Is the Emperor Wearing Clothes? Clinical Trials of Vitamin E and the LDL Oxidation Hypothesis

Jay W. Heinecke

Abstract—A wealth of evidence indicates that oxidized low density lipoprotein (LDL) may be of central importance in animal models of atherogenesis. In recent clinical trials, however, dietary vitamin E supplements have not consistently prevented cardiac events in humans with established coronary artery disease. Such mixed results have led many to question the role of LDL oxidation in human atherosclerosis, although this interpretation assumes that the doses of vitamin E used in the studies inhibited lipid oxidation in vivo. In fact, there is remarkably little evidence indicating that those particular regimens effectively inhibit lipid peroxidation in healthy humans. Moreover, evidence of increased oxidative stress was not a criterion for inclusion in the trials; therefore, vitamin E may have benefited only a subset of the participants. These uncertainties raise doubts about the ability of vitamin E to augment antioxidant defense mechanisms in vivo and leave many questions about LDL oxidation and atherosclerosis unanswered. (Arterioscler Thromb Vasc Biol. 2001;21:1261-1264.)

Key Words: atherosclerosis ■ antioxidant ■ lipid peroxidation ■ oxidative stress ■ oxidized LDL

The hypothesis that oxidation of LDL is a physiologically relevant mechanism for atherogenesis has received considerable experimental support (see reviews1-7). For example, cultured cells from the artery wall oxidize LDL to a form that is recognized by macrophage scavenger receptors. Also, LDLs isolated from human and animal atherosclerotic lesions show clear evidence of oxidative damage. Moreover, antibodies to lipid oxidation products detect epitopes in atherosclerotic lesions, indicating that oxidized LDL is present in the artery wall. Importantly, a variety of lipid-soluble antioxidants retard atherogenesis in animal models of hypercholesterolemia, strongly suggesting that oxidation of LDL lipids and/or proteins is key to atherogenesis.

The oxidation hypothesis suggests that antioxidant strategies might prevent LDL from becoming atherogenic in humans. In the present review, we evaluate recent studies of the ability of one potential antioxidant, vitamin E, to prevent cardiac events in humans with established coronary artery disease, emphasizing the clinical relevance of the oxidation hypothesis.

Many Antioxidants Retard Atherosclerosis in Hyperlipidemic Animals

The ability of structurally unrelated lipid-soluble antioxidants to inhibit atherogenesis in animal models of hypercholesterolemia is one of the strongest lines of evidence for the oxidation hypothesis.4,5,7 For example, probucol, butylated hydroxytoluene, and N,N’-diphenyl-phenylenediamine all inhibited LDL oxidation in vitro8-10 and retarded the progression of atherosclerosis in hypercholesterolemic rabbits.10-12 Probucol also exerted a borderline statistically significant effect on atherogenesis in primates with diet-induced hypercholesterolemia.13

Although these results implicate lipoprotein oxidation in atherogenesis, it is important to note that antioxidants generally have been fed to animals in large amounts, typically as 1% of the diet. Therefore, they reach extremely high concentrations in plasma and tissue. The relevance of such experiments in the prevention of human disease is unclear. Indeed, studies have shown that lower levels of dietary probucol fail to inhibit atherosclerosis in hypercholesterolemic rabbits, even though such concentrations significantly reduce LDL oxidation ex vivo.14

Most animal studies have focused on the effects of antioxidants on the formation of fatty streaks, the earliest cellular lesion of atherosclerosis. The possible contributions of oxidative stress to the progression of atherosclerosis have been much less investigated. Moreover, most clinical events in humans with coronary artery disease are precipitated by the rupture of plaque in intermediate and advanced atherosclerotic lesions, but little is known about the influence of oxidative stress on this dramatic event.4 Such studies are important because they may more closely mimic the influence of antioxidants in humans, in whom atherosclerosis can precede adulthood.

Vitamin E Fails to Consistently Inhibit Atherosclerosis in Animal Models

Despite the impressive ability of other lipid-soluble antioxidants to block atherosclerosis in hypercholesterolemic ani-
mals, vitamin E at doses that fail to lower cholesterol levels has not exerted a consistent inhibitory effect in hypercholesterolemic animals. At the doses used in these studies, LDL isolated from the animals was protected from oxidation ex vivo.

