Regulation of Inflammatory Responses by Oxidized Phospholipids: Structure-Function Relationships

A. Furnkranz and N. Leitinger*

Department of Vascular Biology and Thrombosis Research, University of Vienna, Austria

Abstract: Increasing evidence points to the role of oxidized phospholipids as modulators of inflammatory processes. These modified phospholipids are derived from lipoproteins or cellular membranes and accumulate at sites of inflammation such as atherosclerotic lesions. It has been shown that oxidized phospholipids influence a variety of cellular functions such as chemokine production and expression of adhesion molecules. Furthermore, recent reports indicate that oxidized phospholipids act as ligands for pattern-recognition receptors which detect conserved pathogen-associated molecular patterns during innate immune defense. Thus, the diversity of individual phospholipid oxidation products reflects the many aspects of the inflammatory process they influence. In this review, we focus on structural features used to classify different oxidized phospholipids and how they relate to specific biological responses. As the chemical identification of oxidized phospholipid products proceeds, distinctive structural motifs emerge that can help us to understand the mechanism of action of these unique compounds and how to intervene for therapeutic purposes.

Key Words: Oxidized Phospholipids, Inflammation, Molecular Structure, Innate Immunity.

INTRODUCTION

Since the first publications on the role of oxidative modification of low density lipoproteins in atherogenesis the research interest in biological effects of oxidized lipids has grown exponentially [1]. Concurrently, the role of lipid oxidation has been expanded to conditions other than atherosclerosis, such as rheumatoid arthritis and diabetes [2, 3], and recent reports describe diverse functions of oxidized lipids in innate immunity. While oxidized phospholipids may be regarded as pathologic byproducts towards which an innate immune response has evolved [4], there is also evidence for the intriguing hypothesis that they function as molecular switches, directing the course of an inflammatory / immune reaction [5]. Advances in analytical methods have made possible the chemical identification of distinct molecular species and structural requirements for certain biological actions. Here, we review some of the newer reports covering the role of oxidized phospholipids in inflammatory conditions, focusing on the different classes of phospholipids that have been studied so far and how they exert their biological actions in terms of structure-function relationships.

OXIDATIVE MODIFICATION OF PHOSPHOLIPIDS

The principles of phospholipid oxidation have been reviewed elsewhere [6, 7]. In brief, the *sn*-2 position of the glycerol backbone is of central importance, because it can be linked to a polyunsaturated fatty acyl residue prone to oxidative modification due to the low dissociation energy of carbon-hydrogen bonds adjacent to two olefinic bonds [8]. Hence, oxidation of, e.g., phosphatidylcholines (PCs) results in a number of reaction products with altered *sn*-2 residues. Two types of reactions are of particular interest regarding biological activity. One leads to addition of multiple oxygen atoms to the fatty acyl chain, which in the case of arachidonic acid yields prostaglandin-like structures [9]. Alternatively, carbon-carbon cleavage may occur, resulting in chain-shortened *sn*-2 residues [10, 11]. Depending on the nature of the chemical bond at the *sn*-1 position such modification yields oxidized acyl phospholipids (ester bond), or oxidized alkyl phospholipids (ether bond).

*Address correspondence to this author at the Department of Vascular Biology and Thrombosis Research, University of Vienna, Schwarzspanierstrasse 17, A-1090 Vienna, Austria; Tel: +43-1-4277-62507; Fax: +43-1-4277-9625; E-mail: norbert.leitinger@univie.ac.at

OXIDIZED ALKYL PHOSPHOLIPIDS

Platelet-activating factor (PAF, 1-O-alkyl-2-acetyl-*sn*-glycero-3-phosphorylcholine) is a phospholipid signaling molecule rapidly synthesized by a variety of cell types under inflammatory conditions [12]. Its actions, such as platelet and leukocyte activation, are mediated by a G-protein coupled receptor showing marked specificity towards the *sn*-1 ether bond and a short *sn*-2 acyl residue, as well as the phosphorylcholine headgroup [8]. Besides the tightly controlled synthesis of PAF, an alternative non-enzymatic pathway for the generation of high affinity ligands of the PAF receptor has been proposed. *Marathe* and colleagues could show that oxidation of alkyl arachidonoyl PCs in low density lipoproteins (LDL) creates short chain PAF-analogs (C_4-PAF analogs, PAF-like phospholipids, Fig. **1a**) being 10-fold less efficacious than original PAF [11], hence still potent PAF mimetics. In comparison, diacyl phospholipids with fragmented *sn*-2 residues are several hundred fold less potent than PAF in stimulating its receptor. All of the PAF receptor agonistic activity of oxidized LDL was attributed to these fragmented alkyl phospholipids, because phospholipase A_1 treatment, which only hydrolyzes *sn*-1 ester bonds, did not reduce the PAF-like bioactivity of phospholipids derived from oxidized LDL [11].

1381-6128/04 $45.00+.00 © 2004 Bentham Science Publishers Ltd.

More recently, another alkyl PC oxidation product occurring in oxidized LDL, azelaoyl-PC (Fig. **1b**), has been identified as a ligand for peroxisome proliferator-activated receptor gamma (PPAR) [13]. This transcription factor of the nuclear hormone receptor family governs metabolic and developmental gene expression [14-16] and is involved in atherogenesis [17, 18]. Again, a structural requirement for the *sn*-1 ether bond is demonstrated by the fact that the diacyl homolog of azelaoyl-PC is a poor ligand [13].

