Plasma and Platelet Plasminogen Activator Inhibitor-1 in Patients With Acute Myocardial Infarction

Takeshi Soeki, MD; Yoshiyuki Tamura, MD; Nobuo Fukuda, MD; Susumu Ito, MD*

Several studies have demonstrated an increased level of plasma plasminogen activator inhibitor-1 (PAI-1) in patients with coronary artery disease (CAD). However, the concentration of PAI-1 in platelets, which accounts for more than 90% of the blood PAI-1, is unknown in these patients. The present study evaluated the concentrations of PAI-1 in several fibrinolytic factors in the plasma and platelets of patients with CAD and the serial changes in patients with acute myocardial infarction (AMI). All 72 subjects had coronary angiography and were divided into 3 groups: CAD(−) group without coronary artery stenosis or myocardial ischemia (n=20), CAD(+) group with either stable angina pectoris (n=18) or old myocardial infarction (n=12) with coronary artery stenosis, and the AMI group admitted within 24 h of symptom onset who underwent successful percutaneous transluminal coronary angioplasty (n=22). The concentrations of plasma PAI-1, tissue plasminogen activator (t-PA), and t-PA PAI-1 complex were similar in the CAD(−) and CAD(+) groups, but were greater on day 1 in the AMI group compared with the 2 CAD groups. There were no significant differences between the 3 groups in the plasma concentrations of thrombin–antithrombin III complex (TAT), α2-plasmin inhibitor-plasmin complex (PIC), β-thromboglobulin (β-TG), and platelet factor 4 (PF-4). The platelet PAI-1 concentrations did not differ between the CAD(−) and CAD(+) groups, but was greater on day 1 in the AMI group compared to the CAD groups. The platelet β-TG and PF-4 were similar between the 3 groups. In the AMI group, both the plasma and platelet PAI-1 concentrations were greater on day 1, but the plasma PAI-1 rapidly decreased by day 5 and remained low on day 28 compared with day 1. The platelet PAI-1 concentration gradually decreased by day 5 and was further decreased by day 28. The serial changes of the plasma t-PA and t-PA PAI-1 complex during the course of AMI were similar to those of the plasma PAI-1. A positive correlation was found between the plasma and platelet PAI-1 in all 72 patients, but not in the AMI group alone. These results suggest that the PAI-1 that has accumulated in platelets at the onset of AMI might be released in large amounts into the plasma, resulting in an increase in thrombus formation. (Jpn Circ J 2000; 64: 547–553)

Key Words: Acute myocardial infarction; Plasminogen activator inhibitor-1; Platelet

Coronary artery disease results from progressive atherosclerotic plaque development and subsequent thrombus formation. Imbalances in the coagulation system and the fibrinolytic system lead to fibrin deposition in the arterial wall, which is known to be associated with the plaque fissuring seen in acute coronary syndromes.

Fibrinolysis is stimulated by plasmin, which is converted from plasminogen by tissue plasminogen activator (t-PA) produced and released from endothelial cells. The net fibrinolytic activity in the plasma reflects the balance between t-PA and plasminogen activator inhibitor-1 (PAI-1). Endothelial and hepatic cells are the main sites of PAI-1 synthesis, but it has also been identified in smooth muscle cells and adipose tissue. PAI-1 is then stored and secreted from both endothelial cells and platelets.

Several studies have demonstrated increased plasma PAI-1 in patients with coronary artery disease (CAD) and acute coronary syndromes. However, only one report has examined the PAI-1 activity in platelets, which accounts for more than 90% of the blood PAI-1, in patients with stable CAD and acute coronary syndromes.

The present study evaluated the concentrations of PAI-1 in both the plasma and platelets of patients with stable CAD and the serial changes of the plasma and platelet PAI-1 in patients with acute myocardial infarction (AMI). Furthermore, we measured β-thromboglobulin (β-TG) and platelet factor 4 (PF-4) in both the plasma and platelets and t-PA, t-PA PAI-1 complex, thrombin–antithrombin III complex (TAT), and α2-plasmin inhibitor-plasmin complex (PIC) in the plasma as additional markers of fibrinolytic activity.

