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PHOSPHOLIPASE A_2-MODIFIED LDL IS TAKEN UP AT ENHANCED RATE BY MACROPHAGES

Michael Aviram and Irit Maor

Lipid Research Laboratory, Rambam Medical Center, Rappaport Family Institute for Research in the Medical Sciences, Technion Faculty of Medicine, Haifa, Israel

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SUMMARY: Modification of the low density lipoprotein (LDL) core or surface lipids were shown to affect the cellular uptake of the lipoproteins and hence the formation of foam cell macrophages. In the present study phospholipase A_2 treatment of LDL was shown to produce negatively charged lipoprotein with increased content of lysolechitine. This modified lipoprotein was taken up and degraded by J-774 A.1 macrophage-like cell line at enhanced rate (up to 97% when 10 units/ml of PLase A_2 was used) in comparison to control LDL. This effect of PLase A_2 was enzyme dose dependent. Competition experiments revealed that the uptake of PLase A_2-LDL by the macrophages was specific and was mediated via the LDL receptor. Since PLase A_2 was found to exist in various tissues, thus the production of PLase A_2-LDL under certain pathological conditions can potentially contribute to foam cell formation and accelerated atherosclerosis.

Perturbation of the core of low density lipoprotein (LDL) by triglyceride hydrolysis with lipoprotein or hepatic lipases (1,2) or cholesteryl ester hydrolysis with cholesterol esterase (3), significantly increased or decreased the uptake of the modified lipoproteins by the macrophages respectively.

Abbreviations: LDL, low density lipoprotein(s); PLase A_2 phospholipase A_2; DMEM, Dulbecco's Modified Eagles Medium.

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Similarly, changes in the surface unesterified cholesterol by LDL treatment with cholesterol oxidase (4) or perturbation of the LDL coat phospholipids, following its incubation with phospholipases (5-8) resulted in the formation of modified lipoproteins which demonstrated increased uptake by macrophages. PLase C-modified LDL was shown to be aggregated and was taken up by macrophages at enhanced rate by a phagocytic process that was mediated via the LDL receptor (5,6). PLase D-modified LDL was not aggregated and demonstrated increased uptake by macrophages via an LDL receptor mediated endocytosis (8).

PLase A_2, unlike PLase C and PLase D, do not attack the phosphoryl group, but rather the fatty acids at the sn-2 position of the diacylglycerol phospholipid, was shown to cause changes in LDL physicochemical characterization following lipoprotein incubation with PLase A_2 (7). In human skin fibroblasts, PLase A_2-modified LDL was shown to bind nonspecifically to the cells and demonstrated very limited cellular degradation by the fibroblasts (7). Since macrophages, unlike human fibroblasts, possess in addition to the LDL receptor, also other receptors for modified lipoproteins (9-11) and since even the LDL receptor on macrophages is distinct from the classical LDL receptor on fibroblasts (12), we analyzed in the present study the cellular uptake of PLase A_2-modified LDL by J-774 A.1 macrophages, a cell line which was shown to possess all these various receptors for lipoproteins (11,12).

METHODS

Cells. J-774 A.1 murine macrophage-like cell line was purchased from the American Tissue Culture Collection (ATCC). Cells were plated at 2.5×10^5 cells/16mm dish in DMEM supplemented with 10% fetal calf serum (FCS). The cells were fed every 3 days and were used for experiments within 7 days of plating.

Lipoproteins. LDL was prepared from human plasma derived from fasted normolipidemic volunteers. LDL was prepared by density gradient ultracentrifugation as described previously(13). LDL was iodinated by the method of McFarlane as modified for lipopoproteins (14). LDL protein was measured by the method of Lowry et al. (15). Vitamin E and carotenoids were

determined as previously described (16) and the fatty acids distribution was analyzed by gas liquid chromatography (17).

Phospholipolysis. Phospholipase A_2 (PLase A_2) from bee venom was used (Boehringer Mannheim GmBH, Germany). LDL (1mg of protein/ml) was incubated with an equal volume of a buffer (180 mM Tris, 150mM NaCl,pH=8) in the presence of 3mM $CaCl_2$ and 0.6% fatty-acid free albumin,for 10 min at 37°C. PLase A_2-LDL was prepared by incubation of the LDL solution with 5 units/ml of PLase A_2 for 1h at 37°C. The reaction was stopped by the addition of 1mM EDTA and refrigeration. The modified LDL was separated from excess enzyme by passage on Sephadex G-100 minicolumn (4). For phospholipid analysis, lipid extracts of LDL were prepared using chloroform: methanol solution (2:1,v:v). Phospholipid subclasses were separated by thin layer chromatography (TLC) on silica gel plates, using a developing solution of chloroform: methanol: ammonium hydroxide (60:35:8,v:v:v). Iodine vapors were used to visualize the lipid spots on the TLC plates. The appropriate spots were then scraped and ananlyzed for their phosphourous content (18).

