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OxLDL stimulates lipoprotein-associated phospholipase A_2 expression in THP-1 monocytes via PI3K and p38 MAPK pathways

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Aims Lipoprotein-associated phospholipase A_2 (lp-PLA_2) has been detected in human and rabbit atherosclerotic lesions, where it co-localizes with its substrate, oxidized LDL (oxLDL). Here, we investigated whether oxLDL may exert a regulatory effect on lp-PLA_2 expression.

Methods and results Using human monocytic THP-1 cells as a model system, we found that oxLDL up-regulated the expression of lp-PLA_2 while another substrate of the enzyme, platelet activating factor, had no such effect. The up-regulatory effect of oxLDL could be conferred by its oxidized phospholipids (oxPCs, the exact substrates of lp-PLA_2), but not their hydrolyzed products, lysophosphatidylcholines (lysoPCs). OxLDL induced the activation of p38 mitogen-activating protein kinase (MAPK) through phosphatidylinositol 3-kinase (PI3K). Inhibition of either PI3K or p38 MAPK completely blocked oxLDL-induced lp-PLA_2 expression. In addition, inhibition of lp-PLA_2 activity in the conditioned medium significantly decreased lipid accumulation in macrophages as detected by oil red staining.

Conclusion The present study shows that oxLDL, and more specifically its unhydrolyzed oxidized phospholipids, can up-regulate lp-PLA_2 expression in monocytes through the PI3K and p38 MAPK pathway. In turn, lp-PLA_2 promotes lipoprotein uptake in macrophages. Our results uncover a new link between oxLDL and lp-PLA_2, and may provide insight into this interaction in the context of atherosclerosis.

Keywords Atherosclerosis • lp-PLA2 • oxLDL • p38 • PI3K

1. Introduction

The development of atherosclerotic vascular disease has been linked to the formation of bioactive lipid mediators and accompanying vascular inflammation,[1] but the complex question of how lipids and inflammation cooperate in the progression of atherosclerosis has not yet been fully elucidated. Developing a new understanding of the link between oxLDL and lp-PLA_2 may form a bridge between the lipid- and inflammation-based hypotheses for explaining atherothrombotic complications.[1,2]

Modified low density lipoprotein (LDL), especially oxidized LDL (oxLDL), has long been recognized as playing a central role in the progression of atherosclerosis. OxLDL exerts many atherogenic effects, including induction of inflammatory genes, cytotoxicity to vascular cells, and formation of foam cells.[3]

Lp-PLA_2, also named platelet activating factor acetylhydrolase (PAF-AH; E.C. 3.1.1.47), is a Ca^{2+}-independent phospholipase that associates with the apoB-containing lipoproteins, and primarily with LDL in humans.[4] A small proportion of the enzyme associates with HDL.[5] Lp-PLA_2 acts preferentially on water-soluble polar phospholipids with oxidatively truncated sn-2 chains, but has little activity on naturally occurring long-chain fatty acids in the phospholipids of cellular membranes.[5] Many clinical and epidemiological studies have indicated that lp-PLA_2 is a marker for

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cardiovascular risk, with higher plasma lp-PLA_2 levels (mass or activity) correlating with a higher risk for cardiovascular events. The risk estimates of death, coronary events, or stroke remain statistically significant even after full adjustment for several risk factors.[6–10]

Lp-PLA_2 is thought to play an atherogenic role by hydrolyzing oxidized phospholipids in oxLDL, resulting in the generation of two bioactive lipid mediators, lysophosphatidylcholines (lysoPCs) and oxidized non-esterified fatty acids (oxNEFAs), both of which play important roles in the recruitment of inflammatory cells to lesion-prone areas, and in local increases of inflammatory cytokines.[11–13] The bioactive lipids generated by lp-PLA_2 are also cytotoxic to macrophages, and may facilitate the formation of a necrotic lipid core in advanced atherosclerotic lesions. Inhibition of lp-PLA_2 can reduce the cytotoxicity of oxLDL to vascular cells and attenuate oxLDL-induced expression of inflammatory genes.[11–13] It should be noted that the role of is Lp-PLA_2 still controversial, as the enzyme on HDL seems to be anti-atherosclerosis. HDL-Lp-PLA_2 significantly contributed to the HDL-mediated inhibition of cell stimulation induced by oxLDL *in vitro*.[14] Adenoviral transfer of Lp-PLA_2 gene in apoE$^{-/-}$ mice increased the enzyme activity and it was associated with a reduction in macrophage homing *in vivo*.[15]

Previous studies have shown that lp-PLA_2 exists at human and rabbit atherosclerotic lesions. There, two main sources of lp-PLA_2 can be identified, namely that brought into the intima bound to LDL, and that synthesized *de novo* by plaque inflammatory cells.[16–18] Many inflammatory stimuli, such as lipopolysaccharide (LPS), up-regulate the expression of lp-PLA_2.[19] However, to our knowledge, no previous study has examined whether oxLDL, a substrate of lp-PLA_2 that co-localizes with the enzyme in atherosclerotic coronary and carotid plaques, has any effect on the expression of lp-PLA_2.

