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Oxidized Phospholipids: Biomarker for Cardiovascular Diseases

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Abstract

Biologically active oxidized phospholipids can initiate and modulate many of the cellular events attributed to inflammation leading to atherosclerosis. Produced by enzymatic or non-enzymatic processes, these molecules interact with various cells via specific receptors and in general give rise to inflammatory signals. There is considerable evidence that oxidized phospholipids accumulate *in vivo* and play significant roles in atherosclerosis and thrombosis, suggesting that oxidized phospholipids could be biomarkers that reflect the global extent of these diseases *in vivo*. Thus, understanding the biosynthetic pathways, receptor specificity and signaling processes of oxidized phospholipids is important in understanding atherosclerosis, thrombosis and related inflammatory diseases.

Keywords

Oxidized Phospholipids; Atherosclerosis; Cardiovascular Diseases; Inflammation

1. Introduction

Inflammation and oxidative stress are key factors in atherogenesis and are associated with lipid peroxidation and the formation of bioactive lipids. Many bioactive lipids have been identified in atherosclerotic plaques and in circulation, including oxidized phospholipids (oxPL), short chain reactive aldehydes, platelet activating factor (PAF), oxidized cholesteryl esters, oxidized free fatty acids, lysophosphatidyl-choline, oxysterols and isoprostanes. There is considerable evidence that oxidized phospholipids of oxidized LDL accumulate *in vivo* (Podrez et al., 2002a; Watson et al., 1997), and play a significant role in atherosclerosis (Berliner and Watson, 2005) and thrombosis (Podrez et al., 2007). Plasma bioactive oxidized phospholipids are elevated in two major mouse models of atherosclerosis: ApoE(−/−) and LDLR(−/−) mice on the Western diet (Forte et al., 2002) and in humans with low HDL levels (Podrez et al., 2007). Studies on two HDL-associated enzymes, serum paraoxonase (PON1) and PAF-acetylhydrolase (PAF-AH), which are responsible for hydrolysis of plasma oxidized phospholipids (Forte et al., 2002), provide evidence for the role of oxidized phospholipids in atherosclerosis. Mice deficient in PON1 accumulate oxidized phospholipids and develop more lesions than control mice (Shih et al., 1998). In humans, polymorphisms in the PON-1 and PAF-AH genes have been associated with risk for coronary artery disease. Another important marker of oxidative stress is the association of oxidized phospholipids with the apolipoprotein

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B-100 particle (OxPL/apoB) of LDL. Increased levels of OxPL/apoB are implicated in coronary artery disease, progression of carotid and femoral atherosclerosis and the prediction of new cardiovascular events (Tsimikas et al., 2005). Other studies have demonstrated increased levels of auto-antibodies against oxPL in patients with hypertension and myocardial infarction. Thus, oxPL could be a biomarker for cardiovascular diseases.

2. Structure

Oxidized phospholipids are generated when LDL or cellular phospholipids containing polyunsaturated fatty acids (PUFA) undergo oxidative attack resulting in either addition of an oxygen atom to the sn-2 fatty acid residue or fragmentation of the sn-2 fatty acid chain (Figure 1). Enzymes like cyclooxygenase, lipoxygenase and cytochrome P450 can facilitate this reaction under certain physiological conditions to produce prostaglandins, leukotrienes and other arachidonic acid metabolites that are potent mediators of inflammation. In the absence of enzymes, lipid peroxidation can also occur by free radical attack. A common consequence of such attack is oxidative fragmentation of the sn-2 chain. Another class of products generated during the peroxidation of PUFA consists of free short chain reactive aldehydes: 4-hydroxy-2-alkenals (HNE) and 4-hydroxy-2-hexenal (HHE). These aldehydes may rapidly react with proteins forming covalent adducts.

Mild oxidation of LDL results primarily in phospholipid oxidation, the products of which seem to be proinflammatory (Leitinger, 2003; Watson et al., 1997). Oxidation of LDL lipids also causes the formation of large amounts of cytotoxic substances, such as oxysterols, malondialdehyde and other reactive carbonyls that appear to have roles in the later stages of atherosclerosis. Chromatographic separation of the many products formed by the oxidation of 1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphorylcholine (PAPC) led to the identification of 1-palmitoyl-2-(5-oxovaleroyl)-sn-glycero-3-phosphatidylcholine (POVPC), 1-palmitoyl-2-glutaroyl-sn-glycero-3-phosphatidylcholine (PGPC) and 1-palmitoyl-2-(5,6-epoxyisoprostane E₂)-sn-glycero-3-phosphatidylcholine (PEIPC) as potent lipid mediators of inflammation.

