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COMMENTARY

Oxidized Low-Density Lipoprotein, a Two-Faced Janus in Coronary Artery Disease?

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ABSTRACT. The word antioxidant has become a household term, and every day we are bombarded with claims of antioxidant protection against a host of diseases. Atherosclerosis, cancer, gastric ulcers, memory loss, rheumatoid arthritis, endometriosis, pregnancy complications, hypertension, stroke, and a host of other diseases have been suggested to be induced by oxidative stress, and antioxidants have been suggested to be beneficial in the prevention and treatment of these disorders. While some of these may be exuberant claims, atherosclerosis is one disease in which the oxidation hypothesis has taken firm roots. The oxidation of low-density lipoprotein (LDL) has been suggested to be a key step in the initiation of the early atherosclerotic lesion. A number of proatherogenic effects have been described for both the protein and lipid components of oxidized low-density lipoprotein. In this commentary, a brief description of the involvement of oxidation and the potential for antioxidant treatment for cardiovascular disease will be provided. However, there are innumerable questions plaguing the hypothesis; this commentary, therefore, will also serve as a devil's advocate and propose that some form of oxidation might actually be beneficial. BIOCHEM PHARMACOL **56**;3:279–284, 1998. © 1998 Elsevier Science Inc.

KEY WORDS. oxidation; antioxidant; low density lipoprotein; atherosclerosis; autoantibody; biological effects

Why is oxidized LDL† implicated in atherosclerosis? Plasma cholesterol, particularly that associated with LDL, has been suggested to be an important risk factor in the development of coronary artery disease [1]. The cholesterol that accumulates in the atherosclerotic plaque is localized as cytoplasmic lipid droplets in macrophages and is derived predominantly from plasma LDL, which is internalized by cells usually via the LDL receptor [1]. However, animals and humans that lack the LDL receptor develop severe forms of atherosclerotic lesions, and *in vitro* incubations of macrophages with LDL fail to develop lipid-engorged foam cells [1]. These observations prompted Brown *et al.* [2] to propose the modified LDL hypothesis, which, simply stated, suggests that LDL has to undergo some type of modification before it can be internalized by macrophages via alternate receptors. These alternate pathways are commonly referred to as scavenger pathways, and a number of scavenger receptors have now been identified [3–6].

PROATHEROGENIC ROLE OF LDL

A plethora of studies have suggested that oxidized LDL may be one such modified lipoprotein. Early studies by Steinberg and associates showed that LDL incubated with endothelial cells or other cell types is internalized avidly by macrophages, in contrast to LDL not previously exposed to cells [7–9]. They demonstrated that during this incubation large amounts of decomposition products of lipid peroxidation are generated, and antioxidants such as vitamin E, butylated hydroxytoluene, and probucol inhibit such generation of oxidized LDL [8–10]. Other investigators also showed that such modified LDL could be generated by *in vitro* oxidation of LDL using a number of different oxidation systems [9]. Based on these observations, it was suggested that oxidized LDL might represent a biologically relevant modified lipoprotein. The proatherogenic properties of oxidized LDL and the biologically active components are listed in Table 1.

Oxidation is not the only modification that generates an LDL that is avidly degraded by macrophages; LDL that has been exposed to certain enzymes such as phospholipase C, elastase, lipoxygenases, and peroxidases, as well as LDL that is subjected to aggregation, are also degraded by macrophages, resulting in foam cell formation [9].

Although the initial studies focused on the ability of oxidatively modified LDL to be taken up by macrophages, there are substantial reasons to believe that the oxidized LDL may have other proatherogenic properties. At present, over 30 different proatherogenic effects have been ascribed to oxidized LDL [9, 11].

Oxidation of LDL involves a number of chemical and physical changes on the lipoproteins. Conceptually, different stages of oxidation could be envisioned, and an early oxidation product, designated as mm-LDL or minimally modified LDL, has gained enormous attention due to the pioneer-

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† *Abbreviations:* LDL, low density lipoprotein; and WHHL, Watanabe heritable hyperlipidemic.

