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Pleiotropic effects of oxidized phospholipids

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ABSTRACT

Oxidized phospholipids (OxPLs) are increasingly recognized to play a role in a variety of normal and pathological states. OxPLs were implicated in regulation of inflammation, thrombosis, angiogenesis, endothelial barrier function, immune tolerance and other important processes. Rapidly accumulating evidence suggests that OxPLs are biomarkers of atherosclerosis and other pathologies. In addition, successful application of experimental drugs based on structural scaffold of OxPLs in animal models of inflammation was recently reported. This review briefly summarizes current knowledge on generation, methods of quantification and biological activities of OxPLs. Furthermore, receptor and cellular mechanisms of these effects are discussed. The goal of the review is to give a broad overview of this class of lipid mediators inducing pleiotropic biological effects.

1. Introduction

In addition to their structural role in membranes and function as an energy source, lipids are well recognized as messengers of information. In order to transduce information, a lipid must have structural characteristics that allow receptors to discriminate it from “normal” lipids present in our body. In case of fatty acids a common modification mechanism transforming them to mediators is oxidation. Existing paradigm includes three major postulates, namely that i) lipid messengers are generated enzymatically, ii) they are generated from unesterified PUFAs and iii) they act as unesterified molecules. These principles have a simple biochemical background: enzymatic mechanisms provide proper stereochemistry necessary for high-affinity recognition by receptors, while PUFAs in unesterified form have easier access to lipid-modifying enzymes and, after oxidation, to receptors. A logical consequence of this paradigm is that oxidized PUFAs within complex lipids, and especially those generated by non-enzymatic oxidation, are generally regarded as a biological waste playing little role in physiology and pathology. However, accumulating evidence suggests that this concept may be an oversimplification. More and more data show that oxidized PUFAs that are esterified in triglyceride, cholesterol ester or phospholipid molecules are capable of inducing biological effects potentially relevant to different types of pathology. The volume of information on biological activities of oxidized phospholipids is growing especially fast. More than 100 papers per year were published in the last decade on the topic “oxidized phospholipids”. The goal of this review is to help the readers to orientate themselves in this rapidly evolving field. To this end, we describe the basic facts on the major biological activities of OxPLs and provide citations of original publications and reviews where the readers can get more detailed information.

Abbreviations: ATF4, activating transcription factor; AzPAF, azelaoyl-PAF; AzPC, azelaoyl-PC; CL, cardiolipin; COX, cyclooxygenase; CRP, C-reactive protein; cyt *c*, cytochrome *c*; DC, dendritic cell; DMPC, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine; EC, endothelial cell; ESR, electrophilic stress response; G-PC, glutaroyl-phosphatidylcholine; HAEC, human aortic endothelial cell; HDL, high-density lipoprotein; HPAEC, human pulmonary aortic endothelial cell; HO-1, heme oxygenase-1; HUVEC, human umbelical vein endothelial cell; IL-8, interleukin-8; MFG-E8, milk fat globule epidermal growth factor 8; LBP, LPS-binding protein; LDL, low-density lipoprotein; LOX, lypoxygenase; Lp-PLA2, lipoprotein-associated PLA2; LPS, lipopolysaccharide (endotoxin); mAb, monoclonal antibodies; MM-LDL, minimally modified LDL; NOX, NADPH oxidase; OV-PC, oxovaleroyl-phosphatidylcholine; OxCL, oxidized cardiolipin; OxLDL, oxidized LDL; OxPAPA, oxidized 1-palmitoyl-2-arachidonoyl-*sn*-glycero-3-phosphate (phosphatidic acid); OxPAPC, oxidized 1-palmitoyl-2-arachidonoyl-*sn*-glycero-3-phosphocholine; OxPAPE, oxidized 1-palmitoyl-2-arachidonoyl-*sn*-glycero-3-phosphoethanolamine; OxPAPG, oxidized 1-palmitoyl-2-arachidonoyl-*sn*-glycero-3-phosphoglycerol; OxPL, oxidized phospholipid; OxPLPC, oxidized 1-palmitoyl-2-linoleoyl-*sn*-glycero-3-phosphocholine; PAcPC, 1-palmitoyl-2-acetyl-*sn*-glycero-3-phosphocholine; PAF, platelet activating factor; PAzPC, 1-palmitoyl-2-azealoyl-*sn*-glycero-3-phosphocholine; PBPC, 1-palmitoyl-2-butanoyl-*sn*-glycero-3-phosphocholine; PONPC, 1-palmitoyl-2-oxononanoyl-*sn*-glycero-3-phosphocholine; POVPC, 1-palmitoyl-2-(5-oxovaleroyl)-*sn*-glycero-3-phosphocholine; PGPC, 1-palmitoyl-2-glutaroyl-*sn*-glycero-3-phosphocholine; OxPOPC, oxidized 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine; OxPS, oxidized phosphatidylserine; PAF, platelet activating factor; TLR, toll-like receptor; VEGF, vascular endothelial growth factor

