Inhibition of endothelium-dependent arterial relaxation by oxidized phosphatidylcholine

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Abstract

Formation of oxidized phosphatidylcholine (ox-PC), oxidatively fragmented phosphatidylcholine (PC) containing a short-chain and/or polar oxidative residue at the sn-2 position, in the process of LDL oxidation as well as its existence in atherosclerotic lesions has been demonstrated. To clarify the pathophysiological role of ox-PC in the vascular reactivity, we investigated the effects of various ox-PCs on the isometric tensions in rabbit thoracic aortas. Ox-PCs, which were produced upon oxidation of sn-2 polyunsaturated fatty acid (PUFA)-containing PCs, dose-dependently inhibited endothelium-dependent relaxation (EDR) evoked by acetylcholine or substance P. On the other hand, neither native PUFA-containing PCs nor an oxidative product of monounsaturated fatty acid-containing PC showed an inhibitory effect. None of ox-PCs affected endothelium-independent relaxation to nitroglycerin. The PC-headgroup fraction, but not the oxidized fatty acids fraction, was responsible for the inhibition of EDR by ox-PC. EDR was reduced by 2-(5-oxovaleroyl)-PC, one of the secondary oxidative products of PCs that contains a short chain aldehydic residue at the sn-2 position, but not by PC hydroperoxide, the primary oxidative product. Although the possibility could not be completely ruled out that lysophosphatidylcholine rather than ox-PC may be responsible for inhibitory effects on EDR, these results suggest a novel vascular activity of ox-PCs generated from sn-2 PUFA-containing PCs which may be implicated in the pathophysiology of vascular tone. © 2000 Elsevier Science Ireland Ltd. All rights reserved.

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1. Introduction

The vascular endothelium releases nitric oxide (NO), which is considered as an endothelium-derived relaxing factor, and plays a key role in the regulation of vascular tone [1]. NO is considered as an antiatherogenic molecule because NO has bioactivities such as inhibitions of monocyte adhesion to endothelium [2], platelet aggregation [3], and smooth muscle cell proliferation [4]. In atherosclerosis and hypercholesterolemia, endothelial dysfunction including decreased NO release (reviewed in Ref. [5]) and NO inactivation via increases in superoxide production [6,7] has been demonstrated. The impairment of endothelium-dependent relaxation (EDR) associated with reduced NO activity may facilitate the atherogenic process.

Oxidized LDL has been implicated as a critical factor in atherosclerosis [8], and its presence in atherosclerotic lesions has been demonstrated [9]. Oxidative modification of LDL results in the conversion of phosphatidylcholine (PC) to lysophosphatidylcholine (LPC), which acts as an important mediator of the atherogenic effects of oxidized LDL, by intrinsic phospholipase A2 (PLA2) activity [10,11]. Oxidized LDL and LPC specifically interact with endothelial cells and promote alterations of a variety of endothelial functions including induction of gene expression of cytokines, adhesion molecules and growth factors [12–14]. Furthermore, we [15,16] and others [17,18] have clearly shown that oxidized LDL and LPC inhibit EDR. These observations suggest that oxidized LDL and LPC play a role in endothelial dysfunction in atherosclerotic arteries.
Most fatty acids exist in phospholipids and cholesterol esters in LDL. The first step in oxidative process of LDL is the formation of monohydroperoxy derivatives of fatty acids [19,20]. Peroxidation of fatty acids binding to PCs has been demonstrated in LDL oxidation, and hydroxy fatty acids and oxysterols have been found in human atheroma [21] and aortas of cholesterol-fed rabbits [22]. In particular, the polyunsaturated fatty acids (PUFAs) in phospholipids and cholesterol esters may be the initial sites of oxidative attack during modification of the LDL particle [23,24], and oxidation of PUFAs results in the alteration to short chain fatty acids. Recently, oxidized PC (ox-PC), oxidatively degraded PCs containing short chain fatty acids which are formed from PUFA as a result of oxidative fragmentation, has been identified in oxidized LDL [25] and human coronary atherosclerotic lesions by means of a monoclonal antibody against ox-PC [26]. Although ox-PC has been shown to have a variety of bioactivities such as cytotoxicity [27], hypotensive effect [28,29], and platelet-activating factor (PAF)-like effects to stimulate platelets aggregation [30], neutrophil adhesion [31], and vascular smooth muscle cell proliferation [25] through the PAF receptor, the effect of ox-PC on vasomotor function of endothelium including EDR remains unknown. In the present study, we demonstrate that ox-PC inhibits EDR of rabbit thoracic aortas. This is the first report that has investigated the vascular reactivity of ox-PC and gives us a new insight to clarify the mechanism by which EDR is reduced in atherosclerotic arteries.

