Transient Increase of Plasma Lipoprotein(a) in Patients With Unstable Angina Pectoris
Does Lipoprotein(a) Alter Fibrinolysis?
Shuichi Oshima, Kagehiro Uchida, Takanori Yasu, Kikuya Uno, Hiroshi Nonogi, and Kazuo Haze

It has been shown that lipoprotein(a) (Lp[a]) may interfere with the fibrinolytic system and that the Lp(a) level in an individual remains constant. To evaluate the effects of Lp(a) on the fibrinolytic system in patients with unstable angina, we measured plasma levels of Lp(a), the α₂-plasmin inhibitor–plasmin complex, and the thrombin–antithrombin III complex. The latter is a marker of thrombin generation, and the α₂-plasmin inhibitor–plasmin complex is an indicator of plasminogen activation. Venous plasma samples were taken from 18 patients with unstable angina and 18 patients with stable exertional angina who had been matched for clinical variables. On admission, plasma levels of Lp(a) were significantly higher in patients with unstable angina than in those with stable exertional angina (319 ±193 mg/l versus 191 ±141 mg/l, respectively; p<0.05). On admission, plasma levels of the α₂-plasmin inhibitor–plasmin complex and of the thrombin–antithrombin III complex were also significantly higher in patients with unstable angina than in those with stable exertional angina (0.78±0.42 μg/ml and 3.6±1.3 ng/ml versus 0.41±0.13 μg/ml and 1.9±0.5 ng/ml, respectively; p<0.01). In nine of the 18 patients with unstable angina, serial changes of plasma levels of Lp(a), the α₂-plasmin inhibitor–plasmin complex, the thrombin–antithrombin III complex, and the acute-phase proteins C-reactive protein and α₁-antitrypsin were examined for 3 weeks after admission. Plasma Lp(a) levels were increased significantly 7 and 14 days after admission (p<0.001) but had started to decrease by the 21st day. Plasma levels of the α₂-plasmin inhibitor–plasmin complex and of the thrombin–antithrombin III complex remained unchanged. Plasma levels of C-reactive protein and α₁-antitrypsin remained within the normal ranges during the study period. In conclusion, plasma levels of Lp(a) are increased transiently without any change in the levels of acute-phase reactants in unstable angina. This transient increase in Lp(a) levels is not accompanied by a significant decrease in the α₂-plasmin inhibitor–plasmin complex levels. (Arteriosclerosis and Thrombosis 1991;11:1772–1777)

Lipoprotein(a) [Lp(a)], first demonstrated in human plasma by Berg as a genetic variant of low density lipoprotein, is believed to be transmitted as an autosomal dominant trait. This unusual lipoprotein has been considered an independent risk factor for the development of cardiovascular and cerebrovascular disease, and its level in an individual remains remarkably constant. The actual function of Lp(a) and the mechanism of its atherogenicity, however, remain to be defined. The most exciting aspect of the biology of Lp(a) is the recent observation that Lp(a) is strikingly homologous to plasminogen. This structural similarity between an atherogenic lipoprotein and a proteolytic enzyme important for cleaving fibrin thrombi provides the first potential molecular evidence for a mechanistic link between atherosclerosis and thrombosis. There has been much speculation in the literature about the possibility that Lp(a) can interfere with the fibrinolytic system because of a structural similarity to plasminogen. To date, however, little has been published to support this hypothesis. Recently, several investigators have demonstrated that Lp(a) does, indeed, interfere with the normal mechanisms of fibrinolysis and, as such, may promote a prothrombotic diathesis that contributes to the evolution and clinical expression of atherothrombotic disease independent of its effects on cholesterol metabolism. However, the influence of Lp(a) on the fibrinolytic system remains controversial.
Intracoronary thrombus formation has been thought to play an important role in the genesis of unstable angina and of acute myocardial infarction. On the other hand, the plasmin-dependent fibrinolytic pathway is an important physiological mechanism for the removal of vascular fibrin deposits and for the prevention of thrombosis. Fibrinolysis is initiated by the release of tissue plasminogen activator (t-PA) from the endothelium. Because t-PA and its substrate, plasminogen, have a selective binding affinity for fibrin, the physiological activation of plasminogen to plasmin by t-PA takes place preferentially within the fibrin clot. Impaired endothelial-dependent fibrinolysis, therefore, is associated with an increased risk of thrombosis. Several investigators have reported that impaired endothelial fibrinolysis is a characteristic of subjects with coronary artery disease. It has recently been demonstrated that Lp(a) competes with plasminogen and t-PA for fibrin binding. In vitro studies have also shown that Lp(a) attenuates clot lysis by t-PA. Little is known, however, about the influence of Lp(a) on the fibrinolytic system in vivo, especially in coronary artery disease. We examined serial changes in the levels of Lp(a) in patients with unstable angina or stable exertional angina to evaluate the effects of Lp(a) on the fibrinolytic system in this syndrome. We also measured levels of the $\alpha_2$-plasmin inhibitor–plasmin complex (PIC) as an indicator of plasminogen activation and of the thrombin–antithrombin III complex (TAT) as a marker of thrombin generation.

