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Cholesteryl ester oxidation products in atherosclerosis

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Abstract

Lipid oxidation products are formed at sites of increased oxidant stress and have been shown to accumulate in atherosclerotic lesions. Although recent studies have focused on the formation and metabolism of oxidized lipids, very little is known about their biological activities and possible (patho)physiological functions. Oxidation of cholesteryl esters containing unsaturated fatty acids leads to the formation of hydroperoxides that are either reduced to alcohols or degrade into biologically active “core-aldehydes”. In this review, the mechanisms of formation and metabolic fate of oxidized cholesteryl esters, their occurrence, as well as possible biological activities are discussed. Based on the current knowledge, cholesteryl ester oxidation leads to the formation of biologically active substances, which could actively contribute to the progression of atherosclerotic lesions and their resulting complications.

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1. Introduction

Oxidative modification of lipids has been implicated in the pathogenesis of various diseases including atherosclerosis, diabetes, cancer, and rheumatoid arthritis as well as aging (Halliwell, 1989). During atherogenesis, low density lipoproteins (LDL) accumulate in the artery wall and become atherogenic as a result of oxidation (Lusis, 2000; Glass and Witztum, 2001; Berliner and Heinecke, 1996). Various forms of oxidized lipids including oxidation products of cholesteryl esters have been detected

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in human atherosclerotic lesions where they exert a variety of biological activities. Monocyte adhesion to the endothelium is an initiating crucial event in atherogenesis. Adherent monocytes subsequently transmigrate into the subendothelial space and mature into macrophages (Glass and Witztum, 2001; Lusis, 2000), which take up oxidized LDL through scavenger receptors and become so called “foam cells” that accumulate lipids such as cholesterol esters. In the necrotic core of an atherosclerotic lesion dying foam cells can release their content and oxidized cholesterol esters and other lipid oxidation products accumulate in the extracellular space. However, little is known about the pathophysiological role of these lipid oxidation products.

2. Formation of cholesteryl ester core aldehydes

It has been shown that polyunsaturated fatty acids are especially prone to oxidative modification. Lipid peroxidation leads to the generation of lipid hydroperoxides (Porter et al., 1995), which undergo carbon–carbon bond cleavage resulting in the formation of short chain, unesterified aldehydes (Terao, 1990; Esterbauer et al., 1987) and aldehydes still esterified to the parent lipid, termed core-aldehydes (Kamido et al., 1992, 1993, 1995). However, in most studies investigating oxidative modification of cholesteryl esters, lipid hydroperoxides were measured as indicators of lipid oxidation. Reduction of these lipid hydroperoxides leads to the corresponding hydroxides that seem to be biologically inactive.

Cholesteryl esters are present in the hydrophobic core of the LDL particle. In some studies it has been shown that these compounds are oxidized to hydroperoxides more rapidly than the polyunsaturated phospholipids in the outer monolayer of the LDL particle (Stocker et al., 1991; Noguchi et al., 1993, 1998), while other studies propose that oxidation of LDL first affects the outer shell of the particle (Meyer et al., 1996).

Studies by Kamido et al. have shown that oxidation of LDL, HDL, or isolated cholesteryl esters in vitro using copper or *tert*-butyl hydroperoxide yields cholesteryl ester hydroperoxides as well as cholesteryl ester core aldehydes that consist of sterols and oxysterols esterified to scission products of oxidized fatty acids containing terminal carbonyl groups (Kamido et al., 1992, 1993). The major aldehyde products from peroxidation of cholesteryl esters were 9-oxononanoyl (9-ONC), 8-oxooctanoyl, and 5-oxovaleroyl esters of cholesterol and 7-ketocholesterol. Core aldehydes were estimated to account for 1–2% of the consumed linoleate and arachidonate esters (Kamido et al., 1995). In HDL, α -tocopherol promoted the formation of cholesteryl ester hydroperoxides and cholesteryl ester hydroxides when peroxy radicals, copper or soybean lipoxygenase were used as oxidizing agents (Garner et al., 1998a,b).

It has been shown that the rate of oxidation of cholesteryl esters in LDL using copper or ferrylmyoglobin is a function of unsaturation, meaning that first cholesteryl arachidonate (Ch20:4) would be depleted, followed by cholesteryl linoleate (Ch18:2), cholesteryl oleate (Ch18:1), and finally free cholesterol (Brown et al., 1996).

