Biologically Active Oxidized Phospholipids*  

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Oxidants and free radicals are deleterious in many ways, and organisms employ numerous approaches to block their production or limit their damage. Hydrogen atoms adjacent to olefinic bonds are susceptible to oxidative attack and those between unconjugated olefinic bonds especially so. Lipids are a rich source of these bonds and so are a primary target for oxidative reactions. Lipid oxidation is problematic as the many oxidative chemical reactions are not controlled and constrained by enzymes and may show exponential reaction rates, and some of the products of the attack are highly reactive species that modify proteins and DNA. This review summarizes current information about another outcome of the uncontrolled attack on cellular and circulating phospholipids, the generation of potent biologically active compounds that activate components of the immune and inflammatory systems.

The biologically active species discussed here include oxidized phosphatidylcholines with biologic activity similar to platelet-activating factor (PAF).1 oxidized phosphatidylcholines that stimulate responses through ways other than the PAF receptor, and the lysophosphatidylcholines that result from the enzymatic metabolism of these modified phospholipids. Oxidation products that mimic the properties of a wide variety of arachidonate metabolites are discussed in a separate review in this series (FitzGerald et al. (85)), as is a study of the effects of cholesterol oxidation (Chisolm et al. (86)). We will briefly mention the role of PAF acetylhydrolase in the catabolism of oxidized phospholipids. With the structures of some biologically active oxidation products deciphered, there is physical evidence to show that oxidatively modified phospholipids accumulate in vivo. This suggests that potent biologic mediators can arise from uncontrolled chemical reactions when the antioxidant defenses of the organism are overwhelmed. One area where such newly formed species subvert physiologic events is atherosclerosis, but exposure to cigarette smoke, reperfusion injury, and stroke are also likely candidates for inappropriate events initiated or propagated by biologically active oxidized phospholipids.

Lipid Oxidation

Oxidative reactions of free fatty acids have been defined (e.g. Refs. 1–3), and oxidation of fatty acyl residues esterified in phospholipids appears to proceed in a similar fashion. The initial oxidative attack on polyunsaturated fatty acids generates alkyl radicals and then with the addition of oxygen, alkoxy radicals and peroxydes. Arachidonate when oxidized enzymatically generates hydroperoxyeicosatetraenoates, hydroxyeicosatetraenoates, pros-taglandins, and leukotrienes. A larger series of stereo and positional isomers known as isoprostanes, isothromboxanes, and isoleukotrienes (4, 5) is produced when arachidonate is non-enzymatically oxidized by a series of competing chemical reactions. Oxidation of fatty acyl residues occurs even when they are esterified in phospholipids, and this proceeds by a similar series of reactions to give phosphatidylcholine hydroperoxides (6), epoxides, and hydroxides (7). Oxidation of intact phosphatidylcholine shows little dependence on its physical state as the oxidation of solubi-lized and membranous phosphatidylcholine (8) generates similar products.

Oxidation of phosphatidylcholine generates a large series of phospholipids where the polyunsaturated sn-2 residue is no longer intact. When free fatty acids are oxidized the volatile (9) and soluble products may be lost during the workup; oxidation of phosphatidylcholines, on the other hand, retains the proximal oxidation fragment as a new sn-2 residue (Fig. 1). Carbon–hydrogen bonds are weakened when the adjacent carbon atom is doubly bonded, and the weakest carbon–hydrogen bonds in a fatty acid are the bissalicylic ones adjacent to two olefinic bonds. Abstraction of, for instance, the hydrogen from carbon 7 of arachidonate is facile as both carbon 6 and 8 are doubly bonded. This produces an alkyl radical centered at carbon 7, but rearrangement of the first double bond is favored as a conjugated system can form between carbons 6 and 9. This leaves the radical at position 5 (or 9 or 15 if abstraction initially occurred at position 10 or 13, although attack at the end of the series of double bonds in arachidonate is favored). Attack of molecular oxygen at the alkyl radical therefore yields an alkoxyl radical at carbon 5 that can break the fatty acid chain on either side of this atom. Thus, oxidative fragmentation of phosphatidylcholines yields a series of homologous phospholipids with sn-2 residues ranging primarily from four to nine carbon atoms long without or with ω-aldehydeic, ω-hydroxy, or ω-carboxy functions (10, 11). These are derived from β-scission of alkyl radicals at, or adjacent to, the position of the original olefinic bond so that fragmented, but undervolatilized, fatty acyl fragments are one methylene shorter than the fragments possessing an oxy function. Products from sn-2 arachidonoyl phosphatidylcholine, where the proximal olefinic bond is between carbons 5 and 6, include the four-carbon butanoyl fragment (12) or the five-carbon glutaroyl fragment with an ω-carboxylate function (12, 13). Phosphatidylcholine containing an sn-2 docosahexanoyl residue, with a proximal 4,5 double bond, generates homologs one carbon atom shorter (12), whereas the proximal 9,10 olefinic bond of linoleoyl-containing phosphatidylcholines generates longer octyl (14) and azelaoyl (nonanedioyl) residues (11). Phosphatidylcholines with short sn-2 residues were originally identified in extracts of bovine brain (15, 16) that were similar to products expected from phospholipid oxidation (17). This large family of oxidation products has now also been observed in oxidized LDL (14, 18–20), human plasma (21), and food products (22). They are found in atherosclerotic lesions (13) and in the blood of animals (23) and humans (24) exposed to cigarette smoke. The appearance of unusual fatty acyl residues not normally associated with fatty acid metabolism as a significant (21) component of plasma phospholipids, coupled with their appearance after oxidation of synthetic phosphatidylcholine (12–14, 18), suggests all such phospholipids with these unusual sn-2 residues isolated from natural sources are products of lipid oxidation. Overall, oxidation generates a host of new phospholipid species that correspondingly have new types of actions; some disrupt membrane bilayer integrity (11, 25) and some modify proteins (13, 26, 27), whereas others are biologically active. In each case, their genetics follows an unregulated chemical attack on cellular and lipoprotein phospholipids.