Cullular uptake of lipoproteins. Degradation of ^{125}I-LDL or PLase A-^{125}I-LDL was measured following their incubation with J-774A.1 macrophages for 5h at 37°C. The hydrolysis of the LDL protein was assayed in the incubation medium (19). Cellular cholesterol esterification rates were measured as previously described (4).

RESULTS

Upon incubation of LDL (1mg of protein/ml) with PLase A_2 (5 units/ml) for 1 hours at 37°C, followed by reseparation of the modified lipoportein (by ultracentrifugation), its lysolechitine content increased from 16±3 to 95±10 µg/mg LDL protein (n=3). The electrophoretic mobility of PLase A_2-LDL on cellulose acetate was increased and it migrated 14±3 mm from the origin in comparison to migration of 9±2 mm obtained for native LDL ($p<0.01$,n=3). PLase A_2-LDL demonstrated reduced content of linoleic (C-18:2) and arachidonic (C-20:4) acids with a reduction in their relative content from 40±6% and 6±2% to 32±4% and 3±1% of total LDL fatty acids respectively ($p<0.01$,n=3). The LDL antioxidants,vitamin E and carotenoids, also decreased from 0.18±0.04 and 1.8±0.3 to

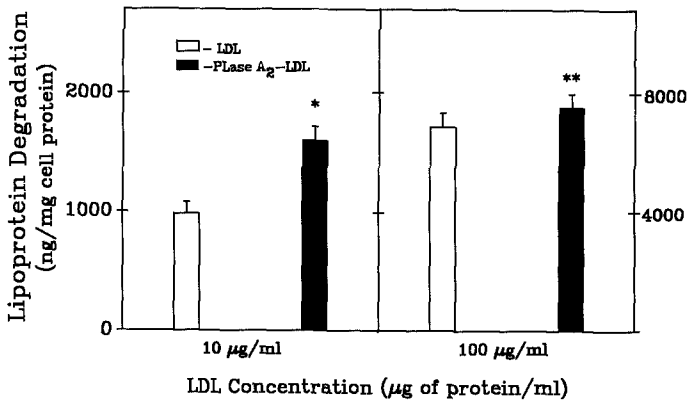

Fig. 1. Macrophage degradation of LDL and phospholipase A_2-modified LDL (PLase A_2-LDL) J-774 A.1 macrophages were incubated with ^{125}I-LDL or with PLase A_2-^{125}I-LDL (10 and 100 μg of protein/ml) for 5 hours at 37°C prior to analysis of the lipoprotein cellular degradation. *p<0.01, ** p<0.05 (vs. LDL,n=3).

0.13±0.04 and 1.3±0.2 μg/mg LDL protein respectively (p<0.01, n=3). PLase A_2-LDL was not aggregated and no fragmentation of the LDL apo B-100 could be found on sodium dodecyl sulfate polyacrylamide gel electrophoresis (dat not shown).

J-774 A.1 macrophages were incubated with native ^{125}I-LDL or PLase A_2-^{125}I-LDL for 5 hours at 37°C, prior to analysis of lipoprotein cellular degradation (Fig 1). At 10μg of LDL protein/ml, the modified lipoprotein showed 64% enhanced cellular degradation, wherease at 100μg of LDL protein/ml, only 10% increment in PLase A_2-LDL degradation was noted in comparison to native LDL (Fig 1).

At a concentration of 10μg of protein/ml,native LDL and PLase A_2-LDL (incubated for 18h at 37°C with J-774 A.1 macrophages) increased cellular cholesterol esterification rate from 0.3±0.1 nmol/mg cell protein in control cells (incubated without lipoproteins) to 0.7±0.2 and 1.2±0.3 nmol/mg cell protein receptively (n=3). PLase A_2 dose response revealed that macrophage degradation of the modified lipoprotein was increased when PLase A_2-LDL was prepared by incubation with increasing concentrations of the enzyme with up to 97% increment when 10U/ml PLase A_2 was used (Fig 2).

