Phospholipase A₂–Modified Low-Density Lipoprotein Activates the Phosphatidylinositol 3-Kinase-Akt Pathway and Increases Cell Survival in Monocytic Cells

Dmitry Namgaladze, Bernhard Brüne

Objective—Monocyte survival is an important determinant in the development of atherosclerotic lesions. We investigated the influence of phospholipase A₂-modified LDL (PLA-LDL), a pro-atherogenic factor, on activation of the pro-survival kinase Akt and cell death in monocytic cells.

Methods and Results—PLA-LDL induced robust phosphorylation and activation of Akt in THP1 cells. It also attenuated oxidative stress-induced cell death, an effect abolished by phosphatidylinositol 3-kinase (PI3K) inhibition. In addition, PLA-LDL increased survival of human monocytes. We noticed that lipid products derived from LDL phospholipolysis are mediators of PLA-LDL–induced Akt activation. Arachidonic acid, which is released on phospholipase treatment of LDL, induced Akt phosphorylation and increased cell survival, whereas lysophosphatidylcholine, another compound generated by LDL phospholipolysis, induced only transient Akt phosphorylation and was cytotoxic.

Conclusions—Our data indicate that PLA-LDL induces activation of the PI3K-Akt pathway and promotes monocytic cell survival, which may contribute to the pro-atherogenic effects of phospholipase A₂-modified LDL. (Arterioscler Thromb Vasc Biol. 2006;26:2510-2516.)

Key Words: apoptosis ■ atherosclerosis ■ low-density lipoprotein ■ monocytes ■ phospholipase A₂

Monocytes play a crucial role in the development of atherosclerotic lesions. In response to atherogenic factors, monocytes migrate from the blood into the intima where they differentiate into macrophages, take up modified low-density lipoprotein (LDL), and progress to foam cells. As lesions advance, these cells eventually die, forming a necrotic core, one of the hallmarks of rupture-prone plaques.

Death of phagocytic cells is considered pivotal for lesion progression. It is widely accepted that macrophage cell death in the advanced lesion may contribute to plaque instability and therefore increases the risk of clinical events. In the early stages of the disease, however, cell death can reduce the number of phagocytes accumulating in the lesion and thus impair atheroma progression. Supporting evidence for this scenario was obtained in recent studies in transgenic mice. Thus, deletion of the macrophage survival factor AIM (apoptosis inhibitor expressed in macrophages) increased macrophage apoptosis and reduced early atherosclerotic lesions in LDL receptor knockout mice.

Conversely, deletion of the pro-apoptotic protein Bax increased lesion size in LDLR⁻/⁻ mice. Therefore, factors controlling monocyte death or survival in the development of atherosclerotic lesion are of considerable clinical importance.

Akt/protein kinase B is a major cellular kinase with a distinct pro-survival action. It is often activated in human malignancies, mostly because of mutations of its negative regulator PTEN. The mechanism of Akt activation involves its membrane translocation and phosphorylation in response to phosphorylation of inositol lipids by phosphatidylinositol 3-kinase (PI3K). Activated Akt phosphorylates several proteins regulating apoptosis/survival, including Bad, caspase-9, Foxo3a, or hexokinase. Activation of Akt was shown to be critical for monocyte survival in various experimental-settings.

LDL accumulation in the vessel wall and its consequent modification is a major driving force of atherosclerosis development. Hydrolysis of LDL phospholipids by secretory phospholipase A₂ (sPLA₂) is observed in addition to other modification such as oxidation, aggregation, or proteolysis during lesion progression. sPLA₂ enzymes are synthesized by all major cell populations in the vessel wall and their levels increase during inflammation. LDL modification by sPLA₂ enhances its uptake by macrophages and concomitant foam cell formation. It also provokes increased LDL aggregation and retention in the arterial wall via enhanced binding to proteoglycans. In addition, expression of human group IIA sPLA₂ in macrophages of LDL receptor-deficient mice was shown to result in an increase of lesion size and macrophage cell numbers. However, little is known about signal transduction pathways activated by sPLA₂-modified LDL.
(PLA-LDL). It was demonstrated that PLA-LDL induces arachidonic acid release and increases tumor necrosis factor-α secretion in human THP-1 monocytes, although underlying mechanisms remain unknown.

