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Effects of Oxidized Low-Density Lipoprotein on Vascular Contraction and Relaxation: Clinical and Pharmacological Implications in Atherosclerosis

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I. Introduction

Elevated plasma levels of low-density lipoprotein (LDL) have been associated with the development of atherosclerosis for more than 20 years (Kannel et al., 1971), and lipid-lowering therapy is a beneficial approach to slowing the progression of this disease (Tyroler, 1987). More recently, modification of LDL, especially oxidation of this lipoprotein, has become recognized as important to many of the atherogenic actions ascribed to LDL (Steinberg et al., 1989; Young and Parasarathy, 1994). Indeed, oxidized LDL exerts many effects in vitro that could contribute to the progression of atherosclerosis if they occur in vivo. Thus, the "oxidation

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Abbreviations: LDL, low-density lipoprotein; EDRF, endothelium-derived relaxing factor; NO, nitric oxide; EDR, endothelium-dependent relaxation; 5-HT, 5-hydroxytryptamine (serotonin); mRNA, messenger ribonucleic acid; NOS, nitric oxide synthase; iNOS, inducible NOS; lyso PC, lysophosphatidylcholine; PLA_2, phospholipase A_2; PKC, protein kinase C; PTX, pertussis toxin; SOD, superoxide dismutase.

hypothesis" of atherosclerosis proposes that oxidative modification of LDL (and possibly other lipoproteins) is a pivotal event in the initiation and progression of atherosclerosis.

Oxidized LDL also has direct effects on the vasomotor properties of blood vessels and may play an important role in the alterations in vascular contractility observed in hypercholesterolemia and atherosclerosis. Vascular dysfunction, especially inappropriate or enhanced vasoconstriction, may be involved in the genesis of several clinical manifestations of atherosclerosis, including stable and unstable angina, acute myocardial infarction, and sudden cardiac death (Zeiher and Schachinger, 1994; Kalsner, 1995). To the extent that oxidized LDL is involved in enhanced vasoconstriction, pharmacological intervention that inhibits either the formation or actions of oxidized LDL may normalize vascular function in hypercholesterolemia and atherosclerosis and could be beneficial in the therapeutic management of these pathologies. Additionally, such pharmacological intervention at a vascular level may greatly augment the beneficial effects of therapies currently used in the treatment of atherosclerosis, including the pharmacological lowering of serum lipid levels.

This review focuses on the vasomotor actions of oxidized LDL, their potential contribution to the clinical manifestations of atherosclerosis, and pharmacological strategies that may inhibit these actions. For a broader discussion of the formation, physiochemical properties, and atherogenic actions of oxidatively modified LDL, the reader is referred to several recent reviews (Steinberg et al., 1989; Penn and Chisolm, 1994; Holvoet and Collen, 1994).

II. Discovery and Characteristics of Oxidized Low-Density Lipoprotein

Realization of the importance of LDL modification to its oxidized form in atherosclerosis was primarily the result of several apparently incongruent observations (Steinberg et al., 1989): (*a*) increased serum LDL cholesterol led to the formation of early atherosclerotic lesions enriched in lipid-laden, macrophage-derived foam cells (Fowler et al., 1979); (*b*) isolated macrophages have a very limited ability to take up native LDL, and incubation of macrophages with native LDL did not lead to foam cell formation (Goldstein et al., 1979); and (*c*) hypercholesterolemia led to atherosclerotic lesion formation, even in animals and humans with deficiencies in LDL receptors (Brown and Goldstein, 1986). From these observations, it was suggested that LDL must undergo some type of modification to be recognized and taken up by macrophages and induce foam cell formation.

In 1979, Goldstein and coworkers discovered that modification of LDL by acetylation resulted in a greatly enhanced capacity for it to be taken up and degraded by macrophages, resulting in the formation of foam cells (Goldstein et al., 1979). This chemically modified LDL, termed acetyl-LDL, lost the ability to bind to the classical LDL receptor, but gained the ability to bind to a novel receptor that mediated the uptake into macrophages, termed the scavenger receptor. Although acetylation of LDL was not likely to occur in vivo, these studies were soon followed with the demonstration that LDL could also be modified by cultured endothelial cells, resulting in LDL that competed with acetyl LDL for binding to the scavenger receptor and was avidly taken up by macrophages (Henrickson et al., 1981). These data provided a potential mechanism by which LDL may be modified in vivo to acquire the ability to form foam cells and contribute to atherosclerotic lesion formation. Subsequent studies demonstrated that the nature of the modification mediated by cultured cells involved an oxidative process (Steinbrecher et al., 1984; Morel et al., 1984): hence, the term "oxidized LDL" was born.

LDL is comprised of a cholesterol ester core surrounded by phospholipids and lipophilic antioxidants, including α-tocopherol and β-carotene. Embedded within the phospholipid coat is one molecule of the apo-B100 protein, a portion of which serves as the ligand for the LDL receptor (fig. 1). LDL can be oxidized in vitro by exposure to either cultured cells as described above, or by incubation with copper ion (Parthasarathy et al., 1989), resulting in profound changes in the physiochemical properties of the LDL particle (fig. 1). Initially, the lipophilic antioxidants ward off modification of the LDL particle. However, after depletion of the antioxidants, polyunsaturated fatty acids of the phospholipids undergo oxidation, resulting in their depletion and the creation of reactive hydroxy fatty acids (Steinbrecher et al., 1984; Morel et al., 1984). At this stage of oxidation, with low levels of oxidized lipid products and a relatively intact apo B100 protein, the LDL particle is considered "minimally modified" (Berliner et al., 1990). However, if the process is allowed to continue and oxidized lipid products accumulate to higher levels in the particle, they begin to react with and covalently modify amino acid residues on the apo B-100 protein, resulting in an increase in negative charge of the LDL particle and a fragmentation of the protein component of LDL (Fruebis

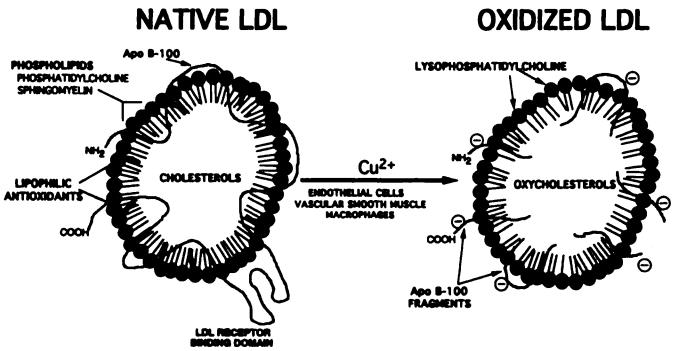

FIG. 1. Schematic representation of the conversion of native LDL to oxidized LDL and the accompanying physiochemical changes that occur.

et al., 1992; Steinbrecher, 1987). These oxidative modifications of the apo-B100 protein lead to the loss of the LDL receptor binding ligand, explaining the inability of oxidized LDL to bind to the classical LDL receptor, but result in new binding epitopes that allow interaction with the scavenger acetyl-LDL receptor. Oxidation also results in an extensive conversion of phosphatidylcholine to lysophosphatidylcholine (lyso PC) (Parthasarathy et al., 1985), as well as to the production of oxysterols from the cholesterol esters in the lipid core of the particle (Zhang et al., 1990).

Thus, oxidative modification of LDL leads to alterations in both the lipid and protein components of the lipoprotein particle. The modified lipoprotein was studied extensively to determine its effects on macrophages and other cell types thought to be involved in atherosclerosis. Indeed, oxidized LDL exerted a myriad of effects that would be atherogenic if they occurred in vivo, the majority of which were not duplicated by native, unoxidized LDL, including chemotaxis of monocytes, inhibition of macrophage motility, foam cell formation, up-regulation of endothelial adhesion molecules, stimulation of growth factor and chemokine expression, and proliferative effects on smooth muscle and monocytes (Holvoet and Collen, 1994). In concert, these effects of oxidized LDL may contribute to the alterations in blood vessel morphology, characterized most prominently by fatty streak formation and intimal thickening, that occur during the initiation and progression of atherosclerosis. However, more recent work has revealed that oxidized LDL also exerts acute effects on the vasomotor properties of blood vessels. These findings suggest that oxidized LDL may be involved not only in the morphological alterations associated with atherosclerosis, but also in the well-known alterations in vasoactivity that occur in this disease.

