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Review

Oxidized phospholipids: From molecular properties to disease

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Abstract

Oxidized lipids are generated from (poly)unsaturated diacyl- and alk(en)ylacyl glycerophospholipids under conditions of oxidative stress. The great variety of reaction products is defined by the degree of modification, hydrophobicity, chemical reactivity, physical properties and biological activity. The biological activities of these compounds may depend on both, the recognition of the particular molecular structures by specific receptors and on the unspecific physical and chemical effects on their target systems (membranes, proteins). In this review, we aim at highlighting the molecular features that are essential for the understanding of the biological actions of pure oxidized phospholipids. Firstly, their chemical structures are described as a basis for an understanding of their physical and (bio)chemical properties in membrane- and protein-bound form. Secondly, the biological activities of oxidized phospholipids are discussed in terms of their unspecific effects on the membrane level as well as their potential interactions with specific targets (receptors) affecting a large set of (signaling) molecules. Finally, the role of oxidized phospholipids as important mediators in pathophysiology is discussed with emphasis on atherosclerosis.

Keywords: Phospholipid oxidation; Membrane; Receptors; Inflammation; Apoptosis

1. Structure and biological origin of oxidized glycerophospholipids

Oxidation of fatty acids linked to the *sn*-1 and *sn*-2 positions of glycerophospholipids leads to many different reaction products, depending on chain length and degree of unsaturation. Enzyme-catalyzed and non-enzymatic reactions may be involved in (phospho)lipid oxidation in vivo. Lipid oxidizing enzymes include lipoxygenase, myeloperoxidase, and NADPH oxidase, the latter being a ROS generating enzyme that is involved in innate immune defense and cell growth. A considerable amount of phospholipid oxidation products detected in biological samples (tissues and fluids) are likely to be formed by non-enzymatic reactions, since the oxidized lipid isolates are mostly enantiomeric. PC is the main phospholipid in all mammalian cells (40–50%) and lipoprotein particles and thus, most oxidized phospholipids detected in mammalian tissues contain the choline moiety. However, recently oxidized PE has been found in the retina, which is a tissue that contains very high amounts of ethanolamine lipids [1]. In addition, there are also reports providing evidence for the presence of oxidized PS in the surface of apoptotic cells [2].

In eukaryotic phospholipids, the *sn*-1 position is either linked to an acyl residue via an ester bond or an alkyl residue via an ether bond, whereas the *sn*-2 position almost exclusively contains acyl residues. The highly oxidizable polyunsaturated fatty acids are preferably bound to the *sn*-2 position of glycerophospholipids. Thus, most of the oxidized phospholipids are modified at this position. At the *sn*-1 position of glycerol a saturated fatty acid is usually bound. By contrast, plasmalogens (alkenylacylglycerophospholipids) contain a vinyl ether bond in position *sn-1* and, as a consequence, they are also susceptible to oxidative modifications at the *sn*-1 position.

1.1. Formation of oxidized diacyl glycerophospholipids

In mammalians, the *sn*-2 position of diacylglycerophospholipids is frequently linked to polyunsaturated fatty acids (PUFAs) that are prone to oxidative modification. The dissociation energy of their bisallylic carbon–hydrogen bonds is low and thus, a hydrogen atom can easily be removed followed eventually by reaction with molecular oxygen [3]. The PUFAs found in

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mammalian glycerophospholipids not only comprise essential fatty acids like AA and LA, but also all long-chain fatty acids generated endogenously from AA or LA including docosahexaenoic acid or eicosapentaenoic acid. Oxidation of a single PUFA in a glycerophospholipid like PA-PC results in a plethora of different reaction products. The first steps in lipid peroxidation consist of hydrogen abstraction, rearrangement of double bonds and addition of triplet oxygen [4,5] leading to highly reactive peroxyl radicals (Fig. 1). These radicals can undergo a large variety of consecutive reactions including further hydrogen abstraction, fragmentation generating truncated phospholipids and different types of low molecular weight aldehydes (e.g. HNE, MDA), or reactions via the isoprostane pathways (Fig. 1). Finally, the oxidatively modified acyl chains of glycerophospholipids can be released from the glycerol backbone by intracellular and plasma phospholipases including PAF-acetyl hydrolases [6].

1.1.1. Formation of glycerophospholipids containing peroxy fatty acids

Hydrogen abstraction of phospholipid-bound PUFAs, e.g. by alkyl peroxyl radicals, leads to formation of hydroperoxide fatty acyl derivatives. These compounds subsequently decompose to various products including 11-HETE or 15-HETE that are generated from AA. Different hydroperoxy fatty acids derive from other PUFAs, e.g. oxidation of LA that leads to 9-HODE and 13-HODE. Although fatty acid hydroperoxides are unstable and tend to decompose by forming multiple reaction products (see below), they can be found in human atherosclerotic plaques (Table 1). In addition to free radical-mediated reactions, phospholipid hydroperoxides also can be generated through direct oxidation of PUFA-PCs with singlet oxygen (Fig. 1).

1.1.2. Formation of truncated phospholipids and low molecular weight aldehydes

Fatty acid hydroperoxides in PC are easily reduced. Fragmentation of the formed hydrodienes is a common reaction leading to so-called γ-hydroxyalkenal PCs. γ-Hydroxyalkenal PCs such as HOOA-PC, KOOA-PC, HOdiA-PC, and KOdiA-PC [7,8] are not only characterized by truncated *sn*-2 acyl chains, but also contain a terminal γ-oxygenated-α,β-unsaturated aldehyde or carboxyl group function (Fig. 1). These aldehydes share this structural motif with the well-known fatty

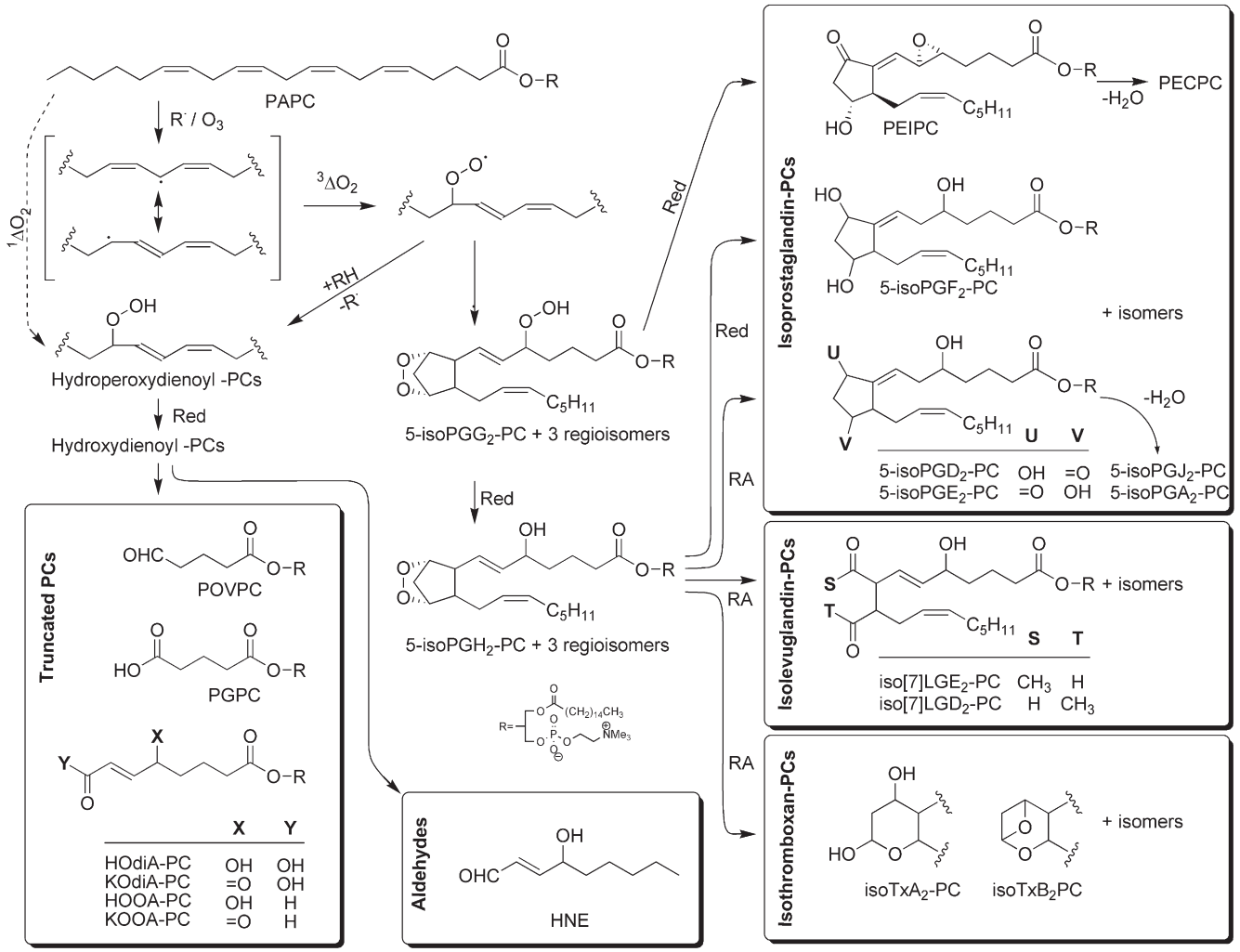

Fig. 1. Free radical-induced oxidation of 1-hexadecanoyl-2-eicosatetra-5′,8′,11′,14′-enoyl-*sn*-glycero-3-phosphocholine (PA-PC) leads to many different products such as short-chain aldehydes, truncated oxidized phospholipids, isoprostanes, isolevuglandins, and isothromboxanes. RA, reaction involves rearrangement; Red, reaction involves reductive steps.

acid peroxidation product HNE [9], another prominent fragmentation product of lipid peroxidation. Further fragmentation of γ-hydroxyalkenal PCs leads to OV-PC and G-PC [8], which have been found in atherosclerotic lesions at similar concentrations compared with γ-hydroxyalkenal PCs (Table 1). In addition to truncated phospholipids that are generated from arachidonoyl PC, degradation products of linoleoyl PCs were also found in biological material. The concentrations of these PL-PC-derived γ-hydroxyalkenal analogs have also been found to be higher in atherosclerotic tissue than in normal tissue. However, the absolute amounts of these γ-hydroxyalkenal PCs were lower as compared to their more highly oxidized relatives ON-PC and ND-PC [8].

