Antioxidant therapy for atherosclerotic vascular disease: the promise and the pitfalls

AHMAD SHIHABI,1 WEI-GEN LI,1,2 FRANCIS J. MILLER, JR.,1 AND NEAL L. WEINTRAUB1
Departments of 1Internal Medicine and 2Biochemistry, University of Iowa
College of Medicine, Iowa City, Iowa 52242

THE POTENTIAL VALUE OF ANTIOXIDANTS in treating conditions associated with oxidative stress is well known to scientists and clinicians and is of immense interest to patients. Oxidative stress is a term used to describe an imbalance between the production and destruction of reactive oxygen species (ROS), such as superoxide anions (O$_2^-$) and hydrogen peroxide (H$_2$O$_2$), thereby leading to cellular and tissue injury. The basic properties of oxygen are responsible for the destructive power of free radicals, in particular, their high reactivity. Humans consume ~250 g of oxygen every day, and of this ~3–5% is converted to O$_2^-$ and other reactive species (38).

The damage inflicted by ROS on cellular and extracellular targets such as membrane lipids, proteins, and DNA clearly contributes to tissue and organ dysfunction in many pathological states. In particular, the oxidation of low-density lipoproteins (LDL) in the vascular wall is widely accepted to play a fundamental role in the pathogenesis of atherosclerosis (5, 40, 44, 56). It stands to reason, then, that antioxidants should be beneficial in preventing atherosclerosis and its complications in humans. Indeed, observational and epidemiological studies, although unable to establish a cause-and-effect relationship, suggest that increased dietary intake of naturally occurring antioxidant vitamins is associated with lower risk of cardiovascular disease (7, 8, 22, 24, 26, 39, 43, 46).

Why, then, have primary and secondary prevention trials of antioxidant regimens yielded less than encouraging results (14, 21, 32, 37, 45, 51, 57)? Some trials have been criticized because of insufficient dosing regimens or durations of antioxidant therapy, harmful interactions between the antioxidant agents, and flaws in enrolling or excluding subsets of patients, among other factors. Nevertheless, these simple explanations do not explain the negative results of all carefully conducted trials. The lack of proven benefit of antioxidants, in conjunction with a recently described detrimental effect on lipid metabolism (4), has led some to suggest that the use of supplemental antioxidant vitamins could even be hazardous to patients who are taking lipid-lowering medications (25).

The apparent lack of efficacy of supplemental antioxidant vitamins to prevent atherosclerosis in humans should be noteworthy not only to clinicians and patients but also to scientists who study the basic mechanisms of vascular disease. In this regard, we discuss several concepts that have emerged from the “antioxidant paradox” that might provide insight into the role of ROS in the pathogenesis of atherosclerotic vascular disease and into the pitfalls of conventional antioxidant therapy.

DIFFERENCES BETWEEN IN VITRO AND IN VIVO OXIDATION OF LDL

Studies performed in vitro suggest that LDL particles are resistant to oxidation by Cu$^{2+}$ until α-tocopherol is consumed, in keeping with the putative function of vitamin E as a chain-breaking antioxidant (9). Tocopherol radicals formed during Cu$^{2+}$-induced LDL oxidation can react with each other or with lipid peroxyl radicals, thereby yielding nonradical products and effectively terminating the peroxidation process. Alternatively, the tocopherol radicals can be scavenged by vitamin C, resulting in regeneration of α-tocopherol. How can it be, then, that substantial amounts of oxidized lipids are present together along with relatively large amounts of α-tocopherol and ascorbate in human atherosclerotic lesions (47)? One possibility is that under some circumstances (for example, at low radical fluxes in the absence of co-antioxidants), vitamin E could promote, rather than terminate, lipid peroxidation in LDL particles (reviewed in Ref. 53). Whether or not tocopherol-mediated peroxidation actually occurs in vivo, however, remains to be demonstrated. Alter-
natively, the ROS that oxidize LDL in vivo may not be efficiently scavenged by vitamin E.