III. Effects of Oxidized Low-Density Lipoprotein on Vascular Motility

A. Inhibition of Endothelium-Dependent Relaxation

The endothelium is involved in vasodilation by releasing multiple factors that diffuse to the underlying smooth muscle and cause relaxation, including endothelium-derived relaxing factor (EDRF), believed to be nitric oxide (NO) or an NO-containing compound (Palmer et al., 1987; Feelisch et al., 1994), prostacyclin (Siegel et al., 1989), and possibly an endothelium-derived hyperpolarization factor (Hecker et al., 1994). Of these endothelium-derived mediators, however, NO has been suggested to be most important in the regulation of both basal tone and vasodilation produced by stimuli such as acetylcholine and shear stress in humans in vivo (Haynes et al., 1993; Quyyumi et al., 1995). NO is generated in endothelial cells by the oxidation of L-arginine to l-citrulline in a reaction catalyzed by endothelial NO synthase (eNOS) (Palmer et al., 1988). NO produced by eNOS in endothelial cells then diffuses to the smooth muscle and mediates relaxation by stimulating the activity of soluble guanylate cyclase and increasing the level of cyclic GMP within the smooth muscle cells (Rapoport and Murad, 1983). NO generated by the endothelium also inhibits platelet aggregation and platelet adhesion to the blood vessel wall (Radomski et al., 1987). Thus, NO plays an important role in the cardiovascular system by regulating both vasomotion and hemostasis.

Exposure of isolated blood vessels to LDL oxidized by incubation with either copper ion or cultured endothelial cells inhibited endothelium-dependent relaxation (EDR), including relaxation to acetylcholine in precontracted rabbit aorta (Kugiyama et al., 1990; Yokohama et al., 1990), and to 5-hydroxytryptamine (5-HT) (serotonin), thrombin, and aggregating platelets in precontracted pig coronary artery (Tanner et al., 1991; Simon et al., 1990; Ohgushi et al., 1993; Murohara et al., 1994). This effect was selective for oxidized versus unoxidized LDL and was mediated by concentrations of oxidized LDL (10–100 μg/ml) that are physiologically and pathologically relevant (see section IV.D). Although oxidized LDL can be toxic to cultured endothelial cells with prolonged incubation (Hessler et al., 1979), oxidized LDL did not inhibit EDR to all relaxant agonists. Oxidized LDL had little effect on bradykinin-induced relaxation in porcine coronary arteries (Tanner et al., 1991), although this response was dependent upon the presence of functional endothelium (Tanner et al., 1991; Cox et al., 1995). Thus, acute incubation of blood vessels with oxidized LDL does not produce a generalized cytotoxic effect on the endothelium. Furthermore, oxidized LDL does not inhibit smooth muscle relaxation nonselectively, because relaxation to NO donors such as nitroglycerin and 3-morpholinosydnonimine was unaffected (Kugiyama et al., 1990; Tanner et al., 1991; Plane et al., 1992). Taken together, these observations indicate that oxidized LDL has a selective effect on the endothelium to inhibit vascular relaxation to some agonists.

Oxidized LDL inhibited most effectively vascular relaxation mediated by agonists that release EDRF(NO) from the endothelium. For example, oxidized LDL markedly inhibited coronary arterial relaxation to 5-HT and thrombin (Tanner et al., 1991; Ohgushi et al., 1993), two agents whose relaxation is highly sensitive to NO synthase inhibitors (Nagao and Vanhoutte, 1992), whereas oxidized LDL was less effective in inhibiting relaxation to bradykinin and the calcium ionophore A23187 (Tanner et al., 1991). EDR to the latter two agonists was also less sensitive to NO synthase inhibitors (Nagao and Vanhoutte, 1992; Holzmann et al., 1994; Cox et al., 1995), suggesting that these mediators do not depend on NO release to cause relaxation, or can activate alternative mechanisms when the NO pathway is inhibited (Kilpatrick and Cocks, 1994). These observations may be explained by an effect of oxidized LDL to either inhibit the production and/or bioactivity of NO, or to increase the rate of NO degradation.

The inhibitory effect of oxidized LDL on endothelium-dependent, NO-mediated relaxation in blood vessels has prompted intense study of the effect of oxidized LDL on the EDRF(NO) pathway in various systems. These studies have suggested multiple mechanisms by which oxidized LDL may inhibit NO-mediated vasorelaxation, including: (*a*) direct inactivation of NO after its release via a direct interaction with oxidized LDL without an alteration in the amount of NO produced (Galle et al., 1991; Chin et al., 1992), (*b*) alteration in the bioactivity of the NO that is released (Myers et al., 1994) or (*c*) a decrease in endothelial NO synthase mRNA levels, leading to decreased NO synthase protein and to decreased NO production (Liao et al., 1995). However, more recent studies suggest a more complex effect of oxidized LDL on NO synthase levels. For example, lower concentrations of oxidized LDL (1–10 μg/ml) actually increased NO synthase messenger ribonucleic acid (mRNA) and protein levels, whereas higher concentrations (100 μg/ml) decreased these parameters in bovine aortic endothelial cells (Hirata et al., 1995b), although data demonstrating functional consequences of these changes in NOS levels were not presented in this study. Nevertheless, the mechanism by which oxidized LDL interferes with NO-mediated EDR is not yet clear, and may involve a combination of acute and chronic effects. In any case, inhibition of EDRF(NO)-mediated vasorelaxation is likely to be a major mechanism for the vascular effects of oxidized LDL.

It should also be noted that NO is produced in other cell types and tissues by isoforms of NOS distinct from that found in endothelial cells (Hattori et al., 1994). A neuronal NOS has been characterized in the brain and peripheral nerves that produces NO as a neurotransmitter (Schmidt et al., 1992). Furthermore, an inducible NOS is expressed in macrophages (Yui et al., 1991a), leukocytes (Yui et al., 1991b), and vascular smooth muscle cells (Busse and Mulsch, 1990) upon stimulation by cytokines or bacterial endotoxin. Unlike the endothelial and neuronal NOS isoforms, which are regulated by the concentration of intracellular free calcium via calmodulin, the inducible NOS is produced in a fully active form not regulated by calcium and capable of generating large amounts of NO. NO produced by iNOS is thought to play an important role in host defense mechanisms by acting as a cytotoxic agent released by neutrophils and macrophages (Hibbs et al., 1987). Thus, in addition to its role in regulating vascular tone, NO has also a regulatory role in the nervous and immune systems. The possibility exists that oxidized LDL may also affect NO-mediated responses in these systems, as well.

B. Enhanced Arterial Contraction

The fact that oxidized LDL can alter relaxant responses by an effect on endothelial NO-related mechanisms may translate into effects of oxidized LDL on the contractile responses of blood vessels to many agonists. Although oxidized LDL exerts its most prominent effects on responses to relaxant agonists that activate endothelial NO release, oxidized LDL may also affect basal NO release and produce direct effects on vascular smooth muscle. All of these actions of oxidized LDL may contribute to its diverse effects on vascular contractility.

Oxidized LDL has little effect on isolated blood vessels in the absence of added tone, but contracts blood vessels that are pretreated with a threshold concentration of contractile agonist. For example, perfused segments of rabbit femoral artery, pretreated with norepinephrine to provide tone, contracted in response to oxidized LDL in a manner that was both concentration-dependent and dependent on the extent to which the lipoprotein was oxidized (Galle et al., 1990). Oxidized LDL also contracted porcine coronary arterial rings precontracted with the thromboxane mimetic U46619 (Simon et al., 1990; Murohara et al., 1994). Comparable concentrations of unoxidized LDL were without effect on blood vessel tone in both tissues. Because arterial blood vessels possess basal tone in vivo due to sympathetic innervation, these in vitro data suggest that oxidized LDL may contract blood vessels and increase vascular tone in the intact vasculature.

In addition to direct contractile actions, oxidized LDL potentiated the contractile response of blood vessels to other stimuli. For example, contractile effects of norepinephrine, 5-HT, and potassium chloride were all enhanced in perfused rabbit femoral arteries treated with oxidized but not unoxidized LDL (Galle et al., 1990). Contraction of porcine coronary arteries by 5-HT was also enhanced after treatment with oxidized LDL (fig. 2*A*). Thus, oxidized LDL may not only inhibit the ability of blood vessels to relax, but may also make vessels more prone to vasospasm in response to contractile stimuli, effects that may be interrelated.

