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COMMENTARY

LIPOPROTEIN OXIDATION AND GENE EXPRESSION IN THE ARTERY WALL

NEW OPPORTUNITIES FOR PHARMACOLOGIC INTERVENTION IN ATHEROSCLEROSIS

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Pharmacologic intervention for atherosclerosis, the major cause of heart disease and stroke, has thus far been directed primarily at systemic factors, particularly blood lipoproteins and hypertension. Lipid lowering agents have targeted intestinal lipid absorption, hepatic lipid metabolism, and lipoprotein–enzyme interactions. Blood pressure lowering agents have targeted ion transport and hormonal pathways. With the increased understanding of the molecular and cellular interactions underlying atherosclerosis at the level of the artery wall, new potential targets for pharmacologic intervention have been revealed (Fig. 1). In particular, a large body of evidence now implicates lipoprotein oxidation in lesion development. Also, many of the cytokines, growth factors, chemotactic factors and adhesion molecules likely to be important in leukocyte recruitment and smooth muscle cell (SMC§) proliferation have been defined. There are promising strategies for inhibiting lipoprotein oxidation and specifically modifying gene expression in the artery wall. Thus, the opportunities for new pharmacologic intervention in atherosclerosis, bypassing the standard systemic risk factors, are promising. We consider here some of the possibilities, with a focus on the role of lipoprotein oxidation.

Molecular and cellular interactions in atherosclerosis. Figure 1 depicts the pathways thought to contribute to the development of atherosclerotic lesions (reviewed in Refs. 1–4). The artery wall consists of a monolayer of endothelial cells (EC) that form tight junctions, insulating the blood from the underlying intima and SMC layers. The intima, very thin in a normal artery, is separated from the layers of smooth muscle by the internal elastic lamina (IEL). During atherogenesis it greatly increases in thickness as a result of the accumulation of excessive plasma-derived lipids (primary cholesterol), connective tissue, monocyte/macrophages and SMC. Myocardial infarctions appear to occur primarily as a result of connective tissue necrosis at the plaque base, leading to plaque rupture and thrombosis.

High levels of plasma low density lipoproteins (LDL) or other atherogenic lipoproteins are a prerequisite for most forms of atherosclerosis. Such lipoproteins enter the artery wall, where they accumulate as a result of interactions with collagen and other matrix components of the intima. Such lipoprotein accumulation triggers the binding of monocytes to the endothelial layer, followed by recruitment into the artery wall, proliferation, and differentiation to macrophages. These macrophages subsequently take up large amounts of modified lipoproteins to give rise to cholesterol engorged "foam cells," that are the hallmark of "fatty streaks," the first histologically recognizable stage of atherogenesis. Some of the foam cells exit the artery wall, but many remain to eventually die and leave behind a massive amount of cholesterol.

The observation that native LDL is not taken up sufficiently rapidly by macrophages to generate foam cells provided a starting point for studies linking lipoprotein accumulation with lipoprotein oxidation and monocyte recruitment. Extensive modification of the apolipoprotein B (apoB) component of LDL by acetylation resulted in a particle rapidly taken up by the macrophage "scavenger receptor," which has now been isolated and cloned [5]. Subsequently, it was shown that extensive oxidation of LDL, a biologically relevant modification, by metal ion treatment or cellular oxidation, also produced a particle capable of producing foam cells. Biochemical analyses as well as immunohistochemical studies with antibodies specific of oxidized LDL then revealed the presence of similarly modified forms of LDL in the lesions of animal models and humans. In the last few years it has become clear that oxidized LDL exhibits several properties, in addition to rapid

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§ Abbreviations: SMC, smooth muscle cells; EC, endothelial cells; LDL, low density lipoprotein; MM-LDL, minimally modified LDL; MCP-1, monocyte chemotactic protein 1; M-CSF, macrophage colony stimulating factor; PDGF, platelet-derived growth factor; IL-1, interleukin-1; TGF-β, transforming growth factor β; bFGF, basic fibroblast growth factor; ACE, angiotensin converting enzyme; ANP, atrial natriuretic peptide; DPPD, *N,N'*-diphenyl phenylenediamine; TNF, tumor necrosis factor; and Lp(a), lipoprotein(a).

