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Original research article

Hydrolysis of lipoproteins by $sPLA_2$'s enhances mitogenesis and eicosanoid release from vascular smooth muscle cells: Diverse activity of $sPLA_2$'s IIA, V and X

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ABSTRACT

Mitogenesis of Vascular Smooth Muscle Cells (VSMC) plays an important role in atherogenesis. Until recently, the effect of lipid subfractions has not been clarified. Secretory phospholipases A_2 ($sPLA_2$'s) hydrolyse glycerophospholipids and release pro-inflammatory lyso-lipids, oxidized and non-oxidized fatty acids and isoprostanes. They localize in the vascular wall. We hypothesized that structurally similar $sPLA_2$'s may exert different impact on VSMC. The influence of $sPLA_2$'s, IIA, V, X, HDL, LDL, and hydrolysis products was tested on mitogenesis of VSMC, i.e., the early effect on the cell membrane phospholipids, and on PGE_2 and LTB_4 release, i.e., late effect of Cyclooxygenase and 5-lipooxygenase activity in VSMC. Mitogenesis was significantly enhanced by HDL and LDL, and by products of $sPLA_2$ hydrolysis. Hydrolysis of HDL or LDL enhanced mitogenic activity in order V > X > IIA. The release of PGE_2 was enhanced by group X $sPLA_2$ and by HDL hydrolyzed by groups V and X. LDL and its hydrolysis products enhanced the release of PGE_2 in order X > V > IIA. The release of LTB_4 was markedly increased by LDL and HDL, and by hydrolytic products of group V and X, but not group IIA $sPLA_2$. Our study demonstrates a diverse interaction of pro-inflammatory $sPLA_2$'s with HDL and LDL affecting both mitogenesis and eicosanoid release from VSMC, therefore potentially enhancing their pro-atherogenic activity.

1. Introduction

Substantial progress has been made in the investigation of the pathogenic mechanisms of atherosclerosis (A-S) with emphasis on inflammatory [1–3] and immunological [4] factors, and on the interaction of cellular components in vascular wall with lipoproteins [5,6] and secretory phospholipases A_2 ($sPLA_2$) [7–9]. Vascular smooth muscle cells (VSMC) while quiescent under physiological conditions, are becoming proatherogenic when exposed to a variety of insults [10]. VSMC participate in the lipid overload and handling of modified LDL, cholesterol and other elements that play a major role in the development of atheromas [10]. Mitogenic activation followed by proliferation and migration of (VSMC) plays a major role in the development of atherosclerosis [10–12]. Previous work [13] has demonstrated the mitogenic effect of native plasma lipoproteins and the effects of their hydrolysis by group IIA $sPLA_2$. However, the effect of lipolysis products generated by the more extensive activity of group V and X $sPLA_2$s upon VSMC mitogenesis has not been previously reported. Unreported has also remained the effect of V and X $sPLA_2$ on biogenesis and extracellular release of PGE_2 and LTB_4 promoters of late stages of pro-inflammatory activity of VSMC [14].

Herein we report that native human lipoproteins, as well as $sPLA_2$'s, IIA, V, and X and especially $sPLA_2$ induced lipolytic products of HDL, HDL_3 and LDL induce mitogenic activity and extracellular

Abbreviations: ALDH, aldehyde dehydrogenase; COX-2, cyclooxygenase-2; DHA, docosahexaenoic acid; DNA, deoxyribonucleic acid; EPA, eicosapentaenoic acid; ESI, electrospray ionization; GroPCho, glycerophosphocholine; GSH, glutathione; HDL, high density lipoprotein; HNE, 4-hydroxy-*trans*-2-nonenal; IL-1β, interleukin β; LC/ESI-MSHNE, liquid chromatography/ESI-mass spectrometry; LDH, lactic dehydrogenase; LDL, low density lipoprotein; lysoPtdCho, lysophosphatidylcholine; LTB4, leukotriene B4; MAPK, mitogen activated protein kinase; NF-κβ, nuclear factor kappa-beta; PGE_2, prostaglandin E2; PGI_2, prostacyclin I2; PKC, protein kinase C; PtdCho, phosphatidylcholine; PUFA, polyunsaturated fatty acids; TBARS, thiobarbituric acid reactive substances; $sPLA_2$s, secretory human phospholipases A_2; VSMC, vascular smooth muscle cells.

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release of PGE_2 and LTB_4 from human VSMC. Marked differences were detected in the impact of groups IIA, V and X $sPLA_2$'s.

2. Materials and methods

2.1. Cell culture

Human VSMC of male and female origin were cultured and maintained as described [13]. The source of VSMC and the methods for making VSMC quiescent and tested for morphology, size, shape, detachment and viability were previously reported in detail [13]. Before treatment with any agents, VSMC were made quiescent by culturing them for 48 h (with one medium change after 24 h) in serum-free medium containing FFA free 0.1% BSA, insulin-transferrin-selenium supplement (ITS) (Gibco BRL., Burlington, Ontario, Canada), 0.1 mM vitamin C, 100 U/mL penicillin, 100 ug/mL streptomycin, and 0.25 ug/ml amphotericin B. In each experiment LDH release was also tested. Only viable, morphologically normal cells from passages 6 and 7 were used for the experiments.

