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-$\beta_1$. 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.

(Circulation. 1998;98:353-359.)

**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...
Lysophosphatidylcholine and SMC Migration

<table>
<thead>
<tr>
<th>Selected Abbreviations and Acronyms</th>
</tr>
</thead>
<tbody>
<tr>
<td>AM = adenomedullin</td>
</tr>
<tr>
<td>bFGF = basic fibroblast growth factor</td>
</tr>
<tr>
<td>ET-1 = endothelin-1</td>
</tr>
<tr>
<td>ETA = endothelin A-type receptor</td>
</tr>
<tr>
<td>FGF = fibroblast growth factor</td>
</tr>
<tr>
<td>IBMX = 3-isobutyl-1-methylxanthine</td>
</tr>
<tr>
<td>lyso-PC = lysophosphatidylycerine</td>
</tr>
<tr>
<td>PDGF = platelet-derived growth factor</td>
</tr>
<tr>
<td>SmBM = smooth muscle cell basal medium</td>
</tr>
<tr>
<td>SMC = smooth muscle cell</td>
</tr>
<tr>
<td>TGF-β1 = transforming growth factor-β1</td>
</tr>
</tbody>
</table>

(phalmitoyl), phosphatidylcholine (1apalmipitoiy), lysophosphatidilinositol (palmitoyl), lysophosphatidylerine, and BSA were purchased from Sigma Chemical Co. Type I, II, III, or V collagen was purchased from Koken Inc. Flasks and multwell plates were purchased from Becton Dickinson Co. The cAMP assay kit was purchased from Yamasa Shoyu Co. Ltd. Diff-Quick staining solution was purchased from Green-cross Corp. Forskolin was provided as a gift by Nihon Kayaku Co. Ltd. ETα, antagonist, BQ-123, was provided as a gift from Nippon Kayaku Co. Ltd. ETα, antagonist, BQ-123, was provided as a gift from New Drug Discovery Research Laboratorys, Tsukuba Research Institute, Banyu Pharmaceutical. Neutralizing antibody to bFGF (mouse monoclonal IgG1K) and human bFGF (recombinant) were purchased from Upstate Biotechnology, Inc. Neutralizing antibody to TGF-β1 (rabbit polyclonal antibody) was purchased from Santa Cruz Biotecnology, Inc.

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% PCS, 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 (Neuro Probe Inc) and polycarbonate filters (Nucleopore Corp), as previously reported. 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 ~5.0×10⁵ 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 10⁻², 5×10⁻², 10⁻⁴, 5×10⁻⁴, 10⁻⁶, 5×10⁻⁶, 10⁻⁸, or 5×10⁻⁸ 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 lysophosphatidylcholines structurally similar to lyso-PC, such as phosphatidylcholine, lysophos-
phosphatidylcholine, induced migration in a concentration-
dependent manner and without causing apparent morphological
injury (Figure 2).

Table 1 shows the effects of other lysophospholipids structurally similar to lyso-PC on SMC migration. When these phospholipids were tested at two comparable concentrations, phosphatidylcholine was without significant activity, and lysophosphatidylglycerol and lysophosphatidylserine appear to be much less effective than lyso-PC.

**Effects of Neutralizing Antibody to bFGF on Lyso-PC–Induced SMC Migration**

To clarify the role of endogenous bFGF in lyso-PC–induced SMC migration, cells were treated with antibody that neutralize bFGF. The results of these experiments are shown in Figure 3. bFGF slightly but significantly stimulates SMC migration in a concentration-dependent manner (Figure 3A).

The addition of neutralizing antibody to bFGF to the upper chamber clearly abolished bFGF-induced SMC migration (Figure 3A). The addition of 1 μg/mL of neutralizing antibody to bFGF to the upper chamber significantly inhibited lyso-PC–induced migration. When 10 μg/mL of antibody was added, the effect was even greater (Figure 3B). By contrast, neither 1 μg/mL nor 10 μg/mL of neutralizing antibody to TGF-β1 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

**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>5×10⁻⁶ mol/L</th>
<th>5×10⁻⁵ mol/L</th>
</tr>
</thead>
<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>Lysophosphatidylglycerol</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>
</tbody>
</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 lysophosphatidylglycerol.
§P<0.05 vs level of lysophosphatidylserine.

