PLASMA LIPOPROTEIN(a) LEVELS AND FIBRINOLYTIC ACTIVITY IN PATIENTS WITH UNSTABLE ANGINA

HIROTO OKUBO, M.D., HIROFUMI YASUE, M.D., HISAO OGAWA, M.D.
IKUO MISUMI, M.D., TAKEHISI MASUDA, M.D., YUJI MIYAO, M.D.
AND TOMOHIRO SAKAMOTO, M.D.

The relationships between plasma Lipoprotein(a) (Lp(a)) levels and plasminogen activator inhibitor (PAI) activity and tissue plasminogen activator (t-PA) antigen levels were studied in 15 patients with unstable angina. Plasma levels of Lp(a) (mg/dl) were significantly higher in patients with unstable angina after treatment 2 weeks than on admission (19.7 ± 2.8 vs 14.6 ± 2.3, p < 0.01). On the other hand, the plasma levels of PAI activity (IU/ml) and t-PA antigen (ng/ml) in patients with unstable angina were significantly higher on admission than after treatment (PAI activity: 11.4 ± 1.4 vs 7.7 ± 1.5, t-PA antigen: 8.7 ± 0.9 vs 7.0 ± 0.9, p < 0.01). We conclude that patients with unstable angina have reduced fibrinolytic capacity, as indicated by increased PAI activity, and that the plasma Lp(a) level may be decreased due to binding with fibrin during the acute stage of unstable angina. 

(Jpn Circ J 1993; 57: 947–954)

There is an increasing body of evidence to indicate that coronary thrombosis plays an important role in the pathogenesis of unstable angina. For example, increased platelet activity and blood coagulability have been demonstrated in patients with unstable angina. Furthermore, thrombus formation has been suggested to be caused by decreased fibrinolysis. However, little is known regarding the role of the fibrinolytic system in patients with unstable angina. The central component of the fibrinolytic system is plasmin, which dissolves fibrin or thrombus, and which is converted from plasminogen by tissue-type plasminogen activator (t-PA). In blood, however, t-PA is rapidly inhibited by plasminogen activator inhibitors (PAIs), mainly PAI type 1 (PAI-1). Thus, fibrinolytic function in blood is determined by the balance between t-PA and PAIs.

Lipoprotein(a) (Lp(a)) is a low density lipoprotein (LDL)-like particle which contains apolipoprotein(a) (apo(a)) in addition to apoB, and which has been shown to be an independent risk factor for atherosclerosis. Apo(a) shares extensive structural homology, and is linked genetically, with plasminogen. Therefore, Lp(a) has been suggested to be involved in the fibrinolytic system although no clinical data implicate Lp(a) in the impairment of fibrinolytic function.

The present study was designed to examine plasma fibrinolytic function and the possible role of Lp(a) in the fibrinolytic function and the possible role of Lp(a) in the fibrinolytic system in patients with unstable angina during treatment by measuring plasma levels of PAI activity, t-PA antigen, and Lp(a).

Key words:
Lipoprotein(a)
Plasminogen activator inhibitor
t-PA
Unstable angina

(Received November 27, 1992; accepted March 31, 1993)
The Division of Cardiology, Kumamoto University School of Medicine, Kumamoto Japan
Mailing address: Hirofumi Yasue, M.D., Division of cardiology, Kumamoto University School of Medicine, 1 Honjo, Kumamoto City 860, Japan

Japanese Circulation Journal Vol. 57, October 1993 947

NII-Electronic Library Service
TABLE 1  PATIENT CHARACTERISTICS

<table>
<thead>
<tr>
<th></th>
<th>Unstable angina</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>15</td>
<td>14</td>
</tr>
<tr>
<td>Age (yr) (Mean ± SEM)</td>
<td>62.8 ± 2.7</td>
<td>60.0 ± 1.6</td>
</tr>
<tr>
<td>Range</td>
<td>48 – 81</td>
<td>50 – 71</td>
</tr>
<tr>
<td>Male/Female</td>
<td>13/2</td>
<td>10/4</td>
</tr>
<tr>
<td>Previous myocardial infarction (n)</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Hypertension (n)</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Smokers (n)</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Diabetes mellitus (n)</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Obesity (n)</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>T-Ch (mg/dl) (Mean ± SEM)</td>
<td>201 ± 9</td>
<td>186 ± 12</td>
</tr>
<tr>
<td>TG (mg/dl) (Mean ± SEM)</td>
<td>129 ± 10</td>
<td>158 ± 16</td>
</tr>
</tbody>
</table>

