Effects of LDL Lipids on Activity of Group IIA Secretory Phospholipase A2

E. V. Samoilova, A. A. Pirkova, N. V. Prokazova, and A. A. Korotaeva

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 150, No. 7, pp. 45-47, July, 2010
Original article submitted July 28, 2009

Effects of phosphatidylcholine, oxidized phosphatidylcholine, sphyngomyelin, cholesterol, and cholesterol esters incorporated in LDL on activity of group IIA secretory phospholipase A2 from human cardiac myxoma were studied. Liposomes containing radioisotope-labeled phosphatidylethanolamine served as the substrate for group IIA secretory phospholipase A2. Oxidized phosphatidylcholine significantly stimulated activity of group IIA secretory phospholipase A2, while phosphatidylcholine in the same concentrations did not modify enzyme activity. Sphyngomyelin incorporated in LDL inhibited group IIA secretory phospholipase A2 activity. Cholesterol and cholesterol esters virtually did not modify enzyme activity. The results indicate that LDL phospholipids and their oxidized forms can be involved in regulation of group IIA secretory phospholipase A2. Study of the mechanisms regulating the proinflammatory group IIA secretory phospholipase A2 can promote the development of new approaches to the diagnosis and treatment of inflammatory processes.

Key Words: *secretory phospholipase A2; oxidized phosphatidylcholin; sphyngomyelin; cholesterol; cholesterol esters*

Group IIA secretory phospholipase A2 (secPlA2-IIA) is a proinflammatory enzyme actively involved in initiation and progress of cardiovascular diseases [4]. Recent studies showed that high level of secPlA2-IIA in blood plasma is an independent risk factor of a variety of unfavorable cardiovascular events [6]. High activity of blood secPlA2-IIA in patients after acute coronary syndrome is a predictor of repeated myocardial infarction or lethal outcome [10]. Increase of enzyme activity after coronary angioplasty is associated with the development of restenosis [7]. Active search for pharmacological inhibitors of secPlA2-IIA, effective antiatherogenic drugs, is now in progress. Inhibition of secPlA2-IIA with varespladib led to a 24% shrinkage of atherosclerotic injury in guinea pigs [9]. Specific inhibitor PX-18 prevented cardiomyocyte apoptosis and significantly reduced the myocardial infarction zone in rats [12]. Clinical studies showed that statins reduce the level and activity of secPlA2-IIA in the blood of cardiovascular patients [2,5].

Study of the mechanisms of secPlA2-IIA stimulation can promote the development of new approaches to the diagnosis and therapy of cardiovascular diseases. Our previous studies showed that secPlA2-IIA activity is inhibited by native and stimulated by oxidized LDL [1].

Here we studied the effects of lipids in native and oxidized LDL on activity of secPlA2-IIA.

MATERIALS AND METHODS

The following reagents were used in the study: L-3-phosphatidylethanolamine, 1-acyl-2-(1-^{14}C)arachidonoyl (59 mCi/mol) ([^{14}C]-PE; Amersham), 1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphorylcholine (PC), cholesterol (CS), cholesterol esters (CSE),

Russian Cardiology Research-and-Production Complex, Federal Agency for Health Care and Social Development, Moscow, Russia. ***Address for correspondence:*** a.korot@cardio.ru. A. A. Korotaeva

0007-4888/10/1501-0039

sphyngomyelin (SM), arachidonic acid (Sigma), oxidized 1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphorylcholine (oxPC; kind gift from Dr. V. P. Bochkov, Medical University of Vienna, Austria); secPlA2-IIA from human heart tumor (myxoma) was a kind gift from Dr. G. L. Khaspekov from Gene Engineering Laboratory of Cardiology Center [3,11]. Specific activity of the enzyme was 2 μmol hydrolyzed PE/min/μg protein. Chromatographic DC plates with silica gel (Merck) were used.

In order to prepare liposomes, [^{14}C]-PE (5 nmol per sample) and cold PC (7.5 nmol per sample) were mixed in chloroform and dried under nitrogen flow, dissolved in 1 ml diethyl ether, and again dried under nitrogen. An appropriate volume of Tris-HCl buffer (pH 8.0) with 100 mM Tris, 2 mM $CaCl_2$, 0.15 M NaCl was added to dry lipids and the preparation was US-treated 3 times (1 min each time) at 4°C in a sonicator (model 450, Branson Ultrasonic Corp).