In this work we studied the effects of PLA-LDL on Akt signaling and survival in THP1 monocyte cells and in human monocytes. We present evidence that PLA-LDL induces robust Akt activation and increases cell survival, which is PI3K/Akt-dependent. We also provide information that non-esterified fatty acids (NEFA) rather than lysophosphatidylcholine may mediate pro-survival effects of PLA-LDL.

Materials and Methods

Materials

Cell culture medium and supplements were from PAA Laboratories GmbH (Coelbe, Germany). A protein assay kit was from Bio-Rad (Munich, Germany). Protease inhibitor mixture came from Roche Diagnostics (Mannheim, Germany). Nitrocellulose membrane, ECL detection system, and horseradish peroxidase-labeled antimouse or antirabbit secondary antibodies were from GE Healthcare (Munich, Germany). LY294002 was from Alexis (Lausen, Switzerland). Snake venom phospholipase A2 (Naja mossambica mossambica, P7778), Methyliethaolylidiphenyl-tetrazolium bromide (MTT, M2128), lysophosphatidylcholine (L4129), arachidonic (A9673), and linoleic acid (L1376) were from Sigma (Taufkirchen, Germany). Primary antibodies against phospho-Akt Ser473 (#9271), Thr308 (#9272), phospho-GSK3 Ser9 (#9336) were from Cell Signaling Technology (Beverly, Mass). Human recombinant IL-4 (M2122), IL-10 (M1020), IL-13 (M3124), TNF-α (M406), IL-1β, IL-6 (M600), and IL-8 (M664) were from PeproTech (London, England). Secondary antibodies were from Thermo Fisher (Waltham, Mass). primary antibodies against phospho-Akt Thr308 (9274), Akt (9272), Akt (P52), phospho-PI3K (2098), Akt (9272), phospho-GSK3 (9336), and α-actin (13079) were from Cell Signaling Technology (Beverly, Mass). Human recombinant group V PLA2 was a kind gift of Dr Wonhwa Cho (University of Illinois at Chicago, Ill).

LDL Isolation and Treatment

Human LDL (d=1.02 to 1.06 g/mL) was isolated from plasma of healthy volunteers (DRK-Blutspendedienst Baden-Württemberg-Hessen, Institut für Transfusionsmedizin und Immunhämatologie Frankfurt am Main, Frankfurt, Germany) by sequential ultracentrifugation. It was maintained in the presence of 0.2 mmol/L EDTA to prevent oxidation. Endotoxin content of the preparations was <0.05 ng/mg LDL (Cambrex Biosciences, Verviers, Belgium). Phospholipidosis was performed by incubating LDL with 0.5 U/mL phospholipase A2 and 10 mmol/L CaCl2 for 2 hours at 37°C or with 20 mmol/L human group V PLA2 and 2 mmol/L CaCl2 for 16 hours at 37°C. The reaction was stopped by adding 10 mmol/L EGTA. Aggregated LDL was prepared by vortexing LDL for 1 minute. Lipid extraction was performed by adding twice the volume of methanol/chloroform (1:1) to PLA-LDL in the presence of 10 mmol/L HCl. The chloroform phase was evaporated under nitrogen followed by resuspension in phosphate-buffered saline (PBS). For ApoB proteolysis PLA-LDL samples were incubated for 16 hours at 37°C with 20 μg/mL proteinase K and the extent of ApoB proteolysis was controlled by gel electrophoresis and Coomasie staining. For bovine serum albumin (BSA) treatment PLA-LDL was incubated for 1 hour at 37°C with 10% BSA and LDL was re-isolated by flotation centrifugation. The NEFA content of LDL samples was analyzed using NEFA colorimetric detection kit (Wako Chemicals, Neuss, Germany). Oxidized LDL was prepared by incubating LDL with 5 μmol/L CuSO4 at room temperature for 24 hours followed by dialysis against PBS with 100 μmol/L EDTA.