Very recently, it was reported that a subset of γ-hydroxyalkenal PCs can react intramolecularly via a hemiacetal intermediate to form a new class of oxidized phospholipids containing a terminal furyl moiety at their *sn*-2 position [10]. This type of oxidized phospholipids was not only generated in vitro but, more importantly, also found ex vivo in brain tissues following cerebral ischemia.

1.1.3. Non-enzymatic isoprostane formation

Enzymatic formation of prostaglandins by cyclooxygenases exclusively leads to compounds with *trans* stereochemistry of the long-chain substituents in the prostane ring and no region- or

Table 1
Content of different PA-PC oxidation products found in atherosclerotic lesions of humans and other mammalian atherosclerosis models

HETEs	Oxidized phospholipid	Control/healthy	Atherosclerosis	Tissue type	Reference
		[ng/μg arachidonic acid]			
	11-HETE	–	0.84±0.24	Human lesions	[144]
	15-HETE	–	0.66±0.24	Human lesions	[144]
Truncated oxidized phospholipids		Chow diet[a]	Atherogenic diet[a]		
		[ng/mg wet tissue weight]			
	POV-PC	41.6±7.0	183.1±54.5	NZW rabbit aortas	[48]
		23.1±3.4	63.7±11.9	rabbit aortas	[145]
	PG-PC	35.3±5.5	104.2±26.3	NZW rabbit aortas	[48]
		18.6±2.0	45.4±11.3	rabbit aortas	[145]
		WHHL <3 months	WHHL >6 months		
		[ng/mg PAPC]			
	POV-PC	0.92±0.18	3.5±0.39	WHHL rabbit aortas	[83]
	PG-PC	0.82±0.48	2.3±0.05	WHHL rabbit aortas	[83]
	HOOA-PC	0.62±0.21	3.2±0.72	WHHL rabbit aortas	[83]
	KOOA-PC	0.59±0.21	3.1±0.89	WHHL rabbit aortas	[83]
	HOdiA-PC	0.26±0.18	1.4±0.10	WHHL rabbit aortas	[83]
	KOdiA-PC	0.05±0.05	0.62±0.07	WHHL rabbit aortas	[83]
Isoprostanes		[pmol/μmol phospholipids]			
	15-*iso*-PGF_2	0.045–0.115	1.31–3.45	Human lesions	[146]
	$F_{2\alpha}$ isoprostane[b]	0.16–0.44	5.6–13.8	Human lesions	[146]
		[pg/mg dry tissue weight]			
	F_2-isoprostanes	11.4±6.2	75.9±29.3	Human lesions	[147]
		Chow diet[a]	Atherogenic diet[a]		
		[ng/mg wet tissue weight]			
	PEI-PC	29.2±3.1	141.5±45.2	NZW rabbit aortas	[48]
		18.9±1.4	58.6±12.9	rabbit aortas	[19,145][c]
Isolevulglandins		Healthy subjects	Subjects with CHD		
		Plasma protein adduct level [pmol/mL]			
	iso[7]LGD_2	3500±100	8500±3100	Human	[26]
	isoLGE_2	121	306	Human	[148]
	iso[4]LGE_2	1691±252	2224±439	Human	[26]

WHHL is Watanabe hereditable hyperlipidaemic rabbit; NZW is New Zealand White rabbit.

[a] All values recalculated to 1-palmitoyl-*sn*-phospholipids and summed up. For values of the PLPC-derived truncated phospholipids see ref. [83] and for linoleic acid-derived hydroperoxide containing fatty acids see ref. [144].

[b] Derived from eicosapentaenoic acid.

[c] Compound detected in [145], but structure was determined doubtlessly later by [19].

stereoisomers. In contrast, non-enzymatic formation of isoprostanes always yields derivatives that contain the thermodynamically more stable *cis* stereochemistry and racemic mixtures of various isomers. Free PUFAs as well as PUFAs esterified to phospholipids appear to serve as substrates for non-enzymatic formation of isoprostanes in vivo [11]. In contrast, phospholipid-bound PUFAs are not modified by cyclooxygenases since these enzymes only accept free fatty acids as substrates [12]. Addition of two molecules of oxygen to one molecule of arachidonoyl phospholipid generates four isomers of bicyclic endoperoxide intermediates. This is due to nonregioselective abstraction of hydrogen atoms from arachidonoyl PC forming three regioisomeric pentadienyl radicals from which four regioisomeric peroxyeicosatetraenoyl radicals are generated by reaction with oxygen. Subsequently four structurally isomeric endoperoxide intermediates are formed (Fig. 1), namely 5-isoPGG$_2$-PC, 8-isoPGG$_2$-PC, 12-isoPGG$_2$-PC, and 15-isoPGG$_2$-PC. Although the probability of abstraction is the same for the three bisallylic hydrogen atoms in AA, very different amounts of the four isoPGG$_2$-PC regioisomers were found in rat liver [13]. The 5- and 15-isoPGG$_2$-isomers, which are more abundant, are generated from endoperoxides that cannot undergo further oxidation or cyclization and thus, accumulate in biological fluids and tissues [14]. Reduction of the isoPGG$_2$-PCs yield four regioisomeric endoperoxide isoPGH$_2$-PCs that can be further reduced to form isoPGF$_2$-PCs, each consisting of eight racemic diastereomers. Alternatively, the four isoPGH$_2$-PCs can undergo various rearrangements, leading to isothromboxanes or E$_2$/D$_2$-isoprostanes (Fig. 1). F$_2$-isoprostanes are formed together with E$_2$/D$_2$-isoprostanes in a competitive manner. Recently, it has been shown that this process depends on the redox status of the cell. Under conditions of oxidative stress, the formation of both types of isoprostanes is more pronounced than under normal conditions, but depletion of cellular antioxidants favors the formation of E$_2$/D$_2$-isoprostanes as compared to F$_2$-isoprostanes [15]. Consequently, the ratio of E$_2$/D$_2$-isoprostanes to F$_2$-isoprostanes is a good measure for the reducing capacity in that tissue. F$_2$-isoprostanes are stable compounds and thus, represent in vivo markers of lipid peroxidation in biological fluids and tissues. Except for conditions of severe oxidant injury, free E$_2$/D$_2$-isoprostanes are undetectable in normal plasma, but can easily be measured in tissues. E$_2$/D$_2$-isoprostanes are not the final products of the isoprostane pathway. They can be dehydrated to give reactive cyclopentenone A$_2$/J$_2$-isoprostanes [16] (Fig. 1). These highly reactive electrophiles form Michael adducts with cellular thiols that become rapidly metabolized by glutathione transferases. Water-soluble glutathione conjugates are generated that can be quantified in human urine [17].

In addition to the endoperoxide core, the allylic hydroperoxide group of isoPGG$_2$-PC can also undergo dehydration thus generating allylic epoxides [18] from which the 5,6-epoxyisoprostane E$_2$ derivatives are obtained. These reaction products are commonly known as PEI-PC [19]. They also can be dehydrated to the corresponding A$_2$-isoprostane (PEC-PC). High amounts of PEI-PC have been found in atherosclerotic lesions that are comparable to the concentrations of the truncated phospholipids OV-PC and G-PC in the diseased vascular tissue (Table 1).

Recently, it has been shown that 15-isoPGE$_2$ undergoes keto-enol tautomerization in aqueous buffer leading to the formation of the prostaglandin PGE$_2$ [20]. From this observation it was concluded that prostaglandins also can be generated via the non-enzymatic isoprostane pathway in vivo.

1.1.4. Formation of isolevuglandins

A completely different rearrangement of isoPGH$_2$-PC, involving migration of one of the bridgehead hydrides in combination with cleavage of the endoperoxide O − O and a C − C bond (bridge carbon), yields one of two isomeric, acyclic, keto-aldehyde-PCs (isolevuglandin-PCs, Fig. 1) [21]. These phospholipids can be generated from all four isomeric isoPGH$_2$-PCs, thus giving rise to the formation of eight highly reactive diastereomeric oxidation products [22]. This type of rearrangement reaction may also occur in vivo at rates comparable to endoperoxide decomposition leading to isoprostanes. While many free isoprostanes can easily be isolated from biological fluids and tissues, the highly reactive isolevuglandins have been overseen in biological samples for a long time and were only discovered as protein-adducts using an immunological approach [22]. Isolevuglandins rapidly form Schiff bases [23] and pyrroles [24] with the ε-amino groups of protein lysyl residues, that are eventually oxidized, generating lactam and hydroxylactam end products [25]. The mean levels of all protein-adducts of isolevuglandins appear to be highly increased in plasma from individuals with advanced atherosclerosis as compared to controls [26]. Isoprostanes, which are rapidly cleared from the blood stream, may be considered reliable "snapshots" of oxidative stress. In contrast, isolevuglandins rapidly bind to proteins in a covalent manner. Since many plasma proteins have half-lives of several weeks [27], isolevuglandins accumulate over days or weeks and therefore, have been suggested as a cumulative index for oxidative injury [26].