The possible receptor involved in the cellular uptake and degradation of PLase A_2-LDL was studied by competition

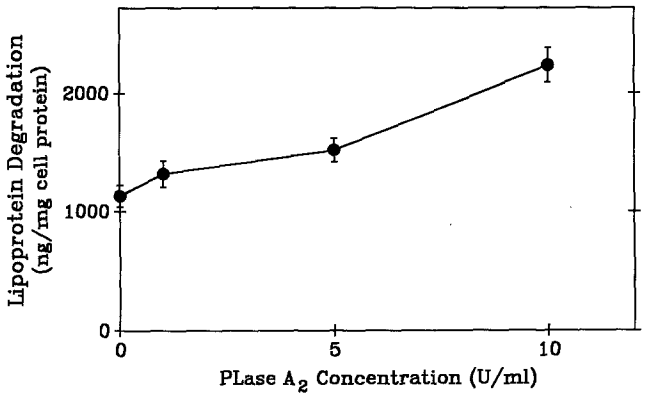

Fig. 2. Dose response of PLase A_2 modification of LDL. LDL (1mg of protein/ml) was incubated with increasing concentrations of PLase A_2 for 1 hour at 37°C. The cellular degradation of the modified lipoproteins was assessed after 5 hours of lipoprotein incubation with J-774 A.1 macrophages.

experiments with excess concentrations of unlabeled competitors (Fig 3). Both LDL and PLase A_2-LDL at 50 fold excess concnetration substantially reduced macrophage

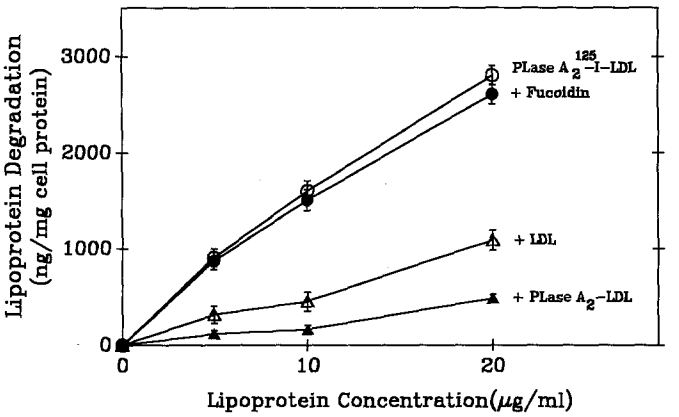

Fig. 3. Involvement of the macrophage LDL receptor in the cellular uptake of PLase A_2-LDL. J-774 A.1 macrophages were incubated with 5,10 or 20μg of protein/ml of ^{125}I--labeled PLase A_2-LDL in the absence or presence of 50 fold excess concentrations of unlabeled LDL or PLase A_2-LDL or with 50μg/ml of fucoidin. Cellular degradation of the PLase A_2-^{125}I-LDL was determined as described under Methods.

degradation of PLaseA_2-^{125}I-LDL (Fig 3), suggesting that PLaseA_2-LDL was taken by macropahges via the LDL receptor. Fucoidin, on the other hand, which is known to block binding to the scavenger receptor did not affect cellular degradation of PLaseA_2-LDL (Fig 3).

DISCUSSION

The present study demonstrated that LDL modification by its incubation with PLase A_2 resulted in the formation of PLase-A_2-LDL which was taken up via the LDL receptor and degraded by macrophages at enhanced rate. These results contradict those found by Kleinman et. al. (7) who have used human skin fibroblasts and not macrophages. Since in our study the cellular uptake of PLase A_2-LDL was shown to be mediated via the macrophage LDL receptor and since on human fibroblasts only LDL receptors, but not scavenger receptors were demonstrated (20),it is suggested that the LDL receptor on macrophages is different from that found on human skin fibroblasts. In fact, it was previously demonstrated that the macrophage LDL receptor possesses different characteristics than the "classical" LDL receptors on human skin fibroblasts (9-11). The macrophage LDL receptor was shown to be involved in the cellular uptake of PLaseA_2-LDL. Since the ability of excess concentrations of unlabeled LDL to compete with PLase A_2-LDL for cellular degradation was lower than that of excess unlabeled PLase A_2-LDL, it is suggested that the modified LDL has a higher affinity for the LDL receptor in comparison to native LDL. Another possibility is that a non-receptor component for the uptake of PLase A_2-LDL may be also involved. Macrophage cholesterol accumulation and foam cell formation, the hallmark of the atherosclerotic lesion, can be achived by several different mechanisms. These mechanisms include cellular uptake of LDL modified in its core triglycerides, or in its surface phospholipids,or in its unesterified cholesterol (1-3). Another mechanism for foam cell formation may involve macrophage uptake of various forms of oxidized LDL (21-23). PLase A_2 exist in various tissuse as well as in LDL itself (24,25) and has an essential role in endothelial cell-mediated oxidation of LDL. The present study thus, demonstrated an additional phospholipid

modification of LDL which can potentially contribute in vivo to the atherogenic process.

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