Lipid oxidation in atherogenesis: an overview

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Abstract

The ‘oxidation theory’ for atherosclerosis proposes that lipid and/or protein oxidation products are responsible for lesion formation/development. The major target for oxidation is suggested to be intimal low-density lipoprotein. This idea was stimulated by the pro-atherogenic properties of in vitro oxidized lipoproteins, such as stimulation of chemotaxis and sterol accumulation in macrophages, adhesion molecule expression on endothelial cells and apoptosis of several cell types. It was supported by detection of oxidation products in lesion lipoproteins, although these are (in general) less heavily oxidized and their biological activity is less rigorously defined than for their in vitro oxidized counterparts. Lesion lipids contain products generated by both enzymic and non-enzymic oxidation reactions; the majority are generated non-enzymically. The type and source of oxidant involved has been the subject of much speculation and is not resolved. The oxidation theory predicts that appropriate antioxidants will protect against atherosclerosis. Vitamin E has been used in several animal and human studies, but to date has shown little evidence of anti-atherosclerotic potential. However, lack of knowledge of the oxidant(s) driving lesion oxidation and the complexity of the anti- and pro-oxidant properties of vitamin E may explain its disappointing track record to date. These subjects require more rigorous study before the oxidation theory can be fairly tested.

The basis of the oxidation hypothesis

Atherosclerosis is a complex disease that develops slowly over decades. We know that key events early in lesion development are intimal accumulation of plasma lipoproteins, increased expression of adhesion molecules on the endothelium at sensitive sites in the vessel wall, and monocyte margination, migration across the endothelium and accumulation in the intima, where they accumulate large intracellular deposits of lipoprotein-derived cholesterol. These events underpin the formation of the earliest detectable lesion, or ‘fatty streak’, that is the precursor of more advanced atheroma.

Yet, while we have a clear picture of these events, the biochemical mechanisms that drive them remain uncertain. For example, we know that cellular cholesterol homeostasis employs several strategies to prevent its accumulation under normal circumstances, and yet cholesterol-loaded macrophage ‘foam cells’ are present in the earliest detectable lesions. Normally, receptor-mediated uptake of low-density lipoprotein (LDL), the major carrier of plasma cholesterol, is suppressed through down-regulation of LDLR (LDL receptor) expression on endothelial cells and apoptosis of several cell types, including macrophages and smooth muscle cells, also mediate this oxidative modification, sufficient to generate a ‘high-uptake’ form of LDL (reviewed in [2]). The degree of oxidation required for this conversion can be achieved by cultured cells within approx. 24 h in a medium containing low micromolar copper and/or iron, or even in a cell-free medium with rather higher metal concentrations (≈10 μM Cu²⁺).

The average LDL particle contains 1 molecule of apolipoprotein B (apoB), 600 molecules of free cholesterol, 1600 molecules of cholesteryl ester, 700 molecules of phospholipid, 180 molecules of triacylglycerol and approx. 10 molecules of α-tocopherol (αTOH), the major endogenous antioxidant. During cell-mediated conversion of LDL into a high-uptake particle, all of these components are subject to oxidation (reviewed in [3]). Cholesterol is oxidized predominantly at the 7-position to oxysterols, initially 7-hydroxycholesterol, and later 7-ketocholesterol (7KC). Both free and esterified cholesterol are oxidized. The
polyunsaturated fatty acid groups (mainly arachidonate and linoleate) of steryl esters, phospholipids and triacylglycerols are initially oxidized to hydroperoxides. These acyl hydroperoxides then undergo C–C bond cleavage to form reactive short-chain (3–9-carbon) aldehydes, such as malondialdehyde, hexanal and 4-hydroxynonenal. Such products formed in esterified lipids (cholesteryl ester or phospholipid) are called ‘core aldehydes’. Up to 90% of steryl esters in high-uptake oxidized LDL contain such extensively oxidized acyl groups. Non-enzymatic peroxidation of arachidonic acid also generates prostaglandin-like compounds, isoprostanes and epoxyisoprostanes [4]. Isoprostanes also undergo rearrangement to isolevuglandins. Reactive lipid-oxidation products such as short-chain aldehydes and isolevuglandins form stable adducts with apoB, masking reactive lysine residues and increasing the negative charge on the protein. Reaction of apoB with bifunctional aldehydes can also lead to intermolecular cross-linking and aggregation of lipoprotein particles. It is these changes to apoB charge and configuration that lead to its increased uptake by macrophages.

