Photooxidation Generates Biologically Active Phospholipids That Induce Heme Oxygenase-1 in Skin Cells

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Heme oxygenase-1 (HO-1) is a key enzyme in the cellular response to tissue injury and oxidative stress. HO-1 enzymatic activity results in the formation of the cytoprotective metabolites CO and biliverdin. In the skin, HO-1 is strongly induced after long wave ultraviolet radiation (UVA-1). Here we show that UVA-1 irradiation generates oxidized phospholipids derived from 1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphorylcholine (PAPC) that mediate the expression of HO-1 in skin cells. Using EO6 antibodies that recognize oxidized phospholipids, we show that UVA-1 irradiation of dermal fibroblasts generates oxidation-specific epitopes. Irradiation of arachidonate-containing phospholipids with UVA-1 led to formation of defined lipid oxidation products including epoxyisoprostane-phosphatidylcholine that induced HO-1 expression in dermal fibroblasts, in keratinocytes, and in a three-dimensional epidermal equivalent model. In addition, we demonstrate that the oxidation of PAPC by UVA-1 is a singlet oxygen-dependent mechanism. Together, we present a novel mechanism of UVA-1-induced HO-1 expression that is mediated by the generation of biologically active phospholipid oxidation products. Because UVA-1 irradiation is a mainstay treatment of several inflammatory skin diseases, structural identification of UVA-1-generated biomolecules with HO-1-inducing capacity should lead to the development of drugs that could substitute for irradiation.

Cells of the skin express HO-1 in response to the oxidative stress imposed by long wavelength UV radiation (UVA) (1–3). Evidence suggests that the generation of singlet oxygen (1O2) by UVA-1 (340–390 nm) and subsequent oxidation of intracellular membrane lipids (4, 5) are involved in this process. However, neither the mechanisms nor the structures of responsible lipid oxidation products have been described so far.

HO-1 plays a general role in cutaneous wound repair, and the resolution of inflammation is strongly up-regulated after skin injury and declines to basal levels after completion of wound healing (6). HO-1 converts free heme and heme moieties of proteins to carbon monoxide, iron, and biliverdin, which is rapidly converted to bilirubin by biliverdin reductase. CO and bilirubin have well described antioxidant and anti-inflammatory properties (7, 8), suggesting that induction of HO-1 is a general mechanism that protects the cell against oxidative damage. Accordingly, the adaptive response of skin fibroblasts after repeated UVA irradiation, which protects them against further membrane damage, is mediated by induction of HO-1 (9).

The phospholipid 1-palmityl-2-arachidonoyl-sn-glycero-3-phosphorylcholine (PAPC) is a major component of cell membranes and lipoproteins. Its oxidation products are found in cells during inflammation (10), in membranes of apoptotic cells (11), as well as in oxidized low density lipoprotein (12). Polyunsaturated fatty acyl residues at the sn-2 position of the glycerol backbone, such as the arachidonoyl moiety in PAPC, are especially prone to oxidative modification. Thus, the oxidation of PAPC (OxPAPC) leads to the addition of oxygen atoms as well as fragmentation of the arachidonate moiety. The type of oxidative modification determines the biological activity of these oxidized phospholipids (13). OxPAPC was shown to induce HO-1 expression in several cell types of the vasculature and the immune system (14).

Here we report that photooxidation of PAPC or irradiation of skin cells results in formation of biologically active lipid oxidation products that induce HO-1 expression. Using mass spectrometry and EO6 antibodies that detect oxidation products of PAPC, we identify epoxyisoprostane-phosphatidylcholine, a known inducer of HO-1, among these oxidation products, and demonstrate their intracellular formation. Because UVA-1 phototherapy is successfully used for treatment of
inflammatory skin diseases (15), identification of UV-generated biomolecules with protective effects will lead to the development of novel drugs that could be used to substitute for UVA-1 phototherapy.

**EXPERIMENTAL PROCEDURES**

**Cell Culture**—Neonatal human epidermal keratinocytes (KCs) derived from foreskin were obtained from Clonetix (San Diego, CA). Keratinocytes were cultured in keratinocyte growth medium up to the fifth passage. Human neonatal skin fibroblasts (FB) were obtained from Cascade Biologies (Portland, OR) and grown in Dulbecco’s modified Eagle’s medium (Invitrogen) supplemented with 10% fetal calf serum and penicillin/streptomycin (1000 units/ml; Invitrogen) to subconfluence.