TABLE 1. Components of oxidized LDL and associated proatherogenic effects*

Component	Effect
A. Protein component of oxidized LDL	1. Increases uptake by macrophages via the scavenger receptor. 2. Immunogenic effects generating autoantibodies. 3. Induces interleukin-1 synthesis and secretion by foam cell macrophages.
B. Lyso-phosphatidylcholine	1. Inhibits/activates nitric oxide synthesis. 2. Chemotactic to monocytes. 3. Chemotactic to T-lymphocytes. 4. Induces/inhibits endothelial cell migration. 5. Induces PDGF synthesis. 6. Induces the synthesis of FGF. 7. Increases transport of macromolecules. 8. Induces the synthesis and expression of leukocyte adhesion molecules. 9. Induces smooth muscle cell proliferation.
C. Oxidatively tailored phospholipids	1. Affect PAF-mediated effects. 2. Induce the synthesis of MCP-1.
D. Oxidized fatty acid products	1. Induce the synthesis and expression of adhesion molecules. 2. Induce the synthesis and secretion of interleukin-1. 3. Induce cell-signaling events such as phospholipases and protein phosphorylation.
E. Aldehydes	1. Modify amino acids. 2. Inactivate enzymes. 3. May generate antigenic epitopes. 4. Induce the synthesis and secretion of interleukin-1.
F. Cholesterol oxidation products	1. Induce endothelial cell cytotoxicity.
G. Oxidized LDL	(In addition to effects listed above) 1. Inhibition of smooth muscle cell chemotaxis. 2. Induction of monocyte differentiation. 3. Stimulation of platelet aggregation. 4. Stimulation/inhibition of prostaglandin production. 5. Increase in smooth muscle cell proliferation. 6. Induces apoptosis in cultured cells. 7. Induces or affects the synthesis and secretion of interleukins, heat shock proteins, and other cytokines.

*Abbreviations: PDGF, platelet-derived growth factor; FGF, fibroblast growth factor; PAF, platelet activating factor; and MCP-1, monocyte chemotactic protein-1.

ing studies of Fogelman, Berliner, Navab, and associates [12]. They demonstrated potent biological properties associated with mm-LDL, predominantly attributable to peroxidized phospholipids [13]. It is due to their efforts that intracellular signaling effects involving the transcription factor NFκB came into focus in relation to specific gene activation [11].

However, oxidized LDL is not a homogeneous chemical entity. The single polypeptide that constitutes LDL, namely apoprotein B_{100} of over 4000 amino acids, is heavily damaged during the oxidative process [14, 15]. It is oxidatively proteolyzed into a number of yet undefined fragments. Some of them may be cross-linked by products of oxidation as well as by specific amino acid oxidation [15]. In addition, there are changes that involve the oxidation of specific amino acids; for example, some of the histidines are oxidized to ascorbic acid residues [15]. More important and well-studied changes include derivatization of specific amino acids, such as lysines and histidines, by products of lipid peroxidation [14, 15]. Some or all of these changes in proteins could be antigenic and could be responsible for the generation of autoantibodies *in vivo*. In addition to these modifications, Fruebis, *et al.* [16] have described a direct modification of the apoprotein B_{100} by lipid peroxides.

EVIDENCE FOR OXIDIZED LDL FORMATION *IN VIVO*

Many of the early studies on oxidized LDL focused on the demonstration of the presence of such LDL *in vivo*. A major contribution to this effort came from the studies of Witztum, Palinski, Parthasarathy and associates, who developed antibodies to proteins that are modified by products of lipid peroxidation [17–19]. Using these antibodies, they demonstrated the presence of antigenic epitopes that resembled *in vitro* oxidized LDL or LDL that is modified by products of lipid peroxidation *in vitro* in the macrophage-rich atherosclerotic lesions. They also demonstrated that LDL carefully extracted from the atherosclerotic lesion resembled LDL that was oxidized *in vitro*. Witztum and coworkers [17, 20, 21] also demonstrated the presence of autoantibodies to oxidized LDL, which suggests that antigenic epitopes that are present in oxidized LDL must be generated *in vivo*. While polyclonal antibodies to oxidized LDL have been