Monocyte Isolation

Human monocytes were isolated from buffy coats (DRK-Blutspendedienst Baden-Württemberg-Hessen, Institut für Transfusionsmedizin und Immunhämatologie Frankfurt am Main, Frankfurt, Germany) using Ficoll density centrifugation (LSM 1077; PAA Laboratories GmbH, Coelbe, Germany) followed by magnetic separation with negative selection (Monocyte Isolation Kit II; Miltenyi Biotec, Bergisch Gladbach, Germany). Purity of monocytes was assessed by flow cytometry using CD14 staining.

Cell Culture

The THP1 monocyte cell line was maintained in RPMI 1640 medium containing 100 U/mL penicillin, 100 μg/mL streptomycin, and 10% heat-inactivated fetal calf serum. Before experiments the medium was changed for fetal calf serum-free medium.

Western Blot Analysis

Cells were incubated for 16 hours in serum-free medium, treated, pelleted, lysed in 200 μL of buffer A (50 mmol/L Tris, 150 mmol/L NaCl, 5 mmol/L EDTA, 0.5% NP-40, 10 mmol/L NaF, 1 mmol/L Na3VO4, 1 mmol/L PMSF, and protease inhibitor mixture [pH 7.5]), sonicated, followed by centrifugation (15 000g, 10 minutes); 40 μg of protein were loaded onto and resolved on 10% SDS-polyacrylamide gels. Proteins were blotted onto nitrocellulose, and the membrane was blocked and incubated with primary antibodies according to manufacturer instructions. Membranes were washed 3 times for 5 minutes each with TTBS (10 mmol/L Tris, 150 mmol/L NaCl, 0.05% Tween 20, pH 7.5). For protein detection, blots were incubated with a horseradish peroxidase-labeled secondary antibody for 1 hour and washed 3 times for 5 minutes each with TTBS, followed by ECL detection.

Cell Death Detection

Cells were incubated for 16 hours in serum-free medium, treated, and processed for cell death detection by flow cytometry (fluorescence-activated-cell sorter [FACS]; Canto, BD Biosciences, Heidelberg, Germany) using Annexin V-fluorescein isothiocyanate (FITC) apoptosis detection kit (Beckman Coulter, Krefeld, Germany) according to manufacturer instructions. Cell death was determined as the sum of apoptotic and necrotic cells. Additionally, mitochondrial membrane potential was measured by flow cytometry using the mitochondrial fluorescent dye DiOC6 (40 nmol/L, 30 minutes) and cell death was determined as the percentage of cells showing reduced mitochondrial membrane potential.

Monocyte Viability Determination

Monocytes were plated on 96-well plates in serum-free RPMI 1640 medium at 2×10⁴ cells/well and incubated for 18 hours. Then medium was changed and the cells were further cultivated in serum-free medium with or without PLA-LDL and/or H2O2 for 48 hours. Finally, MTT (1 mg/mL) was added and cells were incubated for 4 hours before cell lysis and determination of formazan formation by measuring absorbance at 560 nm. Relative cell viability was presented as ratio of A560 of treated cells to untreated controls.

Statistical Analysis

Data are expressed as mean±SEM. Two treatment groups were compared by the independent Student test. Results were considered statistically significant with a value of P<0.05.

Results

To investigate the impact of modified LDL on Akt activation in THP1 cells we monitored phosphorylation of Akt at S473, located in its hydrophobic tail and on T308, found in its activation loop using Western blot with site-specific phospho-antibodies. Phosphorylation on both residues is required for full Akt activation. As shown in Figure 1A, PLA2-treated LDL (PLA-LDL) induced phosphorylation of Akt on both S473 and T308, whereas native LDL and PLA2 alone had no effect. The membrane in this and further experiments was stripped and re-probed with a pan-Akt antibody to verify equal amounts of Akt protein. It was reported that PLA2-treated LDL has a tendency to aggregate, and aggregated LDL has an increased capacity to

[References and data analysis]
induce foam cell formation. To test whether LDL aggregation per se might activate Akt in THP1 cells, we prepared aggregated LDL by vortexing native LDL and investigated its effect on Akt phosphorylation. However, aggregated LDL did not affect Akt phosphorylation (Figure 1A).