**Skin Equivalents**—In vitro reconstructed skin equivalents were generated as described previously (16). Briefly, 1.3 × 10⁶ KCs were added on top of a collagen gel containing fibroblasts. After overnight incubation, the medium from the upper chamber was removed, thus putting the KC at air-liquid interface. Afterward, skin equivalents were cultured in serum-free KC-defined medium, which is KC growth medium without bovine pituitary extract, supplemented with 1.3 mM calcium, 10 µg/ml transferrin, 50 µg/ml ascorbic acid, and 0.1% bovine serum albumin.

**Quantitative Real Time PCR (qPCR)**—RNA was isolated using TRIzol reagent (Invitrogen). 900 ng of total RNA were reverse-transcribed with murine leukemia virus reverse transcriptase (Applied Biosystems, Foster City, CA) and oligo(dT) primers. The following forward (F) and reverse (R) primers were used: HO-1: F, 5′-AAGATTGCCAGAAGC-3′; R, 5′-AACGTGCTGCCAGAAGCTGAG-3′; β2-microglobulin: F, 5′-GATGAGATGCTGCCGTG-3′; and R, 5′-CAATCCAAATGCGGCATCT-3′.

qPCR was performed using LightCycler technology and the Fast Start SYBR Green I kit (Roche Applied Science). In all assays, cDNA was amplified using a standardized program (10 min denaturing step; 55 cycles of 5 s at 95 °C, 15 s at 65 °C, and 15 s at 72 °C; melting point analysis in 0.1 °C steps). Quantification of target gene expression was performed using a mathematical model by Pfaffl (17). The expression of the target molecule was normalized to the expression of β2-microglobulin.

**Western Blot Analysis**—Bound HO-1 antibodies (SPA-896; Stressgen) and control IgG antibody as described previously (20). The cells were counterstained using an antibody against active Caspase 3 (rabbit IgG, 0.5 mg/ml, R & D Systems, Minneapolis, MN) and control IgG. Formalin-fixed paraffin sections of skin equivalents were stained for HO-1 (SPA-896; Stressgen).

**Lipid Oxidation**—PAPC was oxidized by exposure of the dry lipid to air for 72 h to generate OxPAPC. PapC dried to a thin film on a glass support was irradiated with UVA-1 with 80 J/cm² to generate UV-PAPC. For treatment with singlet oxygen, all lipids were vortexed in phosphate-buffered saline containing 90 µM (final concentration in the culture medium was 9 µM) Rose Bengal (Sigma-Aldrich) and irradiated with 10 J/cm² UVA-1 (see Fig. 5A) or under a commercial 35 W halogen lamp at a distance of 30 cm for 30 min (see Fig. 5, B and C). The extent of oxidation was monitored by ESI-MS as described previously (12).

**Lipid Extraction**—Total lipids were extracted from dermal fibroblasts using chloroform/methanol (2:1, v/v) in the presence of 0.01% butyral hydroxytoleule and 0.17 m formic acid as described (12).

**Thin Layer Chromatography**—TLC analysis of lipids was performed on Silica gel 60 TLC plates (Merck) using a mixture of chloroform-methanol-water (100:50:10, v/v/v) as a developing solvent. Lipid spots were visualized after treatment with 10% copper sulfate in an 8.5% aqueous solution of orthophosphoric acid and subsequent heating at 180 °C.

**Mass Spectrometry**—Mass spectrometry was performed on a PE Sciex API 365 triple quadrupole mass spectrometer (Applied Biosystems, Foster City, CA) equipped with an electrospray source. Flow injection experiments were performed by injecting 20-µl aliquots of lipid samples dissolved in 200 µl of methanol-water-formic acid (80:20:0.1, v/v/v) into a stream of the same solvent mixture, delivered by an HPLC system (HP1100; Agilent Technologies, Waldbronn, Germany). Spectra were acquired in the positive mode in the range of 400–900 Da. Liquid chromatography-MS and liquid chromatography-mass spectrometry-MS/MS was performed using an Agilent Zorbax Eclipse XDB-C8 column (150 × 4.6 mm, 5 µm). Samples were dissolved in mobile phase A (10 mM ammonium acetate in methanol-water, 80:20, v/v). Analytes were eluted using a gradient from 25% mobile phase B (10 mM ammonium acetate in methanol) to 100% B in 30 min, followed by an isocratic step at 100% B for 30 min. MS and tandem MS detection was performed in the scan mode (400–900 Da) or the multiple reaction monitoring mode detecting the phosphatidylcholine-specific fragment at m/z 184.1 Da produced from various precursor ions (m/z 594, 610, 782, 814, 828, 846, 878 [MH]+) at a collision energy of 35 eV. Quantification of the peak areas for the multiple reaction monitoring transition of 828 > 184 in the elution time range of 7–13 min was performed using Analyst software (version 1.4, Applied Biosystems).
UVA-1 Generated Active Phospholipids

