Chapter 17

Oxidized Phospholipids: Introduction and Biological Significance

Mohammad Z. Ashraf and Swati Srivastava

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1. Introduction

Phospholipids containing polyunsaturated fatty acids are highly prone to modification by reactive oxygen species. They tend to undergo lipid peroxidation to form OxPLs which induce cytotoxicity and apoptosis and plays a significant role in inflammation. There are reports that provide insights for involvement of OxPLs in interleukin transcription, phenotype switching of smooth muscle cells and apoptotic mechanisms of the modified phospholipids. Thus peroxidation greatly alters the physiochemical properties of membrane lipid bilayers and consequently induces signaling depending upon the formation or reorganization of membrane domains or specific molecular binding (Deigner et al, 2008). Distinct OxPLs species may interact with specific binding sites and receptors leading to the activation of individual signaling pathways. The most prevalent human coronary atherosclerosis is a chronic inflammatory disease that occurs due to lipid abnormalities. Pro-inflammatory oxidized low-density lipoprotein (OxLDL) has been suggested to be a link between lipid accumulation and inflammation in vessel walls. Increased levels of phospholipids' oxidation products have been detected in different organs and pathological states, including atherosclerotic vessels (Watson et al 1997, Subbanagounder et al 2000), inflamed lung (Yoshimi et al 2005, Nakamura et al 1998), non-alcoholic liver disease (Ikura et al 2006), plasma of patients with coronary artery disease (Tsimikas et al 2005), as well as in apoptotic cells (Huber et al 2002, Chang et al 2004), virus-infected cells (Van Lenten et al 2004) and cells stimulated with inflammatory agonists (Subbanagounder et al 2002). Moreover, studies have been done 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) thereby providing evidence for their role in atherosclerosis. Another important marker of oxidative stress is the association of OxPLs with the apolipoprotein B-100 particle (OxPLs/apoB) of

LDL. Increased levels of OxPLs/apoB are implicated in coronary artery disease, progression of carotid and femoral atherosclerosis and the prediction of cardiovascular events (Tsimikas et al 2005).

2. Formation of OxPLs

OxPLs are generated by the oxidation of polyunsaturated fatty acid residues, which are usually present in the phospholipids at the *sn*-2 position. Oxidation of phospholipids is initiated either enzymatically by lipoxygenases or by reactive oxygen species and propagates *via* the classical mechanism of lipid peroxidation chain reaction. This implies that the production of OxPLs cannot be regulated by adjusting the amount or activity of enzymes. Hence there is a probability of the uncontrolled generation of OxPLs during oxidative stress. Several evidences suggest that OxPLs are formed from Poly Unsaturated Fatty Acids (PUFAs) at the sn-2 position (Bochkov et al 2007, Podrez et al 2002). Bioactive oxidized phospholipids may contain fragmentation products of PUFA, such as 1-palmitoyl-2-oxovaleroyl-*sn*-glycero-3-phosphorylcholine and 9-keto-10-dodecendioic acid ester of 2-lyso-phosphatidyl choline (KOdiA-PC); prostaglandins, such as 15 deoxy-delta 12, 14 prostaglandin I2 (PGI2) and 1-palmitoyl-2-(5,6-epoxyisoprostane E2)-*sn*-glycero-3-phosphoryl choline (PEIPC); and levuglandins. These molecules exhibit different biological activities. Chromatographic separation of many products formed by 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-epoxyisopropane E_2)-*sn*-glycero-3-phosphatidylcholine (PEIPC) as potent lipid mediators of inflammation. High structural variation may explain why OxPLs demonstrate a remarkable variety of biological activities (FIGURE-1).

Enzymatic and non-enzymatic reactions, free-radical, and radical-free processes are capable of initiating wide spectrum of reactions causing oxidation of PUFAs. Majority of these reactions produce identical primary oxidation products (i.e., peroxyl radicals and hydroperoxides). Subsequent oxidation of OxPLs is an enzyme-independent stochastic process producing a wide spectrum of OxPLs. Peroxidation products thus generated proceeds according to several mechanisms such as oxidation of PUFA residue, cyclization of peroxyl radical or oxidative fragmentation of esterified PUFAs generating either full-length residues incorporating several oxygen atoms, or shortened fatty acid residues. Introduction of additional oxygen atoms into PUFAs is a common mechanism that increases complexity of OxPLs mixtures however biological activities of poly-oxygenated PLs are still not characterized. On the other hand, cyclization of peroxyl radical produces cyclic peroxide, which undergoes re-arrangements yielding bicyclic endoperoxide, or oxidation introducing additional non-cyclic or cyclic peroxide group. Cyclization of peroxyl radical is only possible for FAs having three or more double bonds (Salomon et al 2005).