Low-density lipoproteins (d=1.019-1.063 g/ml) were isolated from donor plasma by successive ultracentrifugation. Before analysis LDL were dialyzed against PBS (pH 7.4). Protein concentration was measured by Lowry's method. LDL were used within 1 week after isolation.

Low density lipoproteins with CS, ECS, SM, PC, and oxPC were prepared as follows: different doses of these lipids were dissolved in chloroform, dried under nitrogen flow, 50 μl PBS was added, and the preparations were ultrasonicated. Fresh LDL (500 μg protein) were added to the resultant suspension and the mixture was incubated at 37°C for 40 min at stirring. The LDL were separated from free lipids by centrifugation of the mixture in 1.006 g/ml density (8 h, 35,000 rpm in a Type 65 rotor (Beckman). Before analysis LDL was dialyzed against PBS (pH 7.4) and protein concentrations in them were measured by Lowry's method.

In order to evaluate secPlA2-IIA activity, LDL (20 μg protein) with different content of incorporated lipids (PC, oxPC, SM, CS, and CSE) were incubated with secPlA2-IIA (enzyme activity in the reaction mixture 30 pmol hydrolyzed PE/min), 10 μl [^{14}C] labeled liposomes, 100 mM Tris-HCl buffer (pH 8.0), 2 mM $CaCl_2$, and 0.15 M NaCl in the final volume of 500 μl. The reaction was carried out for 40 min at 37°C at constant stirring and stopped by adding 1.5 ml chloroform-methanol (2:1 vol/vol) mixture. The lipids extracted into a chloroform layer were dried under nitrogen flow, dissolved in 80 μl chloroform, and separated by thin layer chromatography on silica gel plates using the hexane-diethyl ether-acetic acid solvent system (85:15:1 vol/vol/vol). Purified arachidonic acid served as the reference for each chromatogram. Lipid spots were visualized by iodine vapor. Fractions corresponding to free fatty acids were scraped out into vials with 7 ml scintillation fluid. Radioactivity was measured on a liquid scintillation counter. Catalytic activity was evaluated by the volume of cleaved off labeled arachidonic acid and expressed as the percentage of hydrolyzed PE *vs*. the control sample, incubated with LDL without incorporated lipids and taken for 100%.

The results were processed using Statistica 6.0 software with Student's *t* test. The data were presented as $M \pm m$. The differences were considered significant at $p<0.05$.

RESULTS

The most abundant phospholipid of plasma LDL is PC. It can transform into oxidized forms (oxPC) in oxidized LDL during inflammation. We previously demonstrated opposite effects of native and oxidized LDL on secPlA2-IIA activity [1]. In order to compare the effects of PC and oxPC on secPlA2-IIA activity, we incorporated different concentrations of PC and oxPC (0.5, 1, 2, and 4 nmol) in native LDL. Activity of secPlA2-IIA was not changed by PC, while the same amount of oxPC incorporated in LDL stimulated enzyme activity in a dose-dependent manner (Fig. 1). Incorporation of 4 nmol oxPC in LDL led to a more than 3-fold increase of secPlA2-IIA activity.

Apart from PC, SM, CS, and CSE are present in high amounts in LDL. It has been shown that SM incorporated in the liposomes and added to secPlA2-IIA inhibits enzyme activity [8]. The effect of SM in lipoproteins on secPlA2-IIA is however unknown. We evaluated the effect of SM incorporated in different concentrations in LDL on activity of secPlA2-IIA. The

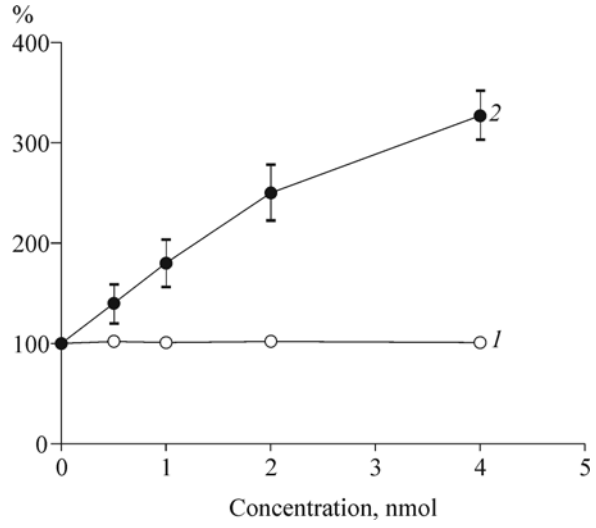

Fig. 1. Effects of PC (1) and oxPC (*2*) incorporated in LDL on secPlA2-IIA activity.

