Implication of lipoprotein associated phospholipase A_2 activity in oxLDL uptake by macrophages

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Abstract Recognition and uptake of oxidized LDL (oxLDL) by scavenger receptors of macrophages and foam cell formation are mediated by the oxidatively modified apolipoprotein B (ApoB) and lipid moiety of oxLDL. A great amount of oxidized phosphatidylcholine (oxPC) of oxLDL is hydrolyzed at the sn-2 position by lipoprotein associated phospholipase A_2 (Lp-PLA_2) to lysophosphatidylcholine and small oxidation products. This study examines the involvement of Lp-PLA_2 in the uptake of oxLDL by mouse peritoneal macrophages. LDL with intact Lp-PLA_2 activity [LDL (+)] and LDL with completely inhibited Lp-PLA_2 activity [LDL (-)] were subjected to oxidation with 5 μM $CuSO_4$ for 6 h [moderately oxLDL (MoxLDL)], or 24 h [heavily oxLDL (HoxLDL)] and peritoneal macrophages were incubated with these preparations. The uptake of MoxLDL(-) was about 30% increased compared with that of MoxLDL(+), and HoxLDL(-) uptake was about 20% increased compared with that of HoxLDL(+). Inhibition of Lp-PLA_2 activity had no effect on the uptake of ApoB-liposomes conjugates with ApoB isolated from MoxLDL(-), MoxLDL(+), HoxLDL(-), and HoxLDL(+). Liposomes prepared from the lipid extract of MoxLDL(-), MoxLDL(+), HoxLDL(-), and HoxLDL(+) exhibited a similar pattern to that observed in the uptake of the corresponding intact lipoproteins. This study suggests that the progressive inactivation of Lp-PLA_2 during LDL oxidation leads to an increased uptake of oxLDL by macrophages, which could be primarily attributed to the increased uptake of the oxidized phospholipids enriched lipid moiety of oxLDL.—Markakis, K. P., M. K. Koropouli, S. Grammenou-Savvoglou, E. C. van Winden, A. A. Dimitriou, C. A. Demopoulos, A. D.Tselepis, and E. E. Kotsifaki. **Implication of lipoprotein associated phospholipase A_2 activity in oxLDL uptake by macrophages.** ***J. Lipid Res.*** **2010.** 51: **2191–2201.**

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Oxidation of LDL is considered as a prerequisite event in the atherogenic process. LDL oxidation is mediated by endothelial cells, smooth muscle cells, and macrophages in the arterial intima and oxidized LDL (oxLDL) are found in abundance into atherosclerotic lesions (1–6). Oxidative modification of LDL includes *i*) peroxidation of the lipids, *ii*) conversion of PC to lysophosphatidylcholine (lysoPC) (up to 50% depending on the extension of the oxidation), *iii*) an increase in density, *iv*) an increase in net negative charge resulting in increased electrophoretic mobility, *v*) breakdown of apolipoprotein B (ApoB) into smaller peptides, and *vi*) binding of oxidized lipids on ApoB (2, 7, 8). Oxidation of LDL has various atherogenic effects such as increased uptake by macrophages and smooth muscle cells leading to foam cell formation, increased production of adhesion molecules, induction of monocytes/macrophages and smooth muscle cell prolif-

Abbreviations: ApoB, apolipoprotein B; [^{14}C]DPPC, phosphatidylcholine L-a-dipalmitoyl[dipalmitoyl-1-^{14}C]; [^{14}C]PAPC, phosphatidylcholine L-a-1-palmitoyl-2-arachidonyl [arachidonoyl-1-^{14}C];CHOL,cholesterol; DPPC, dipalmitoyl-phosphatidylcholine; FITC, fluorescein isothiocyanate; LDL(+), LDL with intact Lp-PLA_2 activity; LDL(-), LDL with inhibited LpPLA_2 activity; LDL + pef, LDL preincubated with pefabloc; HoxLDL, heavily oxidized LDL; [^{3}H]PAF, 1-O-alkyl-2-[^{3}H-acetyl]sn-glycero-3-phosphocholine;Lp-PLA_2, lipoprotein associated phospholipase A_2; lysoPC, lysophosphatidylcholine; MoxLDL, moderately oxidized LDL; OG, octyl glucoside; oxLDL, oxidized LDL; oxPAPC, oxidized 1-palmitoyl 2-arachidonoyl-phosphatidylcholine; oxPC, oxidized phosphatidylcholine; PAF, platelet activating factor; PAPC, 1-palmitoyl-2-arachidonoyl-phosphatidylcholine; pBPB, phospholipase inhibitor 2,4'-dibromoacetophenone; PLPC, 1-palmitoyl-2-lineloyl-phosphatidylcholine; POVPC, 1-palmitoyl-2-(5-oxovaleroyl)-phosphatidylcholine.

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eration, impairment of endothelium-dependent arterial relaxation, cytotoxic action on endothelial cells, and chemoattraction of circulating monocytes (6, 9–13).

Foam cell formation is considered to be an early and important step in the development of the atherosclerotic plaque (5, 14, 15). OxLDL are incorporated into macrophages via scavenger receptors. Several scavenger receptors, such as SR-AI, SR-AII, SR-AIII, CD36, LOX-1 have been described so far (16). Initially it was thought that oxLDL could exclusively be recognized via its protein moiety. ApoB isolated from oxLDL after lipid extraction was shown to bind to macrophages and also compete for the binding of intact oxLDL (7). It was later demonstrated that lipids extracted from oxLDL and reconstituted into liposomes could also bind to macrophages and compete for the binding and the uptake of intact oxLDL (17, 18). It was further shown that liposomes containing oxidized 1-palmitoyl 2-arachidonoyl-phosphatidylcholine (oxPAPC) inhibited the uptake of oxLDL (18). A specific product of oxPAPC, namely 1-palmitoyl-2-(5-oxovaleroyl)-phosphatidylcholine (POVPC), has been isolated as a component of oxLDL (19). POVPC was shown to inhibit the macrophage binding of intact oxLDL, the binding of liposomes prepared from oxLDL lipids, and that of ApoB isolated from oxLDL (20). Moreover, the oxidation of several lipids including phosphatidylcholines such as 1-palmitoyl-2-arachidonoyl-phosphatidylcholine (PAPC) or free fatty acids such as arachidonic and linolenic, leads to the production of molecules that can bind to ApoB. The amino group derivatization resulted from that process is likely to modify the interaction of ApoB with macrophage receptors (8, 21, 22). A conjugate of POVPC with serum albumin was shown to inhibit binding of intact oxLDL and of both the apoprotein and the lipids prepared from oxLDL. When a monoclonal antibody against oxidized phospholipids was used, the same inhibitory effects were observed (17, 20). It is, therefore, acknowledged that the lipid moiety plays a significant role in the recognition of oxLDL by macrophage receptors mediating the binding of oxLDL either independently of the ApoB or covalently linked to the ApoB.