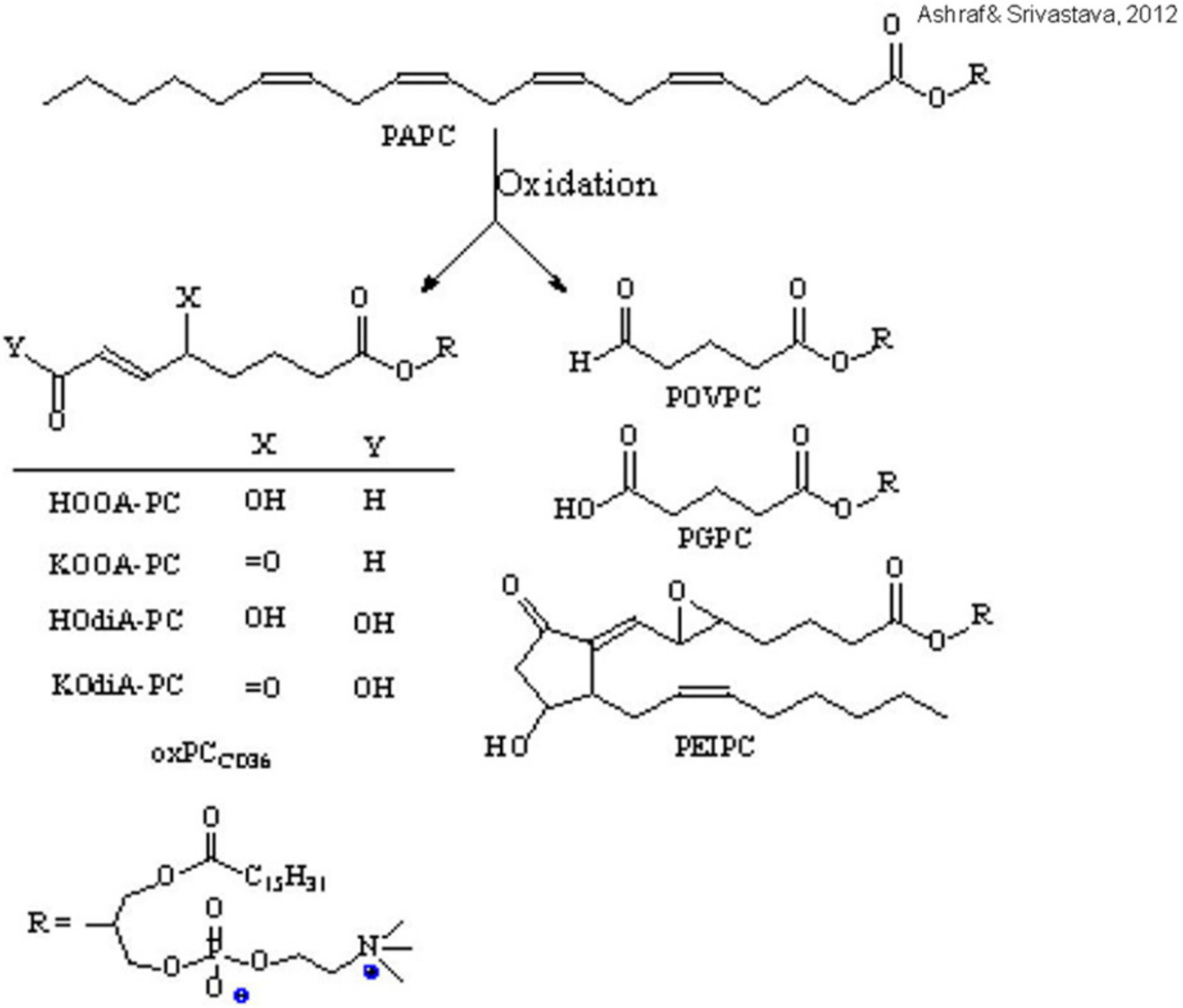

Figure 1. Representative chemical structures of oxidized phospholipids formed during oxidation of PAPC.

2.1. Oxidative cleavage and generation of fragmented OxPLs species

Peroxides/ peroxyls are transformed into advanced oxidation products by fragmentation of hydroperoxides. γ-Hydroxy (or oxo) a,b-unsaturated PLs with terminal aldehyde groups are produced from hydroperoxides via oxidation/fragmentation or polymerization/cleavage. Oxidative fragmentation of hydroperoxides occurs via several mechanisms including b-scission, Hock rearrangement, or cyclization of alkoxy radical produced from hydroperoxide (Gugiu et al 2006). γ-Hydroxy (or oxo)-α,β-unsaturated aldehyde PLs are highly reactive compounds, that are able to covalently link to amino groups of proteins, as well as thiol groups of biomolecules (Hoff et al 2003). On the other hand, peroxyl radical can cross-react with double bonds present in hydroperoxides yielding peroxydimers, these are unstable products and spontaneously break down forming either new radicals or α,β-unsaturated aldehydes (Schneider et al 2008). In addition to these products, saturated fragmented species containing terminal carbonyl groups are produced by oxidative fragmentation of PUFA-PLs, most common amongst which are oxononanoate and azelaoate formed from linoleic acid, oxovaleroate, and glutaroate generated from arachidonic acid, or oxobutyrate and succinate produced from docosahexaenoic acid (Gu et al 2003, Podrez et al 2002). Saturated fragmented OxPLs can be formed by further oxidation of γ-hydroxy (or oxo)-α, β-unsaturated PLs in addition to direct formation from hydroperoxides, (Podrez et al 2002). Saturated fragmented OxPLs l lack double bonds and hence they are resistant to further oxidation as the absence of double bonds within fragmented chains results in reduced reactivity of aldehyde containing saturated OxPLs as compared to α,β-unsaturated fragmented OxPLs.

2.2. Non-enzymatic oxidation of PL-PUFAs

This process is initiated by free radicals or non-radical reactive oxygen species (ROS). Free radical-mediated chain reaction is initiated by the formation of carbon-centered radicals and/or hydroperoxides of PUFAs (peroxidation of PUFAs). Due to the presence of methylene groups located between double bonds (bisallylic methylene groups), PUFAs are more susceptible to oxidation as compared to saturated FAs. As a result they are characterized by weakened hydrogen-carbon bonds. Free radicals can abstract hydrogen from bisallylic methylene leading to the formation of carbon-centered radicals within PUFAs. Now occurs the initiation step of lipid peroxidation, Carbon-centered radicals rapidly react with molecular oxygen, producing peroxyl radicals. These Peroxyl radicals react with bisallylic methylene groups in other PUFA molecules, leading to the transformation of peroxyl radicals to hydroperoxides and generation of new carbon-centered radicals. Thus, additional cycles of peroxidation are initiated. PUFA hydroperoxides in turn produce reactive alkoxyl and hydroxyl radicals via iron or copper-catalyzed Fenton-like reactions, further propagating the chain reaction (Bochkov et al 2010).