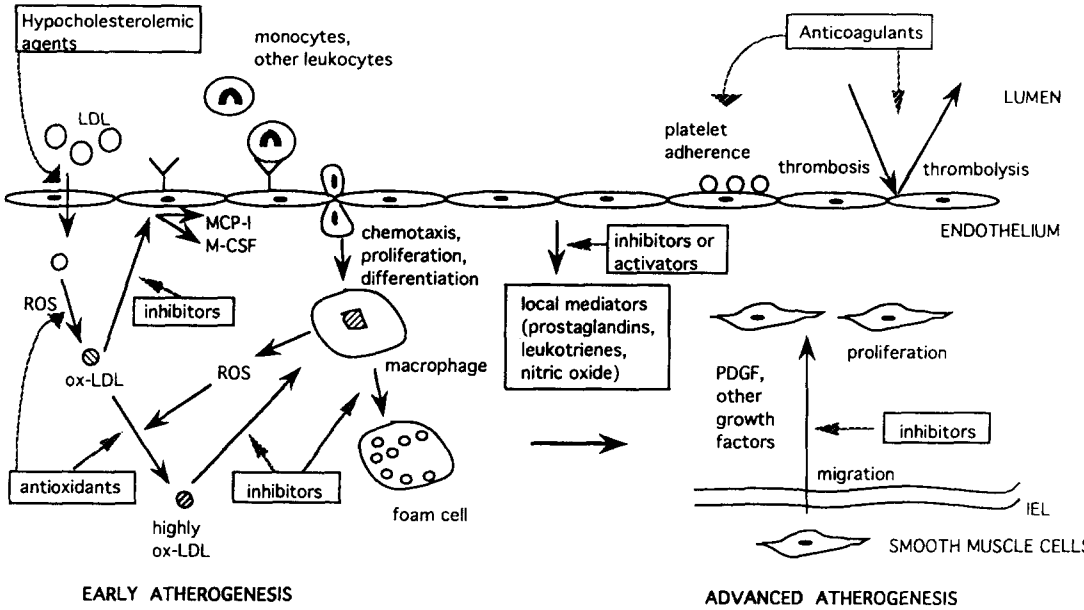


Fig. 1. Potential sites for pharmacologic intervention in a proposed model for atherogenesis.

uptake by macrophages, that are likely to contribute to atherogenesis (reviewed in Ref. 6). In particular, LDL that is only minimally oxidized, referred to here as minimally modified LDL (MM-LDL), is capable of inducing a number of inflammatory genes likely to contribute to monocyte recruitment and differentiation. These include adhesion molecules for monocyte binding to the endothelium, monocyte chemotactic protein 1 (MCP-1) and macrophage colony stimulating factor (M-CSF). A pharmacologic target of particular interest is the adhesion molecule (or molecules) responsible for the endothelial binding of monocytes in atherosclerosis. Certain evidence has implicated VCAM-1, a member of the immunoglobulin gene superfamily [7]. Definitive answers should come from gene targeting studies of adhesion molecules in mice, now underway in several laboratories.

With time, some, but clearly not all, fatty streaks develop into fibrous plaques characterized by a "fibrous cap" overlying the lipid rich core. The formation of this cap appears to involve: (1) a phenotypic modulation of medial SMC from a contractile to a proliferative state; (2) migration of SMC across the internal elastic lamina into the intima; and (3) proliferation in the intima accompanied by deposition of collagen, proteoglycans, and elastin. The link between fatty streaks and fibrous lesions is poorly understood. Undoubtedly of importance are changes in the endothelium. Under normal conditions, EC produce eicosanoids rather than growth factors, resulting in the maintenance of SMC quiescence. Under conditions of activation or injury, however, eicosanoid production is reduced and growth factor production is stimulated [4]. It is likely that foam cells also produce growth factors and cytokines, such as platelet-derived growth factor (PDGF) and interleukin-1 (IL-1) that may participate in triggering SMC migration and proliferation [8, 9].

Several growth factors and circulating vasoactive peptides have been implicated in vessel wall growth. Both *in vitro* studies and balloon catheter studies, in which the vessel wall of experimental animals is injured, indicate that PDGF, transforming growth factor β (TGF-β) and basic fibroblast growth factor (bFGF) are likely to be involved. For example, antibodies to either bFGF and PDGF dramatically inhibited SMC proliferation following angioplasty [10]. The vasoconstrictive hormone angiotensin II (AII) also promotes SMC proliferation *in vitro* and the angiotensin converting enzyme (ACE) inhibitor captopril apears to prevent SMC growth to a greater extent than can be explained by its effect on blood pressure [11]. Recent epidemiologic studies suggest that genetic variations of the ACE gene contribute to susceptibility to atherosclerosis by mechanisms independent of blood pressure [12]. In contrast to AII, the vasorelaxant atrial natriuretic peptide (ANP) inhibits vascular hypertrophy [13].

It is likely that the expression of eicosanoids and nitric oxide (EC-derived relaxing factor) by vascular cells contributes importantly to the expression of growth factors, cytokines and adhesion molecules (reviewed in Ref. 4). Certain eicosanoids, such as PGI_2 and PGE_2, appear to maintain the endothelium in a quiescent state, characterized by a nonadhesive, nonthrombotic surface. They also appear to participate in maintaining SMC in a contractile state and to influence cholesterol metabolism in both macrophages and SMC. On the other hand, leukotrienes, such as LTB_4 and LTC_4, appear to have proatherogenic effects. The influx of macrophages, which express glucocorticoid-regu-

lated prostaglandin synthase and a nitric oxide synthase, is likely to alter homeostatic processes mediated by eicosanoids and nitric oxide. Injection of oxidized LDL into hamsters resulted in enhanced binding of leukocytes to arteriole endothelium, and this could be blocked by the leukotriene biosynthesis inhibitor MK-886 [14]. Studies with an inhibitor of thromboxane A_2 (TXA_2) synthesis, resulting in a shift in metabolism from TXA_2 to PGE_2, suppressed lesion development in hypercholesterolemic rabbits [15]. Endothelium-dependent relaxation, mediated by nitric oxide, is impaired in atherosclerotic arteries, and nitric oxide has an antiproliferative effect on SMC in culture. Strong evidence for the importance of nitric oxide in atherogenesis was provided recently by a study showing that dietary supplementation of L-arginine, the substrate for synthesis of nitric oxide, reduced both the size and thickness of aortic lesions in cholesterol-fed rabbits [16]. Advanced glycosylation end-products, associated with diabetes, block the cytostatic effect of nitric oxide on SMC, providing a possible link between diabetes and heart disease [17].