Extent of coronary vessel (>75% stenosis)
- 0-vessel: 7 (Unstable), 14 (Control)
- 1-vessel: 2 (Unstable), 0 (Control)
- 2-vessel: 1 (Unstable), 0 (Control)
- 3-vessel: 5 (Unstable), 0 (Control)

Ejection fraction <50% (n)
- 4 (Unstable), 0 (Control)

Medication used (n)
- Beta-blockers: 3 (Unstable), 0 (Control)
- Long-acting nitrates: 8 (Unstable), 0 (Control)
- Calcium antagonists: 4 (Unstable), 4 (Control)
- Aspirin: 3 (Unstable), 4 (Control)
- Digitalis: 0 (Unstable), 0 (Control)
- Diuretic: 0 (Unstable), 0 (Control)

T-Ch = serum total cholesterol; TG = total triglycerides

METHODS

Patient population
We studied 29 patients (24 male and 5 female, mean age, 62.6 ± 2.7 years, range 40 – 81 years) who were divided into 2 groups: unstable angina group and control group. The unstable angina group consisted of 15 consecutive patients with unstable angina who had been admitted to our hospital. Eleven patients had a crescendo pattern of chest pain (including 9 patients with angina at rest) and 4 patients had prolonged chest pain that was only slightly relieved by nitroglycerin. All of the patients with unstable angina presented with chest pain associated with reversible electrocardiographic changes consisting ST segment elevation of more than 2.0 mm or horizontal or downsloping ST segment depression of more than 1.0 mm 80 msec after the J point, lasting form 1 to 30 min. Patients with ST changes which lasted for more than 30 min or those with plasma creatine kinase or creatine kinase MB levels that were more than twice the normal levels were excluded from the study.

The control group consisted of 14 patients who had atypical chest pain not accompanied by ECG changes and who were negative for ischemia on a treadmill exercise test. The controls had no coronary organic stenosis and no coronary spasm.

None of the patients were treated with thrombolytic agents, such as urokinase or streptokinase, or tissue plasminogen activator. Furthermore, none of the patients had thromboembolism or collagen disease, disseminated intravascular coagulation, advanced liver disease, renal failure, malignant diseases, septicemia or other inflammations. None had a prosthetic heart valve or a pacemaker.

Written informed consent was obtained from each patient, and the study was performed in accordance with the guidelines approved by the Kumamoto University School of Medicine ethics committee.

Treatments for patients with unstable angina
Patients in the unstable angina group were treated with the following drugs from admission to 2 weeks after admission either larger doses or the addition of beta-blockers in 2 patients, calcium antagonists in 8 patients,
TABLE II  CHANGES IN SEVERAL PROTEIN LEVELS IN THE UNSTABLE ANGINA GROUP

<table>
<thead>
<tr>
<th></th>
<th>admission</th>
<th>after treatment</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>T-Ch</td>
<td>201±9</td>
<td>179±9</td>
<td>NS</td>
</tr>
<tr>
<td>TG</td>
<td>129±10</td>
<td>134±14</td>
<td>NS</td>
</tr>
<tr>
<td>HDL-Ch</td>
<td>41±3</td>
<td>40±3</td>
<td>NS</td>
</tr>
<tr>
<td>LDL-Ch</td>
<td>133±8</td>
<td>112±8</td>
<td>NS</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>333±16</td>
<td>293±25</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are Mean±SEM, mg/dl. NS = not significant
T-Ch = serum total cholesterol; TG = serum triglycerides; HDL-Ch = HDL-cholesterol; LDL-Ch = LDL-cholesterol

long-acting nitrates in 5 patients and aspirin in 2 patients. Unstable angina was controlled in all 15 patients by these treatments.

Blood sampling and assay
Blood was drawn in the fasting state at 7:00 AM on admission by a clean venipuncture of an antecubital vein using a 21 gauge needle by specially trained physicians (H.O., T.M. and I.M.).

Blood sampling in patients with unstable angina was also performed at 7:00 AM after 2 weeks of treatment. A mean of 18 h had elapsed between the last episode of chest pain and blood sampling on admission in patients with unstable angina. The first 3 ml of blood was discarded, and then 4.5 ml of blood was drawn directly into a glass tube containing 0.5 ml of 3.8% buffered citrate solution. The blood was immediately centrifuged (4°C, 3,000 rpm, 15 min) to obtain platelet-poor plasma and the plasma was stored at -80°C until used.