2. Materials and methods

2.1. Materials

1-Palmitoyl-2-linoleoyl-sn-glycero-3-phosphocholine (linoleoyl PC) and 1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphocholine (arachidonoyl PC) were purchased from Avanti Polar Lipids (Alabaster, AL). 1-Palmitoyl-2-oleoyl-sn-glycerol-3-phosphocholine (oleoyl PC) was from Sigma (St Louis, MO). All other chemicals were the best grade available from commercial sources.

2.2. Peroxidation of PCs

PCs were peroxidized by the method previously described [26,32]. Briefly, oleoyl PC, linoleoyl PC and arachidonoyl PC in a mixture of chloroform and methanol were placed in glass tubes and solvents were dried under a stream of N2 gas. Each PC was suspended in phosphate-buffered saline (PBS) with a vortex mixer and the suspension was sonicated in a probe type sonicator for 30 s to make a 0.4-mM suspension. Each PC in PBS was incubated with 40 μM FeSO4 and 0.4 mM ascorbic acid for 3 h at 37°C and the reaction was stopped by butylated hydroxytoluene (final 0.2 mM). Ox-PCs in PBS were diluted with Krebs bicarbonate buffer to make 1–20-μM suspension, added into organ baths and used for EDR experiment. To clarify the profiles of ox-PCs, we subjected the lipid extracts from oxidative product of arachidonoyl PC (ox-arachidonoyl PC) to thin-layer chromatography (TLC) (silica gel G; Merck, Darmstadt, Germany) as described previously [34]. Ox-PC showed a broad spot corresponding between native arachidonoyl PC and the origin, which represented a significantly different pattern from a narrow single spot of native arachidonoyl PC or LPC (data not shown). TLC showed that there was only a weak zone in the LPC-corresponding area, suggesting that only a small amount of LPC was produced in ox-PC products. In some experiments, the lipids were extracted from the LPC-corresponding zone by the method of Folch et al. [35] and tested on EDR. Ox-PC was generated on the day of measurement of EDR and immediately used in the experiments.

2.3. Fractionation of ox-PC by column chromatography

To further investigate whether PCs were responsible for the inhibitory effect of oxidative products of PUFA-containing PCs, we fractionated ox-arachidonoyl PC into the PC-headgroup fraction and the other oxidized fatty acids fraction by silica gel column chromatography by the modified method of Hanahan et al. [36]. Total lipids of ox-arachidonoyl PC were extracted by the method of Bligh and Dyer [33] and the chloroform layer was evaporated under a stream of N2 gas. The extract was dissolved by a small amount of chloroform and applied to a silica gel column. The column was eluted sequentially three times with a column volume of chloroform (oxidized fatty acids fraction) and then three times with a column volume of chloroform/methanol, 1:3 (v/v) (PC-headgroup fraction). After drying under a stream of N2 gas, lipids were redissolved in Krebs bicarbonate buffer and tested on EDR.

2.4. Synthesis of PC hydroperoxide (PC-OOH)

PC hydroperoxide (PC-OOH) was produced by incubation of arachidonoyl PC with soybean lipooxygenase in 10 mM deoxycholate at 37°C for 15 min [37]. The reaction mixture was applied to a Sep-pak column (Waters Associates, Milford, MA) and first eluted by water and successively by methanol. The same volume of water and chloroform was added to the methanol eluate and the reaction mixture was centrifuged (1500 rpm × 10 min). The chloroform layer was recovered and evaporated under a stream of N2 gas. PC-OOH...
was purified by TLC with a mixture of chloroform, methanol and water (5:10:0.5, v/v/v) as the mobile phase and extracted by the method of Bligh and Dyer [33].

