Local Production of Lipoprotein-Associated Phospholipase A₂ and Lysophosphatidylcholine in the Coronary Circulation
Association With Early Coronary Atherosclerosis and Endothelial Dysfunction in Humans

Shahar Lavi, MD; Joseph P. McConnell, PhD; Charanjit S. Rihal, MD; Abhiram Prasad, MD; Verghese Mathew, MD; Lilach O. Lerman, MD, PhD; Amir Lerman, MD

Background—Lipoprotein-associated phospholipase A₂ (Lp-PLA₂) is a novel marker and participant in vascular inflammation. Inflammation also is associated with coronary atherosclerosis. We tested the hypothesis that local coronary production of Lp-PLA₂ is enhanced in patients with early coronary atherosclerosis and associated with local endothelial function.

Methods and Results—Coronary angiography, blood flow, flow reserve, endothelial function assessment, and intravascular ultrasound with volumetric analysis were performed in 15 patients with mild coronary atherosclerosis and in 15 control subjects. Plasma samples were collected simultaneously from the left main coronary artery and coronary sinus for measurement of Lp-PLA₂, lysophosphatidylcholine (a product of Lp-PLA₂), and C-reactive protein. Hemodynamic parameters and cholesterol were similar in both groups. Arterial Lp-PLA₂ levels were similar in patients and control subjects: 225 ng/mL (interquartile range [IQR], 196 to 273 ng/mL) versus 221 ng/mL (IQR, 177 to 294 ng/mL). Lp-PLA₂ net production in the coronary circulation was higher in patients compared with control subjects: 519 ng/min (IQR, 198 to 1276 ng/min) versus 529 ng/min (IQR, 872 to 79 ng/min; \( P = 0.001 \)) and correlated with percent atheroma volume \( (r_s = 0.37, P = 0.04) \). Net production of lysophosphatidylcholine was higher in patients compared with control subjects: 199 ng/min (IQR, −592 to 470 ng/min) versus −505 ng/min (IQR, −1119 to 0 ng/min; \( P = 0.03 \)) and correlated with coronary endothelial dysfunction \( (r_s = 0.5, P = 0.005) \). C-reactive protein was not significantly different between the groups.

Conclusions—Early coronary atherosclerosis in humans is characterized by local production of Lp-PLA₂. Local coronary production of lysophosphatidylcholine, the active product of Lp-PLA₂, is associated with endothelial dysfunction. These results support the role for Lp-PLA₂ in the mechanism of regional vascular inflammation and atherosclerosis in humans. (Circulation. 2007;115:2715-2721.)

Key Words: atherosclerosis • endothelium • inflammation • vasculature

Inflammation has been recognized as a major component in the pathogenesis of atherosclerosis. Inflammation may occur locally in the vessel wall, but signs of inflammation often are found in the systemic circulation. Increased circulating levels of inflammatory biomarkers are found in patients with established coronary artery disease and during acute coronary syndromes. In patients without clinical manifestations of atherosclerosis, levels of inflammatory biomarkers may predict future cardiovascular events. However, in the early stage, coronary atherosclerosis may be associated with a more regional inflammatory process. The increased levels of inflammatory biomarkers in these patients may reflect a direct role of inflammation in the disease process.

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In addition to inflammation, an important manifestation of early atherosclerosis is endothelial dysfunction. Endothelial dysfunction is characterized by an imbalance between the endothelium-dependent vasodilator and vasoconstrictor activity, as well as by altered antiinflammatory and anticoagulant properties of the endothelium. In fact, inflammatory mediators may well contribute to endothelial dysfunction in early atherosclerosis. For example, the association between inflammation and endothelial dysfunction may involve the participation of lipoprotein-associated phospholipase A₂ (Lp-PLA₂). Lp-PLA₂, a member of the phospholipase A₂ family of enzymes, is a 45.4-kDa protein produced by macrophages, T...
lymphocytes, and mast cells. Recently, we have demonstrated that systemic Lp-PLA₂ levels are associated with coronary endothelial dysfunction independently of other cardiovascular risk factors. Increased systemic levels of Lp-PLA₂ also are an independent predictor of coronary events. Lp-PLA₂ is found in human atherosclerotic plaques and hydrolyzes the sn-2 fatty acids of oxidized phospholipids to yield oxidized fatty acid and lysophosphatidylcholine (lysoPC), which are proinflammatory particles. LysoPC plays an important part in the effect of Lp-PLA₂ on endothelial dysfunction. It causes an increase in oxidative stress, downregulation of endothelial nitric oxide synthase mRNA expression in endothelial cells and inhibition of endothelial cell migration to sites of endothelial damage. Thus, Lp-PLA₂ may play a significant role in early coronary atherosclerosis in humans, mainly through production of lysoPC.