We then investigated PLA-LDL-induced Akt activation in further detail. Figure 1B shows that PLA-LDL caused robust Akt phosphorylation, which lasted at least for 8 hours. To examine whether increased Akt phosphorylation is associated with an increased enzymatic activity we monitored phosphorylation of GSK3, an established Akt protein target. Compared with controls phosphorylation of GSK3 increased after PLA-LDL treatment (Figure 1B), confirming that PLA-LDL treatment not only phosphorylated but also activated Akt.

Looking at the concentration dependency of PLA-LDL-induced Akt phosphorylation revealed an extreme sensitivity. Akt phosphorylation was detected at 0.2 μg/mL PLA-LDL and maximum effects were reached at 1 μg/mL PLA-LDL (Figure 1C).

In many cases Akt phosphorylation follows the activation of PI3K. PI3K generates PIP₃, which is necessary for Akt translocation to the plasma membrane where Akt is phosphorylated and activated. To investigate whether Akt phosphorylation requires PI3K enzymatic activity we used 2 PI3K inhibitors, wortmannin and LY294002. As shown in Figure 1D, both inhibitors abolished PLA-LDL-induced Akt phosphorylation, suggesting that PI3K activity is necessary for PLA-LDL to activate Akt.

Although most of the effects attributed to PLA₂-treated LDL described in the literature resulted from PLA₂ enzymatic activity, it is possible that PLA₂ may exert activity-unrelated effects by, eg, activating PLA₂ receptors on the cell surface. To prove that indeed PLA-LDL requires PLA₂ enzymatic activity to phosphorylate Akt we exposed LDL to PLA₂ in the presence of 10 mmol/L EGTA instead of calcium. As shown in Figure 2A, this treatment greatly reduced the ability of PLA-LDL to phosphorylate Akt. Thus, enzymatic activity of
PLA₂ is necessary to confer an Akt-activating property to LDL.

PLA₂ treatment of LDL generates NEFA, preferentially linoleic (LA) and arachidonic (AA) acids, and lysophospholipids, mostly lysophosphatidylcholine (LPC). Phospholipolysis also changes the physico-chemical characteristics of LDL, including an increased density and negative charge. In addition, phospholipolysis changes ApoB conformation, which causes an altered interaction of LDL with proteoglycans and probably enhances its uptake by macrophages. To distinguish whether PLA-LDL provokes Akt activation via its lipid or protein moiety, we extracted lipids from PLA-LDL and tested their influence on Akt phosphorylation. Figure 2B demonstrates that lipid extracts from PLA-LDL mimicked the effect of authentic PLA-LDL particles. Furthermore, we performed proteolysis of ApoB using proteinase K (PK). Coomassie staining of authentic and PK-treated PLA-LDL subjected to gel electrophoresis confirmed the absence of intact ApoB in the proteolyzed sample (data not shown). Interestingly, proteolyzed PLA-LDL phosphorylated Akt comparably to PLA-LDL (Figure 2B), suggesting that the intact ApoB moiety of PLA-LDL is dispensable for Akt activation.

Lipid products of PLA-LDL phospholipolysis, such as LPC and NEFA, are reported to execute a broad spectrum of physiological actions, including modulation of PI3K-Akt. To obtain information, which lipid product might mediate the PLA-LDL effect on Akt phosphorylation, we incubated cells with relevant fatty acids present in LDL particles, such as AA, as well as LPC. Both, AA and LPC induced Akt phosphorylation after 10 minutes of incubation (Figure 2C). While phosphorylation induced by AA lasted for 8 hours, LPC-induced Akt phosphorylation decreased by 4 hours and dephosphorylation was observed after 8 hours. These data indicate that NEFA rather than LPC may transmit the Akt-activating effect of PLA-LDL.

Another possibility to test whether lipid products generated by LDL phospholipolysis are responsible for Akt phosphorylation by PLA-LDL is their depletion from PLA-LDL particles by incubation with fatty acid-free BSA and subsequent re-isolation of PLA-LDL. Performing these experiments, the NEFA content of PLA-LDL was reduced from 0.77 ± 0.01 to 0.05 ± 0.02 μmol/mg as determined by using a colormetric NEFA detection kit. Figure 2D shows that BSA-treated PLA-LDL induced comparably less Akt phosphorylation than PLA-LDL incubated with PBS instead of BSA. This information supports the idea that lipid products generated during phospholipolysis may be involved in PLA-LDL-induced Akt phosphorylation.