Thrombosis is likely to be important in the development of complex lesions, and myocardial infarctions usually result from the formation of large thrombi following plaque rupture [18]. Advanced plaques frequently contain areas of surface erosion to which platelets bind. Moreover, immunohistochemical studies have revealed platelet antigen and fibrin deposits within, as well as on top of, fibromuscular caps. This has led to the concept that medium sized and microscopic thrombi may contribute to episodic lesion growth. The data linking lipoprotein(a) (Lp(a)) levels, atherogenesis, and thrombosis provide support for this possibility.

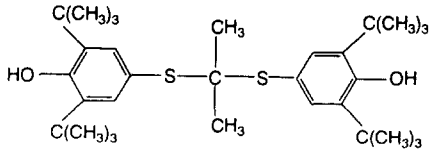
Advanced lesions can be grouped into four types: (1) concentric plaques (covering the entire circumference of the artery) that are primarily fibrous, (2) concentric plaques that are lipid rich, (3) eccentric plaques (in which only part of the wall is involved) that are primarily fibrous, and (4) eccentric plaques that are lipid rich. Although eccentric, lipid-rich plaques account for only a small fraction of the total, they are the major cause of myocardial infarctions. The edges of such plaques, where the fibrous cap joins the normal intima, frequently contain foam cells. Perhaps as a result of lysis of collagen by macrophage-derived proteases, such sites are particularly prone to rupture. These edges are also likely to be involved in plaque growth. Thus, the macrophage appears to play a significant role in all stages of atherosclerosis. These findings also suggest that while inhibition of SMC proliferation is likely to be beneficial in angioplasty, it may actually destabilize other types of plaques. This illustrates the complexity of pharmacologic intervention in atherosclerosis.

Advanced plaques frequently exhibit a number of changes involving all three layers of the artery wall (*intima, media, and adventitia*). These include thinning of the media, fibrosis, neovascularization and lymphocytosis of the adventitia, and calcium deposition around the lipid core and fibrous cap of the intima. The significance of these changes for plaque development and stability is unclear.

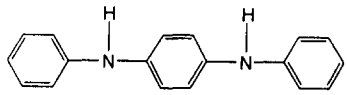
Pharmacologic intervention in lipoprotein oxidation. Several lines of evidence indicate that oxidized lipoproteins play a critical role in atherosclerosis: (1) LDL isolated from human and animal lesions exhibits many of the immunological, physicochemical and biological properties of LDL oxidized *in vitro*; (2) immunohistochemical studies with antibodies specific for oxidized LDL have revealed the presence of such epitopes in atherosclerotic lesions and their colocalization with apoB, the major protein of LDL; (3) autoantibodies to oxidized LDL occur commonly in human and animal sera; (4) prolonged incubation of LDL with vascular cells in culture can yield both minimaly modified LDL and highly oxidized LDL with properties similar to those produced by transition metal ion treatment or enzymatic oxidation; (5) epidemiologic studies [19] have suggested that the incidence of myocardial infarction is inversely correlated with the levels of certain naturally occurring antioxidants (vitamin E, vitamin A) and directly correlated with the levels of the prooxidant iron; and (6) most convincingly, lipophilic antioxidants have been shown to reduce lesion development in animal models [6, 20, 21].

The oxidation of LDL trapped in the artery wall probably initially leads to the formation of a minimally modified particle that is a potent inducer of monocyte binding to EC, monocyte chemotaxis, and monocyte proliferation and differentiation. With further oxidation, the LDL loses the above biologic activities, but becomes a ligand for macrophage receptors capable of generating foam cells, the hallmark of fatty streak lesions. Oxidized LDL also exhibits a number of other properties that may promote atherosclerosis (reviewed in Ref. 6). It is chemotactic for monocytes but it inhibits macrophage mobility, providing a possible explanation for the failure of foam cells to exit the artery wall. Oxidized LDL is cytotoxic and thus may contribute to EC damage and vascular necrosis. It inhibits endothelium-dependent relaxation, mediated by the synthesis of nitric oxide. It can promote coagulation and induce tissue factor. And finally, oxidized LDL is immunogenic, which could contribute to its uptake by macrophages and to the recruitment of lymphocytes. It is important to realize that "oxidized LDL" is not a single entity but a heterogeneous group of particles exhibiting varying levels of lipid oxidation and protein modification. Some of the above properties are attributes of minimally modified LDL (e.g. cytokine induction), whereas others are attributes of highly modified LDL (e.g. cytotoxicity).