Methods

Patients

The subjects consisted of 72 patients who underwent coronary angiography, excluding those with severe liver disease, sepsis, disseminated intravascular coagulation, or cardiogenic shock. The subjects were divided into 3 groups: (i) CAD(−) group: 20 patients who complained of chest pain but did not have coronary artery stenosis or evidence of myocardial ischemia (10 men, 10 women, mean age 60.6 years); (ii) CAD(+) group: 30 patients with stable angina pectoris (n=18) or old myocardial infarction (n=12) with at least 1-vessel coronary artery stenosis of 75% or more according to the American Heart
Table 1 Patient Demographics and Clinical Characteristics

<table>
<thead>
<tr>
<th></th>
<th>CAD(−) group (n=20)</th>
<th>CAD(+) group (n=30)</th>
<th>AMI group (n=22)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malefemale</td>
<td>10/10*</td>
<td>18/12*</td>
<td>19/3</td>
</tr>
<tr>
<td>Age (years)</td>
<td>60±4.9†</td>
<td>64±6.3</td>
<td>65±6.3</td>
</tr>
<tr>
<td>Smoking</td>
<td>5 (25%)</td>
<td>6 (20%)</td>
<td>12 (55%)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>5 (25%)</td>
<td>12 (40%)</td>
<td>8 (36%)</td>
</tr>
<tr>
<td>Diabetes Mellitus</td>
<td>3 (15%)</td>
<td>6 (20%)</td>
<td>6 (27%)</td>
</tr>
<tr>
<td>TC-cholesterol (mg/dl)</td>
<td>209±39°</td>
<td>210±40</td>
<td>190±37</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>124±52</td>
<td>147±54</td>
<td>129±59</td>
</tr>
<tr>
<td>HDL-cholesterol (mg/dl)</td>
<td>56±10*</td>
<td>45±15*</td>
<td>38±7</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>22.7±0.7</td>
<td>23.1±5.4</td>
<td>23.3±7.9</td>
</tr>
</tbody>
</table>

Data are expressed as the mean±standard deviation. *p<0.05 vs AMI group; †p<0.01 vs CAD(+) group. AMI, acute myocardial infarction; CAD, coronary artery disease; HDL, high-density lipoprotein; T, total.

Table 2 Hematologic Parameters

<table>
<thead>
<tr>
<th></th>
<th>CAD(−) group (n=20)</th>
<th>CAD(+) group (n=30)</th>
<th>AMI group (n=22)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma PAI-1 (ng/ml)</td>
<td>11.6±1.0*</td>
<td>14.7±1.3*</td>
<td>38.7±1.9</td>
</tr>
<tr>
<td>Plasma t-PA (ng/ml)</td>
<td>7.6±1.0*</td>
<td>7.9±0.3*</td>
<td>18.3±1.6</td>
</tr>
<tr>
<td>Plasma t-PA/PAI-1 complex (ng/ml)</td>
<td>25.6±9.7*</td>
<td>30.6±10.6*</td>
<td>65.9±21.4</td>
</tr>
<tr>
<td>Plasma TAT (ng/L)</td>
<td>3.0±1.1</td>
<td>3.1±0.6</td>
<td>3.8±0.8</td>
</tr>
<tr>
<td>Plasma PIC (mg/ml)</td>
<td>1.1±0.5</td>
<td>1.2±0.5</td>
<td>1.0±0.5</td>
</tr>
<tr>
<td>Plasma β-TG (IU/ml)</td>
<td>50.8±3.3</td>
<td>45.8±3.3</td>
<td>61±5.7</td>
</tr>
<tr>
<td>Plasma FF-4 (UI/ml)</td>
<td>11.3±2.2</td>
<td>10.6±5.1</td>
<td>7.4±4.7</td>
</tr>
<tr>
<td>Platelet PAI-1 (ng/5×10^9/plt)</td>
<td>240.9±43.9*</td>
<td>251.7±10.8*</td>
<td>347.1±12.2</td>
</tr>
<tr>
<td>Platelet β-TG (IU/5×10^9/plt)</td>
<td>1959±3702</td>
<td>1872±4789</td>
<td>1841±2439</td>
</tr>
<tr>
<td>Platelet FF-4 (IU/5×10^9/plt)</td>
<td>1064±4176</td>
<td>922±3882</td>
<td>832±6385</td>
</tr>
<tr>
<td>Whole blood platelet count (10^12/μl)</td>
<td>23.0±3.8</td>
<td>22.8±5.1</td>
<td>21.4±6.2</td>
</tr>
</tbody>
</table>