2.3. Enzymatic oxidation of PL-PUFAs

1, 4-pentadiene motifs are recognized within unsaturated fatty acids by lipoxygenases (LOXs) and molecular oxygen with high stereoselectivity is introduced. The majority of lipoxygenases oxidize only unesterified PUFAs. Only one group (12/15-LOX) amongst all known LOXs is capable of oxidizing PL-esterified fatty acids. This class of enzymes is present in different biological species and includes mouse, rat, rabbit, bovine, and porcine leukocyte-type 12- LOX, rabbit and human reticulocyte-type 15-LOX, and soybean LOX (Huang et al 2008, Wittwer et al 2007). Switching of activity of electron transport in mitochondria to peroxidation by cytochrome c (cyt c) has been suggested by Kagan et al (2005). This transformation begins when cyt c binds to negatively charged cardiolipin (CL), leading to conformational changes and subsequent release of PL-protein complex from mitochondria into cytosol. The complex of cyt c with CL activated by traces of PUFA-OOH or H_2O_2 acquires the ability to oxidize CL, PS, or PI, with formation of PL-OOH (Kagan et al 2009).

Alternatively, OxPLs are also generated by re-esterification of free oxidized PUFAs into lyso-PLs. Several types of OxPLs have been found to be generated by this mechanism both *in vivo* and *in vitro* (Arai et al 1997, Birkle et al 1984).

2.4. Detoxification of reactive OxPLs

Detoxification of OxPLs comprises the mechanisms that terminate peroxidation chain reaction and inactivate chemically reactive toxic groups produced by oxidation. Hydroxides are characterized by significantly lower chemical reactivity and therefore are considered to be stable and non-toxic compared to hydroperoxides (Spiteller et al 1997). Most commonly, the enzyme catalyzing the reduction of hydroperoxides to hydroxides is glutathione peroxidase (GPx). Lipid hydroperoxides are reduced in a reaction that involve selenocysteine residue of GPx and glutathione thus generating lipid hydroxide and oxidized glutathione. With respect to membrane-bound hydroperoxides of PL esterified PUFAs, PL glutathione peroxidase (GPx4) has the highest activity amongst GPx enzymes (Savaskan et al 2007).

A variety of products containing aldehyde and keto functional groups are formed upon oxidation of OxPLs which are further reduced by aldo-keto reductases to respective hydroxyl groups. Apart from playing physiological role in metabolism of sugar aldehydes, aldo-keto reductases also play a role in detoxification of toxic phospholipid aldehydes (Jin et al 2007).

Another aspect of detoxification is OxPLs cleavage. Platelet activating factor acetylhydrolase (PAF-AH) has been recognized for its ability to cleave and thus inactivate PAF (McIntyre et al 2009).The enzyme was shown to hydrolyze fragmented saturated OxPLs (Stremler et al 1991), as well as long-chain OxPLs, including esterified F2-isoprostanes, PC-hydroperoxides and PEIPC (Kriska et al 2007, Davis et al 2008).

3. Mechanism of action

Specific receptor binding of OxPLs is the subject of an ongoing debate. Available evidence suggests that OxPLs interact with various signal transduction receptors and pattern recognition receptors present on the cell surface. Most commonly known receptors include CD36, SRB1, EP2, VEGFR2 and the PAF receptor (Bochkov et al 2007, Zimman et al 2007). It has been demonstrated that when present in vesicles, truncated oxidized fatty acids at the sn-2 position move from the hydrophobic interior to the aqueous exterior of the vesicle. this would allow their recognition by cell surface receptors Earlier models of isoprostane-containing phospholipids have suggested that they are highly twisted and may distort membrane areas in which they are present (Morrow et al 1992). Moumtzi et al (2007) have shown that phospholipid oxidation products can integrate into lipid membranes of cells and lipoproteins; they can either act as ligands or may cause local membrane disruption. Besides, peroxidation of phospholipids leads to the accumulation of lysoforms as a result of both non-enzymatic decylation and enzymatic hydrolysis reactions catalyzed to a large extent by lipoprotein-as-associated phospholipase A_2 (also known as PAF acetylhydrolase), which has high substrate selectivity toward polar phospholipids, including the oxidized forms (Zalewski et al 2005). Some lysophospholipids bind and activate G protein-coupled receptors (GPCR). Parhami et al (1993 & 1995) explained that oxidized phospholipids act by binding to a G protein-coupled receptor. These authors demonstrated that minimally modified LDL stimulated a putative Gs-coupled receptor, thus increasing cyclic AMP (cAMP) levels in endothelial cells. Lysophosphatidylcholine and lysophosphatidic acid triggered the activity of G2A and LPA1-LPA4 receptors respectively (Tomura et al 2005, Anliker et al 2004). In addition to GPCR, OxPLs also activate other classes of receptors such as peroxisome proliferator-activated receptors (PPAR). Thus, phospholipid peroxidation may induce the generation of lysophospholipids that are known to accumulate in LDL (OxLDL) and atherosclerotic lesions (Siess et al 2004, Tselepis et al 2002).

Prostaglandin receptors have been recently implicated into OxPLs-induced inflammation. OXPAPC and its component lipid PEIPC are able to stimulate prostaglandin E_2 and D_2 receptors (EP2 and DP respectively) and to compete with receptor binding of radio labeled prostaglandin E_2 (Li et al 2006). Previously, it was observed that POVPC binds to human macrophages via the PAF receptor (PAF-R). Occupancy of the PAF-R by the OxPLs modifies the transcription levels of pro-inflammatory genes such as IL-8 (Pegorier et al 2006).