As mentioned above, during LDL oxidation a significant amount of PC is degraded to lysoPC. This phospholipid hydrolysis is catalyzed by lipoprotein associated phospholipase A_2 (Lp-PLA_2) (23). Lp-PLA_2, formerly called platelet-activating factor acetylhydrolase as it was first described as the enzyme that catalyzes PAF hydrolysis, belongs to the PLA_2 superfamily and circulates in plasma mainly bound to LDL (approximately 70–85%), while the rest is bound to HDL (24, 25). However, its distribution varies, depending on the extent of its glycosylation (26). Lp-PLA_2 preferably hydrolyses phospholipids with short acyl chains, up to 6 carbon atoms in length, at the sn-2 position (23, 27, 28). During LDL oxidation, the fragmentation of polyunsaturated sn-2 fatty acyl groups in LDL phosphatidylcholine produces molecules with short acyl chains at the sn-2 position, such as POVPC, that can serve as substrates to Lp-PLA_2 (25, 28). It has to be noted that Lp-PLA_2 is gradually inactivated during oxidation (29, 30). Lp-PLA_2 is present in atherosclerotic lesions where it is produced by macrophages (31–33). In recent years, several epidemiological studies indicate that the level of circulating Lp-PLA_2 is an independent predictor of coronary artery disease incidents (34, 35). It is not yet elucidated whether Lp-PLA_2 is just an indicator of vascular inflammation, which is characterized by macrophage accumulation and increased Lp-PLA_2 production, or it is a causative factor of vascular inflammation and plaque formation (25, 33). In the present study, we focus on the effect of Lp-PLA_2 on the recognition of oxLDL by macrophages, as it seems plausible that the modification of the lipid moiety of LDL caused by Lp-PLA_2 activity, may affect the ability of oxLDL to interact with macrophage receptors.

MATERIALS AND METHODS

Materials

1-O-alkyl-2-[^{3}H-acetyl]sn-glycero-3-phosphocholine ([^{3}H]PAF) was purchased from New England Nuclear. Phosphatidylcholine L-a-dipalmitoyl[dipalmitoyl-1-^{14}C] ([^{14}C]DPPC) and phosphatidylcholine L-a-1-palmitoyl-2-arachidonyl [arachidonoyl-1-^{14}C] ([^{14}C]PAPC) were from American Radiolabeled Chemicals. Dipalmitoyl-phosphatidylcholine (DPPC), PAPC, cholesterol (CHOL), 1-O-palmityl-2-acetyl-sn-glycero-3-phosphocholine (PAF), 4-(2-aminoethyl) benzenesulfonyl fluoride (Pefabloc), and thioglycollate medium were obtained from Sigma. RPMI 1640 medium, fetal bovine serum, penicillin-streptomycin, and HBSS were from BioWhittaker. n-Octyl-β-D-glucopyranoside [octyl glucoside (OG)] was supplied by Alexis. Fluorescein isothiocyanate (FITC) was from Calbiochem. DEAE-Sepharose CL-6B was supplied from Pharmacia Biotech. Resource Q HPLC column (6.4 × 30 mm, 15 μm) was from Amersham Biosciences and Zorbax Eclipse XDB-C18 column (4.6 × 150 mm, 5 μm) was from Agilent Technologies. HPLC solvents were purchased from Lab-Scan Analytical Sciences. Materials for Western plot analysis were purchased from Bio-Rad and Chemilucent ECL Detection System from Chemicon. Rabbit anti-human polyclonal antibody for Lp-PLA_2 was from Cayman Chemical. Goat anti-rabbit IgG HRP was purchased from Chemicon International. The detergent compatible protein assay kit was from Bio-Rad. All other chemical and solvents were reagent grade and were supplied by Sigma, Merck, or Serva.

This study has been approved by the Review Board of the Medical School of the University of Athens.

Cultured cells

Elicited mouse peritoneal macrophages were isolated from female BALB/c mice by peritoneal lavage with PBS 4 days after an intraperitoneal injection of sterile 2.98% thioglycollate solution. The animal procedures were in accordance with the principles of the Guide for the Care and Use of Experimental Animals. The macrophages were plated in 12-well plates at a density of 1×10^6 cells/well in RPMI 1640 medium containing 10% fetal bovine serum, 100 units/ml penicillin, and 100 μg/ml streptomycin. Nonadherent cells were removed after 4 h and the adherent macrophages were used in the experiments.

Isolation and oxidation of LDL

Human LDL (density = 1.019–1.063 g/ml) was isolated from fresh normolipidemic plasma collected in EDTA (1 mg/ml) by sequential ultracentrifugation (Beckman, rotor Ti-75, 40000 rpm, 10 h) (36). Written consent was obtained from the volunteers who participated in this study. The LDL were extensively dialyzed against PBS, pH 7.4, containing 0.2 mM EDTA, and the

protein concentration was measured as described by Lowry (37). The LDL was used within 1–2 week of isolation. The LDL was dialyzed prior to use against PBS, pH 7.4, to remove EDTA. LDL oxidation with copper was performed by incubation of LDL (0.2 mg of LDL protein/ml) with 5 μM $CuSO_4$ in PBS, pH, 7.4 for 6 h [moderately oxLDL (MoxLDL)] or 24 h [highly oxLDL (HoxLDL)] at 37°C. Oxidation was terminated with the addition of 0.2 mM EDTA and refrigeration. Lipid peroxidation was estimated by determination of malondialdehyde concentration with an HPLC method previously described using a reverse phase Zorbax Eclipse XDB-C18 column (4.6 × 150 mm, 5 μm) (38). HPLC was performed on an Agilent 1100 model HPLC system, and fluorescence was monitored with a fluorescence detector (G1321A) that was set at an excitation wavelength of 515 nm and emission wavelength of 553 nm. For experiments with labeled LDL, the LDL was fluorescence labeled with FITC as previously described (39).

Assay of Lp-PLA_2 activity

Lp-PLA_2 activity was determined by a method similar to that described by Blank et al. (40) using [^{3}H]PAF as a substrate. Each sample (plasma, lipoprotein solution, lipoprotein eluate fractions, or purified Lp-PLA_2 preparations) was incubated with 10 μM [^{3}H]PAF in PBS, pH 7.4, for 10 min at 37°C. The lipids were extracted at the end of incubation by the method of Folch et al. (41), and the amount of radioactivity recovered in the aqueous phase was determined by liquid scintillation. Pefabloc (4-[2-aminoethyl] benzensulfonyl fluoride) was used as an Lp-PLA_2 inhibitor (42). For inhibition of Lp-PLA_2 activity, LDL (0.5 mg/ml) was incubated with 1 mM pefabloc in PBS, pH 7.4, for 1 h at 37°C. After incubation, LDL was dialyzed overnight against PBS, pH 7.4.

Phospholipid analysis

Total lipids of LDL or oxLDL were extracted as described above. The phospholipids were separated by TLC on silica gel G plates with chloroform/methanol/water (65/35/6, v/v) and visualized with iodine vapor. The bands corresponding to phosphatidylcholine and lysoPC were scraped from the plates and assayed for phosphorus content (43).

