

Oxidized Phospholipids Stimulate Angiogenesis Via Autocrine Mechanisms, Implicating a Novel Role for Lipid Oxidation in the Evolution of Atherosclerotic Lesions

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Abstract—Angiogenesis is a common feature observed in advanced atherosclerotic lesions. We hypothesized that oxidized phospholipids (OxPLs), which accumulate in atherosclerotic vessels can stimulate angiogenesis. We found that oxidized 1-palmitoyl-2-arachidonoyl-*sn*-glycero-3-phosphocholine (OxPAPC) stimulated the formation of sprouts from endothelial cell spheroids and promoted growth of capillaries into Matrigel plugs in mice. OxPLs stimulated expression of vascular endothelial growth factor (VEGF) in vivo and in several normal and tumor cell types in vitro. In addition, OxPAPC upregulated cyclooxygenase (COX)-2 and interleukin (IL)-8. COX-2 inhibitors, as well as blocking antibodies to IL-8 suppressed activation of sprouting by OxPAPC. We conclude that OxPAPC stimulates angiogenesis via autocrine mechanisms involving VEGF, IL-8, and COX-2-generated prostanoids. Our data suggest that accumulation of OxPLs may contribute to increased growth of blood capillaries in advanced lesions, thus leading to progression and destabilization of atherosclerotic plaques. (*Circ Res.* 2006;99:900-908.)

Key Words: oxidized phospholipids ■ atherosclerosis ■ angiogenesis ■ plaque destabilization

The adventitial layer of human coronary arteries contains a network of microvasculature, termed *vasa vasorum*, that delivers oxygen and nutrients to the outer layers of the arterial wall. In contrast to the adventitia, the intima and the inner media of normal large arteries usually do not contain capillaries. However, during atherosclerotic plaque formation, microvessels appear in the thickened intima and media of more than 40% of lesions.^{1,2} These neovessels originate mainly from the adventitial *vasa vasorum* and support growth of atherosclerotic intima beyond the critical thickness limited by diffusion from the major arterial lumen.³

It has been widely hypothesized that neovascularization is a causative factor for atherosclerotic plaque growth and destabilization. Although these issues are difficult to address in a direct experiment (discussed in Khurana et al⁴), there is much indirect evidence suggesting that neovascularization can influence the evolution of atheroma via several mechanisms. The density of *vasa vasorum* strongly correlates with the number of infiltrating mononuclear cells, suggesting that neovessels are an important route for the entry of leukocytes into advanced lesions.^{2,5} Furthermore, intraplaque microvas-

cular hemorrhages provide blood cell-derived lipids that deposit in the lipid core.^{6,7} Microvessels are functionally important in atherogenesis as illustrated by the ability of angiogenesis inhibitors angiostatin and TNP-470 to reduce angiogenesis and inhibit the development of lesions in apoE knockout mice.^{5,8} In contrast, angiogenic stimuli such as vascular endothelial growth factor (VEGF) promote lesion development.⁹ Such data strongly suggest that formation of *vasa vasorum* stimulates the progression of atherosclerotic lesions.

In addition to a role in plaque growth, the formation of neovessels potentially can decrease the stability of atheroma because of digestion of plaque tissue by metalloproteases secreted by growing capillaries. Indeed, unstable and ruptured atherosclerotic plaques are characterized by an increased density of plaque microvessels.^{10,11}

Hypoxia is among the strongest known angiogenic stimuli also thought to play a role in the development of intimal neovascularization.¹² However, additional microenvironmental factors independent of lesion thickness may also be important for plaque angiogenesis. In apoE-deficient mice,

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neovascularization was shown to be frequently localized to discrete areas of lesions.⁵ This occurrence of intimal capillaries as “hot spots” strongly suggests that local factors in the plaque may regulate angiogenesis.¹²

Atherosclerotic plaques are known to contain oxidized phospholipids (OxPLs) that are formed by nonenzymatic oxidation of esterified polyunsaturated fatty acids.¹³ OxPLs demonstrate a number of activities relevant for atherosclerosis, including upregulation of mononuclear cell-specific chemokines and cell adhesion molecules on endothelial cells (ECs).^{14–16} We hypothesized that OxPLs accumulating in atherosclerotic lesions play a role in activation of plaque angiogenesis. Here we show that OxPLs elicit proangiogenic effects *in vitro* and *in vivo*. Furthermore, we present evidence that angiogenic effects of OxPLs are mediated via autocrine stimulation by VEGF, interleukin (IL)-8, and cyclooxygenase (COX)-2 products. These findings further advance our knowledge on the mechanisms of deleterious actions of OxPLs on the progression and destabilization of atheroma.

Materials and Methods

Materials

Sources of chemicals are described in the expanded Materials and Methods section, which is available in the online data supplement at <http://circres.ahajournals.org>.