Some effects of OxPLs are probably not mediated by signal transducing receptors. Modulation of cellular cholesterol depots has been suggested as a non-receptor mediated mechanism of OxPLs sensing by cells. It is well illustrated that OxPAPC induces depletion and re-distribution of cellular cholesterol reserves finally leading to the activation of a transcription factor SREBP, a well recognized sensor for cellular cholesterol contents. In turn, SREBP activates IL-8 production (Yeh et al 2004).The human aortic EC gene expression was found to be stimulated by PAPC. 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). Leitinger et al (2003) and Watson et al (1997) have described a possible role of toll-like receptors (TLRs) in OxPLs-induced inflammation. Studies have confirmed that Asp299Gly-TLR4 polymorphism plays a protective role in attenuation of atherosclerosis.

Mitogen activated protein kinase phosphatase-1 (MKP-1) was reported to be involved in OxPAPC-induced MCP-1 production. Also activation of eNOS by OxPAPC is regulated via a phosphatidylinositol-3-kinase/Akt-mediated mechanism, OxPAPC-induced SREBP activation is significantly reduced with eNOS inhibition (Berliner and Gharavi, 2008).

Chen et al (2007) reported that LDL-associated phosphatidylcholine esterified with *sn*-2-azelaic acid at the *sn*-2 position is readily taken up by cells. This compound, one of the main phospholipid oxidation products in LDL, induces apoptosis of HL60 cells at low micromolar concentrations. Since the intact phospholipid is required for signaling, this effect can be prevented by over-expression of PAF acetyl hydrolase known for oxidizing phospholipids with polar residues at the *sn*-2 position.

Another biologically active phospholipid described is platelet activating factor (PAF) having various inflammatory actions such as platelet aggregation, hypotension, anaphylactic shock and increased vascular permeability (Prescott et al 2000). PAF is structurally identified as 1-0-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine. Atherogenic effects are also induced by PAF by activating monocytes and stimulating smooth muscle cell growth. In contrast to the tightly regulated physiological generation of PAF, uncontrolled processes of free radical oxidation generate analogs of PAF *in vivo* and *in vitro*. As a result of this uncontrolled chemical reaction, fragmentation of the residue at sn-2 position occurs and 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).

Other oxidized phospholipids such as POVPC and PGPC have also been shown to play major roles in activation of endothelial cells and induction of leukocyte binding. They are identified as abundant products in oxidized LDL. The effect of POVPC is protein kinase-A dependent leading to the stimulation of the cAMP-mediated pathway (Berliner and Gharavi, 2008).

OxPLs also induces autocrine mediators such as vascular endothelial growth factor (VEGF), which works through activation of transcription factor-4 (ATF4) (Oskolkova et al 2008).

4. OxPLs receptors

It has been shown that OxPLs stimulate a number of signal-transducing receptors located on the cell surface or in the nucleus, including G protein-coupled receptors, receptor tyrosine kinases, Toll-like receptors, receptors coupled to endocytosis, and nuclear ligand-activated transcription factors such as PPARs.

4.1. Prostaglandin receptors

OxPCs containing esterified PEIPC activate receptors recognizing prostaglandins E2 and D respectively (Li et al 2006). Activation of EP2 receptor on ECs results in activation of integrins and increased binding of monocytes.

4.2. Scavenger receptors

OxPLs comprise a major group of ligands for scavenger receptors. Different classes of Scavenger receptors range from Class A, B, D, E and F depending upon the nature and type of ligand (FIGURE-2). CD36 have been described as the major receptor expressed on macrophages and involved in the process of atherogenesis and apoptosis. The role of CD36 has been shown to be responsible for recognition of free oxidized phospholipids (Boullier et al 2000, Podrez et al 2000). Also Boullier et al (2000) and Watson et al (1997) have pointed out that oxidized phospholipid is covalently linked to apolipoprotein B-100 in extensively oxidized LDL (e.g. Cu^{2+} -oxLDL) and serve as ligand for CD36. Scavenger receptor- ligand interaction initiates signaling cascades that regulate macrophage activation, lipid metabolism and inflammatory pathways which may influence the development and stability of atherosclerotic plaque. Recent studies have demonstrated the expression of scavenger receptors especially CD36 and SR-BI on platelets suggesting their critical role in platelet hyper-reactivity in dyslipidemia and atheroprogression.