Partial purification of Lp-PLA_2 from LDL

LDL was isolated from 40 ml of fresh human normolipidemic plasma. Tween 20 was added to the LDL solution to a final concentration 0.1, and then the LDL solution was extensively dialyzed against 10 mM potassium phosphate buffer (pH 6.8) containing 0.1% Tween 20 and 10 μM butylated hydroxytoluene. Afterwards, the LDL was used for the partial purification of Lp-PLA_2 on a batch DEAE-Sepharose CL-6B, and an HPLC separation was performed using an anion exchange Resource Q column (6.4 × 30 mm, 15 μm). These two purification steps were carried out as described by Stafforini et al. (24). All procedures were carried out at 4°C. The effluent fractions were assayed for enzyme activity, as described, and protein content was estimated using a detergent compatible protein assay kit based on the method described by Lowry (Bio-Rad). The active fractions were pooled and then concentrated on Amicon Ultra-15 (10000 MWCO) centrifugal filter devices. Western blot analysis on a 16% SDS-polyacrylamide gel, incubating with a 1:2,000 dilution of the primary rabbit anti-human Lp-PLA_2 antibody and a 1:2,000 dilution of the secondary goat anti-rabbit IgG HRP antibody, revealed the presence of a 45 kDa band corresponding to the Lp-PLA_2. Finally, the partially purified enzyme was resuspended in a 5 mM Tris-HCl buffer (pH 7.5) containing 0.5 M KCl, 25 mM OG, and 10 μM butylated hydroxytoluene (buffer A). The latter was accomplished by washing twice the concentrated HPLC eluate with buffer A (the concentrated HPLC eluate was diluted 1:60 with buffer A and then concentrated again). The supernatant was assayed for enzyme activity and protein content and then stored at −20°C. No significant loss of activity was noticed upon storage for at least 2 months.

Preparation of ApoB-liposome conjugates

ApoB was initially isolated from LDL or oxLDL through an extraction with ice-cold methanol/chloroform (1/1, v/v) and then was washed twice with ice-cold water and once with ice-cold acetone as described previously (7). ApoB-liposome conjugates were prepared by a detergent solubilization and removal method. A mixed micelle solution containing DPPC, CHOL, and OG was prepared in a way similar to that used by Lundberg et al. (44) for the preparation of ApoB-liposome conjugates. OG was used as a detergent, because it does not affect the binding of ApoB from oxLDL to macrophage receptors (7). [^{14}C]DPPC and CHOL were mixed with OG [DPPC:CHOL:OG weight ratio 5:0.5:30] in 2.5 mM HEPES, 75 mM NaCl, 50 μM EDTA (pH 7.4) with a final concentration of OG 25 mM. The mixture was sonicated until the solution was completely clear. After a final wash of delipidated ApoB with ice-cold water, the mixed micelles solution was added to ApoB in the proportion 5:1 (weight ratio) between DPPC and ApoB. The mixture was vortexed for 5–10 min and then was immediately dialyzed extensively against PBS (pH 7.4) for 24 h at 4°C. The solution obtained by dialysis was centrifuged at 400 *g* for 10 min for any insoluble material to be removed. The average diameter of the ApoB-liposome conjugates, after they were extruded 8–10 times through 0.2 μm polycarbonate membranes at 37°C, was 180–220 nm as estimated by light scattering. Corresponding unconjugated liposomes were prepared using the same method, without the addition of delipidated ApoB. Solubilized lipid-free ApoB from LDL or oxLDL was prepared using the same procedure, with the addition of OG, instead of DPPC-CHOL-OG mixture to delipidated ApoB. The [^{14}C]ApoB-liposome conjugates and the solubilized lipid-free ApoB were subjected to ultracentrifugation at a density of 1.14 using KBr solution (36). The recovery of protein in both cases and the radioactivity of [^{14}C]ApoB-liposome conjugates at the upper layer were then determined. It has to be noted that the storage of the ApoB-liposome conjugates at 4°C resulted in the formation of sediment, probably due to some degree of aggregation. Because of that, the ApoB-liposome conjugates were used immediately after their preparation.

Liposome preparation

Liposomes were prepared from the lipids of native or oxLDL as previously described (18). HCl was added to the LDL or oxLDL preparations to a final concentration of 10 mM, and the lipids were extracted with methanol/chloroform (1/1, v/v). After centrifugation at 1.800 *g*, the chloroform phase was removed and dried under nitrogen. The lipids were resuspended in PBS (pH 7.4), and a microemulsion was prepared after the lipid suspension was vortex mixed and subsequently sonicated for 10 min. These microemulsions were then extruded 8–10 times through 0.1 μm polycarbonate membranes at 37°C. Liposomes were also prepared following the same procedure using synthetic phospholipids and CHOL [total phospholipids/CHOL 2:1 (mol/mol)] as previously described (18). Liposomes with different phospholipid content were prepared using DPPC, PAPC, or oxPAPC. DPPC-liposomes contained exclusively DPPC, and PAPC or oxPAPC-liposomes were prepared substituting PAPC or oxPAPC, respectively, for one- third of DPPC (molar ratio). Oxidation of PAPC was carried out with incubation of PAPC suspensions (0.5 μmol/ml) in PBS (pH 7.4) with 5 μM $CuSO_4$ for 24 h at 37°C. After the incubation was completed, phospholipids were

extracted as described above. In all cases, the final preparations were clear or slightly opalescent, and the average diameter of liposomes was 120–160 nm as estimated by light scattering. For experiments with labeled liposomes, [^{14}C]DPPC was added to the chloroform phase before resuspension in PBS. The concentration of liposomes was determined by measuring the phosphorus content of extracted lipids and estimating the recovery of β-radioactivity after the extrusion.

Binding of oxidized phosphatidylcholine and products of hydrolysis of oxidized phosphatidylcholine to ApoB

LDL or LDL preincubated with pefabloc (LDL + pef) at a concentration of 100 μg/ml and 1-palmitoyl-2-[1-^{14}C]arachidonylphosphatidylcholine, [^{14}C]PAPC, at a concentration of 25 nmol/ml, were cooxidized with 5 μM $CuSO_4$ for 24 h at 37°C. At the end of incubation, ApoB was isolated and washed as already described in the preparation of ApoB-liposome conjugates. After a final wash with ice-cold water, ApoB was solubilized with the addition of 0.5 ml of 0.2M NaOH and 0.1% SDS, and then aliquots were taken for determination of protein content and radioactivity by liquid scintillation. In a different set of experiments, 0.5 μmol/ml [^{14}C]PAPC was oxidized in PBS (pH 7.4) with 5 μM $CuSO_4$ for 24 h at 37°C. At the end of the incubation ox[^{14}C]PAPC was extracted by the method of Bligh Dyer (45). The chloroform phase of the extraction was dried under nitrogen and ox[^{14}C]PAPC was resuspended in PBS pH 7.4. Aliquots of 0.5 μmole ox[^{14}C]PAPC were incubated with or without the addition of 200 units of Lp-PLA_2 (1 unit hydrolyses 1 nmol of PAF in 1 h, 1 unit = 1 nmol of PAF/1 h) in a final volume of 250 μl for 24 h at 37°C. After the incubation was completed, the radioactively labeled sn-2 acyl chains released by Lp-PLA_2 (low molecular weight products of hydrolysis) were separated from the remaining substrate by a Bligh-Dyer extraction as previously described (27). The upper phase containing the low molecular weight products of hydrolysis was washed once with equal volume of chloroform. The upper phase was collected again and subsequently dried under nitrogen (this step did not result in any change in radioactivity content) and finally the low molecular weight products of hydrolysis derived from incubation of 0.5 μmol ox[^{14}C]PAPC with Lp-PLA_2 were resuspended in 250 μl of PBS (pH 7.4). The same procedure was also followed for samples of ox[^{14}C]PAPC, which were incubated without the addition of Lp-PLA_2. The concentration of ox[^{14}C]PAPC or low molecular weight products of hydrolysis in each solution was estimated by determination of radioactivity content. LDL, LDL + pef, oxLDL, or ox(LDL + pef) at the concentration of 100 μg of LDL protein/ml were incubated with either 25 nmol/ml low molecular weight products of hydrolysis or ox[^{14}C]PAPC for 24 h at 37°C. After the incubation, ApoB was isolated and solubilized as described and aliquots were used for protein quantification and radioactivity measurement. Extraction of intact ox[^{14}C]PAPC (without prior incubation with Lp-PLA_2) resulted in recovery of a small amount of the radioactivity in the upper phase (<2%). Incubation of this upper phase with LDL was performed as negative control.