**Methods**

**Study Population**

The study population consisted of 36 inpatients, 18 with unstable angina and 18 with stable exertional angina, plus 10 outpatients with stable exertional angina. The patients with unstable angina were hospitalized in our coronary care unit within 48 hours after an episode of chest pain at rest with reversible electrocardiographic changes. Exclusion criteria included the development of new Q waves on an electrocardiogram or creatine phosphokinase or creatine phosphokinase-MB values greater than twice the upper limit of the normal range. The 18 inpatients with stable exertional angina had typical exertional angina and $\geq 70\%$ narrowing of the right and/or left coronary artery. The two inpatient groups were matched for age and sex. The 10 outpatients with stable exertional angina (eight men and two women, mean $\pm$ SD age 62.2 $\pm$ 12 years) served as controls for comparison with the inpatient groups. Antianginal medication previously administered on a chronic basis was continued, and additional pharmacological treatments were initiated in patients with unstable angina if they had recurrent chest pain. All patients with unstable angina were given aspirin after the first blood sampling on admission if aspirin had not been administered previously. Patients were excluded if any of the following conditions were present: malignancy, infection, liver disease, inflammatory disease, or noncardiac thrombus. Patients with a prosthetic heart valve or a pace-maker and patients who received thrombolytic therapy or coronary angioplasty during the study period were also excluded. Written informed consent was obtained from each patient.

**Blood Samples and Assays**

In both inpatient groups, blood was drawn at the time of admission by specially trained personnel using a 21-gauge needle for clean venipuncture of an antecubital vein without stasis. In the outpatients, blood was drawn using the same technique at the time of a hospital visit. After the initial 2 ml blood was discarded, venous blood was drawn into siliconized, evacuated tubes (4.5 ml blood and 0.5 ml of 0.13 mol/l sodium citrate). The blood was centrifuged for 10 minutes at 3,000g at 4°C, and the plasma was stored at $-80^\circ$C until assayed. The assay was performed within 1 month.

Lp(a) was measured using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (TintElize 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) concentrations of 40 and 300 mg/l were 8.5% and 3.6%, respectively, and intra-assay coefficients of variation at Lp(a) concentrations of 40 and 300 mg/l were 3.8% and 2.1%, respectively.

TAT was measured using a commercially available ELISA kit (Enzygnost-TAT kit, Behringwerke, Mannheim, FRG) with two different antibodies directed against human thrombin and antithrombin III. The key event in the coagulation of blood is the conversion of prothrombin to thrombin. Once generated, this enzyme converts fibrinogen to fibrin with the release of fibrinopeptides A and B. However, thrombin may be inhibited by antithrombin III, the most relevant physiological inhibitor of this serine protease. Inhibition by antithrombin III leads to the formation of an inactive protease–inhibitor complex. Therefore, determination of TAT reflects the functional state of the clotting system and represents a useful diagnostic tool for the detection of thrombotic disorders. In our laboratory, the normal value for TAT was 1.5 $\pm$ 0.4 ng/ml, and the interassay and intra-assay coefficients of variation were 9.8% and 5.3%, respectively.

Fibrinolysis occurs as a result of activation of fibrin-bound plasminogen by fibrin-bound activators and is inhibited most efficiently by $\alpha_2$-plasmin inhibitor (PI). Thus, an increase in the PIC level may indicate ongoing or recent plasminogen activation and accelerated fibrinolysis. Recently, a sensitive ELISA for PIC has been developed. In the present study, PIC was measured with a TD-80C kit (Teijin Co., Tokyo, Japan), which is based on the one-step sandwich method. This kit uses immunopurified polyclonal rabbit antibody to human plasminogen fixed to
polystyrene balls as the solid-phase antibody and peroxidase-conjugated monoclonal antibody to human PI as the liquid-phase antibody.32 The method has been shown to be specific, sensitive, reproducible, and appropriate for routine clinical application.33 The normal value for PIC (mean±SD) in our laboratory was 0.48±0.13 μg/ml; interassay and intra-assay coefficients of variation were 10% and 6%, respectively. Blood samples in the present study were assayed in duplicate.