Lipids

Oxidation and analysis of phospholipids were performed as described.¹³ 1-Palmitoyl-2-(5-oxovaleryl)-*sn*-glycero-3-phosphocholine (POVPC) and 1-palmitoyl-2-glutaryl-*sn*-glycero-3-phosphocholine (PGPC) were synthesized from PAPC or lyso-PC, respectively.^{13,17} Concentrations of phospholipids were determined by lipid phosphorus assay.¹⁸

Animals

Experiments were performed according to Austrian animal rights law using female C57BL/6J mice (Institut fuer Versuchstierzucht und Haltung, Himberg, Austria).

Cells

Origin and culturing conditions are described in the expanded Materials and Methods section of the online data supplement. Stimulation with OxPLs was performed in growth medium containing either reduced serum (2%) or serum-free to avoid degradation of OxPLs by serum enzymes.

Spheroid Assay of Angiogenesis In Vitro

Sprouting assay using endothelial cell spheroids embedded into fibrin gel was performed as described,¹⁹ with some modifications as described in the expanded Materials and Methods section.

Microcarrier Bead Sprouting Assay

This modification of endothelial sprouting angiogenesis assay was performed using HUVECs seeded onto Cytodex-3 microcarrier beads (Sigma-Aldrich, Vienna, Austria), as described by us previously.²⁰

EC Migration Assay

Migration assay was performed in modified 96-well Boyden microchamber (MBA96, Neuro Probe Inc, Gaithersburg, Md). Details of the procedure are described in the expanded Materials and Methods section.

Real-Time PCR Analysis of Gene Expression

HUVECs seeded in 6-well plates were stimulated with agonists as indicated in figure legends. Isolation of RNA, reverse transcription, and real-time PCR analysis were performed as described by us previously.²¹ The expression of target molecules was normalized to the expression of β_2 -microglobulin. Primer sequences are available on request.

Ribonuclease Protection Assay

MCF7, Hep 3B, and HeLa cells were treated for 2 or 6 hours with 100 μ g/mL OxPLs in minimum essential medium (MEM) containing 2% FBS. After the extraction of total RNA, different splice variants of VEGF mRNA were quantified by ribonuclease protection assay as described.²²

Small Interfering RNA-Induced Inhibition of Gene Expression

HUVECs were transfected with TLR4 small interfering RNA (siRNA) or control scrambled RNA (both at 100 nmol/L, Stealth siRNA, Invitrogen) using PEI reagent²³ in serum-free medium 199 (M199). After 4 hours, the medium was changed to M199 containing 20% FBS. The cells were stimulated 48 hours after the transfection.

VEGF Immunofluorescence

Immunofluorescent staining of HUVECs for VEGF was performed as described in the expanded Materials and Methods section.

VEGF Enzyme-Linked Immunosorbent Assay

VEGF protein released by cells into culture medium was measured after 24 hours of incubation of cells with 100 μ g/mL of 1-palmitoyl-2-arachidonoyl-*sn*-glycero-3-phosphocholine (OxPAPC) in serum-free medium using ELISA according to the protocol of the manufacturer (R&D Systems, Minneapolis, Minn).

6-Keto-Prostaglandin F_{1 α} Immunoassay

6-Keto-prostaglandin F_{1 α} (6-keto-PGF_{1 α}), which is a stable product of the nonenzymatic hydration of PGL₂, was quantitated in HUVEC culture media using a competitive enzyme immunoassay (R&D Systems). Cross-reactivity of OxPAPC in the assay was less than 0.01%.

Microarray Analysis of mRNA Expression

mRNA from HUVECs treated with OxPAPC was reverse transcribed and hybridized with GEArray Q Series Human Angiogenesis Gene Array (SuperArray Bioscience Corporation, Frederick, Md). Details of the procedure are described in the expanded Materials and Methods section.

Western Blotting

After stimulation as described in the figure legends, cells were lysed in Laemmli buffer and further processed as described by us previously.²⁴

Analysis of Gene Expression in Mouse Carotid Arteries

OxPAPC was mixed with carrier Pluronic F-127 gel and applied to the adventitia of common carotid artery as described by us previously.¹⁵ Six hours later, the animals were euthanized and perfused with PBS via the left ventricle and the treated parts of carotid vessels were removed and analyzed for gene expression.¹⁵

In Vivo Matrigel Plug Assay

Mice were injected subcutaneously near the left/right mid-abdomen with 500 μ L of ice-cold Matrigel with or without 300 μ g/mL OxPAPC or 300 ng/mL VEGF. These experiments were performed as described previously,²⁵ with minor modifications, as described in the expanded Materials and Methods section.

Statistical Analysis

Unless otherwise indicated, all results are expressed as mean \pm SD from triplicate measurements performed in 2 to 4 independent experiments giving similar results. The data were analyzed by 2-tailed Student's *t* test. Statistical significance of differences is indicated in the figures as follows: **P*<0.05, ***P*<0.01, *** *P*<0.001.