The procontractile actions of oxidized LDL may be related to its ability to inhibit the release or activity of NO from the endothelium. Indeed, inhibitors of NO synthase such as L-N^G-nitro-L-arginine and N^G-monomethyl-L-arginine contracted isolated blood vessels (Palmer et al., 1988; Rees et al., 1990) and enhanced the contractile response to other agonists (Moncada et al., 1991) via this mechanism. In fact, blockade of NO release by endothelial removal or pretreatment with N^G-monomethyl-L-arginine prevented the contractile action of oxidized LDL (Simon et al., 1990; Murohara et al., 1994), and attenuated the ability of oxidized LDL to enhance 5-HT-induced contraction in porcine coronary arteries (fig. 2*B*). These data are consistent with the hypothesis that the procontractile effects of oxidized LDL are a consequence of its inhibitory effects on the release or activity of NO rather than a direct effect on vascular smooth muscle.

Oxidized LDL may also enhance arterial contraction by stimulating the release of contractile factors from the endothelium. Indeed, oxidized LDL stimulated an in-

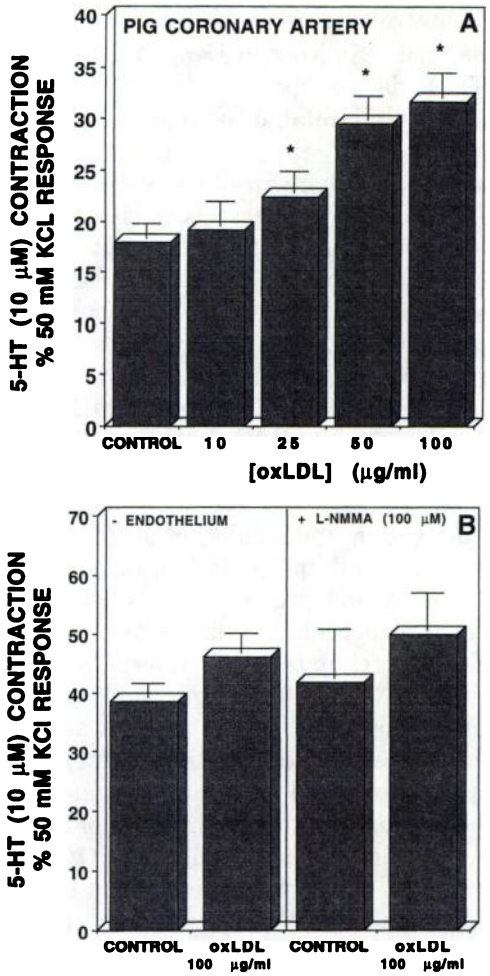

FIG. 2. Effect of oxidized LDL on maximal 5-HT-induced contraction in porcine coronary artery. (*A*) Porcine coronary arterial rings were incubated 45 min with indicated concentrations of copper-oxidized LDL, followed by cumulative addition of increasing concentrations of 5-HT. (*B*) Endothelium was removed from porcine coronary arterial rings by gentle rubbing. NO synthase was inhibited by pretreating rings 30 min with N^G-monomethyl-L-arginine (100 μM). Rings were then incubated with oxidized LDL (100μg/ml) and challenged with 5-HT as described above.

crease in mRNA expression and release of the potent vasoconstrictor peptide endothelin-1 from endothelial cells isolated from porcine and human aortae (Boulanger et al., 1992). Because low concentrations of endothelin-1 potentiated contractile responses of human arteries to norepinephrine and 5-HT (Yang et al., 1990), the effect of oxidized LDL to enhance endothelin-1 release from the endothelium may act in concert with a decrease in EDRF(NO)-mediated vasodilation to promote vasospasm.

It is possible that in some tissues, oxidized LDL may have additional direct effects on smooth muscle to enhance contractile actions of some agonists. The ability of oxidized LDL to contract rabbit femoral artery was not dependent on the presence of a functional endothelium (Galle et al., 1990). Furthermore, contractile responses of rabbit femoral artery and aorta to various stimuli were enhanced by oxidized LDL in an endothelium-independent manner (Galle et al., 1990; Niu et al., 1995). These data suggest that oxidized LDL enhanced agonist-induced vasoconstriction in rabbit peripheral arteries via a direct interaction with vascular smooth muscle.

C. Role of Lysophosphatidylcholine in the Vasomotor Effects of Oxidized Low-Density Lipoprotein

Several studies suggested that the active component mediating many of the in vitro effects of oxidized LDL resides in the lipid rather than the protein component of the lipoprotein and is not present in the lipid fraction of native, unoxidized LDL. One of the lipid components unique to oxidized LDL, lysophosphatidylcholine, mimicked many of the potentially proatherogenic effects of oxidized LDL, including increased monocyte motility (Quinn et al., 1987), decreased macrophage motility (Quinn et al., 1985), and increased expression of adhesion molecules on endothelial cells (Sugiyama et al., 1994). Several lines of evidence suggest that lyso PC may also be involved in the vasomotor effects of oxidized LDL, including the following: (*a*) authentic lyso PC mimicked the vascular effects of oxidized LDL, including inhibition of EDR in the rabbit aorta (Kugiyama et al., 1990; Yokohama et al., 1990) and pig coronary artery (Ohgushi et al., 1993; Murohara et al., 1994), and contraction of pig coronary artery (Murohara et al., 1994); (*b*) oxidized LDL depleted of lyso PC by incubation with defatted albumin or phospholipase B lost the ability to inhibit EDR and cause contraction (Ohgushi et al., 1993; Murohara et al., 1994); (*c*) native LDL incubated with exogenous phospholipase A_2 (PLA_2) to generate lyso PC in the lipoprotein imparted upon unoxidized LDL the ability to inhibit EDR (Yokohama et al., 1990); and (*d*) lyso PC inhibited phosphoinositide hydrolysis and calcium mobilization, mechanisms that are both involved in agonist-stimulated NO release, in human (Inoue et al., 1992) and bovine (Kugiyama et al., 1992) endothelial cells.

Although the bulk of the data support an important role for lyso PC in the vascular effects of oxidized LDL, other factors may also be involved. In fact, some studies have not shown a close association between the ability of oxidized LDL to inhibit EDR and its content of lyso PC (Plane et al., 1992; Hayashi et al., 1994), and Tanner et al. (1991) reported that lyso PC (10 μM) did not mimic the effect of oxidized LDL to inhibit 5-HT-induced EDR in porcine coronary arteries. Furthermore, chronic (8–12 hours) exposure of isolated endothelial cells to lyso PC up-regulated NOS mRNA and protein levels in isolated endothelial cells (Hirata et al., 1995b; Zembowicz et al., 1995), in contrast to its acute effect to inhibit EDR. These data suggest that other components of oxidized LDL may also contribute to its vascular effects. For example, oleic acid, a major fatty acid constituent of oxidized LDL, was recently shown to inhibit acetylcho-

line-induced EDR in rabbit aorta, mimicking the effect of oxidized LDL (Niu et al., 1995).

In summary, it is clear that oxidized LDL, perhaps in part via lyso PC, sensitizes isolated blood vessels to contractile stimuli, although multiple cellular sites of action may be involved, depending upon vascular bed and species. The cellular mechanisms of the vascular effects of oxidized LDL remain ill-defined, but several possibilities are currently under investigation (fig. 3).

IV. Cellular Mechanisms for Vasomotor Effects of Oxidized Low-Density Lipoprotein

A. Activation of Protein Kinase C

Inhibition of EDR, phosphoinositide hydrolysis, and calcium mobilization by oxidized LDL or lyso PC were all blocked by protein kinase C (PKC) inhibitors (Ohgushi et al., 1993; Kugiyama et al., 1992). Furthermore, lyso PC activated PKC in human endothelial cells (Kugiyama et al., 1992) and PKC purified from porcine brain (Oishi et al., 1990). These data suggested that activation of PKC by lyso PC in oxidized LDL may play a role in the impaired EDR induced by these agents, although activation of PKC by oxidized LDL has not yet been reported. Nevertheless, phorbol esters inhibited EDR in blood vessels isolated from several vascular beds (Rubanyi et al., 1989; Weinheimer et al., 1986) and inhibited NO release from porcine and bovine aortic endothelial cells (Smith and Lang, 1990; Hirata et al., 1995a). These data support a role for PKC in the regulation of agonist-stimulated NO release from endothelial cells.