Since the most abundant cholesteryl ester in LDL is cholesteryl linoleate, measurement of its hydroperoxides is a good indicator for lipid peroxidation during LDL oxidation. Oxidation of LDL using the azo compound 2,2'-azobis(2-amidinopropane)dihydrochloride led to the formation of regioisomeric cholesteryl linoleate hydroperoxides. In the presence of α -tocopherol, 9- and 13-hydroperoxides with *trans,cis* conjugated dienes were formed, while after consumption of α -tocopherol *trans,trans* conjugated dienes began to form. Since HDL contains fewer antioxidants, the *trans,trans* and *trans,cis* conjugated diene were formed simultaneously (Kenar et al., 1996). Regioisomeric hydroperoxides derived from the oxidation of arachidonate-containing cholesteryl esters were Ch-5-HPETE, Ch-8-HPETE, Ch-9-HPETE, Ch-11-HPETE, Ch-12-HPETE, and Ch-15-HPETE and recently the formation of isoprostane bicyclic endoperoxides from oxidation of cholesteryl arachidonate has been reported (Yin et al., 2002).

2.1. Enzymes involved in the formation of cholesteryl ester oxidation products

Although it is speculated that most of the oxidation of cholesteryl esters is mediated by free radicals (Mashima et al., 2000), several studies point to an essential role of lipoxygenases in cholesteryl ester modification during LDL oxidation (Kuhn et al., 1994a; Belkner et al., 1991, 1993, 1997, 1998).

Incubation of LDL with cells overexpressing 15-lipoxygenase resulted in a significant increase in cholesteryl ester hydroperoxides but only a small increase in fatty acid hydroperoxides and phospholipid hydroperoxides (Ezaki et al., 1995). It turned out that mammalian 15-lipoxygenase indeed preferentially oxidizes cholesteryl esters in LDL (Belkner et al., 1998). Interestingly, enzymatic oxidation of free fatty acids seems to precede the oxidation of cholesteryl esters (Upston et al., 1996; Lass et al., 1996) and increasing LDL's free fatty acid content facilitated 15-lipoxygenase-induced cholesteryl ester oxidation (Upston et al., 1997). Analysis of S and R stereoisomers of oxidized lipids isolated from human atherosclerotic plaques indicated that lipoxygenase contributed significantly to oxidative modification of cholesteryl esters, implying an important role for this enzyme during atherogenesis (Folcik et al., 1995; Kuhn et al., 1992, 1994b).

A role for lecithin:cholesterol acyltransferase (LCAT) in the formation of oxidized cholesteryl esters was also proposed since decomposition of phospholipid hydroperoxides resulted in the formation of oxidized cholesteryl esters and this reaction could be blocked by an LCAT inhibitor. In addition, transfer of fatty acid hydroxides from phospholipids to cholesterol was observed only in HDL, but not in LDL (Nagata et al., 1996).

3. Metabolism of cholesteryl ester oxidation products

Lipid hydroperoxides are relatively easily decomposed to reactive radical species such as peroxy and alkoxy hydroperoxy radicals, which can propagate the oxidative process, leading to oxidation products that might have deleterious biological

activities. Thus, enzymatic reduction of lipid hydroperoxides to hydroxides may represent an important protective mechanism in settings of increased oxidative stress.

3.1. Processing in plasma

It has been shown that cholesteryl ester hydroperoxides were present in human and rat plasmas while phospholipid hydroperoxides were undetectable. This was likely due to the enzymatic (plasma glutathione peroxidase) and the non-enzymatic (apolipoproteins A and B100) reducing activities of phospholipid hydroperoxides in plasma, and to the enzymatic conversion of phospholipid hydroperoxides to cholesteryl ester hydroperoxides by LCAT in HDL (Nagata et al., 1996; Yamamoto and Niki, 1989).

Investigation of plasma from healthy fasting donors revealed that most of the oxidized core lipoprotein lipids were carried on HDL, while peroxide levels in LDL were relatively low (Bowry et al., 1992). This preferential accumulation of lipid hydroperoxides in HDL is explained by the lack of antioxidants in HDL compared to LDL particles. LDL-associated cholesteryl ester hydroperoxides can be transferred to HDL and a role for CETP was suggested in this process (Christison et al., 1995). However, if this is also the case for different cholesterol ester oxidation products such as core aldehydes needs further investigation. HDL-associated cholesteryl ester hydroperoxides are rapidly reduced to the corresponding hydroxides by an intrinsic peroxidase-like activity (Sattler et al., 1995) and oxidation of specific residues in apoAI and apoAII plays a significant role in this process (Garner et al., 1998a,b).

