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Oxidized Glycerophosphocholines as Biologically Active Mediators for Ultraviolet Radiation-Mediated Effects

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Abstract

Ultraviolet light radiation (UVR) has profound effects upon human skin. Yet, the exact targets for UVR are unclear. Inasmuch as UVR is a known pro-oxidative stressor, one potential target for UVR could be oxidatively modified glycerophosphocholines (GPC). Importantly, recent studies demonstrate that these oxidized GPCs (ox-GPC) are potent agonists for the platelet-activating factor receptor and peroxisome proliferator-activated receptor gamma. This review discusses these new biologically active lipids and their down-stream receptor targets that provide a unique system of biosensors for detecting and responding to UVR photo-oxidation.

Keywords

Oxidation; ultraviolet rays; peroxisome proliferator-activated receptor gamma; platelet activating factor receptor; glycerophosphocholines

INTRODUCTION

UV radiation has profound effects upon human skin. Both UVB (290–320 nm) and UVA (320–400 nm) are important environmental carcinogens, and skin is the major target (reviewed in ^{1, 2}). In addition to its ability to damage DNA, UVA and UVB can modify epithelial gene expression. The known UV response consists of the rapid activation of numerous genes with reparative, protective or apoptotic effects, as well as genes encoding cytokines and other signaling molecules with potent immunomodulatory effects ^{3, 4}. The exact mechanisms by which UVA and UVB exert these effects are not entirely clear.

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Potential targets for UVR include DNA and *cis*-urocanic acid⁵. The ability of both UVA and UVB to act as a pro-oxidative stressor could also provide additional targets for UV-mediated effects. Amongst the cellular molecules that could be modified by oxidative stress to form biologically active agents are lipids, especially polyunsaturated fatty acids incorporated into phospholipids⁶. The important role that oxidized phospholipids play in pathologic processes is possibly best understood in relation to cardiovascular disease, in which oxidized low density lipoproteins (LDL) are thought to be key mediators of atherogenesis (reviewed in^{7,8}).

Glycerophosphocholine (GPC) is an important structural lipid in all cellular membranes. 1-alkyl GPC with a polyunsaturated fatty (such as arachidonate) esterified to the *sn*-2 position is found in reasonable abundance in epithelial cells⁹. These molecules are precursors of bioactive lipid platelet-activating factor (1-alkyl-2-acetyl GPC), which is formed upon the actions of enzymes phospholipase A₂ and PAF acetyltransferase. Esterified polyunsaturated fatty acids, especially arachidonate, are susceptible to oxidation due to the presence of bisallylic hydrogens which serve as hydrogen donors for radical attack. Oxidation of esterified fatty acyl residues introduces oxy functions, rearranges bonds and fragments carbon-carbon bonds by β -scission¹⁰ that generate a myriad of reaction products. Among these are a series of phospholipids with oxidatively fragmented *sn*-2 acyl residues that terminate with either an ω -oxy function or a methyl group. If the oxidatively modified *sn*-2 acyl residue is a 1-alkyl GPC, then the resultant products can act as potent PAF receptor or peroxisome proliferator-activated receptor gamma (PPAR- γ) agonists. Recent studies have demonstrated that UVB can generate both PAF and PPAR γ agonists via its ability to act as a pro-oxidative stressor^{11,12}. The significance of these novel biologically-active GPCs will be discussed in this review (see Figure 1).

Platelet-activating Factor and Keratinocyte Function

PAF is an inflammatory phospholipid mediator that exerts its effects through a single, specific G-protein coupled receptor, the PAF receptor (reviewed in¹³). The PAF receptor is expressed by cells of the innate immune system, but also by keratinocytes of the skin¹⁴. PAF is the most potent phospholipid agonist yet identified where high affinity recognition depends on its *sn*-1 ether bond and a short *sn*-2 acetyl residue. PAF activates some cells at sub-picomolar levels and as would be anticipated, its synthesis is closely controlled.

PAF is synthesized in response to diverse stimuli including cytokines, endotoxin, and Ca⁺⁺ ionophores. Of note, direct damage to keratinocytes by either heat or cold stimuli result in significant PAF production¹⁵. The synthetic pathway consists of two enzymes; a phospholipase A₂ that generates the lysolipid backbone by releasing the *sn*-2 fatty acyl residue from alkyl phosphatidylcholine and PAF acetyltransferase activity that transfers an acetyl residue from acetyl-CoA to this newly generated lysolipid. Both activities are tightly regulated, with increased intracellular Ca⁺⁺ being the premier agent for their activation.

