of the SMC oxidase, the Rac1 GTPase, may also regulate signaling independently of ROS generation by directly interacting with and activating STAT proteins.

The relevance of such interactions, as demon-
stated in the study by Schieffer et al., provides additional justification for the complete characterization of the SMC oxidase.

Regardless of how ROS are generated, it is easy to overwhelm their production with reducing agents and antioxidants in experimental studies, which is the crux of the oxidative paradox of atherosclerosis. The data regarding the use of antioxidants (eg, vitamin E) for prevention of atherosclerosis do not presently support their use in cardiovascular diseases (see Table). This conundrum poses perhaps the greatest challenge to vascular biologists interested in the role of ROS in atherogenesis. How can there be such a divergence between clinical data and studies in experimental models? It is worth considering the following possibilities.

First, the vitamin preparations may not be efficient oxidant scavengers in vivo. Redox reactions are complicated, and the potential exists for paradoxical electron transfer by antioxidant vitamins that results in additional oxidant generation. In addition, some vitamin formulations available for human use (vitamin E in particular) are relatively inefficient in their antioxidant functions in vivo. Our understanding of how the antioxidants available for human use affect oxidative stress in the circumstances described by Schieffer et al. is still at a relatively superficial level.

Second, ROS that are detrimental to vascular function may be compartmentalized at designated sites within the cell. For example, production of ROS occurs by different mechanisms in the cytoplasm and mitochondria, and distinct cellular mechanisms exist to detoxify ROS present in these two compartments. Accumulation of oxidative injury in mitochondria may be particularly detrimental to vascular cells.

Third, antioxidants may have subtle toxicities that mask their beneficial effects on vascular functions in vivo. Cells have evolved means to generate ROS for a reason, and quenching them randomly may have undesired effects, especially when administered on a chronic basis. Cell type–specific delivery of antioxidants may be one means to maximize the therapeutic benefit of these agents.

Fourth, similar to hypertension or hyperlipidemia, increased oxidative stress may be a risk factor for only a subset of patients with atherosclerosis, albeit a subset we presently have no easy way to identify. Measuring markers of oxidative injury, such as circulating lipid peroxides or mitochondrial DNA damage, is a promising, but as yet unproven, means to identify patients who might benefit from antioxidant therapy.

Although several important studies, including the present work by Schieffer et al., emphasize the role ROS play in cellular events critical to atherosclerosis, we need to recognize that our understanding of the relationship between oxidative stress and vascular lesion formation is still fairly naive. We are far enough along to recognize that there is an oxidative paradox but not far enough along to solve it.

### References


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<th>Study (Reference)</th>
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<td>Patients at high risk for cardiovascular events</td>
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<td>GISSI-Prevenzione Investigators (12)</td>
<td>Patients surviving MI</td>
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<td>2 years</td>
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<td>5.3 years</td>
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<td>Angiographically proved coronary artery disease</td>
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MI indicates myocardial infarction.


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