**Other Determinations**

Quantitative determination of C-reactive protein (CRP) and α₁-antitrypsin (α₁-AT) was performed by turbidimetric electroimmunoassay. The normal value for CRP was <0.3 mg/dl, and the normal value for α₁-AT (mean±SD) was 255±71 mg/dl.

In the unstable angina group, blood tests were repeated 7, 14, and 21 days after admission. In six of the 18 inpatients with stable exertional angina and in the 10 control patients, blood tests were repeated 14 days after the first test. Coronary angiography was performed on the inpatients by the Judkins technique within 2 weeks after the last blood test.

**Statistical Analyses**

The clinical and angiographic characteristics of the stable and unstable angina groups were compared using the unpaired Student's t test for continuous data and the χ² test for group data. Plasma Lp(a), TAT, and PIC levels of the patients with unstable angina and those with stable exertional angina were compared using the unpaired t test. The statistical significance of changes over time in patients with unstable angina was evaluated using analysis of variance for repeated measurements. The paired Student's t test was used to compare the Lp(a) levels at baseline and those at 14 days after the first blood test in patients with stable exertional angina and in the controls. Probability levels of less than 0.05 were considered significant. Data are expressed as mean±SD.

**Results**

**Characteristics of Study Groups**

Most clinical characteristics of the patients with unstable angina and those with stable exertional angina were similar (Table 1). Although there were a few more patients in the unstable angina group with a history of previous myocardial infarction and a greater frequency of hypertension among patients with stable exertional angina, these differences were not significant. Other coronary risk factors were equally distributed in the two groups, and the angiographic characteristics were also similar. All patients with unstable angina but only half of those with stable exertional angina had been treated with aspirin (p<0.01).

**Table 1. Characteristics of Study Groups**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Unstable angina (n=18)</th>
<th>Stable angina (n=18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>62±7.5</td>
<td>59±8.8</td>
</tr>
<tr>
<td>Mean±SD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>42–73</td>
<td>45–75</td>
</tr>
<tr>
<td>Sex (male/female) (No.)</td>
<td>162</td>
<td>162</td>
</tr>
<tr>
<td>Previous myocardial infarction (No.)</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>Hypertension (No.)</td>
<td>9</td>
<td>12</td>
</tr>
<tr>
<td>Smokers (No.)</td>
<td>13</td>
<td>12</td>
</tr>
<tr>
<td>Diabetes mellitus (No.)</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>Serum cholesterol (mean±SD mg/dl)</td>
<td>204±37.8</td>
<td>208±42.7</td>
</tr>
<tr>
<td>Serum triglycerides (mean±SD mg/dl)</td>
<td>136±64.3</td>
<td>134±52.6</td>
</tr>
<tr>
<td>No. &gt;70% stenosed vessels (mean±SD)</td>
<td>1.9±0.8</td>
<td>1.7±0.8</td>
</tr>
<tr>
<td>Medications used (No.)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>β-Blockers</td>
<td>7</td>
<td>9</td>
</tr>
<tr>
<td>Long-acting nitrates</td>
<td>17</td>
<td>16</td>
</tr>
<tr>
<td>Calcium antagonists</td>
<td>15</td>
<td>16</td>
</tr>
<tr>
<td>Aspirin</td>
<td>18*</td>
<td>9</td>
</tr>
</tbody>
</table>

*p<0.01 different from stable angina.

**Plasma Lipoprotein(a), Thrombin–Antithrombin Complex, and α₁-Plasmin Inhibitor–Plasmin Complex Concentrations on Admission**

The levels of Lp(a) on admission were significantly higher in the patients with unstable angina than in those with stable exertional angina (Table 2, p<0.05). Plasma levels of TAT and PIC were also significantly higher (p<0.01) in the patients with unstable angina.