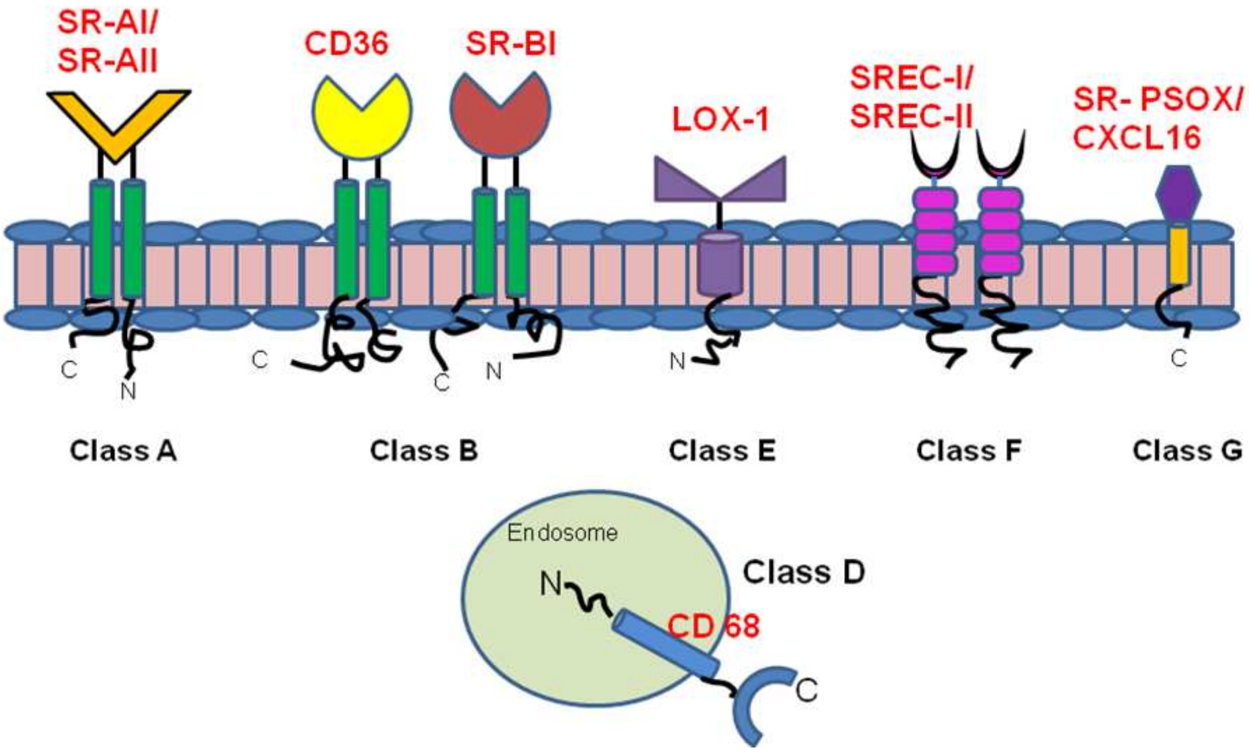

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Figure 2. Schematic representations of different class of scavenger receptors involved in OxPLs binding.

4.3. PAF receptors

OxPLs initiate activation of receptor specific for PAF, which act as an important lipid mediator of inflammation and platelet aggregation. It recognizes alkyl-acyl-phosphatidylcholines specifically and contains an ether bond at the sn-1 position in combination with unusually short sn-2 acetyl residue. Oxidative fragmentation of sn-2 PUFAs in alkyl-PCs generates products such as 1-alkyl-2-butenoyl and 1-alkyl-2-butanoyl that are recognized by PAF receptor (Androulakis et al 2005, Marathe et al 1999). However, the role of the PAF receptor in the overall biological activity of OxPCs is not characterized.

4.4. VEGF receptors

It has been demonstrated that phosphorylation (activation) of VEGFR2 is enhanced within the first minutes of incubation with OxPAPC (Zimman et al 2007). They hypothesized that trans-activation of VEGFR2 in OxPAPC-treated cells was mediated by c-SRC.

4.5. Sphingosine-1-phosphate (S1P) receptor 1

It has shown that OxPAPC stimulates the recruitment of S1P1 to caveolin-enriched membrane microdomains, and induces its phosphorylation (activation) by AKT. Transactivation of S1P1 by OxPAPC plays a role in barrier-protective function of OxPLs.

4.6. Toll-like receptor 4

TLR4 plays a role in OxPAPC-mediated induction of IL-8 in HeLa cells. OxPAPC also induces lung injury and IL-6 production by mouse lung macrophages via the TLR4–TRIF–TRAF6 pathway (Imai et al 2008). On the other hand various classes of OxPLs do not influence the basal levels of E-selectin, ICAM-1, VCAM-1, TNFa, IL-6, IL-1a, IL-1b, and COX-2 in whole blood or individual cell types, including human umbilical vein ECs, blood monocytes, macrophage cell line, or fibroblasts (Bochkov et al 2002, Erridge et al 2008).

4.7. PPARα and PPARγ

Peroxisome proliferator-activated receptors (PPARs) are intracellular ligand-activated transcription factors. Diacyl-OxPLs stimulated a PPAR response element-driven reporter construct in transfected HAECs and the effect of OxPAPC, POVPC, and PGPC was mediated by PPARα as indicated by the activation of the ligand binding domain of PPARα, but not PPARγ or PPARδ (Lee et al 2000).

Second messengers up-regulated by OxPLs: Apart from the above described receptors , minimally modified Low Density Lipoproteins (MM-LDL) also induces elevation of Ca^{2+} in ECs (Honda et al 1999) and also OxPAPC was shown to induce rapid and reversible Ca$_2$þ-responses in ECs (Bochkov et al 2002). MM-LDL causes a saturable dose-dependent increase in cAMP levels in aortic ECs that may arise due to activation of Gs and inhibition of Gi heterotrimeric G-protein complexes (Parhami et al 1995).

5. Biological function

Many cellular events are initiated and modulated by biologically active oxidized phospholipids. OxPLs were initially characterized as an active principle of minimally modified LDL (MM-LDL), responsible for its ability to stimulate EC to bind the leukocytes (Watson et al 1995). MM-LDL and OxPLs has the characteristic feature of inflammatory agonist i.e., their ability to activate binding of monocytes but not neutrophils (Watson et al 1997). In contrast to lipopolysaccride (LPS), tumor necrosis factor α (TNFα), or interleukin 1 (IL-1), MM-LDL does not up-regulate the expression of ICAM-1, VCAM-1 and E-Selectin on EC (Kim et al 1994), but promotes surface deposition of CS-1-containing variant of fibronectin (CS-1 FN) serving as ligand for the α4β1 (VLA-4) integrin expressed on the surface of monocytes (Shih et al 1999). Similar to MM-LDL, OxPLs selectively stimulate adhesion of monocytes by CS-1 FN-dependent mechanism. Likewise other inflammatory agonists, OxPLs also stimulate the production of cyto- and chemokines. OxPLs are known to up-regulate expression IL-6, IL-8, MCP-1, GROα, MIP-1α, MIP-1β and CXCL3 (Subbanagounder et al 2002, Furnkranz et al 2005, Lee et al 2000, Reddy et al 2002, Kadl et al 2002, Gargalovic et al 2006, Huo et al 2001).