Here, we investigated the effect of oxLDL on the expression of lp-PLA_2 in monocytic cells, which are a primary source of the enzyme. We also partially characterized the intracellular signalling pathways through which oxLDL may regulate lp-PLA_2 expression. Furthermore, we also explored the effects of lp-PLA_2 on LDL uptake in macrophages, an important process in the development of atherosclerosis.

2. Methods

2.1 Materials

The RPMI1640 cell culture medium was obtained from Gibco Inc., USA. The SB203580 and other signal pathway inhibitors were obtained from Calbiochem Inc., USA. The primers for reverse transcription and PCR were synthesized by Sangon Inc., China. Other PCR-related reagents were purchased from Tiangen Inc., China. All other reagents, unless specified, were purchased from Sigma.

2.2 Lipoprotein isolation and oxidation

Human LDL ($d = 1.019–1.063$ g/mL) and HDL ($d = 1.1–1.21$ g/mL) were separated from pooled plasma by ultracentrifugation,[20] and then sequentially dialyzed at 4°C against dialysis buffer (0.02 M Tris–HCl, 0.85% NaCl, 0.02% EDTA, pH 7.4) and PBS (pH 7.4) to remove the NaBr and EDTA, respectively. Oxidation was performed by incubation of LDL (1.5 mg protein/mL) or HDL (1.5 mg protein/mL) with 10 μM $CuSO_4$ at 37°C for 20 or 48 h, respectively. For lp-PLA_2 inhibitor-treated oxLDL preparation, 1 μM of SB435495 (IC_{50} for human lp-PLA_2 is 0.06 nM)[21] was added to LDL 2 h before oxidation. The extent of LDL and HDL oxidation was followed by measuring the formation of thiobarbituric acid-reacting substances (TBARS). The TBARS values were 3.8 ± 0.4, 1.9 ± 0.5, 18.7 ± 0.8, 18.4 ± 0.8, and 15.7 ± 0.9 nmol/mg protein for LDL, HDL, oxLDL, SB435495-treated oxLDL (SB-oxLDL), and oxHDL, respectively. The lp-PLA_2 activity in oxLDL or SB-oxLDL was measured by a radioactive assay using [^{3}H] PAF as the substrate, as previously described.[22]

2.3 Lipid mass spectrometry

Equal protein concentrations of LDL, oxLDL, and SB-oxLDL (1.5 mg protein/mL) were diluted 10-fold with distilled water, and 200 μL of each sample was separately mixed with 600 μL of methanol. After vortexing, the samples were subjected to centrifugation at 15 000 *g* for 3 min. The supernatant layer was injected into a Linear Ion Trap Quadrupole Mass Spectrometer (3200 Q TRAP, AB Sciex Instruments) for analysis.

2.4 Cell culture

Monocytic THP-1 cells were maintained in RPMI1640 medium supplemented with 10% foetal bovine serum (Hyclone) at 37°C in a humidified atmosphere of 5% CO_2. Before experiments, the medium was changed to fresh serum-containing medium unless indicated.

2.5 Reverse transcription-PCR (RT–PCR)

Total RNA was isolated from cultured cells using the TRNZOL reagent (Tiangen Inc., China). First-strand cDNAs were synthesized from 4 μg of total RNA using M-MLV Reverse Transcriptase (Promega) and oligo$(dT)_{18}$. The cDNAs for lp-PLA_2 and GAPDH were amplified using the same primers and conditions as Wu *et al.*'s.[19] The PCR products were subjected to electrophoresis on 1% agarose gels and visualized with ethidium bromide.

2.6 Western blot analysis

After various treatments, cells were washed twice with PBS and lysed in lysis buffer (40 mM Tris, PH 7.5, 150 mM NaCl, 2 mM EDTA, 1% Triton X-100, 1 mM PMSF, 1 mM NaF, 1 μg/mL aprotinin, and 1 μg/mL leupeptin) on ice for 30 min. The resulting cell lysates were clarified by centrifugation at 12 000 *g* for 15 min at 4°C. The supernatant was subjected to 10% SDS–PAGE, and then transferred to nitrocellulose membranes. The membranes were blocked with 3% non-fat milk in TBST buffer (50 mM Tris, PH 7.5, 250 mM NaCl, 0.1% Tween 20) and then probed with β-actin, lp-PLA_2, p38, phosphorylated p38, akt, or phosphorylated akt antibodies overnight at 4°C. After five washes in TBST, the membranes were exposed to the appropriate secondary antibodies for 2 h at room temperature. Immunoreactive bands were visualized using chemiluminescent detection reagents, according to the manufacturer's instructions.

2.7 Oil Red O staining

Monocytic THP-1 cells were pre-treated with Phorbol-12-myristate-13-acetate (PMA, 100 nM) for 72 h to differentiate into macrophages, and then washed with PBS. Cells were incubated with LDL (50 μg/mL) alone or combined with either SB435495 (100 nM) or SB480848 (100 nM) in the serum-free medium for another 48 h. Then the cells were washed with PBS again for three times and fixed with 10% formalin for 10 min. After washing with PBS, the cells were incubated with Oil Red O solution

(0.6 g/L in 60% isopropanol) for 30 min and washed with 70% methanol for 10 min. The ORO-positive cells were counted at 20 × powered field.