Cell uptake studies

Cells were washed three times with 1 ml of HBSS prior to the assay. After 30 min of preincubation in RPMI 1640, the cells were incubated with the indicated concentrations of FITC-labeled lipoproteins or [^{14}C]DPPC-labeled ApoB-liposome conjugates or [^{14}C]DPPC-labeled liposomes, with or without unlabeled competitors in RPMI 1640 for 4 h at 37°C. At the end of incubation, the cells were washed three times with 1 ml of HBSS. When FITC-labeled lipoproteins were used, the cells were scraped gently from the bottom of the wells into 1 ml PBS containing 0.1% BSA, and the mean fluorescence intensity of 10,000 events per sample was measured by flow cytometry (FACScalibur, Becton Dickinson) and analyzed with CELLQUEST software (Becton Dickinson). When [^{14}C]DPPC-labeled ApoB-liposome conjugates or [^{14}C]DPPC-labeled liposomes were used, the cells were solubilized by adding 0.5 ml of 0.2M NaOH and 0.1% SDS and then aliquots were taken for determination of radioactivity by liquid scintillation and protein content. The specific uptake of lipoproteins, ApoB-liposome conjugates or liposomes was calculated by subtracting nonspecific uptake (values obtained in the presence of a 25-fold excess of unlabeled preparation).

Statistics

Statistical analysis was performed using a two-tailed Student's *t*-test.

RESULTS

Lp-PLA_2 activity reduces the uptake of oxLDL by peritoneal macrophages

The aim of this study was to investigate the implication of Lp-PLA_2 in the uptake of oxLDL by macrophages. For this purpose, LDL with intact Lp-PLA_2 activity [LDL(+)] and LDL with inhibited Lp-PLA_2 activity [LDL(-)] were used. Inhibition of Lp-PLA_2 activity was achieved by incubation of LDL with 1 mM pefabloc. It is known that during the LDL oxidation the Lp-PLA_2 activity progressively decreases (29, 30). Consistent with the previous findings, in our experiments, during LDL oxidation with 5 μM $CuSO_4$, the remaining Lp-PLA_2 activity after 6 h was approximately 40–50%, and after 24 h, only 10–15% of the initial activity was preserved (data not shown). Also the lysoPC/PC molar ratio was ~0.05 at native LDL and after 6 h or 24 h oxidation was ~0.24 and ~0.36, respectively (data not shown). These two time intervals were used, and LDL were oxidized for 6 h (MoxLDL) or 24 h (HoxLDL) to assess the possible difference between a shorter oxidation with the major percentage of Lp-PLA_2 activity present and a longer oxidation during which Lp-PLA_2 activity was significantly attenuated. Pretreatment with pefabloc did not affect the extent of lipid peroxidation as estimated by determination of malondiandehyde concentration (data not shown). Inhibition of Lp-PLA_2 activity prior to oxidation resulted in greater uptake of MoxLDL or HoxLDL (**Fig. 1**). More specifically, MoxLDL(-) revealed greater uptake compared with that of MoxLDL(+) by 26.06 ± 16.59% ($P < 0.005$), 30.45 ± 15.85% ($P < 0.001$), and 31.72 ± 11.50% ($P < 0.001$) at concentrations of 2.5, 5, and 10 μg of LDL protein/ml, respectively (values given are means ± 95% confidence limits of differences). HoxLDL(-) also exhibited greater uptake compared with that of HoxLDL(+) by 19.81 ± 13.52% ($P < 0.005$), 21.84 ± 14.00% ($P < 0.005$), and 17.37 ± 9.85% ($P < 0.005$) at concentrations of 2.5, 5, and 10 μg of LDL protein/ml, respectively. It seems that the increase of uptake caused by inhibition of Lp-PLA_2 activity prior to oxidation was less in the case of HoxLDL, although this trend was not statistically significant. Incubation with pefabloc at the end of the oxidation did not alter the uptake of MoxLDL or HoxLDL oxidized in the presence of Lp-PLA_2 activity,

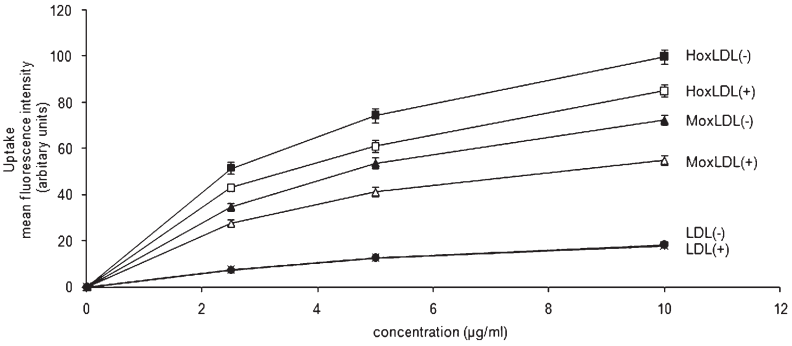

Fig. 1. Uptake of fluorescence-labeled oxLDL oxidized in the presence or in the absence of Lp-PLA2 activity. FITC-labeled LDL with intact Lp-PLA_2 activity [LDL(+)], LDL + pef [LDL(-)], MoxLDL(+) or MoxLDL(-), and HoxLDL(+) or HoxLDL(-) were incubated with macrophages at the indicated concentrations (μg protein/ml) for 4 h at 37°C. The cells were then washed, scraped, and the mean fluorescence intensity of 10,000 events measured by using flow cytometry. Values given are means ± SE of triplicate determinations from a set of three separate experiments. The mean value of the uptake of HoxLDL(-) was taken as 100.

indicating no direct effect of pefabloc on oxLDL uptake other than the inhibition of Lp-PLA_2 activity (**Fig. 2**).

Lp-PLA_2 does not affect the uptake of oxLDL via the ApoB moiety

Provided that macrophage receptors recognize both the protein and the lipid moieties, our next goal was to distinguish which of the two moieties is responsible for the increased uptake of oxLDL(-). The implication of ApoB was examined assessing the uptake of ApoB-liposome conjugates containing ApoB isolated from oxLDL. ApoB from oxLDL was incorporated into ApoB-liposome conjugates consisting of DPPC, CHOL, and ApoB in the proportion 10:5:2 (weight ratio). ApoB-liposome conjugates were prepared by a detergent solubilization and removal method using as detergent OG, as it has been shown that treatment of ApoB with OG does not affect the binding of ApoB from oxLDL to macrophage receptors (7). To examine if the ApoB was conjugated to the liposomes, the [^{14}C]DPPC-ApoB liposome conjugates were subjected to ultracentrifugation at a density of 1.14. The recovery of both the protein and the [^{14}C]PAPC radioactivity at the upper layer was greater than 95%, whereas the recovery of the protein was only 25% when ApoB solubilized only with the use of OG without the addition of [^{14}C]DPPC and CHOL (data not shown). The uptake of these [^{14}C]ApoB-liposome conjugates by macrophages was concentration dependent. When ApoB from oxLDL was used, the uptake was by far greater compared with that of [^{14}C]ApoB-liposome conjugates containing ApoB from native LDL, while the uptake of corresponding unconjugated liposomes was negligible (data not shown). As shown in **Fig. 3**, the uptake of [^{14}C]ApoB-liposome conjugates containing ApoB from oxLDL was inhibited in a concentration-dependent manner by oxLDL, while native LDL had only minor inhibitory effect. No significant difference was observed between the uptake of [^{14}C]ApoB-liposome conjugates containing ApoB from MoxLDL(+) or MoxLDL(-) and the same was observed when [^{14}C]ApoB-liposome conjugates containing ApoB from HoxLDL(+) or HoxLDL(-) were used (**Fig. 4**). The uptake of [^{14}C] ApoB-liposome conjugates containing ApoB from MoxLDL or HoxLDL was unaffected by treatment with pefabloc at the end of oxidation. This finding was in agreement with the results mentioned above concerning the oxLDL uptake, indicating once again no direct effect of pefabloc on receptor binding.