Later studies showed that some of the products formed at early stages of LDL oxidation have significant biological activities. This comparatively less oxidized or ‘minimally modified’ LDL (MM-LDL) is very different in composition from the heavily oxidized high-uptake form. Cholesterol is still the predominant sterol, apoB of MM-LDL still binds to the LDLR, and incubation of macrophages with MM-LDL does not result in foam-cell formation. However, a significant proportion of the unsaturated acyl chains of cholesteryl esters and phospholipids in MM-LDL have been oxidized to hydroperoxides, isoprostanes and short-chain aldehydes [5] that have potent biological effects (see below).

On the basis of the effects of these in vitro oxidations, the ‘oxidation hypothesis’ of atherosclerosis proposed that intimal oxidation of lipids/lipoproteins generates biologically active products that are causal in atherogenesis.

**Biological activities of oxidized lipids/lipoproteins and their relevance to atherogenesis**

The initial interest in a role for lipid oxidation in the development of atherosclerotic lesions was in its ability to modify LDL sufficiently to promote its uptake by macrophages. The oxidation necessary for this is quite significant: by the stage at which particles bind to receptors such as SR-A or CD36, up to 50% of the cholesterol is converted into 7KC and other oxysterols, most of the unsaturated fatty acyl groups are oxidized to a complex mixture of products and apoB is extensively fragmented, derivatized and cross-linked. Not surprisingly, these modifications impact on the metabolism of high-uptake LDL and on the site and nature of lipid deposition in the foam cells that result [3]. Oxidized LDL-loaded cells contain large amounts of oxysterols, distributed into cell membranes and into cytoplasmic lipid droplets after esterification. A large proportion of the oxidized LDL remains trapped as oxidized lipids and ceroid (undegradable lipid–protein complex) in the lysosomal compartment [6]. This is probably due to the intrinsic resistance of some oxidized components to lysosomal hydrolases and/or hydrolase inactivation by some oxidation products. Overall, uptake of oxidized LDL produces a macrophage containing relatively few typical cytoplasmic cholesteryl ester droplets, but significant lysosomal deposits of oxidized lipid and protein. This is more characteristic of advanced lesion foam cells, where up to 70% of cell lipids are stored within lysosomes, together with ceroid (reviewed in [7]), and is not consistent with a role for oxidized LDL in early lesion foam-cell formation.

As well as its potential role in foam-cell lipid accumulation, oxidized LDL has numerous other biological activities (reviewed in [8]). Oxidized LDL stimulates the expression of VCAM-1 (vascular cell adhesion molecule-1) on endothelial cells, is directly chemotactic for monocytes, increases the expression of MCP-1 (monocyte chemotactic protein-1) by vascular cells, and stimulates macrophage proliferation. These properties could promote the early inflammatory infiltration of monocytes into the vascular wall. In addition to its uncontrolled uptake, oxidized LDL also inhibits cholesterol export from macrophages [9] due both to direct effects of oxysterols on the cholesterol efflux machinery [10] and to accumulation of undegradable cholesteryl esters in lysosomes [6]. This could also contribute to foam-cell formation. Oxidation of LDL also increases its sensitivity to aggregation and to modification by sphingomyelinase, promoting further the likelihood of its intimal modification and uptake by macrophages. Oxidized LDL attenuates endothelium-dependent relaxation of blood vessels, and can cause injury, apoptosis and necrosis of vascular cells. This may lead to the release of lipids and lysosomal enzymes into the intima, promoting the progression of atherosclerotic lesions, such as the development of the acellular lipid core. In addition, oxidized LDL can promote macrophage and smooth muscle cell proliferation, and the expression and secretion of a variety of growth factors and cytokines from vascular smooth muscle cells, endothelial cells and macrophages. Components of oxidized LDL are also immunogenic, and may additionally contribute to the chronic inflammatory component of lesion development [11].

During the oxidation of LDL, many of the new lipid species generated have been specifically identified as bioactive and contribute to the effects described above. For example, several specific phospholipid oxidation products in which the sn-2 fatty acid has undergone oxidation have been identified as active components of MM-LDL and oxidized LDL in promoting protein expression and secretion by vascular cells [5]. 7β-Hydroperoxycholesterol is one of the most toxic oxysterols formed [12], whereas 7-ketocholesterol is a major contributor to the inhibition of cholesterol export from oxidized LDL-loaded macrophages [10]. A major challenge will be to identify which of these lipids are present in atherosclerotic lesions (see below) and their contribution to the overall atherogenic process.
Recently, interest has grown in the role of a family of ligand-activated nuclear receptors in regulation of lipid metabolism. Among these, the liver-X receptor (LXR) and peroxisome-proliferator-activated receptors (PPARs) are activated by oxysterols and oxidized fatty acids respectively. Both LXRs and PPAR-γ are highly expressed in macrophages, and are present in atherosclerotic lesions [13,14]. LXRα controls transcription of several anti-atherogenic genes, including those encoding ABCA1 (ATP-binding cassette transporter A1) and apoE, both of which stimulate cell cholesterol export. However, the most potent ligands for LXRα are sterols oxidized enzymically at carbons 22–27 of cholesterol. In contrast, oxysterols generated during cell-mediated LDL oxidation are poor or ineffective ligands for LXR [15].