2.8 Statistic analysis

Each assay was performed at least three times and the representative results were shown in the context. Data in the context were shown as mean ± SD. Differences between two groups were compared using the student's *t*-test and a value of $P < 0.05$ was considered significant.

3. Results

First, we used RT–PCR to examine the mRNA expression of lp-PLA$_2$ in THP-1 cells treated with oxLDL or LDL. As shown in *Figure 1A* and *B*, unstimulated and LDL-treated THP-1 cells failed to produce detectable levels of lp-PLA$_2$, while oxLDL-treated cells produced lp-PLA$_2$ mRNA in a concentration- and time-dependent manner. Consistent with this, lp-PLA$_2$ protein was detected weakly by western blot 24 h after oxLDL treatment (*Figure 1C*).

For comparison, we evaluated the effects of another lp-PLA$_2$ substrate, PAF, and its corresponding hydrolyzed product, lysoPAF, as well as the purchased pure phospholipid, lysophosphocholine (lysoPC, 1-palmitoyl-sn-glycerol-3-phosphocholine), on lp-PLA$_2$ expression since they have similar structures with those phospholipids in oxLDL. Unlike oxLDL, neither PAF and lysoPAF nor lysoPC could induce lp-PLA$_2$ expression (*Figure 2*). Furthermore, the up-regulation of lp-PLA$_2$ by oxLDL was not affected by the presence of these three reagents (*Figure 2*). As HDL is also the substrate of lp-PLA$_2$ and associates with the enzyme, we also tested the effect of HDL and oxHDL on lp-PLA$_2$ expression. Consistent with LDL, oxHDL could induce lp-PLA$_2$ expression while HDL could not (see Supplementary material online, *Figure S1*).

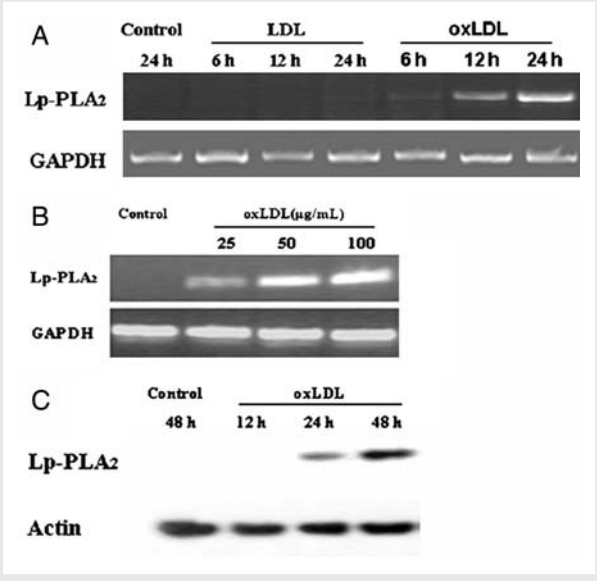

Figure 1 OxLDL induced lp-PLA$_2$ expression in THP-1 cells. (*A*) Cells were treated with 50 μg/mL LDL or oxLDL for the indicated times. The mRNA expression levels of lp-PLA$_2$ and GAPDH were determined by RT–PCR. The result is representative of five independent experiments. (*B*) Cells were treated with different concentrations of oxLDL for 24 h, and the mRNA expression levels of lp-PLA$_2$ and GAPDH were determined. The result is representative of five independent experiments. (*C*) Cells were treated with 50 μg/mL oxLDL for the indicated times, then lp-PLA$_2$ and ß-actin protein levels were determined by western blot and the result is representative of three independent experiments.

That lysoPC could not induce lp-PLA$_2$ expression led us to find the exact up-regulatory factors of lp-PLA$_2$ expression in oxLDL. Since oxLDL is known to be a complex containing various oxidized phospholipids, some of which are substrates of lp-PLA$_2$ and some of which are products of the enzyme. We used a well-studied inhibitor of lp-PLA$_2$, SB435495, to study this issue. We found that SB435495-treated oxLDL (SB-oxLDL) had nearly undetectable lp-PLA$_2$ activity (see Supplementary material online, *Figure S2*) and contained less lysoPCs than untreated oxLDL (*Figure 3A*). THP-1 cells were treated with these two kinds of oxLDL for 24 h, and then lp-PLA$_2$ mRNA levels were examined. As shown in *Figure 3B*, SB-oxLDL stimulated more lp-PLA$_2$ mRNA expression than oxLDL. These results indicated that those unhydrolyzed oxidized phospholipids (oxPCs) in oxLDL may contribute to the up-regulatory effect of oxLDL on lp-PLA$_2$ expression, since SB-oxLDL contained more oxPCs than oxLDL did as oxPCs were hydrolyzed less in SB-oxLDL. To further confirm this hypothesis, we repeated these experiments using a synthesized oxidized phospholipid (oxPC, 1-palmitoyl-2-(3-carboxypropanoyloxy)-sn-glycerol-3-phosphocholine), and found that oxPC obviously up-regulated lp-PLA$_2$ mRNA expression in a dose-dependent manner (*Figure 3C*).

