Published in final edited form as:
Methods Mol Biol. 2010 ; 610: 403–417. doi:10.1007/978-1-60327-029-8_24.

Oxidized Low-Density Lipoprotein

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Abstract

Oxidized low-density lipoprotein (Ox-LDL) has been studied for over 25 years. Numerous pro- and anti-atherogenic properties have been attributed to Ox-LDL. Yet, Ox-LDL has neither been defined nor characterized, as its components and composition change depending on its source, method of preparation, storage, and use. It contains unoxidized and oxidized fatty acid derivatives both in the ester and free forms, their decomposition products, cholesterol and its oxidized products, proteins with oxidized amino acids and cross-links, and polypeptides with varying extents of covalent modification with lipid oxidation products, and many others. It seems to exist in vivo in some form not yet fully characterized. Until its pathophysiological significance, and how it is generated in vivo are determined, the nature of its true identity will be only of classical interest. In this review, its components, their biological actions and methods of preparation will be discussed.

Keywords

Atherosclerosis; oxidative stress; lipid peroxides; antioxidants; aldehydes

1. Introduction

The concept that oxidative stress and the oxidation of low-density lipoprotein (LDL) might play a role in atherosclerosis originated over 25 years ago (1–3). The concept originated from a simple observation that in vitro incubation of macrophages with oxidized LDL and not with native LDL led to cholesterol ester accumulation (2, 4). The oxidation of LDL is a complex process during which both the protein and the lipids undergo oxidative changes and form complex products. For Example, non-enzymatic oxidative changes in amino acids as well as proteolysis and cross-links of apoprotein B (apo B) occur that result in extensive alteration in the protein composition and structure (5). In addition, the peroxidized lipids decompose generating both free and core aldehydes and ketones that covalently modify ε-amino groups of lysine residues of the protein moiety (6). The latter not only generates Schiff's bases, thus modifying charges on the amino acids, but also results in both intra- and intermolecular cross-links between proteolyzed apo B. No wonder the LDL that is damaged to such an extent is avidly scavenged and degraded by macrophages. In fact, the oxidative modification is not unique to LDL as other lipoproteins such as very low-density lipoprotein (VLDL), beta very low-density lipoprotein (β-VLDL), and even high-density lipoprotein (HDL) have been suggested to undergo similar oxidative changes (7-18) with accompanying changes in their pro- or anti-atherosclerotic behavior (19-23).

What is Ox-LDL? The definition depends on the purpose of the question. The original concept defined Ox-LDL as “oxidatively modified LDL” that contained protein components that were “modified” presumably by aldehyde products creating net negative charges that were essential for its interaction and uptake by macrophages (3, 24). However, during the past 25 years myriads of protein-independent pro-atherogenic effects have been attributed to

the lipid components of oxidized LDL that one has to redefine oxidized LDL. In order to define oxidized LDL, one needs to know what components are usually associated with it.

Table 24.1 is a list of products that are generated during in vitro oxidation of LDL.

The formation of all of the above products or the changes in the properties of circulating LDL are not guaranteed during the oxidation of LDL as many are secondary products of oxidation and their formation might depend on the type of oxidant, the extent of oxidation, and the presence or absence of other agents such as redox metals. Also, some products, such as malondialdehyde, readily diffuse out of Ox-LDL. More importantly, the fatty acid profile plays a major role in the formation of many of the products and of Ox-LDL (25-27). Generally, polyunsaturated fatty acids favor the oxidation of LDL while monounsaturated fatty acids are less conducive to its formation (28, 29). In addition, the protein component of the LDL (apo B) might also determine how the oxidation is propagated within the particle. Specific amino acids might generate and propagate oxidation (30-34).

In light of these, Ox-LDL can be defined as follows: A particle derived from circulating LDL that may have peroxides or their degradation products generated within the LDL molecule or elsewhere in the body associated with the particle. This would include minimally oxidized LDL that may have lipid peroxides or their degradation products (for Example, oxovaleryl PtdCho) but no apoprotein changes, MDA modified particles with MDA arising from platelets or elsewhere, and others. However, to this date, LDL particles with oxidized apo B amino acids without lipid changes have not been described. Figure 24.1 describes some of the potential Ox-LDL particles that may be of relevance.