We have previously identified critical structural elements of endogenous oxidized phospholipid ligands that promote high-affinity binding to class B scavenger receptors. Four major structurally related phospholipids with CD36 binding activity (oxPC_{CD36}) were identified from oxidized PAPC and four corresponding structural analogs were identified from oxidized 1-hexadecanoyl-2-octadecadi-9',12'-enoyl-sn-glycero-3-phosphocholine (PLPC) (Podrez et al., 2002a; Podrez et al., 2002b).

3. Expression, activation and turnover

Oxidized phospholipids interact with signal transduction receptors and with different pattern recognition receptors present on the cell surface. OxPL also act by binding to G protein-coupled receptor (GPCR) which increases cyclic AMP (cAMP) levels in endothelial cells (Berliner and Gharavi, 2008). Studies have also suggested occupation of the PAF-receptor by oxidized phospholipids (Prescott et al., 2000).

CD36 recognition of specific oxidized phospholipids (oxPC_{CD36}) is involved in macrophage foam cell formation and platelet hyper-reactivity in dyslipoproteinemia. The critical structural element which is required to serve as a ligand for CD36 is a truncated sn-2 acyl group that incorporates a terminal γ -hydroxy (or oxo)- $\alpha\beta$ -unsaturated carbonyl. Subsequent studies have shown that all classes of oxidized phospholipids examined thus far (phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine and phosphatidic acid) can harbor a high-affinity motif for CD36 (Podrez et al., 2002a; Podrez et al., 2002b). The amino acids 160–168 in CD36 represent the core of the oxPC_{CD36} binding site with lysines 164 and 166 being

indispensable for binding. These oxidized phospholipids also interact with another class B receptor, scavenger receptor-BI, and compete with HDL binding due to the close proximity of their binding sites. This closeness inhibits selective cholesteryl ester uptake from HDL in hepatocytes, potentially promoting accelerated atherosclerosis (Ashraf et al., 2008).

A possible role of toll-like receptors (TLRs) in oxPL-induced inflammation has also been suggested (Leitinger, 2003; Watson et al., 1997). Different studies demonstrated a protective role of the Asp299Gly-TLR4 polymorphism, which seemed to result in an attenuation of atherosclerosis. Endothelial cells isolated from mouse strain C3H/HeJ (which carries a point mutation in the TLR4 gene) show a reduced inflammatory response not only to lipopolysaccharide (LPS), but also to oxidized lipids. Furthermore, oxPAPC may bind to a 37kDa glycosylphosphatidylinositol anchored protein, which interacts with TLR4 to induce interleukin-8 (IL-8) transcription (Walton et al., 2003).

Oxidized PAPC upregulates a spectrum of inflammatory cytokines including IL-8 and the chemokine monocyte chemoattractant protein-1 (MCP-1) in endothelial cells (Gargalovic et al., 2006). Induction of IL-8 transcription by oxidized phospholipids is mediated by activation of c-src and its downstream effector, STAT3. OxPAPC also independently induces activation of JAK2 (Gharavi et al., 2007). Mitogen-activated protein kinase phosphatase-1 (MKP-1) was reported to be involved in oxPAPC-induced MCP-1 production. Whereas activation of eNOS by oxPAPC is regulated through a phosphatidylinositol-3-kinase/Akt-mediated mechanism, oxPAPC-induced SREBP activation is significantly reduced with eNOS inhibition (Berliner and Gharavi, 2008). Co-immunoprecipitation and pull-down assays showed that the scavenger receptor CD36 associates with a signaling complex containing Lyn and MEKK2. The MAP kinases, JNK1 and JNK2, are specifically phosphorylated in macrophages exposed to oxidized phospholipids.

