Induction by Lysophosphatidylcholine, a Major Phospholipid Component of Atherogenic Lipoproteins, of Human Coronary Artery Smooth Muscle Cell Migration

Masakazu Kohno, MD; Koji Yokokawa, MD; Kenichi Yasunari, MD; Mieko Minami, MD; Hiroaki Kano, MD; Takao Hanehira, MD; Junichi Yoshikawa, MD

Background
—The objectives of the present study were (1) to determine whether lysophosphatidylcholine (lyso-PC), a prominent component of oxidatively modified LDL, induces migration of human coronary artery smooth muscle cells (SMCs) and, if so, to clarify the mechanism, and (2) to investigate the possible interactions of lyso-PC and platelet-derived growth factor (PDGF)-BB, endothelin-1 (ET-1), adrenomedullin (AM), or vitamin E on SMC migration by the Boyden’s chamber method.

Methods and Results—Lyso-PC induced SMC migration in a concentration-dependent manner between $10^{-6}$ and $5 \times 10^{-5}$ mol/L. By contrast, phosphatidylcholine was without significant activity, and lysophosphatidylinositol and lysophosphatidylserine were much less effective than lyso-PC. Lyso-PC increased basic fibroblast growth factor (bFGF) production in a concentration-dependent manner between $10^{-6}$ and $5 \times 10^{-5}$ mol/L in these cells. Furthermore, lyso-PC–induced SMC migration was inhibited by neutralizing antibody to bFGF but not by neutralizing antibody to transforming growth factor-β1. Lyso-PC–induced migration was significantly enhanced by PDGF-BB or ET-1 but was clearly inhibited by human AM and vitamin E.

Conclusions—These results indicate that (1) lyso-PC induces human coronary artery SMC migration at least in part through release of endogenous bFGF and (2) this lyso-PC–induced migration can be further induced by PDGF-BB and ET-1 and can be inhibited by human AM and vitamin E. Lyso-PC may recruit medial SMCs during the process of coronary atherosclerosis in part by releasing bFGF in concert with PDGF-BB or ET-1 in vascular tissues. This lyso-PC–induced SMC migration may be suppressed by AM and vitamin E under certain pathological conditions.

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Key Words: lysophosphatidylcholine ■ growth substances ■ adrenomedullin ■ endothelin ■ migration ■ cells

Elevated levels of LDL cholesterol are an important risk factor for coronary atherosclerosis and cardiovascular morbidity. However, oxidized LDL is shown to possess more atherogenic properties than native LDL. Actually, several lines of evidence have indicated that oxidatively modified LDL plays a key role in atherogenesis. In oxidized LDL particles, the content of lyso-PC, a polar phospholipid, is found to be dramatically increased. Actually, Parthasarathy and coworkers have demonstrated that as much as 40% of the phosphatidylcholine of LDL is converted to lyso-PC during oxidized modification. In addition, the concentration of lyso-PC is shown to be increased in atherosclerotic arterial lesions in animals fed an atherogenic diet.

On the other hand, the migration of coronary artery medial SMCs into the intima is proposed to be an initial process of intimal thickening in coronary atherosclerotic lesions. Here, we show for the first time that lyso-PC, not phosphatidylcholine, potently induces migration of human coronary artery SMCs and that this lyso-PC–induced migration can be further induced by a potent mitogen, PDGF-BB, and a vasoconstrictive and growth-promoting peptide, ET-1.

Recently, Chai and coworkers demonstrated that the mechanism of enhanced DNA synthesis by oxidized LDL and lyso-PC at least in part involves an autocrine or paracrine action of release of endogenous bFGF. Therefore, we examined whether this lyso-PC–induced SMC migration is caused by the release of endogenous bFGF.

Finally, we show the interaction of lyso-PC and AM, a novel endothelium-derived vasorelaxant peptide, or vitamin E, a potent antioxidant, on the migration of human coronary artery SMCs.