In contrast, it has been convincingly demonstrated recently that dietary vitamin E inhibits atherosclerosis in mice deficient in apolipoprotein E. The intervention also lowered tissue levels of isoprostanes, which are sensitive and specific markers of lipid oxidation. Using a dose of vitamin E that inhibited lipid peroxidation (0.2% of the diet, approximately 8 IU per mouse per day) was key to the study of Pratico et al. These observations emphasize the importance of documenting that a proposed antioxidant intervention actually inhibits oxidative reactions in vivo.

Recent studies of mice with genetically engineered vitamin E deficiency indicate that vitamin E has a modest impact on atherosclerosis in this animal model. Plasma levels of vitamin E in mice with targeted disruption of the \( \alpha \)-tocopherol transport protein were less than 10% of the levels found in wild-type mice, but the genetically altered animals exhibited only 30% more atherosclerosis and a moderate 2-fold increase in tissue levels of F2-isoprostanes, markers of lipid oxidation. Moreover, these biological effects might be independent of proposed antioxidant properties, as \( \alpha \)-tocopherol exerts potent effects on monocyte/macrophage function, protein kinase C activity, smooth muscle cell proliferation, and platelet aggregation.

Vitamin E Exerts Antioxidant and Pro-Oxidant Effects In Vitro

Lipid peroxidation is initiated by radical abstraction of a hydrogen atom from a bis-allylic position on a polyunsaturated fatty acid. Subsequently, the pentadienyl radical reacts with oxygen to form a peroxyl radical intermediate. This species then attacks another polyunsaturated fatty acid molecule, amplifying the peroxidative process.

In a classic study, Burton and Ingold showed that vitamin E interrupts lipid peroxidation by scavenging peroxyl radical intermediates. In the process, it is converted to a tocopherol radical. The structure and low oxidation potential of the tocopherol radical make it relatively unreactive with polyunsaturated fatty acids. Its usual fate is to react with a lipid peroxyl radical or another tocopherol radical to form nonradical products. The tocopherol radical is also scavenged by co-antioxidants, such as vitamin C. In this reaction, reduction of tocopherol radical by the co-antioxidant regenerates vitamin E. Under these conditions, vitamin E is an effective inhibitor of lipid oxidation.

Vitamin E can behave differently when it is incorporated into lipoproteins. When LDL is exposed to a low radical flux in the absence of co-antioxidant, the tocopherol radical has a long half-life. It can then promote lipid peroxidation by attacking polyunsaturated fatty acids. Compelling evidence indicates that this mechanism, termed tocopherol-mediated peroxidation, oxidizes LDL lipid in many oxidation systems in vitro. Whether tocopherol-mediated peroxidation is biologically relevant has yet to be determined. However, these observations illustrate that the behavior of a compound as an antioxidant or oxidant depends on reaction conditions and available substrates.

Does Vitamin E Prevent Human Atherosclerosis?

A wide range of epidemiological studies has suggested that increased intake of dietary antioxidants lowers the risk of atherosclerosis. This finding suggests that high dietary antioxidant intake might prevent premature vascular disease. A major limitation of such studies is the possibility that confounding factors account for the decreased risk.

The best test of the therapeutic effectiveness of an antioxidant is a prospective, double-blind, placebo-controlled trial. Several such trials have been conducted over the past few years (Table). The results of the Cambridge Heart Antioxidant

### Randomized Prospective Clinical Trials of Vitamin E in the Prevention of Cardiovascular Disease

<table>
<thead>
<tr>
<th>Trial</th>
<th>Vitamin E, IU/d</th>
<th>Subjects, n</th>
<th>Follow-Up, y</th>
<th>Prevention†</th>
<th>Cardiovascular Risk</th>
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<tr>
<td>ATBC</td>
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<td>Primary</td>
<td>No effect‡</td>
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<tr>
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*Studies have used the natural (RRR isomer) or racemic (mixture of 8 isomers) form of vitamin E. Regimens are reported in terms of IU and were calculated by assuming that 1 mg racemic vitamin E equals 1 IU vitamin E. Refer to discussions of biological significance of various isomers of vitamin E. In all studies except ATBC and HOPE were carried out in subjects with established coronary artery disease. All subjects in the ATBC trial were male smokers without known atherosclerosis. The HOPE trial was composed of subjects at high risk of vascular disease with established coronary artery disease or diabetes. The SPACE trial involved patients with renal failure. †Primary end point, lung cancer (cardiovascular disease death monitored as secondary end point). ‡Primary end point, composite of cardiovascular death, cardiovascular events, or stroke. §§Primary end point, composite of cardiovascular death, cardiovascular events, or stroke.

