**Oxidant Stress as a Marker for Cardiovascular Events**

**Ox Marks the Spot**

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During the past decade, numerous experimental and clinical studies have demonstrated that many common conditions predisposing to atherosclerosis, such as hypercholesterolemia, hypertension, diabetes, and smoking, are associated with a reduced vascular availability of nitric oxide (NO\(^*\)). Nitric oxide not only produces vasodilation but also has potent antiatherogenic properties. These properties include inhibition of platelet aggregation, prevention of smooth muscle cell proliferation, reduction of lipid peroxidation, and inhibition of adhesion molecule expression. Thus, the loss of NO\(^*\) observed in these various conditions not only alters vascular tone but also may explain in part why these conditions are risk factors for atherosclerosis.

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Given this apparent link between loss of nitric oxide and atherosclerosis, several groups have been interested in the concept that endothelium-dependent vasodilation, a surrogate factor for NO\(^*\) bioavailability, may predict cardiovascular events. Indeed, Suwaidi et al\(^1\) followed 157 patients with mildly diseased coronary arteries for an average of 28 months and observed cardiac events only in the patients with the lowest tertile of coronary vasodilation to acetylcholine. Similarly, in a study of 147 patients, Schächinger et al\(^2\) used 3 different stimuli for endothelial release of NO: acetylcholine, cold pressor testing, and increased blood flow. The authors showed that responses to each of these stimuli were independent predictors of cardiovascular events during a follow-up period of \(\approx 8\) years. Pernicone et al\(^3\) also demonstrated that endothelial dysfunction in the forearm circulation predicts cardiovascular events in hypertensive subjects.

There have been several explanations for why the various risk factors impair endothelial function. One that has received substantial attention is increased production of reactive oxygen species within the vessel.\(^4\) In particular, superoxide (O\(_2^*\)) reacts rapidly with NO\(^*\), resulting in the formation of the peroxynitrite anion and loss of NO\(^*\) bioactivity. Recently, it has been recognized that reactive oxygen species, especially peroxynitrite, can oxidize tetrahydrobiopterin, a critical cofactor for nitric oxide synthase.\(^5\) Accordingly, endothelial dysfunction is at least partially reversed by administration of several structurally unrelated antioxidants, including membrane-permeable superoxide dismutase,\(^6\) probucol,\(^7\) vitamin C,\(^8,9\) and glutathione.\(^10\)

In the present issue of *Circulation*, Heitzer et al\(^11\) have further defined the relationship between endothelial function, cardiovascular prognosis, and oxidative stress. The authors examined forearm blood flow in response to acetylcholine in 276 patients with coronary artery disease. In addition, in a subset of these subjects, the authors repeated the testing of acetylcholine responses during intra-arterial infusion of vitamin C. These subjects were then followed for \(\approx 80\) months. As in the earlier studies described above, Heitzer et al\(^11\) found that the amount of increase in forearm blood flow in response to acetylcholine was an excellent prognostic indicator; ie, the subsequent event rate was high in those with blunted responses to acetylcholine. The truly novel aspect of this study is that the abnormal responses to acetylcholine could be dramatically improved by the intra-arterial administration of vitamin C only in the group with cardiovascular events. In contrast, vitamin C infusion had minimal effect on forearm blood flow responses in the subjects that subsequently had no cardiovascular events. These relationships held true even when correction was made for other, more classic risk factors.

The authors’ interpretation of their data is that vitamin C infusion corrects oxidative stress, and when the response to vitamin C is large, a large amount of oxidant stress must be present. If this assumption is correct, a high level of oxidant stress portends a poor prognosis from the standpoint of cardiovascular disease. There is a large amount of data in the experimental literature to support this concept. Reactive oxygen species promote lipid oxidation, stimulate smooth muscle cell growth, and initiate expression of proinflammatory genes. Reactive oxygen species have been shown to activate matrix metalloproteinases, which may lead to plaque instability and rupture.\(^12\) In addition, NO\(^*\) has many vasoprotective properties, as noted above, and its loss because of oxidative inactivation very likely contributes to atherosclerosis.