TABLE 2. Antigenic determinants and antibodies to oxidized LDL

Epitope	Antibodies
1. Aldehyde modification of lysine residues	1. Malondialdehyde-lysine. 2. 4-Hydroxynonenal-lysine. 3. Acrolein-lysine.
2. Lipid peroxide-modified lysine	1. Lysine modified by linoleic acid hydroperoxide.
3. Oxidatively modified lipids	1. Oxidatively tailored phospholipids. 2. Phosphatidylethanolamine modified internally by peroxidized fatty acid.
4. Carbonyl groups	1. Antibody against 2,4-dinitrophenylhydrazine-derivatized carbonyl groups.
5. Miscellaneous unidentified epitopes.	1. A number of polyclonal antibodies recognizing epitopes presented above and others.

available for a long time, Witztum and colleagues [19] have described a number of monoclonal antibodies that detect not only specific changes in apoprotein as a result of interaction with aldehyde products of lipid peroxidation, but also specific oxidatively modified phospholipids. Some of the antibodies that are available and that detect epitopes suggested to be present in oxidized LDL are shown in Table 2.

A critical piece of evidence in demonstrating that oxidation may play a major role in *in vivo* came from the studies of Carew and coworkers. They demonstrated that probucol, a powerful antioxidant that was already in use as a hypocholesterol agent, inhibited the development of atherosclerotic lesions in WHHL rabbits [22, 23]. More importantly, they showed that the degradation of LDL by arterial macrophages was inhibited by probucol, and this was taken as evidence to suggest that probucol, acting as an antioxidant, inhibited the oxidation of LDL that is required for uptake by macrophages [23]. These very early animal studies are now supported by a plethora of more recent studies that have used several species of animals such as cholesterol-fed rabbits, WHHL rabbits, apoprotein-E knockout mice, hamsters, monkeys, and quail (Table 3). However, one has to exercise caution in the overinterpretation of these studies. There have been reports of failure of antioxidants to retard the progression of experimental atherosclerosis in the literature. More importantly, studies in which antioxidants failed to retard or even exacerbated the severity of atherosclerosis were underreported or not reported at all. In contrast to these animal studies, antioxidant trials in humans are limited. However, there is evidence to suggest that supplementation with antioxidants such as vitamin E may retard the progression of atherosclerosis even in human subjects.

PRESENCE OF OXIDIZED LDL IN THE PLASMA

One of the puzzling questions is whether oxidized LDL circulates in the plasma and whether one could correlate its presence to the disease process. Evidence for and against the presence of oxidized LDL in the plasma exists in the literature. Three different approaches were taken to suggest the presence of oxidized LDL in the plasma: 1) studies that

TABLE 3. Animal studies on antioxidants and atherosclerosis*

Antioxidant	Animal model	Effects
Vitamin E	1. Cholesterol-fed rabbits 2. WHHL rabbits 3. Hypercholesterolemic hamsters 4. Cholesterol-fed guinea pigs 5. Primates 6. Hyperlipidemic chicken 7. Cholesterol-fed rats 8. Japanese quail	1. Decreased atherosclerotic lesions. 2. Increased resistance of isolated LDL to oxidation. 3. Preservation of endothelial functions. 4. Lipid lowering.
Probucol	1. Cholesterol-fed rabbits 2. WHHL rabbits 3. Cholesterol-fed monkeys 4. Hypercholesterolemic rats 5. Modified WHHL rabbits 6. Hypercholesterolemic Japanese quail	1. Decreased atherosclerotic lesions. 2. Increased resistance of isolated LDL to oxidation.
Butylated hydroxytoluene	1. Cholesterol-fed rabbits	1. Decreased lesions.
Diphenylphenylenediamine	1. Cholesterol-fed rabbits 2. Apoprotein-E knockout mice	1. Decreased lesion size.

*In addition to these studies, vitamin C, pantothene, and chemical analogs of various known antioxidants have been tested in various animal models.

actually tried to isolate and establish the presence of oxidized LDL in the plasma based on the physico-chemical nature [24–26] of *in vitro* oxidized LDL; 2) studies that used immunological and biochemical approaches [19, 27–29] to demonstrate the presence of epitopes that are represented in oxidized LDL; and 3) studies that indirectly suggested the presence of oxidized LDL or an oxidative process in the plasma [30–32].