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‖Primary end point, composite of cardiovascular death or nonfatal myocardial infarction.

#Primary end point, composite of myocardial infarction, ischemic stroke, peripheral vascular disease, or unstable angina.
Vitamin E and LDL Oxidation

Does Vitamin E Lower Oxidative Stress In Vivo?

The mixed results of these trials have led many to question the role of LDL oxidation in human atherosclerosis. This negative interpretation assumes that vitamin E at the doses used in the trials can inhibit lipid oxidation in vivo. But is the emperor wearing clothes?

Despite intense interest in the contributions of oxidative stress to the pathogenesis of human disease, there is surprisingly little evidence that compounds that display antioxidant activities in vitro actually inhibit oxidative reactions in vivo. Two major problems have been (1) the difficulty of monitoring reactive intermediates, which are short-lived and difficult to detect directly, and (2) the lack of sensitive and specific methods for quantifying oxidation products.

Studies of arachidonic acid oxidation have attempted to address these issues. In vitro oxidation of arachidonic acid yields a family of prostaglandin F2-like compounds, the F2-isoprostanes. These compounds are readily quantified in biological material by isotope dilution gas chromatography–mass spectrometry, a sensitive and specific method, and their levels in plasma and urine have been extensively investigated in animal models and in humans. The results provide strong evidence that F2-isoprostanes are reliable markers of lipid peroxidation in vivo.

There is remarkably little information about the influence of vitamin E supplementation on lipid oxidation in humans. An early study suggested that isoprostane levels are elevated in hypercholesterolemic subjects. Levels of the oxidation products were reduced by dietary supplementation for 4 weeks with 100 to 600 IU vitamin E per day. In contrast, a recent study of healthy humans taking dietary supplements as high as 2000 IU/d for 8 weeks found no change in levels of the lipid oxidation products 4-hydroxynonenal and two different isoprostanes, suggesting that vitamin E did not inhibit lipid peroxidation in these individuals. Other studies have suggested that the vitamin lowers isoprostane levels under some, but not all, conditions thought to be associated with oxidative stress.

Future Directions

Evidence that vitamin E augments antioxidant defense mechanisms in vivo is not compelling. It is noteworthy that the vitamin E doses used in the clinical trials have not been convincingly shown to inhibit lipid peroxidation in humans. It may also be significant that animal studies of antioxidants have focused on early events in atherosclerosis, whereas the human clinical trials involved individuals with advanced disease. Most acute coronary events in humans with advanced disease are precipitated by the rupture of atherosclerotic plaque. However, there are no well-accepted animal models for plaque rupture, and we know almost nothing about the effects of antioxidant interventions on this process. These observations leave many questions about LDL oxidation and human atherosclerosis unanswered.

To examine the oxidation hypothesis, suitable test subjects and optimal antioxidant regimens need to be identified. Such a trial should involve subjects with evidence of increased oxidative stress, just as the statin trials studied subjects with high cholesterol levels rather than the general population. This will be especially important for primary prevention trials because the low absolute risk of coronary artery disease necessitates large numbers of subjects and a long period of intervention. Also, the optimal regimen for inhibiting lipid peroxidation in humans needs to be determined so that test compounds can be administered in effective antioxidant regimens. For example, it is possible that higher doses of vitamin E and/or longer periods of supplementation would inhibit lipid peroxidation in vivo and therefore be more appropriate for clinical trials.

One powerful approach to identifying physiologically relevant reactions is to quantify specific lipid and protein oxidation products in tissues and biological fluids by using sensitive and specific methods. This strategy has pointed myeloperoxidase as one pathway that promotes LDL oxidation in the human artery wall. Because the utility of an antioxidant depends critically on the nature of the oxidant that inflicts tissue damage, interventions that specifically inhibit physiologically relevant pathways would be logical candidates for clinical trials.
trials. Such a rational approach to therapy has worked well in other fields and is likely to accelerate progress in the treatment of oxidative stress and coronary heart disease.

References
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