Although not depicted in the picture, major changes in apo B might occur in addition to aldehyde modification and proteolysis, depending on the type of oxidant used. As a result, countless antigenic epitopes are possible (35, 36). As mentioned before, these changes are not unique to LDL and other lipoprotein might undergo similar changes (37, 38). However, the author has not seen any proteolysis of HDL apoproteins as a result of oxidation but has noted aggregation changes.

In summary, Ox-LDL may contain a specific oxidation product (*see* Table 24.2). The simplest Example may be lipoxygenase-treated LDL. The lipoprotein subjected to such treatment may contain varying amounts of phospholipid and cholesterol ester hydroperoxides. Ox-LDL may also contain limited amounts of a variety of degraded oxidized lipid products. “Minimally modified LDL” as described by Berliner, Fogelman and associates may qualify for such a criteria (39, 40). There is also Ox-LDL that is recognized by macrophages (41, 42). While it was believed for a long time that protein changes are essential for macrophage recognition, at least some studies seem to suggest that oxidized lipids might mediate the whole Ox-LDL uptake (43-45). There are, however, Ox-LDL preparations that do not share the properties of conventionally oxidized lipoproteins. For Example, LDL oxidized by photooxidation in the presence of dyes, such as Rose Bengal, showed massive increase in electrophoretic mobility as a result of oxidation of histidine residues (46). However, other than a loss of phospholipase A2 activity, it did not bear any resemblance to oxidized LDL.

Ox-LDL, thus, might represent the elephant that is described by blind men. It is without description and yet distinct; it is a complex mixture of chemical entities and yet they are measurable, and individual investigator is responsible for setting forth the definition that suits a set of criteria. These definitions should be the basis for experimental validation and scientific reproducibility.

Not all the oxidation mechanisms are comparable or lead to similar products even under in vitro conditions. For Example, peroxidase-mediated oxidations require co-oxidants such as H_2O_2 or lipid peroxides. Some damage the proteins more readily than others. For Example, treatment of LDL with 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH), a radical generator or peroxynitrite, Results in more protein oxidation than lipid peroxidation (65, 80, 82, 83). Similarly, peroxidase-catalyzed oxidation generate very little aldehyde products as compared to metal-catalyzed oxidations (84). Lipoxygenase reactions which are exclusively intracellular might require additional reactions or transfers before or after lipoxygenase reactions occur (85, 86). To our knowledge, no one has characterized oxidized LDL isolated from animals or humans to the extent that mechanistic insights could be derived, although there are numerous claims of the presence of an electronegative LDL (presumably minimally oxidized LDL) in human and animal plasma (87-95).

While all these reactions are oxidative in nature, they are not uniformly amenable to inhibition by traditional antioxidants. Vitamin E or simple phenols such as tyrosine or estradiol actually enhance peroxidase-mediated oxidation of LDL (96-98). This has been taken as evidence for an intermediate prooxidant role for antioxidants. Similarly, many thiols actually enhance the oxidation of LDL depending on the peroxide content of LDL (99).

The detection of Ox-LDL, at least in vitro, has been quite easy, although earlier work, before the discovery of the involvement of oxidative process, revolved around the increase in net negative charge and increase in buoyant density. The presence and amounts of MDA has always been used as a yard stick for minimally or terminally oxidized LDL (100-103), although numerous publications concluded a short-term oxidation always resulted in a “mildly” oxidized LDL as opposed to long-term oxidations (104–107). Yet, enzymatic (lipoxygenase, peroxidase) reactions and treatment with peroxynitrite that generated lower amounts of MDA were always considered fully oxidized LDL due the ability of macrophages to engulf such LDL.

The measurement of lipid oxidation has been a great boon, not only to the understanding of the process of LDL oxidation but also in providing numerous serendipitous discoveries and methodologies. The formation of conjugated dienes during lipid peroxidation was successfully exploited to generate the “lag time” concept by Esterbauer and associates (108) that is still used as a yard stick for measuring the oxidizability of LDL. Similarly, the discovery of isoprostanes by Roberts, Morrow and others (109) also created tremendous excitement and opportunity to look for such LDL in vivo (110).