Although circulating inflammatory mediators have been linked to cardiovascular disease, information is lacking on the regional production or extraction of inflammatory markers, especially in the early stage of atherosclerosis. The present study was designed to test the hypothesis that in patients with early coronary atherosclerosis, as determined by intravascular ultrasound, Lp-PLA₂ and lysoPC production increases across the coronary circulation and has local effects on endothelial function.

Methods

Patient Population

The study was approved by the Mayo Clinic Institutional Review Board, and informed consent was obtained. The study group consisted of 30 patients who underwent diagnostic coronary angiography for the evaluation of coronary artery disease and did not have significant epicardial coronary artery stenoses (ie, stenosis <30%). Patients and control subjects were prospectively defined according to clinical characteristics of the patients. Manual contour detection of the lumen and the media-adventitia interface was performed in cross sections spaced 0.8 mm apart. Atheroma area was measured in cross sections spaced 0.8 mm apart. Atheroma area was

Study Protocol

The study protocol was performed at the same stage of the diagnostic coronary angiography. Routine blood samples were obtained before the study and included cholesterol and lipoprotein(a) measurements. 1716 Circulation

LysoPC was measured with liquid chromatography tandem mass spectrometry with an electrospray triple quadruple mass spectrometry (SCIEX API 3000, AME Bioscience, Concord, Ontario, Canada). Plasma was extracted with butanol after the addition of 17:0 lysoPC as internal standard. The dried-down extract was reconstituted in methanol for analysis with a triple quadruple mass spectrometry (SCIEX API 3000) with a Turbo Ion Spray Source. Chromatographic separation was achieved with a Phenomenex C8 column. Mobile phase consisted of a mixture of methanol (90%), 100 mmol/L ammonium acetate (10%), and formic acid (0.05%). Complete analysis time was 3 minutes with lysoPC eluting at approximately 1.5 minutes. Selective reaction monitoring was used to determine 16:0 lysoPC. Inter-assay CVs were 2.0% at 131 μmol/L and 12.0% at 45 μmol/L.

Serum CRP concentrations were measured on a Hitachi 912 automated chemistry analyzer using a high-sensitivity polystyrene particle–enhanced immunoturbidimetric assay from DiaSorin (Stillwater, Minn). Intra-assay coefficients of variation were 8.8%, 1.1%, and 0.4% at 0.028, 0.20, and 1.15 mg/dL, respectively. Interassay coefficients of variation were 8.0%, 2.0%, and 1.0% at 0.05, 0.30, and 1.86 mg/dL, respectively.

The gradients of Lp-PLA₂, lysoPC, and CRP across the coronary circulation were calculated by subtracting the aortic from the coronary sinus concentration. Net production of each substance in the left anterior descending artery territory was then calculated as the gradient times coronary blood flow.

After blood samples were obtained, 5000 U heparin was given systemically. For calculating the degree of production of Lp-PLA₂ and CRP, coronary blood flow was assessed. A Doppler guidewire (Flowire, Volcano Therapeutics Inc, Rancho Cordova, Calif) was positioned in the mid portion of the left anterior descending coronary artery, and measured velocities were used to calculate coronary blood flow. Velocity signals were instantaneously obtained from the Doppler wire by an online fast Fourier transform, and average peak velocity was determined. Coronary artery diameter was measured by an independent investigator in the segment 5 mm distal to the tip of the Doppler wire offline with a quantitative coronary angiography program (Medis Corp, Leiden, the Netherlands) as previously described. Coronary blood flow was calculated from the Doppler-derived time velocity integral and vessel diameter as follows: \( \pi \times (\text{coronary artery diameter/2})^2 \times \text{(average peak velocity/2)} \).