OXIDIZED ACYL PHOSPHOLIPIDS

The finding of *Berliner* and colleagues that mildly oxidized LDL (minimally modified LDL, MM-LDL) activates endothelial cells (EC) to bind monocytes but not neutrophils [19] ultimately led to the structural identification of several biologically active phospholipid species responsible for this effect [9, 10]. These compounds are derived by oxidation of 1-acyl-2-arachidonoyl-phosphatidylcholine of lipoprotein, cellular or synthetic origin [3, 9, 10, 20] and have been detected *in vivo* in atherosclerotic lesions [10, 21]. The specific structure at the *sn*-2 position, being either a truncated and functionalized 5-carbon residue or an epoxyisoprostane, determines the type of biological response [20]. Neither the saturated long chain fatty acid at the *sn*-1 position, nor the phospholipid head group confer specificity, since a change from palmitate to stearate, or phosphorylcholine to phosphorylethanolamine, respectively, does not affect the biological function [20].

The first members of this class of oxidized phospholipids were identified as POVPC and PGPC (Fig. **1c**) [10]. The difference in the terminal functions at the *sn*-2 residues (aldehyde / carboxy group) has a great impact on biological activity [22]. POVPC activates EC to specifically bind monocytes *in vitro*, while PGPC leads to monocyte and neutrophil binding. The involved adhesion molecules also differ: CS-1 containing fibronectin mediates POVPC-induced, while E-selectin and VCAM-1 mediates PGPC-induced leukocyte adherence. Moreover, POVPC inhibits PGPC-induced neutrophil binding and E-selectin expression. The latter effect may account for the observation that in a mixture of phospholipids derived by the oxidation of synthetic 1-palmitoyl-2-arachidonoyl-*sn*-glycero-3-phosphorylcholine (OxPAPC), where POVPC and PGPC are present at approximately equal concentrations, the effect of POVPC on leukocyte adherence dominates [22]. Another differential effect of POVPC and PGPC is observed on the coagulation system. OxPAPC exerts procoagulant activity on endothelial cells by enhanced expression of tissue factor (TF) [23] and reduced expression of the anti-coagulant thrombomodulin [24]. Of two purified lipid components tested, PGPC, but not POVPC mediated TF activation [23]. More recently, another biologically active phospholipid in MM-LDL and OxPAPC could be identified as an epoxyisoprostane derivative of PAPC, 5,6-PEIPC (Fig. **1c**) [9]. This compound has an approximate 10 fold higher potency in the activation of monocyte binding then POVPC and PGPC [9].

OxPAPC has been shown to induce the chemokines monocyte chemotactic protein 1 (MCP-1) and interleukin 8 (IL-8) in EC [25]. A functional role of these chemokines in monocyte recruitment to atherosclerotic lesions has been well documented [26-29]. The component lipids in OxPAPC with the highest potency to induce IL-8 and MCP-1 have been identified as several PEIPC isomers and their cyclopentenone dehydration products. The common structural motif in all these molecules is a , -epoxy, , -unsaturated enone moiety [3] (Fig. **1c**).

Given the considerable amount of data available for biological actions of oxidized acyl phospholipids, which receptor(s) mediate these effects is less clear. It has been shown that a PAF receptor inhibitor can block monocyte binding to endothelial cells stimulated with POVPC or PEIPC [30, 31]. However, several lines of evidence suggest that these lipids do not primarily act via the PAF receptor, but rather via one or more still to be identified receptors targeted by this inhibitor [22]. The most compelling observation is that PAF itself did not induce endothelial cells to bind monocytes [30, 31]. Furthermore, *Xenopus laevis* oocytes expressing human CFTR, a cAMP-dependent chloride channel, respond to POVPC in patch clamp studies, but do not endogenously express the PAF receptor [22]. Effects of PGPC are unaffected by the PAF receptor inhibitor, indicating the involvement of different signaling mechanisms as also evidenced by its different functions. Peroxisome proliferator-activated receptor (PPAR) has been shown to be involved in the induction of MCP-1 and IL-8 by OxPAPC [3, 25]. However, rather than moving to the nucleus and binding to PPAR directly, oxidized phospholipids have been suggested to activate a second messenger pathway, such as the lipoxygenase pathway, to generate PPAR ligands [25]. Finally, a very recent report points to the role of Toll-like receptor 4 (TLR4) in mediating OxPAPC-induced effects [32] (see below). Also in this work, evidence was presented that scavenger receptors such as CD36 are not required for OxPAPC-induced signaling.

Hydroxy Alkenal Phospholipids

The hydroxy alkenal PCs HOOA-PC and HODA-PC (Fig. **2a**) are derived by oxidation from arachidonoyl or linoleyl PC and characterized by a truncated *sn*-2 residue with a terminal -hydroxy- , -unsaturated aldehyde [33], a structural motif they share with 4-hydroxynonenal, a well known breakdown product of lipid peroxidation [34]. This moiety readily forms covalent adducts with cysteine or lysine residues of proteins, thereby altering their function. As a result, these lipids reduce the ability of macrophages to degrade internalized macromolecules, concomitant with a reduction in cellular cathepsin B activity [33]. Such an effect was previously noted with oxidized LDL (OxLDL) [35], suggesting that hydroxy alkenal phospholipids in the outer shell of OxLDL contribute to this effect. In addition, HOOA-PC has been shown to exert similar effects on endothelial cells as POVPC, regarding induction of chemokines and monocyte binding [36]. Finally, HOOA-PC and HODA-PC are members of a broader class of oxidized phospholipids with high affinity binding to the scavenger receptor CD36 (see below).