Deppressor Lipids

The first identification (28) of biologically active phospholipids generated from non-enzymatic oxidative reactions, although not immediately appreciated as products of such a process, was of potent vasopressors in acetone extracts from a wide range of sources. Of interest, the hypotensive activity was originally described as being present in the oxidized form of the extract but was hypertensive in its reduced form. Shortly after this, an anti-hypertensive lipid, now known as PAF, was determined (29) to possess...
the same structure as a phospholipid isolated and identified based on its ability to aggregate platelets (30) and as a potential effector of endotoxic shock (31). Once the structure of PAF was known and an inhibitor was synthesized, the depressor extract was found to contain at least two types of activities (32). One was a PAF-like activity, but there also was non-PAF-like activity present. The latter depressor lipids, identified as fragmented diacyl phosphatidylcholines (16), act via undefined mechanisms.

**PAF-like Lipids**

PAF was the first phospholipid autacoid to be structurally identified (see Refs. 33 and 34), to have specific receptor antagonists identified, and to have its receptor cloned (35). Despite pharmacologic evidence to the contrary (36), only a single PAF receptor has been identified to date. PAF is an ether phosphatidylcholine with a short acetyl residue at the sn-2 position, and biologic samples are mainly a mixture of 1-O-hexadecyl-2-acetyl-sn-glycero-3-phosphocholine and 1-O-octadecyl-2-acetyl-sn-glycero-3-phosphocholine. The PAF receptor shows a strong preference for the sn-1 ether bond, the sn-2 acetyl residue, and the choline headgroup (37).

Oxidation of alkyl phosphatidylcholines fragments the sn-2 polyunsaturated fatty acyl residues, yielding a large series of phosphatidylcholines with shortened sn-2 residues with or without an additional oxygen function. Synthesis of some of these oxidation products shows they are able to activate cells expressing the PAF receptor (12, 38, 39). Because the structure of these bioactive lipid products differs from PAF whose sn-2 residue is exclusively derived from acetyl-CoA, these phospholipids are termed “PAF-like.” Much of the PAF-like activity in oxidized phosphatidylcholine comes from species with four-carbon sn-2 residues, but other species contribute to the total activity. Precise quantitation is difficult as oxidation of complex mixtures of phosphatidylcholines generates a large number of modified phospholipid products, many of which do not stimulate the PAF receptor. Moreover, comparison of a series of synthetic PAF-like lipids with one another yields different rank orders of EC_{50} values depending on the assay system even though specific PAF receptor antagonists effectively block each function (39). There is additional data that support the idea that unidentified factors modulate the signaling of the PAF receptor after binding of agonistic ligands. Different types of responses in the same cell show different EC_{50} values for PAF analogs (39), and we find that the binding constants of the PAF receptor do not match the EC_{50} found in several types of biologic assays. The synthesis of PAF is carefully controlled, but the formation of potent mimetics after oxidation of synthetic phosphatidylcholines (10, 12, 38), isolated low density lipoproteins (14, 19, 40), and food stuffs (22) is unregulated. These chemical reactions therefore have the potential to produce high concentrations of potent inflammatory agents.