Coronary vasoreactivity in response to acetylcholine and coronary flow reserve to adenosine were assessed as previously described. In brief, intracoronary bolus injections of incremental doses (18 to 48 μg) of adenosine were administered until maximal hyperemia was achieved or the largest dose was given to evaluate endothelium-independent microvascular coronary flow reserve. Coronary flow reserve was calculated by dividing the average peak velocity after adenosine injection by the average peak velocity at baseline.

Subsequently, to assess endothelium-dependent vasoreactivity, acetylcholine at increasing concentrations (10⁻⁶, 10⁻⁵, and 10⁻⁴ mol/L) was selectively infused for 3 minutes at each concentration into the left anterior descending coronary artery.

After the assessment of coronary flow reserve and coronary endothelial function, 200 μg nitroglycerin IC was given, and IVUS imaging was performed according to previously described methods. A 2.9F IVUS catheter with electronic scanning (Volcano Therapeutics Inc) was inserted into the mid distal left anterior descending artery, and a motorized pullback system at a speed of 0.5 mm/s was used. During pullback, images were obtained at 30 frames per second and were stored digitally on a CD-ROM for later offline 3-dimensional volumetric IVUS analysis. The volumetric IVUS analyses were performed from the distal left anterior descending coronary artery to the left anterior descending coronary artery ostium with customized software (echoPlaque 2, version 2.5, INDEC Systems Inc, Santa Clara, Calif) by an examiner unaware of the clinical characteristics of the patients. Manual contour detection of the lumen and the media-adventitia interface was performed in accordance with the standards of the American College of Cardiology. The external elastic membrane and lumen areas were measured in cross sections spaced 0.8 mm apart. Atheroma area was
TABLE 1. Clinical Characteristics of the Study Groups

<table>
<thead>
<tr>
<th></th>
<th>Early Atherosclerosis (n=15)</th>
<th>Control Subjects (n=15)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age, y</strong></td>
<td>56 ± 2</td>
<td>45 ± 2*</td>
</tr>
<tr>
<td><strong>Male gender, n (%)</strong></td>
<td>5 (33)</td>
<td>2 (13)</td>
</tr>
<tr>
<td><strong>Hypertension, n (%)</strong></td>
<td>9 (60)</td>
<td>6 (40)</td>
</tr>
<tr>
<td><strong>Diabetes mellitus, n (%)</strong></td>
<td>2 (13)</td>
<td>0</td>
</tr>
<tr>
<td><strong>Smoking, n (%)</strong></td>
<td>1 (7)</td>
<td>1 (7)</td>
</tr>
<tr>
<td><strong>Hyperlipidemia, n (%)</strong></td>
<td>10 (67)</td>
<td>8 (53)</td>
</tr>
<tr>
<td><strong>Body mass index, kg/m²</strong></td>
<td>28.7 ± 1.4</td>
<td>31.7 ± 1.4</td>
</tr>
<tr>
<td><strong>Cholesterol, mmol/L</strong></td>
<td>5.05 ± 0.23</td>
<td>4.79 ± 0.23</td>
</tr>
<tr>
<td><strong>LDL, mmol/L</strong></td>
<td>2.9 ± 0.2</td>
<td>2.74 ± 0.2</td>
</tr>
<tr>
<td><strong>Lipoprotein(a), μmol/L</strong></td>
<td>1.28 ± 0.28</td>
<td>0.6 ± 0.28</td>
</tr>
<tr>
<td><strong>Statin use, n (%)</strong></td>
<td>6 (40)</td>
<td>4 (27)</td>
</tr>
<tr>
<td><strong>Postmenopausal women, n (%)</strong></td>
<td>7 (50)</td>
<td>7 (54)</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SEM when appropriate. *P < 0.05 vs early atherosclerosis.

()`c` calculated as external elastic membrane area minus luminal area. Volumes were calculated according to the Simpson rule as mean atheroma area multiplied by pullback length in millimeters. To compensate for differences in length of the studied vessels segment, each measured volume was normalized to 10 mm of length (volumes divided by the examined segment length and multiplied by 10). The percent atheroma volume was computed as follows: Σ atheroma area×100/Σ external elastic membrane area. Endothelial dysfunction was defined as any decrease in coronary artery diameter in response to acetylcholine.