OXIDIZED PHOSPHOLIPIDS: LIGANDS FOR PATTERN-RECOGNITION RECEPTORS

Pattern-recognition receptors (PRR) are germline-encoded receptors for conserved pathogen-associated molecular patterns, such as lipopolysaccharide (LPS) of

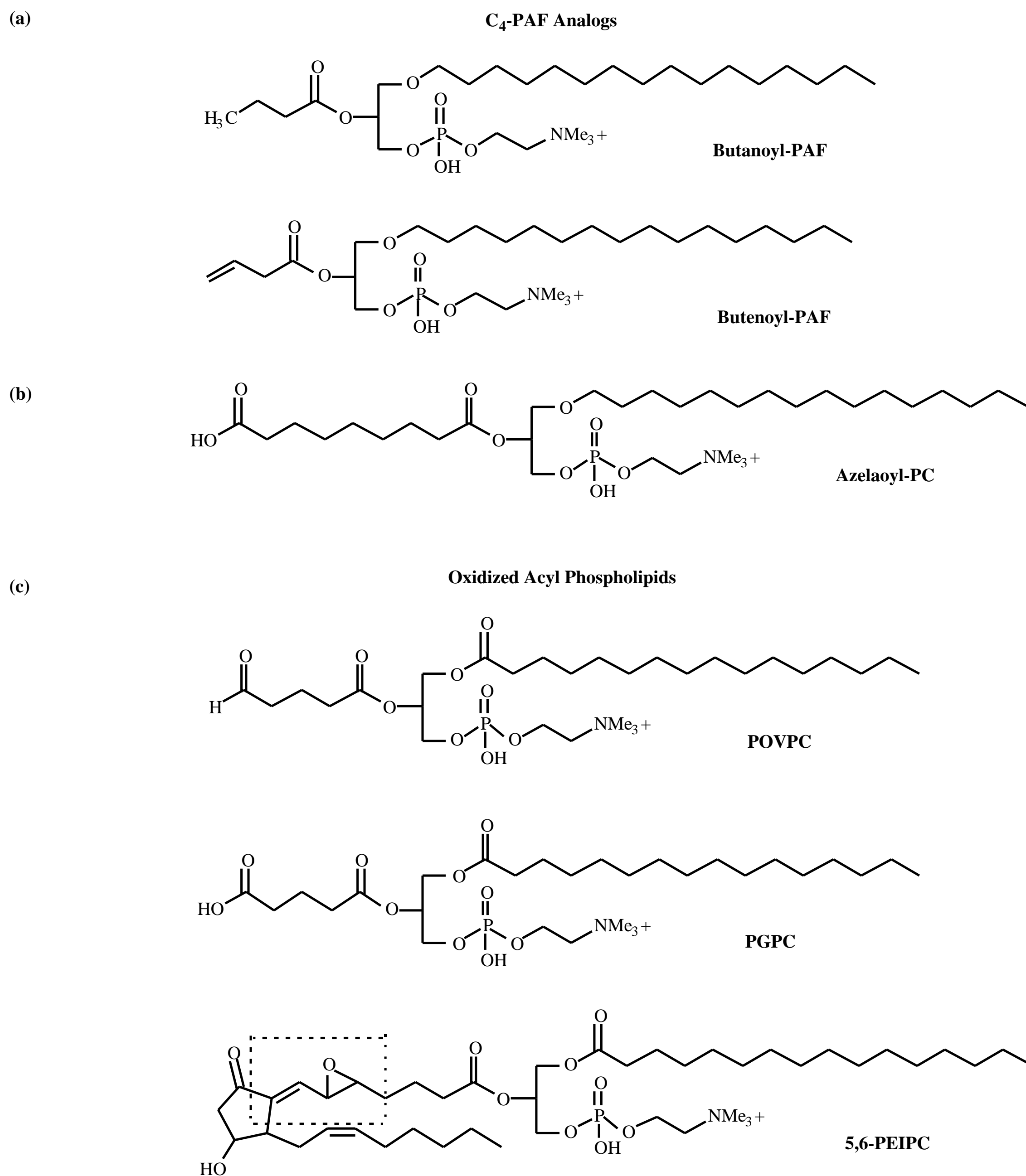

Fig. (1). Please provide figure caption.

gram-negative bacteria. They serve an essential function in first line defense against invading microorganisms or clearance of transformed or otherwise damaged cells [37]. A growing body of evidence indicates that oxidized phospholipids act as ligands for a number of these receptors [4]. This includes PRRs on the cell surface, such as the scavenger receptors CD36 [38] and SR-B1 [39], as well as circulating PRRs such as C-reactive protein (CRP) [40] or LPS binding protein (LBP) and soluble CD14 [41]. In addition, oxidation of phospholipids creates epitopes recognized by natural antibodies known to be germline-encoded antigen receptors against a limited number of phylogenetically conserved structures such as nucleic acids, heat shock proteins and carbohydrates [42, 43]. While we only begin to understand the functional role of oxidized phospholipids in the innate immune system, extensive research has elucidated some of the structural requirements for binding to different PRRs.