Those who strongly believe that oxidized LDL may not exist in the plasma provide two strong lines of evidence in support of their thesis: 1) the presence of even minute amounts of serum completely prevents the oxidation of LDL *in vitro*; and 2) oxidized LDL is cleared very rapidly (within minutes) from circulation by the liver [33].

Strong experimental evidence, at least suggesting oxidation reactions relevant to the progression of atherosclerotic lesions, came from the studies of Fruebis *et al.* [34]. They demonstrated that LDL samples isolated from animals fed a diet containing the powerful antioxidant probucol or an analog of probucol were highly resistant to *in vitro* oxidation. However, probucol, which was readily available at the site of lesion formation, greatly decreased the lesion size, whereas the analog that was not sufficiently present in the lesion site was ineffective in preventing atherosclerosis. These results suggested that oxidation of LDL in the subendothelial space is more important in the context of the disease. These results also suggested that *ex vivo* oxidation of LDL provides no clue as to the disease process or the efficacy of antioxidants to prevent the disease process. Other evidence in support of oxidation in the plasma comes predominantly from the following observations: Avogaro *et al.* [25, 35] have detected and isolated LDL particles with increased negative charge from isolated plasma LDL and have shown them to be somewhat representative of mildly oxidized LDL. While very few studies have confirmed these findings, Shimano *et al.* [36] and Krauss and coworkers [37, 38] have isolated subfractions of LDL that have increased propensity to undergo *in vitro* oxidations. The studies of Krauss and associates are particularly exciting, as they demonstrate that small, dense LDL particles, which represent the LDL phenotype in coronary artery disease-prone subjects, are oxidized faster than large, buoyant LDL [37].

One may wonder whether LDL particles bearing the antigenic epitopes that are present in oxidized LDL have been detected in plasma LDL. Very few, if any, studies have used these antibodies to detect plasma oxidized LDL. Studies by Collen and coworkers [39] have detected the presence of malondialdehyde-modified LDL by western blot techniques in the plasma of unstable angina patients. However, it is likely that in these subjects malondialdehyde was derived from aggregating platelets rather than from the oxidation of LDL lipids.

Studies by Salonen *et al.* [20] demonstrated that the titer of the autoantibodies to oxidized LDL highly correlated with the disease process, suggesting novel means of correlation of the disease process with oxidation markers. Palinski *et al.* [40] immunized rabbits against oxidized LDL and raised the titer of these antibodies in experimental animals.

TABLE 4. Evidence for the involvement of cells in the oxidation of LDL

1. LDL samples incubated with cells are modified more extensively than LDL incubated in the absence of cells. Various cell types such as activated monocytes, U937 cells, HL60 cells, rat mesangial cells, macrophage-derived foam cells, endothelial cells, smooth muscle cells, endocardial cells, and macrophages have been shown to modify LDL.
2. Cell-conditioned media alone could not sustain oxidation when incubated subsequently with LDL. Studies by Heinecke *et al.* [41] showed that the continuous presence of cells is essential in the oxidation of lipoprotein. If lipoproteins were removed from the cells and the incubation was continued in their absence, the oxidation did not proceed to completion and was lower than in the undisturbed incubations continued for the same duration.
3. Studies by Parthasarathy *et al.* [42] also showed that LDL separated from cells by a dialysis membrane was not oxidized.

To their surprise, when they followed the extent of atherosclerotic lesions, they observed that animals with high titers of antibodies against oxidized LDL were more protected against atherosclerosis. They interpreted these findings to indicate that an antigen–antibody complex in plasma might be cleared by an immune mechanism(s), thus affording protection against oxidized LDL-induced changes in the atherosclerotic artery.

HOW IS OXIDIZED LDL GENERATED *IN VIVO*?

An understanding of the oxidation process and the molecular mechanism(s) involved would have the following advantages. First, such an understanding might lead to the development of more specific inhibitors that would not interfere with physiological oxidations. Second, we can begin to understand the factors leading to the cellular malfunction of the oxidative pathway and can then devise preventive strategies. And, third, site-specific inhibitor therapy can be initiated if abnormalities of metabolic functions are confined, for instance, to just the artery.