Binding of oxidized PAPC and products of hydrolysis of oxidized PAPC to ApoB

To examine the possibility that products of oxLDL hydrolysis by Lp-PLA_2 can bind on ApoB, we used as a substrate 1-palmitoyl-2-[1-^{14}C]arachidonoyl-phosphatidylcholine, [^{14}C] PAPC. LDL contains a significant amount of PAPC, the oxidation of which results in the production of powerful ligands for scavenger receptors and oxPAPC molecules serve as substrates of Lp-PLA_2 (27, 28, 46). Ox[^{14}C] PAPC was treated with Lp-PLA_2 purified from LDL, and the radioactive low molecular weight products of hydrolysis released by Lp-PLA_2 from the sn-2 position were isolated. Incubation of these low molecular weight products of hydrolysis with non-oxLDL(+) or LDL(-) revealed their ability to bind to ApoB in a comparable degree to that observed after incubation with ox[^{14}C]PAPC (**Table 1**). The radioactivity was only 10.2% ($P < 0.05$) and 13.76% ($P < 0.05$) less than that recovered after incubation of ox[^{14}C]PAPC with non-oxLDL(+) or LDL(-), respectively. Consistent with this, the radioactivity

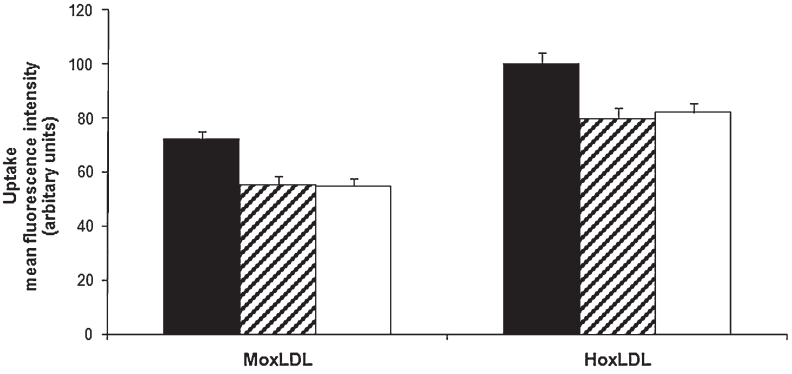

Fig. 2. Assessment of the direct effect of Pefabloc on oxLDL uptake. Macrophages were incubated with FITC-labeled MoxLDL or HoxLDL treated prior to oxidation with pefabloc (black bars) or oxidized in the presence of Lp-PLA_2 activity while pefabloc was added at the end of the oxidation (hatched bars) or oxidized in the presence of Lp-PLA_2 activity without addition of pefabloc at any time (white bars) at a concentration of 5 μg protein/ml for 4 h at 37°C. The uptake was quantitated as described for Fig. 1. Values given are means ± SE of triplicate determinations from a set of three separate experiments.

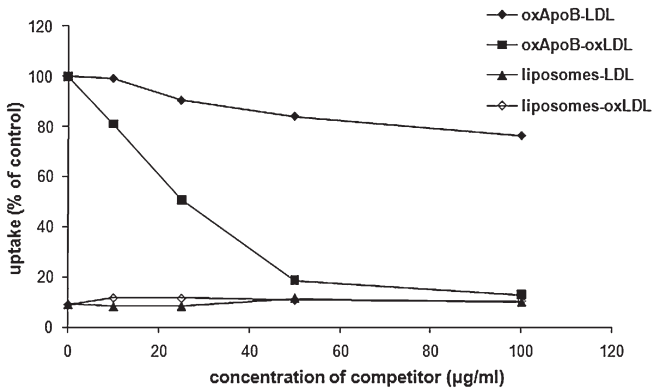

Fig. 3. Uptake of [^{14}C]oxApoB-liposome conjugates in the presence of unlabeled intact native LDL or oxLDL. OxApoB-liposome conjugates (ApoB:[^{14}C]DPPC:CHOL weight ratio 1:5:0.5) were prepared using oxApoB from oxLDL, oxidized with 5 μM $CuSO_4$ for 24 h at 37°C. [^{14}C]oxApoB-liposome conjugates (oxApoB) at the concentration of 5 μg ApoB/ml or [^{14}C]unconjugated liposomes (liposomes) at the corresponding concentration of 25 μg DPPC/ml were incubated with macrophages in the presence of unlabeled intact native LDL or oxLDL as a competitor at the indicated concentrations for 4 h at 37°C. The cells were then washed and lysed and the uptake was quantitated by measuring [^{14}C] radioactivity per mg of cell protein as described in "Methods." Values given are means of triplicate determinations from a set of two separate trials and are expressed as a percentage of the uptake versus that observed in the absence of competitor.

bound to ApoB was decreased by 14.2% ($P < 0.01$) when ox[^{14}C]PAPC was added to LDL(+) compared with that observed after the addition of ox[^{14}C]PAPC to LDL(-). Furthermore, cooxidation of [^{14}C]PAPC with LDL(+) led to a 14.5% decrease of the radioactivity bound to ApoB compared with that observed after cooxidation of [^{14}C]PAPC with LDL(-) ($P < 0.05$). Finally, the addition of either low molecular weight products of hydrolysis or ox[^{14}C]PAPC to oxLDL(+) or oxLDL(-) resulted in much lower values of binding, probably because the responsible sites of ApoB had been already occupied by molecules generated during the oxidation.