Frozen plasma was subsequently analyzed for Lp(a) lipoprotein by an enzyme-linked immunosorbent assay (ELISA) kit (Tint Elize TM Lp(a) kit, Biopool AB, Umeå, Sweden). The assay, which uses polyclonal antibodies raised against purified Lp(a), has been shown to be specific, sensitive, reproducible and appropriate for routine clinical application. Interassay coefficients of variation at Lp(a) levels of 4 and 30 mg/dl were 8.5% and 3.6%, respectively. Intra-assay coefficients of variation at Lp(a) levels of 4 and 30 mg/dl were 3.8% and 2.1%, respectively. The normal lipoprotein(a) level in our laboratory (n=30) was 14.4±1.8 (Mean±SEM) mg/dl.

PAI activity was determined by a chromogenic by a substrate assay with the Spectrolyse/PL reagent kit (Biopool, Umeå, Sweden). The results were expressed in international units per milliliter Intra-assay and interassay coefficients of variation were 9.4% and 11.4%, respectively. The normal PAI activity in our laboratory (n=33) was 5.2±0.5 (Mean±SEM) IU/ml.

The t-PA antigen level was measured by an enzyme-linked immunosorbent assay with an ASSERACHROM TPA reagent kit (Diagnostica Stago, Inc., Franconville, France). Results were expressed in nanograms per milliliter. Intra-assay and interassay coefficients of variation were 2.4% and 4.7%, respectively. The normal t-PA antigen level in our laboratory (n=33) was 5.6±0.3 (Mean±SEM) ng/ml. Serum total cholesterol (T-
Unstable angina (n=15)  

Fig.2. Plasma PAI activity levels on admission and after 2 weeks of treatment in patients with unstable angina, and in the control group (Mean±SEM). In the unstable angina group, plasma PAI activity levels decreased significantly after 2 weeks of treatment (p<0.01). The levels on admission were significantly higher than those of the control group. The level after 2 weeks of treatment were not significantly different from those of the control group.

Plasma PAI activity levels (IU/ml)

Admission After treatment Control (n=14)

Unstable angina (n=15)  

Fig.3. Plasma t-PA antigen levels on admission and after 2 weeks of treatment in patients with unstable angina, and in the control group (Mean±SEM). In the unstable angina group, plasma t-PA antigen levels decreased significantly after 2 weeks of treatment (p<0.01). The levels on admission were significantly higher than those of the control group. The levels after 2 weeks of treatment were not significantly different from those of control group.

Plasma t-PA antigen levels (ng/ml)

Admission After treatment Control (n=14)

RESULTS

Characteristics of the study group and changes in protein levels in the unstable angina group

The clinical characteristics of patients with unstable angina and the control group are shown in Table I. The two groups were matched for age, sex, and other factors which may affect plasma Lp(a) levels. The levels of T-Ch, TG, HDL-Ch, LDL-Ch and fibrinogen in the unstable angina group did not change significantly after 2 weeks of treatment (Table II).

Coronary arteriography

Coronary arteriography was performed in all of the patients 2 to 4 weeks after admission. Eight patients with unstable angina had significant organic coronary stenosis of the coronary arteries with no change in the size of coronary plaques.
≥90% of the luminal diameter in 2 or 3 major coronary arteries. Seven unstable angina patients who had variant angina had coronary arteries with organic stenosis of 25% to 50% of the luminal diameter. In all of these 7 patients, acetylcholine was injected into the left and right coronary arteries to induce coronary artery spasm. Spasm was induced near the normal sites, as well as in regions of organic stenosis (25% to 50% stenosis) in both the left and right coronary arteries in all 7 of these patients. Thus, all of these patients had multivessel coronary spasm. Coronary artery spasm was not induced by intracoronary injection of acethlcholine in any of the patients in the control group. None of the patients in the control group had significant organic stenosis in their coronary arteries.

In the unstable angina group, plasma Lp(a) levels increased significantly after 2 weeks of treatment (from 14.6±2.3 to 19.7±2.8 mg/dl, p<0.01). The levels on admission and those after 2 weeks of treatment were not significantly different from those of the control group (14.8±2.5 mg/dl) (Fig. 1).

Plasma PAI activity levels
In the unstable angina group, plasma PAI activity levels decreased significantly after 2 weeks of treatment (from 11.4±1.4 to 7.7±1.5 IU/ml, p<0.01). The levels on admission were significantly higher than those of the control group (4.9±1.0 IU/ml). The levels after 2 weeks of treatment were not significantly different from those of the control group (Fig. 2).

Plasma t-PA antigen levels

Japanese Circulation Journal Vol.57, October 1993
In the unstable angina group, plasma t-PA antigen levels decreased significantly after 2 weeks of treatment (from 8.7±0.9 to 7.0±0.9, p<0.01). The levels on admission were significantly higher than those of the control group (4.9±0.4 ng/ml). The levels after 2 weeks of treatment were not significantly different from those of the control group (Fig. 3).