**Data Analysis**

Continuous variables are presented as mean ± SEM or median and interquartile range (IQR) and dichotomous variables as values and percentages. The baseline characteristics of groups and biomarkers levels were compared by the Wilcoxon rank-sum test for continuous variables and by Pearson’s χ² statistic for categorical variables. Single predictor and multivariable linear regression models were used to calculate the association between CRP or Lp-PLA₂ net production and plaque volume. Variables found to show marginal association with net production in the single predictor analysis (P < 0.20) were used in the multivariable model. Because of the known association between low-density lipoprotein (LDL) levels and Lp-PLA₂, LDL levels were added to the model. Pearson’s correlation coefficient and the nonparametric Spearman’s correlation methods were used for correlation analysis, and the linear regression line was added to the figure. Statistical significance was defined at P < 0.05; however, because of multiple comparisons of biomarkers levels, we applied the Bonferroni adjustment and used P < 0.004 as significant for the data for biomarkers levels.

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

**Results**

Patients were divided into 2 groups according to the presence or absence of mild coronary disease: early atherosclerosis (n=15) and control subjects (no evidence of atherosclerosis, n=15). No difference existed in the referring diagnosis between groups. The clinical characteristics of the 2 groups are outlined in Table 1. Patients with early atherosclerosis were older than control subjects. No other significant differences existed in the clinical characteristics, including hyperlipidemia and use of statins, between the 2 groups. Importantly, with regard to Lp-PLA₂ levels, the LDL cholesterol levels were similar in both groups. Lipoprotein(a) levels were slightly but nonsignificantly higher in patients with early atherosclerosis.

**Hemodynamic and 3-Dimensional IVUS Findings**

**Hemodynamic parameters**

<table>
<thead>
<tr>
<th></th>
<th>Early Atherosclerosis (n=15)</th>
<th>Control Subjects (n=15)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean blood pressure, mm Hg</strong></td>
<td>99 ± 3</td>
<td>98 ± 3</td>
</tr>
<tr>
<td><strong>Heart rate, bpm</strong></td>
<td>69 ± 3</td>
<td>69 ± 3</td>
</tr>
<tr>
<td><strong>LAD blood flow, mL/min</strong></td>
<td>43 ± 5</td>
<td>46 ± 5</td>
</tr>
<tr>
<td><strong>Coronary flow reserve to adenosine</strong></td>
<td>2.7 ± 0.1</td>
<td>2.8 ± 0.1</td>
</tr>
</tbody>
</table>

**Morphological parameters**

<table>
<thead>
<tr>
<th></th>
<th>Early Atherosclerosis (n=15)</th>
<th>Control Subjects (n=15)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Segment length, mm</strong></td>
<td>35 ± 4</td>
<td>35 ± 4</td>
</tr>
<tr>
<td><strong>Vessel volume/10 mm, mm³</strong></td>
<td>128 ± 8</td>
<td>109 ± 8</td>
</tr>
<tr>
<td><strong>Lumen volume/10 mm, mm³</strong></td>
<td>90 ± 8</td>
<td>97 ± 8</td>
</tr>
<tr>
<td><strong>Plaque + media volume/10 mm, mm³</strong></td>
<td>38 ± 3</td>
<td>13 ± 3*</td>
</tr>
<tr>
<td><strong>Percent atheroma volume</strong></td>
<td>30 ± 2</td>
<td>12 ± 2*</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SEM. LAD indicates left anterior descending. *P < 0.05 vs early atherosclerosis.

**Relationship Between Lp-PLA₂ and Atherosclerosis**

The IVUS characteristics of the population are presented in Table 2. Patients with early atherosclerosis had slightly but nonsignificantly increased vessel volume and decreased lumen volume, both features of remodeling. Coronary percent atheroma volume and plaque plus media volume were significantly larger in patients with early atherosclerosis. A positive correlation existed between plaque volume and Lp-PLA₂ gradient (r=0.36, P < 0.05) and CRP gradient (r=0.37, P=0.04). Percent atheroma volume also correlated with Lp-PLA₂ gradient (r=0.39, P=0.03) and production (r=0.46, P=0.01; r=0.37, P=0.04; Figure 1). In addition,
percent atheroma volume was significantly larger in the highest tertile of Lp-PLA₂ production compared with the lowest tertile (26.1 ± 4% versus 13.6 ± 4%, respectively; \( P < 0.05 \)). In multivariable analysis, after adjustment for age and LDL, the association between atheroma volume and Lp-PLA₂ production remained significant (\( P = 0.03 \)). No relationship existed between Lp-PLA₂ production and lipoprotein(a) levels, number of risk factors for coronary disease, Framingham risk score, or the degree of coronary calcification by IVUS.