Expression of a number of genes related to angiogenesis, atherosclerosis, inflammation and wound healing are modulated by oxidized phospholipids in human aortic endothelial cells (Berliner and Gharavi, 2008; Gargalovic et al., 2006). Bochkov and colleagues (2002, 2007) have made known that OxPLs counteract the lipopolysaccride (LPS) pathway. Considering anti-inflammatory role of OxPLs, they reported that oxidized 1-palmitoyl-2arachidonoyl-*sn*-glycero-3-phosphocholine (OxPAPC) interfered with the ability of LPS to bind to the LPS-binding protein (LBP) and to CD-14, thus suppressing LPS-induced nuclear factor-κB (NF-κB)-mediated up-regulation of inflammatory genes.

Knapp and coworkers (2007) found that OxPAPC inhibits the interaction of LPS with LPS-binding protein and CD14. This also reduces phagocytotic activity of neutrophils and macrophages by a CD-14-independent mechanism. However, in these experiments, administration of OxPAPC rendered mice highly susceptible to *Escherichia coli* peritonitis, which may cause mortality during gram-negative sepsis *in vivo*. Thus the overall harmful profile of phospholipid oxidation products includes the impairment of host response to bacterial infections.

Recently, Gharavi and colleagues (2007) have reported the activation of JAK2/STAT3 pathway by phospholipids and implicated their role in atherogenesis. 1-Palmitoyl-2-epoxyisoprostane-*sn*-glycero-3-phosphocholine, an oxidation product of -1palmitoyl-2-arachidonoyl-*sn*-glycero-3-phosphocholine, induces c-Src kinase-dependent activation of JAK2 in endothelial cells and synthesis of chemotactic factors, such as interleukin (IL)-8. In turn, STAT3 activation and regulation of IL-8 transcription is dependent on JAK2 leading to the enhanced levels of STAT3 activity in inflammatory regions of human atherosclerotic lesions. Since STAT3 activation is involved in other chronic inflammatory diseases such as rheumatoid arthritis, psoriasis etc, it has been suggested that STAT3 activation by oxidized phospholipids could be an important interventional target for atherosclerosis and other diseases with inflammatory components.

5.1. Regulation of vascular cell function

OxPLs have multiple effects on endothelial cells. After 4h treatment with 50μg/ml of OxPAPC ~1000 genes are regulated amongst which ~600 are up-regulated and ~400 are down-regulated (Gargalovic et al 2006). Also, a major difference in responsiveness to specific effects of Ox-PAPC of endothelial cells from different human donors has been documented (Gargalovic et al 2006). The atherogenic pathways which were found to be upregulated include inflammation, cholesterol synthesis, coagulation and decrease in cell division. Some important effects of OxPAPC on endothelial cell function independent of gene regulation have been reported. OxPAPC has been shown to increase monocytes but not neutrophils binding by activating β-1 integrin (Berlin et al 2008, Leitinger et al 2005).

Many effects of OxPLs are mediated by its interaction with CD36. Several studies have indicated that LDL supplemented with OxPAPC or vesicles supplemented with fragmented α/β unsaturated fatty acids at the sn-2 position, such as KOdiA or HODA PC, bind to CD36 (Podrez et al 2002, Greenberg et al 2006). Another important phagocytic function of macrophages is the uptake of apoptotic cells, which are abundant in atherosclerotic plaques. OxPLs including oxidized phosphatidyl serine and phosphatidyl choline derivatives were shown to serve as ligands for macrophage uptake of apoptotic cells (Chou et al 2008, Greenberg et al 2006).

OxPLs also interact and bind with other recognition receptors in macrophages such as TLRs, CD14, LPS binding protein and C-reactive protein competing with negative ligands (Bochkov et al 2007, Bochkov et al 2002, Erridge et al 2008, Miller et al 2003). Thus, the formation of OxPLs during inflammation may represent an important feedback mechanism to limit further tissue damage. OxPLs have also been shown to activate macrophages. Currently conducted studies have revealed the role of OxPAPC in inducing lung injury and cytokine production by lung macrophages (Imai et al 2008).

The role of OxPLs in adaptive immune response can't be overlooked where they modulate the maturation process of dendritic cells (DCs). OxPLs also regulate innate immunity in human leprosy (Cruz et al 2008). In addition to the effects on DCs, OxPLs have also been shown to affect and induce T-cells (Seyerl et al 2008).

Phenotypic switching of smooth muscle cells (SMCs) involving increased proliferation; enhanced migration and down-regulation of SMC differentiation marker genes play a critical role in atherogenesis. Many studies have shown that OxPLs stimulate differentiation and cell division of SMCs (Heery et al 1995, Pidkovka et al 2007) while others have shown activation of apoptotic signaling pathways (Fruhwirth et al 2008).

5.2. Gene expression

OxPLs have profound effect on gene expression. OxPAPC have been shown to modulate the expression of approximately 1000 genes in human aortic ECs which include both up-regulated and down-regulated mRNAs (Gargalovic et al 2006). OxPLs regulate genes related to inflammation, lipid metabolism, cellular stress, proliferation, and differentiation. These include VEGF-A and IL-8, which are induced by OxPLs independent of their transcription factors.

5.3. Pathophysiological functions

Pathophysiologically OxPLs are involved in various proinflammatory and cardiovascular disorder; details are being described below (FIGURE-3).