The mechanism(s) by which cells may oxidize LDL has been one of the most discussed topics in the study of modified lipoproteins. Numerous mechanisms have been proposed for the oxidation of LDL by cells. Evidence presented in Table 4 would suggest that cells do play a role in the oxidation of LDL or other lipoproteins.

The cell type that may be responsible for the initiation of the oxidation of LDL in the artery remains a matter of conjecture. If the endothelial cells are the important cell type involved in the oxidative process, then the intactness of the endothelium, the predisposition of certain areas of the artery to the development of the lesion, the role of hemodynamic factors, the effects of plasma components, and a host of other factors should be taken into consideration. If monocytes/macrophages are the predominant cell types involved in the oxidation, then the specific nature of these cells, the oxidative enzymes that are present and that can be induced in these cells, and, above all, the constitu-

TABLE 5. Role of cells in the oxidation of LDL

1. Cells may play an active role in the oxidation of LDL. Cells may generate reactive oxygen species or other oxidants that initiate the oxidation of LDL in the presence of metal ions or other reactions that may have peroxidase activity. These may include superoxide radicals, hydrogen peroxide, lipid peroxides, and nitric oxide. The latter may combine with superoxide radicals to generate peroxynitrite, a potent oxidant.
2. Cells may also play a passive role. Cells do not themselves participate in the oxidative process but instead provide substrates that generate extracellular oxidants. This may result from the uptake of oxidized thiols such as cystine followed by its release as cysteine or glutathione, which in turn may generate a variety of oxidants in the presence of redox metals. The specific radicals that are generated in these reactions that may participate in the oxidation of LDL have not been characterized.
3. Cells, in addition to providing the pro-oxidant environment, also participate in the propagation of lipid peroxidation in the medium. Cells may contribute to this process by peroxidase reactions. Extracellular heme and secreted cellular peroxidases may participate in these reactions. The question of whether heme or damaged red blood cells abound in early atherosclerotic lesions has not been addressed satisfactorily. For such reactions to occur, the generation of H_2O_2 or lipid hydroperoxide in the medium is essential.
4. Cells possibly deplete the antioxidants from the lipoprotein, thus increasing the spontaneous rate of lipoprotein oxidation.

ents of the subendothelial milieu should be considered. If these cells play a dominant role in the oxidation of LDL, then one has to also consider the redox factors that lead to their recruitment in the artery wall. Table 5 describes the ways by which cells may contribute to the oxidation of LDL.

There is evidence in the literature for all of the proposed mechanisms. The oxidation of LDL by cells is complex, and a number of issues have to be resolved. The use of freshly isolated lipoproteins, better analytical determination that can measure extremely low levels of peroxides, and "metal-free" media may shed more light on this process. On the other hand, there may be more than one mechanism by which oxidized LDL may be generated *in vivo*.

If oxidized LDL is atherogenic and if oxidation products derived from polyunsaturated fatty acids are deleterious to cardiovascular health, we face a puzzling paradox. Studies from Rudel and others [43, 44] have shown conclusively, at least in experimental animals, that polyunsaturated fat is beneficial as compared with monounsaturated fatty acids or saturated fat. How can fat that has increased propensity to undergo oxidation be beneficial as compared with oxidation-resistant fat? Similarly, the beneficial effects of moderate exercise in preventing cardiovascular disease [45] are so well known that sedentary life style is deemed a risk factor for heart disease. Exercise increases oxygen consumption, depletes antioxidants, and may increase the susceptibility of isolated LDL to *in vitro* oxidation [46–48]. Do the beneficial effects of exercise outweigh its oxidative effects, or do exercisers need increased antioxidant protection? Is it possible that the beneficial effects of polyunsaturated fatty acids and exercise themselves are derived from the intrinsic vulnerability to oxidative stress? Many laboratories are currently addressing these questions, but it will be years before our understanding of the oxidative hypothesis will permit consideration of antioxidants as a viable therapy against heart disease.

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