Although the mechanism of these PKC-mediated effects on EDR and NO release is unclear, activation of PKC was shown to modulate both receptor-effector coupling and effector activity in the EDRF(NO) pathway. For example, activation of PKC with phorbol esters inhibited receptor-mediated phosphoinositol hydrolysis and intracellular calcium mobilization in endothelial cells (Brock et al., 1988; Kugiyama et al., 1992), mimicking the effects of lyso PC. In addition, PKC phosphorylated purified NO synthase, resulting in a rapid decrease in enzyme activity (Bredt et al., 1992). More recently, activation and inhibition of PKC were associated with down- and up-regulation, respectively, of NO synthase mRNA and protein levels of this enzyme in bovine aortic endothelial cells (Ohara et al., 1995a). Thus, PKC may regulate NO synthesis by affecting not only the mechanisms of receptor-coupling to NO synthase, but also by affecting the amount and activity of NO synthase itself. Taken together, these data suggest that lyso PC in oxidized LDL may activate PKC in endothelial cells, leading to an inhibition of NO synthase and a decrease in agonist-stimulated NO release. This scenario may be one of the mechanisms by which oxidized LDL impairs EDR and enhances contractile responses in isolated blood vessels.

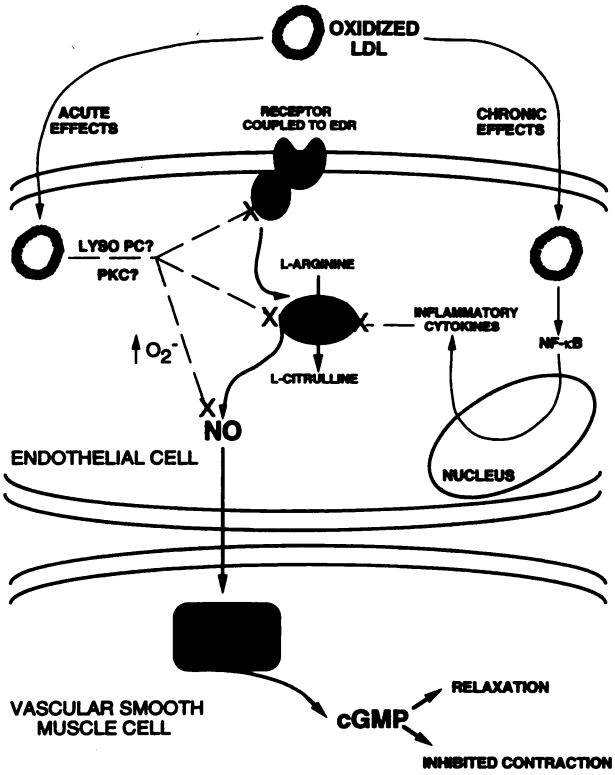

FIG. 3. Potential acute and chronic mechanisms of endothelial dysfunction produced by oxidized LDL. PKC, protein kinase C; PLC, phospholipase C; lyso PC, lysophosphatidylcholine; O_2^-, superoxide anion; NOS, nitric oxide synthase; NO, nitric oxide; cGMP, cyclic guanosine monophosphate.

B. Inhibition of G-Protein Function

Oxidized LDL-induced inhibition of EDR was selective for agonists coupled to a pertussis toxin (PTX)-sensitive G-protein. In the porcine coronary artery, EDR evoked by 5-HT, platelets, and thrombin was inhibited by PTX, whereas EDR evoked by bradykinin and A23187 was PTX-insensitive (Flavahan et al., 1989), a pattern that paralleled the effects of oxidized LDL (Tanner et al., 1991; Cox and Cohen, unpublished observations) and lyso PC (Flavahan, 1993). These data have prompted the hypothesis that oxidized LDL may affect the function of a PTX-sensitive G_i-protein in endothelial cells. Indeed, oxidized LDL down-regulated the level of $G_{i\alpha}$ subunit in bovine aortic endothelial cells and inhibited agonist-induced GTPase activity in concentrations that inhibited EDR in intact blood vessels (Liao and Clark, 1995). Thus, by inhibiting activity of the G_i-protein, oxidized LDL may selectively uncouple certain receptors from mediating EDRF(NO) release and, thus, selectively inhibit EDR. The mechanism by which oxidized LDL inhibits G_i-protein function could be related to changes in the fluidity or lipid composition of the cell membrane (Flavahan, 1992). Alternatively, this action may be related to PKC activation by oxidized LDL, which may phosphorylate G_i and inhibit the activity of this G-protein (Katada et al., 1985).

C. Stimulation of Superoxide Production

Lyso PC increased superoxide anion production from isolated rabbit aorta in a PKC-dependent manner (Ohara et al., 1994). Superoxide anion is generated as a by-product of many cellular reactions and is normally maintained at a very low level by the activity of a radical scavenging system of enzymes consisting primarily of superoxide dismutase (SOD) and catalase (Rubanyi, 1988). Superoxide inactivated NO via a direct interaction (Gryglewski et al., 1986; Rubanyi and Vanhoutte, 1986a), suggesting that an increase in vascular superoxide production could inhibit NO-mediated relaxation. Consistent with this hypothesis, increasing endogenous superoxide by inhibition of vascular SOD activity (Omar et al., 1991) or exogenous generation of superoxide via xanthine and xanthine oxidase (Rubanyi and Vanhoutte, 1986b; Seccombe et al., 1994) inhibited EDR in isolated blood vessels. Interestingly, the pattern of inhibition of EDR produced by superoxide anions in canine coronarary artery was similar to that reported for oxidized LDL and lyso PC in other blood vessels; relaxation evoked by acetylcholine was inhibited, whereas bradykinin-induced relaxation was unaffected (Seccombe et al., 1994). Thus, increased vascular superoxide production is another potential mechanism by which oxidized LDL and lyso PC may inhibit NO-dependent relaxation and vasodilation.

D. Induction of Adhesion Molecules and Inflammatory Cytokines

Although the effects of oxidized LDL described above may be involved in some of the acute and direct vasomotor effects observed in isolated blood vessels, chronic incubation of cultured cells with oxidized LDL has been shown to induce inflammatory gene products in vitro that may exert indirect but profound effects on vasomotion if these effects also occur in vivo. Minimally oxidized LDL induced the expression of monocyte chemoattractant peptide-1 and macrophage colony stimulating factor in endothelial cells (Berliner et al., 1990; Cushing et al., 1990; Rajavashisth et al., 1990), and oxidized LDL and lyso PC stimulated expression of adhesion molecules that play a role in the binding of monocytes and leukocytes to endothelial cells (Kume et al., 1993; Sugiyama et al., 1994). In concert, these effects may be important in the recruitment of monocytes and the conversion of these monocytes to macrophages during early lesion formation in atherosclerosis. In addition, adherence of inflammatory leukocytes to the endothelium also inhibited EDR (Sugiyama et al., 1994), suggesting that enhanced recruitment and retention of monocytes by oxidized LDL may also affect vasomotion. Furthermore, minimally oxidized LDL was also shown to activate NF-κB (Parhami et al., 1993; Peng et al., 1995), a transcription factor involved in the induction of inflammatory cytokines (Collins et al., 1995; Kunsch et al., 1994). Because certain cytokines potently down-regulated the expression of endothelial NO synthase (Yoshizumi et al., 1993; Rosenkranz-Weiss et al., 1994), chronic effects of oxidized LDL on the transcription of inflammatory gene products could produce vasomotor effects related to an inhibition of NO-mediated EDR.

E. Activation of an Oxidized Low-Density Lipoprotein Receptor

The vasoactive effects of oxidized LDL may be independent of receptor interaction. Indeed, lyso PC was passively transfered from oxidized LDL to the cell membrane and into the cytosol via a receptor-independent mechanism (Ohgushi et al., 1993). Although lyso PC may not be the sole mediator responsible for vasoactive effects of oxidized LDL, other active components in oxidized LDL may be similarly transfered to target cell membranes. Alternatively, the binding of oxidized LDL to a cell-surface receptor may also modify cellular function and contribute to its vascular actions.