In addition to enzymatically catalyzed PAF production, PAF receptor ligands are also generated non-enzymatically, and thus not subject to cellular control. The alkyl phospholipid pool is enriched at the *sn*-2 position with arachidonoyl residues, a source of lyso-PAF and arachidonate for prostanoid and leukotriene synthesis. Importantly, these polyunsaturated arachidonoyl residues are highly susceptible to oxidation. Among these are a series of phospholipids with oxidatively fragmented *sn*-2 acyl residues that terminate with either an ω -oxy function or a methyl group. The latter series is one carbon atom shorter than the ω -oxy series, as expected from the β -scission reaction. Among the phospholipid reaction products are those that are ligands for the PAF-receptor. The most potent of the non-enzymatically generated PAF analogs are native PAF, as well as the butanoyl (C₄-PAF) and butenoyl (C_{4:1}-PAF) species that are one tenth as potent as PAF^{16,17}. In addition, alkyl

GPCs found in oxidized LDL with small chain sn-2 groups ending in an ω -oxy function, such as a 4-carbon ω -carboxylic acid (succinoyl-PAF) and 5 carbon ω -aldehydes (5-oxovaleroyl-PAF), have also been shown to serve as PAF-R ligands^{17–20}.

Keratinocytes synthesize PAF in response to numerous stimuli, including cellular damage, ionophores, and cytokines including IL-8^{15, 17, 21}. Keratinocytes also express PAF-receptors and activation of the epidermal PAF-receptor results in the expression of numerous cytokines including IL-1, IL-6, IL-8, IL-10, TNF- α , prostaglandins, and PAF itself^{22–24}. Moreover, the epidermal PAF-R can transactivate the epidermal growth factor receptor which can induce a mild proliferative response²⁵. It should be noted that the phenotype of a transgenic mouse which overexpressed the PAF-R (by using an actin promoter) included a hyperproliferative dermatitis²⁶.

PAF System and UVR

UVB generates PAF-receptor ligands^{11, 24}, and stimulation of the PAF-receptor of epithelial cells mimics many aspects of UVB effects on human keratinocytes including tumor necrosis factor- α , interleukin-8, and cyclooxygenase-2 (COX-2) production. Expression of the PAF-receptor in the PAF-receptor-negative epithelial cell line KB results in an augmentation of UVB-mediated cytokine production and cytotoxicity^{11, 27}. UVB is immunosuppressive and facilitates progression of non-melanoma skin cancer, and it has been proposed²⁴ that PAF and/or PAF-like lipids mediate this systemic immune suppression via PAF-receptor-mediated IL-10 production. Moreover, topical psoralen + UVA-mediated keratinocyte cytotoxicity has been reported to be decreased in PAF-receptor deficient mice in comparison to wild-type counterparts²⁸.

The mechanisms utilized by the PAF system in mediating UVB irradiation-induced systemic immunosuppression have been explored in recent studies²⁹. The importance of the PAF-R in photoimmunosuppression is demonstrated by the ability of CPAF (injected ip into C57BL6 mice) to mimic the ability of UVB to inhibit delayed type hypersensitivity reactions³⁰. In contrast, UVB irradiation (or ip injection of CPAF) of PAF-R-deficient C57BL6 mice does not result in an inhibition of delayed type contact hypersensitivity reactions. However, it was as yet unclear whether immune cells or keratinocytes mediated the PAF-R-dependent effects on photoimmunosuppression. Bone marrow transplantation studies to produce mice chimeric with respect to the PAF-R demonstrated that the PAF-R cells responsible for UVB-mediated immunosuppression are bone-marrow-derived³⁰. The model that is emerging is that UVB irradiation results in keratinocyte-mediated production of PAF-R agonists that then act upon immunocytes to inhibit delayed type hypersensitivity responses through an IL-10-dependent process. These studies indicate that PAF-R activation could be an important mediator of UV-mediated systemic immunosuppression, with regulated PAF ligand production occurring enzymatically in response to cellular damage and non-regulated PAF ligand occurring as a byproduct of oxidized GPC.