CELLULAR AND ENZYMATIC SOURCES OF ROS IN VASCULATURE

Figure 1 shows some of the key mechanisms of ROS formation, interaction, and degradation within the vasculature. Vascular ROS formation is, in large part, initiated by the one-electron reduction of molecular O$_2$ to O$_2^\cdot$, the production of which is increased in atherosclerosis (30, 31, 55). Potential cellular sources of O$_2^\cdot$ in blood vessels include infiltrating phagocytic cells, which contain the high-capacity O$_2^\cdot$-generating flavoenzyme NADPH oxidase, as well as vascular endothelial cells, smooth muscle cells (SMC), and fibroblasts. An earlier report suggested that endothelial cells may be responsible for the increased production of O$_2^\cdot$ in hypercholesterolemic blood vessels (31). Using confocal microscopy to examine the cellular localization of O$_2^\cdot$ in situ, Miller et al. (29) demonstrated that SMC are a major source of ROS in blood vessels from atherosclerotic rabbits. Similar studies also suggest that much of the O$_2^\cdot$ in atherosclerotic human coronary arteries is contained in the media and adventitia, where it is presumably generated by SMC and fibroblasts (Fig. 2).

It was shown recently that a nonphagocytic NAD(P)H oxidase is a major source of ROS in cultured vascular cells (15–17), although xanthine oxidase, nitric oxide synthase, cytochrome P-450, and the mitochondrial electron transport chain may be important sources of ROS in specific situations. Oxidation of LDL by endothelial cells in vitro can be attenuated by overexpressing superoxide dismutase (SOD) in the cells, suggesting a role for O$_2^\cdot$ and/or its reaction products in this process (10). On the other hand, although vitamin E rapidly reacts with lipid peroxyl radicals, it does not efficiently scavenge O$_2^\cdot$. Moreover, the localization of ROS within the deep layers of the blood

![Fig. 1. Potential enzymatic sources of radical oxygen species (ROS) production in the vasculature and the locations (cytoplasmic, mitochondrial, or peroxisomal) of some of the important endogenous antioxidant enzymes. NAD(P)H, reduced nicotinamide adenine dinucleotide phosphate; NO, nitric oxide; MPO, myeloperoxidase; SOD, superoxide dismutase; EcSOD, extracellular SOD; MnSOD, manganese SOD; Cu/ZnSOD, copper/zinc SOD.](http://ajpheart.physiology.org/)

![Fig. 2. Examination of O$_2^\cdot$ in situ in human blood vessels by confocal microscopy. Segments of coronary arteries were removed from freshly explanted hearts. Frozen sections of the vessel segments were incubated with dihydroethidium (HE; fluorescent probe that specifically reacts with O$_2^\cdot$) and examined by confocal scanning microscopy, as previously described (29). The coronary artery shown at left was obtained from a 42-yr-old woman with dilated cardiomyopathy. Note the lack of plaque formation, indicating the absence of significant atherosclerosis. The coronary artery shown at right was from a 69-yr-old man with atherosclerosis (note the large plaque within the subendothelial layer). Marked HE fluorescence is observed, particularly within the media and adventitia. The figures shown were obtained using identical conditions and laser settings and are representative of multiple arterial sections.](http://ajpheart.physiology.org/)
vessel wall suggests that even if antioxidant vitamins were efficient scavengers of \( O_2^- \), delivery to sites where they are most needed may be problematic.

\( O_2^- \) that exits from, or is produced outside of, vascular cells can be converted by extracellular SOD (ECSOD) to \( H_2O_2 \). In addition, \( O_2^- \) produced within cells is converted by Cu/Zn SOD and Mn SOD to \( H_2O_2 \), which can readily cross cellular membranes. \( H_2O_2 \) present in the extracellular space is, in turn, converted to the highly reactive species \( HOCl \) by the enzyme myeloperoxidase (Fig. 1). Recent studies suggest that myeloperoxidase is localized to phagocytic cells in human atherosclerotic lesions (13, 48). Moreover, analysis of amino acid oxidation products in LDL isolated from the human arterial wall suggests that \( HOCl \) is an important modifier of LDL in vivo (20). In vitro, \( HOCl \) reacts rapidly with apolipoproteins in LDL, yielding secondary reaction products, such as chloramines, that induce lipid peroxidation (18). Additionally, myeloperoxidase converts \( L-\)tyrosine to tyrosyl radical, which can also induce lipid peroxidation in LDL particles (41). Interestingly, in vitro, vitamin E failed to protect LDL against protein oxidation by myeloperoxidase, and the \( HOCl \)-induced secondary lipid peroxidation proceeded less rapidly when the lipoproteins were depleted of \( \alpha \)-tocopherol (18, 19). These studies suggest that vitamin E may not afford protection against myeloperoxidase-dependent oxidative modification of LDL.