Relationship With Endothelial Dysfunction and LysoPC

A positive correlation existed between percent atheroma volume and lysoPC levels in the coronary sinus (\( r = 0.43 \), \( P = 0.02 \)) or the production of lysoPC in the coronary circulation (\( r = 0.36 \), \( P < 0.05 \)). Because lysoPC may affect endothelial function, we also divided the patients into 2 groups according to the presence or absence of epicardial endothelial dysfunction (decrease in coronary artery diameter in response to acetylcholine). LysoPC gradients and net production in patients with and without endothelial dysfunction were 2 ng/mL (IQR, −16 to 11 ng/mL) versus −17 ng/mL (IQR, −30 to −10 ng/mL; \( P = 0.01 \)) and 87 ng/min (IQR, −609 to 427 ng/min) versus −590 ng/min (IQR, −959 to −282 ng/min; \( P = 0.03 \)), respectively. LysoPC net production also correlated with the degree of coronary artery diameter response to acetylcholine (Figure 2). A trend was noted toward higher degree of endothelial dysfunction (epicardial and microvascular) with larger atheroma volume. No direct correlation existed between endothelial dysfunction and the production of Lp-PLA₂ or between the production of lysoPC and the coronary blood flow response to acetylcholine (a parameter of microvascular endothelial dysfunction).

Discussion

The present study demonstrates for the first time that early coronary atherosclerosis in humans is associated with local coronary production of Lp-PLA₂ and lysoPC. The production of Lp-PLA₂ across the coronary circulation significantly correlated with the degree of coronary atherosclerosis, whereas the production of its product lysoPC also correlated with coronary endothelial dysfunction. These observations support a role for Lp-PLA₂ as a participant in regional

TABLE 3. Biomarker Levels

<table>
<thead>
<tr>
<th></th>
<th>Early Atherosclerosis (n=15)</th>
<th>Control Subjects (n=15)</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lp-PLA₂ (arterial), ng/mL</td>
<td>225 (196 to 273)</td>
<td>221 (177 to 294)</td>
<td>0.81</td>
</tr>
<tr>
<td>Lp-PLA₂ (coronary sinus), ng/mL</td>
<td>241 (221 to 287)</td>
<td>212 (153 to 282)</td>
<td>0.09</td>
</tr>
<tr>
<td>Lp-PLA₂ gradient, ng/mL</td>
<td>16 (7 to 29)</td>
<td>−10 (−36 to −4)</td>
<td>0.0007</td>
</tr>
<tr>
<td>Lp-PLA₂ net production, ng/min</td>
<td>519 (198 to 1276)</td>
<td>529 (−872 to −79)</td>
<td>0.001</td>
</tr>
<tr>
<td>lysoPC (arterial), ng/mL</td>
<td>135 (119 to 157)</td>
<td>125 (114 to 131)</td>
<td>0.14</td>
</tr>
<tr>
<td>lysoPC (coronary sinus), ng/mL</td>
<td>130 (119 to 148)</td>
<td>110 (93 to 126)</td>
<td>0.0037</td>
</tr>
<tr>
<td>lysoPC gradient, ng/mL</td>
<td>2 (−17 to 12)</td>
<td>−16 (−34 to 0)</td>
<td>0.07</td>
</tr>
<tr>
<td>lysoPC net production, ng/min</td>
<td>199 (−592 to 470)</td>
<td>−505 (−1119 to 0)</td>
<td>0.03</td>
</tr>
<tr>
<td>CRP (arterial), mg/L</td>
<td>1.9 (0.4 to 7.7)</td>
<td>4.6 (0.8 to 6.3)</td>
<td>0.44</td>
</tr>
<tr>
<td>CRP (coronary sinus), mg/L</td>
<td>2 (0.5 to 6.1)</td>
<td>4 (0.8 to 5.4)</td>
<td>0.49</td>
</tr>
<tr>
<td>CRP gradient, mg/L</td>
<td>0 (−0.1 to 0.1)</td>
<td>−0.2 (−0.7 to 0)</td>
<td>0.06</td>
</tr>
<tr>
<td>CRP net production, ( \mu g/)min</td>
<td>0 (−2.8 to 5.2)</td>
<td>−4.2 (−21 to 0)</td>
<td>0.06</td>
</tr>
</tbody>
</table>

Values in parentheses represent IQR.