Structural Requirements for Antibody Recognition and CRP-Binding

Circulating autoantibodies to oxidized LDL have been demonstrated in the plasma of animals and humans [44]. *Hörkkö* and colleagues have shown that some of these antibodies are directed against epitopes created by oxidation

of phospholipids [45]. Cloning of a set of antibodies to oxidized LDL, present in high titers in the plasma of ApoE-deficient mice on a high fat diet, provided a tool for in-depth investigation of the corresponding epitopes [44]. Binding of a representative antibody of this group, EO6, to oxidized LDL was abrogated by OxPAPC liposomes, but not by liposomes created from native PAPC [46]. It was further shown that the antigen recognition domain of this set of antibodies was identical to that of classic T15 natural autoantibodies directed against the phosphorylcholine moiety in the capsular polysaccharide of *Streptococcus pneumoniae* [47]. It was thus hypothesized that in native LDL the phosphorylcholine headgroup is a cryptic epitope that is revealed after oxidation-induced conformational changes in phospholipids of the outer shell [40]. Phosphorylcholine, either covalently bound to keyhole limpet hemocyanin (PC-KLH) or as a free salt, was found to be a good antigen for EO6, as was the glycerophosphocholine backbone alone, or substituted with two short chain fatty acids [48]. POVPC, containing a long chain fatty acyl residue at the *sn*-1 position, exhibited high antigenicity toward EO6 when present as a protein adduct created by Schiff-base formation between POVPC and lysine residues, or as an aldol self-condensation product, both reactions requiring the aldehyde at the oxidatively modified *sn*-2 residue [48].The authors therefore suggest that similar Schiff-base adducts on apolipoprotein B (ApoB), or aldol condensation products are formed when LDL undergoes oxidation, rendering it immunogenic [48, 49]. EO6 was also reported to recognize PEIPC and its cyclopentenone dehydration products [3].

Very similar characteristics were found for the binding of CRP to oxidized LDL [40]. Accordingly, in the presence of EO6 this binding was reduced to about 40%, while CRP binding to PC-KLH was abolished. OxPAPC, but not oxidized phosphatidylserine or phosphatidylethanolamine competed for the binding of CRP to OxLDL. Together, this indicates that the phosphorylcholine moiety when exposed through oxidation-induced changes can serve as a ligand for natural antibodies as well as CRP [40].

CD36 Binding Phospholipids

A novel class of acyl phospholipids has recently been defined through the ability to bind to the scavenger receptor CD36 [38]. These ligands are derived by oxidation from PAPC or 1-palmitoyl-2-linoleyl-*sn*-glycero-3-phosphorylcholine (PLPC), the two most abundant phosphatidylcholines in LDL [50]. The structural requirement for high affinity binding to CD36 was a truncated *sn*-2 residue with a terminal -hydroxy(or oxo)- , -unsaturated aldehyde or carboxyl (Fig .**2**b) [38]. In contrast, a specific long chain fatty acid at the sn-1 position (palmitate *versus* oleate) was not required for CD36-binding [38]. These phospholipids, when inserted into unilamellar vesicles or liposomes containing neutral LDL-derived lipids, can function as ligands for CD36 on murine macrophages, resulting in the internalization of the entire particle and cholesterol loading [21]. This indicates a role for these lipids in CD36-mediated recognition of oxidized lipoproteins, senescent or apoptotic cells, and foam cell formation, which is underlined by enrichment of this class of oxidized phospholipids in atherosclerotic lesions in rabbits [21] and humans [33].

Schiff-base adducts of POVPC to bovine serum albumin (POVPC-BSA) have been shown to inhibit the binding of OxLDL to CD36-transfected cells [51]. A similar effect was noted in the presence of the monoclonal antibody EO6. Moreover, POVPC-BSA and EO6 inhibited the binding of both ApoB and lipids isolated from OxLDL to CD36, suggesting that structural motifs mediating antibody and CRP-binding also serve as ligands for CD36 [4].

Oxidized Phospholipids and Toll-Like Receptors

LBP and CD14 mediate cellular responses to LPS by transporting and presenting LPS to Toll-like receptor 4 (TLR4) [52, 53]. Additionally, LBP and CD14 may have a physiologic function as phospholipid transfer molecules [52]. We have shown recently that OxPAPC inhibits effects of LPS *in vitro* and *in vivo*, involving inhibition of LPS binding to LBP and CD14 [41]. Accordingly, activation of NF B and p38 MAP kinase, signaling events downstream of TLR4, were also inhibited. Oxidation-induced changes were crucial for this effect, because OxPAPC, but not native PAPC or dimyristoyl-PC (DMPC) inhibited E-selectin upregulation on endothelial cells by LPS. DMPC is resistant to oxidation due to the presence of two saturated fatty acyl residues. Furthermore, the presence of a modified residue at the *sn*-2 position, such as the oxovaleroyl group of POVPC, is important because lysophospatidylcholine did not show any inhibitory effect [52]. In contrast, LPS-inhibition seems not to rely on a specific head group, due to oxidized PAPS or PAPE being good inhibitors [20 and *Bochkov et al*., unpublished observation]. In addition, *Walton* and colleagues presented data to suggest a CD14-independent mechanism in LPS-inhibition by OxPAPC, involving interference with LPS-induced redistribution of TLR4 to the caveolar membrane [54]. In these studies KOdiA-PC (Fig. **2c**), a component lipid of OxPAPC identified as a high affinity ligand for CD36 [38] (see above), was the strongest inhibitor. However, LPS-inhibition did not depend on CD36. Furthermore, the authors provide evidence that OxPAPC also inhibits the effect of TLR2 ligands [54]. As sites of acute inflammation due to bacterial infection exhibit high oxidative stress, generation of OxPAPC species could serve as a negative feedback mechanism to modulate the course of an acute inflammation in this setting [41, 54].

