Oxidative stress, resulting from a local imbalance between the ubiquitous formation of reactive oxygen species (ROS) and the equally ubiquitous antioxidant defenses, is thought to be an important contributor to atherogenesis (Figure). Many of the players are known. The biochemical pathways generating superoxide, hydrogen peroxide, hydroxyl radicals, and lipid peroxides have been identified, as have the most important oxygen radical–scavenging enzymes (superoxide dismutase, catalase, glutathione peroxidase, and thioredoxin reductase). Many endogenous and exogenous contributors to oxidative stress are also known, such as hypercholesterolemia, hyperglycemia, hypertension, and shear stress. Leukocytes responding to intimal signals—frequently resulting from increased oxidation—are also an important contributor to ROS formation. In fact, ROS released during the respiratory burst, an innate immune defense against bacterial pathogens, may promote lipid peroxidation and therefore enhance further leukocyte recruitment and differentiation. In addition to antioxidant enzymes, arterial cells express enzymes promoting ROS formation, including, cyclooxygenase, NADPH oxidase, cytochrome P450 epoxygenases, myeloperoxidase, lipoxygenase, and inducible nitric oxide synthase. To what extent these enzymes account for ROS formation and oxidation of LDL in arteries, however, remains unknown. Dietary factors, natural and synthetic antioxidants, and hypocholesterolemic agents, including statins, also directly and indirectly influence ROS. and synthetic antioxidants, and hypocholesterolemic agents, including statins, also directly and indirectly influence ROS.

Conjunct Regulation of Aortic Antioxidant Enzymes During Atherogenesis

Wulf Palinski

Overall, inflammation is thought to promote atherogenesis, but specific immune responses may actually be protective. Subsequently, it was recognized that ROS may play an even more important role by modulating many oxidation-sensitive signaling pathways that regulate the expression of genes that influence cell recruitment, differentiation, proliferation, activity, and death. Most prominent among the oxidation-sensitive pathways is the nuclear transcription factor κB system, which regulates leukocyte adhesion molecules and chemokines, growth-promoting and antiapoptotic factors, but also some proinflammatory and prothrombotic factors. Another prominent pathway is that of the peroxisome proliferator–activated receptor γ, which upon activation upregulates scavenger receptors but downregulates proinflammatory genes and promotes reverse cholesterol transport. The third oxidation-sensitive pathway of importance for atherogenesis is that regulating the expression of caspases, the main effectors of apoptosis. Because transcription factors involved in these pathways are intricately linked and their effect is potentially antagonistic, the consequences of increased ROS on atherosclerosis via specific genes are difficult to predict. It is also noteworthy that many of the above findings were obtained by exposing cells to oxLDL, which may act either through membrane receptors or by increasing intracellular ROS.

The third focus of interest has been the interaction of ROS with nitric oxide (NO). ROS interfere with the vasorelaxation induced by NO generated by endothelial nitric oxide synthase (eNOS), yet eNOS also promotes superoxide formation, which in turn reacts with NO to form peroxynitrite, another potent oxidant. In fact, several studies in eNOS knockout mice indicate a reduction of advanced atherosclerosis, whereas initial lesion formation may be accelerated.

Given the complexity of these mechanisms and the likelihood that knockout of individual ROS-generating or scavenging enzymes are compensated for by synergistic ones have made it very difficult to assess their impact on atherosclerosis. Progression of atherosclerosis is also associated with substantial changes in cellular composition and activity, which are bound to impact enzyme expression, and expression of antioxidant enzymes may change with age. It is therefore obvious that measurements of individual enzymes and at a single time are unlikely to shed much light and that a more comprehensive approach is needed.

In this issue of Circulation Research, ’t Hoen and colleagues report a systematic determination of the expression of the major pro- and antioxidant enzymes in the aorta of apolipoprotein E–deficient and wild-type mice over time. Aortic gene expression was assessed by real-time PCR, and immunohistochemical determination of CD68–positive mac-
rophages in the aorta and cross-sectional lesion areas in the aortic origin were used as surrogate measures of aortic atherosclerosis. In the aortic arch, lesions became apparent between the age of 6 to 12 weeks and rapidly increased in size until 34 weeks. Contrary to expectation, all major antioxidant enzymes showed a significant upregulation before lesion formation, reaching a peak roughly coinciding with the onset of intimal thickening, but were markedly downregulated thereafter. Wild-type mice showed lower enzyme expression and little variation, as did the abdominal aorta, where atherogenesis occurred later and less extensively than in the arch.