Of the known UV-mediated effects in keratinocytes, the PAF system has also been implicated in the early gene expression induced by UVB. Recently our group has examined the role of the epidermal PAF-R in UVB-mediated early gene expression. Affymatrix gene arrays of the PAF-R-negative epithelial cell line KB transduced with the PAF-R (KBP) versus control KB (KBM) cells 1 h following treatment with the PAF-R agonist carbamoyl-PAF resulted in statistically significant differences in the expression of many genes; 187 genes were up-regulated and 59 genes were down-regulated at least 2-fold³¹. Genes altered by PAF-R activation included genes involved in adherens junctions, apoptosis, calcium signaling, cell cycle regulation, cytokines, extracellular matrix receptor interactions, JAK-STAT and MAPK signaling pathways. In particular, CPAF treatment induced mRNA encoding cytokines TNF- α (8.6 fold), IL-6 (11.3 fold), IL-8 (55.4 fold), and IL-11 (12.5

fold). In addition, activation of the PAF-R strongly induced mRNA encoding chemokines CCL20 (173.9 fold), CCL2 (24.8 fold), CXCL1 (30.7 fold), CXCL2 (24.8 fold), and CXCL3 (36.6 fold). CPAF treatment of KBP cells also induced anti-apoptotic genes including BIRC2 and BIRC3, TRAF-1 and MCL1. Consistent with the lack of functional PAF-Rs, treatment of control KB cells with CPAF did not result in significant changes in mRNA levels for any genes. UVB irradiation of PAF-R-positive KBP and control KBM cells revealed that 11 genes were selectively upregulated in KBP over KBM, including cytokines CCL20 and TNF- α . UVB irradiation of the skin of wild-type versus PAF-R-deficient mice on a C57BL6 background resulted in significantly decreased levels of CCL20 and TNF- α mRNA and protein levels in the PAF-R-deficient mice³¹. These studies indicate involvement of the PAF system in UVB-mediated early gene expression, especially inflammatory cytokines.

Peroxisome Proliferator-Activated Receptors (PPAR)

PPARs are transcription factors belonging to the nuclear hormone receptor (NHR) family (reviewed in^{32, 33}). PPARs were first identified as NHRs which are activated by diverse lipid species and hypolipemic xenobiotic agents. The nomenclature for this new class of NHRs was based on the fact that many of these agents were known to induce proliferation of hepatic peroxisomes³⁴. Three different PPARs have been identified (α , β/δ , and γ) that differ in ligand specificity, tissue expression, and transcriptional targets. In addition, four distinct PPAR γ transcripts ($\gamma 1$ - $\gamma 4$) are produced by the use of alternative mRNA splicing leading to two different protein products: PPAR $\gamma 1$, PPAR $\gamma 3$, & PPAR $\gamma 4$ all encode a single protein product³⁵. While PPAR $\gamma 1$ is ubiquitously expressed, PPAR $\gamma 2$ and PPAR $\gamma 3$ are expressed in primarily in adipose tissue³⁵. Tissue-specific expression of PPAR $\gamma 4$ is unknown³⁵. Importantly, PPAR α , PPAR β/δ and PPAR γ are all expressed in keratinocytes and in human and rodent epidermis^{32, 33, 36}.

The ability of PPARs to regulate gene transcription is complex, utilizing ligand-dependent transactivation or ligand-independent target gene repression (reviewed in³⁷). In classic ligand-dependent transactivation, ligand binding induces a conformational change in protein structure that stabilizes heterodimerization with the 9-*cis* retinoic acid receptor (RXR). This in turn triggers loss of co-repressor binding, and facilitates recruitment of co-activators. The active PPAR:RXR complex mediates target gene transcription by binding to specific hexameric DNA repeat sequences termed peroxisome proliferator response elements (PPRE). PPARs can also influence the expression of genes lacking a PPRE. In this case, PPARs act through ligand-dependent transrepression to inhibit the functions of other transcription factors, most notably nuclear factor- κ B (NF- κ B) and activator protein-1 (AP-1)³⁷. This occurs through multiple mechanisms, including direct interaction of PPAR with the transcription factor, competition for co-activator recruitment, and regulation of mitogen-activated protein kinase (MAPK) family member activity³⁷.

