

Oxidized phospholipids as modulators of inflammation in atherosclerosis

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Purpose of review

This review will summarize recent evidence demonstrating that biologically active phospholipid oxidation products modulate inflammatory reactions.

Recent findings

Structural identification of new biologically active oxidized phospholipids and the finding that they can also be formed at inflammatory sites other than the atherosclerotic lesion have expanded the potential role of these compounds in inflammation beyond atherogenesis. Various signaling pathways are induced by oxidized phospholipids, leading to the expression of inflammatory genes by mechanisms that differ from those mediated by the classic inflammatory agonists tumor necrosis factor or lipopolysaccharide. Furthermore, oxidized phospholipids can bind to pattern recognition molecules and thus potentially influence inflammation and immune responses during host defense.

Summary

During inflammatory processes biologically active lipid oxidation products accumulate that modulate the inflammatory process and may determine the fate and outcome of the body's reaction in acute inflammation during host defense. Oxidized phospholipids may induce and propagate chronic inflammatory processes; however, evidence is accumulating that cells and tissues respond towards these oxidatively formed stress signals also by activation of anti-inflammatory, cytoprotective reactions.

Keywords

apoptosis, atherosclerosis, free radicals, host defense, inflammation, oxidized phospholipids, signal transduction

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Abbreviations

CREB	cAMP response element-binding protein
EGR-1	early growth response protein 1
HAEC	human aortic endothelial cell
HO-1	heme oxygenase 1
HUVEC	human umbilical vein endothelial cell
MCP-1	monocyte chemoattractant protein 1
NF- κ B	nuclear factor kappa B
OxPAPC	oxidized 1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphorylcholine
PAF	platelet-activating factor
PGPC	1-palmitoyl-2-glutaroyl-sn-glycero-3-phosphorylcholine
POVPC	1-palmitoyl-2-(5-oxovaleroyl)-sn-glycero-3-phosphorylcholine
PPAR	peroxisome proliferator-activated receptor
RAR β	retinoic acid receptor beta
RXR α	retinoid X receptor alpha
TLR-4	Toll-like receptor 4

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Introduction

It has become evident in recent years that the underlying mechanisms that lead to atherosclerotic changes in the vessel wall are of inflammatory origin. However, what kind of an inflammatory process are we talking about? A very rough distinction between the two major forms of inflammation is that 'acute' inflammation is mainly characterized by the infiltration of polymorphonuclear granulocytes to the site of injury or infection, whereas the hallmark of 'chronic' inflammation is the accumulation of mononuclear cells. Given the ongoing accumulation of mononuclear cells in the vessel wall, atherosclerosis can be regarded as a chronic inflammatory disease. In search of the culprits that cause atherosclerosis, evidence is accumulating that lipid oxidation products could play a major role in inducing and propagating monocytic inflammation, as well as other inflammatory reactions, essentially at all stages of the disease process [1,2]. Indeed, oxidized lipids can mimic *in vitro* major events in the initiation and propagation of chronic inflammation, and may even contribute to the pro-coagulatory phenotype in advanced lesions that would ultimately lead to thrombosis. These findings were the basis of the current view of oxidized lipids as pro-inflammatory agents in atherogenesis – the concept of 'lipid-induced inflammation'. On the other hand, oxidized phospholipids also exert anti-inflammatory and cytoprotective effects.

Biological activity of oxidized phospholipids: relation to chemical structure

The structures and biological activities of oxidized acyl phospholipids were described using oxidized 1-palmi-

toyl-2-arachidonoyl-*sn*-glycero-3-phosphorylcholine (OxPAPC), which is present in minimally modified LDL. The biological activities of OxPAPC and minimally modified LDL include the ability to stimulate endothelial cells to bind monocytes and to secrete monocyte chemoattractant protein 1 (MCP-1) and IL-8, suggesting a role for oxidized phospholipids in atherogenesis [1]. Individual lipids identified in OxPAPC include 1-palmitoyl-2-(5-oxovaleroyl)-*sn*-glycero-3-phosphorylcholine (POVPC), 1-palmitoyl-2-glutaroyl-*sn*-glycero-3-phosphorylcholine (PGPC) [3], and epoxy-isoprostane-PC [4] (Figure 1).