The addition of neutralizing antibody to bFGF to the upper chamber clearly abolished bFGF-induced SMC migration (Figure 3A). The addition of 1 μg/mL of neutralizing antibody to bFGF to the upper chamber significantly inhibited lyso-PC–induced migration. When 10 μg/mL of antibody was added, the effect was even greater (Figure 3B). By contrast, neither 1 μg/mL nor 10 μg/mL of neutralizing antibody to TGF-β1 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

**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 μg/mL) was added to upper chamber 30 minutes before addition of 20 ng/mL bFGF to lower chamber. bFGF significantly stimulated SMC migration, and Ab-FGF clearly abolished bFGF-induced SMC migration. *P<0.05 vs control. B, Effect of neutralizing antibody to bFGF or TGF-β1 on lyso-PC–induced SMC migration. Two concentrations (1 and 10 μg/mL) of neutralizing antibody to bFGF or TGF-β1 were added to upper chamber 30 minutes before addition of 5×10⁻⁵ mol/L lyso-PC to lower chamber. IgG represents an irrelevant neutralizing antibody, Ab-FGF represents a neutralizing monoclonal antibody against bFGF, and Ab-TGF represents a neutralizing polyclonal antibody against TGF-β1. Ab-FGF significantly inhibited SMC migration stimulated with lyso-PC. Irrelevant antibodies (IgG and Ab-TGF) did not significantly change migration activities. *P<0.05 vs control.

**Figure 2.** Micrographs of human coronary artery SMCs after a 6-hour migration period (magnification ×400). A 200-μL volume of SMC suspension was placed in upper chamber, and 40-μL volumes of medium containing various concentrations (b–e) of lyso-PC or 5×10⁻⁵ 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, 10⁻⁵ mol/L lyso-PC; e, 5×10⁻⁵ mol/L lyso-PC; and f, 5×10⁻⁶ mol/L phosphatidylcholine.
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.

![Figure 4](image4.png)  
**Figure 4.** Effects of lyso-PC on bFGF production in cultured human coronary artery SMCs. Cells were exposed to different concentrations ($10^{-2}$ to $5 \times 10^{-5}$ mol/L) of lyso-PC for 6 hours. Values are expressed as mean±SD of four measurements. $^*P<0.05$ vs nonstimulated controls.

![Figure 5](image5.png)  
**Figure 5.** Effects of vitamin E on lyso-PC and bFGF–induced migration in cultured human coronary artery SMCs. Vitamin E ($5 \times 10^{-5}$ mol/L) was added to lower chamber with or without values concentrations ($10^{-2}, 10^{-3}$, and $10^{-4}$ mol/L) of lyso-PC. $^*P<0.05$ vs baseline.

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).

![Figure 6A](image6A.png)  
**Figure 6A.** Effect of PDGF-BB on lyso-PC–induced SMC migration. Two concentrations (0.5 and 2 ng/mL) of PDGF-BB were added to lower chamber with $5 \times 10^{-6}$ mol/L lyso-PC. $^*P<0.05$ vs control; $^†P<0.05$ vs lyso-PC alone; $^‡P<0.05$ vs PDGF (0.5 ng/mL) alone; $§P<0.05$ vs PDGF (2 ng/mL) alone.

![Figure 6B](image6B.png)  
**Figure 6B.** Effect of ET-1 on lyso-PC–induced SMC migration. Two concentrations ($10^{-2}$ and $10^{-3}$ mol/L) of ET-1 were added to lower chamber with $5 \times 10^{-6}$ mol/L lyso-PC. $^*P<0.05$ vs control; $^†P<0.05$ vs lyso-PC ($5 \times 10^{-6}$ mol/L) alone; $^‡P<0.05$ vs ET-1 ($10^{-6}$ mol/L) alone; $§P<0.05$ vs ET-1 ($10^{-7}$ mol/L) alone.

