Phospholipase A₂ activity in ox-LDL-stimulated mesangial cells and modulation by α-tocopherol

Mituru Ozaki, Yoichi Yamada, Katsumi Matoba, Haruhisa Otani, Masatoshi Mune, Susumu Yukawa, and Wataru Sakamoto

Third Department of Internal Medicine, Wakayama Medical College, Wakayama, and Department of Biochemistry, School of Dentistry, Hokkaido University, Sapporo, Japan

Phospholipase A₂ activity in ox-LDL-stimulated mesangial cells and modulation by α-tocopherol.

Background. Oxidized LDL increases the production of both prostaglandin E₂ (PGE₂) and thromboxane B₂ (TXB₂) in rat mesangial cells. These increases were suppressed by antioxidants such as α-tocopherol (α-Toc) or probucol.

Methods. We investigated the mechanism by which oxidized LDL leads to an increase in PGE₂ production using rat mesangial cells in culture. We also examined how α-Toc suppresses this augmentation, by measuring intracellular calcium ion ([Ca²⁺] i) and phospholipase A₂ (PLA₂) activity.

Results. In rat mesangial cells, oxidized LDL increased PLA₂ activity by increasing the intracellular calcium ion content, which resulted in the induction of PGE₂ production. On the other hand, pretreatment of cells with α-Toc, which resulted in a large uptake of α-Toc in cell membranes, markedly suppressed the augmentation of PGE₂ production and PLA₂ activity by oxidized LDL in a dose-dependent manner. However, cytosolic PLA₂ partially purified from mesangial cells was not inhibited by α-Toc despite an increase of α-Toc.

Conclusion. These results suggest that the augmentation of PLA₂ activity in mesangial cells by oxidized LDL is a result of oxidative stresses, and that the antioxidant action of α-Toc is responsible for the suppression of augmentation of PLA₂ activity observed in mesangial cells exposed to oxidized LDL.

Oxidized low density lipoprotein (LDL), which is involved in the formation of foam cells in the early lesions of arteriosclerosis, affects eicosanoid metabolism in several kinds of cultured cells. Eicosanoids modulate inflammatory reactions and participate in the proliferation and differentiation of these cells [1]. Previously, we reported that oxidized LDL increases production of eicosanoids such as prostaglandin E₂ (PGE₂) or thromboxane B₂ (TXB₂) in rat mesangial cells, and that this function of oxidized LDL is suppressed by antioxidants such as α-tocopherol (α-Toc) and probucol [2]. To clarify the effect of oxidized LDL on mesangial cells, we investigated the changes in PGE₂, cytosolic PLA₂ and intracellular calcium ion content ([Ca²⁺] i) after treatment with oxidized LDL. The effects of α-Toc and probucol upon these parameters were also studied in cultured rat mesangial cells.

METHODS

Culture of mesangial cells with human LDL

Rat mesangial cells (1 to 3 × 10⁶ or 3 to 5 × 10⁷ cells) were incubated for more than 24 hours in serum-free RPMI-1640 medium with various concentrations (0, 50, 100, to 200 μg/ml) of native LDL or LDL oxidized by Cu²⁺.

Determination of PGE₂

Mesangial cells (1 to 3 × 10⁶ cells) were incubated in 2 ml of Hanks solution containing 1 μmol/ml arachidonic acid for 60 minutes. PGE₂ released into the medium was then measured by radioimmunoassay using a PGE₂ radioactive iodine RIA kit (PGE₂ [¹²⁵I]–RIA kit; Dupont de Nemours Inc., Wilmington, DE, USA).

Determination of PLA₂ activity

Rat mesangial cells (3 to 5 × 10⁶ cells) were suspended in 50 mm Tris-HCl buffer (pH 7.5, containing 0.1 m NaCl, 1 mm EDTA, 1 mm PMSF, 1 mm benzamidine, 1 μg/ml leupeptin), homogenized with a polytron homogenizer, and centrifuged at 100,000 g at 4°C for 60 minutes. PLA₂ activity was measured in the supernatant (soluble fraction) obtained from this centrifugation using a modification of the method reported by Dole [3].

Determination of intracellular calcium ion content

Intracellular calcium ion ([Ca²⁺] i) was estimated by monitoring the fluorescence of fura-2 using dual-excitation wavelength spectrofluorometry. Rat mesangial cells were incubated in balanced buffer solution containing Fura-2/AM at 37°C for 60 minutes. Fura-2 loaded mesangial cells

Key words: oxidized low density lipoprotein, eicosanoid, inflammation, intracellular calcium, oxidative stress.

© 1999 by the International Society of Nephrology
were centrifuged at 1500 r.p.m. for 10 minutes, thereby excluding extracellular dye from the buffer solution. 

\[ [\text{Ca}^{2+}]_i \] was estimated both before and after stimuli [4].

**Determination of α-tocopherol**

Rat mesangial cells in culture (3 to 5 × 10^7 cells) were homogenized in 4 ml of 20 mM Tris-HCl buffer (pH 7.4) and centrifugated. The supernatant was saved as the cytosol fraction, and the residue was dissolved by mixing in buffer solution containing 0.3% Triton X-100 at 4°C for 60 minutes. Following centrifugation, the supernatant was removed and saved as the particulate fraction. The residue was used as the residue fraction. The concentration of α-Toc in these three fractions was measured by using high-performance liquid chromatography (HPLC).