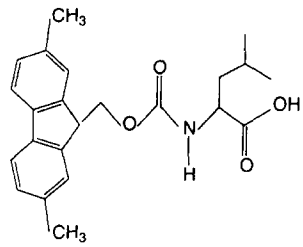
Several antioxidants with the capacity to bind to LDL have been shown to effectively inhibit lesion development in experimental animals. The drug probucol (Fig. 2) is used clinically to lower plasma cholesterol levels, but it is also a hydrophobic antioxidant that binds to LDL and inhibits its oxidation *in vitro* as well as *ex vivo*. A number of studies have now shown that probucol significantly retards the development of atherosclerotic lesions in hypercholesterolemic rabbits and monkeys [20, 22]. Probucol also inhibits neointimal thickening and macrophage accumulation following balloon injury in cholesterol-fed rabbits [23]. It has been



Probucol



DPPD



Leumedin (NPC 15669)

Fig. 2. Compounds capable of inhibiting LDL oxidation.

argued convincingly that the antiatherogenic effects of probucol are a result of its antioxidant properties rather than its effects on lipoprotein levels, although an analogue of probucol, as effective in inhibiting LDL oxidation as probucol, was considerably less effective in inhibiting the development of atherosclerotic lesions [24]. Studies with the antioxidant *N,N'*-diphenyl phenylenediamine (DPPD) are consistent with the concept that LDL oxidation is important in atherosclerosis [25]. DPPD (Fig. 2), a much more potent antioxidant than probucol, prevented LDL oxidation and substantially retarded lesion development in hypercholesterolemic rabbits, but had little effect on lipoprotein levels. Unfortunately, DPPD is a mutagen and not of potential therapeutic utility. The antioxidant butylated hydroxytoluene (BHT) also retarded lesion development in hypercholesterolemic rabbits.

A multilayer coculture of human aortic EC and SMC has proved particularly valuable for the study of lipoprotein oxidation [26]. SMCs are grown on a polycarbonate filter and allowed to produce a large quantity of extracellular matrix. On top of this are then seeded ECs, which are allowed to grow to confluency. The binding and entry of added blood monocytes into the coculture can be stimuated about 5-fold by the addition of MM-LDL. This is due, at least in part, to the induction of MCP-1, since monocyte entry can be blocked by antibody specific for the protein. Moreover, prolonged incubation (48 hr) of native LDL with the coculture, in the presence of serum, results in the production of bioactive MM-LDL, which can be recovered from the medium. It appears that the subendothelial microenvironment in these cocultures is depleted of antioxidants found in serum, thus allowing lipid peroxidation resulting from interaction with reactive oxygen species (ROS) produced by vascular cells. The MM-LDL produced by cocultures is remarkably similar to native LDL in its physical properties. Recent physical separations have suggested that the bioactive component in such MM-LDL is an oxidized phospholipid [27].

The induction of monocyte entry into cocultures incubated with LDL provides a rapid and sensitive assay for factors affecting LDL oxidation. These studies have revealed that a wide range of antioxidants, including probucol and tocopherol, partially or totally block LDL oxidation. Subfractions of high density lipoproteins (HDL), including HDL_2 but not HDL_3, also effectively blocked monocyte entry, suggesting that *in vivo* HDL may function in preventing LDL oxidation as well as reverse cholesterol transport. These studies have also revealed that a new class of anti-inflammatory compounds termed "leumedins" (Fig. 2) may be useful in inhibiting lipoprotein modification [28]. In the coculture system, they block LDL-induced monocyte transmigration almost totally at concentrations of 15–30 μM. Like probucol, leumedins bind strongly to LDL, and LDL isolated from animals injected with leumedins was remarkably resistant to modification.

It should be noted that antioxidants may also inhibit lipoprotein oxidation by preventing the release of cellular reactive oxygen species. In the coculture system, inhibitors of the cycloxygenase pathway (aspirin and indomethacin) and the anti-inflammatory agent dexamethasone partially blocked LDL modification. Similarly, preincubation of cells with leumedin prior to addition of LDL blocked monocyte transmigration [28]. It is likely that the extensive oxidation of LDL *in vivo* is due, in part, to monocyte/macrophages that have entered the artery wall since activated macrophages produce relatively large amounts of ROS. The identification of cellular or lipoprotein-associated proteins mediating lipoprotein oxidation is of considerable interest, since this would make feasible the design and screening of potential pharmacologic inhibitors. For example, studies have suggested that 15-lipoxygenase may produce lipid peroxides capable of promoting LDL oxidation [6]. Also, the enzyme 5-lipoxygenase metabolizes arachidonic acid to various leukocyte chemotactic agents. Based on current knowledge of the mechanism of 5-lipoxygenase, several classes of inhibitors of the enzyme have been proposed [29].