Data are expressed as the mean±standard deviation. *p<0.05 vs AMI group. AMI, acute myocardial infarction; CAD, coronary artery disease; PAI-1, plasminogen activator inhibitor-1; PF-4, platelet factor 4; PIC, α2-antiplasmin inhibitor-plasmin complex; TAT, thrombin antithrombin III complex; PA, platelet microplatelet activator; β-TG, β-thromboglobulin.

Association criteria (18 men, 12 women, mean age 64.6 years); and (iii) AMI group: 22 consecutive patients with AMI admitted within 24h of symptom onset who underwent successful percutaneous transluminal coronary angioplasty (PTCA) (19 men, 3 women, mean age 65.8 years). AMI was diagnosed based on typical chest pain lasting at least 30 min with ST-T segment elevation of more than 0.2 mV in 2 or more contiguous leads on a standard 12-lead electrocardiogram, in addition to an increase in the creatine kinase MB fraction of at least twice the upper limit of normal. The patients in the AMI group did not undergo thrombolytic therapy with t-Pa. All patients with AMI were treated with a 5,000–10,000 U bolus of heparin at the time of the PTCA, and heparin infusion was maintained at 400–800U/h for 48h. Aspirin was given to all AMI patients orally at a dose of 88 mg/day, and other coronary dilating agents such as nitrates or calcium antagonists were administered from the 2nd day after admission throughout the rest of the hospitalization. The patients' hemodynamics were monitored by a Swan-Ganz catheter immediately after the PTCA for at least 24h.

Antiplatelet drugs including aspirin, ticlopidine, and oripride were administered to 10 of 20 CAD(−) patients and 15 of 30 CAD(+) patients. In all AMI patients, antiplatelet drugs were not administered until the 2nd hospital day. Furthermore, none of the AMI patients underwent cardiopulmonary resuscitation, countershock, intraaortic balloon pumping, or percutaneous cardiopulmonary support during the hospitalization. None of the AMI patients had a large hematoma at the sheath site in the femoral artery.

Blood Samples

In the CAD(−) and CAD(+) groups, fasting venous blood samples were drawn without stasis after 15 min of supine rest at 07:00h before coronary angiography. In the AMI group, the samples were obtained immediately after PTCA and at 07:00h in the fasting state on the 5th and 28th days after admission. Samples were collected into siliconized glass tubes containing ETP solution (78 mmol/L EDTA, 10 mmol/L theophylline, 0.33% mg/ml protargol and ETP as an anticoagulant, placed on ice and centrifuged immediately. Platelet-free plasma was prepared from the ETP samples by centrifugation at 2,500 G for 30 min at 4°C and stored at −70°C until assayed. Platelet-rich plasma was prepared by centrifugation in accordance with the Jokls' method of centrifugation at 220 G for 10 min at 4°C and again at 110 G for 15 min at 4°C to sediment remaining red and white cells. The platelets were sedimented by centrifugation for 800 G for 15 min at 4°C. The platelets were then adjusted to a concentration of 5×10^10 platelets/ml with Tyrode's solution using a Coulter Counter (NE-4500,Sysmex,Japan). A 1-ml 5×10^10 platelet sample was lysed with 1% Triton X-100 for 30 min at room temperature and centrifuged at 2,500 G for 15 min, and the supernatant was stored at −70°C until assayed.

Biochemical Analysis

The plasma and platelet PAI-1, β-TG and PF-4 concentrations were determined using a commercially available enzyme-linked immunosorbent assay kit (Diagnostica Stago, Asnieres-Svvr-Scine, France). The plasma t-PA, t-PA-
PAI-1 complex, TAT and PIC were determined using a similar method (t-PA, Biopool AB, Umea, Sweden; t-PA-PAI-1 complex and PIC, Teijin Ltd, Osaka, Japan; TAT, Behringwerke, Marburg, Germany).