PPAR γ and keratinocyte function

PPAR γ is an essential nuclear factor probably best understood in relationship to its role in controlling the expression of a number of regulatory genes involved in lipid metabolism and insulin sensitization (reviewed in³⁸). More recently, evidence suggests that PPAR γ plays an important role in regulating epithelial proliferation, atherogenesis, carcinogenesis, immune cell function, and inflammation^{8, 38-41}. Within the epidermis, much less is known about the function, mechanisms of activation and target genes for PPARs (reviewed in^{32, 36}). However, it is clear that keratinocytes and epithelial cell lines express all three PPAR subtypes, and that PPAR γ likely plays an important role in regulating keratinocyte proliferation and differentiation^{32, 36, 42, 43}. In addition, recent evidence indicates that PPAR γ agonists may act as potential therapeutic agents in inflammatory skin diseases

like psoriasis⁴⁴⁻⁴⁶. However, it is not entirely clear that keratinocyte PPAR γ is the target of this anti-psoriatic activity. PPAR γ has been shown to have potent effects on immune cell function (reviewed in³⁹); thus, it is also possible that dendritic cell or inflammatory cell PPAR γ may also serve as targets in regulating the effects of PPAR γ agonist therapy in inflammatory hyperplasia.

Evidence that Oxidized GPC are native PPAR γ ligands

PPAR γ was initially isolated in mammalian cells as an “orphan” receptor, so named because there were as yet no known endogenous ligands⁴⁷. It was later determined that thiazolidinedione-type anti-diabetic agents act as potent PPAR γ ligands⁴⁸. This was soon followed by the discovery of the first endogenously produced PPAR γ ligand, the prostaglandin J₂ metabolite, 15-deoxy- $\Delta^{12,14}$ -PGJ₂ (15d-PGJ₂)^{49, 50}. However, the small amounts of this product measured in biological systems, the relatively low affinity of 15d-PGJ₂ (EC₅₀ = 1 μ M)⁴⁹, and recent reports that 15d-PGJ₂ regulates a number of biochemical pathways independent of PPAR γ have brought the potential role of this compound as a true endogenous ligand into question⁵¹⁻⁵⁵. This was followed by studies demonstrating that lipoxygenase products of arachidonic acid and linoleic acid found in oxidized LDL, 9- and 13-Hydroxyoctadienoic acids (HODE) and 15-hydroxytetraenoic acid (15-HETE), also exhibited the capacity to activate PPAR γ ^{51, 56}. However, these compounds have other effects, including regulating the activity of PPAR γ through MAPK-mediated phosphorylation⁵⁷. In addition, high (micromolar) concentration of these lipids are needed to exert PPAR γ effects; thus, this has brought their biological relevance into question⁵⁷.

Recent studies examining the ability of oxidized LDL to activate PPAR γ in atherogenesis have resulted in the discovery of the first high-affinity endogenous ligands for PPAR γ . This was first demonstrated by Davies *et al*⁵⁸, who demonstrated that non-enzymatically oxidized alkyl phospholipids present in oxidized low density lipoproteins (LDL), are high affinity ligands for PPAR γ . One of these species was identified as 1-hexadecyl-2-azelaoyl phosphatidylcholine (azPC), which had a K_d for binding to the PPAR γ receptor of approx. 40 nM⁵⁸.

Given that oxidized GPCs with PPAR γ activity are present in oxidized LDL, our group sought to determine whether UVB-induced photo-oxidation could result in PPAR γ ligand production, in addition to the PAF-R ligand activity already described above. Our studies demonstrate that UVB irradiation of epithelial cells and purified 1-hexadecyl-2-arachidonate-GPC (HAPC) also results in PPAR γ activity¹². It should be noted that high-performance liquid chromatography separation of lipids derived from UVB irradiation of epithelial cells or purified HAPC revealed numerous fractions with significant PPAR γ activity¹². Mass spectrometric analysis of these lipids found small amounts of azPC. It is important to note that the ability of UVR to induce not only PPAR γ ligand activity, but also the PAF-R ligand activity described above, was blocked by anti-oxidants and by UV irradiation in an argon environment; thus, this demonstrates that these UVR-mediated effects were dependent on photo-oxidation^{11, 12}. Subsequently, we demonstrated that treatment of epithelial cells with other oxidative stressors result in the production of PPAR γ ligand activity⁵⁹. It should also be noted that though these findings indicate that direct oxidation by UVB results in the production of numerous oxidized GPC with PPAR γ activity, only one of these species has been identified (AzPC); the structural characterization of the numerous remaining oxidized GPC which constitute this activity have yet to be accomplished.