Akt signaling usually provokes a pro-survival response. To test the hypothesis that PLA-LDL–induced Akt activation affects survival of THP1 cells, we subjected cells to oxidative stress using 1 mmol/L H₂O₂. Cell viability was measured using Annexin V/propidium iodide staining. In addition, we measured mitochondrial membrane potential by DiOC₆ staining as a second, independent parameter of cell death. H₂O₂ caused loss of cell viability in 25% to 30% of cells after 8 hours (Figure 3A and 3B). When PLA-LDL was added together with H₂O₂, cell death was significantly reduced whereas in cells treated with native LDL cell death proceeded comparably to controls. Moreover, PLA-LDL reduced cell death in serum-free medium. In addition, PLA-LDL protected cells against cell death induced by high concentrations of oxidized LDL (supplemental Figure I, available online at http://atvb.ahajournals.org).

To verify that pro-survival effects of PLA-LDL are not limited to cell lines we determined cell viability using the MTT assay in primary human monocytes. PLA-LDL increased viability of monocytes in serum-free medium (Figure 3C). In addition, it protected monocytes against cytotoxic actions of H₂O₂, thereby confirming that PLA-LDL is also cytoprotective in primary cells.

To exclude the possibility that the pro-survival effect of PLA-LDL is related to any particular feature of snake venom PLA₂ we performed phospholipolysis of LDL with recombinant human group V PLA₂, which was shown to be present in atherosclerotic plaques. As shown on Figure 3D, phospholipolysis of LDL by group V PLA₂ confers a similar cytoprotective property to LDL as hydrolysis by snake venom PLA₂.

To question PI3K-Akt signaling in transmitting the pro-survival effect of PLA-LDL, we pre-incubated cells with LY294002, a PI3K inhibitor, before treatment with PLA-LDL and H₂O₂. As shown in Figure 4, H₂O₂-induced cell death was significantly reduced in the presence of PLA-LDL. LY294002 reversed protection elicited by PLA-LDL, whereas it had little effect on its own. These results indicate that PI3K-Akt signaling contributes to the cytoprotective action of PLA-LDL.

We also wanted to know whether lipid products of LDL phospholipolysis, such as NEFA and LPC, affect THP1 survival. Figure 5 shows that AA alone left cell survival unaltered, whereas LPC increased cell death. When AA was added together with H₂O₂, cell death was significantly reduced. In contrast, LPC did not protect against H₂O₂ toxicity. These data support the notion that NEFA mediate the pro-survival effect of PLA-LDL.

It is known that modified LDL, such as oxidized LDL, can be both cytoprotective at low and cytotoxic at high concentrations. To investigate whether PLA-LDL could be cytotoxic, we treated the cells with increasing concentrations of PLA-LDL and measured cell death by Annexin/PI labeling. PLA-LDL became cytotoxic at concentrations of 50 μg/mL and higher doses (supplemental Figure II).

Discussion

Mechanisms controlling monocyte survival are central in the development of atherosclerosis. In this study we show that the pro-atherosclerotic factor PLA₂ modified LDL to increase monocyte cell survival under conditions of oxidative stress via activation of the PI3K-Akt pathway. PLA-LDL was also cytoprotective in primary human monocytes, underscoring the physiological significance of PLA-LDL effect. Because phagocyte survival enhances the development of atherosclerosis, at least in its early stage, PLA-LDL–induced survival appears to be one of the mechanisms explaining a pro-atherogenic action of sPLA₂. Studies in animal models showed that sPLA₂ overexpression in macrophages increases...
the number of macrophages in the lesion. Decreased cell death may account for this effect.