Results

OxPLs Stimulate Angiogenesis and EC Migration In Vitro

To test effects of OxPLs on angiogenic reactions of ECs, we used an EC spheroid sprouting assay, which is a well-described in vitro model of angiogenesis.¹⁹ Spheroids formed by HUVECs were embedded into a fibrin gel supplemented with native or oxidized 1-palmitoyl-2-arachidonoyl-*sn*-glycero-3-phosphocholine (PAPC and OxPAPC, respectively¹³). OxPAPC, but not native PAPC, strongly stimulated growth of EC sprouts increasing the total length and number of sprouts, which were also much better defined than in control PAPC-containing gels (Figure 1A). Computer image analysis showed that sprout length, but not sprout number, continued to increase after day 1, suggesting that OxPAPC stimulated elongation, but not sprout branching (Figure 1B and 1C). Similar induction of sprout growth was observed on a cell line derived from microvascular endothelium HMEC-1 (supplemental Figure 1A). Stimulation of sprout formation by OxPAPC was also observed in HUVECs grown on Cytodex-3 beads and embedded into fibrin gel (supplemental Figure 1B). The EC sprouting induced by OxPAPC was more prominent than that induced by VEGF, and was observed in the presence of saturating concentrations of VEGF (Figure 1D and 1E), suggesting that OxPAPC is a strong angiogenic stimulus acting at least partially via VEGF-independent mechanisms.

Stimulation of EC migration is a characteristic property of angiogenic cytokines.²⁶ Therefore, we tested effects of OxPAPC on migration of HUVECs using Boyden chamber assay. In the absence of serum, OxPAPC did not stimulate migration, although it promoted directed migration of HUVECs toward the chamber containing 5% FBS (supplemental Figure 1IA). This result suggests that OxPAPC stimulated general EC motility, rather than induced directed chemotaxis. Nonoxidized phospholipids did not significantly influence cell migration (supplemental Figure 1IB and 1IC).

OxPLs Stimulate Production of VEGF by Endothelial, Blood Mononuclear, Skin, and Tumor Cells

We hypothesized that OxPAPC stimulates production of angiogenic factors, which then would activate HUVECs in an autocrine manner. To test this possibility, we measured expression of key angiogenic cytokines by reverse transcription/real-time PCR (RT-PCR). OxPAPC selectively induced expression of VEGF (Figure 2A). The levels of VEGF mRNA started to increase after 2 hours of treatment (Figure 2B), suggesting that upregulation of VEGF was an early event in OxPAPC-induced angiogenesis. In contrast to VEGF, the levels of fibroblast growth factor-2 (FGF2), platelet-derived growth factor-B (PDGFB), and angiopoietins 1, 2, and 4 mRNA in OxPAPC-treated cells tended to be

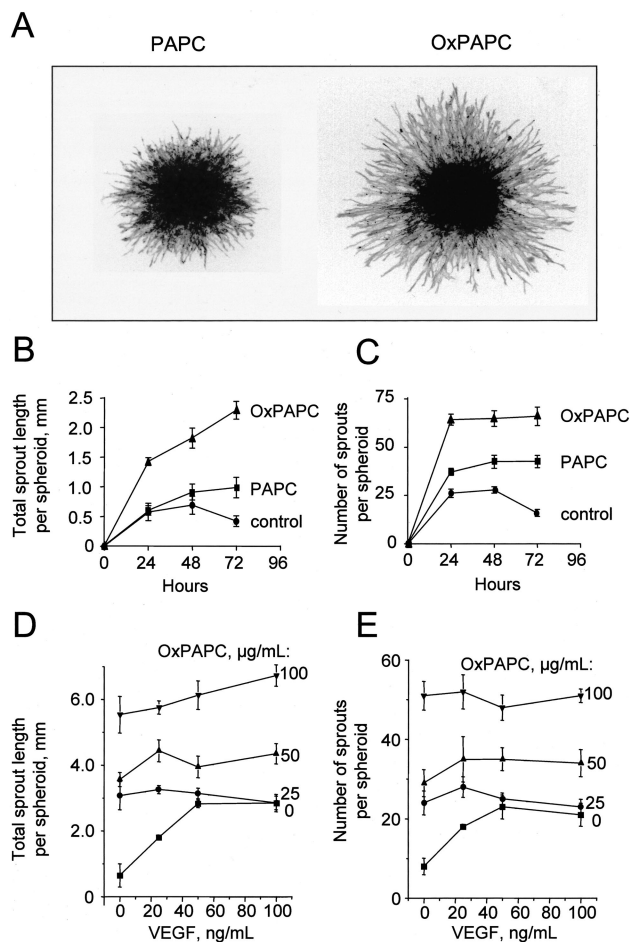


Figure 1. OxPLs stimulate angiogenic response in vitro. A, HUVEC spheroids in fibrin gels were incubated for 72 hours in the presence of medium containing 300 μ g/mL PAPC or OxPAPC and then stained with tetramethyl rhodamine isothiocyanate (TRITC)-conjugated phalloidin. Negative black-and-white images are presented. B and C, Kinetics of sprout outgrowth (length and number) in the presence of 300 μ g/mL PAPC or OxPAPC. D and E, Length and number of sprouts induced by increasing concentrations of VEGF in the presence of indicated concentrations of OxPAPC after 72 hours.

