Clinical Significance of Preheparin Serum Lipoprotein Lipase Mass in Coronary Vasospasm

Takashi Hitumoto, MD; Kunio Yoshinaga, MD; Hirofumi Noike, MD; Masahito Kanai, MD; Kohji Shirai, MD*

The present study investigated the clinical significance of preheparin serum lipoprotein lipase (LPL) mass in coronary vasospasm by examining its relationship with the acetylcholine-induced coronary artery response in patients without angiographically demonstrable atherosclerotic coronary artery disease (CAD). The subjects were 39 men who had suspected CAD and who underwent coronary angiography. Coronary vasospasm was defined as a marked luminal narrowing or total occlusion provoked by the intracoronary administration of acetylcholine. Preheparin LPL mass was lower (p<0.05) in 25 subjects in whom vasospasm was induced by the acetylcholine provocation test than in the 14 subjects with a negative response. As regards preheparin LPL mass, the subjects with multiple vessel spasm had significantly low concentrations (p<0.05) compared with single vessel spasm, although serum lipid levels were not significantly different. Multiple regression analysis revealed only preheparin LPL mass had a significant absolute t-value (2.016) among the coronary risk factors. Low preheparin LPL mass is interpreted as reflecting an impaired acetylcholine-induced coronary relaxation in coronary vasospasm and preheparin LPL mass may be useful as a marker of early stage coronary atherosclerosis that is not detectable by angiography. (Jpn Circ J 2001; 65: 539–544)

Key Words: Acetylcholine; Coronary angiography; Coronary vasospasm; Preheparin serum lipoprotein lipase mass

Lipoprotein lipase (LPL) catalyzes hydrolysis of triglyceride (TG) in circulating lipoproteins1 and exists in preheparin serum, even though lipase activity is scarcely detected. Because preheparin serum LPL mass may be involved in the progression of coronary atherosclerosis,2 the correlation between them needs to be examined.

Acetylcholine (Ach) has been used clinically in catheterization laboratories as a standard spasm provocation test. The Ach-induced vascular relaxation is mediated by nitric oxide (NO) released from the endothelium, and an impaired relaxation response reflects endothelial dysfunction3,4. Endothelial function plays an important role in the regulation of circulation and impaired endothelium-dependent dilatation has been reported in patients with risk factors for vascular disease such as old age, dyslipidemia, hypertension, diabetes mellitus, and smoking.5–9 Previous studies have shown that endothelial dysfunction is an early event in atherosclerosis and precedes occlusive vascular disease in both experimental primate models and human heart transplant recipients.10,11 Kugiyama et al reported that deficiency of endothelial NO activity plays an important role in the pathogenesis of coronary vasospasm12 and a recent clinical study using intravascular ultrasound demonstrated early signs of atherosclerosis at the site of focal and diffuse spasms caused by intracoronary administration of Ach.13,14

Thus, impaired ACh-induced relaxation in coronary vasospasm reflects coronary endothelial dysfunction and early stage coronary atherosclerosis.

We examined the relationship between preheparin serum LPL mass and the ACh-induced coronary artery response in patients without angiographically significant coronary artery stenosis, and elucidated the clinical significance of preheparin LPL mass in regard to coronary endothelial dysfunction and early stage coronary atherosclerosis by comparing it with total cholesterol, TG, high density lipoprotein-cholesterol (HDL-C), low density lipoprotein-cholesterol (LDL-C) and other coronary risk factors.

Methods

Subjects

Thirty-nine men with suspected coronary artery disease (CAD) diagnosed from their symptoms as well as from non-invasive procedures (ECG abnormality, treadmill exercise test, thallium-201 myocardial scintigraphy) underwent coronary angiography after giving informed consent at the Cardiovascular Center of Sakura Hospital. The average age of the men was 56 years (standard deviation: ±25; range, 22–79 years). Blood samples were drawn upon fasting just before heparin injection preceding coronary angiography. The samples necessary for LPL mass measurement were frozen at –80°C within 1h of sampling.

Angiographic Study

Selective coronary angiography was performed using either the Judkins method with a transfemoral approach15 or the Sones method with a transbrachial approach16 in the morning after overnight fasting and without anti-angina medications, except in patients for whom it was judged dangerous to discontinue the therapy. Two experienced
angiographers reviewed all coronary angiograms unaware of the patient's clinical data. None of the coronary arteries had a stenotic lesion and the presence of a subtle irregularity of the arterial wall was classified as nearly normal. Coronary vasospasm was defined as a marked luminal narrowing or total occlusion provoked by intracoronary administration of ACh in at least one coronary vessel. Acetylcholine was dissolved in physiological saline, and incremental doses were injected (20 μg and 40 μg into the right coronary, and 40 μg and 80 μg into the left coronary artery). The coronary vasospasm group was divided into 2: Group I = single vessel spasm, and Group II = multiple vessel spasms. The normal coronary group was defined as showing neither change nor extensive reaction to dilatation following intracoronary administration of ACh.