3.1.1. Enzymatic reduction of cholesteryl ester hydroperoxides in plasma

HDL is not only the major carrier of cholesteryl ester hydroperoxides; it also appears to have the prolonged capacity to decrease the total amount of lipid peroxides generated on LDL. These effects cannot be explained by the action of chain-breaking antioxidants present in HDL but rather seem to involve enzymatic mechanisms (Mackness and Durrington, 1995). One of the HDL-associated enzymes, paraoxonase 1 (PON1), was demonstrated to degrade oxidized phospholipids, but also cholesteryl ester hydroperoxides (Aviram et al., 1998), the catalytic mechanism of which has not been elucidated yet (Laplaud et al., 1998). It has been shown that PON1 contains esterase as well as peroxidase-like activity (Aviram et al., 2000). Cholesteryl ester hydroperoxides as well as cholesteryl ester hydroxides that had been isolated from human atherosclerotic lesions were hydrolyzed by PON1. Furthermore, linoleic acid hydroperoxides were reduced to hydroxides by the action of PON1 (Aviram et al., 2000). Recently, it was demonstrated that aldose reductase leads to reduction of phospholipid core aldehydes (Srivastava et al., 2001). Thus, the activities of these enzymes may be potentially protective by preventing the formation of biologically active products derived from oxidized cholesteryl esters.

3.2. Cellular processing

Cholesterol- and cholesteryl ester-loaded macrophage-derived foam cells are a characteristic feature of atherosclerotic lesions. Loading of macrophages with oxi-

dized LDL leads to accumulation of oxidized cholesteryl esters in lysosomes (Kritharides et al., 1998; Brown et al., 2000). It has been shown that inactivation and detoxification of cholesteryl ester hydroperoxides can be mediated by macrophages extracellularly by secreting products that reduce the lipid hydroperoxides. Cholesteryl linoleate hydroperoxides were degraded to corresponding hydroxides as major products, as well as cholesteryl keto-octadecadienoate as minor products (Baoutina et al., 2000). The mechanisms by which macrophages exert antioxidant properties have been reviewed recently (Baoutina et al., 2001).

Furthermore, cholesteryl ester oxidation products form complexes with serum proteins as evidenced by 9-ONC reacting with epsilon amino groups of lysines (Hoppe et al., 1997). This lipid–protein complex formation renders the oxidized cholesteryl esters less susceptible to degradation and may lead to ceroid formation within the atherosclerotic plaque.

It has been shown that oxidized forms of cholesteryl esters were less susceptible to hydrolytic degradation by macrophage lysosomes as compared to native (unoxidized) cholesteryl esters (Hoppe et al., 1997; Brown et al., 2000). Oxidized lipids may decrease hydrolytic activity by directly inhibiting neutral and acidic cholesteryl ester hydrolase activity (Maehira, 1994). On the other hand, it has been reported that oxidized cholesteryl esters are preferred substrates as compared to native cholesteryl esters for macrophage cholesteryl ester hydrolases and hormone-sensitive lipase when presented in a 1:1 molar ratio (Belkner et al., 2000a,b).

3.2.1. Enzymatic reduction of cholesteryl ester hydroperoxides in cells

Among enzymes involved in reducing cholesteryl ester hydroperoxides, selenium-dependent enzymes have been described. Phospholipid-hydroperoxide glutathione peroxidase (PHGPx), which has broad substrate specificity, was shown to reduce cholesteryl ester hydroperoxides without prior hydrolysis of the fatty acid (Sattler et al., 1994; Thomas et al., 1990; Pushpa-Rekha et al., 1995). Another selenium-dependent enzyme, thioredoxin reductase, which can be secreted from cells, also has been shown to reduce lipid hydroperoxides to the corresponding alcohol (Bjornstedt et al., 1995). Moreover, apoB has been reported to reduce lipid hydroperoxides, including cholesteryl ester hydroperoxides (Mashima et al., 1999). It remains speculative, however, to which degree these enzymes contribute to prohibition of the formation of core aldehydes by reduction of cholesteryl ester hydroperoxides to hydroxides.