lower than in control (Figure 2A and 2B; supplemental Figure 1IIA and 1IIB). Induction of VEGF expression by OxPAPC was not EC specific, because, in addition to upregulation of VEGF protein in HUVECs (Figure 2C), we also observed elevation of VEGF protein levels in cultures of peripheral blood mononuclear cells and monocyte-derived macrophages exposed to OxPAPC (Figure 2D). Furthermore, OxPAPC upregulated VEGF mRNA in fibroblasts, keratinocytes, lung epithelial cells, and epithelial tumor cell lines of different tissue origin (supplemental Figure 1IVA and 1IVB).

OxPLs Stimulate Production of IL-8, COX-2, and ADAMTS-1

The additivity of the effects of VEGF and OxPAPC (Figure 1D and 1E), suggests that in addition to VEGF, other stimuli may be involved in OxPL-induced angiogenesis. We searched for additional OxPAPC-induced angiogenic stimuli by analysis of mRNA expression using a microarray containing probes for 96 selected human genes modulating the

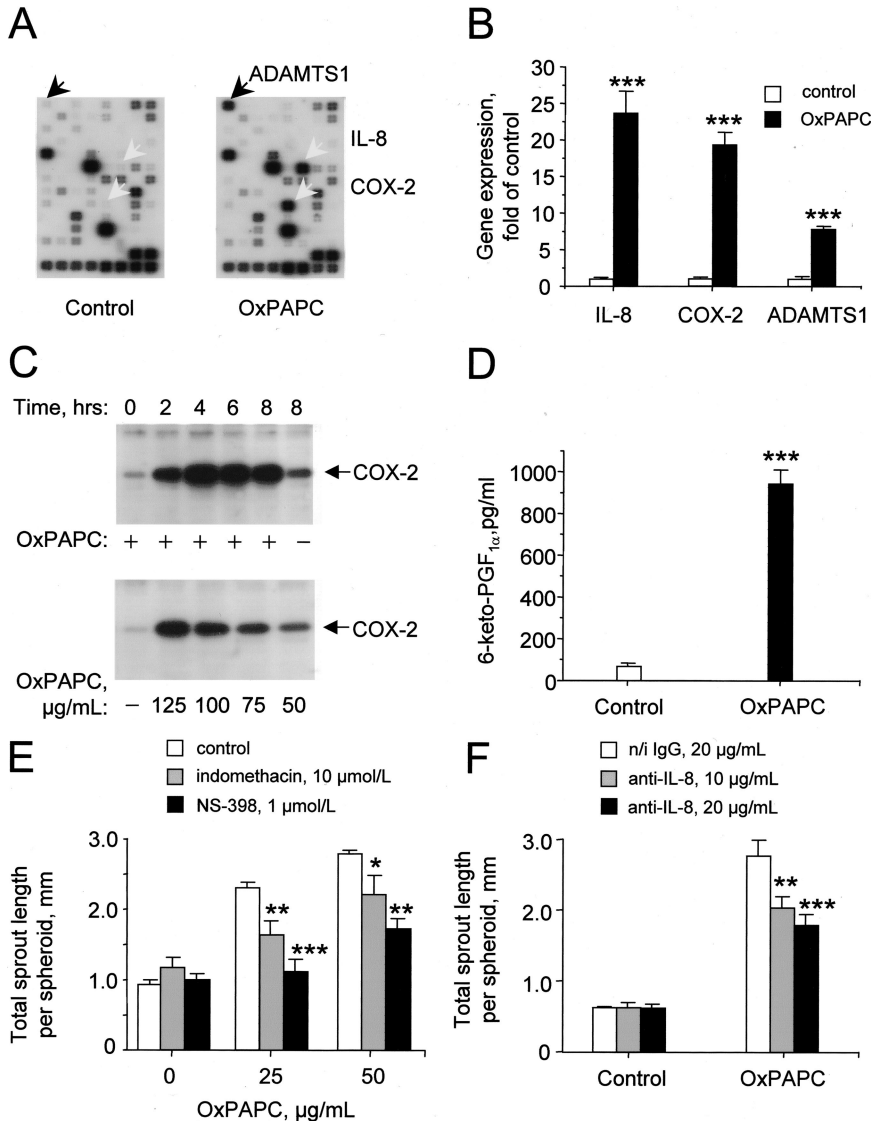


Figure 3. OxPLs stimulate production of additional angiogenic stimuli. A, HUVECs were stimulated with 100 μg/mL OxPAPC for 6 hours or with the medium without OxPAPC (control), and then total RNA was extracted, labeled during the reverse transcription, and hybridized to the Human Angiogenesis Gene Array, followed by chemiluminescent detection. Arrows indicate genes demonstrating the strongest induction by OxPAPC. B, Results from A were confirmed by RT-PCR using RNA from an independent experiment. C, Lysates of HUVECs stimulated for the indicated time with 100 μg/mL OxPAPC (top), or for 6 hours with the indicated concentrations of OxPAPC (bottom), were subjected to the Western blotting analysis using antibodies against COX-2. D, HUVECs were incubated without or with 100 μg/mL OxPAPC for 8 hours, and then concentration of 6-keto-PGF_{1α} in the medium was determined using an ELISA. E and F, Spheroid sprouting assay was performed in gels covered with medium containing 300 μg/mL OxPAPC and indomethacin or NS-398 (E) or blocking antibodies against IL-8 (F). Length of sprouts was estimated after 72 hours.