Oxidized 1-palmitoyl-2-linoleoyl-*sn*-glycero-3-phosphorylcholine (PLPC) also induced monocyte-endothelial interactions [5]. Recently, new structures of oxidized phospholipids were identified. Using a myeloperoxidase- H_2O_2 - NO_2 system, Podrez *et al.* [6•] oxidized PAPC and PLPC and structurally identified ligands for CD36. For high affinity binding to CD36 a chain shortened acyl group at the *sn*-2 position was required, which incorporates a terminal γ -hydroxy(or oxo)- α,β -unsaturated carbonyl group [6•]. In an accompanying paper, the authors showed that these identified lipids were also present in atherosclerotic lesions [7]. Moreover, phospholipid hydroxyl alkenals have been detected in atherosclerotic lesions, both unbound and as covalent adducts [8]. Subbanagounder *et al.* [9] showed that hydroxyl alkenal phospholipids in OxPAPC induce the expression of MCP-1 and IL-8 in human aortic endothelial cells

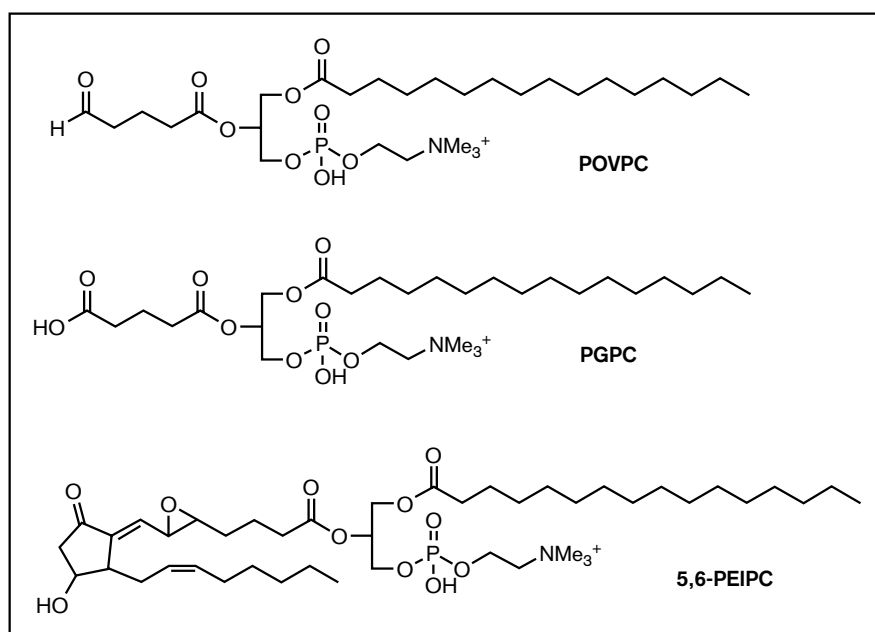
(HAECs), and also inhibit lipopolysaccharide-induced inflammatory gene expression, thus mimicking the biological action of OxPAPC. Moreover, epoxyisoprostane and epoxycyclopentenone phospholipids have been identified in OxPAPC that also induced MCP-1 and IL-8 in endothelial cells [10•].

Phospholipid oxidation products were shown to exert different biological activities depending on the chemical bond at the *sn*-1 position [1,11]. Phospholipids containing alkyl residues (ether bond) at the *sn*-1 position upon oxidative fragmentation of the unsaturated fatty acid at the *sn*-2 position become agonists for the platelet-activating factor (PAF) receptor [12]. An ether bond at the *sn*-1 position is required for high affinity binding to the PAF receptor [13], and oxidized alkyl short chain phospholipids ('PAF-like lipids') were shown to activate polymorphonuclear leukocytes [14], but also platelets and monocytes via the PAF receptor [15].

Some of the ester-containing oxidized phospholipids (acyl phospholipids) derived from OxPAPC can also be inhibited by PAF receptor antagonists; however, their actions cannot be mimicked by PAF, and it is thus proposed that specific receptors for these compounds exist [16]. Furthermore, the PAF receptor shows a preference for phosphatidylcholine. As oxidized ester phospholipids show similar activity irrespective of the head group [17,18], a mechanism of cell activation independent of the PAF receptor seems likely [18,19].

Figure 1. Structures of biologically active oxidized phospholipids derived from oxidation of 1-palmitoyl-2-arachidonoyl-*sn*-glycero-3-phosphorylcholine

POVPC, 1-palmitoyl-2-(5-oxovaleroyl)-*sn*-glycero-3-phosphorylcholine; PGPC, 1-palmitoyl-2-glutaroyl-*sn*-glycero-3-phosphorylcholine; PEIPC, epoxy-isoprostane-PC.



However, cellular receptors mediating the pro-inflammatory actions of oxidized acyl phospholipids are not known. There are indications that the effects of some of these lipids might be mediated by currently still unidentified G protein-coupled receptors [20]. Alternatively, oxidized phospholipids could interfere with membrane integrity and thereby induce intracellular signaling or migrate through membranes and bind to intracellular receptors.