2.2. Lipoproteins

Pure human HDL and LDL were purchased from Sigma–Aldrich (Toronto, ON, Canada) or received as a gift from Dr. F. C. De Beer. Purity and lack of oxidation were tested, as recorded [13]. The content of lioneic acid in HDL was 1.31 μmol/L and in LDL 5.5 μmol/L, while oleic acid made up 0.43 μmol/L and 1.8 μmol/L, respectively. Only the lipid subfractions of highest purity, free from oxidized products as tested by chromatographic analysis were used. The content of linoleic acid in HDL was 1.31 μmol/L and in LDL 5.5 μmol/L, while oleic acid made up 0.43 μmol/L and 1.8 μmol/L, respectively. These concentrations were found to be non-toxic to the cells [14]. The molecular species composition of the glycerophospholipids has been described in detail elsewhere [15,16]. Linoleic, oleic and arachidonic acids and lysoPtdCho were purchased from Sigma–Aldrich, and tested by chromatography for purity and oxidation state in our laboratory.

2.3. Phospholipases

Recombinant human IIA $sPLA_2$ was provided by Dr. J. Browning, Biogen, Cambridge, MA, USA. Recombinant human group V $sPLA_2$ was prepared by Dr. Kwang Pyo Kim, University of Chicago, IL, US. Recombinant human group X $sPLA_2$ was prepared by Dr. G. Lambeau, Center National de la Recherche Scientifique, Sophia Antipolis, France.

2.4. Hydrolysis of lipoproteins

HDL and fresh non-oxidized LDL (25–100 μg/mL) were incubated for 1 to 24 h with $sPLA_2$'s (1.0–50 ng/mL) as described [13]. The products of enzyme hydrolysis as well as those of lipid peroxidation were identified by LC/ESI-MS as described [15,17].

2.5. Mitogenesis and cellular proliferation

[^{3}H] Thymidine incorporation was measured as previously described [13] to determine the effect of exposure of human VSMC to $sPLA_2$'s, IIA, V and X, and to native and $sPLA_2$—digested lipoproteins. Each experiment was performed in triplicate, and repeated as described in the results.

2.6. PGE_2 and LTB_4 release

VSMC were grown to confluence as described [13], plated in 24-well plates, 6 × 104 cells per well in 1 mL of the medium for 24 h and then made quiescent as reported [13]. VSMC were incubated with each lipoprotein alone, each $sPLA_2$ alone, or with each lipoprotein hydrolyzed by each $sPLA_2$ for various periods of time. In the incubations with VSMC, the lipoprotein hydrolysates were utilized together with the $sPLA_2$s used to produce them, as well as the albumin bound free fatty acids and lysophospholipids.

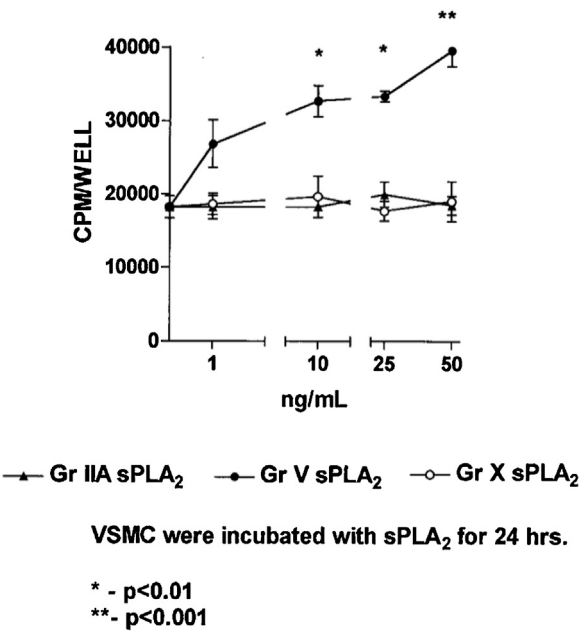

Fig. 1. Mitogenic activity of VSMC induced by Gr IIA, V and X $sPLA_2$'s.

Leukotriene B_4 (LTB_4) was determined by enzyme immunoassay EIA system and Prostaglandin E_2 (PGE_2) by EIA system (Amersham Pharmacia Biotech, Canada). Optical density (450 nm) was measured on microtiter plate reader (Diagnostics Pasteur LP400). Concentrations of released LTB_4 and PGE_2 were measured in the supernatant. The results were normalized to 1 × 106 cells. The range of PGE_2 sensitivity was 0.84–100 ng/10^6 cells and of LTB_4 94–12000 pg/10^6 cells.

Experiments were done in triplicate and repeated 3 or more times. The initial and final cell counts and microscopy were done and LDH concentration was tested in the medium by cytotoxicity Detection Kit (Roche Diagnostics, Mannheim, Germany). Microscopic assessment of the size and shape of cells and of their detachment was made.

2.7. Statistical analysis

Analysis of all experiments was performed by Student *t*-test, Tukey–Kramer multiple comparison test and Bartlett's test for homogeneity of variances.

3. Results

3.1. Exposure of VSMC to $sPLA_2$'s

Incubations of VSMC with $sPLA_2$'s ranged from 1 h to 24 h. Results of 24 h incubation at concentrations from 1 ng/mL to 50 ng/mL are shown in Fig. 1 and show concentration related increase in mitogenic activity (Fig. 1).

Exposure to $sPLA_2$ group IIA or to group X, up to the concentration of 50 ng/mL, did not significantly increase the mitogenic activity of VSMC, (104 ± 3%) regardless of the duration of incubation. In contrast, exposure to $sPLA_2$ group V invariably resulted in dose related increase in mitogenic activity. VSMC exposed to