Activation of PPAR-γ has the potential to be pro- or anti-atherogenic. For example, it stimulates CD36 expression, which could promote oxidized LDL uptake. However, PPAR-γ also inhibits expression of inflammatory genes [e.g. interleukin-1, TNF-α (tumour necrosis factor-α) and iNOS (inducible nitric oxide synthase)] and SR-A, and stimulates expression of LXRα, which could promote cholesterol export [16]. Ligands for PPAR-γ include oxidation products of several fatty acids [9-HODE (where ‘HODE’ is hydroxyoctadecenoic acid), 13-HODE, 15-HETE (where ‘HETE’ is hydroxyeicosatetraenoic acid), 5-HETE, 12-HETE, azoPC (azo-phosphatidylcholine) and lysophosphatidylcholine] [17]. Some of these are present in oxidized LDL, or are generated enzymically through the action of 15- and 5-lipoxygenases, both of which are expressed in lesion macrophages. Overall, studies so far suggest that activation of PPAR-γ appears likely to be anti-rather than pro-atherogenic (reviewed in [18]).

Evidence for lipid oxidation in vivo
A prerequisite for the oxidation hypothesis is that oxidized lipids are indeed generated and detectable in lesions in vivo. There is convincing evidence that oxidized lipids are present in human lesions. This includes many of the products generated during in vitro oxidation of LDL, such as fatty acid peroxides and hydroxides, oxysterols, core aldehydes and oxidation products of αTOH [19–22]. Cholesteryl ester hydroperoxides and hydroxides are the major lipid oxidation products present in human lesions, reaching ≈ 2–3% of parent lipid in the most severe lesions, but are undetectable in normal arteries [23]. The major oxysterol in lesions is the enzymically generated 27-hydroxycholesterol; of non-enzymic oxidation products, 7-oxygenated sterols predominate, reaching ≈ 0.5% of parent cholesterol in the most advanced lesions [24].

There is also immunohistochemical evidence of protein-bound adducts of malon dialdehyde and other end-stage lipid oxidation products in human lesions (reviewed in [25]).

In a detailed study, the temporal progression of lipid oxidation in lesion lipoproteins was measured, grading lesion severity within the same human aortic tissues to minimize inter-subject variability [23]. It was shown that cholesteryl ester hydroperoxides and hydroxides accumulated progressively with lesion severity. However, unoxidized lipid accumulation preceded that of oxidized lipids, raising the suspicion that lipid oxidation is not required for early lesion development.

αTOH is present at all stages of lesion development, yet lipid oxidation still occurs [23]. This could be explained by repeated depletion and repletion of αTOH during lesion formation. Alternatively, αTOH may be inaccessible to and/or unable to protect against the oxidants active in vivo. A further possibility is that αTOH does not prevent, and may in fact promote, formation of lipid peroxides without net consumption of αTOH-mediated peroxidation [26]. The dominant isomer products of oxidized cholesteryl linoleate in human lesions [cis,trans-cholesterol linoleate hydro(per)oxides] are consistent with non-enzymic oxidation in the presence of αTOH.

The nature of the oxidizing conditions largely determines the efficacy of αTOH as an antioxidant. Although it can be effective against radical (one-electron) oxidants, it does not protect against two-electron oxidants, such as hypochlorous acid (HOCl) or peroxynitrite. The spectrum of αTOH products that is formed during its oxidation reflects the nature of the oxidant. Those that predominate in human lesions (α-tocopherolquinone) suggest involvement of two-electron oxidants in early lesion development [27]. HOCl is increasingly implicated in atherosclerosis. It can be formed in vivo through the action of macrophage-derived myeloperoxidase, which is expressed and active in human lesions. Protein oxidation products and epitopes specific for HOCl-modified proteins are present, particularly in more advanced lesions [28,29].