In a recent study [21], both oxidized alkyl as well as acyl phospholipids containing oxovaleroyl residues at the *sn*-2 position were isolated from human atherosclerotic lesions. The oxidized alkyl phospholipids induced platelet aggregation via the PAF receptor whereas the acyl lipids (POVPC) induced platelet shape change. On the other hand, both groups of lipids inhibited endothelium-dependent arterial relaxation [21].

Taken together, whereas oxidized acyl phospholipids activate cells via unknown mechanisms, oxidized alkyl phospholipids exert proinflammatory activities by activating the PAF receptor. As new structures of oxidized phospholipids are being identified, novel biological functions also emerge for this class of lipid oxidation products.

Formation of oxidized phospholipids during inflammation and apoptosis

Oxidized phospholipids have been shown to accumulate in atherosclerotic lesions [22]. However, the atherosclerotic lesion is not the only place where oxidized phospholipids are formed. In general, inflammation is accompanied by increased oxidative stress. Particularly during antibacterial defense, reactive oxygen species generated by activated leukocytes kill bacteria but also modify host molecules. It has been demonstrated that myeloperoxidase promotes lipid peroxidation by the generation of tyrosyl radicals [23,24] and nitric oxide-derived oxidants, such as nitrous oxide [25,26]. The impact of myeloperoxidase in lipid peroxidation is further underlined by studies using leukocytes from individuals lacking this enzyme, demonstrating that lipid oxidation was significantly reduced [27•,28].

It has been shown that oxidized phospholipids may also play a role in lung diseases such as acute respiratory distress syndrome and asthma [29,30]. Oxidized phospholipids are generated by ozone treatment of lung surfactant, which is 90% composed of phospholipids [31], and lipid peroxidation was shown to occur *in vivo* in lungs [32,33]. It was recently shown that the vinyl ether bonds of plasmalogens are targets for reactive brominating species produced by the eosinophil peroxidase system. The resulting α -bromo fatty aldehyde, which was also produced by phorbol ester treatment of

eosinophils, was demonstrated to be a phagocyte chemoattractant [34].

Additional evidence for a role of oxidized phospholipids in general inflammation *in vivo* was recently presented. Using a mouse pleurisy model, Silva *et al.* [35•] showed that PAF-like lipids isolated from oxidized LDL, which were selected by their ability to activate neutrophils *in vitro* via the PAF receptor, induced neutrophil as well as eosinophil extravasation that could be blocked by PAF receptor antagonists. They further showed that the expression of MCP-1 and 5-lipoxygenase and its products were required for the induced inflammatory response. Using RNase protection assays, it was shown that other chemokines such as RANTES (regulated upon activation: normal T cell expressed/secreted), macrophage-inflammatory protein (MIP) types 1 α and β , macrophage-inflammatory protein type 2, and interferon- α -inducible chemokine (IP-10) were also upregulated. In addition, MCP-1 was required for a complete inflammatory response as cytokine expression, leukotriene production and leukocyte accumulation was significantly decreased in MCP-1 null mice [35•]. It was demonstrated also that the formation of oxidized phospholipids in the kidney as a result of ischaemia induced inflammatory responses [36•,37].

These results suggest that oxidized phospholipids are generated during inflammation in various tissues and organs as a general mechanism. Indeed, the formation of oxidized phospholipids as a result of inflammation-induced oxidative stress has been confirmed by experiments *in vitro* showing that IL-1 β induced phospholipid oxidation in cultured endothelial cells [10•].

Oxidized phospholipids in plasma

It is believed that biologically active oxidized phospholipids accumulate locally, especially in inflamed tissue. Although the presence of these lipids has been reported in plasma [38–42], their biological activity may be significantly repressed by the action of plasma enzymes (serum phospholipase A₂, paraoxonase, PAF-acetylhydrolase) that degrade these lipids [43,44•,45,46]. It was recently shown that paraoxonase, PAF-acetylhydrolase and lecithin:cholesterol acyltransferase activities were decreased in atherosclerosis-susceptible mice such as apolipoprotein E or LDL-receptor null mice, which was accompanied by elevated plasma levels of oxidized phospholipids [47]. To investigate whether increased plasma levels of oxidized phospholipids would indeed lead to the expression of inflammatory genes *in vivo*, mice were injected intravenously with OxPAPC, and gene expression was analyzed in various tissues as well as blood. In that study, Kadl *et al.* [48] showed that JE, the mouse homologue of MCP-1, heme oxygenase 1 (HO-1) and early growth response protein 1 (EGR-1)

were upregulated in various tissues including the liver and heart. The studies further imply that increased plasma levels of oxidized phospholipids may contribute to the early atherogenic phenotype seen in atherosclerosis-prone mice.