Methods

Materials
SmBM and human coronary artery SMCs were purchased from Clonetics Corp. FCS, trypsin, and Versine were purchased from Gibco Laboratories. Synthetic human AM (1–52) and ET-1 were purchased from Peptide Institute. 8-Bromo-cAMP, IBMX, lyso-PC

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Lyophilophosphatidylcholine and SMC Migration

<table>
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<th>Selected Abbreviations and Acronyms</th>
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<tr>
<td>AM = adrenomedullin</td>
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<td>bFGF = basic fibroblast growth factor</td>
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<td>ET-1 = endothelin-1</td>
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<td>ET-A = endothelin A-type receptor</td>
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<td>FGF = fibroblast growth factor</td>
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<td>IBMX = 3-isobutyl-1-methylxanthine</td>
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<td>lyso-PC = lysophosphatidylcholine</td>
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<tr>
<td>PDGF = platelet-derived growth factor</td>
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<tr>
<td>SmBM = smooth muscle cell basal medium</td>
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<td>SMC = smooth muscle cell</td>
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<td>TGF-β1 = transforming growth factor-β1</td>
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(palmitoyl), phosphatidylcholine (dipalmitoyl), lysophosphatidylsitol (palmitoyl), lysophosphatidylserine, and BSA were purchased from Sigma Chemical Co. Type I, II, III, or V collagen was obtained from Sigma Chemical Co. The cAMP assay kit was purchased from Yamasa Shoyu Co, Ltd. 8-Bromo-cAMP and forskolin on lyso-PC-induced SMC migration, these cAMP-elevating agents were added to the lower chamber in addition to 5×10^{-3} mol/L lyso-PC.

Culture of Human Coronary Artery SMCs

Human coronary artery SMCs were cultured in SmBM containing human epidermal growth factor (0.5 ng/mL), human FGF (2 ng/mL), insulin (5 μg/mL), 5% FCS, 50 μg/mL gentamicin sulfate, and 50 μg/mL amphotericin-B. Cells were identified as SMCs according to their morphological and growth characteristics. Cultures were maintained at 37°C with atmospheric air and 5% CO2. Cells were subcultured after treatment with 0.25% trypsin and 0.02% EDTA. Subconfluent SMCs between the fourth and eighth passages were used for the experiments.

Migration Assay

Migration of SMCs was assayed by a modification of the Boyden’s chamber method using microchemotaxis chambers (Nucleopore Corp) and polycarbonate filters (Nucleopore Corp), as previously reported. In all experiments, collagen-coated filters were used. Briefly, the membranes were treated with 0.5N acetic acid and then incubated for 48 to 72 hours at 25°C in a collagen solution (100 μg/mL type I collagen in 0.5N acetic acid). They were then air-dried. Type I collagen was used to coat the filters in the chemotaxis chambers, because the chemotactic effect of lyso-PC was greater when type I collagen was used as a substrate than when type II, type III, or type V collagen was used (type I>type III>type V>type II).

Cultured SMCs were trypsinized and suspended at a concentration of 5×10^5 cells/mL in SmBM. The cell number was counted with an electronic cell counter (model ZB1, Coulter Electronics). A volume of 200 μL of SMC suspension was placed in the upper chamber, and 40 μL of medium (0.4% BSA containing 10^{-7}, 5×10^{-7}, 10^{-6}, 5×10^{-6}, 10^{-5}, 5×10^{-5}, 10^{-4}, or 5×10^{-4} mol/L lyso-PC) was placed in the lower chamber. The chamber was incubated at 37°C under 5% CO2 in air for 6 hours.

Migration activity is calculated as the mean number of migrated cells observed in four high-power fields, as previously reported. Four different wells were studied at the same day on the same cell preparation. This procedure was performed in four independent experiments.

In addition, we examined the effects of lysophosphatidylsitol and lysophosphatidylserine on human coronary artery SMC migration.

In experiments to determine the effect of endogenous bFGF or TGF-β1 on lyso-PC-induced SMC migration, two concentrations (1 and 10 μg/mL) of neutralizing antibodies to bFGF and TGF-β1 were added to the upper chamber 30 minutes before the addition of lyso-PC to the lower chamber. Neutralizing antibody to bFGF used here reacts with bovine, human, rat, and mouse bFGF and does not cross-react with bovine acidic FGF. The neutralizing antibody to TGF-β1 used here reacts with mouse, rat, and human TGF-β1 and does not cross-react with either TGF-β1 or TGF-β2.

Effects of PDGF-BB or ET-1 on lyso-PC-induced SMC migration were also examined. Two concentrations of PDGF-BB (0.5 and 2 ng/mL) or ET-1 (10^{-8} and 10^{-7} mol/L) were added to the lower chamber with 5×10^{-6} mol/L lyso-PC. In an experiment to determine the effect of the ET_A receptor antagonist BQ-123 on ET-1-induced migration, BQ-123 and ET-1 were added to the lower chamber with lyso-PC.