Next, we examined the signalling pathways potentially involved in oxLDL or oxPCs-induced lp-PLA$_2$ expression in THP-1 cells. Many inhibitors of several signal transduction molecules were tested and finally we found that the inhibitors of PI3K,

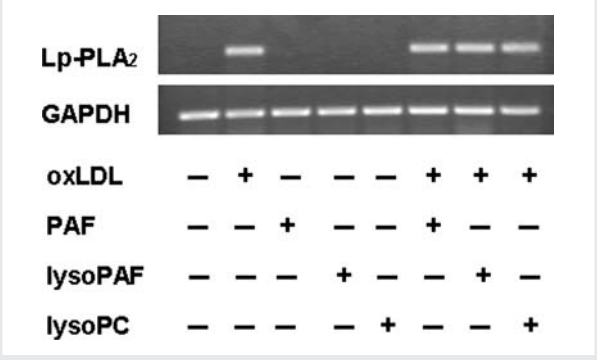

Figure 2 PAF, lysoPAF, and lysoPC had no up-regulatory effect on lp-PLA$_2$ expression. Cells were treated with PAF (500 ng/mL), lysoPAF (500 ng/mL), lysoPC (50 μM), or oxLDL (50 μg/mL) for 24 h, or pre-treated with PAF (500 ng/mL) or lysoPAF (500 ng/mL) for 30 min before oxLDL (50 μg/mL) stimulation for 24 h. The mRNA expression levels of lp-PLA$_2$ and GAPDH were determined by RT–PCR. The figure is representative of five independent experiments.