Statistical Analysis
All results are expressed as the mean ± standard deviation. The baseline concentrations of various lipid parameters and hematologic parameters among the 3 groups were evaluated by one-factor analysis of variance, and if it was significant, a test of Fisher's Protected Least Significance Difference was performed. The differences in the ratios of the other baseline characteristics among the groups were compared using the chi-square test. The statistical significance of changes in the plasma PAI-1, t-PA, t-PA-PAI-1 complex, TAT, PIC, and platelet PAI-1 were evaluated by repeated measure analysis of variance, and the differences of those between each test day were analyzed by the paired t test. The correlation coefficients were calculated by linear regression analysis. A p<0.05 was considered significant.

Results

Patient Characteristics
The clinical and biochemical characteristics of the CAD(−), CAD(+) and AMI groups are shown in Table 1. The AMI group had more males than either of the CAD groups, and there were more smokers in the AMI group than in the CAD(+) group. High density lipoprotein (HDL)-cholesterol was lower in the AMI group than in the CAD groups, and lower in the CAD(+) group than in the CAD(−) group. The groups did not differ in age, incidence of hypertension or diabetes mellitus, serum total cholesterol and triglyceride concentrations, and body mass index. The body mass index did not correlate with the plasma and platelet PAI-1 concentrations in the overall sample.

Plasma Fibrinolytic and Clotting Factors and Platelet-Activating Factors (Table 2)
The mean plasma PAI-1, t-PA, and t-PA-PAI-1 complex concentrations were similar in the CAD(−) and CAD(+) groups, but were greater on day 1 in the AMI group than in the CAD groups. There were no differences in the concentrations of TAT, PIC, β-TG or PF-4 between the 3 groups.

Platelet PAI-1 and Activating Factors in Each Group (Table 2)
The mean platelet PAI-1 concentration was similar in both the CAD groups, but was greater on day 1 in the AMI group. The platelet count and platelet β-TG and PF-4 concentrations were similar in the 3 groups.

In the CAD(+) group, there were no differences between those receiving and not receiving antiplatelet drugs with respect to the concentrations of the plasma and platelet PAI-1, β-TG, PF-4 or plasma t-PA, t-PA-PAI-1 complex and TAT. The PIC concentration was lower in the group receiving antiplatelet drugs (1.4±0.7 vs 1.6±0.3 mg/ml, p<0.05).

Correlations Between the PAI-1, β-TG, and PF-4 Concentrations in Plasma and Platelets
The plasma PAI-1 concentration correlated with the platelet PAI-1 concentration in the entire patient cohort (r=0.373, p<0.01), but no correlation was found between the two in any one group (Fig 1). Neither was any correlation noted between the plasma and platelet β-TG concentrations or between the plasma and platelet PF-4 concentrations overall or in any one group. A positive correlation was found between the plasma β-TG and plasma PF-4 concentrations in all the patients (r=0.415, p<0.01) as well as in each patient group (CAD(−): r=0.469, p<0.05; CAD(+): r=0.428, p<0.05; AMI: r=0.589, p<0.05). There was no significant correlation between the plasma PAI-1 and plasma β-TG or between the plasma PAI-1 and plasma PF-4 concentrations in any group or overall. A positive correlation was noted between the platelet β-TG and platelet PF-4 concentrations in the overall sample (r=0.635, p<0.01) and in each group (CAD(−): r=0.760, p<0.01; CAD(+): r=0.443, p<0.05; AMI: r=0.884, p<0.01). However, no significant correlation was found between the platelet PAI-1 and platelet β-TG concentrations or between the platelet PAI-1 and platelet PF-4 concentrations.
Serial Changes in the Plasma and Platelet PAI-1 Concentrations in Patients With AMI

The plasma PAI-1 concentration (ng/ml), which was elevated on day 1 of AMI (38.7±13.9) compared with the CAD groups, decreased by day 5 (11.9±6.5) and remained low on day 28 (18.2±11.6) (Fig 2). The platelet PAI-1 concentration (ng/5x10^6 platelets), which was elevated on day 1 of AMI (347.1±132.1), decreased on day 5 (283.5±73.9) and further decreased on day 28 (218±47.4) (Fig 3).