Apoptosis is accompanied by phospholipid oxidation

Apoptosis of various cell types was shown to be accompanied by the oxidation of all major phospholipid classes by reactive oxygen species generated by NADPH oxidase [49,50–52]. The externalization of oxidized phospholipids was shown to be required for macrophage clearance of apoptotic cells [53], and oxidized phospholipid epitopes of apoptotic cells were recognized not only by specific antibodies [54] but also by C-reactive protein [55]. Furthermore, we were able to show that membrane parts shed from apoptotic cells (apoptotic blebs) contained biologically active oxidized phospholipids that activated endothelial cells to bind monocytes [56]. Apoptotic cells are thus an additional source of oxidized phospholipids and may actively contribute to inflammation.

Lipid peroxidation also leads to the loss of phospholipid asymmetry in plasma membranes, causing membrane vesiculation [57]. It was shown that microvesicles released from tert-butyl-hydroperoxide (t-BuOOH)-treated endothelial cells contained the oxidized phospholipids [57] POVPC and PGPC, and induced monocyte-endothelial interactions [56]. Such microvesicles originating from different cell types were demonstrated in various pathological settings, and the oxidative modification of microvesicles *in vivo* seems likely. Oxidized microvesicles may thus play a role in the initiation and amplification of chronic inflammatory processes.

Signaling pathways induced by oxidized phospholipids

Oxidized phospholipids activate several different signaling pathways in target cells (Figure 2). Signaling mechanisms activated by oxidized phospholipids in endothelial cells include the elevation of cyclic AMP [58], an increase in cytosolic calcium levels [59] and the activation of mitogen-activated protein kinase cascades [59], but also the induction of mitogen-activated protein kinase phosphatase 1 [60]. This results in the activation of transcription mediated by EGR-1 and NFAT (nuclear factor of activated T-cells) [59], CREB (cAMP response element-binding protein)(manuscript submitted), peroxisome proliferator-activated receptor (PPAR) α [61,62] and PPAR γ [63]. Recently, the ability to activate PPAR α in endothelial cells was described for phosphatidylcholines containing epoxyisoprostane and epoxycyclopentenone at the *sn*-2 position [10]. In addition, we could show that OxPAPC does not induce the nuclear factor kappa B (NF κ B) signaling pathway in human umbilical vein endothelial cells (HUVECs) [59,64] (Figure 2, Table 1).

The activation of the redox-sensitive NF κ B signaling pathway by oxidized LDL thus probably results from the presence of lipid hydroperoxides, which are present in very low amounts in OxPAPC.

Gene expression induced by oxidized phospholipids

Oxidized phospholipids activate various cell types to express a specific set of proteins that is involved in inflammatory reactions.

Gene expression in human aortic endothelial cells

In order to screen for genes that would be induced by the treatment of HAECs with OxPAPC, Reddy *et al.* [69] used the method of suppression subtractive hybridization. Genes induced by OxPAPC, but not by native PAPC, included MCP-1, IL-8, or GRO- α (growth related oncogene), and a pattern of genes that may play important roles in atherosclerosis, angiogenesis, and general inflammation [70] (see Table 2).