FIGURE 1. OxPAPC induces HO-1 expression in skin cells. A, levels of HO-1 mRNA expression in dermal fibroblasts 4 h after stimulation with 40 J/cm² UVA-1, 20 J/cm² UVB, or 100 μg/ml OxPAPC. Relative expression of HO-1 mRNA was determined using qPCR normalized to expression of the housekeeping gene β2-microglobulin. The data represent the means ± S.D. B and C, keratinocytes (B) and dermal fibroblasts (C) were incubated with the indicated concentrations (μg/ml) of OxPAPC, and the induction of HO-1 mRNA was measured after 4 h by qPCR. The insets show corresponding HO-1 protein levels as determined by Western blot (WB) after 6 h of treatment. D, primary keratinocytes differentiated in a three-dimensional epidermal equivalent were incubated with OxPAPC at the indicated concentrations for 4 days and analyzed for HO-1 expression using immunohistochemistry and qPCR. ctrl, control.

RESULTS

OxPAPC Induces HO-1 Expression in KCs and Dermal FB—We and others have recently shown that HO-1 expression in vascular endothelial and smooth muscle cells is strongly up-regulated by OxPAPC, which had been generated by air exposure (14, 22, 23). Here we examined whether OxPAPC had similar effects on skin cells. As it has been reported previously (5), treatment of FB with UVA-1 (40 J/cm²), but not UVB (20 ml/cm²), leads to a strong induction of HO-1 mRNA expression. This effect was mimicked by the addition of OxPAPC (100 μg/ml) (Fig. 1A). When OxPAPC was added at different concentrations to cultures of primary human epidermal KC and FB, HO-1 mRNA and protein expression were strongly induced in both cell types in a dose-dependent manner (Fig. 1, B and C). Strong induction of HO-1 mRNA and protein was also observed when epidermal equivalent cultures, which mimic more closely the in vivo situation, (16) were exposed to OxPAPC for 4 consecutive days (Fig. 1D). HO-1 protein expression was most pronounced in the epidermal layers immediately below the stratum corneum. These data demonstrate that like UVA-1, OxPAPC is able to strongly stimulate HO-1 expression in skin cells.

HO-1 Inducing Activity Is Contained in "Long Chain" but Not in "Short Chain" Oxidized Phospholipids—OxPAPC contains several oxidation products of PAPC that can be grouped into long chain oxidation products, which result from insertion of oxygen into the arachidonic acid moiety, and short chain oxidation products, which result from oxidative fragmentation (21).

Distinct biological properties have been attributed to compounds of either group (24, 25). To identify HO-1-inducing compounds within OxPAPC, we tested long chain, short chain, and a polar lipid fraction that had been separated by thin layer chromatography. As shown in Fig. 2A, HO-1 was strongly induced in fibroblasts by the long chain fraction, whereas the other fractions had virtually no effect. This was confirmed when the synthetic short chain compounds 1-palmitoyl-2-(5-oxovaleroyl)-sn-glycero-3-phosphorylcholine (POVPC), 1-palmitoyl-2-glutaroyl-sn-glycero-3-phosphorylcholine, and 1-palmitoyl-2-lyso-sn-glycero-3-phosphorylcholine (lysoPC) failed to induce HO-1 expression, as did unoxidized PAPC and di-myristoyl-phosphorylcholine (Fig. 2B). These data demonstrate that the capacity to induce HO-1 resides within the long chain oxidation products of PAPC.

To examine a possible contribution of lipid hydroperoxides, which are present in the OxPAPC preparation, to the induction of HO-1 expression, we incubated fibroblasts with the ROS scavengers β-carotene, vitamin C, and vitamin E prior to exposure to OxPAPC. This treatment did not significantly reduce OxPAPC-mediated HO-1 induction (supplemental Fig. S1), suggesting that secondary peroxidation of cellular lipids caused by OxPAPC was not involved in the induction of HO-1 expression.

