Association between Preheparin Serum Lipoprotein Lipase Mass and Acute Myocardial Infarction in Japanese Men

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A sensitive immunoassay system using a specific monoclonal antibody against lipoprotein lipase (LPL) recently demonstrated the presence of an LPL mass in preheparin serum. We reported that a preheparin serum LPL mass (pre-LPL mass) reflected the level of functioning LPL activity in the whole body and could be deeply involved in the progression of coronary atherosclerosis of stable organic angina pectoris. We examined the relation between the pre-LPL mass and acute myocardial infarction (AMI). We studied 44 males with AMI (AMI group) and 16 males with a normal coronary artery (NCA group), and measured the pre-LPL mass by enzyme-linked immunosorbent assay. Coronary risk factors including the pre-LPL mass were compared between the two groups and multiple regression analysis was performed for AMI. There were no significant differences in the lipid data, but the pre-LPL mass level was significantly low in the AMI group (52 ± 16 vs 41 ± 14 ng/ml, $p = 0.01$), and a low pre-LPL mass concentration was observed in the small sized LDL group and/or the Midband positive group. Multiple regression analysis revealed that a low pre-LPL mass and hypertriglyceridemia were independent risk factors for AMI (t value = 2.1, 2.4). The result indicates that a low pre-LPL mass may be an important risk factor for AMI and stable organic angina pectoris. *J Atheroscler Thromb*, 2002; 9: 163–169.

Key words: Preheparin serum lipoprotein lipase mass, Lipoprotein lipase activity, Coronary risk factor, Acute myocardial infarction

Introduction

Lipoprotein lipase (LPL) catalyzes the hydrolysis of triglyceride in circulating lipoproteins (1). This enzyme exists in preheparin serum, even though little lipase activity is detected. Recently, a sensitive immunoassay system using a specific monoclonal antibody against LPL demonstrated the presence of an LPL mass in preheparin serum (2-4). We reported that the preheparin serum LPL mass (pre-LPL mass) reflected the level of functioning LPL ac-

tivity in the whole body and could be deeply involved in the progression of coronary atherosclerosis of stable organic angina pectoris (5). The clinical manifestation of stable organic angina pectoris depends on the presence of advanced coronary atherosclerosis, which predictably causes a reduction in myocardial oxygen supply relative to myocardial oxygen demand (6). In contrast, acute coronary syndromes such as myocardial infarction (AMI) and/or unstable angina pectoris are unpredictable and caused by a coronary flow obstruction, which may be due to different pathological mechanisms such as increased vaso-motor tone or thrombus formation following coronary plaque rupture or plaque erosion (7,8). Therefore, acute coronary syndromes should be treated as a different disease from stable organic angina pectoris. In this study, we examined the relation between pre-LPL mass and AMI, and elucidated the clinical significance of the pre-LPL

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mass in AMI by comparing it with total cholesterol, triglyceride, high density lipoprotein (HDL)-cholesterol, low density lipoprotein (LDL)-cholesterol and other coronary risk factors.

Subjects and Methods

Subjects

Forty-four male patients with AMI, who were admitted to hospital between December 1999 and May 2001, and 16 male patients with normal coronary artery (NCA) were enrolled in this study. The average age was 60 years (standard deviation: ± 13), ranging from 31 to 81 years. The diagnosis of definite AMI required two of the following criteria: (1) a clinical history of central chest pressure, pain, or tightness for 30 minutes or more, (2) ST-segment elevation greater than 0.1 mV in at least one standard or precordial lead, and (3) a rise in the serum creatine kinase concentration to greater than twice the normal laboratory value. We also performed coronary angiography for all patients and obtained culprit lesions, which were caused by total or subtotal occlusion. In addition, we immediately performed percutaneous coronary intervention, and procedures were successful in all cases. The pre-LPL mass and lipid data were evaluated in the morning after 12 hours of fasting one month after the subject had suffered AMI. Portions necessary for LPL mass measurement were frozen at -80°C within 1 hour of blood sampling. The normal coronary group was defined as not having a stenotic lesion and showing neither change nor an extensive reaction to dilatation following intra coronary administration of acetylcholine.