Oxidized LDL binds to a variety of cell surface proteins, including the acetyl-LDL receptor (Henrikson et al., 1981), the glycoprotein CD36 (Endemann et al., 1993; Nicholson et al., 1995), the immunoglobulin receptor FcγRII (Stanton et al., 1992), and a recently characterized 94–97 kDa macrophage plasma membrane protein (Ottnad et al., 1995; Sambrano and Steinberg, 1995). Of these, the acetyl-LDL receptor (Stein and Stein, 1980; Voyta et al., 1984) and CD36 (Swerlick et al., 1992) have already been identified on endothelial cells, suggesting that receptors for oxidized LDL are present on endothelial cells that may play a role in the vascular motility effects of this lipoprotein. In support of this possibility, dextran sulfate, a blocker of the acetyl-LDL receptor, blocked the effect of oxidized LDL to inhibit 5-HT relaxation of porcine coronary artery (Tanner et al., 1991). However, fucoidon and polyinosinic acid, two other blockers of the acetyl-LDL receptor, failed to block the effect of oxidized LDL to inhibit thrombin-mediated relaxation of porcine coronary artery (Sugiyama et al., 1994). Additionally, acetylated LDL failed to mimic the effect of oxidized LDL on thrombin-mediated relaxation in this tissue (Sugiyama et al., 1994). Thus, further study is required before a role for the acetyl LDL receptor and other oxidized LDL binding sites in the vasomotor effects of this lipoprotein can be established.

In summary, there is clearly much to learn about the multiple cellular and molecular mechanisms by which oxidized LDL may exert vascular effects. However, clarification of these mechanisms may provide opportunities to pharmacologically inhibit or reverse these vascular actions. If oxidized LDL is intimately involved in the development of vascular dysfunction in hypercholesterolemia and atherosclerosis, then inhibiting the vasomotor actions of oxidized LDL in vivo may normalize vascular function in these pathologies. There are multiple

lines of evidence to suggest that oxidized LDL is, indeed, involved in atherosclerosis-induced disturbances in vasomotion.

V. Vascular Effects of Oxidized Low-Density Lipoprotein Associated with Hypercholesterolemia and Atherosclerosis

It is now well-accepted that human atherosclerosis and hypercholesterolemia are associated with alterations in vascular reactivity in vivo related to an impairment in endothelium-mediated vasodilator function (Verbeuren, 1991; Harrison, 1994). An important role of oxidized LDL in these alterations in vascular function is suggested by four major lines of evidence (table 1): (*a*) the striking similarity between the nature of the vascular impairment produced by atherosclerosis in vivo and that produced by oxidized LDL in vitro, (*b*) the ability of elevated serum cholesterol to produce altered vascular function independent of the presence of gross atherosclerotic lesions, (*c*) the ability of antioxidants to reverse or inhibit impairment of endothelial function in hypercholesterolemia and atherosclerosis, and (*d*) confirmation of the presence of oxidized LDL in vivo in human blood vessels with atherosclerosis.

A. Vascular Effects of Oxidized Low-Density Lipoprotein Mimic the Vascular Dysfunction of Atherosclerosis and Hypercholesterolemia

Isolated segments of blood vessels from animals and humans with hypercholesterolemia or atherosclerosis exhibit altered vasomotor properties very similar to those produced by oxidized LDL in vitro. For example, aortic rings obtained from cholesterol-fed rabbits showed impaired ability to relax to the endothelium-dependent vasodilators acetylcholine and adenosine diphosphate, whereas relaxation to the endothelium-independent vasodilator nitroglycerin was unaffected except in the most severely lesioned arteries (Habib et al., 1986; Verbeuren et al., 1986). Similar data were reported for isolated coronary arterial segments from hypercholesterolemic and atherosclerotic pigs, where EDR to 5-HT and aggregating platelets was impaired, whereas EDR to the calcium ionophore A23187 and endothelium-independent relaxation to sodium nitroprusside were both preserved (Cohen et al., 1988; Shimokawa and Vanhoutte, 1989). Human coronary arterial segments with atherosclerosis were also selectively impaired in the ability to relax to acetylcholine, whereas endothelial-dependent relaxation to substance P and histamine were only partially affected, and that to A23187 was completely preserved (Bossaller et al., 1987). Thus, blood vessels from animals and humans with hypercholesterolemia or atherosclerosis exhibited a pattern of impaired EDR that closely resembled that produced by oxidized LDL.

TABLE 1
Summary of evidence for the role of oxidized LDL in the vascular dysfunction of atherosclerosis and hypercholesterolemia

Evidence	References
Atherosclerosis-induced vascular dysfunction is mimicked by treatment of blood vessels with oxidized LDL in vitro.	Tanner et al., 1991 Ohgushi et al., 1993 Cohen et al., 1988 Shimokawa and Vanhoutte, 1989
Atherosclerosis-induced vascular dysfunction is closely associated with elevated serum LDL cholesterol levels.	Celermajer et al., 1992 Zeiher et al., 1991a Harrison et al., 1987 Lamping et al., 1994
Antioxidants can normalize vascular functioning in atherosclerosis and hypercholesterolemia.	Stewart-Lee et al., 1994 Keaney et al., 1994 Anderson et al., 1995
LDL can be oxidized in vivo and exists in human atherosclerotic lesions and plasma.	Daugherty et al., 1988 Hoff and O'Neil, 1991 Yla-Herttuala et al., 1989 Cazzolato et al., 1991

Atherosclerotic blood vessels were also more sensitive to certain contractile stimuli (Verbeuren et al., 1986; Shimokawa and Vanhoutte, 1989; Cohen et al., 1988; Lamping et al., 1994; Miwa et al., 1994). Interestingly, the mechanism of the enhanced contractile response in atherosclerotic blood vessels differs among species and parallels the species differences in the mechanism of oxidized LDL's in vitro vasomotor effects. For example, the enhanced contractile response in atherosclerotic rabbit arteries was independent of the endothelium and caused by direct effects on calcium handling in the vascular smooth muscle (Miwa et al., 1994; Stepp and Tulenko, 1994; Cox and Tulenko, 1995). In contrast, enhanced contraction of coronary arteries produced by atherosclerosis in pigs (Shimokawa and Vanhoutte, 1989), monkeys (Lamping et al., 1994), and humans (Golino et al., 1991) was primarily due to the inhibition of EDRF(NO) release from the endothelium and not related to direct effects on the vascular smooth muscle. The fact that oxidized LDL exhibited very similar species-dependent mechanistic differences in its effect on contractile responses (see section II.B.) strongly supports the hypothesis that oxidized LDL is involved directly in the vasomotor disturbances produced by hypercholesterolemia and atherosclerosis in various species.

The alterations in vascular reactivity observed in vitro in isolated blood vessels from atherosclerotic animals and humans appears to be present in vivo, as well. Quantitative angiography has been utilized to measure changes in human coronary blood flow in vivo after intracoronary infusion of various vasoactive mediators. Whereas infusion of acetylcholine resulted in modest vasodilation in patients without atherosclerosis, this treatment caused vasoconstriction in patients with coronary artery disease (Ludmer et al., 1986). Coronary vasodilation in response to nitroglycerin was normal in patients with atherosclerosis (Ludmer et al., 1986), con-

sistent with the in vitro studies on atherosclerotic blood vessels described above. More recently, a similar phenomenon was demonstrated with 5-HT, which caused coronary vasodilation and increased coronary flow in patients with angiographically normal arteries, but induced vasospasm and decreased flow in patients with angiographic evidence of coronary artery disease (Golino et al., 1991) or symptoms of variant or stable angina (McFadden et al., 1991). Impaired vasodilation was also demonstrated in several regions of the peripheral vasculature in patients with atherosclerosis or hypercholesterolemia (Gilligan et al., 1994b; Casino et al., 1995; Arcaro et al., 1995). Therefore, in vitro and in vivo data from both animal and human studies suggest that hypercholesterolemia and atherosclerosis are associated with vascular dysfunction characterized by a selective inhibition of EDR and enhanced contraction to certain agonists, similar to the in vitro vascular effects of oxidized LDL.