UVA-1 Irradiation of PAPC (UV-PAPC) Leads to Formation of Long Chain Oxidation Products That Induce HO-1 Expression—PAPC is abundant among phospholipids of cellular membranes (26). Because it contains a polyunsaturated fatty acyl side chain (20:4) at the sn-2 position, it is prone to oxidation by UV light, singlet oxygen, or free radicals (27, 28). For the
skin, UVA irradiation is an important inducer of oxidative stress and results in the expression of HO-1 in fibroblasts (2). To investigate the direct effects of UVA-1 irradiation on PAPC oxidation, we irradiated synthetic PAPC in vitro. PAPC was dried to a film on a glass support and irradiated with fluencies up to 80 J/cm² UVA-1. The irradiated PAPC (UV-PAPC) and the sham treated PAPC were analyzed by ESI-MS (Fig. 3, A and B). Fig. 3C shows the formation of mono-, di-, and tri-hydroperoxides of the arachidonic acid moiety of PAPC (m/z 814 (peak b), 846 (peak d), and 878 (peak e)) as well as ions with m/z 828 (peak c). The relative intensity of the nonoxidized [MNa]⁺ ion (peak a) was set as 100%. Generation of these oxidation products was UVA-1 fluency-dependent, whereas irradiation with corresponding fluencies of UVB (up to 120 mJ/cm²) did not lead to formation of these oxidation products (supplemental Fig. S2). The ion with m/z 828 (c) corresponds to 1-palmitoyl-2-(epoxy-isoprostane-E₂)-sn-glycero-3-phosphorylcholine (PEIPC), a strong inducer of HO-1 expression (14). PEIPC was also present in the long chain TLC fraction of OxPAPC (Fig. 2A), as evidenced by ESI-MS and recently described by us (21). Ions (b), (d), and (e) correspond to the mono-, di-, and tri-hydroperoxides of PAPC.

To investigate the respective biological activities, sham treated PAPC and UVA-1-treated (20, 40, and 80 J/cm²) PAPC were added to cultures of FB, and HO-1 mRNA expression was measured after 4 h. UV-PAPC, but neither sham treated nor UVB-treated PAPC (supplemental Fig. S2), induced HO-1 expression (Fig. 3D), confirming that UVA-1-mediated oxidation of PAPC leads to the formation of biologically active lipid mediators.

UV-PAPC consists of a molecule with m/z 828 (PEIPC), hydroperoxides, and trace amounts of hydroxides of PAPC. To investigate which of the oxidation products present in UV-PAPC induce HO-1 expression, we treated dermal FB with synthetic hydroperoxides and hydroxides of PAPC as well as with PEIPC that had been purified from OxPAPC. Although purified PEIPC strongly induced HO-1 expression, neither the hydroperoxides nor the hydroxides were biologically active (supplemental Fig. S3). Thus, we conclude that PEIPC (m/z 828) is the oxidized phospholipid species present in UV-PAPC, which induces HO-1 expression.

**UVA-1 Irradiation Generates Oxidation-specific Phospholipid Epitopes in Human Fibroblasts and Leads to Formation of PEIPC**—To investigate whether UVA-1 would induce phospholipid oxidation in living cells, we irradiated FB for different times and analyzed them for the presence of PAPC oxidation products. In addition to ESI-MS, we used the murine monoclonal IgM antibody EO6, which binds to oxidized phosphorylcholine-containing phospholipids present in OxPAPC, in oxidized low density lipoprotein, and in the cell membranes of apoptotic cells. For instance, EO6 recognizes POVPC and PEIPC, but not lyso-PC (10, 30, 31). Irradiation of FB with UVA-1 resulted in a dose-dependent increase of intracellular EO6 immunoreactivity, demonstrating the formation of phospholipid oxidation products within 10 min after UVA-1 exposure (Fig. 4, A–D). Staining with antibodies detecting active caspase 3 (aC-3) showed that EO6 immunoreactivity was not confined to apoptotic cells (EO6 positive: 45.5%, aC-3 positive: 15.6%, double positive: 8.5% after irradiation with 60 J/cm²) (supplemental Fig. S4). At lower fluencies of UVA-1, apoptosis (as detected with aC-3) in FB did not occur at all (supplemental Fig. S4), whereas EO6 immunoreactivity was found on 12% of cells as compared with 3% of sham treated cells.
Among the lipids present in UV-PAPC, only PEIPC (m/z 828) was reported to be recognized by the EO6 antibody (10). To confirm the structure of the ion with m/z 828 as PEIPC (12), we performed HPLC-tandem MS analysis of OxPAPC and UV-PAPC (Fig. 4, E and F). Multiple reaction monitoring revealed an identical HPLC elution profile for m/z 828 in both OxPAPC and UV-PAPC. In addition, retention times were identical, and relative abundances of individual peaks were highly comparable. Thus, we conclude that the molecule with m/z 828 that is present in UV-PAPC is identical to PEIPC.