2.5. Synthesis of ox-PC containing aldehydic residue (PC-5CHO)

1-Palmitoyl-2-(5-oxovaleryl)-sn-glycero-3-phosphocholine (2-(5-oxovaleryl)-PC, PC-5CHO), one of the ox-PCs that contains aldehyde residues at the sn-2 position, was prepared by ozonolysis as described previously [38]. Briefly, arachidonoyl PC was ozonized and a reducing work-up with dimethyl sulfide performed. Aldehydic PC was purified by TLC with a mixture of chloroform, methanol and water (10:5:1, v/v/v) as the mobile phase. The production of PC-5CHO was quantitated by fluorometric HPLC [38]. PC-5CHO purified by TLC was reacted with a fluorescent reagent, 4-((N,N-dimethylaminosulfonyl)-7-hydrazino-2,1,3-benzoxadiazole (Tokyo Kasei Kogyo, Tokyo, Japan). The fluorescent derivatives of aldehydic PC were fractionated and quantitated by reversed-phase HPLC. The ozonolysis was complete in our experiments, and the purity of PC-5CHO was 100%. There were no contaminations such as C8, C11, and C14 derivatives.

2.6. Measurement of EDR

Strip rings were prepared from rabbits’ aortas as described previously [39], and all procedures were conducted according to the ‘Guidelines for Animal Experiments at Kobe University School of Medicine’. Japanese white rabbits (2.0–2.5 kg) were anesthetized with pentobarbital sodium (30 mg/kg body wt i.v.), and the descending thoracic aortas were isolated and cleaned of surrounding tissue. Aortic rings approximately 2-mm wide were cut and opened. Care was taken not to damage the endothelial surface. For isometric force measurements, transverse aortic strips were suspended in 30-ml organ baths containing Kreb’s bicarbonate buffer of following composition (mM): NaCl 118, KCl 4.0, CaCl2 1.5, MgSO4 1.2, NaH2PO4 1.2, NaHCO3 25 and glucose 5, and equilibrated at 37°C with a 95% O2-5% CO2 gas mixture. One end of the strip was attached to the bottom of the chamber, and the other end was attached to a transducer (Nihon-Kohden, Tokyo, Japan), which was connected to an amplifier (Nihon-Kohden)/recorder (Nippon Denshi Kagaku, Kyoto, Japan) system. An initial preload of 1.5 g was applied, and the strips were allowed to stabilize for 90 min. A test contraction was induced by raising KCl concentration to 40 mM. When the developed tension attained its peak value, the strips were relaxed by rinsing with Krebs buffer. Then the strips were contracted with phenylephrine (PE, 0.3 μM) and subsequently relaxed by cumulative additions of acetylcholine (ACh, final concentration 1 nM–3 μM), substance P (SP, 10 pM–10 nM), or nitroglycerin (NTG, 0.1 nM–3 μM). After wash-out and equilibration, the strips were incubated with selected concentrations of ox-PC for 30 min, and then the contraction-relaxation cycle was repeated as above. In all the experiments, butylated hydroxytoluene was added in a buffer (final 10 μM). In certain experiments, the endothelium was removed mechanically by rubbing the intimal surface with filter paper moistened with the buffer. Relaxation values were expressed as percent decreases of the PE-induced constrictor tone.

2.7. Statistical analysis

Data are expressed as mean ± S.E.M. The significance of the difference between group means was analyzed by one-way ANOVA and the Bonferroni test for samples. Values of P < 0.05 were considered statistically significant.