Preheparin LPL mass assay

The pre-LPL mass was measured by a sandwich enzyme-linked immunosorbent assay (ELISA) using a specific monoclonal antibody against lipoprotein lipase as described by Kobayashi *et al.* (2). For the assay, a kit available from Daiichi Pure Chemicals Co., Ltd. (Tokyo, Japan) was used. The linearity of this assay system was observed from 5 to 400 ng/ml. The within-run coefficient of variation was 2.8%. The between-day coefficient of variation was 4.3%. Interference by serum triglyceride from 50 mg/dl (0.55 mmol/L) to 1500 mg/dl (16.5 mmol/L) and serum HDL-cholesterol from 5 mg/dl (0.13 mmol/L) to 120 mg/dl (3.09 mmol/L) was not observed.

Lipid analysis

Total cholesterol and triglyceride concentrations were measured enzymatically using a kit available from Nippon Shoji Co., Ltd. (Osaka, Japan) and an automatic analyzer (HITACHI 7150 available from Hitachi, Ltd., Tokyo, Japan). HDL-cholesterol (HDL-C) was measured by the selective inhibition method (Daiichi Pure Chemicals, Co. Ltd., Tokyo, Japan) (9). LDL-cholesterol was calculated with the

Friedwald equation (total cholesterol-HDL-cholesterol-triglyceride/5).

Evaluation of the LDL particle size and Midband

The LDL particle size and Midband were evaluated by polyacrylamide gel electrophoresis using the LipoPhor system (Quantimetrix, CA; Jyohkoh, Tokyo, Japan) (10). The pattern was recorded with a densitometer (Densitron 20-HR; Jyohkoh). As shown in Fig. 1, LDL particle size was evaluated using the LDL migration index (LDL-MI) ratio (11). According to this method, the moving ratio of LDL to that of HDL negatively correlates with the particle (Fig. 1) and we determined that the LDL particle was small when this reading was > 0.35 . We concluded that the Midband was positive when the individually eluted lipoprotein patterns showed a peak between the LDL and the very low density lipoprotein (VLDL) band discs on electrophoresis or a shoulder was observed on the VLDL side of the LDL lipoprotein (Fig. 1).

Examination of coronary risk factors

The coronary risk factors examined were total cholesterol, LDL cholesterol, triglyceride, HDL-cholesterol, smoking, family history, diabetes mellitus, hyperuricemia, hypertension, body mass index, age, and pre-LPL mass. Hypercholesterolemia was defined as a total cholesterol level ≥ 220 mg/dl (5.69 mmol/L) and hyper LDL-choles-

Fig. 1. Evaluation of LDL particle size and Midband. The eluted lipoprotein patterns were recorded by polyacrylamide gel electrophoresis using a LipoPhor system. LDL particle size was simply estimated based on an LDL migration index (LDL-MI). The LDL-MI was obtained by dividing the distance from the LDL peak to VLDL peak by the distance from the HDL peak to VLDL peak, and when this reading was > 0.35 , LDL particle size was small. We also determined that Midband was positive when an individual peak was observed between the LDL and VLDL lipoprotein bands or a shoulder was observed on the VLDL side of the LDL lipoproteins.

terolemia as an LDL-cholesterol level ≥ 140 mg/dl (3.59 mmol/L). Hypertriglyceridemia was defined as a triglyceride level ≥ 150 mg/dl (1.65 mmol/L) and low HDL-cholesterol as an HDL-cholesterol level < 40 mg/dl (1.03 mmol/L). Smoking was defined as a current and 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 a history of diabetes mellitus if fasting blood glucose was > 126 mg/dl (6.93 mmol/L) and $\text{HbA}_{1c} > 6.5\%$. Hyperuricemia was defined as serum uric acid level of > 8.0 mg/dl (0.48 mmol/L). Hypertension was defined as a history of hypertension (systolic pressure ≥ 140 mmHg or diastolic pressure ≥ 90 mmHg). Obesity was defined as a body mass index ≥ 25 . An aged patient was defined as a patient aged ≥ 60 years. A low pre-LPL mass was defined by an LPL mass level ≤ 40 ng/ml.