Whereas substantial inhibitory action of oxidized phospholipids towards LPS-induced TLR4 signaling is evident from the above, surprisingly, recent reports indicate that TLR4 may actually be involved in oxidized phospholipid-mediated effects. *Walton* and colleagues provided evidence that IL-8 induction by OxPAPC is dependent on TLR4 [32]. Furthermore, it was demonstrated that CD14 was not involved in this effect. OxPAPC treatment induced a complex of TLR4 and a 37 kD GPI-anchored protein which bound to the EO6 antibody, indicative of a role for this newly found protein in presenting OxPAPC to TLR4 [32]. In a report by *Miller et al.* it was shown that MM-LDL specifically binds to CD14 resulting in TLR4-mediated induction of F-actin polymerization and spreading of macrophages as well as reduced phagocytosis of apoptotic cells [55]. Which component of MM-LDL binds to CD14 remains to be determined but the authors provide evidence that MM-LDL and LPS binding to CD14 occurs via different

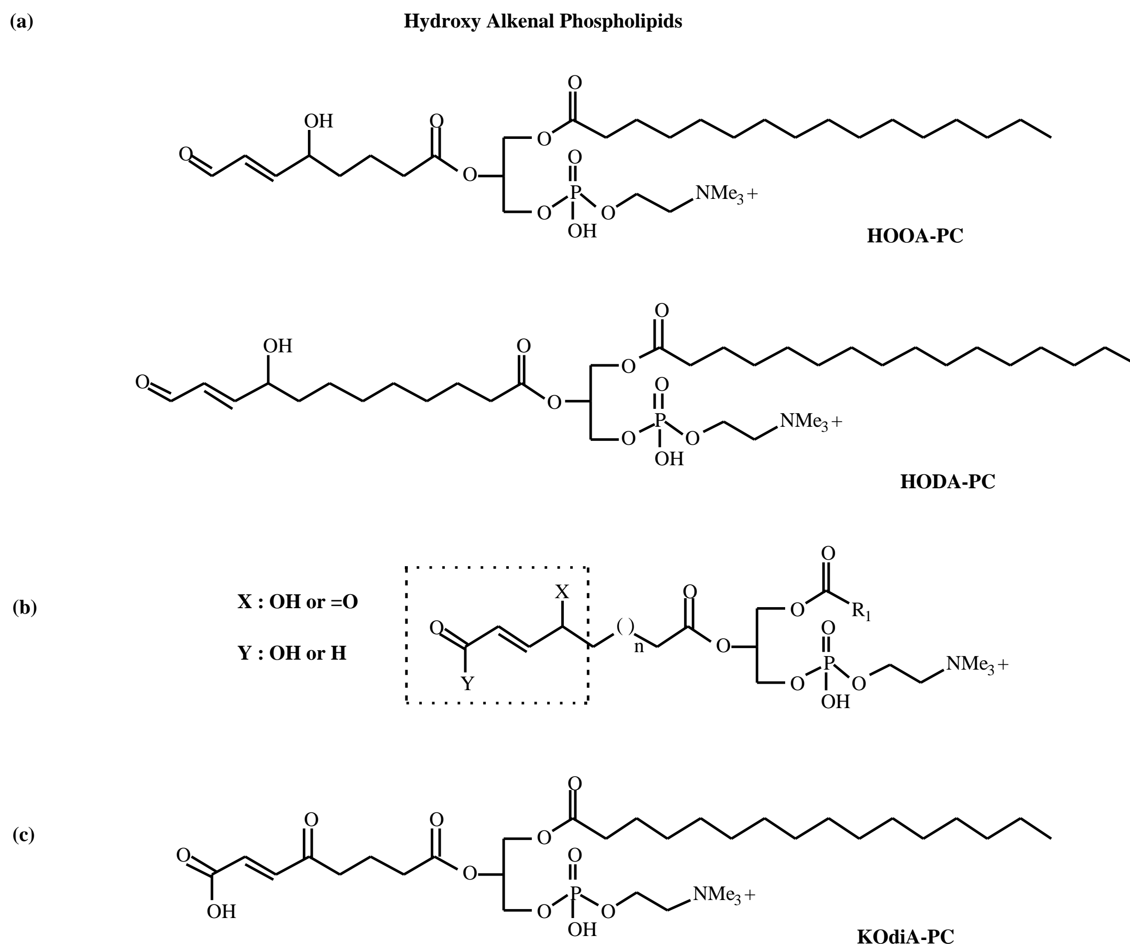

Fig. (2). Please provide figure caption.

sites and that phosphoinositide 3-kinase, but not NF B or p38 MAP kinase, is involved in these effects. Thus, it is proposed that oxidized phospholipids may mediate a hitherto unknown pathway of TLR4 signaling independent of NF B [32].

FUTURE PERSPECTIVES

With the expansion of our knowledge about oxidized phospholipids and structural requirements for certain biological effects we approach the possibility to exploit this information for therapeutic purposes. There is, of course, ongoing effort to inhibit unphysiological lipid peroxidation in the first place. However, results from clinical trials directed at proving the benefits of antioxidants in cardiovascular disease are far from clear [56]. Hence, there is a rationale for interfering more directly with oxidized phospholipid-mediated effects. Clearly, additional research is needed to perform such a task but should ultimately enable us to find proper strategies. Along these lines, a recent report points at the therapeutic potential of enhancing immune reactions to oxidized phospholipids in atherosclerosis [57]. On the other hand, given its LPS inhibiting properties, the structure of POVPC could serve as a template for new anti-endotoxin drugs lacking unwanted proinflammatory side effects [5]. Whatever path we choose, many surprises might still wait for us in this expanding and exciting field.