3. Results

3.1. Effects of ox-PCs on ACh-induced EDR

Representative tracings of responses to ACh in rabbit aortic strips incubated with or without ox-PCs are shown in Fig. 1A. We confirmed that ox-PCs by themselves altered neither the resting tension nor PE-elicited contraction (1.48 ± 0.05 vs. 1.47 ± 0.03 g, mean ± S.E.M., n = 6, before versus after exposure to 20 μM ox-arachidonoyl PC). The tracing showed reduced relaxation to ACh after incubation with ox-linoleoyl PC (center), but not with native linoleoyl-PC (right). As shown in Fig. 1B, the inhibitory effects of oxidative products of PUFA-containing PCs were reversible, as wash-out of oxidative products of PUFA-containing PCs with Krebs buffer containing 0.1% bovine serum albumin restored ACh-induced EDR (maximum relaxation was 73.8 ± 2.0 vs. 63.7 ± 3.2%, mean ± S.E.M., n = 5, before exposure to ox-PCs versus after wash-out), indicating that the functional integrity of endothelial was preserved after exposure to ox-PCs.

The incubation of the strips with oxidative residues of PUFA-containing PCs, namely ox-linoleoyl PC (Fig. 2A) and ox-arachidonoyl PC (Fig. 2B), significantly inhibited EDR to ACh in a dose-dependent manner. Relaxation to ACh (1 μM) was 71.1 ± 3.2% in control, whereas it was reduced to 22.1 ± 3.4 and 14.5 ± 2.9% by ox-linoleoyl PC and ox-arachidonoyl PC (mean ± S.E.M., n = 6, P < 0.001), respectively. However, the incubation of the strips with ox-oleoyl PC (Fig. 2C) did not show significant effect. These results indicate that oxidative products of PUFA-containing PCs, but not of...
Fig. 1. (A) Representative tracings of responses to ACh in rabbit aortic strips incubated with or without ox-PC. The strips were contracted with phenylephrine (0.3 µM), and subsequently EDR was induced by cumulative additions of ACh (1 nM–3 µM). In comparison with control (left), the strip showed reduced relaxation to ACh after the incubation with 20 µM ox-linoleoyl PC (center), but not with 20 µM native linoleoyl PC (right). (B) Representative tracings of responses to ACh in rabbit aortic strips incubated with or after wash-out of ox-PC. After the contraction-relaxation cycle (left), the strip was incubated with 20 µM ox-arachidonoyl PC (center), and then the contraction-relaxation cycle was repeated. After extensive washing of the strip with Krebs buffer containing 0.1% bovine serum albumin, the contraction-relaxation cycle was repeated (right). PE, phenylephrine; W, wash-out.

Fig. 2. Effects of oxidative products of sn-2 PUFA-containing PCs, sn-2 saturated fatty acid-containing PCs, or monounsaturated fatty acid-containing PCs on ACh-induced EDR. Rabbit aortic strips were incubated either with ox-linoleoyl PC (A), ox-arachidonoyl PC (B), or ox-oleoyl PC (C) at concentrations of 1 µM (open square), 10 µM (open triangle) or 20 µM (open circle), or without PC (control, closed circle) for 30 min in Krebs buffer. The strips were contracted with 0.3 µM PE, and subsequently EDR was induced by cumulative additions of ACh (1 nM–3 µM). Symbols represent the mean percent of relaxation ± S.E.M. for six independent experiments. Data were analyzed using one-way ANOVA: * P < 0.05, † P < 0.01, § P < 0.005, // P < 0.001 versus control.
Fig. 3. Effects of ox-PCs on SP-induced EDR. Rabbit aortic strips were incubated with 20 μM ox-oleoyl PC (open triangle), ox-linoleoyl PC (open circle), or ox-arachidonoyl PC (open square), or without PC (control, closed circle) for 30 min in Krebs buffer. The strips were contracted with 0.3 μM PE, and subsequently EDR was induced by cumulative additions of SP (10 pM–10 nM). Symbols represent the mean percent of relaxation ± S.E.M. (n = 6–8). Data were analyzed using one-way ANOVA: *P < 0.01, ‡P < 0.001 versus control.