1.2. Oxidized alkyl glycerophospholipids

Free radical-induced oxidation of 1-*O*-hexadecyl-2-arachidonoyl-PC results in generation of the so-called C$_4$-PAF analogs Butanoyl-PAF and Butenoyl-PAF (Fig. 2), which was first shown by Marathe and colleagues [28]. HAz-PC is another oxidized alkyl phospholipid that was detected in oxLDL after removal of the large excess (99.5%) of diacyl phospholipids by phospholipase A$_1$-catalyzed hydrolysis [29]. Analysis of the remaining phospholipids revealed that besides the C$_4$-PAF analogs, HAz-PC (Fig. 2) was the main oxidation product of alkyl glycerophospholipids in oxLDL.

From plasmalogens (alkenylacylglycerophospholipids), which contain a vinyl ether bond in position *sn-1* of glycerol, oxidation products were found that are modified in the *sn*-1 and/or *sn*-2 positions. 1-Lyso-2-acylglycero-3-phosphocholine and 1-formyl-2-acylglycero-3-phosphocholine [30] are products of oxidative and hydrolytic degradation at the *sn*-1 position. Oxidative modifications of the *sn*-2 acyl position of plasmalogens can be the same as described for diacyl phospholipids (see Section 1.1). The amounts of oxidized

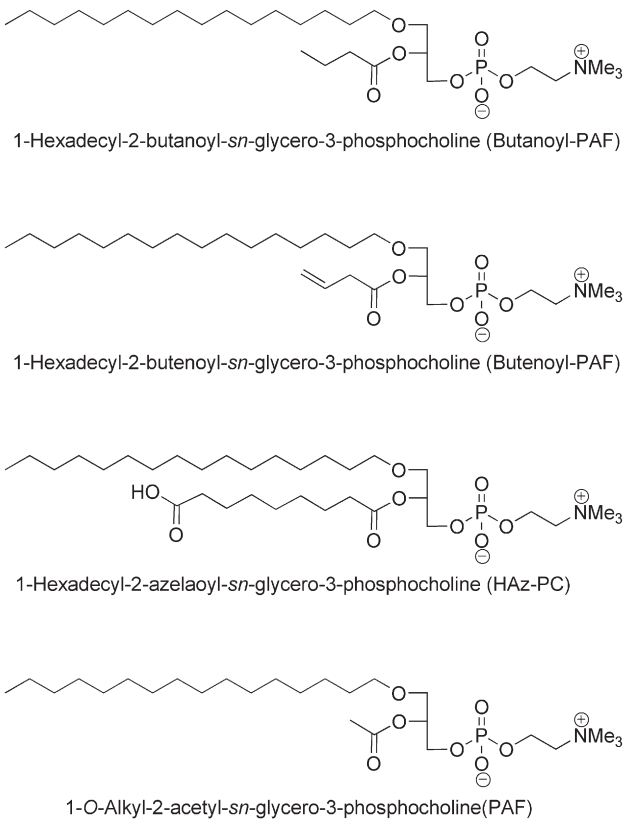

Fig. 2. Chemical structures of platelet-activating factor (PAF) and PAF-like lipids that can be formed during oxidation of alkylacylphospholipids.

ether phospholipids in biological fluids and tissues have not been determined yet.

2. Biophysical properties of phospholipid oxidation products

Biomembranes contain different phospholipid classes (headgroup heterogeneity), subclasses (acyl, alkyl chains) and species (chain length and unsaturation). The differences in chemical structure determine the physical properties of these important membrane and lipoprotein components. PC tends to form bilayers with little curvature, while PE imposes a negative curvature on these lipid bilayers [31]. Conversely, introduction of the micelle-forming LPC into a PC membrane results in a positive curvature. In addition to the polar headgroups the polarity, length and unsaturation of the phospholipid acyl chains have also an impact on physical membrane properties. Thus, phospholipid oxidation products (Fig. 1) are very likely to change the properties of biological membranes, because their polarity and shape may differ significantly from the structures of their parent molecules. Thus, they may unspecifically alter lipid–lipid and lipid–protein interactions and, as a consequence, also membrane protein functions.

Since natural biomembranes are very complex structures, defined biophysical studies on the impact of phospholipid oxidation on membrane properties have been performed using artificial membrane systems including small unilamellar vesicles (SUV), multilamellar vesicles (MLV) or phospholipid monolayers.

Upon oxidation of PC-SUVs in an air atmosphere, the transbilayer movement of PC increases. Information about effects of lipid oxidation on phospholipids mobility in SUVs has been obtained from time-resolved fluorescence anisotropy and excimer/monomer ratios of pyrene labeled phospholipids [32,33]. It was inferred from fluorescence correlation spectroscopy studies that the SUVs containing higher amounts of unsaturated phospholipids had a higher negative curvature thus facilitating the partition of the oxidant into the hydrophobic membrane region. Once the unsaturated phospholipids are oxidized (loss of double bonds), the membrane lipids are more rigid, showing lower lateral diffusion and decreased local lipid mobility.

A recent EPR spectroscopy study used β-*n*-doxylstearoyl-PC as a spin label reports on the detection of phase separations in MLV containing different mixtures of DP-PC, LP-PC and oxidized LP-PC. Oxidized LP-PC induced phase separation in the densely packed DP-PC-rich membranes, whereas lipid mobility is almost unaffected by the oxidized lipids in more fluid LP-PC-rich membranes [34]. Very recently, two oxidatively modified diacyl PCs, namely PAz-PC and PON-PC, have been investigated in DP-PC monolayers. The authors observed phase separation, upfolding of the truncated polar fatty acid chain towards the lipid–water interface and, as a result, monolayer expansion. Most of the oxidized phospholipids partitioned finally into the aqueous subphase [35].

Studies on isolated mitochondria showed that oxidation of membrane lipids destroyed lipid bilayer packing in vitro [36] and in vivo [37]. Oxidized phospholipids also influence the functions of membrane proteins. In this context, impairment of ion transport and negative effects on membrane proteins have been observed [38,39].

3. Biochemical reactivity of phospholipid oxidation products

Depending on their chemical structure, oxidized phospholipids can interact with other biomolecules in a purely physical manner or by chemical reaction. The spectrum of chemical reactivity of phospholipid oxidation products is very broad. Modification reactions may lead to stable (e.g. F_2-isoprostanes) or reactive compounds (e.g. isolevuglandins or γ-hydroxyalkenals). Some of these reactions can be considered relevant for detoxification, but most are leading to modification of important functional molecules and thus, negatively influence their activity within a cell. Incorporation of oxidized phospholipids into cells can be mediated by direct uptake and integration into membranes or via receptor-mediated pathways. In the following sections we separately discuss these basic mechanisms underlying the action of oxidized phospholipids.

3.1. Detoxification of oxidized phospholipids

Most truncated phospholipids and the oxidation products that are formed via the isoprostane pathway contain an oxidatively modified acyl chain at their *sn*-2 position which is more polar than the original PUFA. Phospholipases play an important role in the metabolism of these compounds. Calcium-independent

intracellular (iPLA$_2$), calcium-dependent intracellular (cPLA$_2$), and calcium-dependent secretory (sPLA$_2$) phospholipases were shown to release the modified *sn*-2 carboxylic acids from oxidized phospholipids [40] thus generating 2-lyso-phospholipids and free oxidized fatty acid derivatives. Platelet activating factor acetylhydrolase (PAF-AH, Lp-PLA$_2$) hydrolyzes glycerophospholipids with short acyl chains, including PAF and truncated oxidized phospholipids (e.g. OV-PC, G-PC). PAF-AH might play an anti-inflammatory role in human diseases by preventing the accumulation of oxidized phospholipids [41]. However, the role of PAF-AH in atherosclerosis is controversial because the production of LPC and oxidatively modified free fatty acids can also promote the pathogenesis of atherosclerosis. For instance, Carpenter et al. showed that LPC was more toxic to macrophages than oxidized LDL containing oxPA-PCs and that toxicity and induction of apoptosis was decreased in the presence of a PAF-AH inhibitor [42,43]. Inhibition of LDL-associated PAF-AH in hypercholesterolemic rabbits also suggests that this enzyme might have pro-atherogenic effects [44]. Furthermore, elevated plasma levels of PAF-AH were recently shown to be a novel cardiovascular risk marker and an independent predictor of coronary artery disease in men [45]. On the contrary, overexpression of PAF-AH in Chinese hamster ovary-K1 cells suppressed oxidative stress-induced apoptosis [46]. In addition, data from our laboratory suggest that serum phospholipases, which hydrolyze oxidized phospholipids, protect vascular smooth muscle cells from apoptotic cell death [47]. Furthermore, other studies show that LPC, unlike oxidized phospholipids, is not able to trigger inflammatory transcription events like IL-8 expression [48]. Tselepsis et al. concluded that PAF-AH or PAF-AH-like activity associated with HDL particles plays a predominantly anti-atherogenic and anti-inflammatory role through its reduction of monocyte adhesion to the endothelium, its ability to abrogate the biological activity of minimally modified LDL, and finally its capacity to attenuate phospholipid oxidation and induce reduction in lesion volume via a decrease in macrophage homing in animal models [49]. Thus, depending on its location (LDL-associated vs. HDL-associated), PAF-AH plays a dual role in the pathogenesis of atherosclerosis, serving a protective function against mild inflammatory and oxidative stress but promoting the development of pro-atherogenic states under more severe states in which excess amounts of oxidized phospholipids are produced [41]. Furthermore, it is also dependent on the cell type and the progression of the disease, whether inhibition of PAF-AH exerts anti- or pro-atherogenic effects.

F_2-isoprostanes, which are considered markers for oxidative stress in human body fluids (e.g. urine) are also released from their oxidized glycerophospholipids by the action of PLA$_2$s [6]. Fatty acid hydroperoxides which are released by the action of the same enzymes are eventually reduced and thus detoxified by cytosolic glutathione peroxidase (GPx) [40,50]. In this context, it is noteworthy that oxidized phospholipids and oxLDL enhance the expression of glutamate-cysteine ligase, the key enzyme of glutathione formation [51]. In addition, GPxs which directly reduce phospholipid hydroperoxides were recently detected in mammalian cells [52–54].