We observed that PLA-LDL induced robust and sustained activation of Akt in THP1 cells. Activation of PI3K/Akt signaling is critical for monocyte/macrophage survival and phosphorylation of cell death-related proteins, including Bad or IKKβ, was suggested to attenuate cell demise. We noticed that inhibition of PI3K activity attenuated the cytoprotective effect of PLA-LDL, indicating the key role of PI3K-Akt activation. Further studies need to determine which of the potential cytoprotective targets are activated by PI3K-Akt in THP1 cells.

The impact of modified LDL on monocyte and macrophage viability, particularly the impact of oxidized LDL, has been a subject of several studies. It has been noted that oxidized LDL or minimally modified LDL at low concentrations increases survival and the involvement of PI3K-Akt signaling has been postulated. However, we did not observe any change in the ability of PLA-LDL to activate Akt when phospholipase A2 treatments were conducted in the presence of antioxidant BHA. In addition, oxidized LDL at low concentrations did not increase THP1 cell survival under our conditions, and treatments with phospholipase A2 did not alter this response, which may be attributed to loss of esterified fatty acids from LDL phospholipids on oxidation (data not shown). Thus, we conclude that phospholipolysis represents a distinct type of LDL modification that increases monocyte survival. Furthermore, we noticed that treatment of LDL with group V PLA2, an enzyme present in atherosclerotic plaques and therefore relevant to human disease, confers to LDL similar pro-survival activity as treatment with snake venom PLA2. This further strengthens the hypothesis that enhanced phospholipase A2 activity in the plaque contributes to increased monocyte cell numbers and thus enhanced atherogenesis. Because elevated sPLA2 activity in the intima is likely to be associated with inflammatory events, one may assume that an increased monocyte survival might be part of a positive feedback mechanism, propagating inflammation, and disease progression.

Oxidized LDL was shown to be cytotoxic at high concentrations. Similar to oxidized LDL, we noticed that higher concentrations of PLA-LDL provoked cell death. Our data thus correlate with the observations that LDL treated with phospholipase A2 and 15-lipoxygenase was toxic to monocyctic cells. Cytotoxicity of PLA-LDL can be considered a general phenomenon, because it was also observed in human endothelial and smooth muscle cells, whereas cytoprotection was observed only in monocytes (data not shown). PLA-LDL toxicity may reflect increased cell death during advanced plaque progression, a condition in which modified LDL

Figure 3. PLA-LDL is cytoprotective. THP1 cells were incubated for 8 hours with 10 μg/mL LDL or PLA-LDL in the absence or in the presence of 1 mmol/L H2O2 and cell viability was analyzed by (A) measuring Annexin/PI labeling or (B) mitochondrial membrane potential. C, Monocytes were cultured for 48 hours in the absence or presence of 10 μg/mL PLA-LDL and 100 μmol/L H2O2 and cell viability was measured by MTT assay. *P<0.05 vs control. #P<0.05 vs H2O2 (n=4). D, THP1 cells were incubated in the presence of 10 μg/mL LDL treated with group V PLA2 (gV PLA-LDL) or Naja mossambica PLA2 (Naja PLA-LDL) and 1 mmol/L H2O2 for 8 hours. Cell viability was analyzed by measuring Annexin/PI labeling and mitochondrial membrane potential. *P<0.05 vs H2O2 (n=4).
accumulates at higher amounts, and confirms pro-atherogenic action of PLA-LDL. The specificity of a PLA-LDL cytoprotective action for monocytes compared with endothelial or smooth muscle cells might be caused by the particular receptor-mediated pathways underlying PLA-LDL–induced signaling in monocytes. Thus, for oxidized LDL it was shown that LOX-1 conveys some of its actions in endothelial cells, whereas scavenger receptors SR-A and CD36 may be involved in signaling by oxidized LDL in phagocytic cells. Differences in receptor subtype composition at distinct cells may account for variable responses by lipolytically-modified LDL as well.

We have shown that sPLA₂-generated lipid products of LDL are mediators of Akt activation. It is known that sPLA₂ treatment of LDL may affect its retention in the intima by changing ApoB conformation. Our data on PLA-LDL proteolysis indicate that intact ApoB is not necessary for PLA-LDL-induced Akt activation. In addition, lipid extracts of PLA-LDL were able to activate Akt, supporting the notion that the lipid, but not the protein moiety of PLA-LDL mediates pro-survival effects.

