Lipoprotein oxidation in cardiovascular disease: chief culprit or innocent bystander?

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Oxidation of low–density lipoprotein (LDL) is thought to contribute to atherosclerosis and cardiovascular disease. Consistent with this idea, the antioxidant drug probucol reduces the risk of restenosis, a form of cardiovascular disease, in humans. However, a new study now suggests that the protective effect of probucol depends not on its ability to inhibit lipid oxidation, but on its ability to induce the stress-induced antiinflammatory enzyme heme oxygenase (HO)–1. This might explain why other antioxidants, such as vitamin E, fail to prevent cardiovascular disease in humans.

The LDL oxidation hypothesis

Atherosclerosis, a narrowing of blood vessels caused by the deposition of inflammatory plaques in the vessel wall, is the leading cause of death in industrialized society. One important risk factor for atherosclerosis is an elevation in a particular type of plasma cholesterol called LDL. Oxidation of LDL lipids is thought to render the lipoprotein atherogenic, because oxidized LDL is more readily taken up by macrophages via scavenger receptors (for review see references 1, 2). Lipid-laden macrophages (known as foam cells) are the major component of atherosclerotic lesions. Macrophages also secrete myeloperoxidase, an inflammatory enzyme that generates an array of oxidizing intermediates (2). Oxidation products characteristic of myeloperoxidase have been detected in human atherosclerotic lesions, implicating the enzyme in LDL oxidation in vivo (2). Oxidized LDL attracts monocytes and stimulates the production of monocyte chemoattractant protein (MCP)-1 and other inflammatory cytokines, which might contribute to the accumulation of phagocytic cells in vessel walls (Fig. 1). Subsequently, simple fatty streaks (the earliest stage of the atherosclerotic lesion), which consist exclusively of macrophage foam cells, are converted to more complex lesions characterized by the immigration of smooth muscle cells from the medial layer of the artery wall into the subendothelial space. At this late stage of lesion development, oxidized LDL may convert smooth muscle cells into foam cells and also induce smooth muscle cells to synthesize extracellular matrix.

Early in vitro experiments showed that all of the major cell types found in atherosclerotic lesions (endothelial cells, smooth muscle cells, monocytes, and macrophages) produce reactive intermediates that oxidize LDL (3–5). Modification of LDL in these model systems requires redox-active metal ions in the medium, generates lipid oxidation products, and is inhibited by lipid-soluble antioxidants. These data led to the hypothesis that lipid oxidation is essential for rendering LDL atherogenic (Fig. 2 A), a proposal supported by biochemical and immunological studies showing that oxidized LDL and protein-bound adducts of lipid oxidation are found in atherosclerotic lesions (6, 7). One of the most persuasive arguments supporting the oxidation model is the finding that antioxidants, such as probucol, N,N'-diphenyl–phenylenediamine, and butylated hydroxytoluene, which prevent the oxidation of LDL in vitro, inhibit atherosclerosis in animal models of hypercholesterolemia (8–10).

Probucol lowers LDL in hypercholesterolemic humans, but the drug is no longer used clinically because it had negative side effects, including reductions in high density lipoprotein (HDL; the "good cholesterol"), which protects against atherosclerosis, and potentially dangerous changes in heart rhythm. Probucol dramatically reduces atherosclerosis in most hypercholesterolemic animals (1, 2, 8, 9), and LDL isolated from probucol-treated animals and humans is markedly resistant to oxidation ex vivo (10). LDL from humans treated with other lipid-soluble antioxidants (1, 2) is also resistant to oxidation, suggesting that antioxidants block LDL oxidation and thus inhibit vascular disease. However, a study by Wu et al. (11) in this issue (p. 1117) suggests that the beneficial effect of probucol is unrelated to its antioxidant properties. Instead, the compound appears to exert a wide variety of potent antiinflammatory effects that depend on the induction of the stress–induced enzyme HO-1, which degrades hemoglobin-derived heme to biliverdin, carbon monoxide (CO), and iron. This raises the possibility that related compounds that lack probucol's potentially deleterious side effects may be useful in the prevention of cardiovascular disease in humans, and explains why other antioxidants have not been successful in treating atherosclerosis and cardiovascular disease.