ABBREVIATIONS

POVPC	=	1-Palmitoyl-2-(5-oxovaleroyl)-*sn*-glycero-3-phosphorylcholine
PGPC	=	1-Palmitoyl-2-glutaroyl-*sn*-glycero-3-phosphorylcholine
PEIPC	=	1-Palmitoyl-2-(5,6-epoxyisoprostane E_2)-*sn*-glycero-3-phosphorylcholine
HOOA-PC	=	1-Palmitoyl-2-(5-hydroxy-8-oxooct-6-enoyl)-*sn*-glycero-3-phosphorylcholine
HODA-PC	=	1-Palitoyl-2-(9-hydroxy-12-oxo-10-dodecenoyl)-*sn*-glycero-3-phosphorylcholine
KOdiA-PC	=	1-Palmitoyl-2-(5-keto-6-octendioleyl)-*sn*-glycero-3-phosphorylcholine
PC-KLH	=	Keyhole limpet hemocyanin
CFTR	=	Cystic fibrosis transmembrane conductance regulator
ApoE	=	Apolipoprotein E
ApoB	=	Apolipoprotein B

REFERENCES

[1] Hazen SL. Oxidation and atherosclerosis. Free Radic Biol Med 2000; 28(12):1683-1684.

[2] Berliner JA, Subbanagounder G, Leitinger N, Watson AD, Vora D. Evidence for a role of phospholipid oxidation products in atherogenesis. Trends Cardiovasc Med 2001; 11(3-4):142-7.

[3] Subbanagounder G, Wong JW, Lee H, Faull KF, Miller E, Witztum JL et al. Epoxyisoprostane and epoxycyclopentenone phospholipids regulate monocyte chemotactic protein-1 and interleukin-8 synthesis. Formation of these oxidized phospholipids in response to interleukin-1beta. J Biol Chem 2002; 277(9):7271-81.

[4] Hazen SL, Chisolm GM. Oxidized phosphatidylcholines: pattern recognition ligands for multiple pathways of the innate immune response. Proc Natl Acad Sci U S A 2002; 99(20):12515-12517.

[5] Riedemann NC, Ward PA. Oxidized lipid protects against sepsis. Nat Med 2002; 8(10):1084-1085.

[6] Subbanagounder G, Watson AD, Berliner JA. Bioactive products of phospholipid oxidation: isolation, identification, measurement and activities. Free Radic Biol Med 2000; 28(12):1751-61.

[7] Marathe GK, Harrison KA, Murphy RC, Prescott SM, Zimmerman GA, McIntyre TM. Bioactive phospholipid oxidation products. Free Radical Biology and Medicine 2000; 28(12):1762-1770.

[8] McIntyre TM, Zimmerman GA, Prescott SM. Biologically active oxidized phospholipids. J Biol Chem 1999; 274(36):25189-92.

[9] Watson AD, Subbanagounder G, Welsbie DS, Faull KF, Navab M, Jung ME et al. Structural identification of a novel pro-inflammatory epoxyisoprostane phospholipid in mildly oxidized low density lipoprotein. J Biol Chem 1999; 274(35):24787-98.

[10] Watson AD, Leitinger N, Navab M, Faull KF, Horkko S, Witztum JL et al. Structural identification by mass spectrometry of oxidized phospholipids in minimally oxidized low density lipoprotein that induce monocyte/endothelial interactions and evidence for their presence in vivo. J Biol Chem 1997; 272(21):13597-607.

[11] Marathe GK, Davies SS, Harrison KA, Silva AR, Murphy RC, Castro-Faria-Neto H et al. Inflammatory platelet-activating factor-like phospholipids in oxidized low density lipoproteins are fragmented alkyl phosphatidylcholines. J Biol Chem 1999; 274(40):28395-404.

[12] Zimmerman GA, McIntyre TM, Prescott SM, Stafforini DM. The platelet-activating factor signaling system and its regulators in syndromes of inflammation and thrombosis. Crit Care Med 2002; 30(5 Suppl):S294-S301.

[13] Davies SS, Pontsler AV, Marathe GK, Harrison KA, Murphy RC, Hinshaw JC et al. Oxidized Alkyl Phospholipids Are Specific, High Affinity Peroxisome Proliferator-activated Receptor gamma Ligands and Agonists. J Biol Chem 2001; 276(19):16015-16023.

[14] Barak Y, Nelson MC, Ong ES, Jones YZ, Ruiz-Lozano P, Chien KR et al. PPAR gamma is required for placental, cardiac, and adipose tissue development. Mol Cell 1999; 4(4):585-595.

[15] Kubota N, Terauchi Y, Miki H, Tamemoto H, Yamauchi T, Komeda K et al. PPAR gamma mediates high-fat diet-induced adipocyte hypertrophy and insulin resistance. Mol Cell 1999; 4(4):597-609.

[16] Rosen ED, Sarraf P, Troy AE, Bradwin G, Moore K, Milstone DS et al. PPAR gamma is required for the differentiation of adipose tissue in vivo and in vitro. Mol Cell 1999; 4(4):611-617.

[17] Nagy L, Tontonoz P, Alvarez JG, Chen H, Evans RM. Oxidized LDL regulates macrophage gene expression through ligand activation of PPARgamma. Cell 1998; 93(2):229-40.