Early growth response protein 1

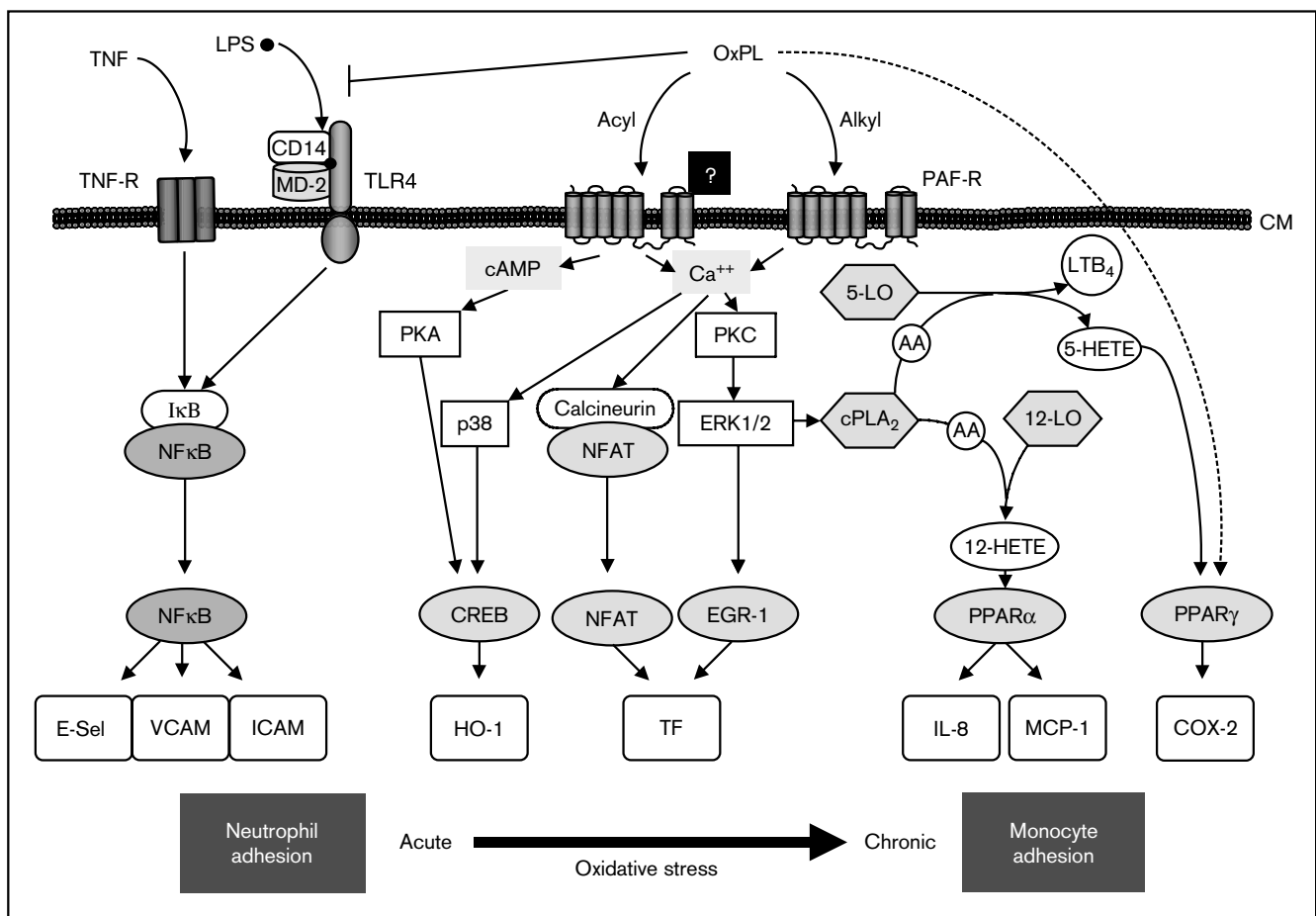
We were able to show that oxidized phospholipids increase the synthesis of EGR-1 *in vitro* [59] and *in vivo* [48]. EGR-1 is a transcription factor rapidly and transiently induced by a variety of extracellular stimuli, including growth factors, cytokines, hypoxia, mechanical stimulation, and injurious stimuli. The induction of EGR-1 by OxPAPC was mediated by the MEK/ERK (met-enkephalin/extracellular signal-related kinase) 1/2 cascade (Figure 2). Many genes that mediate thrombosis and inflammation contain an EGR-1-binding site in their promoter, and as high levels of EGR-1 were found in atherosclerotic lesions, a role for EGR-1 in atherosclerosis was suggested. Oxidized phospholipids may thus contribute to increased levels of EGR-1 in atherosclerotic lesions.

Tissue factor and thrombomodulin

Tissue factor expression induced by OxPAPC was mediated by the protein kinase C/ERK/EGR-1 and calcium/calcieneurin/NFAT pathways, not involving the NF κ B pathway [59] (Figure 2). In this paper, the authors showed that PGPC was the active substance in OxPAPC responsible for tissue factor upregulation. In addition to HUVEC, the upregulation of tissue factor by oxidized phospholipids has also been demonstrated in smooth muscle cells [66,71] and *in vivo* [48].

It has recently been shown that the expression of thrombomodulin, an anticoagulant glycoprotein, is down-regulated in HUVECs by oxidized LDL [67]. The authors showed that oxidized phospholipids, but not other lipid components of oxidized LDL, were responsible for the effect. The transcriptional repression of thrombomodulin expression by OxPAPC was mediated

Figure 2. There are striking differences between cell activation by oxidized phospholipids and other proinflammatory mediators such as TNF or lipopolysaccharide



Activation of the nuclear factor kappa B (NFκB) pathway by TNF or lipopolysaccharide leads to an 'acute' inflammatory response involving neutrophil as well as monocyte recruitment. At the endothelial level, this effect is mainly achieved by the expression of inflammatory adhesion molecules such as E-selectin and vascular cell adhesion molecule 1 and various chemokines, all of which have NFκB binding sites in their promoters. On the other hand, oxidized phospholipids would induce the expression of certain inflammatory genes independent of NFκB signaling, leading to the observed 'chronic', mononuclear-cell-specific response.