Preheparin LPL Mass Assay
LPL mass was measured by a sandwich enzyme-linked immunosorbent assay (ELISA) kit using a specific monoclonal antibody against lipoprotein lipase (Daiichi Pure Chemicals Co, Ltd, Tokyo, Japan), as described by Kobayashi et al.17 The linearity of this assay system was observed from 5 to 400 ng/ml. The within-run coefficient of variation was 2.8%. Between-day coefficient of variation

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<tr>
<th>Table 1 Baseline Clinical Characteristics</th>
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<tr>
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<tr>
<td><strong>Normal coronary</strong> group (n=14)</td>
</tr>
<tr>
<td>Age (years) 54±16</td>
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<tr>
<td>Coronary risk factor</td>
</tr>
<tr>
<td>Hypertension 5 (36%)</td>
</tr>
<tr>
<td>Diabetes mellitus 1 (7%)</td>
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<td>Hyperuricemia 1 (7%)</td>
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<tr>
<td>Obesity* 5 (36%)</td>
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<tr>
<td>Smoking 6 (43%)</td>
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<td>Family history 1 (7%)</td>
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All values are mean±SD followed by the range. *Body mass index≥24.

![Fig 1. Evaluation of the midband. The eluted lipoprotein patterns were recorded by densitometer and when the individual peak was observed between the and pre- lipoprotein bands or the shoulder was observed on the pre- side of the lipoproteins, the midband was designated positive.](image)

![Fig 2. The correlation between preheparin LPL mass and serum lipids was studied in 39 men who underwent coronary angiography. A negative correlation between preheparin LPL mass and triglyceride (r=-0.384, p<0.001) and a positive correlation between preheparin LPL mass and HDL-cholesterol (r=0.416, p<0.001) were observed.](image)
was 4.3%. Interference by serum TG from 50 to 1,500 mg/dl and serum HDL-C from 5 to 120 mg/dl was not observed.

**Lipid Analysis**

Total cholesterol and TG concentrations were measured enzymatically using a kit (Nippon Shoji Co, Ltd, Osaka, Japan) and an automatic analyzer (Hitachi 7150, Hitachi, Ltd, Tokyo, Japan). HDL-C was measured by the selective inhibition method (Daiichi Pure Chemicals)\(^1\) and LDL-C was calculated by the Friedwalds method (total cholesterol – HDL-C – TG/5). The eluted lipoprotein patterns were recorded by densitometer. As shown in Fig 1, when an independent peak was observed between the \( \Phi \) and pre-\( \Phi \) lipoprotein bands or when a shoulder was observed on the pre-\( \Phi \) side of the \( \Phi \) lipoprotein, the midband was interpreted as positive.

**Coronary Risk Factors**

Examined coronary risk factors were age, smoking, family history, hypertension, hyperuricemia, diabetes mellitus, total cholesterol, TG, HDL-C and body mass index. Smoking was positive with a current or past history of cigarette smoking. The family history was positive if angina pectoris and/or myocardial infarction were present in grandparents, parents and/or siblings. Diabetes mellitus was defined as present if fasting blood glucose was >126 mg/dl and hemoglobin A\(_1\)C was >6.5%. Hyperuricemia was defined by a serum uric acid level of >8.0 mg/dl and hypertension was defined as a history of systolic pressure ≥140 mmHg or diastolic pressure ≥90 mmHg.

**Statistical Analysis**

The results were expressed as means±standard deviation. A t test and Mann-Whitney U test were used for group comparisons. P values less than 0.05 were considered significant. Multiple logistic regressions were performed with the SAS computer programs. Eleven explanatory risk factors including preheparin LPL mass were scored and subordinate variable was coronary vasospasm (normal coronary=0, coronary vasospasm=1). According to the analysis, an explanatory factor with a t value of more than 2 was significantly correlated with the dependent variables.

**Results**

**Baseline Clinical Characteristics (Table 1)**

There were no significant differences in the coronary risk factors or medications.

**Preheparin LPL Mass and Serum Lipid Levels in All Subjects Undergoing Coronary Angiography**

A negative correlation between preheparin LPL mass and TG (r=–0.384) and a positive correlation between preheparin LPL mass and HDL-C (r=0.416) were observed (Fig 2A,B). There was not a significant correlation between preheparin LPL mass and either total cholesterol or LDL-C (Fig 2C,D).