Fig. 4. Effects of oxidative products of PUFA-containing PCs on endothelium-independent relaxation to NTG. Rabbit aortic strips whose endothelium was removed, were preincubated with 20 μM ox-linoleoyl PC (open square), or ox-arachidonoyl PC (open triangle), or without PC (control, closed circle) for 30 min in Krebs buffer. The strips were contracted with 0.3 μM PE, and subsequently endothelium-independent relaxation was induced by cumulative additions of NTG (0.1 nM–3 μM). Symbols represent the mean percent of relaxation ± S.E.M. for five independent experiments. Data were analyzed using one-way ANOVA: P = NS versus control.

monounsaturated fatty acid-containing PC, have an inhibitory effect on EDR.

3.2. Effects of ox-PCs on SP-induced EDR

As shown in Fig. 3, arterial relaxations elicited by another endothelium-dependent relaxant, SP, were attenuated by 20 μM oxidative products of PUFA-containing PCs. Relaxation to SP (10 nM) was 42.3 ± 3.7% in control, whereas it was reduced to 26.2 ± 1.9 and 22.8 ± 4.0% by ox-linoleoyl PC (mean ± S.E.M., n = 8, P < 0.01) and ox-arachidonoyl PC (mean ± S.E.M., n = 8, P < 0.001), respectively. In contrast, SP-induced relaxations were not impaired after exposure to 20 μM ox-oleoyl PC.

3.3. Effects of ox-PCs on endothelium-independent relaxation to NTG

In an additional series of experiments, to determine whether the dilator capacity of smooth muscle was altered after exposure to ox-PCs, we studied the effects of oxidative products of PUFA-containing PCs on endothelium-independent relaxation induced by NTG (0.1 nM–3 μM). In these series of experiments, the endothelium was removed mechanically by rubbing the intimal surface with filter paper moistened with the buffer. As shown in Fig. 4, NTG-induced relaxation was not impaired in arteries after incubation with oxidative products of PUFA-containing PCs, indicating that the dilator function of smooth muscle was not influenced by ox-PCs.

3.4. Effects of fractions of oxidative product of arachidonoyl PC on ACh-induced EDR

During oxidation of PC, a PUFA at the sn-2 position of PC is degraded to a short-chain fatty acid, resulting in the production of oxidized free fatty acids which are known to have some bioactivity. For example, F₂-isoprostanes, a family of prostaglandins produced by free radical-catalyzed peroxidation of arachidonoyl PC, have been reported to act as a vasoconstrictor [40]. Therefore, to examine whether degraded PCs or oxidized fatty acids were responsible for the inhibitory effects of oxidative products of PUFA-containing PCs, we fractionated ox-arachidonoyl PC into the PC-headgroup fraction and the other oxidized fatty acids fraction by silica gel column chromatography. As shown in Fig. 5, incubation with the PC-headgroup fraction, but not with the oxidized fatty acids fraction, resulted in the inhibition of EDR.

3.5. Effects of PC-OOH and PC-5CHO on ACh-induced EDR

Peroxidation of PUFA-containing PCs generates PC hydroperoxide, a primary oxidative product of PC [41]. Oxidative breakdown of PC hydroperoxide provides various ox-PCs that contain short-chain carbonyl, alde-
Fig. 5. Effects of fractions of ox-arachidonoyl PC on ACh-induced EDR. Ox-arachidonoyl PC was subjected to silica gel column chromatography and fractionated into the PC-headgroup fraction and the oxidized fatty acids fraction as described in Section 2. After incubation with the PC-headgroup fraction or the oxidized fatty acids fraction, the strips were contracted with 0.3 \( \mu \)M PE, and subsequently EDR was induced by cumulative additions of ACh (1 nM–3 \( \mu \)M). In comparison with control (left), representative tracings showed reduced relaxation to ACh after incubation with the PC-headgroup fraction (center), but not with the oxidized fatty acids fraction (right) of ox-arachidonoyl PC. PE, phenylephrine; W, wash-out.

hydic, or carboxyl moieties as secondary products. To further investigate whether the inhibition of EDR by ox-PCs is caused by the primary oxidative product or the secondary short-chain ox-PC, we tested the effects of incubations of the strips with PC-5OOH and PC-5CHO on EDR evoked by ACh. PC-5CHO (2-(5-oxo-valeroyl)-PC), which contains a short chain aldehydic residues at the sn-2 position and has been reported to exist in ox-arachidonoyl PC [25,30,31], reduced EDR (Fig. 6), however, PC-OOH did not inhibit EDR. These results demonstrated for the first time that ox-PC containing sn-2 short chain fatty acid, a secondary oxidative product, impaired EDR, whereas EDR was not changed by PC-OOH, a primary product. This result suggests that the secondary oxidative products including aldehydic ox-PCs rather than PC hydroperoxide, the primary product, may play a role in the inhibition of EDR by oxidized PUFA-containing PCs.