antioxidant butylated hydroxytoluene did not inhibit OxPAPC-induced upregulation of VEGF (Figure 4C), IL-8, and COX-2 (data not shown). In addition, we tested effects of synthetic POVPC and PGPC,¹³ which are major OxPAPC components that do not contain hydroperoxides or any other groups that would support lipid peroxidation (Figure 4D). Both POVPC and PGPC stimulated production of VEGF, IL-8, COX-2, and ADAMTS-1 (Figure 4E) and promoted sprout formation (Figure 4F). Thus, the ability of OxPLs to induce angiogenic switch does not directly depend on their ability to stimulate lipid peroxidation and generate free radicals.

Several types of receptors have been implicated to mediate the action of OxPLs, including the PAF receptor,³² peroxisome proliferator-activated receptors (PPARs),³³ and Toll-like receptor 4 (TLR4).³⁴ Our data suggest that neither of these receptors was involved in OxPAPC-induced upregulation of VEGF. We found that knockdown of TLR4 using siRNA led to a 90% decrease of TLR4 mRNA expression but did not inhibit OxPAPC-induced upregulation of VEGF (data not shown). Induction of VEGF by OxPAPC was neither

mimicked by PAF used at concentrations saturating the PAF receptor (0.5 μmol/L) nor inhibited by 2 PAF receptor antagonists, CV-3988 or BN 52021 (each at 10 μmol/L; data not shown). Furthermore, neither the PPARα agonist WY-14643 (at 100 and 200 μmol/L) nor the PPARγ agonist rosiglitazone (at 5 and 10 μmol/L) upregulated VEGF mRNA in HUVECs (data not shown).

Oxidized Phospholipids Stimulate Expression of VEGF, IL-8, and COX-2 and Promote Angiogenesis In Vivo

To investigate angiogenic properties of OxPLs in vivo, we first tested whether OxPAPC can stimulate the expression of angiogenesis-related transcripts in mouse vessels. Application of OxPAPC mixed with Pluronic F-127 carrier gel to the adventitial surface of mouse carotid artery¹⁵ resulted in significant upregulation of mRNA levels for VEGF, KC (which is the closest mouse analog of human IL-8), and COX-2 in the vessel wall (Figure 5A).

We next exploited the in vivo Matrigel plug model in mice.²⁵ Addition of OxPAPC to Matrigel stimulated growth