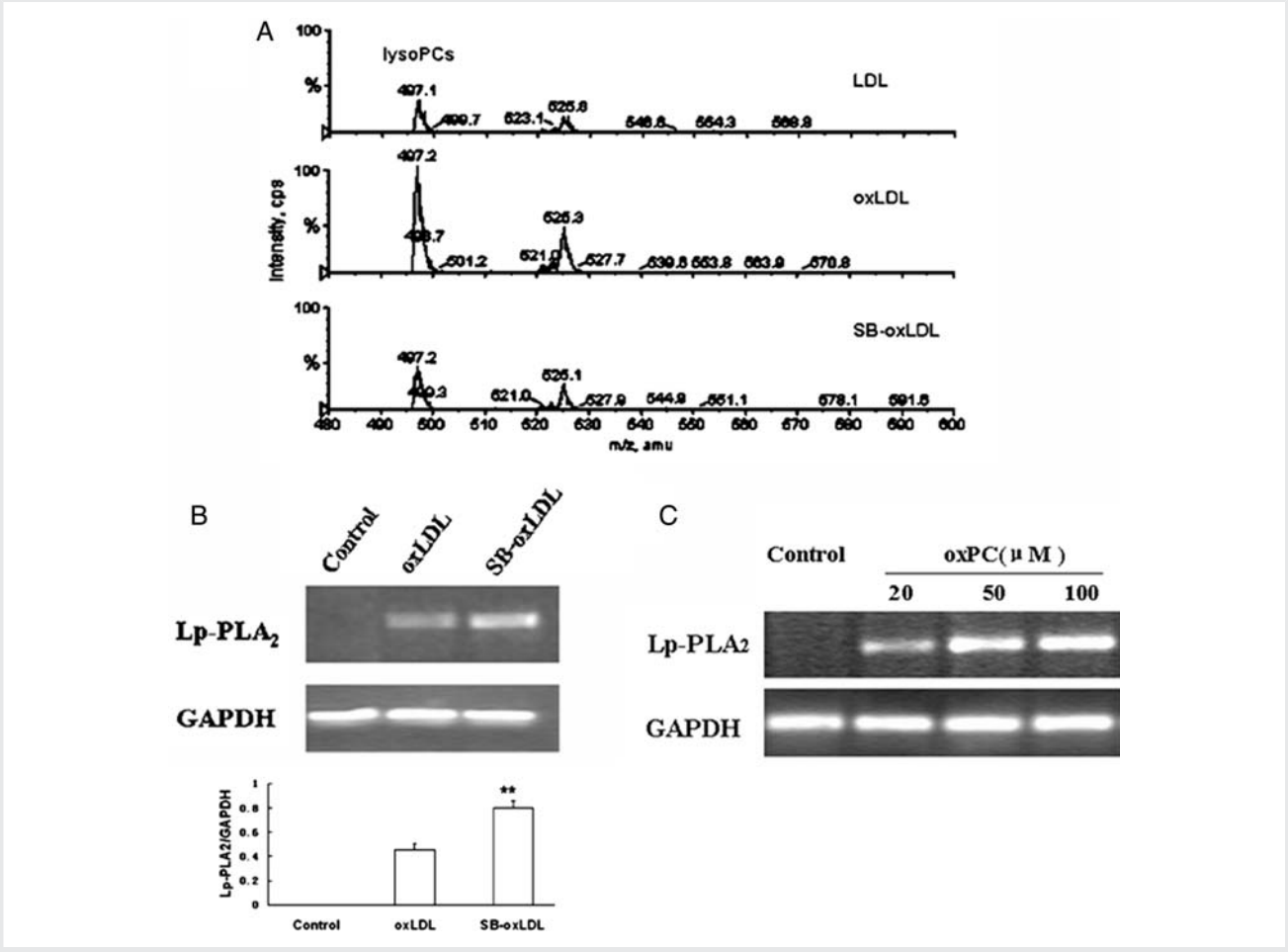

Figure 3 Unhydrolyzed oxidized phospholipids in oxLDL mediated the up-regulatory effect of oxLDL on lp-PLA_2 expression. (*A*) Representative ESI-MS spectra of the lysoPC content in LDL and oxidized LDL in the absence (oxLDL) and presence (SB-oxLDL) of the lp-PLA_2 inhibitor, SB435495. (*B*) Cells were treated with 25 μg/mL oxLDL or SB-oxLDL for 24 h, and the mRNA expression levels of lp-PLA_2 and GAPDH were determined. $^{**}P < 0.01$ vs. oxLDL group. (*C*). Cells were treated with different concentrations of oxPC for 24 h, then the mRNA expression levels of lp-PLA_2 and GAPDH were determined by RT–PCR. The result is representative of five independent experiments.

LY294002, and wortmannin, and the inhibitors of p38 mitogen-activating protein kinase (MAPK), SB203580, and SB202190, could completely block the expression of lp-PLA_2 up-regulated by oxLDL or oxPC (*Figure 4*). These results demonstrated PI3K and p38 might mediate the effects of oxLDL on lp-PLA_2 expression (*Figure 5*). Indeed, oxLDL/oxPC could activate akt, the substrate of PI3K and p38 phosphorylation. However, pre-treatment of cells with each of the four inhibitors prevented p38 activation (*Figure 5A* and *B*), while the two p38 inhibitors could not significantly prevent akt phosphorylation (*Figure 5C* and *D*). These results suggest that oxLDL/oxPC might activate PI3K and p38 MAPK sequentially to up-regulate lp-PLA_2 expression.

To our knowledge, the study of the role lp-PLA_2 in atherosclerosis mainly focuses on the view of inflammation and anti-oxidation. Thus, more direct studies of its role in atherosclerosis should be carried out. As lp-PLA_2 is expressed in atherosclerotic plaques, we finally examined the role of lp-PLA_2 in lipid accumulation, a key step in foam cell formation, using macrophagic THP-1 cells as a model. First, we completely inhibited the lp-PLA_2 enzyme activity in the whole culture media with 100 nM of its two well-known inhibitors, SB435495 or SB480848 (darapladib). Then we assessed the uptake of LDL by macrophagic THP-1 cells. Oil Red O staining indicated that inhibition of lp-PLA_2 in the condition significantly decreased intracellular lipid accumulation (*Figure 6*).

4. Discussion

Lp-PLA_2, which binds primarily with LDL in humans, can hydrolyze oxidative phospholipids in LDL. Given that lp-PLA_2 and LDL coexist in atherosclerotic plaques, we herein examined a potential regulatory relationship between the two, and examined some potential pathways underlying this interaction. We first found that oxLDL could up-regulate lp-PLA_2 expression in monocytic THP-1 cells. Notably, another substrate of lp-PLA_2, PAF, and its corresponding product, lysoPAF, had no such effect. Furthermore, neither PAF nor lysoPAF could antagonize oxLDL-induced lp-PLA_2 expression.