Statistical analysis

The results were expressed as the mean $\pm$ standard deviation. The unpaired *t* test and Mann-Whitney *U* test were used for group comparisons. *p*-values of less than 0.05 were considered significant. Multiple regression analysis was performed to identify the risk factors for AMI. Fourteen risk factors including the pre-LPL mass, small sized LDL and Midband were scored as explanatory factors, and the subordinate variable was AMI (NCA = 0, AMI = 1). According to the analysis, an explanatory factor showing an absolute value of *t*, which is more than 2, is significantly correlated with dependent variables.

Results

Baseline clinical characteristics

The clinical characteristics of the two groups are shown in Table 1. The ages of the NCA group and the AMI group were 56 ± 14 and 61 ± 11 years ($p = \text{NS}$), respectively. There were no significant differences in coronary risk factors such as hypertension, diabetes mellitus, smoking, family history, hyperuricemia, and body mass index.

Serum lipid levels and preheparin LPL mass in the normal coronary group and the AMI group

The serum lipid levels and pre-LPL mass levels were compared between the normal coronary group and the AMI group. There were no significant differences in serum lipid levels between the two groups as shown in Table 2. However, in the AMI group, small sized LDL and Midband were more frequently detected than in the NCA group (small sized LDL, 68% vs 31%, $p = 0.02$, Midband, 80% vs 56%, $p = 0.04$). On the other hand, the pre-LPL mass level was significantly lower ($p = 0.01$) in the AMI group than the NCA group as shown in Fig. 2 (41 ± 14 ng/ml vs 52 ± 16 ng/ml).

Table 1. Baseline clinical characteristics

	NCA group (<i>n</i> = 16)	AMI group (<i>n</i> = 44)	<i>p</i> value
Age (years)	56 ± 14	61 ± 11	NS
Coronary riskfactor			
Hypertension	5/16 (31)	19/44 (43)	NS
Diabetes mellitus	2/16 (13)	13/44 (30)	NS
Smoking	8/16 (50)	30/44 (68)	NS
Hyperuricemia	2/16 (13)	5/44 (11)	NS
Family history	1/16 (6)	5/44 (11)	NS
BMI	23 ± 4	24 ± 3	NS

(): % BMI = body mass index

Table 2. Comparison of serum lipid parameters

	NCA group (<i>n</i> = 16)	AMI group (<i>n</i> = 44)	<i>p</i> value
Total cholesterol (mg/dl)	197 ± 28	197 ± 42	NS
Triglyceride (mg/dl)	145 ± 83	145 ± 63	NS
HDL cholesterol (mg/dl)	49 ± 14	43 ± 14	NS
LDL cholesterol (mg/dl)	120 ± 24	124 ± 40	NS
Small sized LDL	5/16 (31)	30/44 (68)	0.02
Midband	9/16 (56)	35/44 (80)	0.04

Lipid values are the mean $\pm$ SD followed by the range (): %

Fig. 2. Comparison of preheparin LPL mass levels in the normal coronary group (*n* = 16) and the AMI group (*n* = 44). Data represent the mean $\pm$ SD.

Serum preheparin LPL mass in the small sized LDL particle group and the normal sized LDL particle group

The pre-LPL mass levels were compared between the small sized LDL particle group (LDL-MI > 0.35) and the normal sized LDL particle group (LDL-MI ≤ 0.35) in the

AMI group. As shown in Fig. 3, the pre-LPL mass level was significantly lower ($p = 0.02$) in the small than normal sized LDL particle group (37 ± 13 ng/ml vs 48 ± 14 ng/ml).

Serum preheparin LPL mass in the Midband positive group and the Midband negative group

The serum pre-LPL mass levels were compared between the Midband positive group and the Midband negative group in the AMI group. As shown in Fig. 4, pre-LPL mass was significantly lower ($p = 0.04$) in the Midband positive than negative group (38 ± 12 ng/ml vs 49 ± 17 ng/ml).