[18] Tontonoz P, Nagy L, Alvarez JG, Thomazy VA, Evans RM. PPARgamma promotes monocyte/macrophage differentiation and uptake of oxidized LDL. Cell 1998; 93(2):241-52.

[19] Berliner JA, Territo MC, Sevanian A, Ramin S, Kim JA, Bamshad B et al. Minimally modified low density lipoprotein stimulates monocyte endothelial interactions. J Clin Invest 1990; 85(4):1260-6.

[20] Subbanagounder G, Leitinger N, Schwenke DC, Wong JW, Lee H, Rizza C et al. Determinants of bioactivity of oxidized phospholipids. Specific oxidized fatty acyl groups at the sn-2 position. Arterioscler Thromb Vasc Biol 2000; 20(10):2248-54.

[21] Podrez EA, Poliakov E, Shen Z, Zhang R, Deng Y, Sun M et al. A Novel Family of Atherogenic Oxidized Phospholipids Promotes Macrophage Foam Cell Formation via the Scavenger Receptor CD36 and Is Enriched in Atherosclerotic Lesions. Journal of Biological Chemistry 2002; 277(41):38517-38523.

[22] Leitinger N, Tyner TR, Oslund L, Rizza C, Subbanagounder G, Lee H et al. Structurally similar oxidized phospholipids differentially regulate endothelial binding of monocytes and neutrophils. Proc Natl Acad Sci U S A 1999; 96(21):12010-5.

[23] Bochkov VN, Mechtcheriakova D, Lucerna M, Huber J, Malli R, Graier WF et al. Oxidized phospholipids stimulate tissue factor expression in human endothelial cells via activation of ERK/EGR-1 and Ca(++)/NFAT. Blood 2002; 99(1):199-206.

[24] Ishii H, Tezuka T, Ishikawa H, Takada K, Oida K, Horie S. Oxidized phospholipids in oxidized low-density lipoprotein downregulate thrombomodulin transcription in vascular endothelial cells through a decrease in the binding of RAR{beta}-RXR{alpha} heterodimers and Sp1 and 3 to their binding sequences in the TM promoter. Blood 2003;2002-2008.

[25] Lee H, Shi W, Tontonoz P, Wang S, Subbanagounder G, Hedrick CC et al. Role for peroxisome proliferator-activated receptor alpha in oxidized phospholipid-induced synthesis of monocyte chemotactic protein-1 and interleukin-8 by endothelial cells. Circ Res 2000; 87(6):516-21.

[26] Gu L, Okada Y, Clinton SK, Gerard C, Sukhova GK, Libby P et al. Absence of monocyte chemoattractant protein-1 reduces atherosclerosis in low density lipoprotein receptor-deficient mice. Mol Cell 1998; 2(2):275-281.

[27] Boring L, Gosling J, Cleary M, Charo IF. Decreased lesion formation in CCR2-/- mice reveals a role for chemokines in the initiation of atherosclerosis. Nature 1998; 394(6696):894-897.

[28] Boisvert WA, Santiago R, Curtiss LK, Terkeltaub RA. A Leukocyte Homologue of the IL-8 Receptor CXCR-2 Mediates the Accumulation of Macrophages in Atherosclerotic Lesions of LDL Receptor-deficient Mice. J Clin Invest 1998; 101(2):353-363.

[29] Gerszten RE, Garcia-Zepeda EA, Lim YC, Yoshida M, Ding HA, Gimbrone MA, Jr. et al. MCP-1 and IL-8 trigger firm adhesion of monocytes to vascular endothelium under flow conditions. Nature 1999; 398(6729):718-23.

[30] Leitinger N, Watson AD, Faull KF, Fogelman AM, Berliner JA. Monocyte binding to endothelial cells induced by oxidized phospholipids present in minimally oxidized low density lipoprotein is inhibited by a platelet activating factor receptor antagonist. Adv Exp Med Biol 1997; 433:379-382.

[31] Subbanagounder G, Leitinger N, Shih PT, Faull KF, Berliner JA. Evidence that phospholipid oxidation products and/or platelet-activating factor play an important role in early atherogenesis : in vitro and In vivo inhibition by WEB 2086. Circ Res 1999; 85(4):311-8.

[32] Walton KA, Hsieh X, Gharavi N, Wang S, Wang G, Yeh M et al. Receptors involved in the Ox-PAPC-mediated synthesis of IL-8: A role for toll-like receptor 4 and a GPI anchored protein. Journal of Biological Chemistry 2003;M300738200.

[33] Hoff HF, O'Neil J, Wu Z, Hoppe G, Salomon RL. Phospholipid Hydroxyalkenals: Biological and Chemical Properties of Specific Oxidized Lipids Present in Atherosclerotic Lesions. Arterioscler Thromb Vasc Biol 2003; 23(2):275-282.

[34] Esterbauer H, Schaur RJ, Zollner H. Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. Free Radic Biol Med 1991; 11(1):81-128.

[35] Hoppe G, O'Neil J, Hoff HF. Inactivation of lysosomal proteases by oxidized low density lipoprotein is partially responsible for its poor degradation by mouse peritoneal macrophages. J Clin Invest 1994; 94(4):1506-1512.

[36] Subbanagounder G, Deng Y, Borromeo C, Dooley AN, Berliner JA, Salomon RG. Hydroxy alkenal phospholipids regulate inflammatory functions of endothelial cells. Vascular Pharmacology 2002; 38(4):201-209.

[37] Medzhitov R, Janeway CA, Jr. Decoding the Patterns of Self and Nonself by the Innate Immune System. Science 2002; 296(5566):298.