Another ROS implicated in the oxidation of LDL in vivo is peroxynitrite (\( OONO^- \)), the reaction product of \( O_2^- \) and nitric oxide (20). In vitro, \( OONO^- \)-induced protein oxidation was found to be independent of the content of \( \alpha \)-tocopherol in LDL particles (52). Moreover, the magnitude of \( OONO^- \)-induced lipid peroxidation was increased with increasing \( \alpha \)-tocopherol content at oxidant-to-LDL ratios of \(<100:1 \) (52). Thus vitamin E also may not adequately protect against LDL oxidation by \( OONO^- \), an important oxidant species in the blood vessel wall.

EFFECTS OF ROS ON VASCULAR FUNCTION

The reaction between \( O_2^- \) and nitric oxide also leads to diminished endothelium-dependent relaxation, a well-known feature of atherosclerosis (12). One very important contributing factor appears to be that the production of \( O_2^- \) is increased in atherosclerosis (30, 31, 55). In this regard, the NAD(P)H oxidase in vascular cells is activated by cytokines thought to participate in atherosclerosis, such as platelet-derived growth factor (PDGF), angiotensin II, and thrombin (16, 17). Moreover, the activity of NAD(P)H oxidase is upregulated in experimental models of atherosclerosis, hypertension, and balloon denudation, suggesting that increased production of ROS via this pathway may represent a generalized response to vascular injury (1, 16, 17, 29, 34, 42). Not only would reaction of nitric oxide with \( O_2^- \) generate the toxic species \( OONO^- \) and attenuate endothelium-dependent vasorelaxation, but it would also offset the beneficial effects of nitric oxide to inhibit platelet function, leukocyte adhesion, and SMC proliferation, among others. Wagner et al. (54) recently reported that inhibitors of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase diminish endothelial \( O_2^- \) production by preventing the isoprenylation of p21 rac, a small G protein that is an obligate component of NAD(P)H oxidase. This finding may help to explain some of the non-lipid-lowering beneficial effects of HMG-CoA reductase inhibitors on vascular function (6). Likewise, angiotensin II stimulates NAD(P)H oxidase expression and activity (15, 33), and angiotensin-1 receptor inhibition reduced \( O_2^- \) production, improved endothelial dysfunction, and diminished plaque formation in hypercholesterolemic rabbits (55). On the other hand, clinical studies of vitamin E in humans have shown mixed results in regard to improvement in endothelial function in patients with atherosclerosis (reviewed in Ref. 3). Thus, if improvement in endothelial function is important in preventing atherosclerosis and its complications, treatment with vitamin E alone may be of little value. The beneficial effects of vitamin C on endothelial function are more consistent, however, which supports the view that coadministration of vitamin C with vitamin E is likely to be more efficacious (3, 28).

IMPORTANCE OF ROS AS SIGNALING MOLECULES IN VASCULARITY

Traditionally, high levels of ROS have been viewed principally as toxic mediators of cell and tissue injury. More recently, however, low levels of ROS have been identified as important regulators of cellular signaling pathways and gene expression in the vasculature. For example, ROS can induce the release of arachidonic acid (2) and activate tyrosine kinases and mitogen-activated protein kinases (16), which are critical components of many intracellular signaling cascades, including those required for cell survival and growth (36, 49). In addition, many cardiovascular genes are redox sensitive. Redox regulation of gene expression occurs through multiple mechanisms, including activation of upstream signal transduction pathways and modulation of transcription factor binding activity (16). As ROS, nitric oxide, and products formed via interactions among oxidant species (including \( H_2O_2 \)) can mediate signal transduction, it is conceivable that very high doses of antioxidants could, paradoxically, produce harmful effects on cellular signaling processes. In addition, the expression of antioxidant enzymes is upregulated in disease states and by factors such as angiotensin II and tumor necrosis factor-\( \alpha \), indicative of an adaptive response to oxidative stress (50). By altering redox-mediated signaling, it is possible that antioxidant therapy could suppress this adaptive response, which may, paradoxically, increase the vulnerability of the blood vessel to oxidative injury.

ADDITIONAL CONSIDERATIONS

The aforementioned potential pitfalls of antioxidant therapy might be considered theoretical rather than...
pragmatic. Several other more practical considerations should be mentioned. First, although antioxidants are typically given in constant amounts and dosing intervals, oxidative stress is not a continuous, uniform process. For example, marked intensification of oxidative stress occurs transiently after vascular balloon injury, and, most likely, during periods of increased inflammatory activity in atherosclerotic lesions (1). The oxidants may activate signaling cascades and gene expression that, once set in motion, no longer require the presence of ROS. This may explain why antioxidant therapy must be given before balloon angioplasty to be effective (1).