3.2. Chemical reactions of phospholipid oxidation products

Many phospholipid oxidation products contain chemically reactive groups including aldehydo ore keto carbonyls, epoxides or double bonds in conjugation to carbonyl groups (Figs. 1 and 2). As a consequence, oxidized phospholipids can undergo chemical reactions with a large variety of biological molecules containing nucleophilic functional groups. Representative examples are described in the following chapters. In addition, it should again be emphasized here that the reactive carboxylic acids can be released from the parent phospholipid by PLA$_2$s (see Section 3.1). In free form, they will undergo qualitatively the same reactions they would perform in the phospholipid-bound state. However, the target molecules might be different, since the polarities and thus, the preferred cellular compartments are different, too.

3.2.1. Reactions of aldehydes

Aldehydes can form Schiff bases with primary amino groups of e.g. proteins and amino phospholipids (Fig. 3A), thus affecting structure and function of these biomolecules. In addition, aldehydes can be modified by oxidation or reduction leading to formation of carboxylic acids and alcohols, respectively. Studies with specific enzyme inhibitors and cells overexpressing aldose reductase provided evidence that human aldose reductase is responsible for the reduction of free aldehydes such as HNE and the aldehydo phospholipid POV-PC [55,56].

3.2.2. The γ-hydroxyalkenals—much more reactivity!

Members of this group of oxidized phospholipids are either truncated phospholipids or free γ-hydroxyaldehydes. The latter are released from their glycerophospholipid backbone by the action of phospholipases or directly generated by free radical-induced peroxidation of linoleates or arachidonates. HNE is the most extensively studied derivative. It is an aldehyde possessing three reactive groups and undergoes multiple chemical reactions. Since it is a vinyl aldehyde, the carbon atom in β-position relative to the carbonyl group has a partial positive charge which is further enhanced by the inductive effect of a hydroxyl group at the γ-carbon. In addition, all three functional groups may act in concert contributing to its high reactivity. One of the biologically most important reactions of γ-hydroxyalkenals is the addition of thiol or amine nucleophiles to its electrophilic β-carbon atom (Michael addition, Fig. 3B). The resulting Michael adduct as well as the γ-hydroxyalkenal itself contains a γ-hydroxyaldehyde moiety that can further react either with alcohols, thus forming hemiacetals and acetals, or intramolecularly forming cyclic hemiacetals (Fig. 3B). The Michael addition of GSH to HNE is catalyzed by glutathione-*S*-transferases. In vivo, HNE forms the conjugate mercapturic acid with glutathione leading to detoxification and urinary excretion of HNE [57].

The second major reaction of γ-hydroxyalkenals is the formation of Schiff bases with primary amines (Fig. 3B). In a consecutive reaction pyrroles are formed by dehydration and cyclization of the adducts. 2-Pentylpyrroles that are reaction products of HNE with the ε-amino group of protein lysines were found in vivo. The levels of these protein adducts with HNE and

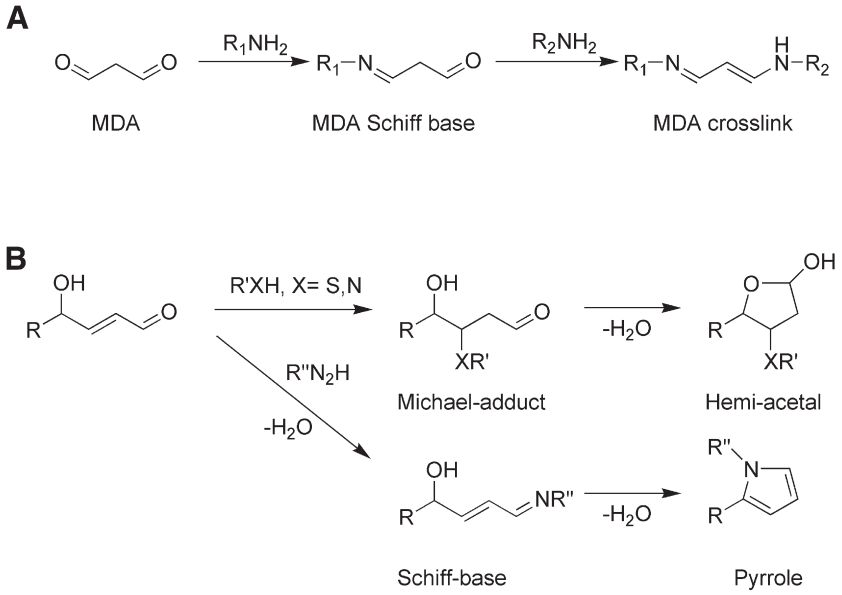

Fig. 3. Reactions of aldehydes formed by phospholipid oxidation. (A) Aldehydes can form Schiff bases with primary amines and bifunctional aldehydes can lead to crosslinking of two primary amines. (B) γ-hydroxyalkenals are much more reactive and can react via various pathways, either with nucleophiles to form hemiacetals (Michael addition), or with primary amines via Schiff bases to form pyrroles.

other γ-hydroxyalkenal phospholipids are significantly elevated in patients with atherosclerosis as compared to healthy individuals [58,59]. Schiff bases of γ-hydroxyalkenals with proteins can subsequently undergo Michael additions to other molecules thus forming protein–protein cross-links. In addition to proteins, amino lipids such as PE can also form Schiff bases with γ-hydroxyalkenals and their corresponding pyrrol end products [60]. Michael addition of the DNA base guanosine gives a tricyclic adduct in vivo that is considered to be at least in part responsible for the gentoxic effect of HNE [61]. Another reactive aldehyde, 2,3-epoxy-4-hydroxynonenal has also been proposed to modify DNA bases, but the mechanism of this reaction is still not fully understood. An etheno guanosine adduct of this aldehyde was found in human lung tissue [62]. From systematic studies in vitro, evidence is available that aldehyde phospholipids such as POV-PC also form addition products with proteins [44]. Lipid–protein adducts have also been found in tissue using antibodies specific for the choline headgroup of the protein associated oxidized phospholipids (see Section 3.3.5).

3.2.3. Reactivity of isolevuglandins and levuglandins

Levuglandins, that are formed enzymatically or nonenzymatically as well as free radical-generated isolevuglandins are γ-dicarbonyl compounds which undergo Paal–Knorr condensation with primary amines leading to the formation of pyrroles [24]. This reaction occurs via Schiff base formation and tautomerization to the corresponding enamine (Fig. 4A). These very efficient reactions are responsible for the formation of LGE_2-protein adducts. The reaction of LGE_2 with albumin is two orders of magnitude faster than the condensation of HNE with the same protein [25]. The condensation products dehydrate to form pyrroles that are slowly oxidized in the presence of oxygen or peroxyl radicals. Lactams and hydroxylactams are generated which are stable end products [22,25]. Alternatively, the Schiff bases that can be stable for minutes may react with a second primary amine leading to aminal formation. The primary amine can either be a low molecular compound or another free lysyl group of a protein (Fig. 4B). The latter reaction of levuglandins and isolevuglandins leads to cross-linking of proteins and formation of high molecular weight protein–protein adducts in vitro [63]. Free radical-induced isoprostane formation and overproduction of prostanoids through the cyclooxygenase pathway have been reported to contribute to pathophysiologic courses by protein cross-linking in vivo [64,65].

3.3. Receptors for oxidized phospholipids

Oxidized phospholipids are largely recognized by a molecular machinery that was evolved to protect an organism from invading microorganisms or transformed and damaged cells. Cell surfaces display germline-encoded receptors for conserved pathogen-associated molecular patterns (PAMP), such as lipopolysaccharide (LPS), peptidoglycans, lipoteichoic acid, or dsRNA [66]. These pattern-recognition receptors (PRR) can reside on the cell surface, like the scavenger receptors [8,67] or Toll-like receptors (TLR), or they circulate in the blood stream like C-reactive protein (CRP) [68], LPS binding protein (LPB) and plasma CD14 [69] (Fig. 5).

3.3.1. Scavenger receptors

The oxidized lipids of oxLDL were supposed to be solely responsible for the cellular uptake and recognition of the particle via CD36, a member of the scavenger receptor class B family [70]. This assumption is supported by the observation that macrophages from CD36 knockout mice were resistant to foam cell formation in vitro and that CD36-dependent signaling cascades were necessary for murine macrophage foam cell