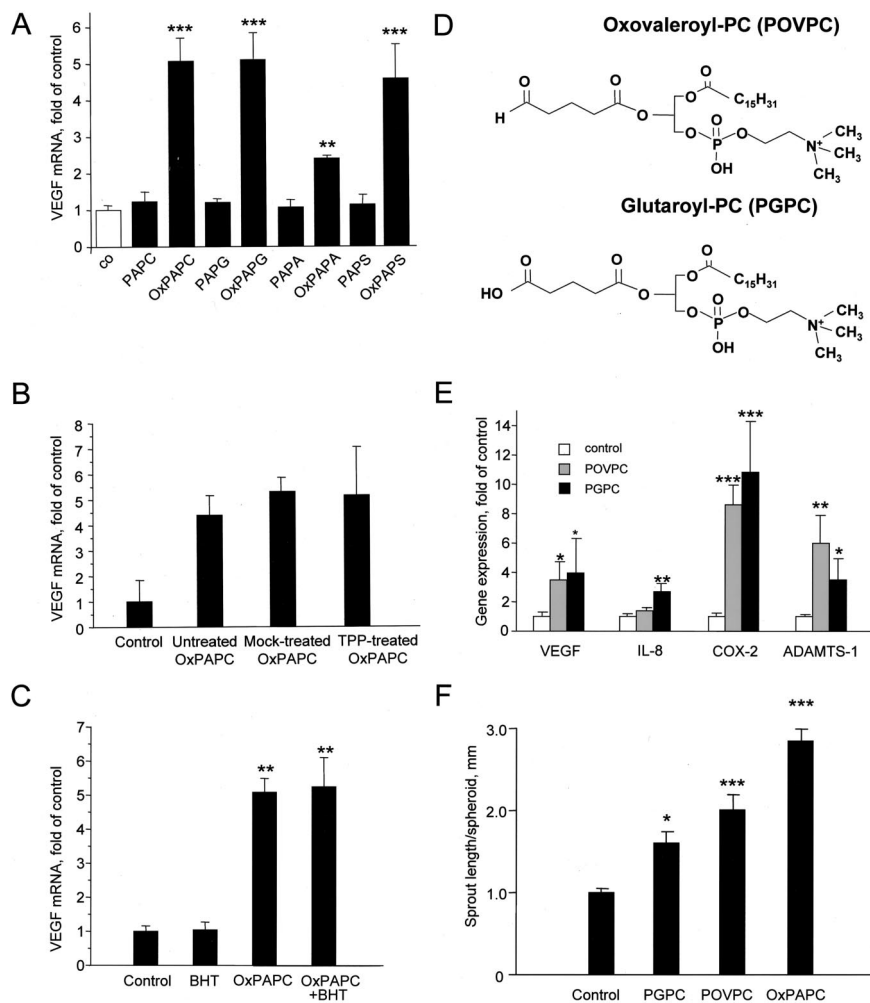


Figure 4. Various OxPL species stimulate generation of angiogenic mediators. A, Expression of VEGF was measured by RT-PCR in HUVECs treated without or with 130 $\mu\text{mol/L}$ of native or oxidized PAPC, phosphatidylglycerol (PAPG), phosphatidic acid (PAPA), and phosphatidylserine (PAPS). This molar concentration corresponds to 100 $\mu\text{g/mL}$ PAPC. B, HUVECs were treated for 6 hours with 130 $\mu\text{mol/L}$ OxPAPC and TPP-treated and mock-treated OxPAPC. C, HUVECs were treated with 100 $\mu\text{g/mL}$ OxPAPC in the presence or absence of 10 $\mu\text{mol/L}$ butylated hydroxytoluene (BHT). Expression of VEGF mRNA was quantitated after 6 hours of incubation. D, Chemical structures of POVPC and PGPC. E, Expression of VEGF, IL-8, COX-2, and ADAMTS-1 was measured by reverse transcription/real-time PCR in HUVECs treated without or with 50 $\mu\text{g/mL}$ of POVPC or PGPC for 6 hours. Expression levels in A, B, C, and E are normalized to β_2 -microglobulin. F, PGPC, POVPC, and OxPAPC (all at 50 $\mu\text{g/mL}$) were tested by HUVEC spheroid sprouting assay. Sprout length was measured after 72 hours.

of new vessels from preexisting skin vessels into the Matrigel plug (Figure 5B and 5C). In the deep layers of the plug, we frequently observed hemorrhages, which were not evident in control plugs, thus suggesting that the newly formed vessels are prone to rupture (Figure 5C). Staining for cell nuclei demonstrated the presence of cells in deep layers of OxPAPC-containing plugs, whereas the internal layers of controls plugs were essentially cell free (Figure 5D and 5E). In Matrigel plugs containing OxPAPC, some of the invading ECs formed vessel-like structures, as illustrated by the presence of CD31-positive strands (Figure 5D, inset). Quantification of angiogenesis using hemoglobin assay showed elevated levels of hemoglobin in OxPAPC-containing plugs as compared with control plugs (Figure 5F). Taken together, the data presented in Figure 5 demonstrate that OxPAPC stimulated production of angiogenic stimuli and promoted angiogenesis *in vivo*.

Discussion

The major finding in our studies is that major classes of phospholipids after oxidation stimulate production of angiogenic stimuli by ECs, monocytes/macrophages, and other cell types, which consequently induce EC migration and sprouting *in vitro*, and formation of new blood vessels *in vivo*. Concentrations of OxPLs in lesions are comparable to those

inducing angiogenic effects in our experiments (Berliner and Watson¹³ and Figure 1D and 1E). Therefore, OxPLs might represent microenvironmental angiogenic stimuli that accumulate within atheroma. Different classes of phospholipids upregulated VEGF, suggesting that general phospholipid structure and especially an oxidized *sn*-2 residue, rather than particular head group, determines the angiogenic activity on oxidation. Our findings correlate well with the ability of oxidant stress to stimulate angiogenesis in carotid arteries of mice overexpressing NAD(P)H oxidase.³⁰ However, the data show that reactive oxygen species and OxPLs induce angiogenesis via different mechanisms because even peroxidation-inert species of OxPLs such as POVPC and PGPC stimulate VEGF production and EC sprouting.