To investigate whether PEIPC is formed in dermal FB upon irradiation, we analyzed total lipid extracts from nonirradiated and UVA-1-irradiated (40 J/cm²) dermal fibroblasts. In Fig. 4G we show that PEIPC is strongly increased in extracts of UVA-1-irradiated cells. To quantify the amounts of PEIPC formed in the cells after irradiation, we measured the peak areas for the multiple reaction monitoring transition 828→11022 in the elution time range of 7–13 min (shaded gray in the diagram in Fig. 4) using Analyst software (version 1.4, Applied Biosystems). We generated a calibration curve (Fig. 4H) where we plotted the area counts versus the amount of OxPAPC loaded onto the column. This was used to calculate the amount of PEIPC in the cell extracts. For this calculation we estimated the cell volume of a human dermal foreskin fibroblast to be 3.5 pl, a cell number of 3.2×10⁶ cells/mg of protein (32) and about 20 mol % of OxPAPC corresponding to the isomers of PEIPC. We found that the intracellular concentration of PEIPC was 0.8 μM in nonirradiated cells and increased to 7.7 μM after irradiation with 40 J/cm² of UVA-1. It was recently shown that PEIPC-induced gene expression in endothelial cells at concentrations as low as 0.1 μM (33, 34). These data demonstrate that UVA-1 irradiation leads to formation of oxidized phospholipids in cells that remain viable and that PEIPC is formed in sufficient amounts to induce expression of HO-1.

Generation of HO-1-inducing Oxidized Phospholipids by UVA-1 Involves Singlet Oxygen and Requires an sn-2 Arachidonic Moiety—Singlet oxygen (1O₂) is an important ROS that mediates photooxidation by UVA-1 (35). To investigate the role of singlet oxygen in UVA-1-induced oxidation of PAPC, the 1O₂ generator Rose Bengal (RB) was added before irradiation. To test whether generation of 1O₂ would enhance the formation of biologically active phospholipids and whether this activity is confined to phospholipids containing an arachidonic acid moiety, we used 1-palmitoyl-2-oleoyl-sn-3-glycerophosphorylcholine (POPC) and 1-palmitoyl-2-linoleyl-sn-3-glycerophosphorylcholine (PLPC), which differ from PAPC in their length and number of double bonds of the sn-2 acyl chain: a single double bond in POPC (18:1) and two double bonds in PLPC (18:2). The structure of their sn-2 acyl chain allows ox-

FIGURE 4. UVA-1 irradiation of dermal fibroblasts leads to intracellular formation of oxidation specific epitopes of PAPC. A–D, dermal fibroblasts cultured in 8-well chamber slides were subjected to increasing fluencies of UVA-1 (sham (A), 20 J/cm² (B), 40 J/cm² (C), and 60 J/cm² (D)), immediately fixed, and stained with EO6 IgM monoclonal mouse antibody. E and F, HPLC elution profile of OxPAPC (E) and UV-PAPC (F) in HPLC-tandem-MS analysis. 

The plot shows HPLC retention times and relative abundances of ions that have an m/z of 828 (=PEIPC) and yield a fragment ion of 184, specific for phosphorylcholine. The range used for preparation of the calibration curve in H is shaded. 

The cellular concentration of PEIPC (μM) in unirradiated and UVA-1 (40 J/cm²) irradiated dermal fibroblasts.
ative formation of hydroperoxides but not of the epoxyisoprostane oxidation product, which can only be formed from PAPC (20:4).

When PAPC was irradiated with UVA-1 in the presence of RB (90 μM), a fluency of 10 J/cm² was sufficient for production of HO-1-inducing oxidation products. On the other hand, equally treated POPC or PLPC did not gain HO-1 inducing activity (Fig. 5A). ESI-MS analysis showed that mono-, di-, and tri-hydroperoxides as well as PEIPC were generated from PAPC by this treatment. Accordingly, irradiation of POPC and PLPC in the presence of RB led to the formation of hydroperoxides, whereas epoxyisoprostane structures were not present. (supplemental Fig. S5). Irradiation with 10 J/cm² of UVA-1 or treatment with RB in the dark did not lead to formation of oxidation products detectable by MS (not shown).