3.3. Processing in the liver

HDL carries lipid hydroperoxides to the liver thereby preventing accumulation of oxidized lipids in the plasma. It has been shown that cholesteryl ester hydroperoxides from HDL are degraded by HepG2 cells due to a rapid, selective uptake of oxidized over unoxidized cholesteryl esters (Sattler and Stocker, 1993). Lipid hydroperoxides were not detectable within HepG2 cells, suggesting efficient detoxification of cholesteryl ester hydroperoxides by these cells. Further studies in rats showed that HDL-associated cholesteryl ester hydroperoxides and hydroxides were efficiently and

selectively removed from the circulation by the liver (Fluiter et al., 1996). It was shown that liver uptake was mediated by parenchymal cells and coupled to a rapid biliary secretion pathway. In addition, perfusion studies in rat liver demonstrated that oxidized cholesteryl esters were efficiently removed by the liver as compared to native cholesteryl esters. Cholesteryl ester hydroperoxides were readily cleared and reduced by the liver when bound to HDL. In contrast, LDL-associated cholesteryl ester hydroperoxides were neither removed nor reduced by the liver in these studies (Christison et al., 1996). These studies show that the liver is capable of efficiently detoxifying circulating core lipid hydroperoxides and they further underline the importance of HDL in this process.

4. Presence of cholesteryl ester oxidation products in vivo

4.1. Oxidized cholesterol esters in plasma

It has to be said that the concentrations of lipid hydroperoxides in plasma in normal healthy subjects are rather low (Thomas et al., 1994). However, cholesteryl ester hydroperoxides were detected at nanomolar levels in the plasma from healthy humans (Yamamoto and Niki, 1989). Analysis of the regioisomeric distribution of cholesteryl ester hydroperoxides and hydroxides indicated that the formation of these lipid oxidation products was mediated by free radical-induced lipid peroxidation rather than enzymatic modification (Mashima et al., 2000), suggesting that oxidation of unsaturated lipids is an ongoing process within healthy individuals.

Increased plasma levels of cholesteryl ester hydroperoxides were detected in patients suffering from subarachnoid hemorrhage and patients with ischemic stroke. In these studies, increased levels of cholesteryl ester hydroperoxides were associated with increased mortality and correlated with clinical outcome scales, suggesting that plasma levels of cholesteryl ester hydroperoxides may be useful as prognostic markers as well as for monitoring therapeutic interventions (Polidori et al., 1997, 1998). Levels of cholesteryl ester hydroperoxides were not increased in patients with hyperhomocysteinemia (Dudman NP, 1993).

4.2. Oxidized cholesteryl esters accumulate in atherosclerotic lesions

An association of lipid peroxidation in atherosclerotic plaques with the progression of lesion development and also plaque stability has been suggested (Nishi et al., 2002; Felton et al., 1997). However, the biological role of the determined lipid oxidation products and their metabolic fate has not been investigated in detail. By monitoring time-dependent changes of the lipid content in aortas of apoE null mice that received a high fat diet, an association of aortic contents of cholesteryl ester hydroperoxides with the progression of atherosclerosis was observed (Letters et al., 1999). Furthermore, in a recent study performed with human aortic lesions, it was shown that accumulation of non-oxidized lipids preceded accumulation of

cholesteryl ester hydroperoxides, which were significantly increased in fibro fatty and in even more complex lesions (Upston et al., 2002; Suarna et al., 1995).

Homogenates of human atherosclerotic plaques contain very large amounts of oxidized lipids, with approximately 30% of the fatty acid moiety of cholesteryl linoleate being present in oxidized forms (Suarna et al., 1995). The most abundant cholesteryl ester quantitated in advanced lesions was cholesteryl linoleate with mean concentrations of approximately 0.50 mol/mol cholesterol (Karten et al., 1998). In a different study, 2–5% of lesion cholesteryl linoleate was present as hydroperoxides or hydroxides (Niu et al., 1999). In advanced human atherosclerotic plaques, 9-oxo-nonanoyl cholesterol (9-ONC), was found one of the most abundant oxidation products derived from cholesteryl linoleate (Kamido et al., 1992, 1995; Karten et al., 1998, 1999; Hoppe et al., 1997). In different plaque samples, 9-ONC was analyzed at mean concentrations of 29 μmol per mol cholesterol (Karten et al., 1998). Since these data represent normalized mean values derived from total tissue samples, local concentrations of oxidized cholesteryl esters could be several folds higher in areas of the lesion where lipids are concentrated.