There are also striking similarities between some of the mechanisms that are thought to be involved in the inhibition of EDR induced by atherosclerosis and hypercholesterolemia and the mechanisms of oxidized LDL's vasomotor effects. Isolated blood vessels exposed to oxidized LDL (Tanner et al., 1991; Flavahan, 1993) and coronary arteries from atherosclerotic pigs were both selectively deficient in EDR mediated by G_i-protein-coupled agonists (Shimokawa et al., 1991). Similarities are also evident in rabbit models of atherosclerosis where, similar to the effect of lyso PC, superoxide anion production was increased in aortae from hypercholesterolemic rabbits (Ohara et al., 1993; Keaney et al., 1995). In fact, the increased production of vascular superoxide anion induced by hypercholesterolemia positively correlated with increased tissue levels of both lyso PC and indices of oxidized lipids (Keaney et al., 1995), suggesting that oxidized LDL was involved in the increase in superoxide production in vivo. Furthermore, hypercholesterolemia-induced enhancement of superoxide anion production was reversed by either dietary normalization of serum cholesterol levels (Ohara et al., 1995b) or concomitant treatment with the antioxidant cholesterol-lowering drug probucol (Keaney et al., 1995). Taken together, these data suggest that the underlying mechanisms responsible for the vascular dysfunction observed in hypercholesterolemia and atherosclerosis are consistent with some of the effects of oxidized LDL on isolated blood vessels, providing further evidence for a role of this modified lipoprotein in pathological vasomotor disturbances.

B. Atherosclerosis-Induced Vascular Dysfunction Is Related Primarily to Elevated Serum Cholesterol

Several lines of evidence suggest that the vascular dysfunction associated with atherosclerosis is due to increased serum cholesterol levels (and presumedly increased levels of oxidized LDL) rather than the formation and progression of vascular atherosclerotic lesions. Endothelial dysfunction was evident in humans with risk factors for atherosclerosis, including hypercholesterolemia, before the development of visible atherosclerotic lesions (Celermajer et al., 1992; Zeiher et al., 1991a; Creager et al., 1990). Furthermore, hypercholesterolemia in several species including human produced impairment of endothelium-dependent responses in blood vessels of the microcirculation, vascular beds that do not develop overt atherosclerotic lesions (Yamamoto et al., 1988; Sellke et al., 1990; Zeiher et al., 1991b). In fact, impaired endothelial function in atherosclerosis was suggested to be systemic in nature, affecting many regions of the vasculature rather than only those that develop visible signs of atherosclerosis (Anderson et al., 1995b). Thus, vascular dysfunction in atherosclerosis was associated more closely with increased serum cholesterol rather than morphological alterations of the blood vessel.

The effectiveness of lowering serum lipid levels in correcting the vascular dysfunction induced by atherosclerosis also supports a role for oxidized LDL in these alterations. Reduction of serum cholesterol via dietary modification completely restored impaired endothelial function in iliac arteries from Cynomolgus monkeys with diet-induced atherosclerosis, whereas only a modest regression of atherosclerotic plaque was observed (Harrison et al., 1987). Reduction of serum cholesterol also normalized the enhanced coronary vasoconstrictive response of intracoronary infusion of 5-HT in the absence of any regression of morphological evidence of atherosclerosis in monkeys (Lamping et al., 1994). Several clinical studies suggest that lipid-lowering therapy also normalized vascular function in humans with hypercholesterolemia and atherosclerosis. In patients with hypercholesterolemia but free of significant coronary lesions as assessed by angiography, abnormal coronary vasoconstriction in response to intracoronary infusion of acetylcholine was reversed after 6 months of treatment with either a combination of diet modification and cholestyramine (Leung et al., 1993) or pravastatin (Egashira et al., 1994), regimens that decreased total serum cholesterol levels by approximately 30% in both studies. More recently, lipid-lowering therapy normalized coronary vasomotor function in patients with hypercholesterolemia and established coronary arterial lesions (Treasure et al., 1995; Anderson et al., 1995a). Most importantly, normalization of endothelial function by lipid-lowering therapy occurred in the absence of any significant regression in lesion size or extent of intimal thickening. Taken together, these data suggest that vascular endothelial dysfunction is related more to increased serum cholesterol rather than the presence of atherosclerotic lesions.

C. Antioxidants Improve Endothelium-Dependent Vasodilation in Atherosclerosis

The role of increased cholesterol levels in the vascular dysfunction produced by hypercholesterolemia and ath-

erosclerosis has also been linked to an important role of oxidative processes. Treatment of rabbits with the antioxidant compound α-tocopherol (vitamin E) prevented the endothelial dysfunction produced by cholesterol feeding (Stewart-Lee et al., 1994; Keaney et al., 1994). Butylated hydroxytoluene, another antioxidant compound, also prevented alterations in microcirculatory function produced by cholesterol feeding in rabbits (Xiu et al., 1994). Antioxidant use may require a long duration of treatment to show clinical benefit, inasmuch as short-term (4 weeks) antioxidant therapy did not reverse endothelial dysfunction in hypercholesterolemic patients (Gilligan et al., 1994a). Indeed, patients with coronary artery disease treated for 6 months with a combination of lovastatin and the antioxidant lipid-lowering drug probucol showed significantly greater improvement in vasomotor function compared with patients treated with either the nonantioxidant combination of lovastatin and cholestyramine or lipid-lowering diet alone (Anderson et al., 1995a). These data suggest that oxidation is an important contributing factor in the vasomotor disturbances produced in hypercholesterolemia and atherosclerosis, and inhibiting oxidation is beneficial in improving vascular functioning in atherosclerosis. One aspect of the oxidation process may involve oxidation of LDL, the likely active species involved in vascular motility changes produced by hypercholesterolemia and atherosclerosis.

D. Oxidized Low-Density Lipoprotein Exists In Vivo

Whether LDL becomes oxidized in vivo and the extent of this oxidation relative to that produced in vitro via incubation with copper ion or cultured endothelial cells have been difficult to establish. However, recent studies have suggested that oxidized LDL is present in rabbit and human blood vessels and is associated with atherosclerotic lesions. Modified lipoproteins have been extracted from atherosclerotic lesions of rabbit and human blood vessels. These lipoproteins possess the physiochemical properties of LDL that has been oxidized in vitro, including an increased electrophoretic mobility relative to native LDL, extensive fragmentation of the Apo B protein, and an increase in peroxidized lipids (Daugherty et al., 1988; Hoff and O'Neil, 1991; Yla-Herttuala et al., 1989). Furthermore, immunohistochemistry using antibodies raised against epitopes of the modified protein component of oxidized LDL has recognized modified lipoprotein in atherosclerotic blood vessels (Palinski et al., 1989; Haberland et al., 1988). In fact, autoantibodies that recognized oxidized LDL were identified in serum of patients with carotid artery atherosclerosis and were predictive of the rate of progression of this disease (Salonen et al., 1992). These data suggest that LDL oxidation does indeed occur in vivo in the arterial wall, and the endogenous oxidized form of LDL is similar to the oxidized form of LDL that is generated and studied in vitro.

In addition to oxidatively modified LDL in atherosclerotic lesions, subfractionation of human plasma has recently suggested that oxidized LDL may also exist in the circulation. Analysis of total LDL from human plasma by ion exchange high-performance liquid chromatography revealed two major subfractions: unmodified, native LDL and a more negatively charged LDL (Cazzolato et al., 1991; Hodis et al., 1994). The more negatively charged LDL (LDL−) possessed many of the characteristics of LDL that was oxidized in vitro or extracted from atherosclerotic lesions, including increased electrophoretic mobility, an increased content of conjugated dienes, oxycholesterols, and peroxidized lipids, and a decreased content of vitamin E. The fraction of total plasma LDL present in this modified form was relatively small, approximately 5% (Cazzolato et al., 1991; Hodis et al., 1994). However, assuming a plasma LDL concentration of 1–2 mg/ml (Creagor et al., 1990; Gilligan et al., 1994b), this fraction would translate to a plasma concentration of modified LDL of 50–100 μg/ml, consistent with concentrations of oxidized LDL that mediate vasomotor effects in vitro (Kugiyama et al., 1990; Simon et al., 1990; Tanner et al., 1991; Murohara et al., 1994). Although these studies did not clarify whether the circulating LDL− originated from the oxidation of LDL in plasma or within the arterial wall, they suggest that vasoactive concentrations of oxidized LDL may exist in the circulation of humans, and would presumedly be elevated in hypercholesterolemia and atherosclerosis.

The extent to which LDL is oxidized in vivo relative to the levels of oxidation produced in vitro remains uncertain. However, the fact that modified LDL extracted from rabbit and human lesions had extensive fragmentation of the apo-B protein suggests a substantial level of oxidation, as opposed to the lower levels of oxidation reported for minimally modified LDL, where apo-B remains intact (Berliner et al., 1990). Quantitative measures of LDL modification in vivo are limited. Nevertheless, the properties of LDL modified in vivo and LDL oxidized in vitro are qualitatively and quantitatively comparable as measured by several independent approaches, including: (*a*) extent of lipid peroxidation as measured by formation of thiobarbaturic reactive substances (Daugherty et al., 1988), (*b*) increased electrophoretic mobility (Daugherty et al., 1988; Hoff and O'Neil, 1991; Yla-Herttuala et al., 1989), and (*c*) increased fluorescence measured at an excitation wavelength of 360 nm (Hoff and O'Neil, 1991). Thus, the functional effects demonstrated with LDL that has been oxidized in vitro, including the vasoactive effects of this lipoprotein, have relevance to the in vivo situation.