A reduction in the levels of oxidized lipoproteins in the intima would also likely be achieved by preventing the excessive transport of LDL (and other atherogenic lipoproteins) to the intima and by reducing the retention of LDL in the intima. Since hyperlipidemia stimulates artery wall lipid accumulation, it is likely that the flux of LDL into the intima is of importance. This can be decreased by reducing the levels of blood lipoproteins by dietary or hypolipidemic drug treatments. The increased permeability of the endothelial barrier to

LDL is also a contributing factor. Studies with animal models have revealed that regions of the normal artery that are likely to develop lesions are especialy permeable to dyes. This permeability is probably determined in part by hemodynamic forces. Also, activated or injured endothelium is more permeable than resting endothelium. In heart transplant atherosclerosis, autoimmune mediated activation of the endothelium is likely to contribute to increased endothelial permeability, as well as altered eicosanoid, growth factor, and cytokine expression. It is possible that the accumulation of oxidized lipids could be slowed by anti-inflammatory agents that decrease endothelial permeability. Animal studies have shown that the retention of LDL in the artery wall is greatly increased in atherosclerotic lesions as compared with normal artery. This may reflect the alteration and increased proliferation of connnective tissue in atherosclerosis. Recent studies involving injection of labeled lipoproteins have revealed that the atherogenic Lp(a) particle accumulates to a much greater extent than LDL both in normal arteries and atherosclerotic lesions of mice, which may explain, in part, its atherogenic potential [30]. Also, homocysteine has been shown to greatly increase the binding of Lp(a) to fibrin, providing a possible explanation for the finding that moderate elevations in blood homocysteine predispose to premature atherosclerosis [31]. Whether similar specific interactions occur between LDL and collagen or other matrix components is unknown.

Another potential pharmacologic target is the metabolism of highly oxidized lipoproteins by intimal macrophages. The precise mechanisms leading to rapid lipoprotein uptake are not yet certain, but substantial evidence suggests the involvement of the scavenger receptor, which recognizes the highly modified forms of LDL. A recent study suggests that SMC can also express the receptor, providing an additional link between oxidized lipoproteins and SMC alterations [32]. The scavenger receptor is modulated by interferon γ (derived from T lymphocytes), and treatment of macrophages with interferon γ prevents foam cell formation *in vitro* [33]. The mobilization of cholesterol following macrophage uptake could potentially be altered by modulation of the activities of acid- and neutral-cholesterol hydrolases, resulting in reduced deposition of cholesterol esters.

The inhibition of lipoprotein oxidation in the artery wall is one of the most attractive strategies for pharmacologic intervention in atherosclerosis. Lipoprotein oxidation does not occur to a detectable extent in normal artery and, thus, inhibition of LDL oxidation is unlikely to have deleterious side-effects. There is also a large body of information relevant to the properties of agents that inhibit LDL oxidation. Moreover, studies with some antioxidants are consistent with the concept that inhibition of lipoprotein oxidation will retard the development of early atherosclerotic lesions. Inhibition of lipoprotein oxidation may also have beneficial effects on advanced atherosclerotic lesions since foam cells appear to contribute to lesion growth and instability. It seems likely that antioxidants more potent than probucol, exhibiting desirable characteristics with respect to absorption, nontoxicity, metabolism, and localization (including within the LDL particle), will be identified in the near future. The coculture system described above may provide a sensitive, semi-physiologic initial screen for such agents. Maximal inhibition of the accumulation of oxidized LDL in the artery wall would probably best be achieved using a combination of pharmacologic agents, such as a hypolipidemic agent (reducing LDL accumulation in the artery) plus an antioxidant.

Pharmacologic intervention in gene expression. The biologically active molecules mediating inflammatory gene activation by MM-LDL appear to be oxidized phospholipids [27], although their identity is unknown. The activity in copper oxidized LDL responsible for the induction of macrophage interleukin-1β appears to be due, in part, to the linoleate oxidation products 9- and 13-hydroxyoctadecadienoic acid (9-HODE and 13-HODE) [34]. Some studies suggest that oxidized lipids are general mediators of inflammation, not restricted to the artery wall. For example, injection of MM-LDL into mice induced inflammatory genes in a variety of tissues, including liver, and hepatic lipid accumulation in response to hyperlipidemia resulted in the activation of the same set of genes as MM-LDL injection [35]. Oxidized LDL is known to induce a large number of inflammatory genes (Table 1), and the list is surely far from complete. Several are "immediate early genes" whose transcription is rapidly activated, even in the presence of inhibitors of protein synthesis. The activation of at least some of the genes appears to be determined, in part, by the transcription factor NF-κB. *Cis*-elements for NF-κB are present in a number of the genes (SAA, MCP-1/JE, tissue factor, M-CSF), and NF-κB is activated by MM-LDL in tissue culture and by hepatic lipid accumulation *in vivo* [35]. NF-κB is normally present as an inactive trimer in the cytoplasm and is activated by an intracellular signalling pathway that involves reactive oxygen species. This results in the dissociation of an inhibitory subunit and the transport of the active dimer into the nucleus. Thus, protein synthesis is not required for transcriptional activation. Strong evidence for the role of NF-κB in lipid peroxide mediated gene induction has been obtained from genetic studies showing a correspondence between NF-κB induction and inflammatory gene activation in reponse to hyperlipidemia among inbred strains of mice [35]. Consistent with this conclusion is the observation that MM-LDL results in cellular oxidative stress as judged by the induction of heme oxygenase and glutathione transferase (Table 1). The signalling pathways by which the various forms of oxidized LDL modulate gene expression are not as yet well understood. Many of the genes induced by MM-LDL are also activated by bacterial lipopolysaccharide (LPS) and tumor necrosis factor (TNF), suggesting the possibility of common signalling pathways. There are,however, some clear differences in gene induction; for example, whereas LPS induces EC to express adhesion molecules for both neutrophils and monocytes, MM-LDL specifically induces monocyte adhesion [36].