Figure 1. Correlation between Lp-PLA₂ net production in the left anterior descending artery territory and percent atheroma volume.

Figure 2. Correlation between lysoPC net production in the left anterior descending artery territory and endothelial dysfunction (coronary artery diameter response [ΔCAD] to acetylcholine).
vascular inflammation and endothelial dysfunction in early atherosclerosis in humans.

A growing body of epidemiological studies reports an association between systemic Lp-PLA₂ levels and cardiovascular events. In a nested case-control study from the West of Scotland Coronary Prevention Study (WOSCOPS), the risk for coronary events in the highest quintile for Lp-PLA₂ was twice the risk for the lowest quintile. The risk was not attenuated after adjustment for other risk factors, including CRP. In the Atherosclerosis Risk in Communities (ARIC) study, in a large cohort of middle-aged men and women, Lp-PLA₂ levels were associated with increased risk for incident coronary heart disease. These associations raise the question of whether Lp-PLA₂ is a marker of or plays an active role in the atherosclerotic process. The independent association between Lp-PLA₂ levels and cardiovascular risk among different populations and across different levels of cholesterol supports the hypothesis that Lp-PLA₂ has a causative role in this process. Further support for the role of Lp-PLA₂ early in the atherosclerotic process comes from the observation that Lp-PLA₂ levels are elevated in patients with subclinical coronary disease and coronary calcifications and from a recent report from our group demonstrating that circulating Lp-PLA₂ is an independent predictor of coronary endothelial dysfunction, an established stage of early coronary atherosclerosis.

The present study extends these previous observations and demonstrates that in patients with minimal coronary atherosclerosis, local net production of Lp-PLA₂ is increased compared with patients without evidence of atherosclerosis as assessed by IVUS. We prospectively included all patients before performing the IVUS, which was done at the end of the protocol. It is likely that using stricter IVUS criteria to define the control subjects and comparing them with patients with atherosclerosis would result in even more robust results. We also were able to quantify the percent atheroma volume by 3-dimensional volumetric method. This established approach enabled us to correlate the level of Lp-PLA₂ net production in the coronary circulation with the atheroma volume, and indeed a direct and significant association between these parameters was found.

The current paradigm is that Lp-PLA₂ is a promoter of vascular inflammation by virtue of generation of oxidized free fatty acids. Initially, Lp-PLA₂ is carried by LDL to lesion-prone segments of the arterial wall. After LDL oxidation, enzymatic hydrolysis by Lp-PLA₂ leads to generation of lysoPC and oxidized nonesterified fatty acids that play a role in homing of inflammatory cells to lesion-prone areas. These inflammatory cells increase the concentration of Lp-PLA₂ in the vessel wall, upregulate intercellular adhesion molecule-1 and vascular cell adhesion molecule-1, and lead to oxidative stress and endothelial dysfunction. Thus, the source of Lp-PLA₂ in the atherosclerotic plaque may be both from the circulation, bound to LDL, and from synthesis by inflammatory cells within the plaque.

The finding in the present study of increased production of lysoPC in the coronary circulation in patients with early atherosclerosis adds support to the aforementioned paradigm. LysoPC plays an important part in the effect of Lp-PLA₂ on endothelial dysfunction; indeed, we found a direct relationship between lysoPC net production and epicardial coronary endothelial function. The absence of correlation between microvascular endothelial dysfunction and lysoPC production may support the concept that the PLA₂ pathway may play a more significant role in the progression and complications of the epicardial vessels. Possible mechanisms by which lysoPC may affect endothelial function include downregulation of endothelial nitric oxide synthase mRNA expression and depletion of nitric oxide, enhanced production of reactive oxygen species and oxidative stress; induction of endothelial cell apoptosis; and blocking of the repair mechanism by inhibition of endothelial cell migration to sites of endothelial damage.

We hypothesize that in areas with increased local oxidative stress and vascular inflammation, an increase in the local production of Lp-PLA₂ will also be found. This process leads to the initiation and progression of atherosclerotic plaque. The local production of lysoPC causes endothelial dysfunction and further enhances vascular inflammation and progression of atherosclerosis.