4. Discussion

The present study demonstrates for the first time that oxidative products of sn-2 PUFA-containing PCs, namely ox-linoleoyl PC and ox-arachidonoyl PC, inhibit EDR in rabbit aortic strips. However, EDR was reduced by neither ox-oleoyl PC nor native PCs. In addition, this inhibitory effect of oxidative products of PUFA-containing PCs was caused by the lipids in PC fraction, but not oxidized fatty acids or LPC existing in the oxidative products of PUFA-containing PCs in our experiments. Moreover, ox-PC with an sn-2 short chain aldehyde, but not PC hydroperoxide, attenuated EDR. Therefore, we conclude that oxidatively degraded PCs reduce EDR and may contribute to the impaired vaso-motion in atherosclerotic arteries.

During oxidative modification of LDL, PUFA-containing PCs change to PC hydroperoxide, a primary product, and further oxidative modification causes the alteration of PUFAs to short chain fatty acids. This modification results in formation of four kinds of PCs with a short-chain dicarboxylate, dicarboxylate semi-aldehyde, monocarboxylate or \( \omega \)-hydroxymonocarboxylate moiety, as secondary oxidative products [34]. For instance, previous reports showed that 2-azelaoyl PC [27], an sn-2 dicarboxylate group, is generated from 2-linoleoyl PC, and 2-(4-hydroxynonenal)PC [42] and 2-(5-oxovaleroyl)PC [43–45] are generated from 2-arachidonoyl PC. 2-(5-oxovaleroyl)PC (PC-5CHO), used in our experiment as a secondary oxidative product, has been reported to exist in oxidized LDL [25] and to have atherogenic effects to stimulate platelets aggregation [30], neutrophil adhesion [31], and vascular smooth muscle cell proliferation [25]. Hörrkō et al. [46] have recently demonstrated that 2-(5-oxo-valeroyl)PC is an important ligand for the recognition of oxidized LDL by macrophages. Watson et al. [47] have recently reported that 2-(5-oxovaleroyl)PC is a bioactive phospholipid in minimally modified LDL and

Fig. 6. Effects of PC-OOH and PC-5CHO on ACh-induced EDR. PC-OOH and PC-5CHO were prepared as described in Section 2. Rabbit aortic strips were incubated with 20 \( \mu \)M PC-OOH (open square), PC-5CHO (open triangle), or without PC (control, closed circle) for 30 min in Krebs buffer. Relaxations were assessed as described above. Symbols represent the mean percent of relaxation ± S.E.M. (n = 5). Data were analyzed using one-way ANOVA: * \( P < 0.01 \), ‡ \( P < 0.001 \) versus control.
is identified in aortas of atherogenic diet-fed rabbits. In the present study, oxidative products of sn-2 PUFA-containing PCs as well as PC-5CHO inhibited EDR, whereas neither native PUFA-containing PCs nor PC-OOH, a primary oxidative product, reduced EDR. We also confirmed that the presence of PC-5CHO in ox-arachidonoyl PC by fluorometric HPLC (data not shown), suggesting that PUFAs at the sn-2 position changed to short chain residues during oxidative modification of PCs. However, PC-5CHO occupies a relatively small constituent of ox-arachidonoyl PC products. In the present study, ox-arachidonoyl PC is more potent than PC-5CHO (Fig. 2B vs. Fig. 6). Since ox-arachidonoyl PC contains diverse degraded PCs other than PC-5CHO, this result suggests that oxidatively degraded products which are more potent than PC-5CHO may be generated in ox-arachidonoyl PC. In addition, it is also suggested that these degraded PCs may also have weak inhibitory effects individually, but they may additionally or synergistically augment the effect of PC-5CHO or themselves. Because oxidatively modified PCs besides PC-5CHO may have diverse profound effects on vascular endothelium, it is unlikely that only PC-5CHO is responsible for the inhibitory effects of ox-PCs. Therefore, although PC-5CHO is likely to be a participant in reduced EDR by oxidized sn-2 PUFA-containing PCs, other secondary oxidative products including PCs with a short-chain dicarboxylate or monocarboxylate may also play a role in the ox-PC-induced inhibition of EDR. The reason why ox-oleoyl PC did not inhibit EDR may be due to the low rate of conversion of a monounsaturated fatty acid to a short chain residue during oxidative modification in our experiment.