In experiments to determine the effects of AM or vitamin E on lyso-PC-induced SMC migration, various concentrations (10^{-8}, 10^{-7}, and 10^{-6} mol/L) of human AM or 5×10^{-5} mol/L vitamin E were added to the lower chamber in addition to lyso-PC.

In separate experiments to determine the effects of 8-bromo-cAMP and forskolin on lyso-PC-induced SMC migration, these cAMP-elevating agents were added to the lower chamber in addition to 5×10^{-3} mol/L lyso-PC. bFGF Measurement

Human coronary artery SMCs were cultured in 35-mm culture dishes, treated with various concentrations of lyso-PC for 6 hours, and then harvested by first rinsing the cultures in Dulbecco’s PBS and then overlaying them with 0.2 mL of Dulbecco’s PBS containing 2×10^{-4} mol/L EDTA, pH 8.0, 2×10^{-3} mol/L N-ethylmaleimide, and 10^{-3} mol/L PMSF, as previously reported. After cells were disrupted by three cycles of freezing and thawing followed by sonication for 1 minute, the homogenate was centrifuged at 25 000g for 30 minutes. The supernatant was stored at −80°C until bFGF quantification was performed with a solid-phase ELISA (Quantikine FGF Basic Immunoassay Kit; R&D Systems, Inc). The average recovery rate of bFGF in cell culture medium was 89%. The minimal detectable dose of bFGF was 1 pg/mL. The interassay variation was 8.7%, and the intra-assay variation was 4.6%.

cAMP Measurement

After preincubation, the cell monolayers were washed twice with PBS and then stimulated for 30 minutes with different concentrations of human AM dissolved in SmBM that contained 5×10^{-6} mol/L IBMX. cAMP levels were determined by radioimmunoassay done with the cAMP assay kit, as previously described.

Calculations and Analysis

The statistical significance of differences in the results was evaluated with an unpaired ANOVA, and P values were calculated by Scheffe’s method. All values were expressed as mean±SD.

Results

Effect of Lyso-PC on SMC Migration

Figure 1 shows concentration-dependent effects of lyso-PC (10^{-6}–5×10^{-5} mol/L) on SMC migration for 6 hours of incubation. Lyso-PC significantly induced migration in a concentration-dependent manner between 10^{-5} and 5×10^{-5} mol/L. By contrast, the migration-stimulatory effects of the higher range of concentrations (10^{-4} and 5×10^{-4} mol/L) of lyso-PC were clearly weak or none (Figure 1).

Micrographs of human coronary artery SMCs taken after a 6-hour migration period clearly show that lyso-PC, not...
lyso-PC or 5 mol/L lyso-PC; d, 10 \(2\) mol/L lyso-PC; e, 5 \(10^{-7}\) mol/L lyso-PC; f, 5 \(10^{-6}\) mol/L lyso-PC.

**Figure 1.** Concentration-dependent effects of lyso-PC on human coronary artery SMC migration. *P<0.05 vs control (no lyso-PC); †P<0.05 vs 10 \(7\) mol/L lyso-PC; ‡P<0.05 vs 5 \(10^{-7}\) mol/L lyso-PC; §P<0.05 vs 10 \(8\) mol/L lyso-PC.

The addition of neutralizing antibody to bFGF to the upper chamber clearly abolished bFGF-induced SMC migration (Figure 3A). The addition of 1 \(\mu\)g/mL of neutralizing antibody to bFGF to the upper chamber significantly inhibited lyso-PC–induced migration. When 10 \(\mu\)g/mL of antibody was added, the effect was even greater (Figure 3B). By contrast, neither 1 \(\mu\)g/mL nor 10 \(\mu\)g/mL of neutralizing antibody to TGF-\(\beta\) to the upper chamber affected lyso-PC–induced migration (Figure 3B).

Furthermore, we examined the effect of lyso-PC on bFGF production in cultured human coronary artery SMCs. Lyso-PC significantly increased bFGF production in a concentration-dependent manner and without causing apparent morphological injury (Figure 2).

**Figure 2.** Micrographs of human coronary artery SMCs after a 6-hour migration period (magnification \(\times400\)). A 200-\(\mu\)L volume of SMC suspension was placed in upper chamber, and 40-\(\mu\)L volumes of medium containing various concentrations (b-e) of lyso-PC or 5 \(10^{-8}\) mol/L of phosphatidylcholine (f) were placed in lower chamber. a, Control; b, 10 \(6\) mol/L lyso-PC; c, 5 \(10^{-6}\) mol/L lyso-PC; d, 10 \(7\) mol/L lyso-PC; e, 5 \(10^{-7}\) mol/L lyso-PC; and f, 5 \(10^{-6}\) mol/L phosphatidylcholine.