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2. Generation, structure, methods of analysis and abundance of OxPLs

2.1. Formation of OxPLs

The second position in the glycerol backbone of phospholipids typically contains esterified mono- or polyunsaturated fatty acids (PUFAs). PUFAs (e.g., arachidonic, docosahexaenoic acid etc.) are prone to oxidation induced by reactive oxygen and nitrogen species. These reactive molecules may be of endogenous (mitochondrial respiratory chain, myeloperoxidase, etc.) or exogenous (air pollution, smoking, etc.) origin and typically form a peroxyl radical of esterified PUFA as a primary oxidation product, which is further transformed to a hydroperoxide. Peroxyl radicals and hydroperoxides are unstable and are further transformed through intramolecular rearrangements, cyclization or oxidative fragmentation. As a result of these diverse reactions, nonenzymatic oxidation of a single molecular precursor of PUFA-PL produces numerous molecular species with different functional groups within the oxidized residues.

The non-enzymatic peroxidation of PL-esterified PUFAs is thought to proceed according to the same basic mechanisms as oxidation of unesterified PUFAs. In contrast, the enzymatic oxidation of free PUFAs differs from that of PL-esterified PUFAs. Only two types of enzymes are capable of direct peroxidizing PLs within cellular membranes or lipoproteins. 12/15-lipoxygenases (e.g. human 15-LOX-1, soybean LOX or murine leukocyte-type 12-LOX) accept PLs as substrates [1–3]. In addition, cytochrome *c* has been shown to predominantly peroxidize PUFAs esterified in cardiolipin [4]. Both types of enzymes insert dioxygen into PUFA residues thus producing hydroperoxides of corresponding PLs.

In contrast to esterified PUFAs, free fatty acids can be directly oxidized by a much broader group of enzymes including 12/15-LOX, 5-LOX, cyclooxygenases-1 and 2 and cytochrome P450. These enzymes oxidize free fatty acids with formation of hydroperoxy- (LOXs), endoperoxy- (COXs) or epoxy- (cytP450) PUFAs. It has been shown that PUFAs that were enzymatically oxidized in unesterified form can be reesterified into phospholipids [5–7] (Fig. 1).

OxPLs can be divided into two structural groups according to the length of carbon chain in comparison to the nonoxidized precursors: non-fragmented (full-chain) OxPLs having the same number of carbon atoms in the oxidized residue as the precursor, or oxidatively fragmented OxPLs that have shorter chains (Fig. 2). Both full-chain and fragmented OxPLs may contain one or more oxygen-containing functional groups such as hydroperoxy-, hydroxy-, keto-, epoxy-, prostane groups as well as aldehydic or carboxylic ω-terminal groups (Fig. 2). Both full-chain and oxidatively fragmented OxPLs can be either inert or chemically reactive, as for example α,β-unsaturated aldehydes or cyclopentenone prostanoids, that are highly electrophilic and rapidly interact with nucleophilic groups (e.g., SH- and NH_2-groups of proteins), which often leads to the damage of biomolecules [8]. Some proteins have enhanced capacity to bind OxPLs covalently through the formation of Schiff-bases or Michael adducts with lysine, histidine, arginine and cysteine amino acid residues. About 85% of all circulating OxPCs are covalently bound to the apo(a) part of Lp(a) [9], namely to two lysines of kringle V domain in apo(a) [10,11]. Other examples of OxPCs-modified proteins found in plasma is plasminogen [12] and albumin [13]. Importantly, protein adducts of OxPLs retain proinflammatory properties of free OxPLs [12,14,15]. It has yet to be elucidated whether other biological activities of OxPLs that are described below are similar between protein adducts and free form.