Cause or effect: antioxidant therapy and animal models
The acid test of a causal relationship between lipid oxidation and atherosclerosis is the capacity of an appropriate antioxidant to inhibit lesion lipid oxidation and prevent disease. This has been tested in both humans and animal models (reviewed in [30,31]).

In humans, both epidemiological and self-reported intake data support a protective role for vitamin E (of which αTOH is the major antioxidant). Consumption of diets rich in vitamin E lowers the risk of cardiovascular disease (CVD) [32], whereas self-reported intake of vitamin E and CVD correlate inversely in several prospective cohort studies [33–35]. However, discrepancies exist between these studies. In particular, modest dietary intake of vitamin E is far more effective that much larger doses of vitamin E taken as a supplement. This suggests that plasma antioxidant levels may be surrogate measures of a healthy diet in which other, presently unidentified nutrients are protective. However, such studies provided the rationale for the use of vitamin E in atherosclerosis prevention studies.

Overall, the outcomes of these studies have been disappointing. Two large primary prevention studies [the Alpha-Tocopherol, Beta-Carotene (ATBC) Cancer Prevention and
PPP (Collaborative Group of the Primary Prevention Project) studies investigated the effect of supplemental vitamin E on myocardial infarction (MI), CVD or stroke at 3–6 years. Neither showed any protective effect of supplementation against cardiovascular risk [36,37]. Two smaller studies [the Antioxidant Supplementation in Atherosclerosis Prevention (ASAP) study and VEAPS (Vitamin E Atherosclerosis Prevention Study)] used intima-media thickness progression as a marker of ongoing CVD. Apart from one subgroup, neither showed any effect of vitamin E on progression of disease in healthy subjects at low risk of CVD [38,39]. Similarly, several prospective double-blind, placebo-controlled studies of the effects of vitamin E supplementation in patients with pre-existing CVD (secondary prevention) also found no benefits of antioxidant supplementation. There are two exceptions to this. The CHAOS study (Cambridge Heart AntiOxidant Study) reported a major reduction in the risk of non-fatal MI (although fatal MI was non-significantly increased; [40]), whereas a significant decrease in acute MI was reported in a small group of haemodialysis patients with supplemental vitamin E (SPACE) [41].

Such mixed results have raised doubts about the role of LDL oxidation in human atherosclerosis. However, this interpretation does assume that the doses of vitamin E used in the studies inhibited lipid oxidation in vivo, but there is little direct evidence for this. Moreover, evidence of increased oxidative stress or vitamin E deficiency was not a criterion for inclusion in these studies, so that supplementation may have benefited only a subset of the participants. This may explain some of the discrepancies reported.

Determination of a causal role for lipid oxidation in atherosclerosis is limited in human studies, which are essentially observational and can be confounded by uncontrolled dietary, genetic or lifestyle factors. Animal models provide a much more controlled way to examine the role of lipid oxidation and the protective effects of antioxidants against atherosclerosis. A large number of antioxidant intervention studies have been conducted in animal models of atherosclerosis (reviewed in [30]) but, as with human trials, have yielded inconsistent results. Although several studies do show protective effects of antioxidant supplementation on lesion formation, many others show no effect or even increased lesion formation. Potential confounding effects in animal studies that may contribute to these discrepancies are the direct lipid-lowering effects of some antioxidants and the high levels of hypercholesterolaemia achieved (relative to humans) in some animal models.

Several recent animal studies have attempted to directly determine whether antioxidant supplementation inhibits lipid oxidation in the vessel wall and to correlate this with effects on lesion formation. Again, the results have been inconsistent. Thus while several studies have shown a positive correlation between lipid oxidation and lesion size and an inverse correlation with atherosclerosis [42,43], in others lipid oxidation and atherosclerosis were dissociated [44,45]. These suggest that, at least in some animal models of atherosclerosis, lipid oxidation and lesion formation are not connected.

Conclusions
Both in vitro studies and epidemiology indicate that oxidized lipids may be involved in atherosclerosis. However, in humans in vivo lipid oxidation appears to progress in the presence of normal vitamin E levels, and antioxidant supplementation does not prevent atherosclerosis. This suggests that the oxidation of lipids in the vessel wall may be independent of, or even stimulated by, vitamin E. However, two-electron oxidants are increasingly implicated in atherosclerosis, against which αTOH is not effective. The increasing evidence of a role for HOCl in atherosclerosis raises the possibility that its primary target, protein oxidation, is a more important contributor to lesion development than lipid oxidation. Further studies are required to identify unequivocally the oxidants involved in lesion oxidation and their primary targets, to allow better design of future antioxidant strategies.

References

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