LDL phospholipolysis results in the formation of NEFA and LPC. Both, NEFA and LPC were assumed to affect cell survival and PI3K/Akt signaling. Whereas LPC was reported to mediate pro-mitogenic effects of oxidized LDL, other studies suggested that it mediates Akt dephosphorylation. Similarly, NEFA were suggested to be pro-apoptotic or pro-survival, depending on test conditions. In our cell culture model, LPC only transiently induced Akt phosphorylation and was itself cytotoxic, whereas AA provoked prolonged Akt phosphorylation and showed a pro-survival effect. Thus, we conclude that NEFA rather than LPC are likely to be mediators of the PLA-LDL pro-survival action. A recent study in LDL receptor-deficient mice that also lack the putative LPC receptor G2A showed increased macrophage numbers and reduced apoptosis, supporting a pro-apoptotic effect of LPC on macrophages.

The mechanisms how NEFA may modulate signaling pathways leading to cell survival are less clear. NEFA were shown to directly modulate the activity of intracellular signaling proteins such as PKC or to act via their metabolism by, e.g., cyclooxygenase or lipoxygenase pathways. In addition, NEFA may activate intracellular signaling via a recently identified family of G protein-coupled receptors, such as GPR40 and GPR120. We did not observe effects of cyclooxygenase (indomethacin) or 5-lipoxygenase (CI-13,610) inhibition on Akt phosphorylation induced by PLA-LDL (data not shown), which makes direct effects of NEFA likely. Further research is needed to elucidate the exact nature of the mechanism mediating the activation of PI3K/Akt pathway in response to NEFA.

In summary, we have shown that PLA-LDL increases monocyte survival via activation of PI3K/Akt. This finding provides a mechanistic explanation for a pro-atherogenic action of sPLA₂, which is confirmed in several clinical and...
experimental studies. Our data suggest that any interference with sPLA₂ activity in the lesion may have beneficial effects during disease progression by reducing monocyte viability and cell numbers, and thus be of therapeutic significance.

Acknowledgments

We greatly appreciate the technical assistance of Elke Dauber and Sabine Knaus. We thank Dr Wonhwa Cho for providing human recombinant group V PLA2.

Source of Funding

This study was supported by a grant from Deutsche Forschungsgemeinschaft (BR999).

Disclosures

None.

References


Phospholipase A2–Modified Low-Density Lipoprotein Activates the Phosphatidylinositol 3-Kinase-Akt Pathway and Increases Cell Survival in Monocytic Cells

Dmitry Namgaladze and Bernhard Brüne

Arterioscler Thromb Vasc Biol. 2006;26:2510-2516; originally published online September 14, 2006;
doi: 10.1161/01.ATV.0000245797.76062.2e
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2006 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/26/11/2510

Data Supplement (unedited) at:
http://atvb.ahajournals.org/content/suppl/2006/09/18/01.ATV.0000245797.76062.2e.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Arteriosclerosis, Thrombosis, and Vascular Biology can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online at:
http://atvb.ahajournals.org//subscriptions/
**Figure I.** PLA-LDL protects THP1 cells against oxLDL-induced cell death. Cells were incubated with 10 µg/mL PLA-LDL and 25 µg/mL oxLDL for 8 hours. Cell viability was analyzed by measuring Annexin/PI labeling and mitochondrial membrane potential. *, $P<0.05$ vs. oxLDL ($n=4$).

**Figure II.** Cytotoxicity of PLA-LDL. THP1 cells were incubated with increasing concentrations of PLA-LDL (20, 50 and 100 µg/ml) for 8 hours and cell viability was measured by Annexin/PI labeling.
Figure I

% cell death

<table>
<thead>
<tr>
<th>oxLDL</th>
<th>PLA-LDL</th>
<th>DiOC6</th>
<th>Annexin</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
<td>-</td>
<td>+</td>
<td>*</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

* denotes statistical significance.
Figure II

![Bar graph showing Annexin/PI positive cells (%) for different conditions: K, PLA-LDL 20, PLA-LDL 50, PLA-LDL 100.](image)