Lp-PLA$_2$ reduces the uptake of oxLDL via the lipid moiety

The role of Lp-PLA$_2$ in the uptake of oxLDL via the lipid moiety was examined using [^{14}C]DPPC-labeled liposomes prepared from the lipid extract of MoxLDL(-), MoxLDL(+), HoxLDL(-), and HoxLDL(+) (**Fig. 5**). The uptake of these liposomes exhibited a similar pattern to that of the corresponding FITC-labeled lipoproteins. Incubation with liposomes from MoxLDL(-) lipids resulted in greater uptake compared with that of liposomes from MoxLDL(+) lipids by 39.70 ± 13.40% ($P < 0.001$), 42.84 ± 14.94% ($P < 0.001$), and 45.32 ± 18.85% ($P < 0.001$) at concentrations of 2.5, 5, and 10 μg of lipids/ml, respectively (values given are means ± 95% confidence limits of differences). Similarly, the inhibition of Lp-PLA$_2$ led to increased uptake, though to a lesser extent, of liposomes from HoxLDL(-) lipids compared with that of liposomes from HoxLDL(+) lipids by 31.19 ± 8.82% ($P < 0.001$), 27.06 ± 9.97% ($P < 0.001$), and 32.84 ± 10.35% ($P < 0.001$) at concentrations of 2.5, 5, and 10 μg/ml, respectively. OxPAPC liposomes very effectively inhibited the uptake of liposomes from MoxLDL(-) lipids, whereas liposomes from LDL(-) lipids, DPPC liposomes, or unoxidized PAPC liposomes had a negligible effect as competitors (**Fig. 6**). Furthermore, liposomes from MoxLDL(-) inhibited in a similar degree the uptake of oxPAPC liposomes, whereas

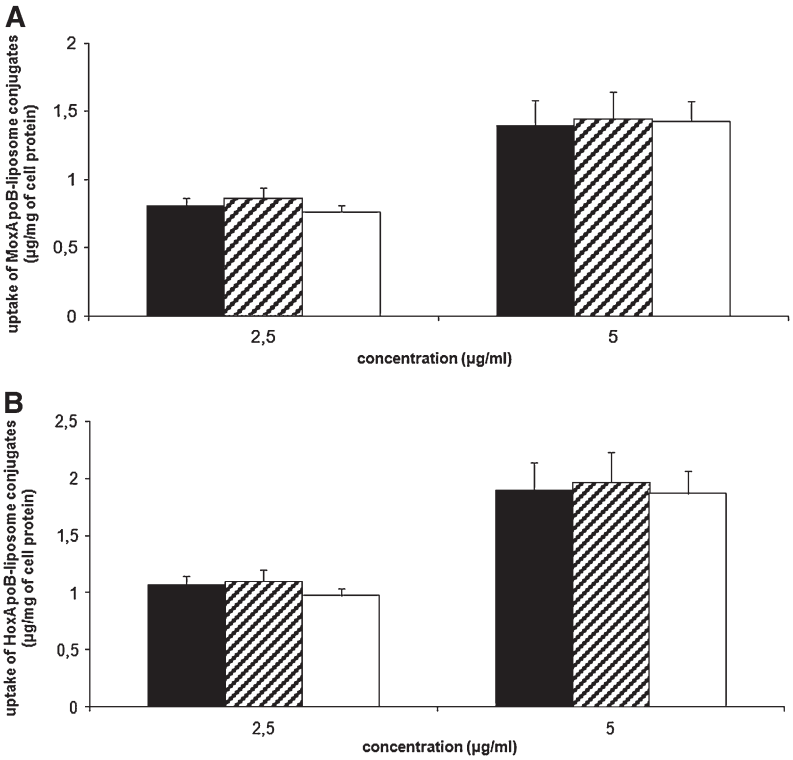

Fig. 4. Uptake of [^{14}C] oxApoB-liposome conjugates prepared with ApoB from oxLDL oxidized in the presence or in the absence of Lp-PLA$_2$ activity. [^{14}C]oxApoB-liposome conjugates were prepared as described in "Methods" using ApoB from oxLDL treated prior to oxidation with pefabloc (black bars), oxLDL oxidized in the presence of Lp-PLA$_2$ activity while pefabloc was added at the end of the oxidation (hatched bars), or oxLDL oxidized in the presence of Lp-PLA$_2$ activity without addition of pefabloc at any time (white bars). Moderately oxidized ApoB (MoxApoB) was obtained from LDL that was oxidized with 5 μM CuSO4 for 6 h (A) while heavily oxidized ApoB (HoxApoB) was obtained from LDL, which were oxidized with 5 μM CuSO4 for 24 h (B). Macrophages were incubated with the [^{14}C]oxApoB-liposome conjugates at the indicated concentrations (μg protein/ml) for 4 h at 37°C. The uptake was quantitated as described for Fig. 2. Values given are means ± SE of triplicate determinations from a set of three separate trials.

TABLE 1. Binding of oxidized [^{14}C]PAPC and products of hydrolysis of oxidized [^{14}C]PAPC to ApoB

	Percent Bound to ApoB (± 95% confidence limits)
ox[LDL + [^{14}C]PAPC][a]	6.640 ± 0.780*
ox[(LDL + pef) + [^{14}C]PAPC]	7.766 ± 0.722*
LDL + low molecular weight products of hydrolysis[b]	3.650 ± 0.573‡
LDL + ox[^{14}C]PAPC	4.064 ± 0.251†¶
(LDL + pef) + low molecular weight products of hydrolysis	3.886 ± 0.400†
(LDL + pef) + ox[^{14}C]PAPC	4.506 ± 0.336†¶
oxLDL + low molecular weight products of hydrolysis	1.067 ± 0.172
oxLDL + ox[^{14}C]PAPC	1.164 ± 0.217
ox(LDL + pef) + low molecular weight products of hydrolysis	1.009 ± 0.175
ox(LDL + pef) + ox[^{14}C]PAPC	1.372 ± 0.250

[a] LDL or LDL + pef at a concentration of 100 μg protein/ml was cooxidized with [^{14}C]PAPC at a concentration of 25 nmol/ml with 5 μM $CuSO_4$ for 24 h at 37°C.

[b] In a different set of experiments, [^{14}C]PAPC was oxidized with 5 μM $CuSO_4$ for 24 h at 37°C and then ox[^{14}C]PAPC was obtained with Bligh Dyer extraction and resuspended in PBS (pH 7.4). Aliquots of 0.5 μmol ox[^{14}C]PAPC were incubated with or without the addition of 200 units of Lp-PLA_2 in a final volume of 250 μl for 24 h at 37°C. The low molecular weight products of hydrolysis were isolated in the upper phase of a Bligh Dyer extraction, while ox[^{14}C]PAPC was isolated in the lower phase, and then they were resuspended in PBS. LDL, LDL + pef, oxLDL, or ox(LDL + pef) was incubated with either low molecular weight products of hydrolysis or ox[^{14}C]PAPC for 24 h at 37°C. The indicated lipoprotein preparation at a concentration of 100 μg/ml was incubated with 25 nmol/ml of either low molecular products of hydrolysis or ox[^{14}C]PAPC. In both cases ([a] and [b]), after the incubation, ApoB was isolated and solubilized as described in "Methods." Aliquots were used for protein quantification and radioactivity measurement. Because the extraction of intact ox[^{14}C]PAPC (without prior incubation with Lp-PLA_2) resulted in the recovery of a small amount of the radioactivity in the upper phase, incubation of this upper phase with LDL as negative control was performed. The results are the mean values ± 95% confidence limits of triplicate determinations from a set of three separate experiments and are expressed as percentage of total radioactivity that was associated with ApoB.

† $P < 0.01$; *, ¶, ‡ $P < 0.05$.

liposomes from LDL(-) lipids, DPPC liposomes, or nonoxidized PAPC liposomes had no effect (data not shown). The same competition between oxPAPC and lipids from oxLDL for common binding sites was revealed when HoxLDL(-) lipids liposomes were used (data not shown).