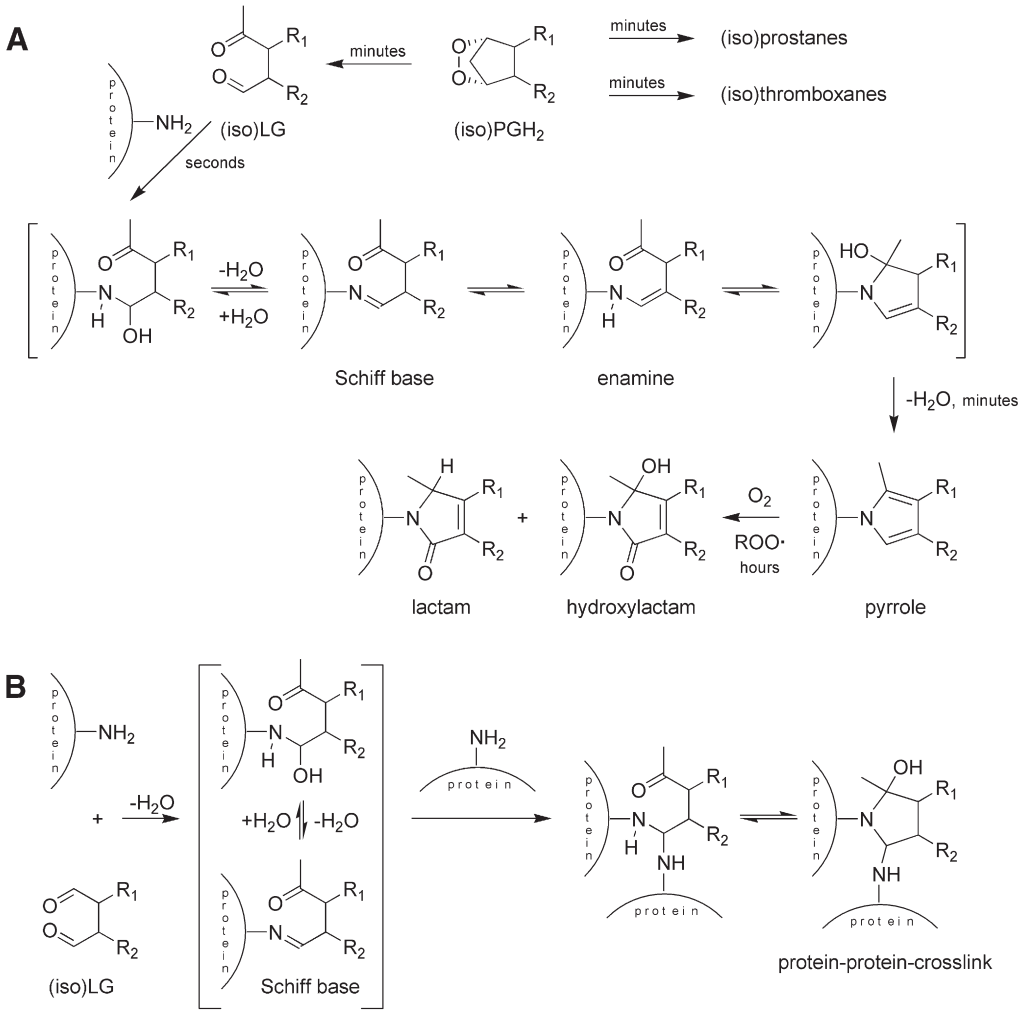

Fig. 4. Formation and reactions of isolevuglandins. (A) Isolevuglandins are formed by decomposition of tricyclic H_2 (iso)prostanes and subsequently can react with proteins to form protein adducts. (B) Isolevuglandins can crosslink proteins.

formation [71]. These mice also developed less atherogenic lesions than wild-type mice [72]. CD36 is a multi-ligand receptor, that recognizes amongst others oxLDL [73,74], thrombospondin [75,76], PS [70,77], and long-chain fatty acids [78]. The binding sites for oxLDL, thrombospondin [79,80] and PS [81] are different. Several studies have identified oxidized phospholipids derived from PL-PC and PA-PC as ligands of CD36. The structural requirement for high affinity binding to CD36 is a γ-oxygenated-α,β-unsaturated aldehyde or carboxyl group at the *sn*-2 position of truncated phospholipids [8]. Keto dicarboxylic monoesters KOdiA-PC and KDdiA-PC (Fig. 1) derived from PA-PC and PL-PC, respectively, are the oxidized phospholipids with the strongest CD36 binding properties. However, the same lipids are not recognized by scavenger receptor A1, except they are bound to apoB [82]. The fatty acid chains (e.g. oleate versus palmitate) at the *sn*-1 position did not influence CD36 binding [8]. Addition of just a few molecules of these keto dicarboxylic monoesters to liposomes prepared from unoxidized LDL lipids already led to macrophage binding, uptake of the entire particle, cholesterol loading and foam cell formation [83].

3.3.2. Toll-like receptors

TLRs are type 1 transmembrane receptors that are highly conserved throughout evolution containing an intracellular Toll/IL-1 (TIR) domain and an extracellular leucine-rich domain. In mammalians, 12 different TLRs have been identified to date. They are critical to the function of both innate and adaptive immunity and are expressed in cells regulating inflammation and immunity such as macrophages, endothelial cells or dendritic cells. TLRs detect and bind PAMPs and their ligands include LPS (recognized by TLR4) and various bacterial lipids, such as lipoteichoic acid, peptidoglycan and lipopeptides that are recognized by TLR2 heterodimers with TLR1 or TLR6. In contrast to these cell surface-expressed TLRs, there are also intracellular TLRs that bind to both viral double stranded RNA and DNA. But, in addition to recognition of bacterial- and viral-derived molecules, various endogenous ligands that are produced in response to stress or tissue injury have recently been suggested [84]. Interestingly, TLR1, TLR2 and TLR4 are expressed in both mouse and human atherosclerotic lesions and are upregulated by oxidized LDL. [85]. Furthermore, phospholipid oxidation

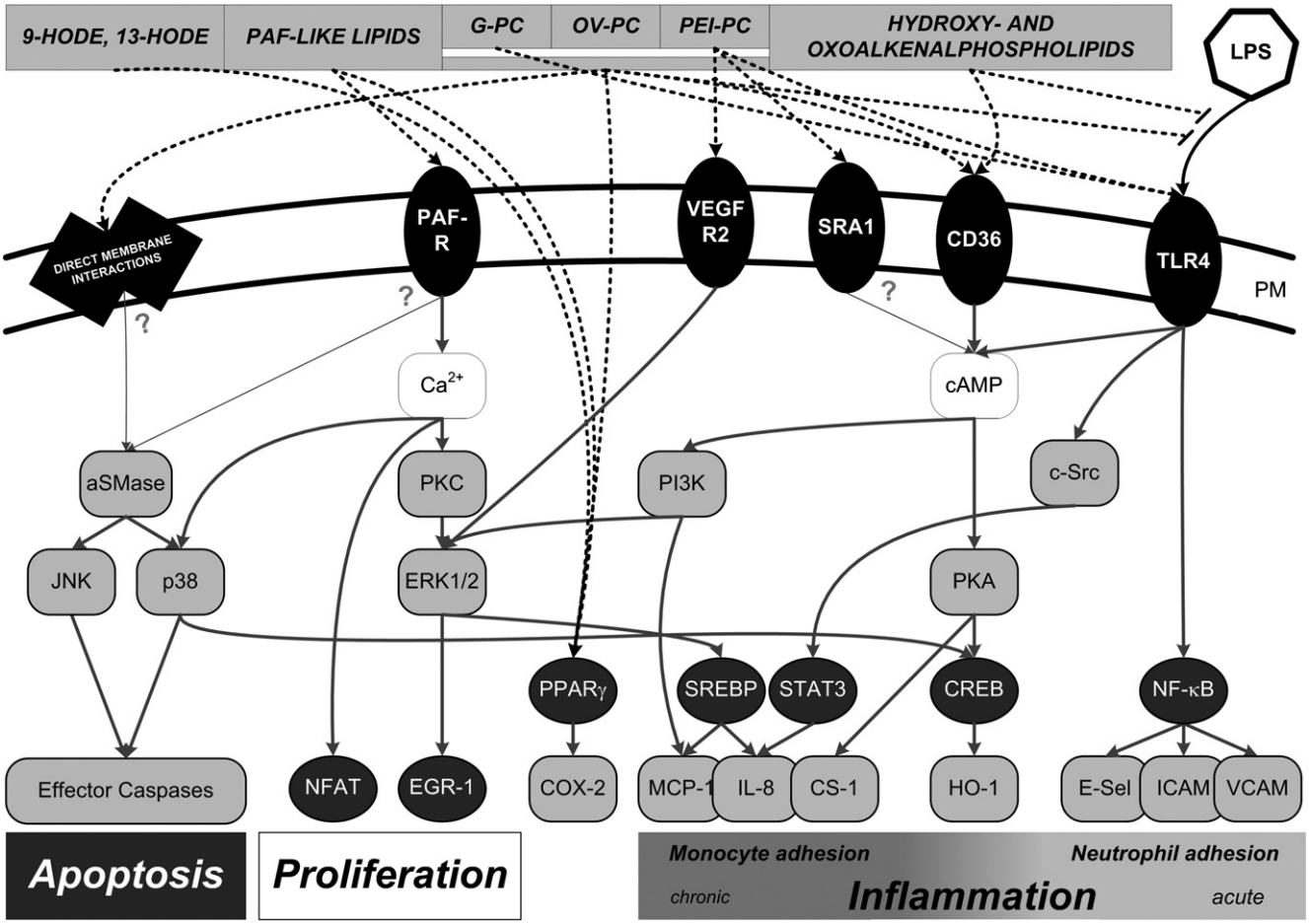

Fig. 5. Different pure oxidized phospholipids activate distinct signaling pathways leading to various inflammatory responses or apoptosis. For simplification, the most relevant pathway components are shown. aSMase, acid sphingomyelinase; COX-2, cyclooxygenase 2; CREB, response element-binding protein; CS-1, connecting segment 1; EGR-1, early growth response protein 1; ERK1/2, extracellular signal-related kinases 1/2; E-Sel, E-selectin; G-PC, glutaric acid ester of LPC; HODE, hydroxyoctadecanoic acid (position of hydroxyl group not specified); HO-1, heme oxygenase 1; ICAM, intercellular adhesion molecule; IL-8, interleukin 8; JNK, c-Jun N-terminal kinase; LPS, lipopolysaccharide; MCP-1, monocyte chemoattractant protein 1; NFAT nuclear factor of activated T-cells; NF-κB, nuclear factor kappa B; OV-PC, 5-oxovaleric acid ester of LPC; p38, p38 mitogen-activated protein kinase; PAF platelet-activating factor; PAF-R, PAF receptor; PEI-PC, 1-hexadecanoyl-2-(5,6-epoxyisoprostane-E2-oyl)-*sn*-glycero-3-phosphocholine; PI3K, phosphatidylinositol-3-kinase; PKA, protein kinase A; PKC, protein kinase C; PM, plasma membrane; PPAR, peroxisome proliferator-activated receptor; SR-A1, scavenger receptor A1; SREBP, sterol regulatory element-binding protein 1; STAT3, signal transducer and activator of transcription 3; TLR-4, Toll-like receptor 4; VCAM, vascular cell adhesion molecule; VEGFR2, vascular endothelial growth factor receptor 2.

products have been shown to interact with TLRs [86]. OxPA-PC inhibits LPS-induced inflammation by interaction with plasma LPB and CD14 in endothelial cells and macrophages. POV-PC and KOdiA-PC were identified as the main components of oxPA-PC that inhibit ligand binding to TLR4 and TLR2 [69]. Interestingly, oxPA-PC itself also shows pro-inflammatory effects by inducing interleukin-8 (IL-8) synthesis via TLR4, but not CD14 [67].