The structural analogs of PAF created by oxidation of phospholipids containing a polyunsaturated residue (41) activate human leukocytes, and this new biologic activity is completely blocked by specific PAF receptor antagonists (19, 38). These newly formed phospholipids also activate PAF receptors on rabbit platelets (12), and they stimulate Ca^{2+} transients (39) and γ-interferon secretion (40) from human monocytes via their PAF receptors. These PAF-like lipids also displace the competitive PAF receptor antagonist [3H]WEB 2086 from Chinese hamster ovary cells expressing human PAF receptors and activate 293 cells expressing these receptors. These oxidatively fragmented PAF-like lipids also induce [3H]thymidine incorporation into smooth muscle cells (19), an event relevant to the smooth muscle hypertrophy of atherosclerosis.

**Non-PAF-like Phospholipids**

Oxidation of LDL creates phospholipids that induce the synthesis of several cytokines and promotes expression of adhesion molecules by endothelial cells and monocytes. The import of this is that
signals from short-lived lipid mediators are converted to longer acting inflammatory cytokines. The identity of the phospholipid mediators responsible for these events is not always apparent; the induction of inflammatory cytokines may proceed via PAF receptors (42, 43), although in some cases cytokine induction can be distinguished from the effects of PAF-like lipids (13, 44). Oxidized LDL induces endothelial cell expression of the adhesion proteins ICAM-1 (45) and P-selectin synthesis (46), and it induces an unidentified monocyte adhesion protein VMAP-1 (47). Oxidized LDL also induces synthesis of GRO chemokines (48), endothelin (49), and MCP-1 by monocytes (50) and endothelial cells and smooth muscle cells (51). These events are induced by lipid components of the lipoprotein particle and are not found in native particles not subjected to oxidation. Higher concentrations (10⁻⁵ M) of oxidized synthetic phosphatidylcholine also induce monocyte-endothelial cell interactions that are PAF-receptor-independent (44). Oxidized LDL induces vascular endothelial growth factor expression, and this cytokine is present in macrophage-rich areas of atherosclerotic lesions (52). Other inflammatory cytokines produced in response to oxidized LDL are present in atherosclerotic lesions, and gene targeting to delete the inflammatory MCP-1 cytokine (53) or cytokine receptors (54, 55) markedly impairs monocyte trafficking in inflammatory and atherosclerotic settings.

Lysophosphatidylcholine

Lysophosphatidylcholine (LPC) is present in human plasma at quite high levels (140–150 μM (56, 57)). Little of this is associated with LDL, although oxidation of LDL increases the content of LPC from 1–5% of the phosphatidylcholine content to 40–50% of this value (58–60). The increase in LPC content is the result of two sequential events: oxidation and fragmentation of the sn-2 residues of phosphatidylcholine, followed by the hydrolysis of the shortened fatty acyl residues by LDL-associated PAF acetylhydrolase (18, 61). The restricted substrate specificity of the PAF acetylhydrolase prevents it from using intact phospholipids as substrates, but fragmentation, and especially addition of an oxy function, generates highly susceptible neosubstrates. Although identified and purified by its ability to hydrolyze PAF, this LDL- and high density lipoprotein-associated enzyme (62) functions as an oxidized phospholipid phospholipase (63). Other activities have also been suggested to contribute to the accumulation of LPC in oxidized lipoprotein particles (64, 65) in conjunction with the PAF acetylhydrolase. A wide range of activities has been ascribed to LPC. These include the induction of growth factor expression by endothelial cells (66) and monocytes (52), the suppression of endothelium-dependent vasorelaxation (67), and chemotraction of monocytes (68). However, for each of these responses maximal activity is achieved only at concentrations around 10–50 μM, so LPC is therefore just a modestly effective agonist. Moreover the 150 μM serum concentration in the blood of normal individuals is vastly higher than its action range, even before LDL oxidation. Thus LPC is the least effective signaling molecule generated by phospholipid oxidation. This conclusion also means that a primary function of the PAF acetylhydrolase in converting oxidized phospholipids to lysophosphatidylcholine and free acyl fragments is a protective one, a conclusion consistent with the protection of cells from oxidative death by overexpression of an intracellular form of PAF acetylhydrolase (69).