We consider that LPC is unlikely to be responsible for the impaired EDR by oxidative products of PUFA-containing PCs because only a small amount of LPC seemed to exist in the oxidative products of PUFA-containing PCs and the LPC fraction obtained after TLC of ox-arachidonoyl PC did not inhibit ACh-induced EDR (data not shown). However, the possibility that ox-PCs may be hydrolyzed to LPC by PLA2 or PAF acetylhydrolase activities of the endothelium in situ could not be excluded in the present study.

Several mechanisms by which ox-PC inhibited EDR are considered. It may be possible that peroxy radicals or other reactive oxygen intermediates are induced by ox-PCs in endothelial cells. However, n-acetylcysteine (1 mM), an antioxidant, did not block the inhibitory effects of ox-PCs on EDR (data not shown), suggesting that reactive oxygen intermediates are unlikely to be involved in the impairment of EDR by ox-PC. In some cell types, ox-PC may function via the PAF receptor since the bioactivities of ox-PC including 2-(5-oxovaleroyl)PC are blocked by the PAF receptor antagonist or diminished by the PAF acetylhydrolase [25,31]. A previous study showed that PAF attenuates EDR in canine coronary artery both in vivo and in vitro at the concentrations of 0.1–1 nM [48]. Thus, it appears that actions via the PAF receptor may be one potential mechanism of the impairment of EDR by ox-PC. However, this possibility is unlikely in the present study on the basis of the following observations. PAF inhibited EDR at rather high concentrations of at least several micromoles per liter, and CV-6209 (10 nM), a specific and powerful PAF receptor antagonist, did not block ox-PC-induced impairment of EDR (data not shown). These results suggest that the inhibition of EDR by ox-PC is not mediated via the PAF receptor in rabbit thoracic aortas.

The third possibility is that ox-PC may cause the impairment of receptor-mediated intracellular signaling like LPC. Flavahan has shown that LPC inhibits a pertussis toxin-sensitive G protein-dependent signaling pathway in porcine endothelial cells [5]. We have previously demonstrated that LPC inhibits the increase in G protein coupled receptor-mediated calcium mobilization in cultured and intact endothelial cells [49,50]. Amphiphilic compounds such as LPC may modulate the activity of membrane-bound enzymes through the increased membrane fluidity and permeability as a result of the alteration of lipid composition of the cell membrane. It is speculated that the inhibitory effect of ox-PC may be related to changes in the endothelial membrane fluidity, which may alter the kinetics of membrane-bound proteins and lead to the impairment of membrane-associated signaling. However, further investigations about the effects of ox-PC on intracellular signal transduction and NO production in endothelial cells are required to clarify the mechanisms of the inhibitory effect of ox-PC on EDR.

In conclusion, ox-PCs generated from PCs containing PUFA at the sn-2 position as well as ox-PC with an sn-2 short chain fatty acid inhibited EDR. It is likely that, in addition to LPC, ox-PC is one of the plausible causes of the impaired EDR in atherosclerotic arteries. In addition to stimulation of smooth muscle cell growth and neutrophil activation, ox-PC may play a role in the progression and pathophysiological conditions in atherosclerosis through the inhibition of bioactivity of endothelium-derived relaxing factor.

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