DISCUSSION

The data presented here demonstrate that inhibition of the Lp-PLA_2 hydrolysis of oxidized phospholipids increases the uptake of oxLDL by macrophages. Specifically, the uptake of MoxLDL(-) was increased about 30% compared with that of MoxLDL(+), and HoxLDL(-) uptake was increased about 20% compared with that of HoxLDL(+). HoxLDL abolished most of the Lp-PLA_2 activity at the end of the 24 h oxidation period, but the difference in the uptake between HoxLDL(-) and HoxLDL(+) should not be expected to be negligible, as in the case of HoxLDL(+), a great amount of oxidized phosphotidylcholine was already hydrolyzed before a severe attenuation of Lp-PLA_2 activity occurred. Moreover, an additional amount of oxidized phosphotidylcholine is hydrolyzed after the first 6 h of oxidation. Indeed, the lysoPC/PC molar ratio was ~0.05 at native LDL and after 6 h of oxidation was ~0.24, and it was further increased to ~0.36 at the end of the 24 h period of oxidation. It also has to be noted that even after 24 h oxidation, 10–15% of the initial activity was preserved. However, we cannot exclude the possibility that the ongoing formation of oxidized molecules after Lp-PLA_2 activity has been significantly reduced tends to attenuate the difference in the uptake between inhibitor treated and untreated preparations on a prolonged oxidation.

In a recent study, it was reported that inhibition of Lp-PLA_2 activity with pefabloc increased oxLDL cellular association in cultured human monocyte-derived macrophages and hepatocytes by 43% and 51%, respectively, whereas the addition of recombinant Lp-PLA_2 decreased the cellular association of oxLDL and oxLp(a) in macrophages

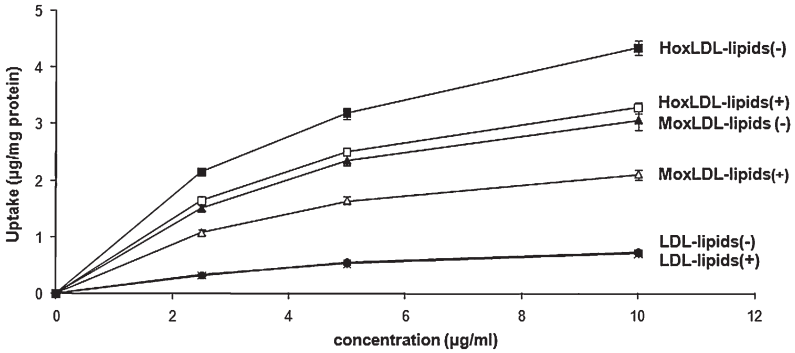

Fig. 5. Effect of Lp-PLA_2 activity on the uptake of [^{14}C]DPPC-labeled oxLDL-lipids liposomes. [^{14}C]DPPC-labeled liposomes prepared from lipid extract of LDL with intact Lp-PLA_2-activity [LDL-lipids (+)], LDL + pef [LDL-lipids (-)], MoxLDL-lipids (+) or MoxLDL-lipids (-), and HoxLDL-lipids (+) or HoxLDL-lipids (-) were incubated with macrophages at the indicated concentrations (nmol phospholipids/ml) for 4 h at 37°C. The cells were then washed and lysed and the uptake was quantitated by measuring [^{14}C] radioactivity per mg of cell protein as described in "Methods." Values given are means ± SE of triplicate determinations from a set of four independent experiments.

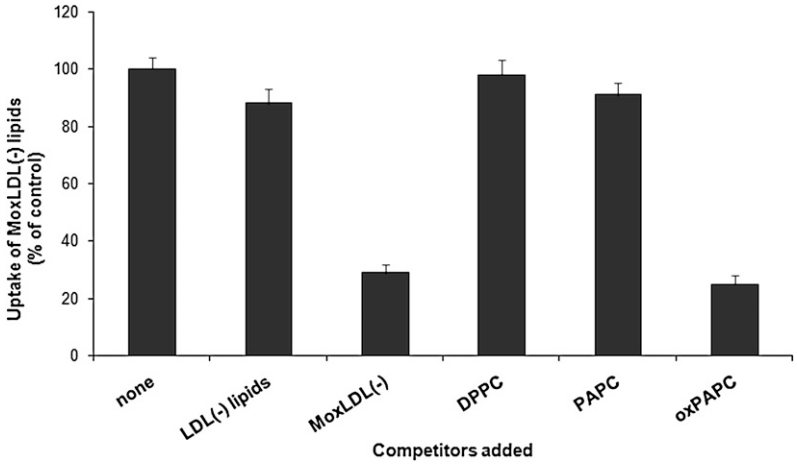

Fig. 6. Uptake of [^{14}C]DPPC-labeled MoxLDL(+) lipids liposomes in the presence of unlabeled liposomes. Macrophages were incubated with MoxLDL(-) lipids liposomes labeled with [^{14}C]DPPC at the concentration of 5 nmol phospholipids/ml for 4 h at 37°C with or without competitors added. Unlabeled liposomes from LDL(-) or MoxLDL(-) lipids and also DPPC, PAPC, and oxPAPC liposomes (prepared as described in Methods) at the concentration of 50 nmol phospholipids/ml were used as competitors. The cells were then washed and lysed and the uptake was quantitated by measuring [^{14}C] radioactivity per mg of cell protein as described in "Methods." Values given are means ± SE of triplicate determinations from a set of three separate experiments and are expressed as a percentage of uptake versus that observed in the absence of competitor.

and hepatocytes by approximately 30–40% (47). These results are close to those found in our study. In another study, an adenovirus encoding human Lp-PLA$_2$ was injected into rabbits, and LDL with 3-fold increased Lp-PLA$_2$ activity was produced (48). Oxidation of this Lp-PLA$_2$ overexpressing LDL resulted in an uptake by RAW 264 macrophages that was decreased to 60–80% compared with oxLDL from LacZ-tranduced control rabbits. Inhibition of Lp-PLA$_2$ with treatment with pefabloc prior to oxidation led to a 2-fold increase in the uptake compared with LacZ control oxLDL.

Incubation of LDL with the phospholipase inhibitor 2,4'-dibromoacetophenone (pBPB) prior to oxidation resulted in the inhibition of both the phospholipid hydrolysis and the degradation of oxLDL by mouse peritoneal macrophages (49). The addition of pBPB exhibited an antioxidant effect, reducing lipid peroxidation and thus making the results obtained with pBPB-treated LDL not completely comparable with those obtained using untreated LDL. In the present study, pefabloc proved to be a proper inhibitor for uptake studies, because it neither altered the degree of lipid peroxidation nor exhibited any direct effect on oxLDL association by macrophages other than the inhibition of Lp-PLA$_2$ activity.