Vitamin E therapy does not prevent cardiovascular disease in humans

The LDL oxidation hypothesis has attracted skepticism in recent years because clinical trials have failed to show that dietary supplementation with vitamin E, or other antioxidants, reduces the incidence of cardiovascular disease. The first prospective trial to test whether antioxidants protect against coronary artery disease suggested that the risk of nonfatal heart attacks in patients with cardiovascular disease was significantly lower in those treated with vitamin E (12). However, this was a relatively small and short-term trial. Two subsequent studies, which included larger numbers

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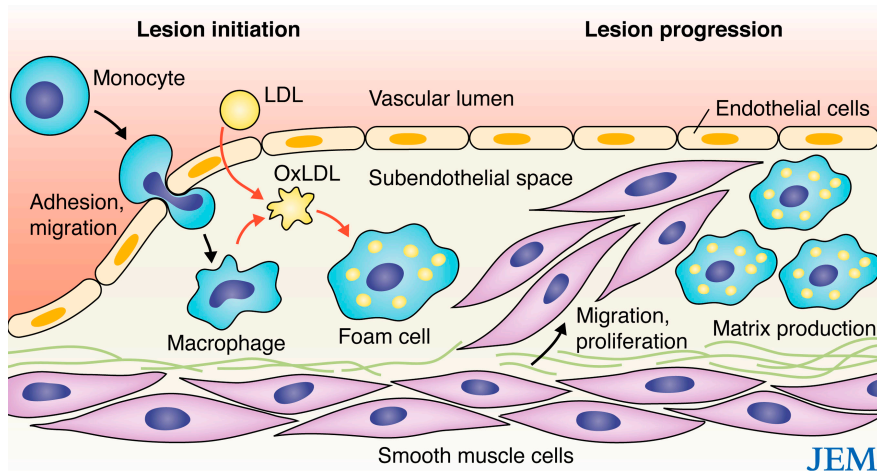J.W.H.: heinecke@u.washington.edu.

Figure 1. Initiation and progression of atherosclerotic lesions. LDL enters the subendothelial space and is oxidized. Oxidized LDL (OxLDL) promotes monocyte adhesion to injured or inflamed epithelium and migration into the artery wall, where the cells differentiate into macrophages. OxLDL also binds to scavenger receptors on macrophages. This binding triggers uptake of OxLDL, converting macrophages into lipid-laden foam cells. Subsequently, smooth muscle cells migrate into the subendothelial space, where they accumulate and produce extracellular matrix, two important components of more advanced atherosclerotic lesions.

of study subjects that were followed for longer periods of time, found that vitamin E supplementation had no statistically significant effect (13, 14). Another clinical trial, which followed ~40,000 apparently healthy women for an average of 10 years, showed that a high dose of natural-source vitamin E taken every other day did not reduce the occurrence of major cardiovascular events, nor did it affect overall mortality (15). Thus, randomized clinical trials strongly suggest that the antioxidant vitamin E does not prevent cardiovascular disease, leading many to question the role of LDL oxidation in human atherogenesis.

LDL oxidation debunked?
In this issue, the findings of Wu et al. challenge the role of antioxidants and LDL oxidation in cardiovascular disease (11). They demonstrate that probucol, and certain probucol analogues, retard vascular disease in three different animal models: apolipoprotein E-deficient mice (a model of hypercholesterolemia-induced atherosclerosis), obese Zucker rats (a model of type 2 diabetes), and rabbits with balloon injury of the carotid artery (a model of restenosis, a thickening of the artery wall caused by accumulation of smooth muscle cells in

the subendothelial space). In all three models, the ability of the drug to inhibit vascular disease depended on the presence of a sulfur-containing di-thioether bridge in the molecule. The presence of the two phenol groups alone, on the other hand, was not protective. This observation is important because the antiatherosclerotic effect of probucol is generally attributed to the ability of its phenol rings to react with and defuse one-electron oxidants such as lipid peroxyl radicals (1, 2).

Casting further doubt on the role of lipid oxidation in these models, Wu et al. found that the degree of lipid oxidation in vascular tissue failed to correlate with the extent of the lesions. In addition, probucol dithiobisphenol—a di-thioether bridge-containing analogue of probucol—protected against atherosclerosis but did not reduce plasma cholesterol levels. These observations strongly suggest that the protective effects of probucol do not involve lipid oxidation or cholesterol reduction.