In summary, there is strong evidence that LDL is modified by an oxidative mechanism in humans and may exist in both the circulation and the arterial wall. In these locales, oxidized LDL may exert effects analogous to those demonstrated in isolated blood vessels in vitro, and thus produce the vasomotor disturbances associated

with hypercholesterolemia and atherosclerosis. Therefore, inhibition of the in vivo actions of oxidized LDL would likely improve or normalize vascular functioning in atherosclerosis. However, would such intervention have any benefit in reducing the clinical manifestations of this disease? Recent studies suggest that the direct role of increased plasma cholesterol levels and the resulting vascular dysfunctioning in acute clinical syndromes of atherosclerosis may have been underestimated.

VI. Role of the Vasomotor Actions of Oxidized Low-Density Lipoprotein in the Clinical Manifestations of Atherosclerosis

Atherosclerosis is clearly a chronic, multifactorial disease process with several regulatory pathways influencing its development and progression. Thus, arguments are presented here to support an important role for oxidized LDL and its vasomotor actions in some of the acute clinical syndromes associated with this disease, while recognizing that this mediator may be only one of several important mechanisms involved in these processes.

Contrary to conventional wisdom, the abundance of data suggests that the incidence of clinical events associated with atherosclerosis is not related to the extent of coronary artery stenosis due to plaque formation and expansion into the arterial lumen. Several angiographic studies demonstrated that the majority of patients undergoing examination following incidents of unstable angina or myocardial infarction had lesions with stenosis of less than 50% (Ambrose et al., 1986; Little et al., 1988), too small to be hemodynamically significant. Furthermore, clinical trials of lipid-lowering therapies demonstrated that although lowering serum cholesterol was associated with little or no regression in the extent of stenosis, these treatments nevertheless resulted in a substantial decrease in the frequency of clinical events (Brown et al., 1990; Watts et al., 1992). Taken together, these studies suggest that the occurrence of clinical events associated with atherosclerosis is not rooted in a decrease in lumen diameter due to plaque formation. Furthermore, the beneficial effects of lowering serum cholesterol cannot be explained by a regression of plaque size or change in the extent of stenosis.

To explain this lack of correlation between clinical events and extent of stenosis, current theory suggests that progression of atherosclerosis from a chronic progressive disease to an acute clinical event such as unstable angina or myocardial infarction involves the rupture of smaller, hemodynamically insignificant plaque (Fuster et al., 1990). Release of the contents of the plaque into the bloodstream results in platelet activation, thrombus formation, and eventual arterial occlusion. The vasomotor disturbances produced by elevated serum cholesterol, and possibly increased levels of oxidized LDL in the circulation or arterial wall, may contribute to the generation of clinical events in patients with atherosclerosis via at least two mechanisms: (*a*) promoting conditions that are favorable for plaque rupture and (*b*) aggravating the arterial response to events subsequent to the rupture.

Oxidized LDL in atherosclerosis may promote plaque rupture both directly and indirectly. Although debated (Kaski et al., 1992), intense vasospasm at the locus of an atherosclerotic plaque has the potential to induce plaque rupture via compression of the lesion against the arterial wall (Leary, 1934; Lin et al., 1988; Ciampricotti et al., 1990). The ability of oxidized LDL to increase the sensitivity of blood vessels to contractile stimuli may promote this mechanism of plaque rupture. In addition, impaired endothelial function produced by oxidized LDL in atherosclerosis may increase hemodynamic shear forces in the coronary circulation, an effect that has also been suggested to weaken plaque stability and promote rupture (Loree et al., 1992). Thus, the vasomotor effects of oxidized LDL may contribute to an environment that increases the likelihood of plaque rupture.

The presence of oxidized LDL within atherosclerotic coronary arteries may also exacerbate the contractile response of these vessels to mediators released from aggregating platelets subsequent to plaque rupture. Indeed, endothelium-dependent relaxation to aggregating platelets was profoundly impaired in atherosclerotic pig coronary arteries (Shimokawa and Vanhoutte, 1989) and in normal arteries incubated with oxidized LDL (Tanner et al., 1991). Oxidized LDL also impaired endothelium-dependent relaxation of pig coronary arteries to platelet-derived mediators such as 5-HT and thrombin (Simon et al., 1990; Tanner et al., 1991). Thus, platelet aggregation in the presence of a dysfunctional endothelium as occurs in atherosclerotic blood vessels may be aggravated by oxidized LDL, resulting in vasospasm, myocardial ischemia and, depending upon the severity of the thrombus, acute myocardial infarction and death.

Oxidized LDL also may be involved in clinical syndromes associated with atherosclerosis that do not necessarily involve plaque rupture. Myocardial ischemia can be triggered in patients with stable angina via a variety of stimuli, including exercise and cold-exposure (Zeiher and Schachinger, 1994). Whereas these stimuli produced vasodilation in normal coronary arteries, both caused constriction of coronary arteries with atherosclerosis due to a loss of normal endothelium-dependent vasodilator function (Gage et al., 1986; Nabel et al., 1988). Furthermore, atherosclerotic coronary arteries were much more sensitive to constriction by infusion of catecholamines (Vita et al., 1992). Thus, loss of normal endothelium-dependent relaxation due, in part, to oxidized LDL in diseased coronary blood vessels may amplify vasoconstriction in response to sympathetic activation and lead to myocardial ischemia associated with stable angina without necessarily involving rupture of atherosclerotic plaque.

VII. Inhibiting the Vasoactive Effects of Oxidized Low-Density Lipoprotein: Potential Pharmacological Strategies and Therapeutic Implications

The mechanism by which lowering serum cholesterol decreases the frequency of clinical manifestations of atherosclerosis in the absence of any substantial change in lesion size or stenosis is not clear (Levine et al., 1995). Although many components of serum cholesterol may be responsible for its detrimental effects, oxidized LDL is likely to play an important role based on the arguments presented above. Lipid lowering may alter the characteristics of atherosclerotic lesions such that they are more resistant to rupture and may improve endothelium function and normalize vasoactivity to account for the clinical benefit. Because much evidence suggests that oxidized LDL is a primary determinant in the vascular dysfunction of atherosclerosis and an important factor in lesion formation and progression (Steinberg et al., 1989), it is reasonable to hypothesize that interventions that either decrease the formation of oxidized LDL, increase its degradation, and/or inhibit its action on target cells will be beneficial in the treatment of this disease (fig. 4).

A. Inhibiting the Formation of Oxidized Low-Density Lipoprotein

Thus far, pharmacological efforts to decrease the formation of oxidized LDL have been limited to the use of antioxidant compounds. Although probucol is used clinically to lower serum cholesterol (Buckley et al., 1989), this compound is also an antioxidant that inhibits the oxidation of LDL in vitro and ex vivo (Parthasarathy et al., 1986). The ability of probucol to slow the progression of atherosclerosis in rabbits (Carew et al., 1987) and monkeys (Sasahara et al., 1994) was at least partially due to its antioxidant activity and independent of its lipid-lowering effects. In fact, some antioxidant compounds slowed the progression of atherosclerosis in the absence of a substantial change in serum lipid levels (Sparrow et al., 1992), consistent with the possibility that decreased oxidized LDL was important in the beneficial effects observed. Indeed, intake of the antioxidant vitamin α-tocopherol increased the resistance of LDL to ex vivo oxidation in humans (Jialal et al., 1995; Princen et al., 1995). However, as discussed above, data on the effects of antioxidants on vascular functioning in atherosclerosis are limited. Although the predicted benefit of antioxidant vitamins in slowing the progression of atherosclerosis has not yet been proven in humans, a significant inverse correlation between serum levels of natural antioxidant vitamins and the frequency of occurrence of cardiovascular disease has been documented (Gey et al., 1991). Additionally, several limited prospective trials have associated intake of antioxidant vitamins with a decrease in clinical events associated with atherosclerosis (Stampfer et al., 1993; Rimm et al., 1993). Because of these preliminary encouraging results with antioxidant vitamins, the search for more potent and safe antioxidant compounds with oral activity is currently underway (Breugnot et al., 1994).