Since the discovery of oxidized LDL, over 5,000 articles have appeared on the topic with over 400 articles appearing every year during the past decade (Fig. 24.2). Combined with epidemiological evidence and success in several animal trials in a number of species using a variety of antioxidants, the hypothesis appeared to be on solid ground until recently. This euphoria of initial success led to clinical trials that were planned mostly to be the “first” to validate the hypothesis. Obviously vitamin E was the chosen antioxidant as the expectation of the general public and scientists was that a natural antioxidant (such as vitamin E) would have less undesirable effects. Accordingly a number of clinical trials were performed (111–114) using vitamin E at pharmacological doses. Some of these trials were piggyback studies on cancer and other diseases (115, 116). These trials with vitamin E have not been overwhelmingly supportive of the hypothesis. Understanding the sequence of events that led to the hypothesis, the molecular basis of antioxidant action, the potential interaction with other drugs, and the stage of the disease process to which oxidation was suggested to contribute, dosage, the transport mechanisms involved in the release and utilization of vitamin E in the body, its interaction(s) with cytochrome systems, and its function as a

prooxidant in peroxidase-mediated oxidations played little role in the design of the study (117, 118). More importantly, little if any attention was paid to factors that would affect and control oxidation in vivo. There have been many reviews written dissecting out the trials that "disproved" the oxidation hypothesis (119–122).

On the other hand, more and more recent publications have described the effects of oxidized lipids on atherosclerosis and associated risk factors (123–130). The prevalence of antibodies that recognize epitopes of Ox-LDL seem to correlate well with the disease process (35, 95, 131–145). These antibodies also detect such LDL in the plasma of subjects who have higher risk associated with atherosclerosis (35, 139, 145–153). Oxidative enzymes such as Myeloperoxidase seem to predict the vulnerable population and seem to correlate with the severity of the disease (154–156). Considering that vitamin E might actually enhance peroxidase-catalyzed oxidations (98), the clinical trials might have actually endorsed the hypothesis rather than shooting it down. Perhaps, when inhibitors of specific oxidases are available, additional trials might be warranted to induce the development of anti-atherosclerotic pharmacological agents.

Acknowledgments

This work was supported by funding from National Institutes of Health, HL-069038 and HL-74239 (SP) and HL74239 (NS).

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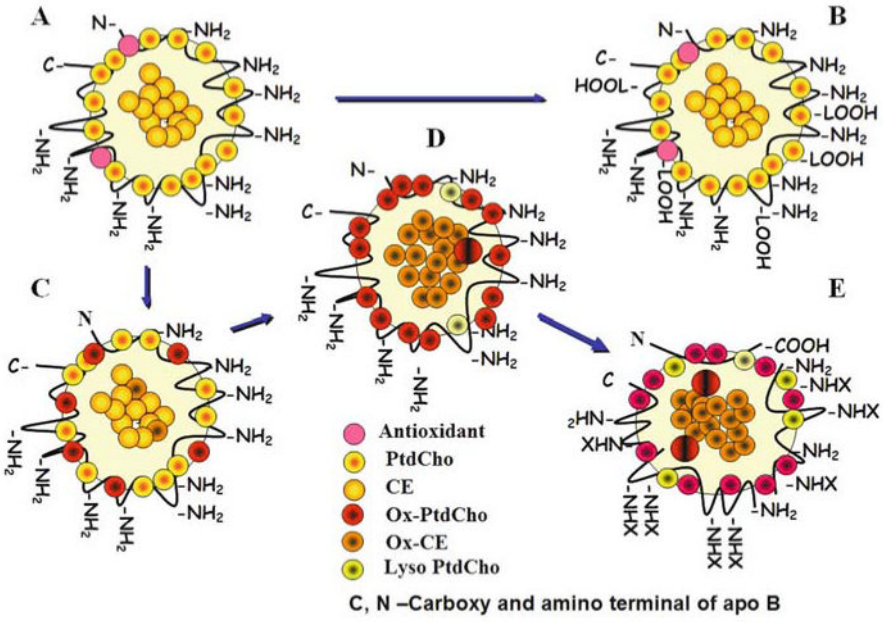

Fig. 24.1.