**Table 2.** Effect of ETα 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 $10^{-6}$ mol/L</th>
<th>ET-1 + lyso-PC + BQ-123 $10^{-7}$ mol/L</th>
<th>ET-1 + lyso-PC + BQ-123 $10^{-8}$ mol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td></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>

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 $5 \times 10^{-6}$ mol/L were added to the lower chamber with or without values concentrations ($10^{-6}, 10^{-7}$, and $10^{-8}$ mol/L) of BQ-123. $^*P<0.05$ 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.
SMC migration, cellular cAMP increased after treatment with AM. In parallel with the inhibition by AM of lyso-PC–induced SMC migration, reduced lyso-PC–induced SMC migration at concentrations 10⁻⁶ through 10⁻³ mol/L AM also inhibited 5 mol/L lyso-PC (Figure 7A and 7B).

**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 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 5×10⁻⁷ mol/L lyso-PC–induced migration in a concentration-dependent manner between 10⁻⁶ 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,³,⁵ 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 coworkers¹² 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.³ Furthermore, both lyso-PC and oxidized LDL are shown to stimulate SMC proliferation.⁹,¹⁸ Several lines of evidence suggest that oxidized LDL⁵ 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.⁵ 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

**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+10⁻³ mol/L adrenomedullin; ‡P<0.05 vs lyso-PC+10⁻⁴ 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.

**Figure 8.** A, Effect of 8-bromo-cAMP (8-Br) on migration of cultured human coronary artery SMCs stimulated with lyso-PC. Various concentrations (10⁻⁴, 10⁻⁵, and 10⁻⁶ mol/L) of 8-bromo-cAMP were added to lower chamber in addition to 5×10⁻⁵ mol/L lyso-PC. *P<0.05 vs lyso-PC alone; †P<0.05 vs 10⁻⁶ mol/L 8-bromo-cAMP. B, Effect of forskolin (FOR) on lyso-PC–induced human coronary artery SMC migration. Various concentrations (10⁻⁷, 10⁻⁶, and 10⁻⁵ mol/L) of forskolin were added to lower chamber in addition to 5×10⁻⁵ mol/L lyso-PC. *P<0.05 vs lyso-PC alone; †P<0.05 vs 10⁻⁷ mol/L forskolin.
SMC migration, which means the synergism of ET-1 with lyso-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 ET 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 ET 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 lyso-PC. However, the precise structural specificity of lyso-PC that has such an effect on SMC migration remains to be clarified at this time.

Then, we showed that human AM strongly inhibits lyso-PC–induced migration of human coronary artery SMCs in a concentration-dependent manner. Actually, 5 × 10⁻⁵ mol/L lyso-PC–induced SMC migration was significantly inhibited by human AM at concentrations of 10⁻⁸ to 10⁻⁹ mol/L. Although human AM (1–52) is the major circulating form of AM, the normal plasma concentrations (∼10⁻¹ⁱ 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 lyso-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 lyso-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, a novel endothelium-derived relaxing factor, and vitamin E, an antioxidant, inhibit lyso-PC–induced human coronary artery SMC migration. However, further studies will be necessary to clarify the exact cellular mechanisms responsible for cellular actions of lyso-PC and the interaction of this phospholipid and other vasoactive substances.

Acknowledgments
This work was supported by a grant-in-aid for scientific research from the Ministry of Education, Science, and Culture, Japan. The authors gratefully acknowledge the technical assistance of Atsumi Ohnishi, Yuka Inoshita, and Miyuki Kitakaze of the Division of Hypertension and Atherosclerosis, The First Department of Internal Medicine, Osaka City University Medical School.

References


Induction by Lysophosphatidylcholine, a Major Phospholipid Component of Atherogenic Lipoproteins, of Human Coronary Artery Smooth Muscle Cell Migration
Masakazu Kohno, Koji Yokokawa, Kenichi Yasunari, Mieko Minami, Hiroaki Kano, Takao Hanehira and Junichi Yoshikawa

_Circulation_. 1998;98:353-359
doi: 10.1161/01.CIR.98.4.353

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1998 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/98/4/353

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Circulation_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Circulation_ is online at:
http://circ.ahajournals.org//subscriptions/