Effect of treatment on plasma levels of Lp(a) and PAI activity in patients with unstable angina.

Plasma Lp(a) levels increased and plasma PAI activity levels decreased after 2 weeks of treatment (Fig. 4).

Effect of treatment on plasma levels of Lp(a) and t-PA antigen in patients with unstable angina.

Plasma Lp(a) levels increased and plasma t-PA antigen levels decreased after 2 weeks of treatment (Fig. 5).

DISCUSSION

In the present study, both plasma PAI activity levels and plasma t-PA antigen levels were significantly higher in patients with unstable angina before treatment than in the control subjects. Thus, PAIs have a greater effect on fibrinolytic capacity than t-PA and plasma fibrinolytic function is impaired in patients with unstable angina. PAIs (mainly PAI-1) are secreted from endothelial cells and platelets. Thrombin and transforming growth factor beta released from platelets are potent stimulants for the synthesis and secretion of PAIs. Intracoronary thrombus has been demonstrated in many patients with unstable angina using coronary arteriography and angioscopy, or at autopsy. Theroux et al reported that plasma levels of fibrinopeptide A (FPA), a marker of thrombin activity, were increased in patients with unstable angina and organic coronary stenosis. We have previously shown that plasma FPA levels are also increased in patients with coronary spastic angina presenting as unstable angina. In the present study, we included 7 patients with coronary spastic angina who had normal or almost normal coronary arteries in the unstable angina group because they had severe and prolonged anginal attacks at rest. In all 7 of these patients, plasma PAI activity levels were also increased. Thus, in patients with unstable angina, including those with coronary spastic angina, fibrinolytic function is impaired and this, together with the increased activity of platelets and the coagulation system, may lead to coronary thrombus formation. Attacks of unstable angina were controlled after 2 weeks of treatment, and increased plasma levels of both PAI activity and t-PA antigen decreased to the levels in the control subjects after treatment. These results support the view that coronary thrombosis plays an important role in the pathogenesis of unstable angina.

Interestingly, plasma levels of Lp(a) increased significantly in association with a significant decrease in plasma levels of PAI activity after treatment. The significance of this finding is not yet clear. Lp(a) is an LDL-like particle which contains apo(a) in addition to apoB, and which has been shown to be an independent risk factor for atherosclerosis. Apo(a) shares extensive structural similarities, and is genetically linked, with plasminogen. Several studies have shown that Lp(a) can compete in vitro with the binding of plasminogen to fibrin under conditions of streptokinase- or t-PA-mediated activation of human plasminogen. It has also been shown that Lp(a) competes with the binding of plasminogen to plasminogen receptors on endothelial cells, macrophages, and platelets. All of these characteristics of Lp(a), if they were to occur in vivo, could lead to an impairment of the fibrinolytic system. It is possible that Lp(a) binds to fibrin in competition with plasminogen or t-PA, and thus impairs fibrinolysis in patients with unstable angina. Intracoronary fibrin or thrombus may have either disappeared or become organized due to treatment. Therefore plasma levels of Lp(a) may have increased due to the lack of fibrin available for binding. Indeed, a recent report showed that apo(a) was co-localized with fibrin in segments of human atheromatous coronary artery. The process by which Lp(a) is produced in the liver is fairly well understood. However, the process by which Lp(a) is degraded is still unknown. Therefore, the factors that determine the plasma levels of Lp(a) are still unclear. Hegele et al

*Japanese Circulation Journal* Vol.57, October 1993
have recently demonstrated that intravenous t-PA administration reduced plasma Lp(a) levels in patients with unstable angina. This result suggests that the concentration of Lp(a) in vivo is not as static as had been believed.\(^\text{30}\)

We conclude that plasma fibrinolytic function is impaired, as indicated by increased levels of plasma PAI activity, in patients with unstable angina and that Lp(a) may be involved in regulation of the fibrinolytic system by competing with plasminogen or t-PA.

**REFERENCES**

7. ROSENSREN A, HILMSEN L, ERIKSSON E, RISBERG B, WEDEL E: Lipoprotein(a) and coronary heart disease: A prospective case control study in a general population sample of middle aged men. *Br Med J* 1990; 301: 1248—1251
25. KARADI L, KOSTNER GM, GRIES A, NIMPF J, ROMICS L, MALLE E: Lipoprotein(a) and plasminogen are immunologically related.
Biochemistry 1989; 28: 2370—2374