**Changes in Plasma Concentrations of Lipoprotein(a), α₁-Plasmin Inhibitor–Plasmin Complex, Thrombin–Antithrombin Complex, C-Reactive Protein, and α₁-Antitrypsin in Patients With Unstable Angina**

In nine of the 18 patients with unstable angina, serial changes of plasma Lp(a), PIC, TAT, CRP, and α₁-AT levels were examined for 3 weeks after admission (Figure 1, Table 3). Plasma Lp(a) levels demonstrated significant increases 7 and 14 days after admission (p<0.001), but Lp(a) levels had decreased

**Table 2. Lipoprotein(a), Thrombin–Antithrombin Complex, and α₁-Plasmin Inhibitor–Plasmin Complex Levels on Admission in Patients With Unstable Angina and Stable Angina**

<table>
<thead>
<tr>
<th>Assay</th>
<th>Unstable angina (n=18)</th>
<th>Stable angina (n=18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lp(a) (mg/l)</td>
<td>319±193*</td>
<td>191±141</td>
</tr>
<tr>
<td>TAT (mg/ml)</td>
<td>3.6±1.3†</td>
<td>1.9±0.5</td>
</tr>
<tr>
<td>PIC (μg/ml)</td>
<td>0.78±0.42†</td>
<td>0.41±0.13</td>
</tr>
</tbody>
</table>

Lp(a), lipoprotein(a); TAT, thrombin–antithrombin III complex; PIC, α₁-plasmin inhibitor-plasmin complex. Values are mean±SD.

* p<0.05, † p<0.01 different from stable angina.
transient increase of Lp(a) in unstable angina

by the 21st day. However, at 21 days the levels were still significantly higher than those on admission ($p<0.05$). Plasma PIC levels remained unchanged during the study period, and plasma TAT levels were slightly decreased 7 days after admission, but this difference was not significant. CRP and a1-AT values remained within the normal ranges during the study period. Of the 18 patients with unstable angina, 16 were stabilized by pharmacological intervention, but two did not stabilize and received urgent coronary angioplasty during the first week of their hospital stay. All nine patients whose Lp(a) levels were examined serially stabilized within 7 days after admission. Ten of the 16 patients who stabilized underwent elective coronary angioplasty, and the other six had coronary bypass surgery after the study period.

Changes of Plasma Lipoprotein(a) Levels in Patients With Stable Exertional Angina and Controls

Serial changes of Lp(a) levels were examined for 2 weeks in six of the 18 patients with stable exertional angina and in the 10 control patients (Table 4). These levels remained unchanged during the study period. Two of the six patients with stable exertional angina and three of the 10 control patients had chest pain attacks within 48 hours before the first blood test. However, plasma Lp(a) levels were not significantly changed during the study period in these patients (136±59 mg/l at the first blood test, 155±55 mg/l after 14 days).

Discussion

It is now established that elevated plasma Lp(a) levels are linked to atherosclerosis of the coronary and cerebral arteries.2-6 It has also been shown that Lp(a) levels are an important predictor of the severity of coronary artery disease.5,34 In the present study, plasma Lp(a) levels on admission were significantly higher in patients with unstable angina than in those with stable exertional angina. Coronary angiographic findings, however, were similar in the two groups. On the other hand, it is known that intracoronary throm-

### Table 3. Changes in Plasma Concentrations of Lipoprotein(a), α1-Plasmin Inhibitor–Plasmin Complex, Thrombin–Antitrombin III Complex, C-Reactive Protein, and α1-Antitrypsin After Admission in Nine of 18 Patients With Unstable Angina

<table>
<thead>
<tr>
<th>Assay</th>
<th>Time of sampling</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Admission</td>
</tr>
<tr>
<td>Lp(a) (mg/l)</td>
<td>327±207</td>
</tr>
<tr>
<td>PIC (µg/ml)</td>
<td>0.73±0.45</td>
</tr>
<tr>
<td>TAT (µg/ml)</td>
<td>3.1±0.8</td>
</tr>
<tr>
<td>CRP (mg/dl)</td>
<td>0.26±0.22</td>
</tr>
<tr>
<td>α1-AT (mg/dl)</td>
<td>196±14.0</td>
</tr>
</tbody>
</table>

Lp(a), lipoprotein(a); PIC, α1-plasmin inhibitor–plasmin complex; TAT, thrombin–antithrombin III complex; CRP, C-reactive protein; α1-AT, α1-antitrypsin; NS, not significant. Values are mean±SD.
bosis plays an important role in the pathogenesis of unstable angina. We found that plasma levels of TAT, a specific indicator of thrombin generation, were higher in the patients with unstable angina than in those with stable exertional angina. Therefore, our data suggest that elevated Lp(a) levels may be associated with activation of the hemostatic system in unstable angina.