Finally, the theme that endogenous PPAR γ ligands are produced as a consequence of oxidative stress is further supported by studies of nitric oxide dependent oxidative

inflammatory reactions. Recent studies indicate that nitroalkene derivatives of unsaturated fatty acids, particularly linoleic acid (nitrolinoleic acid (NLA)) and oleic acid (nitrated oleic acid (OA-NO₂)) are highly potent PPAR γ agonists, with both inducing a significant increase in PPAR γ transcriptional activity at concentrations as low as 100 nM, well below their physiologic concentration ranges^{60, 61}. A lesser degree of PPAR α and PPAR β/δ activation was also noted, but at higher concentrations (> 1 μ M)⁶¹. In addition, as with many of the other proposed PPAR γ ligands, NLA has recently been shown to have PPAR γ -independent activity⁶². It is unclear whether these nitroalkene PPAR γ agonists are relevant to photobiology. However, UV irradiation is known to induce NO production through both enzymatic and non-enzymatic mechanisms^{63, 64}.

PPAR γ and UVR

Given the importance of UVR in photocarcinogenesis and in the treatment of inflammatory hyperplastic disorders, as well as the anti-psoriatic activity of PPAR γ agonists, our recent findings that UVR and other oxidative stressors induce PPAR γ ligand activity suggest a potential role for PPAR γ in photobiology^{12, 59}. Thus, since both keratinocytes and immune cells express PPAR γ protein, and UVR induces PPAR γ activity, the exact role of PPAR γ in cutaneous photobiology is an active area of investigation.

Recent studies in both our laboratory and others have provided an additional mechanism through which UVR-mediated PPAR γ activation can influence epidermal photobiology. A previous study demonstrated that COX-2 contains a PPRE in the promoter region and is a target for PPAR-mediated transactivation in epithelial cells⁶⁵ and immortalized keratinocytes⁶⁶. Importantly, COX-2 induction has been implicated in UV-mediated processes ranging from carcinogenesis to local and systemic immunosuppression^{29, 30, 67–69}. Recent studies by our group demonstrate that cyclooxygenase-2 (COX-2) is a target of UVR-mediated PPAR γ activation. First, treatment of the human epithelial cell line KB with PPAR γ agonists augmented COX-2 mRNA and protein levels in response to various stimuli including IL-1 β and PAF, although PPAR γ agonist alone had little effect¹². Of significance, inhibition of PPAR γ by a dominant-negative PPAR construct or use of the selective and specific PPAR γ antagonist GW9662 inhibited UVB-induced COX-2 expression and resultant PGE₂ production in KB cells¹². Second, PPAR γ protein has been shown to be expressed in primary cultures of human keratinocytes and the sebaceous cell line SZ-95, and in these cell types inhibition of PPAR γ blocks COX-2 expression induced by UVB or the lipid pro-oxidative stressor *tert*-butyl hydroperoxide (⁵⁹, and unpublished data). Thus, these studies indicate that PPAR γ is likely to contribute to photobiology through a COX-2-dependent signaling pathway.

A potential role for a PPAR γ -COX-2 coupled response in UVB-immunoregulation may be inferred from studies by our group³⁰ and by Ullrich and colleagues²⁴: These studies indicate that UVB-induced systemic immunosuppression is mediated by a PAF-R-COX-2 coupled signaling pathway. Insofar as PPAR γ also plays a role in UVB-mediated COX-2 induction, this introduces the possibility that oxidized GPCs may act through both PAF-R and PPAR γ -mediated signaling to regulate photo-immunosuppression. It should also be noted that UVB-induced immunosuppression targets T helper cells polarized to the Th1 phenotype^{70, 71}. Given that PPAR γ specifically inhibits Th1-mediated immune responses in a mouse model of multiple sclerosis⁷², this suggests a potential role for PPAR γ in UVB-induced Th1 suppression. Finally, it is quite possible that UVB-induced PPAR γ activation acts to suppress immune responses independent of COX-2. There is growing evidence that many of the anti-inflammatory actions of PPAR γ are dependent on its ability to block expression of a number of proinflammatory cytokines through several ligand-dependent transrepression mechanisms (reviewed in^{37, 39}).