**Comparison of the Serum Lipid Levels and Preheparin Lipoprotein Lipase (LPL) Mass**

Table 2

<table>
<thead>
<tr>
<th></th>
<th>Midband negative group (n=18)</th>
<th>Midband positive group (n=21)</th>
<th>p value</th>
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<tbody>
<tr>
<td>Age (years)</td>
<td>52±14</td>
<td>59±13</td>
<td>NS</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>196±23</td>
<td>179±33</td>
<td>NS</td>
</tr>
<tr>
<td>Triglyceride (mg/dl)</td>
<td>103±52</td>
<td>186±122</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dl)</td>
<td>56±14</td>
<td>44±1</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dl)</td>
<td>120±26</td>
<td>98±35</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Preheparin LPL mass (ng/ml)</td>
<td>50±17</td>
<td>40±15</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>No. of coronary vasospasms/normal coronary</td>
<td>10/8</td>
<td>15/6</td>
<td>NS</td>
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All values are mean±SD followed by the range. HDL, high density lipoprotein; LDL, low density lipoprotein.

Table 3

<table>
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<th></th>
<th>Normal coronary group (n=14)</th>
<th>Coronary vasospasm group (n=25)</th>
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<tbody>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>192±23</td>
<td>184±31</td>
</tr>
<tr>
<td>Triglyceride (mg/dl)</td>
<td>138±67</td>
<td>184±10</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dl)</td>
<td>48±13</td>
<td>49±14</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dl)</td>
<td>116±26</td>
<td>103±35</td>
</tr>
<tr>
<td>Preheparin LPL mass (ng/ml)</td>
<td>51±16</td>
<td>47±16</td>
</tr>
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All values are mean±SD followed by the range. *p<0.05. HDL, high density lipoprotein; LDL, low density lipoprotein.

**Comparison of the Serum Lipid Levels and Preheparin LPL Mass in the Midband Positive and Negative Groups (Table 2)**

The total cholesterol level did not differ between the 2 groups, but the TG levels were significantly higher in the midband positive group and the HDL-C (p<0.05) and LDL-C was significantly lower (p<0.05 for both) compared with the midband negative group. Preheparin LPL mass was significantly lower (p<0.05) in the midband positive group (40±15 vs 50±17 ng/ml). There were more episodes of coronary vasospasm in the midband lipoprotein-positive group than in the midband lipoprotein-negative group, but not significantly.

**Comparison of the Serum Lipid Levels and Preheparin LPL Mass in the Normal and the Coronary Vasospasm Groups (Table 3)**

The total cholesterol, LDL-C and HDL-C levels did not differ between the 2 groups. The TG level was higher in the coronary vasospasm group, but not significantly. Preheparin
Serum Lipid Levels and Preheparin LPL Mass in the Coronary Vasospasm Group (Table 4)

The coronary vasospasm group was divided into two groups (ie, Group I having single vessel spasm and Group II having multiple vessel spasms) and the total cholesterol and HDL-C levels did not differ between them. The TG level was higher in Group II, but not significantly. LDL-C was significantly lower (p<0.05) in Group II compared with Group I, as was preheparin LPL mass (35±12 vs 48±17 ng/ml; p<0.05).

Multivariate Analysis of the Risk Factors for the Number of Lesions (Table 5)

Among the 11 coronary risk factors, preheparin LPL mass had the highest absolute t value (2.016) and was the only factor that significantly correlated with coronary vasospasm. The important risk factors of coronary vasospasm, such as smoking and serum lipid levels, were not significantly correlated.

Discussion

The present study observed a significantly low preheparin LPL mass in association with coronary vasospasm. Furthermore, the significantly low preheparin LPL mass was observed in the multiple vessel spasm group. Multivariate analysis revealed that among 11 coronary risk factors only preheparin LPL mass had a significant absolute t value. These results suggest that a low preheparin LPL mass without observable lipase activity in vivo reflects the seriousness of the coronary vasospasm, although it is still unclear why, and that it is the most important of the coronary risk factors.

Preheparin LPL Mass and LPL Activity

LPL analysis is conducted using post-heparin plasma, because LPL is detached from the endothelial cells by heparin and released into the bloodstream. Previous clinical study have shown that post-heparin plasma LPL activity is decreased in hypertriglyceridemic subjects and has a strongly positive relation to the HDL-C level. The present study revealed a negative correlation between preheparin LPL mass and TG and a positive correlation with HDL-C (Fig 2), which is consistent with reports by Tronvall et al22 and Watanabe et al23 and suggests that preheparin LPL mass reflects the level of functioning LPL activity in vivo, even though lipase activity is not detected.