Given the results from studies like that by Heitzer et al\(^11\) and the huge amount of basic research suggesting that reactive oxygen species contribute to atherosclerosis, antioxidant therapy would be expected to be beneficial in improving outcome in humans with atherosclerosis. Several recent trials, however, have shown no effect of vitamin E on cardiovascular events. In the Heart Outcomes Prevention Evaluation (HOPE)\(^13\) trial, vitamin E was found to have no benefit over placebo in 9500 high-risk subjects. In the GISSI prevention trial,\(^14\) >11 000 survivors of myocardial infarction were randomized to receive vitamin E, n-3 polyunsaturated fatty acids (PUFAs), both, or neither. Although the n-3 PUFA

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therapy proved beneficial, vitamin E reduced the event rate by only an insignificant amount. In the Study to Evaluate Carotid Ultrasound changes in patients treated with Ramipril and vitamin E (SECURE) (a substudy of HOPE), vitamin E was found to have no effect on the progression of carotid intima-media thickness (CIMT), whereas the ACE inhibitor ramipril markedly reduced progression of CIMT.15

On the surface, these trials would seem to refute a role of oxidative stress in the pathogenesis of atherosclerosis. There are several reasons to believe that this conclusion is not justified but rather that treatment with vitamin E alone is perhaps not the best approach for reducing oxidant stress. First, the rate constant for reactions between vitamin E and superoxide, relevant to many processes, is $5 \times 10^9 \text{M}^{-1} \times \text{sec}^{-1}$, a value that is 6 orders of magnitude less than the rate constant for the reactions of superoxide with NO.16 Given that the increase in plasma and tissue levels of vitamin E is relatively modest during oral therapy, it is possible that oral treatment with vitamin E has no effect on many important biological processes. Second, vitamin E is concentrated in lipid membranes and lipoproteins. It has become clear that many of the oxidative events that may be important in atherosclerosis occur in the cytoplasm and in extracellular space and would not be affected by lipid-soluble antioxidants. Third, after scavenging a radical, vitamin E becomes the tocopheroxyl radical, which can under certain circumstances enhance lipid peroxidation.17 The tocopheroxyl radical can be “regenerated” back to tocopherol by other antioxidants such as vitamin C or co-enzyme Q.18 In the case of vitamin C, the ascorbyl radical is formed; this, however, is converted back to ascorbic acid enzymatically in mammalian cells and has a very low oxidative potential. For this reason, there has been substantial interest in using cocktails of antioxidants: for example, vitamins E and C together. Indeed, in the recent Antioxidant Supplementation in Atherosclerosis Prevention (ASAP) trial, the combination of vitamins E and C produced a substantial reduction in CIMT progression, although when used alone, these vitamins were ineffective.19

Given the above considerations, it is quite possible that use of antioxidant vitamins will never prove to be the best approach to limit vascular oxidant stress. It should be noted that at least 2 widely accepted modes of treatment for atherosclerosis have been shown to potently inhibit production of reactive oxygen species by vascular cells. Lipid lowering dramatically decreases superoxide production.20 The statins have the added effect of preventing geranylgeranylation of the small g-protein Rac-1,21,22 which is a critical component of the NADPH oxidase, a major source of vascular reactive oxygen species. In addition, the NADPH oxidase is potently activated by angiotensin II, and strategies to block angiotensin II production or its receptor dramatically lower superoxide production in experimental animal models.23,24 Thus statins, ACE inhibitors, and angiotensin-receptor antagonists have antioxidant effects not because they scavenge radicals but because they block the production of radicals.

In olden times, Pirates would mark their maps with an X at the site of the buried treasure, with the notation that “X marks the spot.” The article by Heitzer et al11 demonstrates the concept that Ox (oxidative stress) may mark individuals at high risk for subsequent cardiovascular events. Future research should focus on identifying markers of oxidative stress that may prove to be a hidden treasure and will allow more targeted therapy. Such markers might include plasma thiobarbituric acid reactive substances, plasma or red cells’ ratios of reduced glutathione to oxidized glutathione, urinary isoprostanes, or, as used by Heitzer et al,11 the increment in endothelium-dependent vasodilatation achieved by vitamin C administration. Just as we think of hypertension or hypercholesterolemia as a phenotype requiring treatment, we may begin to consider high levels of oxidative stress in a similar fashion. As we enter the era of genomics, it is very likely that we will identify genetic polymorphisms that predispose to oxidative stress. Polymorphisms of superoxide dismutase, NADPH oxidase, the endothelial nitric oxide synthase, the promoter region of catalase, and glutathione peroxidase have already been identified and may contribute to the high oxidative stress phenotype. When and if we begin to identify this phenotype, we will be able to target treatment at a very early stage, perhaps with statins, angiotensin II antagonism, or potent antioxidants, to prevent the development of the earliest stages of vascular disease.

References
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