[38] Podrez EA, Poliakov E, Shen Z, Zhang R, Deng Y, Sun M et al. Identification of a Novel Family of Oxidized Phospholipids That Serve as Ligands for the Macrophage Scavenger Receptor CD36. Journal of Biological Chemistry 2002; 277(41):38503-38516.

[39] Gillotte-Taylor K, Boullier A, Witztum JL, Steinberg D, Quehenberger O. Scavenger receptor class B type I as a receptor for oxidized low density lipoprotein. J Lipid Res 2001; 42(9):1474.

[40] Chang MK, Binder CJ, Torzewski M, Witztum JL. C-reactive protein binds to both oxidized LDL and apoptotic cells through recognition of a common ligand: Phosphorylcholine of oxidized

phospholipids. Proc Natl Acad Sci U S A 2002; 99(20):13043-13048.

[41] Bochkov VN, Kadl A, Huber J, Gruber F, Binder BR, Leitinger N. Protective role of phospholipid oxidation products in endotoxin-induced tissue damage. Nature 2002; 419(6902):77-81.

[42] Binder CJ, Chang MK, Shaw PX, Miller YI, Hartvigsen K, Dewan A et al. Innate and acquired immunity in atherogenesis. Nat Med 2002; 8(11):1218-1226.

[43] Boes M, Prodeus AP, Schmidt T, Carroll MC, Chen J. A Critical Role of Natural Immunoglobulin M in Immediate Defense Against Systemic Bacterial Infection. J Exp Med 1998; 188(12):2381.

[44] Palinski W, Horkko S, Miller E, Steinbrecher UP, Powell HC, Curtiss LK et al. Cloning of Monoclonal Autoantibodies to Epitopes of Oxidized Lipoproteins from Apolipoprotein E-deficient Mice . Demonstration of Epitopes of Oxidized Low Density Lipoprotein in Human Plasma. J Clin Invest 1996; 98(3):800.

[45] Horkko S, Miller E, Dudl E, Reaven P, Curtiss LK, Zvaifler NJ et al. Antiphospholipid Antibodies Are Directed against Epitopes of Oxidized Phospholipids . Recognition of Cardiolipin by Monoclonal Antibodies to Epitopes of Oxidized Low Density Lipoprotein. J Clin Invest 1996; 98(3):815.

[46] Horkko S, Bird DA, Miller E, Itabe H, Leitinger N, Subbanagounder G et al. Monoclonal autoantibodies specific for oxidized phospholipids or oxidized phospholipidûprotein adducts inhibit macrophage uptake of oxidized low-density lipoproteins. J Clin Invest 1999; 103(1):117.

[47] Shaw PX, Horkko S, Chang MK, Curtiss LK, Palinski W, Silverman GJ et al. Natural antibodies with the T15 idiotype may act in atherosclerosis, apoptotic clearance, and protective immunity. J Clin Invest 2000; 105(12):1731.

[48] Friedman P, Horkko S, Steinberg D, Witztum JL, Dennis EA. Correlation of Antiphospholipid Antibody Recognition with the Structure of Synthetic Oxidized Phospholipids. IMPORTANCE OF SCHIFF BASE FORMATION AND ALDOL CONDENSATION. Journal of Biological Chemistry 2002; 277(9):7010.

[49] Gillotte KL, Horkko S, Witztum JL, Steinberg D. Oxidized phospholipids, linked to apolipoprotein B of oxidized LDL, are ligands for macrophage scavenger receptors. J Lipid Res 2000; 41(5):824.

[50] Steinberg D. Low Density Lipoprotein Oxidation and Its Pathobiological Significance. Journal of Biological Chemistry 1997; 272(34):20963.

[51] Boullier A, Gillotte KL, Horkko S, Green SR, Friedman P, Dennis EA et al. The Binding of Oxidized Low Density Lipoprotein to Mouse CD36 Is Mediated in Part by Oxidized Phospholipids That Are Associated with Both the Lipid and Protein Moieties of the Lipoprotein. Journal of Biological Chemistry 2000; 275(13):9163.

[52] Yu B, Hailman E, Wright SD. Lipopolysaccharide Binding Protein and Soluble CD14 Catalyze Exchange of Phospholipids. J Clin Invest 1997; 99(2):315.

[53] Aderem A, Ulevitch RJ. Toll-like receptors in the induction of the innate immune response. Nature 2000; 406(6797):782-787.

[54] Walton KA, Cole AL, Yeh M, Subbanagounder G, Krutzik SR, Modlin RL et al. Specific Phospholipid Oxidation Products Inhibit Ligand Activation of Toll-Like Receptors 4 and 2. Arterioscler Thromb Vasc Biol 2003;01.

[55] Miller YI, Viriyakosol S, Binder CJ, Feramisco JR, Kirkland TN, Witztum JL. Minimally Modified LDL Binds to CD14, Induces Macrophage Spreading via TLR4/MD-2, and Inhibits Phagocytosis of Apoptotic Cells. Journal of Biological Chemistry 2003; 278(3):1561-1568.

[56] Jialal I, Devaraj S. Antioxidants and Atherosclerosis: Don't Throw Out the Baby With the Bath Water. Circulation 2003; 107(7):926.

[57] Binder CJ, Horkko S, Dewan A, Chang MK, Kieu EP, Goodyear CS et al. Pneumococcal vaccination decreases atherosclerotic lesion formation: molecular mimicry between Streptococcus pneumoniae and oxidized LDL. Nat Med 2003.