Because of its structural similarity to plasminogen, Lp(a) has been shown in vitro to compete for plasminogen binding sites on the endothelial membrane, where Lp(a) suppresses fibrinolysis and produces a procoagulant state. It is, however, not known whether Lp(a) has the same effects on the fibrinolytic system in patients with unstable angina. Serial changes in levels of Lp(a), TAT, and PIC, a specific indicator of plasminogen activation or accelerated fibrinolysis, were examined after admission in patients with unstable angina to evaluate the effects of Lp(a) on the fibrinolytic system. Plasma levels of Lp(a), TAT, and PIC on admission were significantly higher in patients with unstable angina than in those with stable exertional angina. Plasma Lp(a) concentrations were increased 7 and 14 days after admission but had decreased by the 21st day. On the other hand, plasma PIC levels remained unchanged during the study period. Our data suggest that angina may be unstable when the clotting system is in the active state with accelerated fibrinolysis. However, we were unable to show that Lp(a) had any effect on the fibrinolytic system, as was previously reported for in vitro studies. The influence of Lp(a) on the fibrinolytic system is still controversial. Furthermore, the inhibitory effect of Lp(a) on fibrinolysis may be a local event associated with the fibrin thrombus, and systemic fibrinolytic activation may not be affected. It is not clear in the present study whether fibrinolytic activation is a local or a systemic phenomenon in patients with unstable angina because the samples studied were drawn from the peripheral blood. Coronary sinus sampling would help resolve this issue. The rate of conversion of plasminogen to plasmin by t-PA and prourokinase is enhanced by the presence of thrombin and fibrin.

Both the rate and extent of fibrinolysis are also regulated by the circulating inhibitors of plasminogen activator inhibitor. We measured only PIC, an inactive complex of plasmin, and PI, as an indicator of plasminogen activation. Thus, much additional investigative effort of plasminogen activator inhibitor or other useful indicators of the fibrinolytic system may be needed to delineate the interference of Lp(a) with fibrinolysis in patients with ischemic heart disease.

It has been well documented that plasma levels of Lp(a) vary widely among individuals in a population, whereas levels in an individual remain remarkably constant. The present study provides evidence that transient increases of plasma Lp(a) concentrations occur in patients with unstable angina. Fluctuations of Lp(a) levels have also been demonstrated during pregnancy and during treatment with the anabolic steroid stanozolol. Maeda et al. recently demonstrated transient elevations of plasma Lp(a) levels after acute myocardial infarction and suggested that Lp(a) has the characteristics of an acute-phase reactant. However, in the present study Lp(a) levels were increased transiently without changes in levels of the acute-phase proteins CRP and α1-AT. It is not known whether stimuli such as pain or hospitalization influence Lp(a) levels. In the present study, plasma Lp(a) levels remained unchanged for 2 weeks in both patients with stable exertional angina who were hospitalized and those who were not hospitalized. Further, five of those patients had chest pain attacks within 48 hours before the first blood test. Our data suggest that stimuli such as pain or hospitalization do not influence Lp(a) levels. Antianginal medication except for aspirin was similar in both patients with unstable angina and those with stable exertional angina. After the first blood test, five of the nine patients with unstable angina who were examined serially for Lp(a) levels were given aspirin, which the others had already received. All nine patients were stabilized by pharmacological interventions within 7 days after admission. However, Lp(a) levels were increased in all nine of these persons. Therefore, it is suggested that aspirin or other antianginal medication does not influence Lp(a) levels and that the increase in Lp(a) levels may not be associated with the activity of unstable angina. In our study, plasma levels of Lp(a), TAT, and PIC were significantly higher in patients with unstable angina than in those with stable exertional angina. Coronary angiographic findings, however, were similar in both groups. Therefore, it is speculated that elevated Lp(a) levels are caused by activation of the hemostatic system or the fibrinolytic system. At present, however, the mechanism of the increase in levels of Lp(a) or its role in unstable angina is not clear.

References


8. Albers JJ, Cabana VG, Warnick GR, Hazzard WR: Lp(a) lipoproteins: Relationship to sinking pre-beta lipoprotein, hyperlipoproteinemia, and apolipoprotein B. *Metabolism* 1975; 24:1047–1054


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