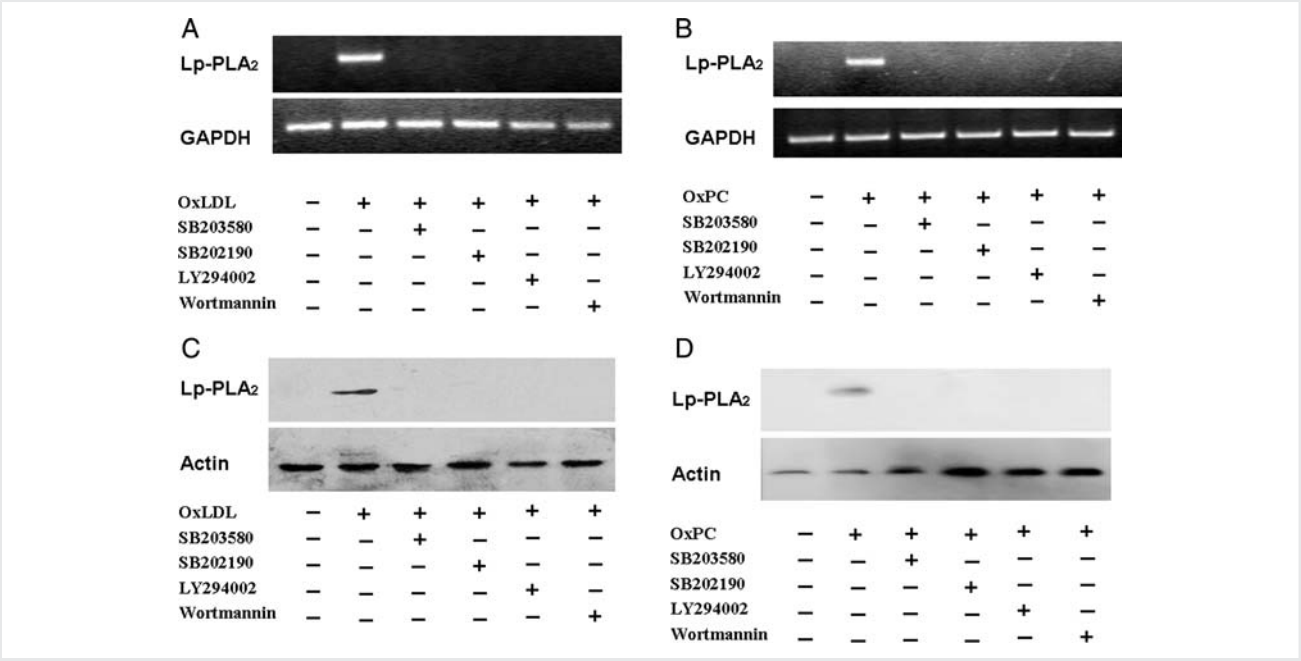

Figure 4 Inhibition of PI3K or P38 MAPK blocked oxLDL-induced lp-PLA$_2$ expression. (*A* and *B*) Monocytic THP-1 cells were pre-treated with the specific p38 inhibitors, SB203580 (20 μM) and SB202190 (20 μM) or PI3K inhibitors, LY294002 (10 μM) and wortmannin (500 nM) for 30 min prior to incubation with 50 μg/mL oxLDL (A) or 50 μM oxPC (B) for 24 h. The mRNA expression levels of lp-PLA$_2$ and GAPDH were determined by RT–PCR and representative results of five independent experiments are shown. (*C* and *D*) Monocytic THP-1 cells were pre-treated with the specific p38 inhibitors, SB203580 (20 μM) and SB202190 (20 μM) or PI3K inhibitors, LY294002 (10 μM) and wortmannin (500 nM) for 30 min prior to incubation with 50 μg/mL oxLDL (C) or 50 μM oxPC (D) for 48 h. The protein levels of lp-PLA$_2$ and β-actin were determined by western blot and representative results of three independent experiments are shown.

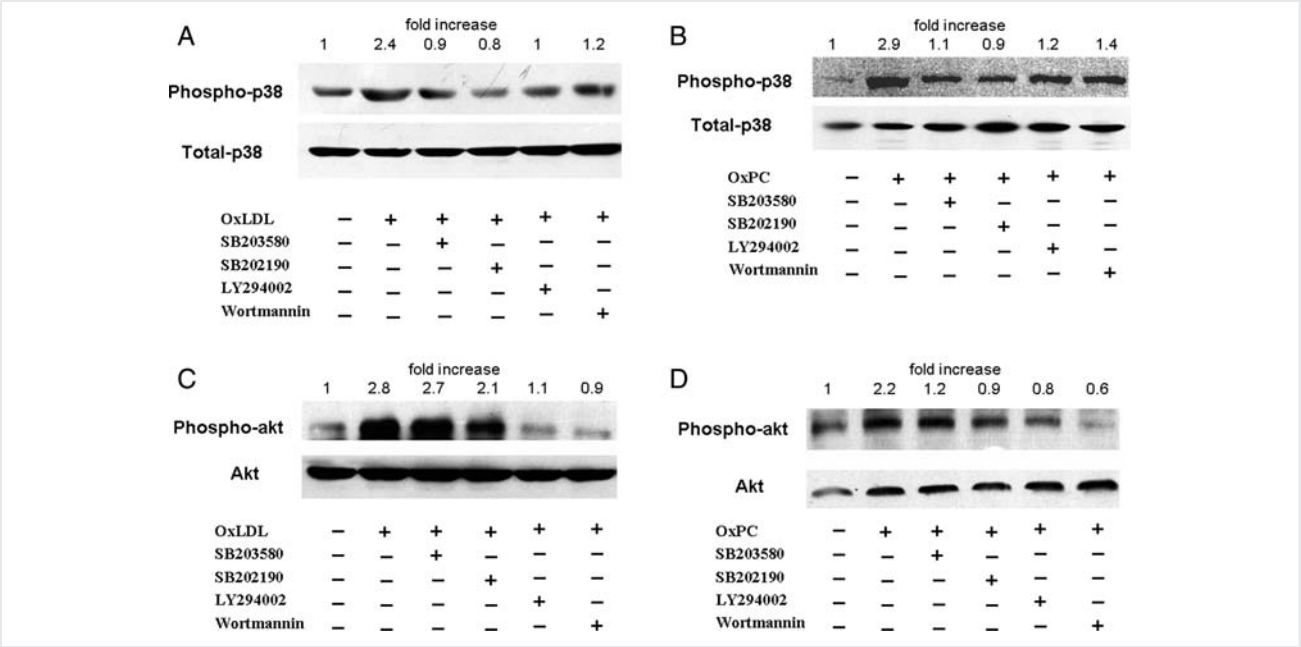

Figure 5 OxLDL and oxPC induced the Akt phosphorylation and p38 phosphorylation in monocytic THP-1 cells. (*A* and *B*) Cells were pre-treated with the specific p38 inhibitors, SB203580 (20 μM) and SB202190 (20 μM) or PI3K inhibitors, LY294002 (10 μM) and wortmannin (500 nM) for 30 min prior to incubation with 50 μg/mL oxLDL (A) or 50 μM oxPC (B) for 6 h. The phosphorylation of p38 was determined by western blot. (*C* and *D*) Monocytic THP-1 cells were pre-treated with the specific p38 inhibitors, SB203580 (20 μM) and SB202190 (20 μM) or PI3K inhibitors, LY294002 (10 μM) and wortmannin (500 nM) for 30 min prior to incubation with 50 μg/mL oxLDL (C) or 50 μM oxPC (*D*) for 10 min. The Akt phosphorylation was determined by western blot. The results are representative of three independent experiments.