4. Biological function

Biologically active oxidized phospholipids can initiate and modulate many cellular events. In human aortic endothelial cells, oxidized phospholipids modulate the expression of a number of genes related to angiogenesis, atherosclerosis, inflammation and wound healing (Berliner and Gharavi, 2008; Gargalovic et al., 2006). In addition, oxPL can activate platelets, induce differentiation of monocytes and de-differentiation of smooth muscle cells - processes related to atherosclerotic plaque formation (Figure 2). They also can modulate the fate of an inflammatory response by intervening in such processes as removal of apoptotic cells and by dampening bacterial-induced inflammation (Bochkov et al., 2002).

Some recently identified bioactive phospholipid species and their key biological activities are described below.

4.1 Ligands for the macrophage class-B scavenger receptors

A variety of scavenger receptors expressed on macrophages has been described to be involved in the processes of atherogenesis and apoptosis. Among these, class A scavenger receptor, SR-A, and class B scavenger receptor, CD36 were demonstrated to be the major receptors responsible for the uptake of modified forms of LDL in a mouse model of atherosclerosis. The role of CD36 as the receptor responsible for the recognition of free oxidized phospholipids has been demonstrated (Boullier et al., 2000; Podrez et al., 2000). Oxidized phospholipid covalently linked to apolipoprotein B-100 in extensively oxidized LDL (e.g. Cu²⁺-oxLDL) have also been shown to serve as a ligand for CD36 (Boullier et al., 2000; Watson et al., 1997). Recent studies have demonstrated that the interactions of platelet CD36 with specific endogenous oxidized phospholipids play a crucial role in the well-known clinical association between dyslipidemia and prothrombotic phenotype (Podrez et al., 2007).

4.2 PAF and PAF mimetics

Platelet-activating factor (PAF) is a biologically active phospholipid, structurally identified as 1-O-alkyl-2-acetyl-sn-glycero-3-phosphocholine. Some of the inflammatory actions of PAF include platelet aggregation, hypotension, anaphylactic shock and increased vascular permeability (Prescott et al., 2000). PAF also induces atherogenic effects by activating monocytes and by stimulating smooth muscle cell growth. In contrast to the tightly regulated physiologic generation of PAF, uncontrolled processes of free-radical oxidation generate PAF analogs *in vivo* and *in vitro*. Since precursors for PAF synthesis frequently contain arachidonate in the sn-2 position, they can also be targets for peroxidation. One outcome of this type of uncontrolled chemical reaction is the fragmentation of the residue at the sn-2 position. These oxidatively generated PAF mimetics stimulate monocytes, leukocytes and platelets. They are found in atherosclerotic lesions and even in blood from individuals exposed to cigarette smoke (Heery et al., 1995).

4.3 Endothelial cell activators

Two specific oxidized phospholipids, POVPC and PGPC, were identified as abundant products in oxidized LDL and were shown to have major roles in the activation of endothelial cells and induction of leukocyte binding. The effect of POVPC is protein kinase-A dependent leading to stimulation of the cAMP-mediated pathway (Berliner and Gharavi, 2008). In endothelial cells, oxPL modulate transcription factors such as peroxisome proliferator-activated receptors (PPAR) alpha and gamma, nuclear factor of activated T cells (NFAT) and Egr-1. OxPL also stimulate angiogenesis in human endothelial cells via induction of autocrine mediators such as vascular endothelial growth factor (VEGF), which works through activating transcription factor-4 (ATF4) (Oskolkova et al., 2008).

5. Possible medical applications

A growing number of studies suggest that, apart from elevated cholesterol levels that are already recognized as risk factors, oxidized phospholipids also play an important role in atherosclerosis. Oxidized phospholipids accelerate atherosclerosis by interacting with specific receptors that mediate atherogenesis, as well as through their reactive groups that can bind covalently to proteins, forming lipid-protein adducts that become dysfunctional. Pro-inflammatory oxidized phospholipids are significant predictors of the presence and extent of carotid and femoral atherosclerosis, development of new lesions, and increased risk of cardiovascular events. Thus, the oxidized phospholipids could be a diagnostic marker of coronary artery disease or may represent a potential target for therapeutic intervention.

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Figure 1.
Representative chemical structures of oxidized phospholipids formed during oxidation of PAPC.

Figure 2. Oxidized phospholipids present in oxidized LDL induce various inflammatory pathways leading to atherosclerosis.