**Partial purification of PLA2 in rat mesangial cells**

Rat mesangial cells in culture (4.6 × 10^9) were homogenized in 10 ml of 50 mM Tris-HCl buffer solution. The supernatant from centrifugation was used as the starting material. Partial purification of PLA2 was then carried out using Heparin-cellulose affinity column chromatography and Sephacryl S-200 gel-filtration.

**RESULTS**

**Oxidized LDL and PGE2 production**

Prostaglandin E2 production by rat mesangial cells was significantly higher in the group treated with 200 μg/ml oxidized LDL (135 ± 4.0 pg/μg protein/60 min) than the group with no LDL in the culture medium (50.4 ± 2.6 pg/μg protein/60 min), or the group treated with 200 μg/ml native LDL (66.4 ± 1.6 pg/μg protein/60 min), indicating that oxidized LDL stimulates PGE2 production.

**Oxidized LDL and PLA2 activity**

In the group with no LDL, PLA2 activity in the soluble fraction obtained from homogenized mesangial cells was 31.8 U/10^7 cells. As shown in Figure 1, PLA2 activity in the group treated with oxidized LDL increased in a dose dependent manner (50 to 200 μg/ml). In particular, 200 μg/ml oxidized LDL increased PLA2 activity by 5.6 times that of the group with no LDL, while 200 μg/ml native LDL increased PLA2 activity 2.6 times that of the group with no LDL.

**Oxidized LDL and intracellular calcium ion**

In the group with no LDL, [Ca^{2+}] in mesangial cells was 109.8 ± 3.0 nm. The addition of 40 μg/ml of oxidized LDL resulted in a significant increase in [Ca^{2+}] to 427.6 ± 4.6 nm. However, 40 μg/ml native LDL only slightly increased [Ca^{2+}] to 165.4 ± 6.5 nm.

**Modulation of rat mesangial cells by α-tocopherol**

Rat mesangial cells were incubated for 24 hours with 50 μM α-Toc. In the group with no α-Toc, the intracellular α-Toc content was 1.5 ng/10^6 cells, while that in the group with α-Toc increased significantly to 133.9 to 139.4 ng/10^6 cells. In particular, a high concentration of α-Toc was detected in the particulate fraction.

**Increase in PGE2 production and augmentation of PLA2 by oxidized LDL and α-Toc**

A 200 μg/ml aliquot of oxidized LDL increased PGE2 production to 112.8 ± 4.8 pg/μg protein/60 min, while pretreatment with α-Toc reduced this increase to 21.1 ± 2.9 pg/μg protein/60 min, and pretreatment with probucol reduced this increase to 21.0 ± 2.8 pg/μg protein/60 min. Oxidized LDL increased PLA2 activity to 5.6 times that in the group with no LDL, while α-Toc treatment reduced this increase to 35.8 ± 2.0% of that in the group with no LDL. Pretreatment with probucol reduced PLA2 activity to 71.6 ± 7.2% (Fig. 2).

**Partial purification of PLA2 and α-Toc**

When the soluble fraction of mesangial cells was subjected to Heparin-cellulose affinity column chromatography, PLA2 activity was detected in both adsorption and nonadsorption fractions. The PLA2 of the nonadsorption fraction was filtrated at a position equal to a molecular weight of 85 kDa, suggesting that this PLA2 was cytosolic PLA2 (cPLA2). Investigation of the direct action of α-Toc on this partially purified cPLA2 revealed that 6 to 480 μM α-Toc had almost no effect on enzyme activity.
Ozaki et al: PLA₂ activity in mesangial cells

S-173

reported that oxidative stresses oxidized the phosphatidylcholine in cell membranes, resulting in changes to the molecular structure of the cell membrane as well as increased combining affinity of phosphatidylcholine to PLA₂, and thereby increased PLA₂ activity. Consequently, we believe that the augmentation of PLA₂ activity by oxidized LDL reflects this mechanism of oxidative stresses and that the antioxidant action of α-Toc strongly suppresses augmentation of PLA₂ activity in oxidized LDL-stimulated mesangial cells. On the other hand, when stimulating macrophages with PMA or the calcium ionophore A23187, α-Toc suppressed Ca²⁺ influx into macrophages as well as the protein kinase C activity of the cells [6]. This suggests that, in mesangial cells, phenomenon other than antioxidation play a role in the suppression of PLA₂ activity by α-Toc in oxidized LDL-stimulated cells, such as inhibition of intracellular Ca²⁺−induced translocation of cPLA₂ or suppression of phosphorylation of cPLA₂ by protein kinase C. Future research will focus on clarifying these issues.

DISCUSSION

The aim of this study was to analyze the effects of α-Toc on increases in PGE₂ production and augmentation of PLA₂ activity in oxidized LDL-stimulated mesangial cells by modulation of mesangial cells with α-Toc. We found that oxidized LDL increased PLA₂ activity in rat mesangial cells in culture by increasing the intracellular calcium ion content and resulted in induction of PGE₂ production. Chakraborti et al have suggested that the affinity of PLA₂ for membrane phospholipids, which are the enzyme’s substrates, was responsible for the augmentation of PLA₂ activity by oxidative stresses [5]. They

Fig. 2. Effects of α-tocopherol (α-Toc) on oxidized low density lipoprotein (LDL)-induced phospholipase A₂ (PLA₂) activity in rat mesangial cells. After 24 hours of preincubation in medium containing 50 μM α-Toc or probucol (0.25% ethanol), rat mesangial cells (1 to 3 × 10⁶ cells) were incubated for 24 hours in medium containing 200 μg/ml of oxidized LDL. Data express mean ± SE.

REFERENCES