These findings are remarkable in two ways. First, they indicate that many antioxidant enzymes are conjunctly regulated. This suggests that common response elements, or at least synchronous responses to different receptor or transcription factor signals, must govern the expression of these enzymes. If so, these may constitute novel targets for intervention. Second, results suggest that antioxidant enzymes respond to hypercholesterolemia with a pronounced initial upregulation, which may constitute a defensive mechanism, whereas the antioxidant defenses weaken significantly once atherosclerosis becomes more extensive, which may accelerate atherogenesis.

The present study does have some weaknesses. Unfortunately, no measurements of the cumulative lesion sizes in the aorta were available that would have permitted the determination of the changes relative to the amount of intimal tissue. Correction of data by CD68-positive macrophages (see their online data supplement, available at http://www.circresaha.org) confirms and strengthens the results obtained for overall expression, but it is no substitute for lesion areas, because macrophages are clearly not the only aortic cells expressing antioxidant enzymes. Ultimately, quantitative PCR of tissues obtained by laser capture microscopy (which permits harvesting of selective lesion areas or cell types) will be required to determine the cell-specific regulation of antioxidant enzymes. However, the fact that aortic thickness increased over time, whereas absolute mRNA expression of enzymes declined, clearly indicates that arteries with extensive atherosclerosis are poorly protected. This presumes, of course, that gene expression corresponds to protein expression and antioxidant activity. Although there is reason to assume so for antioxidant enzymes, as pointed out by the authors, a direct measurement of enzyme activities did not yield sufficient signal intensities.

Nevertheless, the present results are intriguing and consistent with other findings suggesting that antioxidant enzymes are regulated by hypercholesterolemia (or pathogenic effects of hypercholesterolemia) before the onset of atherosclerosis.
For example, fatty streak formation in human fetuses is greatly accelerated in offspring of mothers with chronic or temporary hypercholesterolemia during pregnancy (reviewed in Reference 15). Fetal lesions and plasma contained markedly increased lipid peroxidation products. More importantly, genetically more homogeneous animal models showed that fetal exposure to hypercholesterolemia significantly increases their susceptibility to atherosclerosis later in life. In a mouse model, exposure to maternal hypercholesterolemia and/or fetal lesion formation led to persistent changes in postnatal expression of many aortic genes before lesion formation, including upregulation of SOD3.15 The assumption that the downregulation of antioxidant enzymes in advanced atherosclerosis is not just an epiphenomenon, but that it actually contributes to accelerated lesion formation, is also consistent with a study comparing the activity of 4 such enzymes in human lesions from the fetus to old age.16 In this study, intracranial arteries showed a greater activity of antioxidant enzymes and slower progression of atherosclerosis than extracranial arteries in fetuses, children, and adolescents. When this comparative edge in antioxidant protection of brain arteries was lost in older subjects, their atherogenesis accelerated.

The observations of consistent variations in antioxidant enzymes in humans suggest that the choice of the murine model in the study of ‘t Hoen et al was a good one. Despite the obvious differences in arterial morphology and pathogenic factors (lipoprotein profiles, hemodynamics), lesions in apoE−/− mice are rich in oxidation products and reflect the abundance of eccentric atheromas with large necrotic cores in humans. Given the increasing size of necrotic areas, it seems a foregone conclusion that the expression of many genes relative to the aortic mass will decrease in advanced atherosclerosis. Even if viable intimal cells in atheromas were to express normal levels of antioxidant enzymes, this may be insufficient, because the lipids accumulated in the necrotic core are a prime substrate for oxidation. Thus, it is easy to postulate that decreased antioxidant protection may contribute to increasing plaque vulnerability. Increased prooxidant enzyme activity in macrophages of advanced human lesions may have similar effects.17

The pathogenic role of oxidative stress in vascular dysfunction, inflammation, and atherogenesis is based on a very large body of basic research on their pathogenic effects and the efficacy of antioxidant interventions in animal models. It is, however, overshadowed by the failure of several large clinical trials with natural antioxidants.19 The same caveats regarding such trials19 will have to be considered when the atherogenic role of weakened antioxidant enzyme activity proposed by Van Berkel’s group is tested in vivo. These include the fact that antioxidants tested to date appear to be most efficient in early stages of the disease (could this be because they receive less support from antioxidant enzymes in advanced lesions?). Other caveats are the lack of appropriate measures of the degree of cellular protection achieved by antioxidants and the uncertainty about the overall effect on ROS production of antioxidants inhibiting selective enzymes. Future studies with more powerful synthetic antioxidants or modulating pro- or antioxidant enzyme activities should therefore include comprehensive measurements of enzymatic activities and overall efficacy of antioxidant defenses.

References

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