Potential alternative targets of probucol are two-electron oxidants, which react rapidly with di-thioether bridges (11). This may be important because hypochlorous acid (HOCl), a two-electron oxidant generated by myelo-

peroxidase, has been implicated in oxidative damage in human atherosclerotic lesions (2). Recent studies indicate that HDL isolated from the blood of humans with established cardiovascular disease contains elevated levels of chlorinated tyrosine, a characteristic product of HOCl produced by myeloperoxidase (16, 17). Moreover, in vitro oxidation of the HDL-associated protein, apolipoprotein A-I, impaired the ability of this protein to remove cholesterol from cells. These observations suggest that protein oxidation—rather than lipid oxidation—might be crucial for atherogenesis.

Probucol targets HO-1
Rather than antioxidant activity, Wu et al. trace the antiatherogenic effect of probucol and its analogues to the induction of HO-1. CO, a product of heme degradation by HO-1, has been shown to inhibit endothelial cell apoptosis, prevent smooth muscle cell proliferation, and inhibit macrophage production of inflammatory mediators, all of which may be important in vascular protection (18). Another HO-1 by-product, biliverdin, has also been shown to exert antiinflammatory effects on various cell types. Perhaps because of these effects, the induction of HO-1 is protective in animal models of inflammatory diseases, such as ischemia-reperfusion injury and chronic graft rejection (for review see reference 18). HO-1 is expressed in aortic tissue after injury and was recently shown to help control intimal hyperplasia, another form of vascular disease (19).

Wu et al. found that levels of HO-1 increased in the aortas of hypercholesterolemic rabbits after balloon injury if the animals were treated with probucol or an analogue containing the di-thioether bridge. If the rabbits were treated with an analogue containing only the phenol groups, on the other hand, no HO-1 induction occurred (11). The protection against vascular disease in this model (based on a reduction in intimal hyperplasia) also required the presence of a di-thioether bridge and was independent of the phenol groups. The animals that were protected against disease also had enhanced

aortic reendothelialization, a process required for the repair of injured vessels.

Inhibition of HO-1 with a synthetic inhibitor negated the beneficial effects of probucol treatment, thus directly linking HO-1 induction with the probucol-induced inhibition of vascular injury. Similar results were observed in Zucker rats and in the carotid arteries of rabbits subjected to balloon injury. Thus, in three different models of vessel injury, treatment with probucol or an analogue containing a di-thioether bridge was strongly associated with vascular protection. It is also important to note that vitamin E, which did not induce HO-1, was not protective in these models (11).

Probucol lowers the risk for restenosis in humans

Are these observations relevant to the prevention of human vascular disease? Patients suffering from unstable angina (chest pain resulting from acute blockage of coronary arteries) or extensive chronic blockage of cardiac arteries are often treated with percutaneous angioplasty, a procedure in which a balloon catheter is inserted into the lumen of the occluded artery and expanded to disrupt the atherosclerotic plaque, thus clearing the arterial blockage. A major drawback of this intervention is the rapid rethickening (restenosis) of the treated vessel, which occurs in approximately half of subjects who receive no additional treatment. In a large clinical trial of subjects with coronary artery blockage, probucol therapy reduced the incidence of restenosis by almost 50% (20). In contrast, intervention with an antioxidant "cocktail" of vitamin E, β–carotene, and vitamin C failed to affect the rate of restenosis. Probucol was also protective in another trial, which assessed the extent of coronary artery restenosis in angioplasty patients using intravascular ultrasound (21). In a third study, probucol treatment induced the regression of carotid atherosclerotic lesions in asymptomatic patients with high cholesterol, and also reduced the incidence of major cardiovascular events (22). Thus, probucol, but not vitamin E, appears to inhibit restenosis in people who have undergone angioplasty.
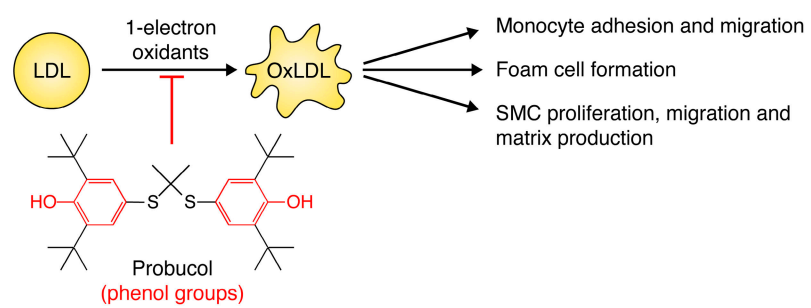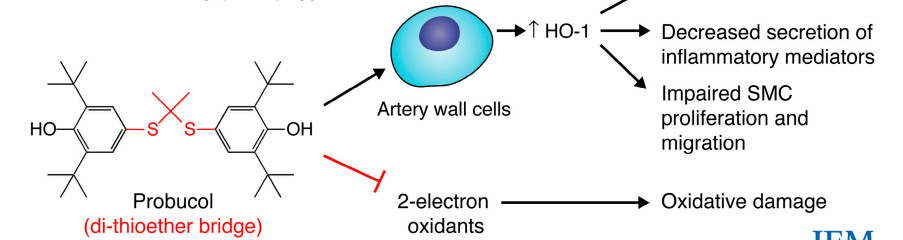
Figure 2. Proposed pathways for the impact of probucol on the pathogenesis of vascular disease. (A) LDL in the intima of the artery wall is oxidized with the generation of proatherogenic lipid oxidation products (OxLDL). The phenol moiety of probucol blocks this process by acting as a scavenger of one-electron oxidants. (B) Vascular injury results in endothelial injury, smooth muscle cell (SMC) proliferation, and an influx of inflammatory cells and SMCs into the intima. Probucol induces the cellular expression of HO-1, presumably by cells of the artery wall. HO-1 reduces intimal hyperplasia and promotes tissue repair by several direct cellular actions. For example, the HO-1 product CO has been shown to inhibit endothelial cell (EC) apoptosis, prevent SMC proliferation, and inhibit macrophage production of inflammatory mediators, all of which may be important in vascular protection. Probucol may also react with two-electron oxidants that play a role in tissue injury.