AA, arachidonic acid; Ca²⁺, calcium ion; CM, cellular membrane; COX-2, cyclooxygenase 2; cPLA₂, cytosolic phospholipase A₂; CREB cAMP response element-binding protein; EGR-1, early growth response protein 1; ERK, extracellular signal-related kinase; E-Sel, E-selectin; HETE, hydroxyeicosatetraenoic; HO-1, heme oxygenase 1; ICAM, intercellular adhesion molecule; IκB, inhibitor of nuclear factor kappa B; LO, lipoxygenase; LPS, lipopolysaccharide; leukotriene B₄, LTB₄; MCP, monocyte chemoattractant protein; NFAT nuclear factor of activated T-cells; NFκB, nuclear factor kappa B; OxPL, oxidized phospholipids; PAF-R, platelet activating factor receptor; PKA, protein kinase A; PKC, protein kinase C; PPAR, peroxisome proliferator-activated receptor; TF, tissue factor; TLR-4, Toll-like receptor 4; VCAM, vascular cell adhesion molecule.

by inhibition of the binding of retinoic acid receptor beta (RARβ)-retinoid X receptor alpha (RXRα) heterodimers as well as Sp1 and Sp3 to their respective binding elements in the thrombomodulin promoter. In addition, OxPAPC reduced the expression of RARβ-RXRα as well as Sp1 and Sp3. Remarkably, it was shown in the study that treatment of cells with the endocytosis inhibitor cytochalasin B suppressed oxidized LDL-induced effects on thrombomodulin downregulation, but not the downregulation of thrombomodulin by OxPAPC. These results indicated that the effects of oxidized LDL were mediated by endocytosis; however, OxPAPC-exerted effects were mediated by either permeation through the

membrane and acting on intracellular receptors, or by acting on membrane receptors [67*].

Taken together, both OxPAPC-mediated upregulation of the procoagulant tissue factor and downregulation of the anticoagulant thrombomodulin in different cell types of the vascular wall make a strong case for OxPAPC as a promoter of a coagulation-inducing vascular surface.

Cyclooxygenase 2

Oxidized alkyl phospholipids were shown to induce cyclooxygenase 2 in monocytes, which resulted in increased prostaglandin E₂ production [65]. The authors

Table 1. Inflammatory genes that are differently regulated by oxidized phospholipids and lipopolysaccharide/TNF

Genes induced during inflammation	Involved signaling pathways and transcription factors		Reference
	Induced by lipopolysaccharide, TNF- α	Induced by oxidized phospholipids	
E-selectin	NF κ B	n.i.	[64]
VCAM-1	NF κ B, AP-1	n.i.	[64]
ICAM-1	NF κ B	n.i.	[64]
IL-8	NF κ B, AP-1	PPAR α	[10*,61]
MCP-1	NF κ B	PPAR α , MKP-1	[10*,60,61]
COX-2	NF κ B	PPAR γ	[65]
Tissue factor	NF κ B	ERK-EGR1, Ca ²⁺ -NFAT, Sp-1	[59*,66]
Thrombomodulin (downregulation!)	Mechanism not clear	Decrease of RAR β -RXR α , Sp1, Sp3	[67*]
HO-1	n.i.	Antioxidant-responsive-element binding proteins	[48,68]

Ca²⁺, calcium ion; COX-2, cyclooxygenase 2; EGR-1, early growth response protein 1; ERK, extracellular signal-related kinase; HO-1, heme oxygenase 1; ICAM-1, intercellular adhesion molecule 1; MCP-1, monocyte chemoattractant protein 1; MKP-1, MAP kinase phosphatase 1; NFAT, nuclear factor of activated T-cells; NF κ B, nuclear factor kappa B; n.i., not induced; PPAR, peroxisome proliferator-activated receptor; RAR β , retinoic acid receptor beta; RXR α , retinoid X receptor alpha; VCAM-1, vascular cell adhesion molecule 1.

Table 2. Genes that were shown to be regulated by oxidized phospholipids *in vitro* and *in vivo*