Lipid Metabolism

An impaired endothelium-dependent vasodilatation response has been reported in hypercholesterolemia and hypertriglyceridemia, but in the present study, the serum cholesterol and TG levels of patients with coronary vasospasm were not significantly elevated (Table 2). Inoue et al reported that remnant-like particle cholesterol (RLP-C) was associated with coronary vasospasm, even though in ACh-positive patients the strongly atherogenic lipids and lipoproteins, such as total cholesterol, LDL-C and lipoprotein...
tein(a), were not as high as in ACh-negative patients. Sakata et al reported that the levels of remnants of or residual TG-rich lipoproteins were more meaningful than TG levels in coronary heart diseases and taken together these reports indicate that TG-rich lipoprotein is the most important factor in coronary vasospasm even though the serum lipids level is not so significantly different. Atherogenesis of TG-rich lipoproteins, such as the remnants, or intermediate density lipoproteins has recently highlighted.

Dysfunction of LPL in retention of TG-rich lipoproteins has been reported as a problem, in addition to apolipoprotein E and the receptors for remnants. Shimada et al revealed a relationship between low LPL production and the development of atherosclerosis in transgenic mice, and Tsutsumi et al also reported the correlation after administering an LPL enhancer NO-1886. However, the mechanism is still not fully clarified. Shimada et al suggested that LPL over-expression decreased serum triglyceride-rich lipoprotein, especially remnant lipoproteins, but Beisiegel et al reported that LPL works as a ligand of the remnants in the uptake of remnants to the liver, in addition to hydrolysis of TG. Based on these observations, preheparin LPL mass may be promoting the clearance of atherogenic TG-rich lipoproteins. This is till controversial because Huff et al reported that inactive LPL does not promote the uptake of TG-rich lipoproteins into the HepG2 liver cells. Thus, LPL works to decrease serum TG-rich lipoprotein, but the real role of human preheparin LPL mass in the uptake of TG-rich lipoprotein has not been clearly delineated.

Significantly elevated TG, lower HDL-C and lower preheparin LPL mass was observed in the midband lipoprotein-positive group (Table 2), which was consistent with the results of Totsuka et al. On the other hand, in the present study there were a greater number of ACh-positive patients in the midband lipoprotein-positive group than in the midband lipoprotein-negative group, but not significantly. These results suggest that preheparin LPL mass may reflect coronary vasospasm with the retention of TG-rich lipoproteins, such as remnant and intermediate density lipoproteins, but it is difficult to explain coronary vasospasm only on the basis of TG-rich lipoprotein.

Insulin Resistance and Preheparin LPL Mass

Recent clinical studies have demonstrated a link between insulin resistance syndrome and coronary vasospasm and Feingold et al proposed that, in the case of insulin resistance syndrome, small-sized dense LDL are present, the same type of LDL that is often associated with coronary heart disease and which Juha et al reported were associated with in vivo impaired endothelial function. Insulin is one of the most important factors for production of LPL in adipose tissues and LPL activity is reduced by obstruction of insulin function. Shirai et al reported that an insulin sensitizer, troglitazone, increased preheparin LPL mass level accompanied by a decrease in TG and an increase in HDL-C levels and an increase in the size of the LDLs. They also reported that preheparin LPL mass reflected some of the LPL produced in the whole body and was related to insulin sensitivity. All these results suggest that low preheparin LPL mass may reflect coronary vasospasm associated with insulin resistant syndrome in the presence of small-sized LDL.

Study Limitations

Several limitations should be noted when interpreting the results of this study.

1. All medications were stopped for at least 12h, but long-acting calcium channel blockers and isosorbide nitrate may have had residual effects during the spasm provocation test.
2. We evaluated the midband visually.
3. The study population was small and so further investigation with a larger population is required.

Conclusions

A significantly low preheparin LPL mass was observed in patients with positive coronary vasospasm. Furthermore, it reflected the seriousness of the vasospasm, which suggests that it may be an important risk factor. Preheparin LPL mass may be a useful marker of early stage coronary atherosclerosis that is not detectable by angiography.

Acknowledgment

We are greatly indebted to Des Takashi Uchi, Masaki Yoshinuma, Takeshi Sakurai and Kanyukei Aoyagi for their assistance in conducting CAG studies. This work was supported by Toho University’s 60th anniversary memorial fund and also by Dai-ichi Pure Chemical Co, Ltd. We also thank Professor Dr Hisao Tomioka and Dr Hidefumi Ohsawa for critical analysis of this manuscript.

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