Forms of oxidized low-density lipoprotein (reproduced from Parthasarathy et al. (157)). (**a**)Unoxidized native LDL with amino groups of lysine residues of apo B and representative lipids. (**b**) Lipid peroxides generated elsewhere associated with such LDL. (**c**) LDL lipids might get oxidized resulting in the generation of cholesterol ester and phospholipid peroxides. (**d**) Such LDL might undergo extensive oxidation leading to protein changes. (e) Extensive protein changes and lipid decomposition might hallmark the end stages of oxidation.

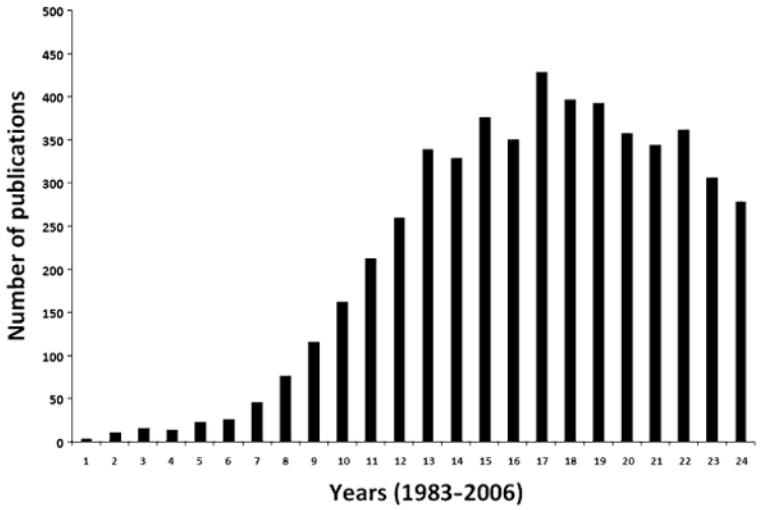

Fig. 24.2.
Number of publications related to Ox-LDL.

Table 24.1
List of lipid/protein oxidation products generated during the oxidation of LDL

- **A.** Fatty acid oxidation products:
  - **i.** Free and esterified fatty acid peroxides, such as 13-hydroperoxylinoleic acid (13-HPODE)
  - **ii.** Free and esterified fatty acid hydroxides, such as 13-hydroxylinoleic acid (13-HODE)
  - **iii.** Prostaglandin-like products, such as isoprostanes in free and esterified forms
  - **iv.** Aldehydes, such as malondialdehyde (MDA), 4-hydroxy nonenal, and hexanal
  - **v.** Core aldehydes that contain esterified lipid backbone, such as oxovaleryl phosphatidylcholine
  - **vi.** Pentane and other hydrocarbons
- **B.** Lipid derived products:
  - **i.** Lysophosphatidylcholine
  - **ii.** Cholesterol oxidation products, such as 7 keto cholesterol
  - **iii.** Internally modified phosphatidyl ethanolamine/serine products
- **C.** Protein oxidation products:
  - **i.** Protein carbonyls
  - **ii.** Non-enzymatic proteolyzed fragments
  - **iii.** Modified cysteine, cystine, histidine, methionine, lysine, arginine, tryptophan, and tyrosine
  - **iv.** Protein cross-links due to tyrosine cross-links as well as due to bifunctional aldehydes
  - **v.** Lipid–protein adducts which could be classified as ceroids (lipofuscins)
  - **vi.** Many of the above changes as well as conformational changes might lead to antigenicity
- **D.** Other changes:
  - **i.** Increased buoyant density
  - **ii.** Increased negative charge
  - **iii.** Loss of characteristic yellow color (human)
  - **iv.** Loss of enzyme activities associated with LDL

Table 24.2
Suggested mechanisms for the oxidation of LDL

Mechanism	References
1. Lipoxygenase reaction	(47–53)
2. Copper and ceruloplasmin-mediated oxidation	(54–58)
3. Iron-mediated oxidation	(54, 59, 60)
4. Peroxidase-mediated oxidation including myeloperoxidase and heme	(61–63)
5. Peroxynitrite-mediated oxidation	(64–66)
6. Thiol-dependent oxidation	(67–70)
7. Xanthine oxidase, NADPH oxidase, and other superoxide generators	(71–78)
8. AAPH or other means of radical generation including cytochromes	(79–81)