Table 1. Genes induced by oxidized LDL

Gene (cell type)	Properties and functions of protein
Monocyte adhesion molecules (endothelial cells)	The identity of the molecules responsible for monocyte binding in response to oxidized LDL is unknown.
M-CSF (endothelial cells, fibroblasts)	A growth and differentiation factor for monocytes, expressed at high levels in atherosclerotic lesions.
MCP-1/JE (endothelial cells, monocytes, fibroblasts, smooth muscle cells)	MCP-1 and its mouse homologue, JE, are encoded by early response genes and are monocyte chemoattractants. MCP-1/JE are expressed at high levels in atherosclerotic lesions.
KC/gro (endothelial cells, fibroblasts)	A primary response gene of unknown function.
IL-1β (macrophages)	A cytokine that plays a role in the induction of many inflammatory responses.
Serum amyloid A (SAA) family (hepatocytes, macrophages)	A family of acute phase reactant proteins that bind to HDL.
Glutathione transferase (multiple)	An enzyme involved in the removal of lipid peroxide by the glutathione pathway.
Granulocyte-macrophage CSF and granulocyte CSF (endothelial cells)	Growth and differentiation factors for granulocyte and macrophage progenitor cells.
Tissue factor (monocytes, smooth muscle cells)	A membrane protein primarily responsible for the initiation of the coagulation cascade.
Heme oxygenase	The rate-limiting enzyme in the degradation of heme, a potent prooxidant.

Of the genes known to be induced by oxidized lipids (Table 1), IL-1β is clearly an attractive target for pharmacologic intervention, since it activates endothelium and promotes SMC proliferation. Inhibition of expression of monocyte adhesion molecules and MCP-1 would also likely inhibit lesion development, since monocytes are likely to contribute to the proinflammatory state. As mentioned above, however, the identity of the relevant adhesion molecules remains to be determined. The effect of inhibition of M-CSF on atherosclerosis is uncertain. One could also consider inhibiting proteins involved in the signalling pathways mediating inflammatory gene induction. For example, inhibition of transcription factor NF-κB expression would likely block the activation of several of the genes known to be induced by oxidized forms of LDL.

Some possible approaches for pharmacological mediation of cytokine functions have been reviewed [37]. In principle, the production of a cytokine could be blocked at any stage in its realization—transcription, translation, cellular release, processing, or action: (1) Transcriptional down-regulation of cytokines could be achieved through the use of natural mediators (for example, glucocorticoids inhibit the expression of certain endothelial adhesion molecules) or the use of synthetic molecules. Gene transcription has also been blocked through the use of double-stranded phosphorothioate oligonucleotides corresponding to important *cis*-elements (for example, oligonucleotides containing the NF-κB consensus sequence could be considered), although it is presently unclear how delivery of such compounds would be achieved. Rapid screening of substances influencing gene transcription has been achieved through the use of cells containing appropriate promoter sequences ligated to sensitive reporter genes such as luciferase. (2) Cytokine translation could be blocked by antisense sequences or ribozymes; for example, antisense oligonucleotides for IL-1 have been shown to block inflammatory responses. Recent studies suggest that phosphorodithioate DNA is likely to have therapeutic potential [38]. (3) A number of cytokines are exported from cells by a novel secretory pathway or require proteolytic processing for activation, providing an opportunity to specifically interfere with the secretion or activation. (4) The action of cytokines could be blocked by antibodies, synthetic receptor derivatives or other substances identified in screening assays. Some of the difficulties associated with the use of antibodies may be circumvented by the development of mice reconstituted with a human immunoglobulin system. It is noteworthy that antibodies have effectively retarded restenosis following balloon injury in experimental animals. (5) Finally, it may be possible to block the action of cytokines by intervention in the signal transduction pathways of target cells.

As techniques for *in vivo* gene insertion into artery wall cells continue to improve, it may be possible to express genes that alter the balance of cytokine–eicosanoid "cross talk" from an atherogenic to an antiatherogenic state. Low efficiency, transient gene insertion into artery wall has been achieved, and new techniques, such as adenovirus based methods, are likely to greatly improve transfection efficiencies [39]. Such measures would only be temporary, since the expression of the transfected DNA would likely not be expressed for more than weeks to months. Permanent insertion, requiring integration into the genome, would probably best be achieved by the use of retroviral vectors (reviewed in Ref. 40). Such retroviral gene insertion may be particularly applicable to the prevention of restenosis following angioplasty or accelerated atherosclerosis following heart transplant. It could be performed

during the surgery, and such local therapy would be advantageous with respect to nonspecific influences and other risks.

Overall, the possibilities for the modulation of expression of genes influencing atherosclerosis appears promising despite many uncertainties. Although the complexity of the interactions in atherosclerosis makes it difficult to predict the outcome, there are many opportunities to affect the homeostatic balance of the artery wall. Most likely, multiple pharmacologic interventions utilized together will prove most effective.