Epidemiological evidence has long associated high fat diets with increased risk for neoplastic development, including cutaneous malignancy^{73, 74}. It has since become apparent that increased cancer risk from high fat diets is due in part to higher levels of oxidized lipid species^{74, 75}. A potential role for PPAR γ in cutaneous carcinogenesis is indicated by a recent study demonstrating that mice deficient in either epidermal PPAR γ or its heterodimer partner, RXR, exhibit increased rates of cutaneous neoplasia following a chemical carcinogenesis protocol⁷⁶. This study is in agreement with an earlier study demonstrating that heterozygous germ-line loss of PPAR γ also augments chemically-induced cutaneous neoplasia⁷⁷. Potential mechanisms for this anti-neoplastic activity are suggested by studies indicating that PPAR γ acts to suppress inflammatory hyperplasia and induce differentiation in the epidermis^{42, 43, 45}. In contrast to the above studies, work by He *et al* indicate that topical or systemic synthetic thiazolidinedione-type PPAR γ agonists (rosiglitazone and troglitazone) have no affect on either photocarcinogenesis or chemical carcinogenesis⁷⁸. However, inasmuch as PPAR γ is already activated by UVR^{12, 59}, this likely would negate the impact of exogenous ligand on PPAR γ -mediated photobiological effects.

While PPAR γ exhibits anti-neoplastic activity in models of cutaneous chemical carcinogenesis, the role of PPAR γ in photocarcinogenesis is at yet unknown. If future studies demonstrate that loss of PPAR γ similarly augments photocarcinogenesis, then it would be difficult to attribute this action to the ability of PPAR γ to induce pro-carcinogenic COX-2 signaling within the epidermis. In this scenario, this would simply indicate that UVR-induced PPAR γ signaling is likely to have important COX-2 independent effects on photobiology. This would not be surprising, given the complexity and diversity of PPAR γ -dependent transcriptional regulation^{37, 39}.

Mechanisms by which UVB generates ox-GPCs

Solar UV radiation can be broken down into 3 separate wavelength spectra, each with its own biological and toxic effects^{1, 2}. However, only long wavelength UVA (320–400 nm) and intermediate wavelength UVB (280–320) are able to penetrate the atmosphere in biologically significant quantities. Because of its long wavelength, UVA light has the greatest penetrating power of the UV spectra, reaching into the dermis. UVB, because of its shorter wavelength, has much less penetrating power, with most UVB being absorbed by the epidermis.

The ability of UVA and UVB irradiation to induce molecular changes is dependent on the first law of photochemistry, or the Grotthuss-Draper law. Simply put, a photon of light must be absorbed by a chemical substance to initiate a photochemical reaction. Once absorbed by the skin, the primary toxicologic effects of UVA radiation are due to production of reactive oxygen species (ROS)⁷⁹. This oxidative stress can occur through multiple mechanisms (reviewed in⁷⁹). However, a key mechanism occurs through photon absorption by endogenous, non-DNA chromophores, that then act as photosensitizers (S*, figure 1) that initiate ROS production⁸⁰. Like UVA, UVB is also able to induce photo-oxidation through similar mechanisms. However, unlike UVA, UVB is also directly absorbed by DNA ($\lambda_{\text{max}}=260$ nm), resulting in direct photoexcitation, molecular rearrangement, and genotoxicity⁸⁰. Because of their much lower absorption maxima, polyunsaturated fatty acids do not absorb either UVA or UVB, thus they do not undergo direct photochemical reactions. UVR mediated changes to phospholipids is therefore thought to occur as a result of photo-oxidation reactions⁸⁰. Recent studies by photobiologists indicate that UVB generates ROS through a series of sequential steps involving: EGFR activation, ROS generation by cytosolic NADPH oxidase, ras/ERK & p38MAPK activation, p53 activation, and finally apoptotic signaling leading to mitochondrial ROS generation^{81–88}. Thus, UVB-mediated ROS are likely involved in the generation of ox-GPCs.