3.3.3. PAF-receptor

A variety of mammalian cells synthesize the lipid mediator PAF under inflammatory conditions. PAF receptor (PAF-R) signaling is linked to various second messenger networks through phospholipases leading to calcium fluxes, PI cycling, and activation of phosphorylation. It shows several hundred-fold specificity for the *sn*-1 ether bond of PAF compared with the 1-acyl analog and a pronounced although lower specificity for a short *sn*-2 acyl chain and the PC headgroup [3]. Oxidation of AA in alkylacyl PCs of LDL leads to formation of short chain PAF-analogs (Fig. 2) which are ten-fold less efficient receptor ligands than PAF, but still potent PAF mimetics [28]. In contrast, diacyl phospholipids with short *sn-2* acyl chains several hundred-folds less efficiently bind to the PAF receptor. All the studies indicated above compared the receptor binding capacities of PAF and oxidized phospholipids indirectly by measuring the induction of intracellular calcium fluxes. Recent binding/displacement experiments in cultured human macrophages were performed using [^{3}H]PAF and POV-PC as competitors. The binding affinity of POV-PC was only 3.4 times lower as compared to PAF [87], although much larger differences in calcium flux were found between PAF and POV-PC as compared to earlier studies [28]. Obviously, the conformational changes induced by POV-PC are not sufficient to activate the receptor in a manner comparable to PAF.

3.3.4. G Protein-coupled receptors

Parhami et al. provided evidence that G protein-coupled receptors (GPCR) may become activated by oxidized phospholipids [88]. They found that mmLDL in which mainly the lipids are oxidized increased cyclic AMP levels in endothelial cells via a putative Gαs-coupled receptor. According to a recent report [89], the mammalian GPCR G2A, which was originally identified as a stress inducible GPCR [90], responds to oxidized free fatty acids. 9-HODE and 11-HETE were the most potent derivatives in mediating intracellular calcium mobilization. 9-HPODE was also a potent ligand, whereas 13-HODE and 13-HPODE showed weaker affinities. In general, only free oxidized fatty acids were potent ligands and therefore it was concluded that hydrolytic release of the oxidized fatty acids from oxidized PC was required for the observed intracellular calcium mobilization via G2A [89]. Very recently, it was shown that the GPCRs prostaglandin E2 receptor (EP2) and prostaglandin D2 receptor (DP) are activated by PEI-PC. EP2 is expressed in endothelial cells, smooth muscle cells, and macrophages, while DP is not expressed in endothelial cells [91]. The epoxyisoprostane structure is required for receptor activation and the most potent activator of EP2 contained such an element.

3.3.5. Recognition by antibodies

The development of atherosclerosis is intimately associated with the immune response [92–94]. A number of potential antigens is present in lesions that could be responsible for the activation or a response of the immune system including bacteria and viruses, heat shock proteins, modifications of arterial wall components and lipoproteins and importantly, oxidatively modified lipoproteins and their products [95–97]. There is now considerable evidence that neo-epitopes generated on oxidized LDL are important and even dominant antigens that drive this immune response. The dominant epitopes in oxLDL are oxidized phospholipids either present in free form or as lipid–protein adducts [98]. Any phospholipid containing a polyunsaturated fatty acid is susceptible to oxidation and can generate an antigenic neo-epitope. Many of the so-called anti-phospholipid antibodies that are associated with rheumatologic diseases or with the primary Antiphospholipid Antibody Syndrome (APS) are antibodies directed against oxidized phospholipids [99]. Cardiolipin is still the standard phospholipid which is usually used for anti-phospholipid antibody screening. Antibodies purified from APS patients bound only to oxidized cardiolipin but not to an unmodified cardiolipin analog that is stable against oxidation. Furthermore, β_2GP1 which is a cofactor required for binding of anti-cardiolipin antibodies is recognized by APS sera only when bound to oxidized cardiolipin [100].

OxLDL-specific E0 antibodies, which were generated from apoE-deficient mice also recognize oxidized phospholipid epitopes [98]. The antibody EO6 recognizes the oxidized phospholipid POV-PC but not unoxidized PA-PC. EO6 specifically is able to bind to an adduct of POV-PC with BSA. Furthermore, EO6 specifically binds to apoptotic but not to viable cells and was able to blocks macrophage phagocytosis of apoptotic cells [101]. Apoptotic cells are rich in oxidized phospholipids and, as a consequence, in protein bound lipid epitopes that are recognized by E06 on the cell surface.

4. Biological effects and disease

Evidence is growing that oxidized phospholipids play a key role in the development of several chronic diseases including atherosclerosis. Oxidized phospholipids have been shown to accumulate in atherosclerotic lesions [102] and show a great variety of biological effects in vivo and in vitro. They are being identified as essential molecular components that are responsible for the pathophysiologic actions of oxidized LDL. This is just one more example showing that the use of chemically defined compounds rather than complex lipoprotein mixtures helps very much to gain a better understanding of the biological functions of complex lipid systems such as lipoproteins and membranes. In this chapter we will focus on how defined oxidized phospholipids can trigger immune response, inflammation, apoptosis and the underlying signaling pathways.

4.1. Oxidized phospholipids, inflammation and inflammatory signaling

Atherosclerosis is a chronic inflammatory disease which is characterized by specific invasion of monocytes and T-cells into the vascular wall, while neutrophils are essentially not present in atherosclerotic lesions. In acute inflammation, cytokines and bacterial products like interleukin-1 (IL-1), tumor necrosis factor α (TNFα) or LPS activate the classical NF-κB pathway. The latter in turn triggers expression of adhesion molecules like vascular cell adhesion molecule-1 (VCAM-1), intracellular adhesion molecule-1 (ICAM-1), or the neutrophil-binding molecule E-selectin, which results in adhesion of both monocytes and neutrophils. In contrast, mmLDL, oxPA-PC and one of its active compounds, pure POV-PC, activate endothelial cells to specifically bind monocytes but not neutrophils, which is a hallmark of chronic inflammation [103]. Furthermore, oxPA-PE and oxPA-PS also were found to induce monocyte adhesion to endothelial cells [48]. Monocyte adhesion to endothelial cells after stimulation with these oxidized lipids involves the activation of an $\alpha_5\beta_1$ integrin and increased deposition of connecting segment-1 (CS-1)-containing fibronectin on the cell surface [104]. It is not mediated by surface expression of the adhesion molecules VCAM-1, ICAM-1 or E-Selectin, or activation of the classical NF-κB pathway. POV-PC and oxPA-PC induce monocyte binding to endothelial cells by a cAMP-coupled pathway involving protein kinase A (PKA), R-Ras and PI3K [105].

In contrast to POV-PC, the more highly oxidized phospholipid PG-PC induces both monocyte and neutrophil binding to the endothelium. PG-PC leads to the expression of VCAM-1 and E-selectin on the endothelial surface, but does not affect CS-1 expression [48]. These data provide evidence that POV-PC, PG-PC and probably also other pure oxidized phospholipids are important regulators of leukocyte–endothelial cell interactions. POV-PC may play a dominant role in chronic inflammation under circumstances where monocyte adhesion

becomes predominant. Ultimately, it will depend on the ratio of POV-PC and PG-PC in the cells whether a chronic (monocytic) or an acute (monocytic and neutrophil) inflammation prevails [106].

In the past decade most of the studies dealing with signaling effects of oxidized phospholipids used oxPA-PC, a mixture of oxidized phospholipids that induces a plethora of biological effects. These include stimulation of tissue factor (TF) expression in human umbilical vein endothelial cells (HUVEC) involving protein kinase C (PKC), extracellular signal-related kinases 1 and 2 (ERK1/2), p38 mitogen-activated protein kinase (p38-MAPK) and increased intracellular calcium, ultimately leading to activation of the transcription factors endothelial growth related-1 (EGR-1) and nuclear factor of activated T-cells (NFAT) [107]. Very recently, in human aortic endothelial cells (HAEC) vascular endothelial growth factor receptor 2 (VEGFR2) was reported to be a major regulator in the ERK1/2-mediated activation of the transcription factor sterol regulatory element-binding protein 1 (SREBP-1) and subsequent transcription of TF, LDL receptor and IL-8 [108]. In addition, this study revealed PEI-PC to be the active component of oxPA-PC-mediated activation of this signaling pathway. Furthermore, induction of chemotactic activity of monocytes via activation of MAPK phosphatase 1 was demonstrated as well as expression of numerous inflammatory genes [109,110]. Very recently, POV-PC was found to activate $cPLA_2$ via the MAPK pathway in HUVEC resulting in the release of free AA [111]. The latter is subsequently used by lipoxygenase but not cyclooxygenase, producing 12-HETE that previously has been shown to stimulate HAEC to bind monocytes [112]. Possible targets for HETEs are the lipid-dependent transcription factors peroxisome proliferator-activated receptor α and γ (PPARα , PPARγ). Indeed, both have been reported to become activated by the products of phospholipid oxidation. PEI-PC and PEC-PC isomers, which are both found in oxPA-PC increase IL-8 and monocyte chemotactic protein-1 (MCP-1) expression in endothelial cells involving activation of PPARα [113,114]. However, although PPARα-null murine aortic endothelial cells did not produce MCP-1/JE in response to oxPA-PC [113], a role for PPARα in oxPA-PC induction of IL-8 transcription in HAEC could not be consistently demonstrated [115]. Importantly, the increase of IL-8 and MCP-1 expression induced by oxidized phospholipids was independent of the classical NF-κB pathway, but involved the activation of c-Src kinase. PEI-PC binds to the TLR4 complex and leads to rapid and transient activation of c-Src kinase that in turn induces phosphorylation and activation of the transcription factor signal transducer and activator of transcription 3 (STAT3) [116]. Subsequently, STAT3 dimerizes, translocates to the nucleus and induces transcription of IL-8. Recently, HDL was reported to inhibit oxidized phospholipid-induced c-Src/STAT3 and SREBP activation as well as endothelial nitric oxide synthase phosphorylation [117] that was previously also associated with the inflammatory effects of oxPA-PC [118]. Interestingly, HDL did not interfere with oxPA-PC-induced expression of antioxidant proteins such as heme oxygenase 1 (HO-1) or the heat-shock protein A1A via the transcription factor NF-E2-related factor-2 (Nrf2) [117]. Thus, high-density lipoprotein (HDL) not only inhibits the pro-inflammatory effects of oxidized phospholipids such as PEI-PC, but also maintains the antioxidant cellular responses to these oxidized lipids.