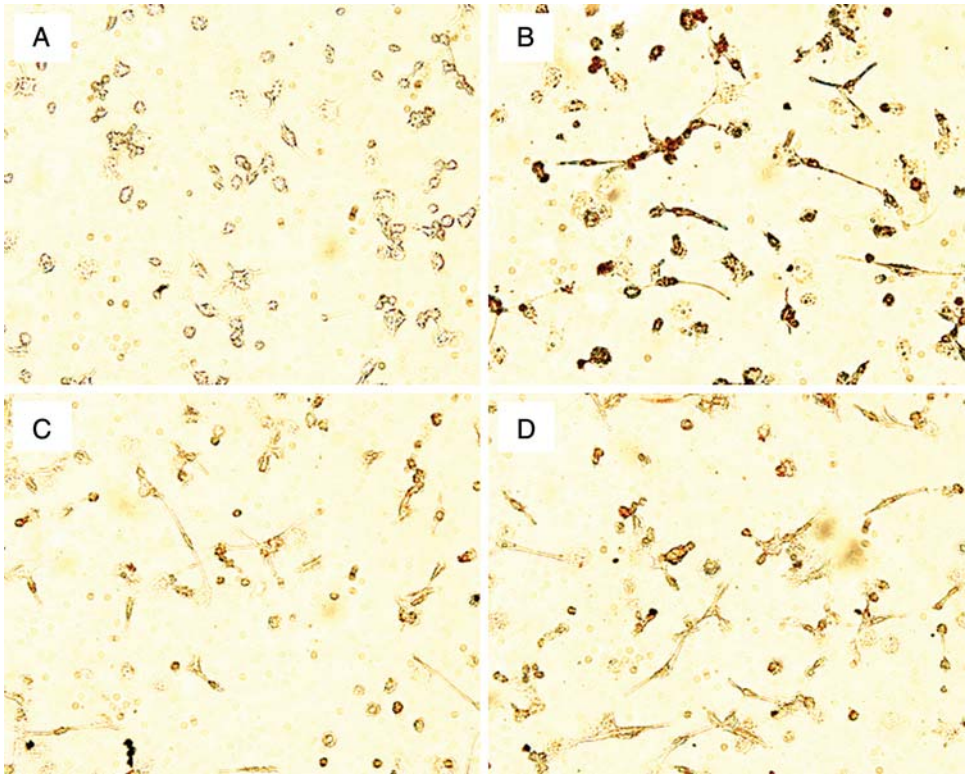

Figure 6 Inhibition of lp-PLA_2 activity in the medium decreased the lipid accumulation in macrophages. THP-1 macrophages were incubated with 50 μg/mL LDL alone or combined with 100 nM either SB435495 or SB480848 in the serum-free medium for 48 h. Lipid accumulation was observed and counted through light microscopy after ORO staining at 20 × magnification. (*A*) Blank; (*B*) LDL; (*C*) LDL + SB435495. (*D*) LDL + SB480848. The pictures are representative of five independent experiments.

The oxPCs in oxLDL and PAF are well-known inflammatory mediators that have been implicated in the progression of atherosclerosis.[5] Lp-PLA_2 can hydrolyze these two phospholipids, suggesting that it may modulate the process of atherosclerosis. However, the precise role of lp-PLA_2 in atherosclerosis is controversial, with previous studies proposing seemingly contradictory anti- and pro-atherogenic functions. Lp-PLA_2 is thought to exert anti-atherogenic effects by degrading pro-inflammatory mediators, such as PAF and oxidized phospholipids. However, recent findings have ascribed several anti-inflammatory properties to oxidized phospholipids.[23,24] In addition, intravenous administration of recombinant lp-PLA_2 failed to alter PAF-mediated responses in patients with asthma or septic shock.[25,26] Furthermore, the pro-atherogenic role of lp-PLA_2 is strongly supported by its ability to hydrolyze oxidized phospholipids in oxLDL, leading to the formation of downstream inflammatory mediators, such as the lysoPCs and oxNEFAs. The lysoPCs can up-regulate expression of adhesive molecules (e.g. VCAM-1) in endothelial cells, and that of inflammatory genes (e.g. MCP-1 and IL-1ß) in smooth muscle cells and monocytes. In addition, both the lysoPCs and the oxNEFAs are cytotoxic to many vascular cells.[25,26] Indeed, the lp-PLA_2 inhibitor, Darapladib, has shown beneficial effects against atherosclerosis in both animal assays and clinical trials. Darapladib exerted an anti-inflammatory action and inhibited progression to advanced atherosclerotic lesions in porcine models.[27] Darapladib also have shown good effects against inflammatory burden and necrotic core expansion, a key determinant of plaque vulnerability.[28,29]