Gene	Cell type/tissue	Phospholipid type	Reference
MCP-1/JE	HAEC, HUVEC, HepG2, liver, heart, monocytes, leukocytes from pleural cavity	OxPAPC, PEIPC, PECPC, HOOA-PC, PAF-like	[9,10*,35*,48,69*]
IL-8/KC	HAEC, HUVEC, liver, monocytes	OxPAPC, PEIPC, PECPC, HOOA-PC	[9,10*,42,48,69*]
GRO- α	HAEC	OxPAPC	[69*]
EGR-1	HUVEC, liver, heart, SMC	OxPAPC	[48,59*,66]
Tissue factor	HAEC, HUVEC, monocytes, U937, lung, blood cells, SMC	OxPAPC, PGPC	[48,59*,71]
MKP-1	HAEC, HUVEC	OxPAPC	[69*]
Annexin II	HAEC	OxPAPC	[69*]
HBGF-1	HAEC	OxPAPC	[69*]
Fe-H chain	HAEC	OxPAPC	[69*]
uPAR	HAEC	OxPAPC	[69*]
ThB4	HAEC	OxPAPC	[69*]
TSP-1	HAEC	OxPAPC	[69*]
CD59	HAEC	OxPAPC	[69*]
FosB	HAEC	OxPAPC	[69*]
LAMB2	HAEC	OxPAPC	[69*]
EF-1	HAEC	OxPAPC	[69*]
ST13	HAEC	OxPAPC	[69*]
HO-1	HAEC, HUVEC, monocytes (U937), liver, heart, aorta, blood cells	OxPAPC	[48,72]
COX-2	HUVEC, SMC, keratinocytes, monocytes	OxPAPC, PAF-like	[65,73*]
Glutamate-cysteine ligase	BAEC	OxPAPC	[74*]
ApoJ, IL-6	HepG2, liver	OxPAPC	[70]
5-LO	Leukocytes from pleural cavity	PAF-like	[35*]
RANTES	Leukocytes from pleural cavity	PAF-like	[35*]
MIP-1 α	Leukocytes from pleural cavity, monocytes	PAF-like	[35*,42]
MIP-1 β	Leukocytes from pleural cavity	PAF-like	[35*]
IP-10	Leukocytes from pleural cavity	PAF-like	[35*]
Thrombomodulin (downregulated)	HUVEC	OxPAPC	[67*]

ApoJ, apolipoprotein J; BAEC, bovine aortic endothelial cells; COX-2, cyclooxygenase 2; EF-1, elongation factor 1; Fe-H, iron hydrogenase; GRO-2, growth related oncogene 2; HAEC, human aortic endothelial cell; HBGF-1, heparin-binding growth factor 1; HO-1, heme oxygenase 1; HOOA-PC, 1-palmitoyl-2-(5-hydroxy-8-oxooct-6-enoyl)-sn-glycero-3-phosphocholine; HUVEC, human umbilical vein endothelial cell; IP, interferon-alpha-inducible chemokine; KC, mouse homolog to human gro; LAMB2, laminin beta2 chain; 5-LO, 5-lipoxygenase; MCP-1, monocyte chemoattractant protein 1; MIP, macrophage-inflammatory protein; MKP-1, MAP kinase phosphatase 1; OxPAPC, oxidized 1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphorylcholine; PAF, platelet-activating factor; PECPC, epoxy cyclopentenone PC; PEIPC, epoxyisoprostane PC; PGPC, 1-palmitoyl-2-glutaroyl-sn-glycero-3-phosphorylcholine; RANTES, regulated upon activation: normal T cell expressed/secreted; SMC, smooth muscle cell; ST13, a tumor suppressor gene; ThB4, tetrahydro-alpha-biopterin; TSP-1, thrombospondin 1; uPAR, urokinase receptor.

showed that oxidized phospholipids isolated from oxidized LDL activated a pathway involving PPAR γ . A synthetic oxidized alkyl phospholipid had previously been shown to be a PPAR γ agonist [63], and both alkyl phospholipids and rosiglitazone induced cyclooxygenase 2 expression and prostaglandin E₂ production. On the other hand, in macrophages it was shown that lipopolysaccharide-induced cyclooxygenase 2 expression was inhibited by OxPAPC [75]. In that study, inhibition was caused by blocking of the NF κ B pathway and ERK activation, whereas interference by PPARs could not be demonstrated. A role for cyclooxygenase 2 was also suggested in immune suppressive action that was attributed to PAF-like lipids (or PAF). Using a footpad-swelling model, systemic immune suppression was detected after the administration of ultraviolet irradiated phospholipids [73 \bullet]. The authors showed that cyclooxygenase 2 expression and subsequent prostaglandin E₂ production are involved in the immune suppression that is induced by the oxidation of phospholipids by ultraviolet light, resulting in the activation of the PAF receptor.

Cytoprotective effects induced by oxidized phospholipids

Although it has been shown that high levels of oxidized lipids are cytotoxic and cause cell death, the treatment of cells with low levels of oxidized phospholipids has been shown to induce various cytoprotective responses. Among these are the upregulation of glutathione synthesis and the induction of heme-oxygenase expression.

Glutathione synthesis

The induction of glutathione synthesis by OxPAPC in endothelial cells was shown to be mediated by increased activity and protein expression of the regulatory subunit of glutamate-cysteine ligase. The prevention of glutathione synthesis caused extensive cell death. Furthermore, the pretreatment of cells with OxPAPC protected cells against the cytotoxicity induced by oxidizing agents. Oxidized phospholipids can thus induce cytoprotective pathways in endothelial cells that resemble the adaptation to oxidative stress. It was shown in that study that lipid hydroperoxides were not responsible for the observed effect, because the addition of lipid hydroperoxides only led to a small increase in glutathione levels. Neither lyso-PC nor oxidized 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphorylcholine (POPC) induced glutathione synthesis, showing that only 1-palmitoyl-2-arachidonoyl-*sn*-glycero-3-phosphorylcholine oxidation products were effective [74 \bullet].