Given the above discussion, it is somewhat surprising that our group^{11, 12} and others⁸⁹ show that UVB irradiation of purified GPCs directly in a cell-free system generates biologically active ox-GPCs. Given that the ox-GPC precursor molecule 1-hexadecyl-2-arachidonoyl-GPC does not absorb UVB, how does the initial photosensitization reaction take place? As shown in figure 2, 1-hexadecyl-2-arachidonoyl-GPC left exposed to room air to allow oxidation results in the formation of products that absorb significantly in the UVB spectrum. These results are also in agreement with an earlier study in which 2-arachidonoyl-phosphatidylcholine was treated with 15-lipoxygenase, then allowed to oxidize in air for several days¹⁹. This oxidation resulted in the presence of more polar species with peak absorption at 235 nm, similar to the peak absorption seen in our studies after air oxidation alone. These findings fit with the notion that any low level amounts of cellular ROS that act upon 1-hexadecyl-2-arachidonoyl GPC could result in the production of direct targets for UVB. Even small amounts of ox-GPC could then serve as a photosensitizer and source of the initial ROS that could then propagate through non-oxidized GPCs in a free-radical reaction chain reaction. This also introduces the possibility that ox-GPCs also may serve as targets for direct photoexcitation and non-ROS-initiated photochemical reactions. Finally, given the ability of GPCs to serve as targets of photooxidation, how does this result in both PAF-R and PPAR γ agonist production? Previous studies indicate that both PPAR γ and PAF-R ligands are derived from intact oxidized alkyl phospholipids with a sn-1 ether linkage rather than the much larger pool of phospholipids with esterified fatty acids at the sn-1 position^{58, 90}. However, given the structure of PAF itself (sn-2 acetyl group), along with less potent succinoyl-PAF, 5-oxovaleroyl-PAF, butanoyl-PAF(C4-PAF) and butenoyl-PAF (C4:1-PAF), available evidence indicates that PAF-R agonists consist primarily of short chain sn-2 acyl groups terminating with either an ω -oxy function or a methyl group (Figure 1)^{11, 18, 19}.

In contrast to ox-GPCs with PAF-R ligand activity, we propose that ox-GPCs with highly potent PPAR γ ligand activity will exhibit intermediate chain cleavage products with ω -oxy functions (Figure 1). This idea is based on a number of observations: First, the structure of AzPC consists of a 9-carbon azelaic acid esterified at the sn-2 position⁵⁸. Second, small chain products, like PAF, exhibit limited capacity to displace binding of the PPAR γ ligand, rosiglitazone⁵⁸. Most importantly, we show that treatment of either AzPC or lipid extracts from UVB-irradiated KB cells with serum PAF acetylhydrolase (PAF-AH) results in loss of PPAR γ -agonistic activities (Figure 3). This is important, as serum PAF-AH exhibits high substrate specificity for phospholipids with short sn-2 acyl chains; enzymatic activity is rapidly lost with saturated acyl chain lengths greater than 2 carbons, with 10-carbon acyl groups demonstrating essentially no activity^{19, 91, 92}. However, as seen in figure 3 for AzPC, serum PAF-AH exhibits potent activity for oxidized phospholipids with sn-2 residues of up to 9 carbons in length that contain ω -oxy functions^{19, 91-93}. Thus, while PAF-AH has been proposed to limit the actions of un-regulated ox-GPC production on PAF-R activation^{19, 92}, it is also quite likely that it serves to limit the ability of ox-GPCs to activate PPAR γ . Finally, there is evidence that short to intermediate chain ox-GPCs are found *in vivo*. Studies by Takumura *et al* demonstrate that 4–9 carbon dicarboxylic acids are found in choline phosphoglycerides isolated from bovine brain, presumably as a result of oxidative cleavage⁹¹.

Therapeutic Potential of OX-GPCs in UVR-mediated effects

Inasmuch as UV irradiation results in the production of ox-GPCs formed by free radical-mediated oxidation with PAF-R or PPAR γ activity, ongoing studies are defining the exact roles of these novel biological lipids in UV-mediated responses. The data to date suggest that the PAF system is involved in production of cytokines including TNF- α , and also mediates systemic immunosuppression. The PPAR γ system appears to be important in

regulating keratinocyte proliferation & differentiation. The PPAR γ system is also important in UVB-mediated production of COX-2 in keratinocytes, which has been linked to immunosuppression and neoplastic development. Inasmuch as UVB-mediated PAF-R and PPAR γ activation likely plays a role in UV-mediated effects, an obvious question would be whether these effects could be tempered with the use of antioxidants. Of interest, McIntyre and colleagues have demonstrated that hamsters fed chow supplemented with vitamin C did not produce ox-GPCs following challenge with the pro-oxidative stressor cigarette smoke⁹⁴. Along those lines, we have demonstrated that mice fed a diet supplemented with 10g/kg vitamin C failed to exhibit a UVB-mediated decrease in contact hypersensitivity to DNFB. However, intraperitoneal injection of CPAF resulted in a similar inhibition of contact hypersensitivity in mice fed standard versus vitamin C-enriched chow (manuscript in preparation).