The oxidized fatty acids 9-HODE and 13-HODE that have also been found in oxLDL were both reported to be activators and ligands of PPAR γ, thereby inducing CD36 scavenger receptors and foam cell formation [119]. Others found that POV-PC also binds to human macrophages via the PAF-receptor followed by stimulation of intracellular calcium signaling which activates the transcription of highly proinflammatory cytokines such as IL-8 [87].

Hydroxy and oxo alkenal phospholipids such as HOOA-PC, KOOA-PC, KOdiA-PC and HOdiA-PC are another group of oxidized phospholipids found in LDL in vivo and are enriched in atherosclerotic lesions [83]. These compounds localize to the surface of oxidized LDL and represent ligands of CD36 on macrophages (see also Section 3). Lipid binding to the receptor is responsible for uptake of the whole particle, intracellular accumulation of cholesterol and foam cell formation [8,83]. HOOA-PC also activates endothelial binding of monocytes, increases formation of MCP-1 and IL-8 [120]. Furthermore, it was shown that various pure hydroxy alkenal phospholipids like KHdiA-PE, KOdiA-PE, KDdiA-PE, HDdiA-PE or HOOA-PC potently inhibit LPS-induced inflammation and thus mimic the actions of oxPA-PE or oxPA-PC [120–122]. KOdiA-PC was the most potent compound inhibiting LPS-induced expression of E-selectin 500-fold more than oxPA-PC. Very recently, it has been shown that this inhibitory effect of KOdiA-PC on LPS induction of IL-8 is mediated, at least in part, by the activation of neutral sphingomyelinase and its product ceramide might induce changes in the assembly of the LPS receptor complex [122]. Furthermore, it was found that there is some overlap in gene expression induced by LPS and oxPA-PC. But it is noteworthy that there are also significant differences [123]. Genes induced by LPS and oxidized phospholipids (oxPA-PC) include IL-6, IL-8, IP-10, and MCP-1. In addition, γ-hydroxyalkenal PCs inhibit a key function of macrophages, namely the processing of internalized material. These compounds reduce cathepsin B activity and the maturation of the fusion protein Rab5a, leading to reduced endosome–phagosome fusion with lysosomes in mouse peritoneal macrophages [7].

In addition to oxidized diacylglycerophospholipids, oxidized alkylacylglycero-phospholipids mimic PAF which is one of the most potent inflammatory mediator [124]. In general, these PAF-like lipids activate leukocytes and can stimulate the growth of smooth muscle cells [125], which is inhibited by a PAF-R antagonist. Furthermore, PAF-like lipids stimulate polymorphonuclear leukocyte adhesion to endothelial cells followed by activation of intracellular calcium fluxes [126]. Activation of platelets and stimulation of γ-interferon secretion from human monocytes is also mediated by the PAF-Rs [3]. Another oxidatively fragmented alkylacylglycerophospholipid of oxLDL is HAz-PC. It is a very potent PPARγ agonist [29], induces cyclooxygenase-2 expression and stimulates prostaglandin E2 secretion in primary human monocytes [127].

In addition to the biological effects of pure oxidized phospholipids, there is a plethora of effects of oxidized phospholipids mixtures, of which the most relevant are summarized

Table 2
Biological effects of selected pure oxidized phospholipids and oxPA-PC in macrophages, endothelial cells and smooth muscle cells

Effects in macrophages	Effects in endothelial cells	Effects in smooth muscle cells	Other effects
POV-PC			
Blocked uptake of oxLDL [98]	Induction of monocyte binding and inhibition of neutrophil binding [103]	Induction of apoptotic pathways, including SMase, p38-MAPK, JNK and caspases [47,139]	Recognized by monoclonal antibodies [19]
Binding to PAF-R and transduction of the signals leading to the intracellular Ca^{2+} fluxes and modification of the transcription levels of numerous pro-inflammatory and pro-atherogenic genes [87]	Specific monocyte binding involves activation of $cPLA_2$ and lipoxygenase [111] Upregulation of CS-1 fibronectin expression [48] Generation of angiogenic mediators via VEGF and COX-2-generated prostanoids [149]	Stimulation of UDP-galactose: glucosylceramide (β1–4) galactosyl transferase, production of lactosylceramide and activation of p44-MAPK and proliferation [150]	
PG-PC			
	Induction of monocyte and neutrophil binding, E-Selectin-expression [103] Upregulation of VCAM-1 [48] Generation of angiogenic mediators via VEGF and COX-2-generated prostanoids [149]	Induction of apoptotic pathways, including sphingomyelinases, p38-MAPK, JNK and caspases [47,139]	
PEI-PC			
	Induction of monocyte/endothelial cell adhesion via activation of cAMP/R-Ras/PI3-K signaling pathway [105] Regulation of MCP-1 and IL-8 synthesis via the c-Src/STAT3 pathway [114,115] Activation of TF and SREBP-1 via VEGFR2 [108] Activates PPARα in murine aortic endothelial cells [113]		Restoration of endothelial barrier function via Cdc42 and Rac [151]
PEC-PC			
	Activates PPARα, increases IL-1 and MCP-1 synthesis [114]		
KOdiA-PC, HOdiA-PC, KOOA-PC, HOOA-PC			
Ligands for CD36 [8,83] Inhibit the processing of internalized material, reduce cathepsin B activity and the maturation of the fusion protein Rab5a [7]	HOOA-PC activates endothelial cells to bind monocytes, causes an increase in MCP-1 and IL-8 Inhibition of LPS-induced expression of E-Selectin [120] via activation of neutral sphingomyelinase [122]		
PAF-like lipids			
Stimulation of γ-interferon secretion from human monocytes via their PAF-R [3]		Growth stimulation [125] Regulation of DNA synthesis and NO production [140] Induction of apoptosis by temporary membrane distortions [141]	Stimulate polymorphonuclear leukocyte adhesion [126] Activate platelets through their PAF-R [3]

Table 2 (*continued*)

Effects in macrophages	Effects in endothelial cells	Effects in smooth muscle cells	Other effects
Az-PC			
Potent PPARγ agonist [29]			
Induction of cyclooxygenase-2 expression and enhanced prostaglandin E_2 secretion in primary human monocytes [127]			
9- and 13-HODE			
Activators and ligands of PPARγ, leading to the induction of CD36 and foam cell formation [119]			
15(S)-HPETE			
		Induction of apoptosis [142]	
oxPA-PC (additional effects only studied as a complex mixture)			
Blocked uptake of oxLDL [98]	Microarray analysis revealed changes in >1000 genes [128]		Recognized by monoclonal antibodies [19]
Inhibition of cyclooxygenase-2 via NF-κB/IκB and ERK2-dependent mechanisms [110,152]	Induction of monocyte chemotactic activity via activation of MAPK phosphatase 1 [109]		Induction of murine MCP-1 homologue JE) in liver and heart [123]
	Activation of MCP-1 and IL-8 synthesis [114,123]		Upregulation of CD62p and CD41 receptors and inhibition of anti-CD36 MoAb binding in platelets [157]
	Depletion of endothelial cholesterol (causing caveolin-1 internalization from the plasma membrane to the ER and Golgi) and activation of SREBP [108,153]		Induction of atherosclerosis-related genes in murine arteries [158]
	Induction of genes involved in signal transduction, extracellular matrix, growth factors and chemokines [110]		
	Stimulation of tissue factor expression via activation of ERK/EGR-1 and Ca^{2+}/NFAT [107]		
	Activation of PKC, PKA, Raf/MEK1,2/ERK-1,2 MAP kinase cascade, JNK Map kinase and transient protein tyrosine phosphorylation [154]		
	Induction of heme oxygenase-1 expression involving activation of CREBP [155]		
	Down-regulation of thrombomodulin transcription by inhibiting the binding of RARβ-RXRα heterodimer and stimulatory proteins (Sp1 and Sp3) to the DR4 and Sp1 binding element in the TM promoter and reduced expression of RARβ, RXRα and SP1 and SP3 in the nucleus [156]		

in Table 2. Recently, it was found by a gene expression network approach that more than 1000 genes were regulated by oxidized phospholipids (oxPA-PC) in HAEC cultures derived from 12 individuals [128]. However, we will not discuss all biological effects of these oxidized phospholipids mixtures in detail, since it is difficult to interpret effects of mixtures on a molecular basis. Thus, it would be desirable to put more emphasis on the biological effects of pure compounds and perform future studies using chemically well-defined oxidized phospholipids.