5.4. Atherosclerosis

Quantification of OxPLs using liquid chromatography coupled with mass spectrometry has indicated that atherosclerotic vessels contain high concentrations of OxPCs. Different species of OxPCs were detected in atherosclerotic vessels including PL-hydroperoxides and hydroxides (Waddington et al 2001). In addition to elevated levels of OxPLs, atherosclerotic vessels express high amounts of proteins known to be induced by OxPLs in vitro. The latter includes MKP-1 (Reddy et al 2004), ATF3, ATF4 (Gargalovic et al 2006), SREBP-1 (Yeh et al 2004), HO-1 and IL-8 (Cheng et al 2009), MCP-1 and COX-2 (Ma et al 2008). OxPLs act on all major cell types involved in atherogenesis including monocytes, endothelial and vascular smooth muscle cells, lymphocytes, and platelets.

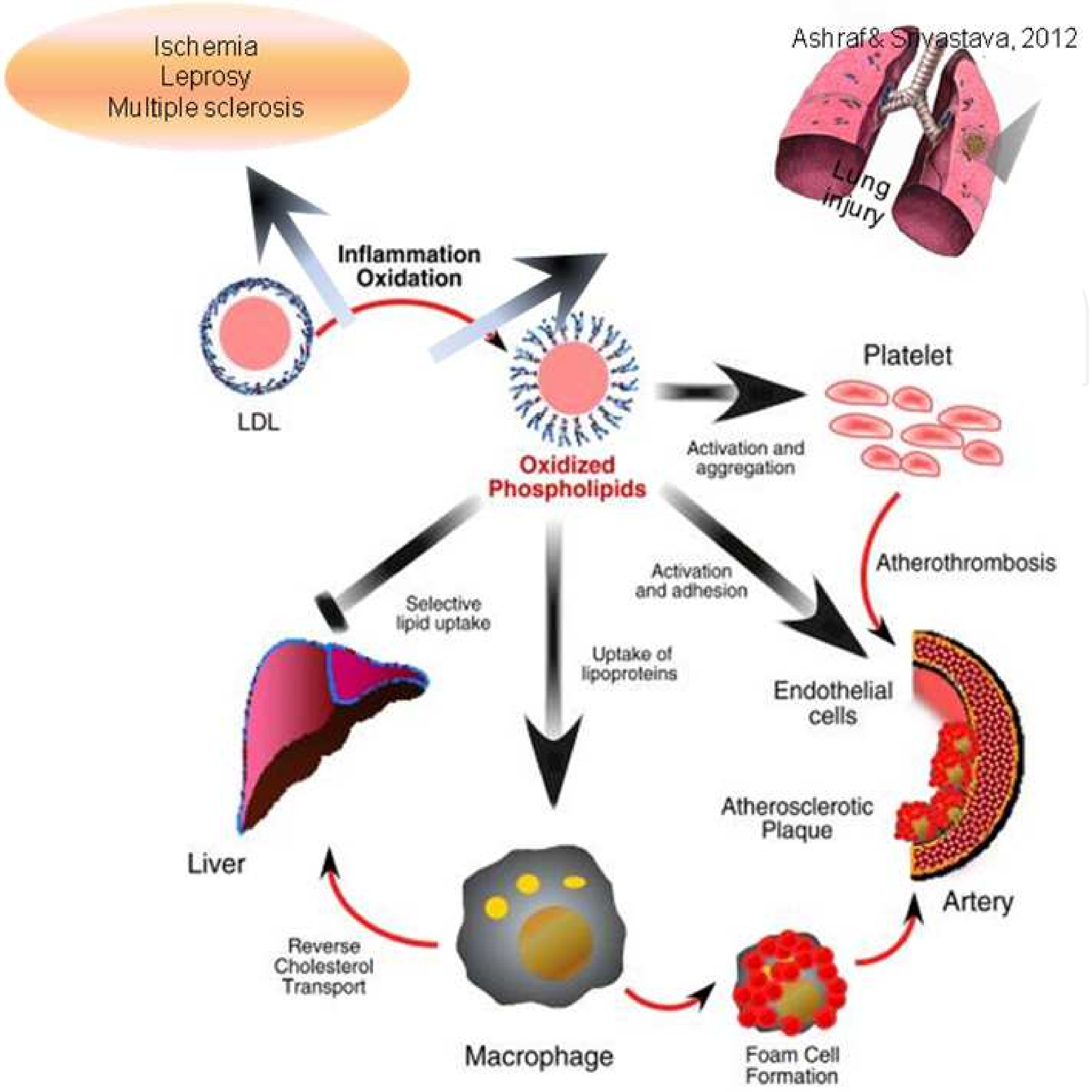

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Figure 3. Oxidized phospholipids present in oxidized LDL induce various Diseases.

5.5. Lung injury

The epithelial lining pulmonary surfactant is permanently exposed to high concentrations of oxygen and other oxidants present in the air. Ozone gas also plays a role in generating oxidatively truncated PLs (Uhlson et al 2002). Under normal healthy conditions surfactant is protected from oxidation by maintaining low contents of PUFAs, antioxidant action of glutathione present in the lining fluid and surfactant proteins A and D (Kuzmenko et al 2004). However, the accumulation of biologically active OxPLs products occurs in pathological states due to the oxidation of surfactant PCs, membrane lipids and apoptosis of bronchial cells. Studies conducted with animal models have shown that OxPLs protect lungs from acute lung injury. Ma et al. (2004) showed that OxPAPC inhibits elevation of TNFa in mice upon intratracheal or systemic administration of LPS or CpG DNA. Hence the available data shows that OxPLs may induce either beneficial or detrimental effects on lungs. The action of OxPLs on the lungs may depend upon their concentrations, lower levels of OxPLs protect endothelial barrier whereas high concentrations of the same OxPLs induce disruptive effects (Birukov et al 2004, DeMaio et al 2006).