**Figure 3.** A, Effect of bFGF on SMC migration and effect of neutralizing antibody to bFGF (Ab-FGF) on bFGF-induced SMC migration. Various concentrations (5, 10, and 20 ng/mL) of bFGF were added to lower chamber. Ab-FGF (10 \(\mu\)g/mL) was added to upper chamber 30 minutes before addition of bFGF. B, Effect of neutralizing antibody to bFGF or TGF-\(\beta\) on lyso-PC–induced SMC migration. *P<0.05 vs control. Ab-FGF represents a neutralizing monoclonal antibody against bFGF, and Ab-TGF represents an irrelevant neutralizing antibody, Ab-FGF represents a neutralizing polyclonal antibody against bFGF, and Ab-TGF represents a neutralizing monoclone antibody against TGF-\(\beta\).

**Table 1.** Effects of Other Lysophospholipids Structurally Similar to Lyso-PC on Human Coronary Artery SMC Migration

<table>
<thead>
<tr>
<th>Migration Activity, Cells/4HPF/6 h</th>
<th>5(\times10^{-6}) mol/L</th>
<th>5(\times10^{-5}) mol/L</th>
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<tbody>
<tr>
<td>Baseline</td>
<td>9.5±1.3</td>
<td>11.3±2.5</td>
</tr>
<tr>
<td>Phosphatidylcholine</td>
<td>8.8±2.8</td>
<td>10.8±2.1</td>
</tr>
<tr>
<td>Lysophosphatidylinositol</td>
<td>13.0±1.8</td>
<td>16.5±2.6†</td>
</tr>
<tr>
<td>Lysophosphatidylserine</td>
<td>13.8±2.4</td>
<td>15.5±1.3†</td>
</tr>
<tr>
<td>Lyso-PC</td>
<td>23.5±3.3§†‡§</td>
<td>36.3±2.6†‡§</td>
</tr>
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</table>

Values are mean±SD of four measurements. Migration activities are expressed as number of cells per four high-power fields (4HPF).

*P<0.05 vs baseline level.
†P<0.05 vs level of phosphatidylcholine.
‡P<0.05 vs level of lysophosphatidylinositol.
§P<0.05 vs level of lysophosphatidylserine.
concentration-dependent manner between $10^{-6}$ and $5 \times 10^{-5}$ mol/L (Figure 4). bFGF concentration caused by $5 \times 10^{-5}$ mol/L were 9-fold greater than nonstimulated controls. These results suggest that lyso-PC induces SMC migration at least in part through release of endogenous bFGF.

To further clarify the mechanism by which lyso-PC induces human coronary artery SMC migration, we examined the ability of vitamin E, a potent antioxidant, to inhibit lyso-PC–induced chemotaxis. Vitamin E ($5 \times 10^{-5}$ mol/L) significantly inhibited SMC migration induced by $5 \times 10^{-5}$ mol/L lyso-PC, whereas vitamin E could not inhibit the nonstimulated and bFGF-induced SMC migration (Figure 5). This suggested that the vitamin E–inhibitable step in the lyso-PC–induced migration was before stimulation by bFGF.

**Effects of PDGF-BB and ET-1 on Lyso-PC–Induced SMC Migration**

Figure 6 shows the effects of PDGF-BB and ET-1 on lyso-PC–induced SMC migration. Lyso-PC–induced migration was significantly enhanced by PDGF-BB. Lyso-PC–induced migration was also enhanced by ET-1. It is of note that ET-1 alone cannot induce SMC migration at the concentrations used in this study (Figure 6B).

**Table 2. Effect of ET A Receptor Antagonist BQ-123 on Enhancement by ET-1 of Lyso-PC–Induced Human Coronary Artery SMC Migration**