Our results further demonstrated that the effect of oxLDL on lp-PLA_2 expression was due to its oxidized phospholipids (oxPCs), but not their hydrolyzed products, the lysophosphatidylcholines (lysoPCs). As LDL invades the vascular wall through injured endothelium and begins the process of oxidation under oxidative stress conditions, oxidized phospholipids form and accumulate in atherosclerotic lesions.[30] Those oxLDL/oxPCs can cause vascular cells to express many genes that are involved in inflammatory reactions, such as MCP-1, CXCR2, and IL-8.[31] Studies have also shown that oxidized phospholipids activate many signalling pathways, such as the MAPK, NFκB, and PPAR pathways.[31]

In this study, we first reported that PI3K participated in the regulation of lp-PLA_2 expression. PI3K has been shown to play a critical role in cell survival.[32] PI3K are also involved in the regulation of gene expression. A recent study revealed PI3K positively regulated lipopolysacchride-induced IL-12 production in human macrophages and dendritic cells.[33]

The results of the present study also suggest that the p38 MAPK pathway participates in oxLDL-mediated modulation of lp-PLA_2

expression. A well-known inflammatory stimulator, lipopolysaccharide, has been demonstrated to upregulate lp-PLA$_2$ expression through the p38 MAPK pathway in monocytic THP-1 and RAW264.7 cells.[19] The ability of LPS to induce transcriptional activation of lp-PLA$_2$ is not due to enhanced Sp1/Sp2 binding to the promoter, but rather involves enhanced transactivating function of Sp1.[19] The p38 MAPK pathway mediates the expression of many genes involved in stress-induced responses, such as IL-1ß, iNOS, and cyclooxygenase-2.[34,35] Here, we examined whether p38 MAPK was involved in the oxLDL-induced up-regulation of lp-PLA$_2$. Indeed, the p38-specific inhibitors, SB203580 reduced p38 phosphorylation and completely blocked oxLDL-induced lp-PLA$_2$ expression in THP-1 monocytes. Our findings and Wu *et al.*'s[19] suggest that p38 plays a key role in the regulation of lp-PLA$_2$ expression. Thus, p38 may be a good target for drugs to intervene in lp-PLA$_2$ production instead of inhibiting the enzyme activity.

Previous reports revealed that PI3K participates in the activation of p38.[36] That inhibition of PI3K reduced p38 phosphorylation activated by oxLDL in our results also confirmed this finding and further demonstrated that the interaction of PI3K and p38 MAPK play a critical role in the regulation of lp-PLA$_2$ expression. OxLDL could up-regulate lp-PLA$_2$ expression through sequential activation of PI3K and p38. Additional intensive investigation is needed to be done to elucidate the precise mechanism how PI3K activate p38 pathway.

Lp-PLA$_2$ is considered to be a marker of cardiovascular risk. Several studies have demonstrated that the mass or activity of lp-PLA$_2$ is higher in those who are susceptible to cardiovascular risk.[6–10] Some other studies have shown that lp-PLA$_2$ is expressed by macrophages in human and rabbit atherosclerotic lesions.[16–18] In a porcine model, lp-PLA$_2$ was found to increase rapidly over time in porcine plasma and coronary arteries.[13] Our present finding that oxLDL can induce lp-PLA$_2$ expression, combined with the prior knowledge that oxLDL accumulates in atherosclerotic plaques, may provide at least part of the mechanism whereby lp-PLA$_2$ expression increases during the development of atherosclerosis.

Inhibition of lp-PLA$_2$ could decrease inflammatory gene expression induced by oxLDL, and attenuate the cytotoxicity of oxLDL to vascular cells.[11–13] In addition, our finding in this context suggested a novel pro-atherogenic role of lp-PLA$_2$ as inhibition of its activity seemed to attenuate the uptake of lipids in macrophages, which is a key step in foam cell formation during the development of atherosclerosis.

In summary, our data demonstrate that oxLDL stimulates lp-PLA$_2$ expression in monocytic THP-1 cells. This up-regulatory effect of oxLDL is due to its oxidized phospholipids but not their hydrolyzed products, the lysoPCs. The oxLDL-induced effect appears to be critically mediated via the PI3K and p38 MAPK pathway. As a model, we suggest that the oxidized phospholipids of oxLDL accumulate in the atherosclerotic lesions and up-regulate the production of lp-PLA$_2$. In turn, the generated lp-PLA$_2$ hydrolyzes the oxidized phospholipids to produce more bioactive lipid mediators, thereby promoting the development of atherosclerosis. In this view, our findings provide some new insights into the relationship between oxLDL and lp-PLA$_2$ in the context of atherosclerosis. In addition, our findings also suggest a new beneficial effect of anti-oxidation therapy on atherosclerosis that may decrease LDL oxidation and subsequent lp-PLA$_2$ production.

Supplementary material

Supplementary material is available at *Cardiovascular Research* online.

Conflict of interest: none declared.

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