The Ghostly Hand of Oxidized Phospholipids

Defining a role for oxidatively modified phospholipids in pathologic events is difficult. Their receptor-mediated bioactivity mimics that of endogenous mediators; their structure is ill defined and many closely related homologs are produced from numerous phospholipid precursors; they are potent so that their effects are produced by trace quantities of materials; and they may be reactive and unstable. Actually the latter property, chemical reactivity, has been a boon in identifying the fleeting presence of oxidatively modified phospholipids in vivo. For instance, some of the antigens in the anti-phospholipid syndrome are against epitopes of oxidized phospholipid-protein adducts (27). Additionally, antibodies against protein-phospholipid adducts recognize epitopes in oxidized LDL (70, 71). Moreover a monoclonal antibody raised against antigens in atheromatous plaque reacts with oxidized LDL, and oxidation of LDL lipids created reactive moieties that derivatized peptides so that they too were recognized by the anti-atheromatous monoclonal antibody (72). That the immune system has been able to generate a response to proteins derivatized with the whole phospholipid backbone suggests that reactive, fragmented phospholipids were presented to the immune system over long periods of time.

Although phospholipid oxidation products are difficult to detect and quantify, phospholipid oxidation does occur in vivo. Lipoperoxides are present in atherosclerotic lesions (73), and the major peroxide in this circumstance is phosphatidylcholine hydroperoxide (74). These are precursors of fragmented phospholipids, and phosphatidylcholine oxidation products have been identified in atherosclerotic lesions (13). Phosphatidylcholine hydroperoxides are also found in plasma (75), and phospholipids that are likely derived from their further oxidation are present in plasma (21). These products can also be found in LDL oxidized in vitro (14, 18), so their origin is exclusively from chemical oxidation.

The subclass of oxidized phospholipids that are biologically active is generated as a consequence of in vivo oxidant stress. PAF-like lipids have been quantitated in animals exposed to the smoke of a single cigarette, and a PAF receptor antagonist blocked the systemic inflammatory changes the smoke induced (23). The smoke-induced inflammation was the direct result of oxidant stress (a single puff is estimated to contain 5 nmol of radicals (76)) as superoxide dismutase (77) or dietary supplementation with the antioxidant vitamin C (78) prevents the smoke-induced systemic inflammation. Dietary vitamin C also prevented the formation of circulating PAF-like lipids in animals subjected to cigarette smoke (23), suggesting a causal link to the system-wide inflammatory response. PAF-like activity is also found in the lipoproteins of human smokers (24), strengthening this association. PAF and/or PAF-like lipids are involved in long term vascular changes as an orally administered PAF receptor antagonist protects against fatty streak formation in an animal model of atherosclerosis (79). The potential for oxidized phospholipids to have participated in these vascular alterations is suggested by the observation that dietary antioxidants also prevent these changes (80). Additional indirect evidence for pathologic effects of the PAF-like lipids is that deficiency of the protective PAF acetylhydrolase correlates with an increased risk of stroke (81) and increased levels of circulating PAF-like lipids (82). Lack of this enzyme also correlates with an increased risk of coronary disease (83). The increased risk of vascular disease in subjects deficient in the plasma PAF acetylhydrolase likely derives, at least in part, from an inability to destroy reactive bioactive oxidatively fragmented phospholipids because overexpression of intracellular PAF acetylhydrolase protects cells from oxidative apoptotic death (69). Overexpression of phospholipid peroxidase (84) also protects cells, so preventing the oxidative fragmentation of phospholipids or efficiently removing them after they form blocks a major route of oxidative cell death.

Summary

A key component in the immediate response to inflammatory signals is the synthesis of PAF, a carefully regulated process. Oxidation of cellular and lipoprotein phosphatidylcholine through unregulated chemical reactions achieves the same end as these oxidative processes result in the formation of mimetics that are also potent activators of the inflammatory PAF receptor. Oxidation of LDL in vitro, where cellular regulation is moot, generates numerous lipid products that display several types of bioactivity. The best defined examples of bioactive neophospholipids are those that act through the PAF receptor. These PAF mimetics are found in vivo shortly after exposure to cigarette smoke, a powerful oxidative stress. Antioxidants or catalysis by the PAF acetylhydrolase counteract the formation of reactive and bioactive oxidized phospholipids, but these are not always sufficient. Oxidation of phosphatidylcholine generates other types of inflammatory mediators that clearly work in ways that do not require the PAF receptor. We anticipate, from the numerous types of products generated by phospholipid oxidation, that other activities and events caused by phospholipid oxidation will soon come to light.

REFERENCES

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