5. Biological activity of oxidized cholesteryl esters

Very little is known about the (patho)physiological role of oxidation products derived from cholesteryl esters. It has been observed that incubation of macrophages with cholesteryl esters caused cell damage that could be prevented by the addition of antioxidants (Reid et al., 1992). Toxicity was related to increasing unsaturation (Hardwick et al., 1997) and a connection to the onset of macrophage necrosis in the atherosclerotic plaque was suggested.

Furthermore, the 9- and 13-hydroxy derivatives of cholesteryl linoleate were identified to inhibit the mitogenic activity of platelet-derived growth factor (PDGF), basic fibroblast growth factor (FGF), and the antiproliferative activity of transforming growth factor beta 1 (TGF β 1), while not influencing the mitogenic activity of epidermal growth factor (EGF). This modulation of effects of growth factors may have important implications during the inflammatory processes in atherogenesis (Van Heek et al., 1998).

Core aldehydes react with lysine groups on apo B and other proteins (Steinbrecher et al., 1984, 1989) and thus may compromise their biological function. Moreover, direct cellular activation by cholesteryl ester core aldehydes has been shown recently. Products derived from the oxidation of cholesteryl linoleate stimulated endothelial cells to specifically bind monocytes, but not neutrophils (Huber et al., 2002). Biologically active components contained in oxidized cholesteryl linoleate were 9-ONC and cholesteryl linoleate hydroperoxides, while 9R-HODE cholesteryl ester and 13R-HODE cholesteryl ester and 9S-HODE- and 13S-HODE cholesteryl esters were not active. However, since cholesteryl ester hydroperoxides decompose into cholesteryl ester-core aldehydes such as 9-ONC, it may be speculated that the biological activity of cholesteryl linoleate hydroperoxides can also be attributed to fragmentation products due to further oxidation.

It was shown that functional groups present in oxidized cholesteryl linoleate were responsible for the biological activity. Reduction of oxidized cholesteryl linoleate and cholesteryl linoleate hydroperoxides with sodium borohydride (which is well documented to reduce hydroperoxides, epoxides, aldehydes and ketones to hydroxides) resulted in a decrease of biological activity evidenced by decreased induction of monocyte adhesion (Huber et al., 2002). Furthermore, reduction of 9-ONC completely abolished its ability to induce monocyte adhesion indicating that reduction of the aldehyde resulted in complete loss of activity.

5.1. Oxidized cholesteryl linoleate and 9-ONC activate a MAP-kinase-dependent signaling pathway

It was shown that oxidized cholesteryl esters stimulated phosphorylation of ERK 1/2 in human umbilical vein endothelial cells (Huber et al., 2002). Activation of ERK 1/2 depends on upstream MEK 1/2, and may require activation of protein kinase C (PKC) (Bowry et al., 1992). Monocyte binding as well as phosphorylation of ERK 1/2 induced by oxidized cholesteryl esters and 9-ONC was blocked by the MEK 1/2 inhibitor PD098059 and the PKC inhibitor bisindolylmaleimide I. These data show that stimulation of endothelial cells by cholesteryl ester oxidation products induces a specific signaling pathway involving activation of protein kinase C, MEK 1/2 and ERK 1/2.

Activation of vascular cells in the atherosclerotic plaque is most likely due to an additive effect of a variety of oxidation products derived from several cholesteryl esters with different polyunsaturated fatty acids, as well as other biologically active lipid oxidation products. However, transportation of these rather hydrophobic lipids to the endothelium and subsequent stimulation thereof remains speculative. In the core region of atherosclerotic lesions, both cholesterol-rich and cholesteryl ester-rich lipid deposits form (Guyton and Klemp, 1994) and intact oxidized cholesteryl esters may be released into the extracellular space due to apoptosis and necrosis of foam cells (Hegyi et al., 1996).

Since oxidized cholesteryl esters have various effects on vascular cells and macrophages, implicating a pathophysiological role for these lipid oxidation products in various diseases, tight control of the formation as well as the metabolism seems essential to keep vascular homeostasis.

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