Given that Lp-PLA$_2$ activity reduced the oxLDL uptake by macrophages in our study, we examined whether this effect was due to ApoB modification, oxLDL-lipid modification, or both. The uptake of ApoB isolated from oxLDL was examined using an ApoB-liposome conjugate method. Inhibition of Lp-PLA$_2$ had no effect on the uptake of these oxApoB-liposome conjugates. It has been demonstrated that the ability of ApoB to be recognized by scavenger receptors results from several chemical modifications that have in common the derivatization of ε-amino groups of lysine residues (8, 21). It has been shown that oxidation products of free fatty acids such as arachidonic, linoleic, and linolenic can bind to ApoB, modifying the lysine residues and leading to rapid uptake by macrophages (21). Fab fragments of monoclonal antibodies, specific for adducts of oxidation products with lysine, prevented the increased uptake of oxidation products-modified LDL (50). The macrophage binding of solubilized ApoB, isolated from oxLDL, was inhibited by a monoclonal antibody against oxidized phospholipids, which could recognize oxPAPC and POVPC, indicating the presence of oxidized phospholipids bound to ApoB (20). It has also been demonstrated that during oxidation of LDL, there is a progressive increase in the amount of phosphorus covalently linked to ApoB (15). Oxidation of 2-[^{14}C]arachidonoylphosphatidylcholine with LDL revealed radioactivity bound to ApoB (8, 22). Theoretically, this could be due not only to the binding of oxPAPC but also to the binding of fatty chains released by Lp-PLA$_2$ from the sn-2 position. A plausible explanation of the similar uptake of ApoB from oxLDL(+) or oxLDL(-) in our study could be that the low molecular weight products of hydrolysis of oxidized phosphatidylcholine (oxPC), released by Lp-PLA$_2$ from the sn-2 position, are also able to sufficiently derivatize ApoB. After incubation of ox[^{14}C]PAPC with Lp-PLA$_2$, the low molecular weight products of hydrolysis were isolated, and it was shown that they could bind to ApoB almost to the same extent as ox[^{14}C]PAPC. This finding may explain, at least in part, the similar uptake of ApoB from oxLDL(+) or oxLDL(-). However, we should consider that these low molecular weight products of hydrolysis are expected to be hydrophilic and the experimental conditions precluded the diffusion of these molecules. This probably does not simulate the actual conditions of LDL oxidation in the arterial intima, where these molecules could diffuse away from LDL and/or may bind to other proteins such as albumin, which is known to have binding sites for fatty acids (29).

Besides ApoB, the lipid moiety of oxLDL is also significant for the binding of oxLDL to macrophage receptors. A number of studies have shown that oxLDL lipids can mediate the binding of oxLDL to macrophage receptors. Liposomes prepared from oxLDL lipids could bind to macrophages and also compete for the binding of intact oxLDL and solubilized ApoB from oxLDL, while lipopsomes containing oxPAPC as well as a conjugate of POVPC with serum albumin proved effective competitors for the binding of intact oxLDL and oxLDL lipids (17, 18, 20). A monoclonal antibody against oxidized phospholipids that could recognize oxPAPC and POVPC, caused a great

inhibition of the binding of oxLDL lipids and the degradation rate of intact oxLDL (20, 51). In the present study, we found that the uptake pattern of liposomes prepared from the lipids extracted from MoxLDL(-), MoxLDL(+), HoxLDL(-), and HoxLDL(+) was similar to that observed in the uptake of the corresponding intact lipoproteins. The uptake of MoxLDL(-) lipids was increased by approximately 40% compared with that of MoxLDL(+) lipids, and HoxLDL(-) lipids uptake was increased by approximately 30% compared with that of HoxLDL(+) lipids. These results, along with the absence of difference in the uptake of ApoB-liposome conjugates, strongly suggest that the lipid moiety is responsible for the increased uptake of oxLDL observed after the inhibition of Lp-PLA_2 activity. It has been previously shown that certain oxidized phospholipids derived from the oxidation of PAPC and 2-linoneloyl-1-palmitoyl-phosphatidylcholine (PLPC) can serve as high affinity ligands for recognition by CD36 scavenger receptor and can compete for the binding of oxLDL by macrophages (17, 18, 20, 46, 52). Among them, POVPC deriving from PAPC oxidation has been mostly studied. The susceptibility of POVPC to Lp-PLA_2 hydrolysis has been well established (27, 28). Moreover, the structure of several high-affinity ligands for CD36 derived from oxidation of PAPC or PLPC has been thoroughly defined (46). These included not only POVPC but also series of molecules with 8 carbon atoms in length chain at the sn-2 position in the case of oxPAPC and 12 atoms carbon in length at the sn-2 position in the case of oxPLPC. We cannot exclude the possibility that some of these molecules could potentially serve as substrates to Lp-PLA_2, especially concerning the mentioned derivatives of oxPAPC; it has been shown that certain oxidized phospholipids with chains longer than 6 carbon atoms in length at the sn-2 position such as 1-palmitoyl-2-(9-oxononanoyl)-sn-glycero-3-phosphocholine are also susceptible to Lp-PLA_2 activity, although less efficiently (27). In the present study, we demonstrated that oxPAPC-liposomes effectively inhibited the uptake of liposomes from MoxLDL(-) lipids, whereas liposomes containing non-oxidized PAPC or exclusively the saturated phosphpolipid DPPC, which is resistant to oxidation, had no effect as competitors. These results are consistent with the previously published studies demonstrating oxPAPC as a strong ligand for macrophage receptors, thus further supporting the role of oxidized phospholipids that can serve as substrates to Lp-PLA_2 in oxLDL binding to macrophage receptors in our experimental setting.

Based on the above-mentioned data, we can assume that the hydrolysis of oxPC molecules, especially those with short fragmented acyl chains at the sn-2 position such as POVPC, is a plausible explanation of the decreased uptake of oxLDL when Lp-PLA_2 activity is present during the oxidation. The preserved recognition via ApoB, the production of oxidized phospholipids with long chains at the sn-2 position (which act as ligands for scavenger receptors but are not sensitive to hydrolysis by Lp-PLA_2), and the progressive loss of Lp-PLA_2 activity during oxidation tend to attenuate the difference in the uptake between oxLDL(+) and oxLDL(-). Nevertheless, the observed effect of Lp-PLA_2 activity on oxLDL uptake could play a role in the evolution of atherosclerotic lesions.

The question that rises is whether this decreased binding of oxLDL caused by the presence of Lp-PLA_2 activity could be considered as protective or harmful. It is beyond the purpose of this study to review the data about the implication of Lp-PLA_2 in the atherosclerotic process, but we can focus on the effect of the degradation of oxPC to lysoPC concerning the macrophages. As mentioned above, molecules derived from the oxidation of PAPC and PLPC are potent ligands for at least some of the macrophage receptors. Several signaling pathways have been described, initiated by scavenger receptors and leading to monocyte adhesion and migration, production of reactive oxygen species, inflammation, and apoptosis (16). OxLDL binding to CD36 induces the activation of the transcription factor nuclear factor κB in macrophages through a mechanism depending on protein kinase C and leads to production of inflammatory cytokines, such as tumor necrosis factor α and β, interleukin-1β and -6, and interferon β and γ (53). On the other hand, lysoPC interacts with macrophages, and it was shown to induce macrophage proliferation in mice and to stimulate the production of interleukin-1β and monocyte chemoattractant protein 1 in human monocytes (13, 54–56). Moreover, lysoPC stimulates the production of reactive oxygen species and was cytotoxic in RAW 264.7 macrophages (57). So far, there are no data indicating which effects on macrophages, those of oxPC or lysoPC, are more important concerning foam cell formation and the progression of atherosclerotic lesions. Regarding the effects of oxPC, we can consider the reduced macrophage recognition of oxLDL, caused by Lp-PLA_2, to be beneficial. On the contrary, if we presume that lysoPC is more harmful, we can assume that the decrease in oxLDL(+) uptake, caused by Lp-PLA_2, is too limited to compensate for the 3- to 5-fold greater amount of lysoPC that oxLDL(+) contains, and, thus, hydrolysis by Lp-PLA_2 can be considered as harmful.

In conclusion, the present study shows that the progressive inactivation of Lp-PLA_2 during LDL oxidation leads to an increased uptake of oxLDL by macrophages, which results from the increased uptake of the oxidized phospholipid-enriched lipid moiety of oxLDL. The biological significance of this role of LDL-associated Lp-PLA_2 needs further investigation.

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