When PAPC was irradiated with white light from a commercial 35 W source at a distance of 30 cm for 30 min in the presence of RB (30 and 90 μM), it gained HO-1 inducing activity. The presence of sodium azide (10 mM) during irradiation prevented this activity (Fig. 5B), further indicating the involvement of 1O₂. Sodium azide alone inhibited the inducibility of HO-1 expression by about 25% in these settings (not shown), whereas ESI-MS analysis demonstrated that the presence of sodium azide potently inhibited 1O₂-mediated PAPC oxidation (Fig. 5C–E). These findings demonstrate that the presence of 1O₂ facilitates the formation of long chain oxidation products of phospholipids and the resulting capacity of PEIPC to induce HO-1 expression.

DISCUSSION

Expression of HO-1 is up-regulated as a cellular response to oxidative stress such as induced by UVA irradiation (reviewed in Ref. 36) or during inflammation (reviewed in Ref. 8). Previous studies have implicated that singlet oxygen-mediated lipid oxidation is involved in the gene regulation of HO-1 after UVA-1 irradiation of skin cells (reviewed in Ref. 37). Here we show that specific oxidation products of PAPC, an abundant membrane phospholipid, induce expression of HO-1 in epidermal KC and dermal FB and that the formation of these oxidation products is induced by UVA-1. Induction of HO-1 as observed during inflammation and in the early phase of wound repair, involves the generation of ROS (6, 38). In the skin, UVA irradiation generates ROS that modify cellular lipids and proteins and induces gene regulation (39–41). Singlet oxygen (1O₂) is an important ROS that leads to activation of signaling pathways and gene regulation (42–45) upon UVA-1 irradiation. Here we identify a UVA-1 photoproduct that is a strong inducer of HO-1. Its formation from the membrane phospholipid PAPC is facilitated by 1O₂. In addition we demonstrate that oxidation-specific epitopes of the phospholipid PAPC are formed by UVA-1 oxidation in cells that remain viable. Finally our data strongly suggest that the HO-1-inducing capacity resides mainly in the PAPC oxidation product PEIPC.

During oxidation of PAPC the target for ROS is the unsaturated sn2-arachidonoyl residue, the oxidation of which results in insertion of oxygen atoms into the chain (long chain products) and subsequent shortening of the chain resulting from fragmentation (short chain products) (46). The long chain products

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**FIGURE 5.** UVA-1 irradiation of common phosphatidylcholine-based phospholipids in the presence of the 1O₂ generating dye RB strongly promotes formation of long chain oxidation products. A, relative mRNA expression of HO-1 after stimulation with sham treated (dark, no RB) and UVA-1/RB irradiated PAPC, POPC, and PLPC determined by qPCR. B, 1 mg/ml PAPC was irradiated with white light (commercial halogen bulb 35 W, 30 min, 30-cm distance) in 30 and 90 μM RB with or without 10 mM NaN₃ in phosphate-buffered saline. Dermal fibroblasts were incubated with medium that contained 100 μg/ml PAPC treated as above for 4 h, +, p < 0.005 using an unpaired Student’s t test. C–E, ESI-MS analysis of PAPC that was unirradiated (C), irradiated with 10 J/cm² UVA-1 in the presence of RB (D), and irradiated with 10 J/cm² UVA-1 in the presence of RB (90 μM) and NaN₃ (10 mM) (E). ctrl, control.
can include hydroxy-, hydroperoxy-, and poly-hydroxy/peroxy forms as well as epoxides and isoprostanes, whereas the short chain products include carboxylic aldehydes and hydroxy-aldehydes (47, 48). Bose and Chatterjee (49) described the products of UVA oxidation of lipids in a dried film state and found conjugated dienes, lipid hydroperoxides, and malondialdehyde. Our data are in line with these findings, because we identified hydroperoxides and isoprostane-like modifications after irradiation of PAPC with UVA-1. OxPAPC generated by autoxidation in atmospheric oxygen was shown to contain numerous different oxidation products (12), making the attribution of biological activities difficult. By contrast, UVA-1 oxidation products of PAPC (UV-PAPC) only consist of a limited number of different molecules, which facilitates tracing of biological effects to individual molecules.