Fig. 3. Comparison of preheparin LPL mass levels in the small sized LDL particle group ($n = 30$) and the normal sized LDL particle group ($n = 14$). Data represent the mean $\pm$ SD.

Multiple regression analysis of the risk factors for AMI

The result of multiple regression analysis is shown in Table 3. Low pre-LPL mass and hypertriglyceridemia had a significantly high absolute t-value (2.1, 2.4) among the coronary risk factors. However, the important risk factors for AMI such as hypercholesterolemia, hyperLDL-cholesterolemia, and smoking were not significantly correlated with AMI. We also performed multiple regression analysis, except for six subjects receiving antihyperlipidemic drugs, and found that only low pre-LPL mass and hypertriglyceridemia had a significantly high absolute t-value among the coronary risk factors (data not shown).

Fig. 4. Comparison of preheparin LPL mass levels in the Midband positive group ($n = 35$) and the Midband negative group ($n = 9$). Data represent the mean $\pm$ SD.

Table 3. Multiple regression analysis of acute myocardial infarction

	Partial regression coefficient (b)	Standard error of b (SE)	t value (b/S.E)	p value
X Hypertriglyceridemia	0.34	0.14	2.4	0.02
Low pre-LPL mass	0.27	0.13	2.1	0.02
Small sized LDL	0.20	0.13	-	-
Low HDL-cholesterolemia	0.17	0.12	-	-
Smoking	0.16	0.12	-	-
Age	0.13	0.12	-	-
Diabetes mellitus	0.12	0.13	-	-
Midband	0.04	0.15	-	-
Hypertension	0.02	0.11	-	-
Hyper LDL-cholesterolemia	0.04	0.22	-	-
Hyper totalcholesterolemia	-0.08	0.23	-	-
Hyperuricemia	-0.13	0.18	-	-
Family history	-0.22	0.20	-	-
Obesity	-0.26	0.15	-	-

Y NCA = 0 AMI = 1

correlation coefficient (R) = 0.56 F value = 2.3 p value = 0.03 (n = 60)

Discussion

The present study revealed a significantly low pre-LPL mass concentration in the AMI group (Fig. 2). According to multiple regression analysis, the low pre-LPL mass and hypertriglyceridemia showed significantly high t-values among the coronary risk factors (Table 3). These results indicate that a low pre-LPL mass and hypertriglyceridemia are important risk factors for AMI as well as stable organic angina pectoris. This study also indicated a significantly low pre-LPL mass concentration in the small sized LDL particle group and the Midband positive group (Figs. 3 and 4).

Low preheparin LPL mass and plaque instability

The rupture of coronary plaques is probably the most important mechanism underlying the sudden onset of acute coronary syndromes (12). In atheromatous plaques, macrophages express scavenger receptors and engulf modified lipoproteins to become foam cells and macrophage-rich atheroma, which are prone to rupture and thrombus formation and result in the onset of acute coronary syndrome (13, 14). Hypercholesterolemia, particularly hyper LDL-cholesterolemia, is considered to be the most atherosclerotic lipoprotein of the circulating lipoproteins and to increase the incidence of atherosclerotic cardiovascular diseases (15, 16). *In vitro* studies, however, suggest that native LDL itself does not induce vascular cell activation and foam cell formation, the features that are related to atherosclerosis. A number of studies showed that a predominance of small LDL particles was associated with the presence of coronary heart diseases (17, 18). Moreover, Austin *et al.* and Stampfer *et al.* reported that small LDL particles were an important risk factor for MI (19, 20). This study also showed small LDL particles were detected at high incidences in the AMI subjects. Small LDL particles have increased susceptibility to oxidation (21), and monocytes subsequently migrate into the intima and are transformed into foam cells. Recently, Ehara *et al.* measured oxidized LDL (ox-LDL) levels with the sandwich ELISA method and reported that they had a significant positive correlation with the severity of acute coronary syndromes and that more severe lesions also contained a significantly higher percentage of ox-LDL-positive macrophages (22). Thus, small LDL particles related to oxidated LDL may be an important lipoprotein to precipitate acute coronary syndromes. Our study showed that a significantly low pre-LPL mass concentration was observed in the small sized LDL particle group and this result is interpreted as meaning a low pre-LPL mass concentration reflects plaque instability with LDL abnormality. However, further study on intravascular ultrasound, coronary endoscopy, cytokines, and high sensitive C-reactive protein is necessary to reveal the implications of pre-LPL and plaque instability.