It is important to point out that the underlying mechanisms of atherosclerosis and restenosis injury are thought to be different. Thus, the earliest cellular event in atherosclerosis is the appearance of macrophage foam cells in the artery wall, with the subsequent accumulation of smooth muscle cells in the subendothelial space. In contrast, the hallmark of restenosis is the selective appearance of smooth muscle cells in the subintimal space with little evidence of macrophage foam cells. However, the observation that probucol appears to be effective in animal models of both types of vascular injury suggests that these diseases might have common pathophysiologic mechanisms—perhaps involving endothelial injury or smooth muscle cell migration and proliferation (Fig. 2 B).

Probucol therapy in humans has potentially deleterious side effects. It markedly reduces levels of HDL, which protects arteries from unregulated cholesterol accumulation and atherosclerosis. It may also predispose humans to dangerous changes in electrical heart rhythms. These factors have precluded its widespread use in human clinical trials. However, the probucol analogue AGI-1067 (which contains the di-thioether bridge) does not cause electrical abnormalities and appears to lower HDL levels only modestly (23). Like probucol, AGI-1067 reduced the extent of coronary artery restenosis in patients (21). It also exerts a wide variety of antioxidant and anti–inflammatory effects in vitro, and inhibits atherosclerosis in hypercholesterolemic animals (for review see reference 23). A large clinical trial is in progress to determine whether AGI-1067 prevents cardiovascular events in humans with established coronary artery disease. A positive result would have major implications for our understanding of atherogenesis and for current approaches to therapy.

Future directions

It is noteworthy that the pathways that promote oxidative stress in animals and humans may differ. For example, myeloperoxidase promotes oxidative damage in the human artery wall (2, 16, 24–26) but is undetectable in lesions of mice that are prone to atherosclerosis because they have high levels of LDL (27). Moreover, vitamin E fails to scavenge hypochlorous acid, a two-electron oxidant and major product of myeloperoxidase that might contribute to vessel damage (28). Thus, to understand the effects of antioxidants on atherosclerosis and vascular injury, it will be important to define the biochemical pathways that promote oxidative reactions in the human artery wall and to determine whether proposed antioxidants block those reactions. It would be of great interest to determine whether probucol and AGI-1067 can interrupt myeloperoxidase-dependent pathways that contribute to oxidative damage of proteins in vivo.

The provocative study by Wu et al. (11) implicates HO–1 as a molecular target for probucol in several different animal models of vascular injury. In future studies, it will be important to explore the role of HO-1 in the pathogenesis of human vascular disease, to establish how probucol induces the expression of the enzyme and how HO-1 exerts its anti-inflammatory actions.

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