Our data indicate that multiple autocrine loops are major mechanisms of the angiogenic activity of OxPLs. Although characterization of the full spectrum of angiogenic stimuli induced by OxPLs was beyond the scope of the study, we found that OxPLs strongly upregulated at least 3 well-characterized inducers of angiogenesis (VEGF, IL-8 and COX-2), both in cultured cells and in mouse carotid artery. Other angiogenic cytokines necessary for correct growth and maturation of neovessels, such as FGF2, PDGFB, and angiopoietin-1, were not induced but, rather, suppressed by OxPAPC, suggesting that capillaries induced by OxPLs

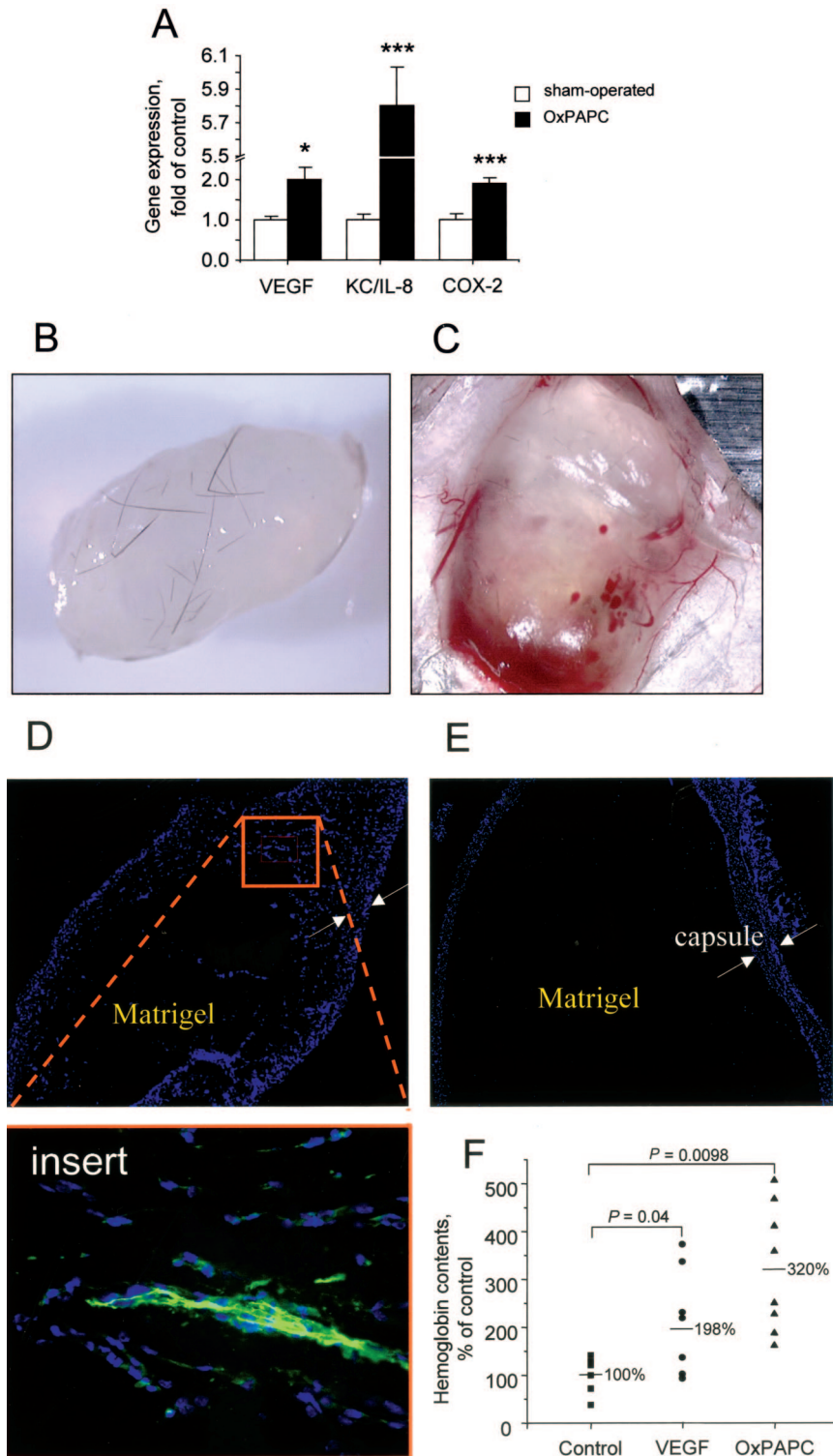


Figure 5. OxPLs upregulate angiogenic proteins and stimulate angiogenesis in vivo. A, OxPAPC mixed with carrier Pluronic F-127 gel were applied onto the adventitial surface of common carotid artery in mice. Sham-operated animals received Pluronic without the OxPAPC. Six hours later, the animals were euthanized and expression of indicated mRNAs was measured in treated parts of the vessels. B, Macroscopic view of a representative control Matrigel plug on the day 12 after injection into mice. C, In situ view of a plug containing 300 µg/mL OxPAPC. The image was produced before extraction of the plug to illustrate growth of new vessels from the preexisting skin vessels into the gel. Note the presence of multiple intraplug hemorrhages. D and E, Staining of cryosections of OxPAPC-containing (D) and control (E) Matrigel plugs with Hoechst 3342 to visualize the nuclei (blue). Note that in addition to capsule cells, multiple cells are present inside of OxPAPC-containing, but not control, plugs. Insert shows combination of Hoechst 3342 (blue) and CD31 (green) staining, demonstrating the presence of vessel-like structures in OxPAPC-containing plugs. F, Matrigel plugs were homogenized in cyanide free hemoglobin reagent, and the amount of plug-associated hemoglobin was quantitated by measuring optical density of the solution as described in Materials and Methods. The data are combined from 2 independent experiments, each including 4 animals. Morphologically visible stimulation of angiogenesis was observed in 2 additional independent experiments.