<table>
<thead>
<tr>
<th>Migration Activity, Cells/4HPF/6 h</th>
<th>Baseline</th>
<th>ET-1</th>
<th>Lyso-PC</th>
<th>Lyso-PC+BQ-123 $10^{-6}$ mol/L</th>
<th>ET-1+lyso-PC</th>
<th>ET-1+lyso-PC+BQ-123 $10^{-4}$ mol/L</th>
<th>ET-1+lyso-PC+BQ-123 $10^{-2}$ mol/L</th>
<th>ET-1+lyso-PC+BQ-123 $10^{-6}$ mol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Values are mean±SD of four measurements. Migration activities are expressed as number of cells per four high-power fields (4HPF). ET-1 $10^{-7}$ mol/L and/or lyso-PC $5 \times 10^{-5}$ mol/L were added to the lower chamber with or without values concentrations ($10^{-4}$, $10^{-2}$, and $10^{-3}$ mol/L) of BQ-123.</td>
<td>10.7±2.2</td>
<td>11.1±2.5</td>
<td>24.1±3.7†</td>
<td>23.4±4.2‡</td>
<td>39.7±4.2‡‡</td>
<td>34.5±4.7‡‡</td>
<td>29.3±4.1§</td>
<td>25.7±3.0‡§</td>
</tr>
</tbody>
</table>

*P<0.05 vs baseline.
†P<0.05 vs ET-1 alone.
‡P<0.05 vs lyso-PC alone.
§P<0.05 vs ET-1 plus lyso-PC.
Figure 7. A, Effects of human adrenomedullin on lyso-PC-induced human coronary artery SMC migration. Various concentrations (10⁻⁹, 10⁻⁸, 10⁻⁷, and 10⁻⁶ mol/L) of human adrenomedullin were added to lower chamber in addition to 5×10⁻⁵ mol/L lyso-PC (●) or 5×10⁻⁶ mol/L lyso-PC (○). *P<0.05 vs lyso-PC alone; †P<0.05 vs lyso-PC+10⁻⁸ mol/L adrenomedullin; ‡P<0.05 vs lyso-PC+10⁻⁶ mol/L adrenomedullin.

B, Effects of human adrenomedullin on cellular cAMP level in cultured human coronary artery SMCs treated with lyso-PC. Cells were exposed to different concentrations of human adrenomedullin for 30 minutes in addition to 5×10⁻⁵ mol/L lyso-PC (●) or 5×10⁻⁶ mol/L lyso-PC (○) in presence of 5×10⁻⁴ mol/L IBMX. Values are expressed as mean±SD of 6 measurements. *P<0.05 vs lyso-PC alone.

Effect of AM on Lyso-PC–Induced SMC Migration

Figure 7A shows effects of various concentrations (10⁻⁹, 10⁻⁸, 10⁻⁷, and 10⁻⁶ mol/L) of human AM on 5×10⁻⁵ mol/L or 5×10⁻⁶ mol/L lyso-PC–induced SMC migration. Human AM significantly inhibited 5×10⁻⁵ mol/L lyso-PC–induced migration at concentrations of 10⁻⁷ and 10⁻⁶ mol/L. Human AM also inhibited 5×10⁻⁵ mol/L lyso-PC–induced migration in a concentration-dependent manner between 10⁻⁸ and 10⁻⁶ mol/L.

The effects of AM on cellular cAMP levels in cells treated with 5×10⁻⁶ or 5×10⁻⁵ mol/L lyso-PC are shown in Figure 7B. In parallel with the inhibition by AM of lyso-PC–induced SMC migration, cellular cAMP increased after treatment with AM (Figure 7A and 7B).

Effects of 8-Bromo-cAMP and Forskolin on Lyso-PC–Induced SMC Migration

To elucidate whether the inhibitory effect of AM on lyso-PC–induced SMC migration is causally linked to the increase in cellular cAMP, we examined the effect of 8-bromo-cAMP, a cAMP analogue, on lyso-PC (5×10⁻⁵ mol/L)–induced SMC migration. Inhibition of lyso-PC–induced SMC migration by AM could be reproduced by this analogue at concentrations of 10⁻⁶ and 10⁻⁴ mol/L (Figure 8A).

Furthermore, the effect of forskolin, an activator of adenylate cyclase, on lyso-PC (5×10⁻⁵ mol/L)–induced SMC migration was examined. The addition of forskolin also reduced lyso-PC–induced SMC migration at concentrations of 10⁻⁷ and 10⁻⁶ mol/L (Figure 8B).