Serial Changes in the Plasma Concentrations of t-PA, t-PA·PAI-1 Complex, TAT, and PIC in Patients With AMI

The plasma t-PA concentration (ng/ml) increased on day 1 of AMI (18.3±12.6), decreased by day 5 (10.0±9.5), and remained low on day 28 (9.8±6.2). The plasma t-PA·PAI-1 complex concentration (ng/ml) also increased on day 1 of AMI (65.9±23.4), decreased by day 5 (33.9±22.1), and remained low on day 28 (35.3±15.1) (Fig 4). The plasma TAT concentration (μg/L) did not change during the course of AMI (3.8±4.4 on day 1, 4.4±3.4 on day 5, and 3.0±3.6 on day 28). Similarly, no significant changes in the plasma PIC concentration (μg/L) were found throughout the course of AMI (1.9±2.5 on day 1, 2.8±0.8 on day 5, and 1.5±0.8 on day 28).

Discussion

Serial Changes of PAI-1 in Plasma and Platelets in Patients With AMI

The net fibrinolytic activity in plasma is determined by the balance between t-PA, which is the rate-limiting enzyme from the endothelium, and PAI-1 secreted from platelets and endothelial cells. Several immunologically distinct plasminogen activator inhibitors have been reported, and it has been demonstrated that the PAI activity in plasma...
depends largely on free PAI-1 bound to vitronectin, which does not complex with t-PA.

In the present study, the plasma PAI-1 was increased in the AMI group compared with the CAD(−) and CAD(+) groups, but did not differ between the 2 CAD groups. In the AMI group, the plasma PAI-1 concentration increased on day 1 but decreased by day 5 following a myocardial infarction. Our results are compatible with previous reports which showed no association between the plasma concentration of PAI-1 and angiographically documented coronary artery disease;25,26 and with prior studies that demonstrated significantly decreased plasma PAI activity several days after the onset of an acute coronary syndrome.8,19

We also found that the platelet PAI-1 concentration increased on day 1 of AMI, compared with the CAD(−) and CAD(+) groups, but was similar in the 2 CAD groups. In the AMI group, the platelet PAI-1 concentration increased on day 1, decreased on day 5, and was further decreased by day 28. Simpson et al studied the platelet PAI-1 in patients with a variety of disorders and found elevated plasma PAI-1, but similar amounts of platelet PAI-1 antigen per platelet, on day 1 in patients with angina and myocardial infarction compared with healthy individuals.25 In their study, only 8 patients had AMI, which may explain the discrepancy between their results and ours. In addition, no detailed characteristics of the myocardial infarction group were presented, which makes analysis of the different results between the 2 studies difficult.

Mechanism for the Elevated Plasma and Platelet PAI-1 in AMI

Whether the increase in platelet PAI-1 during the acute phase of AMI is the cause or the result of an acute coronary syndrome is uncertain. Although several reports have suggested that platelet PAI-1 might be present mainly in its inactive form22,27 a dose-dependent platelet release of PAI-1 activity has been observed.28-30 In fact, it was reported that a 4.8-fold increase in platelets was accompanied by a 3-fold increase in the plasma PAI-1 antigen and a 1.6-fold increase in the plasma PAI-1 activity in patients with essential thrombocytopenia.21 An in vivo study in rabbits showed reactivation of latent PAI-1 and several studies have demonstrated PAI-1 mRNA in human megakaryocytes.32,33 Furthermore, it was reported that a specific form of insulin in plasma was related to the platelet PAI-1 concentration in patients with type 2 diabetes;24 which suggests that insulin might regulate PAI-1 synthesis by bone marrow megakaryocytes. These findings suggest that an increased number of platelets, which contain more PAI-1 stimulated by unknown factors including insulin, may contribute to the increased plasma PAI-1, which may result in thrombus formation during the early stage of an acute coronary syndrome. However, it seems unlikely that there is a transport mechanism of PAI-1 against a large gradient from the plasma to the platelet PAI-1. The present investigation did not measure the plasma insulin concentration, so further study is needed to clarify the relationship between plasma insulin and platelet PAI-1 concentrations in patients with CAD, especially those with AMI. Finally, any unknown stimulators that act in the absence of insulin for PAI-1 synthesis in megakaryocytes need to be identified and characterized.