Conclusions. Clearly, there are exciting possibilities for new pharmacologic intervention at the level of the artery wall. Several opportunities exist for inhibiting lipoprotein oxidation and vascular responses to oxidized lipoproteins, and a number of potentially useful drug screening strategies have been developed. Also, appropriate animal models such as the mouse and the rabbit are available for drug testing. Given the possibility of species specific pharmacologic effects, it seems prudent to study two or more animal species before proceeding with human trials. A major obstacle to the development of drugs acting at the level of the artery wall is likely to be human testing. Unlike trials with hypolipidemic drugs or hypertensive drugs, in which the effects can be quantitated within weeks, the measured endpoint in trials of drugs acting at the level of the artery wall would be lesion development (by quantitative angiography or a related technique) or myocardial infarction. This would require large numbers of subjects and many years. Although differing in some respects from most forms of atherosclerosis, transplant atherosclerosis and restenosis following angioplasty present particularly attractive opportunities for drug intervention and testing.

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REFERENCES

1. Rosenfeld ME, Palinski W, Yla-Herttuala S and Carew TE, Macrophages, endothelial cells and lipoprotein oxidation in the pathogenesis of atherosclerosis. *Toxicol Pathol* **18**: 560–571, 1990.
2. Ross R, The pathogenesis of atherosclerosis: A perspective for the 1990s. *Nature* **362**: 801–809, 1993.
3. Territo M, Navab M and Berliner J, Cellular interactions of the artery wall. In: *Molecular Genetics of Coronary Artery Disease* (Eds. Lusis AJ, Rotter JI and Sparkes RS), pp. 18–34. Karger, Basel, 1992.
4. Hajjar DP and Pomerantz KB, Signal transduction in atherosclerosis: Integration of cytokines and the eicosanoid network. *FASEB J* **6**: 2933–2941, 1992.
5. Brown MS and Goldstein JL, Scavenging for receptors. *Nature* **343**: 508–509, 1990.
6. Parthasarathy S, Steinberg D and Witztum JL, The role of oxidized low-density lipoproteins in the pathogenesis of atherosclerosis. *Annu Rev Med* **43**: 219–225, 1992.
7. Cybulsky MI and Gimbrome MA Jr, Endothelial expression of a mononuclear leukocyte adhesion molecule during atherogenesis. *Science* **251**: 788–791, 1991.
8. Akeson AL, Woods CW, Mosher LB, Thomas CE and Jackson RL, Inhibition of IL-1β expression in THP-1 cells by probucol and tocopherol. *Atherosclerosis* **86**: 261–270, 1991.
9. Fuster V, Badimon L, Badimon JJ and Chesebro JH, The pathogenesis of coronary artery disease and the acute coronary syndromes. *N Engl J Med* **326**: 242–250, 1992.
10. Lindner V and Reidy MA, Proliferation of smooth muscle cells after vascular injury is inhibited by an antibody against fibroblast growth factor. *Proc Natl Acad Sci USA* **88**: 3739–3743, 1991.
11. Owens GK, Differential effects of antihypertensive therapy on vascular smooth muscle cell hypertrophy, hyperploidy, and hyperplasia in the spontaneously hypertensive rat. *Circ Res* **56**: 525–536, 1985.
12. Cambien F, Poirer O, Lecerf L, Evans A, Cambou J-P, Arveiler D, Luc G, Bard J-M, Bara L, Ricard S, Tiret L, Amouyel P, Alhenc-Gelas F and Soubrier F, Deletion polymorphism in the gene for angiotensin-converting enzyme is a potent risk factor for myocardial infarction. *Nature* **359**: 641–644, 1992.
13. Itoh H, Pratt RE and Dzau VJ, Atrial natriuretic polypeptide inhibits hypertrophy of vascular smooth muscle cells. *J Clin Invest* **86**: 1690–1697, 1990.
14. Lehr HA, Hubner C, Fincke B, Angermuller S, Nolte D, Beisiegel U, Kohlschutter A and Messmer K, Role of leukotrienes in leukocyte adhesion following systemic administration of oxidatively modified human low density lipoprotein in hamsters. *J Clin Invest* **88**: 9–14, 1991.
15. Skrinska VA, Konieczkowski M, Gerrity RG, Galang CF and Rebec MV, Suppression of foam cell lesions in hypercholesterolemic rabbits by inhibition of thromboxane A_2 synthesis. *Arteriosclerosis* **8**: 359–367, 1988.
16. Cooke JP, Singer AH, Tsao P, Zera P, Rowan RA and Billingham ME, Antiatherogenic effects of L-arginine in the hypercholesterolemic rabbit. *J Clin Invest* **90**: 1168–1172, 1992.
17. Hogan M, Cerami A and Bucala R, Advanced glycosylation endproducts block the antiproliferative effect of nitric oxide. Role in the vascular and renal complications of diabetes mellitus. *J Clin Invest* **90**: 1110–1115, 1992.
18. Fuster V, Badimon L, Badimon JJ and Chesebro JH, The pathogensis of coronary artery disease and the acute coronary syndromes. *N Engl J Med* **326**: 310–318, 1992.
19. Gey KF and Puska P, Plasma vitamin E and A inversely correlated to mortality from chronic heart disease in cross-cultural epidemiology. *Ann NY Acad Sci* **570**: 260–282, 1989.
20. Carew TE, Schwenke DC and Steinberg D, Antiatherogenic effect of probucol unrelated to its hypercholesterolemic effect: Evidence that antioxidants *in vivo* can selectively inhibit low density lipoprotein degradation in macrophage-rich fatty streaks and slow the progression of atherosclerosis in the Watanabe heritable hyperlipidemic rabbit. *Proc Natl Acad Sci USA* **84**: 7725–7729, 1987.
21. Haberland ME and Steinbrecher UP, Modified low density lipoproteins: Diversity and biologic relevance in atherosclerosis. In: *Molecular Genetics of Coronary Artery Disease* (Eds. Lusis AJ, Rotter JI and Sparkes RS), pp. 35–61. Karger, Basel, 1992.
22. Chisolm GM, Antioxidants and atherosclerosis: A current assessment. *Clin Cardiol* **14**: I25–I30, 1991.
23. Ferns GAA, Forster L, Stewart-Lee A, Konneh M, Nourooz-Zadeh J and Ånggård EE, Probucol inhibits neointimal thickening and macrophage accumulation after balloon injury in the cholesterol-fed rabbit. *Proc Natl Acad Sci USA* **89**: 11312–11316, 1992.
24. Mao SJT, Yates MT, Parker RA, Chi EM and Jackson