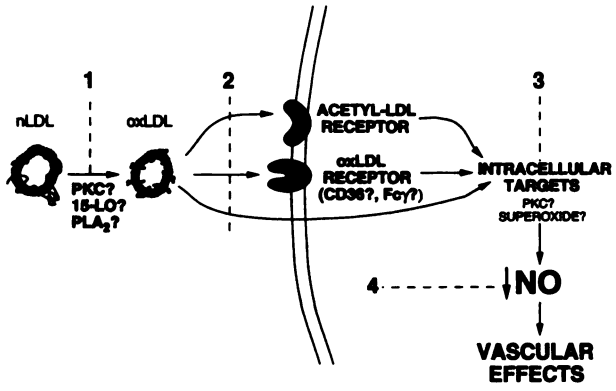

FIG. 4. Potential targets for pharmacological intervention to inhibit or reverse oxidized LDL-induced vascular dysfunction: (*a*) inhibition of oxidized LDL formation, (*b*) inhibition of oxidized LDL interaction with target cells, (*c*) inhibition of intracellular actions of oxidized LDL, (*d*) correction of oxidized LDL-induced EDRF(NO) deficit via augmentation of endogenous synthesis or exogenous supplementation with NO donors. nLDL, native LDL; oxLDL, oxidized LDL; PKC, protein kinase C; 15-LO, 15-lipoxygenase; PLA_2, phospholipase A_2.

Our current understanding of the mechanisms of LDL oxidation in vivo is limited. Elucidation of these mechanisms may reveal novel and perhaps more effective strategies for inhibiting the in vivo formation of oxidized LDL. For example, several recent studies suggested important roles for 15-lipoxygenase (Parthasarathy et al., 1989) and PKC (Li and Cathcart, 1994) in cell-mediated LDL oxidation. In addition, LDL was suggested to possess an intrinsic PLA_2 activity responsible for the extensive conversion of phosphatidylcholine to lyso PC upon oxidation and, indeed, PLA_2 inhibitors attenuated LDL oxidation (Parthasarathy et al., 1985). If similar processes participate in the oxidation of LDL in vivo, then selective inhibitors of these enzymes may inhibit the in vivo oxidation of LDL, and could be of potential benefit in correcting atherosclerosis-induced vascular dysfunction.

B. Inhibiting the Interaction of Oxidized Low-Density Lipoprotein with Target Cells

In addition to inhibiting the in vivo formation of oxidized LDL, blocking its interaction with vascular target cells may also be an effective strategy in reversing atherosclerosis-induced vascular dysfunction. If the vasoactive effects of oxidized LDL were confirmed to be a direct consequence of its interaction with a specific receptor, then this would provide the opportunity for the development of an oxidized LDL "antagonist" with which to block cellular effects of this lipoprotein. However, the nature of oxidized LDL's interaction with target cells is currently unclear. Although several oxidized LDL binding proteins have been described (Endemann et al.,

1993; Stanton et al., 1992; Ottnad et al., 1995), their role, if any, in the vascular effects of oxidized LDL is unknown. Thus, understanding the consequences of the binding of oxidized LDL to these sites on cellular function and biochemistry is clearly an important area for future research and pharmacological intervention.

C. Inhibiting the Cellular Actions of Oxidized Low-Density Lipoprotein

An alternative approach to blockade of oxidized LDL vascular receptors may be to target the cellular pathways by which oxidized LDL mediates its effects. For example, lyso PC has been implicated as one of the components of oxidized LDL with vascular activity, and PKC has been suggested to be an important intracellular target in the actions of both oxidized LDL and lyso PC. The ability of PKC inhibitors to reverse impairment of endothelium-dependent relaxation by oxidized LDL (Ohgushi et al., 1993) suggests that selective inhibition of this intracellular signaling pathway may be beneficial in correcting atherosclerosis-induced vascular dysfunction. However, to date, selective inhibitors of PKC have not been examined for their ability to improve vascular function in hypercholesterolemia or atherosclerosis in vivo. Furthermore, PKC is a heterogeneous family of enzymes that regulates a variety of cellular functions (Parker et al., 1989), and additional research will be required to understand which isoform(s) may potentially regulate the EDRF(NO) pathway. Undesirable physiological effects due to the nonselective nature of currently available PKC inhibitors has hindered the clinical exploration of this strategy to reverse atherosclerosis-induced vascular dysfunction. Therefore, the development and utilization of isoform-selective PKC inhibitors would provide a deeper understanding of the role of PKC in modulating vascular function in atherosclerosis and allow a better evaluation of the clinical utility of these inhibitors in normalizing vascular functioning in this disease.

Oxidized LDL has been shown to affect several other second-messenger pathways in addition to PKC, including phosphatidylinositol hydrolysis (Hamilton et al., 1994) and phospholipase D (Natarajan et al., 1993). Development of selective inhibitors of these pathways will also be important in elucidating their role in the inhibition of endothelial function produced by oxidized LDL.

A role for increased superoxide anion production in the vasomotor actions of oxidized LDL may provide another site of pharmacological intervention to improve vascular function in atherosclerosis. Indeed, treatment of rabbits in vivo with polyethylene-glycolated SOD partially prevented the impairment of EDR produced by atherosclerosis (Mugge et al., 1991). Furthermore, a pharmacological SOD mimetic, SC52608, enhanced NO-mediated vascular relaxation in vitro by increasing the biological half-life of NO (Kasten et al., 1995). Thus, pharmacological scavengers of superoxide anion may potentiate the activity of endogenous NO, inhibit the vasomotor actions of oxidized LDL, and normalize vascular function in atherosclerosis.

D. Augmentation of Endogenous Nitric Oxide Release or Exogenous Replacement of Nitric Oxide

Because the bulk of the data suggest that oxidized LDL and atherosclerosis cause vascular dysfunction via an effect on the EDRF(NO) pathway, another potential strategy to normalize vascular function in atherosclerosis may be either to augment endogenous NO release or to deliver exogenous NO via orally active NO donors. In support of this concept, treatment with the NO precurser L-arginine normalized hypercholesterolemia-induced endothelial dysfunction in both rabbits (Rossitch et al., 1991) and humans (Creager et al., 1992). Furthermore, pentaerythrityl-tetranitrate, an organic nitrovasodilator, also prevented impairment of EDR in cholesterol-fed rabbits (Kojda et al., 1995). However, chronic treatment of rabbits with organic nitrovasodilators (Kojda et al., 1995) and direct NO donors such as molsidomine (Bult et al., 1995), also resulted in a desensitization of EDR due to down-regulation of the EDRF(NO) pathway, an effect that could actually aggravate rather than alleviate atherosclerosis-induced vascular dysfunction. Thus, the utility of NO donors in normalizing pathological vasomotion caused by oxidized LDL requires further study.

In summary, there are several conceptual strategies related to drug development that may prevent or inhibit oxidized LDL-induced endothelial dysfunction and may be beneficial in normalizing vascular function in atherosclerosis. Inhibiting the formation and action of oxidized LDL may have additional benefit beyond normalization of vascular function, including slowing the progression of lesion formation and perhaps decreasing the lipid and foam-cell content of the lesion, thereby decreasing chances of plaque rupture. However, further research into the basic mechanism of oxidized LDL's vasoactive effects is required before we can more fully evaluate the validity of these various strategies as well as the impact of these interventions on the occurrence of clinical manifestations of atherosclerosis.

VIII. Summary and Conclusions

Oxidized LDL exerts profound effects on the vasomotor response of isolated blood vessels to various stimuli that closely mimic the vascular dysfunction associated with hypercholesterolemia and atherosclerosis in humans. The beneficial effect of lipid-lowering therapy in normalizing vascular function and greatly decreasing the frequency of clinical events associated with atherosclerosis, combined with the ability of antioxidants to alleviate vasomotor disturbances in hypercholesterolemia and slow the progression of atherosclerosis, strongly support a causative role of oxidized LDL in mediating vascular dysfunction in vivo and contributing

to the clinical sequalae of coronary artery disease. Further research to understand more fully the mechanisms of oxidized LDL formation and actions in vivo may reveal novel strategies to inhibit these events, and may prove beneficial in the therapeutic management of atherosclerotic disease.

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