might be functionally impaired. These results are in good agreement with previous data showing that oxidized LDL inhibits production of FGF2 by ECs.³⁵ Altogether, these data allow the supposition that OxPL-induced angiogenesis is different from compensatory hypoxia-driven angiogenesis activated at sites of insufficient blood supply.

We also found that OxPLs induced COX-2, an enzyme intimately involved in angiogenesis caused by the ability of prostanoids to induce EC sprouting, migration, tube forma-

tion, and other effects characteristic of neovascularization.³⁶ Furthermore, OxPLs upregulated IL-8 (Subbanagounder et al¹⁶ and this work), known to possess angiogenic activity.²⁸ Selective inhibition of COX-2 or IL-8 action partially suppressed angiogenic effects of OxPLs. Our data demonstrating additive effects of VEGF and OxPAPC suggest that maximal angiogenic stimulation by OxPLs is achieved when multiple autocrine stimuli act in concert. Potential in vivo relevance of VEGF, COX-2, and IL-8 for plaque angiogenesis is sup-

ported by data demonstrating enhanced expression of VEGF,³⁷ COX-2,³⁸ and IL-8³⁹ in atherosclerotic vessels.

An additional mechanism of the angiogenic action of OxPLs may be based on their ability to directly activate signal-transducing systems coupled to angiogenesis. It has been shown that OxPLs stimulate intracellular signaling mechanisms known to be activated by classical angiogenic cytokines. In particular, OxPLs stimulate phosphatidylinositol 3-kinase, protein kinase C, and extracellular signal-regulated kinases (ERKs) and activate early growth response factor-1 (EGR-1) and NFAT-mediated transcription.^{24,40} These events are known to be important for angiogenesis induced by VEGF and other growth factors.^{20,41–43}

Our findings indicate that OxPLs stimulate VEGF expression in a variety of cell types, including skin and lung cells, which are often exposed to the oxidative stress induced by UV irradiation and oxidizing air pollutants such as ozone. Furthermore, we found that OxPLs induced VEGF in epithelial tumor cell lines of different tissue origin, raising a possibility that OxPLs act within the tumor milieu as an angiogenic stimulus generated by oxidative stress accompanying antitumor cytotoxic reactions and apoptosis. These data suggest that the angiogenic properties of OxPLs may also be relevant in a variety of pathological processes other than atherosclerosis, including tumor angiogenesis.

In addition to its key role in angiogenesis, VEGF is known to stimulate migration of monocytes via Flt-1 receptors⁴⁴ and also to promote atherogenesis after systemic injection into mice.⁹ Thus, VEGF represents an additional previously unrecognized cytokine mediator of inflammatory and atherogenic action of OxPLs. This possibility is supported by data showing that anti-Flt-1 suppressed inflammatory infiltration and growth of atherosclerotic lesions.⁴⁵

We show for the first time that OxPLs upregulate expression of ADAMTS-1, a metalloprotease secreted by cells and cleaving matrix proteoglycans.⁴⁶ ADAMTS-1 is known to be upregulated in the intima of atherosclerotic vessels and to enhance intimal hyperplasia in the mouse carotid artery ligation model, probably as a result of its ability to stimulate migration of vascular smooth muscle cells as a result of versican cleavage.⁴⁷ Thus, it was hypothesized that the general role of ADAMTS-1 is proatherogenic,⁴⁷ and therefore induction of ADAMTS-1 by OxPLs might contribute to growth and proteolytic destabilization of plaques.

To summarize, our data show that OxPLs upregulate angiogenic cytokines and enzymes, such as VEGF, IL-8, and COX-2. Furthermore, OxPLs stimulate angiogenesis in vitro and in vivo. Previously, we have shown that in mouse vessels OxPLs also induce a set of proinflammatory genes that lead to leukocyte accumulation.¹⁵ Thus, OxPLs may play a prominent role in the evolution of atheroma by stimulating formation of neovessels and production of leukocyte chemoattractants, together leading to the accumulation of inflammatory cells and growth and destabilization of atherosclerotic plaques. Furthermore, plaque destabilization might be further exacerbated because of the ability of OxPLs to stimulate production of metalloproteases such as ADAMTS-1. Altogether, the results presented herein suggest a novel mechanism of atherogenic action of OxPLs dependent on their

angiogenic properties and implicate OxPLs in destabilization of atherosclerotic plaques.

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Disclosures

None.

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