Commentary

Why do antioxidants fail to provide clinical benefit?

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Abstract

The results of recent randomized trials to test the influence of antioxidants on coronary-event rates and prognosis in patients with coronary-artery disease were disappointing. In none of these studies did the use of vitamin E improve prognosis. In contrast, treatment of coronary-artery disease with angiotensin-converting-enzyme (ACE) inhibitors reduced coronary-event rates and improved prognosis. ACE inhibition prevents the formation of angiotensin II, which has been shown to be a potent stimulus of superoxide-producing enzymes in atherosclerosis. The findings suggest that inhibition of superoxide production at enzymatic levels, rather than symptomatic superoxide scavenging, may be the better choice of treatment.

Keywords: ACE inhibitor, angiotensin II, antioxidants, atherosclerosis, superoxide

The oxidative-stress concept of atherosclerosis: the rationale for the use of antioxidants

The endothelium-derived relaxing factor, recently identified as nitric oxide (NO [1]) or a closely related compound [2], has potent antiatherosclerotic properties. NO released from endothelial cells works in concert with prostacyclin to inhibit platelet aggregation; it inhibits the attachment of neutrophils to endothelial cells and the expression of adhesion molecules. NO in high concentrations inhibits the proliferation of smooth-muscle cells. Therefore, under all conditions where an absolute or relative NO deficit is encountered, the process of atherosclerosis is being initiated or accelerated. The half-life of NO, and therefore its biological activity, is decisively determined by oxygen-derived free radicals such as superoxide (O_2^-) [3]. Superoxide reacts rapidly with nitric oxide (NO) to form the highly reactive intermediate peroxynitrite ($ONOO^-$). The rapid bimolecular reaction between NO and O_2^-, yielding $ONOO^-$ (rate constant 1.9×10^{10}/M/s), is about 5 to 10 times faster than the dismutation of O_2^- by superoxide dismutase. Therefore, $ONOO^-$ formation represents a major potential pathway of NO reactivity depending on the rates of tissue O_2^- production. Peroxynitrite is also a highly reactive intermediate that may cause oxidative damage to lipids, proteins, and DNA.

ACE = angiotensin-converting enzyme; CHAOS = Cambridge Heart Antioxidant Study; GISSI = Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardico; HOPE = Heart Outcomes Prevention Evaluation; LDL = low-density lipoprotein; NADH = nicotinamide adenine dinucleotide; NO = nitric oxide; O_2^- = superoxide; $ONOO^-$ = peroxynitrite.

There is a growing body of evidence that endothelial dysfunction in the setting of hypercholesterolemia, diabetes mellitus, hypertension, or chronic smoking is often a consequence of enhanced NO degradation by oxygen-derived free radicals rather than diminished NO formation due to decreased activity and/or expression of the nitric oxide synthase. Therefore, in many instances the most effective approach to improve NO bioavailability in the presence of risk factors is to lower O_2^- levels, eg by treatment with scavenging agents or by the administration of drugs that specifically inhibit O_2^- production.

Effects of antioxidants in clinical studies

The effects of antioxidants such as vitamin C, α- and β-carotene, or vitamin E on the prognosis in patients with coronary-artery disease are very disappointing. So far, there is just one study showing a reduction in coronary-event rates in patients with angiographically evident coronary-artery disease (the Cambridge Heart Antioxidant Study [CHAOS] trial) after treatment with vitamin E (400–800 IU/day), although the overall prognosis in these patients was not improved [4]. Other randomized trials, such as the Alpha-Tocopherol, Beta Carotene Cancer Prevention Study [5], the Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardico (GISSI-Prevenzione trial) [6], and the Heart Outcomes Prevention Evaluation (HOPE) [7] study, failed to demonstrate any improvement of the prognosis in patients with coronary-artery disease.