Ideally, antioxidant therapies should be judged on the basis of their therapeutic efficacy. Unfortunately, determination of the efficacy of antioxidant therapy is hampered by the lack of available methodology to quantify ROS in tissues and blood vessels in vivo. Surrogate end points, such as assessment of endothelial function or lipid peroxidation products in the plasma, do not adequately reflect the capacity of antioxidants to protect the deeper layers of the blood vessel wall from oxidative injury. Negative results of clinical trials must be interpreted cautiously in the absence of verification that antioxidant therapy successfully reduces vascular oxidant stress.

Antioxidant defense systems are preferentially localized to specific subcellular domains (Fig. 1). The lipophilic vitamin E is partitioned into cell membranes and may not adequately protect against oxidant stress in the aqueous cytosolic environment. Furthermore, vitamin E has eight diastereoisomers, which vary considerably in regards to bioavailability and antioxidant potency. Surprisingly, many clinical studies do not specify which isomer (or mixture of isomers) was administered to patients. Some of the stereoisomers possess important actions aside from their chain-breaking antioxidant effects, such as inhibition of protein kinase C, which could affect vascular function, and, perhaps, O$_2^-$ production (11, 23). This may explain why studies involving dietary sources of vitamin E are more encouraging than studies using a single isomer.

It is important to consider that antioxidants do not inhibit the production of ROS. Conceptually, agents that inhibit oxidant production should be much more effective than scavenger agents (i.e., antioxidant vitamins) in ameliorating oxidative stress. This is highlighted by the finding that the rate of reaction of ROS with antioxidant vitamins is orders of magnitude slower than with other cellular targets. Inhibiting ROS production would be the surest way of preventing oxidant species from inflicting cellular damage. In this regard, the NAD(P)H oxidase of vascular cells, which appears to a major producer of ROS in vascular diseases, may be a novel therapeutic target for vascular research.

Finally, oxidative stress may contribute to vascular diseases only in certain subsets of patients. This possibility is suggested by studies in experimental models of hypertension (27, 35). Genetic polymorphisms in ROS-generating systems or cellular antioxidants may identify subsets of patients who are most prone to oxidative stress. Identification of such at-risk patients would facilitate clinical studies designed to determine whether they might benefit from antioxidant therapy.

CONCLUSIONS

The recognition that supplementation with antioxidant vitamins (in particular, vitamin E) is of little or no benefit in preventing or treating atherosclerosis in large-scale clinical studies in humans raises more questions than it answers. On the one hand, there is ample experimental evidence that ROS play a pivotal role in the pathogenesis of atherosclerotic vascular disease. However, the ROS responsible for in vivo oxidation of LDL, a key factor in the development of atherosclerosis, may not be effectively scavenged by vitamin E. Also, the cellular actions of ROS are quite complex, in that low levels of ROS appear to be essential for cellular signaling. Thus antioxidant supplementation could potentially be harmful to those tissues that are not subjected to substantial oxidative stress. Conversely, for those disorders that are associated with marked increases in ROS production, such as vascular balloon injury, the temporal and spatial characteristics of oxidant production pose great challenges in regard to delivering effective antioxidant therapy. In addition, the methods currently available to assess the degree of oxidative stress, and the efficacy of antioxidant therapy, in vivo are quite limited. Finally, ROS may participate only in certain subsets of vascular diseases and/or in specific patient subpopulations. Thus the recent “negative” trials of antioxidant therapy for atherosclerosis should herald a new beginning, rather than an end, for basic research into the role of ROS in vascular diseases.

This work was supported by National Heart, Lung, and Blood Institute Grants HL-49264 (to N. L. Weintraub) and HL-62984 (to F. J. Miller, Jr., and N. L. Weintraub) and by an American Heart Association grant-in-aid (to W.-G. Li).

REFERENCES

6. Dupuis J, Tardif JC, Cernacek P, and Theroux P. Cholesterol reduction rapidly improves endothelial function after acute coronary syndromes: the RECIFE (Reduction of Cholesterol in


49. Steinberg D, Parhasarathy S, Carew TE, Kho JC, and Witzum JL. Beyond cholesterol: modifications of low-density lipoprotein.