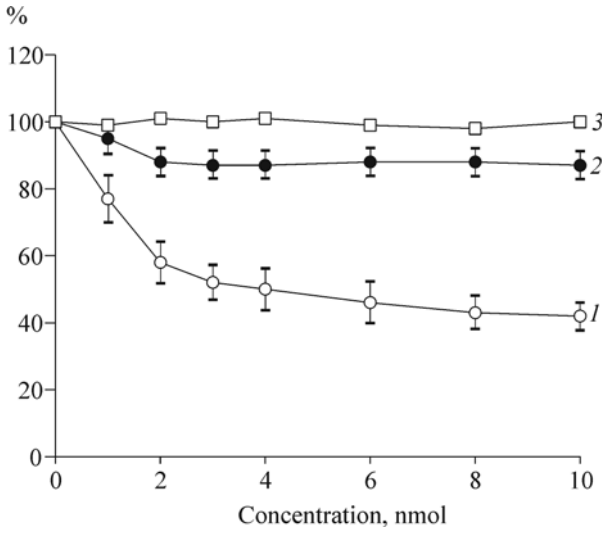

Fig. 2. Effects of SM (*1*), CS (*2*), and CSE (*3*) incorporated in LDL on secPlA2-IIA activity.

LDL enriched with SM inhibit activity of secPlA2-IIA; CS and CSE virtually do not modify enzyme activity (Fig. 2).

The results indicate the involvement of lipids in native and oxidized LDL in the regulation of proinflammatory secPlA2-IIA activity.

The study was supported by the Russian Foundation for Basic Research (grant No. 08-04-00409a).

REFERENCES

1. A. A. Korotaeva, E. V. Samoilova, A. A. Pirkova, *et al.*, *Ros. Fiziol. Zh.*, **95**, No. 5. 476-483 (2009).
2. A. A. Pirkova, E. V. Samoilova, V. A. Amelyushkina, *et al.*, *Kardiologiya*, **47**, No. 4, 37-40 (2007).
3. G. L. Khaspekov, G. F. Sheremetyeva, A. V. Skamrov, *et al.*, *Arkh. Patol.*, No. 2, 31-36 (2008).
4. B. B. Boyanovsky and N. R. Webb, *Cardiovasc. Drugs Ther.*, **23**, No. 1, 61-72 (2009).
5. D. Divchev, C. Grothusen, M. Luchtefeld, *et al.*, *Eur. Heart J.*, **29**, No. 16, 1956-1965 (2008).
6. W. Koenig and N. Khuseyinova, *Cardiovasc. Drugs Ther.,* **23**, No. 1, 85-92 (2009).
7. A. A. Korotaeva, E. V. Samoilova, A. I. Kaminny, *et al.*, *Mol. Cell Biochem.*, **270**, Nos. 1-2, 107-113 (2005).
8. K. Koumanov, C. Wolf, and G. Béreziat, *Biochem. J.*, **326**, Pt. 1, 227-233 (1997).
9. J. O. Leite, U. Vaishnav, M. Puglisi, *et al.*, *BMC Cardiovasc. Disord.*, **9**, 7 (2009).
10. Z. Mallat, P. G. Steg, J. Benessiano, *et al.*, *J. Am. Coll. Cardiol.*, **46**, No. 7, 1249-1257 (2005).
11. A. V. Skamrov, M. A. Nechaenko, L. E. Goryunova, *et al.*, *J. Mol. Cell. Cardiol.*, **37**, No. 3, 717-733 (2004).
12. A. Van Dijk, P. A. Krijnen, R. A. Vermond, *et al.*, *Apoptosis*, **14**, No. 6, 753-763 (2009).