4.2. Oxidized phospholipids, apoptosis and apoptotic signaling

Increasing evidence suggests that apoptosis is a major event in the pathology of atherosclerosis [129]. In primary and secondary lesions apoptosis may help to reduce lesion size [130,131], whereas at later stages apoptosis may contribute to the formation of unstable plaques [132,133].

On the other hand, apoptotic cells are an additional source of oxidized phospholipids and may actively contribute to inflammation [134]. Apoptosis of endothelial cells is associated with oxidation of PC, PS, and PE [135]. Oxidized PC on the surface of apoptotic cells was detected using the monoclonal antibody EO6, which exclusively binds to protein-bound oxidized PC [101]. It has been shown that EO6 can effectively block the uptake of apoptotic cells by macrophages. Thus, in addition to oxidized PS, the presence of oxidatively modified PC is an important signal for phagocytosis and externalization of oxidized phospholipids was shown to be required for macrophage clearance of apoptotic cells [136]. Blebs from apoptotic endothelial cells and biologically active oxidized membrane vesicles contain POV-PC and activate endothelial cells to bind monocytes but not neutrophils [134]. The ability of apoptotic blebs or oxidized vesicles to induce monocyte adhesion was abolished by preincubation with the EO6 antibody recognizing oxidized PC [134]. Lipid peroxidation of biological membranes also leads to the loss of phospholipid asymmetry in plasma membranes of apoptotic cells and causes membrane vesiculation. Microvesicles released from endothelial cells under the influence of *tert*-butylhydroperoxide in vitro contained the oxidized phospholipids POV-PC and PG-PC [137] and induced monocyte–endothelial interactions [134]. In contrast, membrane vesicles from activated endothelial cells failed to bind monocytes [134]. Recently, it was shown that apoptotic blebs derived from T-cells also contain oxidized phospholipids and thus induce monocyte–endothelial interactions [138].

As described above, apoptotic cells can be the source for oxidized phospholipids, but in addition, oxidized phospholipids themselves can induce apoptosis of the cells of the vascular wall.

It was shown in our laboratory, that PG-PC and POV-PC activate sphingomyelinases in arterial smooth muscle cells. The sphingolipid-hydrolyzing enzyme is known to participate in the very early phase of stress signaling. Downstream of acid sphingomyelinase (aSMase) the mitogen-activated protein kinases (MAPK) c-Jun N-terminal kinase (JNK) and p38 which are also involved in induction of stress response and apoptosis were phosphorylated. Further downstream of JNK and p38 MAPK activation of caspase 3 was observed, showing that stimulation of smooth muscle cells with POV-PC and PG-PC was associated with apoptosis [139]. Stimulation of all these enzymes by POV-PC and PG-PC almost perfectly matched the activation of intracellular signaling by mmLDL in the same cells. Obviously, oxidized phospholipids are responsible for the effects of this lipoprotein on apoptotic signaling. In contrast, components of survival and proliferation pathways including NF-κB or AKT-kinase were not activated by POV-PC and PG-PC. Experiments with NB6, which specifically inhibits gene expression of aSMase, showed that this enzyme plays a central role in mediating the apoptotic effects of POV-PC and PG-PC. In addition, oxidized phospholipid-induced apoptosis of arterial smooth muscle cells was also assessed by determining several morphological criteria, the occurrence of DNA fragmentation, and a PS shift towards the outer leaflet of the plasma membrane [47]. In all experiments POV-PC was a more potent apoptotic agent than PG-PC.

Furthermore, PAF-like lipids can regulate DNA synthesis and nitric oxide production without involvement of the PAF-R in vascular smooth muscle cells [140]. Thus, these compounds seem to be able to induce apoptosis of vascular smooth muscle cells also by temporary membrane distortions [141].

Moreover, AA and its oxidation products HPETE, 15(S)-HPETE, HNE and MDA were also shown to induce apoptosis of vascular smooth muscle cells. The apoptotic effects are characterized by annexin V binding, sub-G1 population of cells, cell shrinkage and chromatin condensation. Vascular smooth muscle cells death was attenuated by the antioxidants α-tocopherol and glutathione, the hydrogen peroxide scavenger catalase and the serum proteins albumin and gamma globulin [142].

5. Future perspectives

So far, many studies on oxidized phospholipids have been performed using mixtures of oxidation products that are generated from their polyunsaturated parent compounds, e.g. PA-PC. These preparations contain a large variety of different substances differing in structure, polarity and hydrophobicity. As a consequence, the biological availability, the degree of polarity and amphiphilicity, as well as the reactivities of the individual lipid classes contribute to the observed effects of "oxidized phospholipids" to a greatly different extent. Although in the past years various research groups became interested in the effects of pure oxidized phospholipids rather than complex mixtures, many biological effects are not fully understood or remain unclear. Thus, it will be desirable to concentrate on chemically defined lipid species in the future. In addition, it is mandatory to specify how these compounds are presented to a cell or an individual target molecule. Even the truncated phospholipids are still hydrophobic enough to bind efficiently to lipoproteins, cell membranes or proteins (albumin!). However, their diffusion rates and thus their bioavailabilities are higher as compared to the more hydrophobic dual-chain lipids. Since their action radius is much larger, they do not only act locally in a cell but may also transmit signals within a larger cross section of a tissue. This high exchangeability and mobility challenges the concept of lipid interaction with specific cell surface receptors. This phenomenon is particularly relevant to the more polar oxidized phospholipids.

After they have been generated within a cell or a lipoprotein they may interact with many different target proteins and lipids on the surface of and inside a cell leading to impaired functions of these target molecules. We recently showed that fluorescent analogs of oxidized phospholipids are easily taken up into cells and label a limited fraction of their proteins and lipids [143]. The identification of these target molecules and their downstream effectors will be an important issue in the future, which will help to understand the complex molecular network underlying the biological effects of oxidized phospholipids.

A major focus of current biomedical studies is still on the role of these compounds in classical lipid-associated disorders including atherosclerosis. Other fields such as cancer research and immunology will be emerging in the context of oxidative stress and the role of oxidized lipids in the initiation and progress of related diseases. The interdependence of cell growth, (programmed) cell death and cell function is a key aspect in the context of (patho) physiological lipid activity. Thus, it can be expected that research on the great variety of oxidized phospholipid species will not only lead to a deeper understanding of these compounds but will also provide new insights into the function of lipid mediators.

6. Abbreviations of chemical nomenclature

AA arachidonic acid
DP-PC 1,2-dihexadecanoyl-*sn*-glycero-3-phosphocholine
G-PC and ND-PC glutaric and nonanedioic monoesters of LPC
HAz-PC 1-O-hexadecyl-2-azelaoyl-*sn*-glycero-3-phosphocholine
HDdiA-PC and HOdiA-PC 9-hydroxy-10-dodecenedioic acid and 5-hydroxy-8-oxo-6-octenedioic acid esters of LPC
HETE hydroxyeicosatetraenoic acid (position of hydroxyl group not specified)
HNE 4-hydroxy-trans-2-nonenal
HODA-PC and HOOA-PC 9-hydroxy-12-oxo-10-dodecenoic acid and 5-hydroxy-8-oxo-6-octenoic acid esters of LPC
HODE hydroxyoctadecanoic acid (position of hydroxyl group not specified)
HPODE hydroperoxyoctadecadienoic acid (position of hydroxyl group not specified)
KDdiA-PC and KOdiA-PC 9-keto-10-dodecendioic acid and 5-keto-6-octendioic acid esters of LPC
KHdiA-PE 3-keto-4-hexendioic acid esters of LPE
KODA-PC and KOOA-PC 9-keto-12-oxo-10-dodecenoic acid and 5-keto-8-oxo-6-octenoic acid esters of LPC
LA linoleic acid
LDL low density lipoprotein
LPC 1-acyl-*sn*-glycero-3-phosphocholine (1-acyl group not specified)
LPE 1-acyl-*sn*-glycero-3-phosphoethanolamine (1-acyl group not specified)
LP-PC 1-hexadecadienoyl-2-hexadecanoyl-*sn*-glycero-3-phosphocholine
MDA malondialdehyde
mmLDL minimally modified LDL
OV-PC and ON-PC 5-oxovaleric acid and 9-oxononanoic acid esters of LPC
oxLDL oxidized LDL
oxPA-PC, oxPA-PS, oxPA-PE undefined mixtures of free radically-oxidized PA-PC, PA-PS, PA-PE
PAF platelet-activating factor, 1-O-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine
PA-PC 1-hexadecanoyl-2-eicosatetra-5′,8′,11′,14′-enoyl-*sn*-glycero-3-phosphocholine
Paz-PC 1-hexadecanoyl-2-azelaoyl-*sn*-glycero-3-phosphocholine
PC phosphatidylcholine
PE phosphatidylethanolamine
PEC-PC 1-hexadecanoyl-2-(5,6-epoxyisoprostane A2 oyl)-*sn*-glycero-3-phosphocholine
PEI-PC 1-hexadecanoyl-2-(5,6-epoxyisoprostane E2 oyl)-*sn*-glycero-3-phosphocholine
PI phosphatidylinositol
PL-PC 1-hexadecanoyl-2-octadecadi-9′,12′-enoyl-*sn*-glycero-3-phosphocholine
PON-PC 1-hexadecanoyl-2-(9′-oxononanoyl)-*sn*-glycero-3-phosphocholine
POV-PC, PON-PC, PG-PC and PND-PC 1-acyl group is a hexadecanoic acid ester of OV-PC, ON-PC, G-PC and ND-PC
PS phosphatidylserine

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