Relationship Between Plasma and Platelet PAI-1

In the present study, a weak but significant positive correlation (r=0.373) was found between the plasma and platelet PAI-1 concentrations in the overall sample, but not in any individual group. It has been reported that no correlation exists between the plasma and platelet PAI-1 concentrations34 or between the plasma PAI-1 concentration and the corresponding total platelet count.22 These data indicate that plasma PAI-1 is independent of the platelet
pool of PAI-1 and suggest that some of the plasma PAI-1 may be released by platelets and comes from several sources.

Several recent studies have implicated a direct pathogenic link between obesity and the plasma PAI-1 concentration. Lundgren et al demonstrated that the PAI-1 release from an increased mass of adipose tissue might result in increased plasma PAI-1 activity in obese humans. Shimomura et al demonstrated that enhanced expression of the PAI-1 gene in visceral fat might increase the plasma PAI-1 concentration and have a role in the development of vascular disease in visceral obesity. In the present study, the body mass index did not differ between the 3 groups and did not correlate with the plasma and platelet PAI-1 concentrations in the overall sample. However, in one study the visceral fat accumulation was shown to contribute to the development of CAD in non-obese men. The direct association between the visceral fat accumulation and the PAI-1 in the plasma and platelets was not specifically studied in our work.

Other Fibrinolytic Factors in AMI Patients

The plasma t-PA and t-PA:PAI-1 complex concentrations increased in the AMI group compared with the CAD groups, and the changes during the course of AMI were similar to those of plasma PAI-1. No intergroup difference in the concentrations of TAT or PIC was noted, suggesting that the PAI-1 and t-PA concentrations are more sensitive and more important than the TAT or PIC concentrations as markers of impaired fibrinolysis in patients with acute coronary syndrome.

Influence of Coronary Angioplasty on the Measurement of Fibrinolytic Factors

We did not measure the concentrations of PAI-1 and the other components of the fibrinolytic system in the plasma and platelets prior to PTCA in patients with AMI. A previous report demonstrated that the plasma PAI-1 and t-PA concentrations did not differ immediately before or 1 h after direct PTCA. In addition, a preliminary study in our laboratory showed that the plasma and platelet PAI-1, β-TG, PF-4 and plasma t-PA, t-PA:PAI-1 complex concentrations did not change immediately before or after an elective PTCA with the administration of heparin. From these findings, we believe that the increased concentrations of PAI-1 and other fibrinolytic factors in the plasma and platelets post PTCA are not likely to be caused by the procedure.

Study Limitations

Our study has several limitations. First, we measured the concentration of PAI-1 antigen and not PAI-1 activity, and therefore could not differentiate between the active and latent forms of platelet PAI-1. Second, differences in the drugs administered, the antiplatelet drugs in particular, may have had an effect on the results. However, no differences were detected between the groups receiving and not receiving antiplatelet drugs with respect to the various hematologic parameters, which suggests that antiplatelet drugs had little influence on the results.

Conclusion

Our findings demonstrate that both the plasma and platelet PAI-1 increase during the acute phase of AMI and that the plasma PAI-1 rapidly decreases by day 5 whereas the platelet PAI-1 decreases gradually from day 5 to day 28 post MI. These findings suggest that unknown releasing factors may stimulate the synthesis of PAI-1 in megakaryocytes at the onset of MI and that the PAI-1 stored in platelets may be released in large quantities into the plasma, followed by acceleration of thrombus formation. Further studies are needed to determine the mechanism and the role of the increased platelet and plasma PAI-1 concentrations during the acute phase of AMI.

Acknowledgments

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References