The EO6 antibody recognizes PAPC oxidation products (50) but not unoxidized PAPC or lyso-PC (10). We demonstrate for the first time that upon UVA-1 irradiation epitoypes recognized by EO6 form inside dermal fibroblasts and that this occurs independently of apoptosis induction. These data show that UVA-1, at fluencies that are not cytotoxic, leads to accumulation of intracellular phospholipid oxidation products that are able to induce HO-1. Therefore, our data suggest that these oxidation products at least in part mediate the HO-1 induction observed after UVA irradiation of living cells.

The presence of the \(^1\)O\(_2\)-generating dye RB during the UVA-1 irradiation of PAPC strongly enhanced the formation of the HO-1 inducer PEIPC (14). This treatment also led to formation of phospholipid hydroperoxides in PAPC, POPC, and PLPC. However, in contrast to PAPC, irradiation of POPC and PLPC did not yield any oxidation products that could efficiently induce HO-1 expression. Therefore, we conclude that oxidation products of the arachidonyl residue in PAPC are mainly responsible for this biological activity. This conclusion is further strengthened by our additional finding that reduction of hydroperoxides in OxPAPC with TPP only slightly reduced its capacity to induce HO-1 (not shown), further indicating that lipid hydroperoxides are not involved in HO-1 induction. Furthermore, our demonstration that irradiation of PAPC with white light in the presence of RB also led to the formation of HO-1-inducing lipid oxidation products and that formation of these products could be inhibited by sodium azide strongly indicates the involvement of \(^1\)O\(_2\) (51) in this process.

In inflammation, oxidative modification of lipids results in formation of biologically active molecules that can modulate the inflammatory process (13). Most of the current research is focused on pro-inflammatory or detrimental photoproduts that arise upon UV radiation. Marathe et al. (52) have shown that UVB-mediated lipid oxidation generates lipids that have platelet-activating factor-like activity, and Zhang et al. (53) have demonstrated peroxisome proliferator-activated receptor \(\gamma\) ligand activity of such lipids. Malondialdehyde, a major end product of lipid peroxidation that has mutagenic properties, also has been shown to accumulate upon UVA-1 irradiation (54). Here we focus on the biological effects of phospholipid oxidation products derived from arachidonic acid-containing phospholipids, which are sensed by cells as indicators for oxidative stress and thereby induce an antioxidant response.

Recent findings suggest that besides the inhibition of endotoxin-induced tissue damage (55), the induction of anti-inflammatory and inhibition of pro-inflammatory signaling cascades by oxidized lipids could potentially contribute to resolution of inflammation (reviewed in Ref. 56). Our work demonstrates that some of the oxidized lipid mediators including PEIPC are formed upon UVA-1 irradiation. Therefore, the therapeutic effect of UVA-1, which is used for the treatment of inflammatory skin diseases such as atopic dermatitis, scleroderma, and graft versus host disease (reviewed in Ref. 15) may at least in part be due to the generation of oxidized lipids in the skin. Some of the benefits of UVA-1 phototherapy include a decrease of T cells by UV-induced apoptosis (57) and a decrease in the Th1/Th2 cell ratio in the inflamed tissue (58). In this context it is interesting that we previously demonstrated that oxidized phospholipids limit the capacity of DC to stimulate T cells to initiate Th-1 responses (29). It will be a focus of our further research to investigate the contribution of UVA-1-oxidized lipids in immunomodulatory effects of UVA-1 irradiation. Skin diseases that can be treated by UVA-1 represent prime targets to investigate the beneficial effects of direct application of HO-1-inducing oxidized lipids.

Taken together, we could show that UVA-1 irradiation dose-dependently and directly oxidizes the common membrane phospholipid PAPC, leading to formation of biologically active compounds that up-regulate HO-1 expression in skin cells. We tentatively identify one of these compounds as epoxyisoprostane-containing PC (PEIPC). Furthermore, we show that the oxidation of PAPC by UVA-1 depends on the generation of singlet oxygen. Biologically active oxidized phospholipids generated by UVA-1 may thus contribute to the effect of UVA-1 irradiation on normal and diseased skin. Further analysis will be necessary to delineate whether these lipid mediators mimic the beneficial effects of UVA-1 in therapy of skin diseases while avoiding the potential long term disadvantages of repeated UVA-1 irradiation.

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