Low preheparin LPL mass and coronary vasospasm

Previous reports showed that coronary vasospasm was one of the most important triggers of acute coronary syndromes (23, 24). Moreover, the incidence of coronary vasospasm is higher in Japanese with a recent MI than in Caucasian patients (23, 24). Sakata *et al.* reported that remnant-like cholesterol was the major risk factor for myocardial infarction in the vasospastic angina with a nearly normal coronary artery (25). Recently, the atherogenesis of triglyceride-rich lipoproteins has been pointed out (26, 27). Tsutsumi *et al.* reported that administration of the LPL enhancer NO-1886 inhibited coronary atherosclerosis in mice (28), and Shimada *et al.* reported that the antiarteriosclerotic role of LPL was mainly responsible for the reduction of remnant lipoproteins (29). Thus, LPL is well known to provide protection against atherosclerosis with a reduction of remnant lipoproteins. Our study showed the Midband positive group had a significantly lower pre-LPL mass concentration than the Midband negative group. In addition, we already reported that low pre-LPL mass was an independent risk factor for coronary vasospasm patients without an angiographically demonstrable atherosclerotic coronary artery (30). As we immediately performed percutaneous coronary intervention for all culprit lesions, in which thrombus was probably responsible, we are not certain to what extent coronary vasospasms effected the degree of stenosis and the number of subjects prior to onset of AMI. However, it is possible that low pre-LPL mass reflects the presence of triglyceride-rich lipoproteins such as remnant and intermediate density lipoprotein in the whole body, and may be related to precipitation of AMI due to coronary vasospasms.

Hypertriglyceridemia and insulin resistance

In the present study, hypertriglyceridemia was the most important risk factor for AMI. The Münster Heart Study based on a large population showed that the risks for myocardial infarction or sudden cardiac death increased markedly as triglyceride levels increased (31). Hypertriglyceridemia contributes to the etiology of coronary artery diseases via a direct atherogenic effect of triglyceride-rich lipoproteins such as very low density lipoprotein or remnant lipoproteins (26, 32). Alternatively, hypertriglyceridemia is associated with atherogenic lipoproteins such as low HDL cholesterol, small LDL particles or large apolipoprotein E-enriched VLDL particles. In addition, hypertriglyceridemia enhances thrombogenesis through abnormal alterations in coagulation and fibrinolytic mechanisms (33). Several clinical studies have reported that insulin resistant syndrome is an important risk factor for acute coronary syndromes (34, 35). Insulin resistance is related to coronary vasospasms (36) and thrombus formation, which are caused by plasminogen activator inhibitor-1 (37). Hypertriglyceridemia, low HDL

cholesterol and small LDL particles are often associated with insulin resistant syndromes (38-40). On the other hand, LPL production is known to be controlled by insulin (41, 42). In addition, Shirai *et al.* reported that administration of an insulin sensitizer, troglitazone, elevated the pre-LPL mass level accompanying a decrease in triglyceride, an increase in HDL cholesterol and enlargement of LDL size (43). Thus, a low preheparin LPL mass and hypertriglyceridemia reflect insulin resistance and may be deeply involved with the precipitation of AMI.

Study limitations

Several limitations should be noted when interpreting the results of this study; 1) this was a retrospective study; 2) we evaluated the Midband visually and LDL particle size simply; and 3) our study population was small. Therefore, further investigation with a larger population will be necessary.

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