Highly reactive electrophilic residues of OxPLs can interact with amino-groups of phosphatidylserine and phosphatidylethanolamine. Resulting phospholipids that are covalently modified on their polar head groups were shown to induce various biological effects [16–20].

In summary, oxidation of PLs dramatically increases complexity of lipid mixtures by generating a variety of oxidized molecular species. This diversity of chemical structures and reactivities of OxPLs is apparently one of the major reasons explaining pleiotropic biological activities of OxPLs that will be described in this review. Deeper information on the mechanisms of generation and chemical properties of OxPLs can be found in several reviews [21,22].

2.2. Analytical techniques

High structural heterogeneity and low *in vivo* abundance of OxPLs makes their quantification a challenging task. Two different approaches demonstrate sufficient selectivity and sensitivity for OxPL analysis, namely immune assays and methods based on mass spectrometry.

Antibody-based methods offer a sensitive and technically simple approach to analyse phospholipid oxidation and are widely used in clinical investigations. Although several antibodies are available to detect lipid oxidation products, only three monoclonal antibodies (mAb) are specific for oxidized phospholipids (E06, DLH3 and 509). E06 was cloned from hybridomas generated from apoE-deficient mice that had not been immunized [23]. DLH3 was isolated from BALB/c mice that were immunized with homogenates of human atheromatous plaques of aortae [24]. The mAb 509 was generated by immunizing mice with a mixture of OxLDL and OxPAPC [25]. All these antibodies recognize only oxidized forms of PLs, either in a pure form or as a component of OxLDL or membranes. In contrast to E06 and DLH3, which are specific for phosphatidylcholine moieties, mAb 509 specifically recognizes oxidized phosphatidylethanolamine [25]. E06 is the best characterized PL-specific antibody which recognizes also PL-protein adducts [26] and is widely used in an ELISA to quantify phosphocholin-containing OxPLs on human OxLDL [27]. Although the immunoassay using E06 is sufficiently sensitive for clinical applications and on the other hand allows high throughput analysis, the method has important limitations. The antigen specificity of E06 is broad and only partially characterized. Binding specificities of DLH3 and mAb 509 are even less understood. E06, DLH3 and mAb 509 are phospholipid class-specific antibodies that do not distinguish between individual molecular species of oxidized phosphatidylcholine (E06, DLH3) or phosphatidylethanolamine (mAb 509). Such differentiation may be however quite useful because available data clearly show that individual molecular species often have very different biological activites. For example, fragmented oxidized phosphatidylcholine species such as POVPC or PGPC disrupt endothelial barrier in lung vessels, while molecules with full-length oxidized residues such as PEIPC demonstrate barrier-protective activity [28]. E06, however, can not differentiate between these molecular species. Another apparent weakness of immune methods in analysis of OxPLs is that biological fluids and tissues contain high amounts of proteins that either enzymatically degrade OxPLs within lipoproteins or membranes (e.g., lipoprotein-associated PAF-acetylhydrolase) or opsonize OxPLs (e.g., natural antibodies, C-reactive protein or complement H component) and thus "mask" them from recognition by antibodies [25]. In order to overcome these limitations of immune assays, a variety of methods were developed recently that are based on HPLC separation and mass spectrometry.

Recent developments in liquid chromatography (LC) and mass spectrometry (MS), turned MS-based techniques into the method of choice to analyse phospholipid oxidation. Since PLs and their oxidation products are rather labile biomolecules, low-energy ionisation techniques, like matrix assisted laser desorption ionisation (MALDI) and electrospray ionisation (ESI) are mainly used to analyse these lipids.

Because of complexity and low abundance of OxPLs their quantitative analysis in real biological samples is impossible without prior chromatographic separation. MALDI is not compatible with online separation techniques, which limits its application for analysis of OxPLs in complex samples, although in principle detection of OxPLs by MALDI is possible [29]. An advantage of MALDI is that it allows performing phospholipid imaging studies within tissue sections (re-