How can one explain the lack of benefit from antioxidants despite the known causal role of oxidative stress in the initiation and progression of atherosclerosis? Vitamins scavenge free radicals. However, the rate constant for the reaction of vitamins E and C with O_2^- is much slower (1000- to 10 000-fold) than the rate constant for the reaction between NO and O_2^-. Therefore, in order to scavenge O_2^-, antioxidants must be administered in very high concentrations, to reach compartments where O_2^- is formed (such as endothelial and smooth-muscle cells). When high concentrations of vitamins are used, however, pro-oxidative effects may come into play. For example, low concentrations of vitamin E improved endothelial function in cholesterol-fed animals, while high concentrations of the vitamin had the opposite effects and even worsened endothelial function [8]. One explanation for this is that when vitamin E reacts with a radical, it becomes the vitamin-E radical, ie the tocopheroxyl radical, which itself may participate in pro-oxidative events. These findings may indicate that the optimal dose of antioxidants must be titrated in order to improve rather than worsen vascular function. The phenomenon may at least partly explain the failure of antioxidants to improve prognosis in patients with coronary-artery disease. It is also possible that combinations of antioxidants, such as vitamins C and E, need to be used to prevent accumulation of the vitamin-E radical.

Are angiotensin-converting-enzyme inhibitors the better 'antioxidants'?

The effect of angiotensin-converting-enzyme (ACE) inhibitors on the progression of atherosclerosis and endothelial function has been studied in several animal models and in patients with coronary-artery disease. It has been consistently shown that ACE inhibition slows the progression of atherosclerosis [9] and improves impaired endothelial dysfunction [10] without altering plasma cholesterol levels. In contrast to the published findings in studies of antioxidants, ACE inhibitors seem to have a beneficial influence on the prognosis in patients with coronary-artery disease [11]. How do ACE inhibitors beneficially influence vascular function in atherosclerosis? ACE inhibition prevents formation of angiotensin II, one of the most potent vasoconstrictors. Angiotensin II, in turn, stimulates the release of endothelin-1 from endothelial cells and induces the expression of the preproendothelin gene in endothelial and smooth-muscle cells. Another important aspect is that endothelial kininase II is identical with ACE. Inhibition of kininase II, which is responsible for the breakdown of bradykinin, leads to high local concentrations of bradykinin. This substance, in turn, is a potent stimulus for the release of endothelium-derived hyperpolarizing factor, NO, and prostacyclin. A novel, recently identified, mechanism contributes to the detrimental effects of angiotensin II on vascular superoxide production. Angiotensin II has been shown to increase vascular superoxide production by activating an NADH-driven oxidase [12], one of the most significant superoxide-producing enzymes in endothelial and smooth-muscle cells. Recent studies with hypercholesterolemic animals [13] and patients with coronary-artery disease [14] indicate that activation of this enzyme contributes considerably to endothelial dysfunction and early plaque formation.

The stimulatory effects of angiotensin II on the activity of the HADH-driven oxidase would suggest that in the presence of an activated renin–angiotensin system (local or circulating), vascular dysfunction due to increased vascular superoxide production is likely.

Indeed, there is a large body of literature providing evidence that the renin–angiotensin system is causally linked to the development and progression of atherosclerosis. Incubation *in vitro* of cultured smooth-muscle cells with native low-density lipoproteins (LDLs) increases the expression of the angiotensin II receptor subtype AT1 [15]. Similar phenomena have been observed in animals fed cholesterol [16], in animals that are hyperlipidemic because of an LDL-receptor defect [13], and in patients with hypercholesterolemia [17]. Macrophages in atherosclerotic plaques produce large amounts of angiotensin II. The activity of the ACE is increased in response to oxidative stress. Angiotensin II facilitates the recruitment of monocytes/macrophages into the vessel wall by stimulating

the production of the monocyte chemoattractant protein (MCP-1) and vascular-adhesion molecules.

Further indirect support for the involvement of the renin–angiotensin system in the development of atherosclerosis was provided by studies testing the antiatherosclerotic properties of ACE inhibitors and of AT1-receptor antagonists. These studies have shown that both treatment regimens beneficially influence endothelial function [9], reduce oxidative stress within vascular tissue by inhibiting the vascular NADH oxidase, and, therefore, retard the formation of atherosclerotic plaques [13].

The recently published results of the HOPE study seem to strengthen the idea that inhibition of the renin–angiotensin system [11], rather than treatment with the classical antioxidant α-tocopherol, beneficially affects the prognosis in patients with coronary-artery disease [7]. Treatment with ramipril significantly lowered the risk of cardiovascular mortality and nonfatal cardiovascular events in high-risk patients who had vascular disease or diabetes plus one known cardiovascular risk factor. Thus, it may be concluded that inhibition of the renin–angiotensin system and the subsequent inhibition of superoxide-producing enzymes provide a better antioxidative regimen than the administration of classic antioxidants, which merely scavenge already formed reactive oxygen species.

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