Discussion

In the present study, we show that lyso-PC, a prominent component of oxidized LDL,1,5 induces migration of human coronary artery SMCs. To the best of our knowledge, this is the first demonstration concerning the relationship between lyso-PC and SMC migration. This may support the finding shown by Autio and coworkers17 and subsequently by our group12 that oxidized LDL stimulates the migration of cultured rat or human SMCs, because phosphatidylcholine of LDL is found to be converted to lyso-PC during oxidized modification. Furthermore, both lyso-PC and oxidized LDL are shown to stimulate SMC proliferation.1,8 Several lines of evidence suggest that oxidized LDL19 and its major phospholipid component, lyso-PC, are present in the intima. Therefore, these observations raise the hypothesis that the conversion of phosphatidylcholine into lyso-PC in the intima may render it chemotactic for medial SMCs, thus contributing to plaque formation and atherogenesis in the coronary artery.

The specific mechanisms of action of lyso-PC as a chemotactic factor for human coronary artery SMCs remains to be established, but the present findings at least allow us to say something about the possibilities. bFGF was a weak chemoattractant for human coronary artery SMCs and neutralizing antibody to bFGF–inhibited lyso-PC–induced migration of human coronary artery SMCs. Actually, lyso-PC increased bFGF production in these cells. These results raise the possibility that lyso-PC stimulates human coronary artery SMC migration at least in part through release of bFGF, which is similar to the mechanism involved in stimulation of SMC proliferation by lyso-PC. Furthermore, we showed that vitamin E significantly inhibited lyso-PC–induced SMC migration but could not inhibit the bFGF–induced SMC migration. It is therefore likely that the vitamin E–inhibitable step in the lyso-PC–induced SMC migration is before stimulation by bFGF.

Next, we showed that lyso-PC–induced migration could be enhanced by a potent mitogen, PDGF-BB, and the endothelium-derived vasoconstrictive and growth-promoting peptide ET-1. It is of note that ET-1 significantly enhanced lyso-PC–induced migration even though ET-1 alone had no effects on...
SMC migration, which means the synergism of ET-1 with lys-PC. Actually, a low concentration of exogenous ET-1 is found to potentiate the vasoconstrictive or mitogenic action of norepinephrine, serotonin, or PDGF. In addition, ET-induced enhancement of SMC migration was clearly inhibited by the ET₄ receptor antagonist BQ-123. It is therefore likely that this naturally occurring phospholipid may function in combination with PDGF or ET-1 (possibly mediated by ET₁ receptors) and may contribute to the intimal thickening in coronary atherosclerotic lesions. Conversely, phosphatidylcholine was without significant activity, and lysophosphatidylinositol and lysophosphatidylserine were much less effective than lys-PC. However, the precise structural specificity of lys-PC that has such an effect on SMC migration remains to be clarified at this time.

Then, we showed that human AM strongly inhibits lys-PC–induced migration of human coronary artery SMCs in a concentration-dependent manner. Actually, 5×10⁻⁷ mol/L lyso-PC–induced SMC migration was significantly inhibited by human AM at concentrations of 10⁻⁸ to 10⁻⁶ mol/L. Although human AM (1–52) is the major circulating form of AM, the normal plasma concentrations (≈10⁻¹¹ to 10⁻¹⁰ mol/L) are much lower than those of synthetic human AM that inhibited SMC migration in our study. However, local levels of AM in coronary artery tissues may be much higher than plasma concentrations of AM, because it has been demonstrated that a considerable amount of AM is synthesized in and secreted from vascular endothelial cells. Taking the matter into account, our results suggest that AM, by acting locally as a paracrine agent, inhibits the migration of human coronary artery SMCs. Recently, we showed that AM had a modest antiproliferative effect in cultured rat aortic SMCs. Consequently, these findings may raise the possibility that human AM in local vascular tissues antagonizes the development of intimal thickening during the process of coronary atherosclerosis. However, this experiment was done on cultured SMCs. Therefore, any extrapolation from the present experiment on cultured SMCs to in vivo conditions should be carefully performed.

Finally, we have shown that the inhibitory effect of AM on lys-PC–induced SMC migration is causally linked to the increase in the cellular cAMP. Actually, human AM increased cAMP levels, and this effect paralleled the migration-inhibitory effect of AM. The cAMP-elevating agents 8-bromo-cAMP and forskolin significantly inhibited lys-PC–induced migration. These results suggest that AM inhibits its lyso-PC–induced migration, at least in part, through a cAMP-dependent process.

In conclusion, our results indicate that AM, the normal plasma concentrations of AM, is a novel endothelium-derived relaxing factor, and vitamin E, an antioxidant, inhibit lys-PC–induced human coronary artery SMC migration. Therefore, further studies will be necessary to clarify the exact cellular mechanisms responsible for cellular actions of lys-PC and the interaction of this phospholipid and other vasoactive substances.

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References


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