RL, Attenuation of atherosclerosis in a modified strain of hypercholesterolemic Watanabe rabbits with use of a probucol analogue (MDA 29,311) that does not lower serum cholesterol. *Arterioscler Thromb* **11**: 1266–1275, 1991.

25. Sparrow CP, Doebber TW, Olszewski J, Wu MS, Ventre J, Stevens KA and Chao Y-S, Low density lipoprotein is protected from oxidation and the progression of atherosclerosis is slowed in cholesterol-fed rabbits by the antioxidant *N,N'*-diphenylphenylenediamine. *J Clin Invest* **89**: 1885–1891, 1992.
26. Navab M, Liao F, Hough G, Ross LA, Van Lenten BJ, Rajavashisth TB, Lusis AJ, Laks H, Drinkwater DC and Fogelman AM, Interaction of monocytes with cocultures of human aortic wall cells involves interleukins 1 and 6 with marked increases in connexin43 message. *J Clin Invest* **87**: 1763–1772, 1991.
27. Watson AD, Sevanian A, Parhami R, Navab M, Fogelman AM and Berliner JA, Characterization of phospholipid components of minimally modified LDL. *Circulation* **86**: I-210, 1992.
28. Navab M, Hama SY, Van Lenten BJ, Drinkwater DC, Laks H and Fogelman AM, A new anti-inflammatory compound, leumedin, inhibits modification of low density lipoprotein and the resulting monocyte transmigration into the subendothelial space of cocultures of human aortic wall cells. *J Clin Invest* **91**: 1225–1230, 1993.
29. McMillan RM and Walker ERH, Designing therapeutically effective 5-lipoxygenase inhibitors. *Trends Pharmacol Sci* **13**: 323–330, 1992.
30. Kreuzer J, Lloyd MB, Bok D, Fless GM, Scano AM, Lusis AJ and Haberland ME, Lipoprotein(a) displays increased accumulation compared to low density lipoprotein in the murine artery wall. *Chem Phys Lipids*, in press.
31. Harpel PC, Chang VT and Borth W, Homocysteine and other sulfhydryl compounds enhance the binding of lipoprotein(a) to fibrin: A potential biochemical link between thrombosis, atherogenesis, and sulfhydryl compound metabolism. *Proc Natl Acad Sci USA* **89**: 10193–10197, 1992.
32. Pitas R, Expression of the low-density lipoprotein receptor by rabbit fibroblasts and smooth muscle cells. *J Biol Chem* **265**: 12722–12727, 1990.
33. Geng Y-j and Hansson GK, Interferon-γ inhibits scavenger receptor expression and foam cell formation in human monocyte-derived macrophages. *J Clin Invest* **89**: 1322–1330, 1992.
34. Ku G, Thomas CE, Akeson AL and Jackson RL, Induction of interleukin 1β expression from human peripheral blood monocyte-derived macrophages by 9-hydroxyoctadecadienoic acid. *J Biol Chem* **267**: 14183–14188, 1992.
35. Liao F, Andalibi A, DeBeer FC, Fogelman AM and Lusis AJ, Genetic control of inflammatory gene induction and NF-κB-like transcription factor activation in response to an atherogenic diet in mice. *J Clin Invest* **91**: 2572–2579, 1993.
36. Berliner JA, Territo MC, Sevanian A, Ramin S, Kim JA, Bamshad B, Esterson M and Fogelman AM, Minimally modified low density lipoprotein stimulates monocyte endothelial interactions. *J Clin Invest* **85**: 1260–1266, 1990.
37. Henderson B and Blake S, Therapeutic potential of cytokine manipulation. *Trends Pharmacol Sci* **13**: 145–152, 1992.
38. Marshall WS and Caruthers MH, Phosphorodithioate DNA as a potential therapeutic drug. *Science* **259**: 1564–1570, 1992.
39. Nabel EG, Plautz G and Nabel GS, Site-specific expression *in vivo* by direct gene transfer into the arterial wall. *Science* **249**: 1285–1288, 1990.
40. Lee SW, Kahn ML and Dichek DA, Control of clot lysis by gene transfer. *Trend Cardiovasc Med* **3**: 61–66, 1993.