Interestingly, patients without evidence of atherosclerosis had a reduction in Lp-PLA₂ and lysoPC levels in the coronary sinus compared with the arterial levels and a negative net production of these inflammatory mediators in the coronary circulation. It may be speculated that one of the functions of the normal vessel wall is extraction of substances like Lp-PLA₂, lysoPC, and CRP, and with the development of atherosclerosis, a shift occurs from extraction to production of such substances.

Although we found a correlation between the gradients of Lp-PLA₂ and CRP across the coronary circulation, CRP gradients were similar in patients with early atherosclerosis and control subjects. In contrast, Lp-PLA₂ gradients and net production, and its product lysoPC, were significantly increased in patients with early atherosclerosis. Although CRP is a marker of systemic inflammation and is produced mainly in the liver, Lp-PLA₂ may be more directly related to local vascular inflammation and endothelial dysfunction. These differences suggest that Lp-PLA₂ and CRP may have different pathophysiological mechanisms in the atherosclerotic process. Although Lp-PLA₂ is more associated with vascular inflammation, both Lp-PLA₂ and CRP can mediate increased expression of adhesion molecules and promote vascular inflammation. The positive correlation between CRP and Lp-PLA₂ production in the present study may be consistent with the synergism between these 2 inflammatory mediators and is supported by findings from the ARIC study, which show that patients who had both CRP and Lp-PLA₂ in the highest tertiles had the highest risk for future coronary events and stroke.

Our study is unique in the measurement of Lp-PLA₂ and lysoPC across an area of atherosclerosis. With this approach, variables that affect their levels such as gender and LDL levels do not affect the gradient. This approach enabled us to show that the activity of Lp-PLA₂ and lysoPC is associated with coronary endothelial function and the degree of the atherosclerotic process.
Study Limitations and Clinical Perspectives

The sampling from the coronary sinus was not selective. However, because none of the patients had significant atherosclerosis, we assumed similar levels in the coronary sinus tributaries for the calculations of net production. Although the mechanism of increased net production of Lp-PLA2 in patients with early atherosclerosis is not completely understood, our findings have important clinical implications. Although intensive therapy with statins decreases both cholesterol levels and inflammation, it does not fully eliminate cardiovascular events.41 Atherosclerosis has been considered an inflammatory disease for the past several years; thus, antiinflammatory agents are being tested for the prevention and treatment of coronary artery disease.42 Drugs that inhibit the Lp-PLA2 pathway may be more specific for atherosclerosis by decreasing oxidative stress and vascular inflammation and potentially will further decrease the risk for future cardiovascular events. Inhibitors of Lp-PLA2 have been developed43 and are currently being assessed in clinical trials. These inhibitors may potentially have a role in the treatment of patients with early atherosclerosis.

Conclusions

The present study demonstrates for the first time that humans with early coronary atherosclerosis and endothelial dysfunction are characterized by local coronary production of Lp-PLA2 and lysoPC and supports the role of local oxidative stress and inflammation in early atherosclerosis in humans.

Sources of Funding

This study was supported by grants from the National Institutes of Health (NIH K24 HL-69840, NIH R01 HL-63911, and HL-77131) and from the Mayo Clinic and the University of Minnesota (MAYO-UOFM #4 PROJ1–2).

Disclosures

None.

References

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Lipoprotein-associated phospholipase A₂ (Lp-PLA₂) is an emerging marker for vascular inflammation, atherosclerosis, and future cardiovascular events. We investigated the relationship between local production of Lp-PLA₂ and early coronary disease in patients with mild coronary atherosclerosis and patients without evidence of coronary disease by angiography and intravascular ultrasound. We collected plasma samples from the left main coronary artery and coronary sinus for measurements of Lp-PLA₂, lysophosphatidylcholine, and C-reactive protein; assessed coronary endothelial function; and calculated the production of each of the inflammatory mediators in the coronary circulation. Patients with early atherosclerosis had increased coronary production of Lp-PLA₂ compared with control subjects. Lp-PLA₂ net production in patients with early coronary disease was higher in patients compared with control subjects and correlated with coronary endothelial dysfunction. In contrast, C-reactive protein, a marker of systemic inflammation, did not demonstrate such relationships. Our results support a role for Lp-PLA₂ as a participant in regional vascular inflammation and endothelial dysfunction in early atherosclerosis in humans.
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