Recent studies have used systemic and topical antioxidants in murine model systems to modulate UV effects^{95–98}. The relative favorable safety profile of antioxidants has allowed human studies (reviewed in^{99–101}). As our understanding of the targets for UV radiation and their pathophysiological significance increases, more effective therapies can be designed to modulate the both beneficial and detrimental consequences of this ubiquitous agent.

Future considerations—From the standpoint of future studies, there are several key questions that remain unanswered regarding the role of ox-GPCs in photobiology. First, additional studies are needed to identify the molecular structure of both PAF-R and PPAR γ ligands that are produced as a consequence of UVB irradiation. Once these ligands have been identified, the specific and characteristic structural differences in the oxidized *sn*-2 constituents that differentiate a PAF-R ligand from a PPAR γ ligand should be verified. Finally, identification of these ligands will support further studies to verify that these ligands are produced in physiologically relevant concentrations within not only cultured cell lines, but also intact epidermis. These questions are active areas of investigation within our laboratories.

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Figure 1. Proposed scheme for UVB-induced PAF-R and PPAR γ ligand production from oxidized glycerophosphocholines (ox-GPCs)

Ox-GPCs serve as secondary products of photooxidation initiated by the ability of UV light to shift endogenous chromophores or photosensitizers (S) to the photoexcited state (S*). In the presence of molecular oxygen (O_2), relaxation to the ground state can result in the formation of reactive oxygen species (ROS). This includes superoxide ($O_2^{\cdot-}$), which rapidly undergoes dismutation to form hydrogen peroxide (H_2O_2). In the presence of transition metals (Fe(II), Cu(II)), H_2O_2 is converted to highly reactive hydroxyl radicals ($\cdot OH$). These ROS then act to initiate oxidative extraction of protons from polyunsaturated fatty acids in glycerophosphocholines, like 1-hexadecyl-2-arachidonoyl-glycerophosphocholine (HAPC) (as shown in the box). Bisallylic hydrogens (red dotted arrows) serve as favored sites of proton extraction and eventually introduces oxy functions, rearranges bonds and fragments carbon-carbon bonds by β -scission that generate a myriad of reaction products. OxGPCs with PAFR activity likely have short-chain aliphatic groups ending with a methyl group or an ω -oxy function esterified at the sn-2 position (R1)(PAF, R1=CH₃). OxGPCs with PPAR γ activity likely have intermediate chain aliphatic groups (< 9 carbons) ending with an ω -oxy function esterified at the sn-2 position (R2). Once formed, PAF ligands activate the PAF-R, a G α_i coupled G-protein coupled receptor. PAF-R activation is likely important in mediating UVB-induced cytokine production, cytotoxicity, and photoimmunosuppression. UVB-induced PPAR γ ligands act to regulate gene expression, targeting genes containing a peroxisome proliferator response element (PPRE). PPAR γ activation likely plays a role in

UVB-mediated regulation of keratinocyte growth, differentiation, and cyclooxygenase 2 (COX-2) expression.

Figure 2. Air oxidation of 1-hexadecyl-2-arachidonoyl GPC results in products that absorb in UVB range

The absorbance of 200 $\mu\text{g/ml}$ purified 1-hexadecyl-2-arachidonoyl-GPC (Avanti Polar Lipids) in ethanol was obtained after dried lipid was left at room temperature in the presence of air for one or fourteen days, and the absorbance obtained using a spectrophotometer.

Figure 3. PPAR γ agonistic activity in UVB-irradiated KB cells is abolished by PAF-AH enzyme treatment

KB cells co-transfected with PPRE-luciferase reporter and β -gal plasmids were treated with AzPC (1.5 μ M), or AzPC treated with PAF-AH or PBS control, or lipid extracts from 10^7 KB cells either sham- or 2000 J/m² UVB irradiated treated with PAF-AH or PBS. Cells were harvested 24 hr after treatment for luciferase and β -gal activity assay as previously described ¹²