5.6. Ischemia

Ischemia/reperfusion results in elevated levels of OxPLs both in tissues and systemic levels. PAF like (alkyl-acyl) OxPLs were detected within the first minutes after reperfusion of kidneys after warm ischemia (Lloberas et al 2002). Plasma concentrations of fragmented OxPCs were increased in patients during the reperfusion period after coronary surgery with cardiopulmonary bypass (Frey et al 2000). Hence available data shows that ischemia/reperfusion is a pathological state characterized by elevated local and circulating levels of OxPLs.

5.7. Inflammation

Inflammation is characterized by a massive production of ROS. The elevation of circulating levels of OxLDL in response to inflammatory stimuli has already been shown. The OxPLs production in response to inflammation is induced by different cell types including leukocytes. Phorbol ester-stimulated neutrophils and monocytes incubated with PUFA-PCs produced mono- and bishydroperoxides of PC, as well as isoP–PC, thus suggesting that activated phagocytes can oxidize lipids in the surrounding medium (Jerlich et al 2003).

5.8. Radiation stress

Formation of OxPLs can be activated by visual and UV-light. OxPLs accumulating in retinas serve as ligands for CD36-dependent phagocytosis of shed photoreceptor outer segments by retinal pigment epithelium; this process is necessary for normal function of the retina (Sun et al 2006). Generation of OxPLs by light exposure has also been shown in skin cells. UVA-1-irradiated PAPC containing several OxPLs species induced expression of antioxidant and anti-inflammatory enzyme heme oxygenase-1 in dermal fibroblasts, keratinocytes, and in a three-dimensional epidermal equivalent model (Gruber et al 2007). Therefore, OxPLs are likely to play a protective role in UVA irradiated skin by inducing HO-1.

5.9. Leprosy

Oxidized PCs have been detected in lepromatous (disseminated) leprosy lesions, but not in tuberculous leprosy characterized by stronger host immune response and self-contained infection (Cruz et al 2008). Lepromatous leprosy lesions are characterized by the accumulation of OxPLs, which can counteract innate and specific immune responses, thereby promoting survival.

5.10. Multiple sclerosis

Multiple sclerosis (MS) is an autoimmune disease of the brain that causes neurodegeneration. Role of OxPLs in MS is supported by Qin et al. (2007), demonstrating the presence of OxPLs (alone and conjugated to a 15 KDa protein) in extracts of MS lesions directly by Western blot analyses using the E06 antibody. OxPLs might be promoting the inflammatory process in MS lesions.

6. Medical relevance

Increasing number of studies suggest the role of oxidized phospholipids in development of atherosclerosis by interacting with specific receptors as well as through their reactive groups that can bind covalently to proteins, forming lipid-protein adducts that become dysfunctional. It is a challenge to determine if therapeutic inhibition of the OxPLs interaction with vessel wall cells can inhibit atherosclerosis. Also it will be interesting to identify the lipid oxidation products that activate each response in the various cell types and the receptors or binding molecules and signal transduction pathways activated by these lipids.

Pro-inflammatory oxidized phospholipids are significant predictors of the presence of carotid and femoral atherosclerosis, development of new lesions and increased risk of cardiovascular events (Ashraf et al 2009). Hence oxidized phospholipids could serve as biomarker for diagnosis of coronary artery disease and they could also be used as potential targets for therapeutic intervention.

7. Conclusions

The inflammatory profile of OxPLs combines both pro- and anti-inflammatory effects. OxPLs may show detrimental as well as beneficial cellular effects. OxPLs exert pro-inflammatory effects on different cell types such as endothelium where they induce a shift from antithrombotic and anti-inflammatory state to procoagulant and inflammatory phenotype of EC. Although OxPLs stimulate a number of classical inflammation mechanisms, they are not capable of activating many signaling and adhesion events characteristic of acute inflammation, such as activation of the NFκB pathway, expression of ICAM-1 and E-selectin or adhesion of granulocytes. Several studies have provided evidence that OxPLs play an important role in atherosclerosis. In addition, OxPLs also up-regulate monocytes-specific chemokines and stimulate EC to bind monocytes, thus initiating monocytic inflammation. Thus it can be concluded that OxPLs can stimulate and inhibit inflammation depending upon the biological situation. Advancement in this field can be expected from studies that are based on well defined synthetic and labeled OxPLs species and the modern techniques of system biology. Also advances in the knowledge of signaling pathways and the interaction partners of oxidized phospholipid will increase our understanding of inflammatory processes and molecular mechanisms of various diseases such as atherosclerosis. These studies may also help in playing important role in future therapeutic diagnostics.

Abbreviations

Oxidized phospholipids (OxPL)
Oxidized low-density lipoprotein (OxLDL)
Serum paraoxonase (PON1)
PAF-acetylhydrolase (PAF-AH)

9-keto-10-dodecendioic acid ester of 2-lyso-phosphatidyl choline (KOdiA-PC)
15 deoxy-delta 12, 14 prostaglandin I2 (PGI2)
1-palmitoyl-2-(5,6-epoxyisoprostane E2)-*sn*-glycero-3-phosphoryl choline (PEIPC)
Reactive oxygen species (ROS)
Lipoxygenases (LOXs)
Glutathione peroxidase (GPx)
G protein-coupled receptors (GPCR)
Peroxisome proliferator-activated receptors (PPAR)
Toll-like receptors (TLRs)
Vascular endothelial growth factor (VEGF)
Lipopolysaccride (LPS)
Tumor necrosis factor α (TNFα)
Dendritic cells (DCs)
Smooth muscle cells (SMCs)
Multiple sclerosis (MS)

Author details

Mohammad Z. Ashraf and Swati Srivastava
Genomics Group, Defence Institute of Physiology & Allied Sciences, India

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