Heme oxygenase 1

HO-1 is an enzyme mediating the catabolism of heme into biliverdin, free iron and carbon monoxide [76]. In addition to its antioxidant action, anti-inflammatory

properties of HO-1 have been described [77–80]. HO-1 is highly induced in human endothelial and smooth muscle cells by minimally modified LDL and oxidized phospholipids [68,72]. We recently found that the OxPAPC-induced expression of HO-1 in human endothelial cells was paralleled by the phosphorylation of CREB and the binding of CREB to a responsive element in the HO-1 promoter (Figure 2) (Kronke *et al.*, unpublished). Furthermore, HO-1 was induced in mice after intravenous administration of OxPAPC [48]. Taking into account the antioxidant and anti-inflammatory properties of HO-1, one can speculate that the induction of this enzyme by oxidized phospholipids might play a protective role during inflammation and facilitate the resolution of the inflammatory process [81].

Inhibition of lipopolysaccharide-induced inflammation, nuclear factor kappa B signaling and innate immune response

Several studies have demonstrated the ability of minimally modified LDL and oxidized phospholipids to inhibit the lipopolysaccharide-induced upregulation of cell adhesion molecules in endothelial cells [58,82]. Several oxidized species of phospholipids possess anti-endotoxin activity, such as POVPC and hydroxy alkenal phosphatidylcholine [9,82]. In addition, oxidized phospholipids exerting identical anti-endotoxin activity can be obtained from phospholipid precursors having choline and ethanolamine head groups, and palmitic or stearic fatty acids at the *sn*-1 position [17]. More recently, the mechanism underlying the aforementioned results has been demonstrated. We have shown that OxPAPC inhibits lipopolysaccharide-induced inflammation *in vitro* and *in vivo*, and that the inhibitory effect was selective for lipopolysaccharide compared with other inflammatory agonists, suggesting that oxidized phospholipids inhibited the recognition of lipopolysaccharide by cells [83 $\bullet\bullet$]. It is known that the binding of lipopolysaccharide to its receptor Toll-like receptor 4 (TLR-4) is assisted by two proteins, lipopolysaccharide-binding protein and CD14. Evidence has been obtained suggesting that OxPAPC inhibits the binding of lipopolysaccharide to these proteins presenting lipopolysaccharide to TLR-4 (Figure 2). We suggest that increased oxidative stress during inflammation would stimulate the formation of TLR4-inhibiting oxidized lipid species, and thus prevent the propagation of inflammatory reactions. We therefore hypothesize that the formation of oxidized phospholipids could be a negative feedback mechanism in acute Gram-negative inflammation. On the other hand, the presence of oxidized phospholipids in some settings of chronic inflammatory conditions or in the elderly, in whom increased oxidative stress has been reported, would result in a diminished innate immune

response and thus in insufficient host defense reactions (immune senescence).

Conclusion

As novel sources for oxidized phospholipids are discovered and new structures of phospholipid oxidation products continue to be described, the pathological processes in which these products may play a role increase in number. Recent findings point to an important role of lipid oxidation products not only in atherogenesis, but in general inflammatory processes in which increased free radical production has been reported.

Accumulating evidence suggests that oxidized phospholipids exert both pro and anti-inflammatory effects depending on the biological context. It is apparent that the anti-inflammatory action of lipid oxidation products is to a large extent mediated by inhibition of the NF κ B pathway. It thus seems likely that the anti-inflammatory properties of oxidized phospholipids are more important in the course of acute bacterial inflammation, when the NF κ B pathway plays a major role. As the acute inflammation blunts, or under conditions of chronic inflammation, the pro-inflammatory activity of lipid oxidation products may become more pathologically relevant (Figure 2). It seems as if different transcriptional mechanisms are induced during acute and 'lipid-induced' chronic inflammation. Indeed, while transcription induced by inflammatory cytokines and lipopolysaccharide is mainly mediated by the NF κ B pathway, oxidized phospholipids do not activate NF κ B, but rather alternative signaling pathways that lead to the activation of PPARs, NFAT and EGR-1 (Table 2). In addition, oxidized lipids can activate glutathione production and the transcription of protective genes, such as HO-1, which may play important roles in the resolution of acute inflammation. Oxidized phospholipids may thus switch transcriptional mechanisms of inflammation and promote a shift from acute inflammation to a chronic inflammatory process.

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- of outstanding interest

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