Plasminogen Activator Inhibitor Activity in Diabetic and Nondiabetic Survivors of Myocardial Infarction

Rosaire P. Gray, David L.H. Patterson, and John S. Yudkin

Recent studies suggest that plasminogen activator inhibitor (PAI-1) may be a risk factor for recurrent myocardial infarction. We measured PAI-1 activity and antigen and tissue-type plasminogen activator (t-PA) antigen in 35 (20 nondiabetic and 15 diabetic) subjects with no clinical or electrocardiographic evidence of ischemic heart disease and in 74 (50 nondiabetic and 24 diabetic subjects) who had survived a myocardial infarction in the preceding 6–24 months. Levels of PAI-1 activity (18.7±5.6 versus 12.0±3.8 arbitrary units [AU] per milliliter, p=0.001) and t-PA antigen (7.0±1.9 versus 4.6±2.0 ng/mL, p=0.001) were significantly higher in diabetic compared with nondiabetic control subjects. Survivors of myocardial infarction had higher levels of PAI-1 activity and antigen and t-PA antigen than control subjects, and the diabetic survivors had higher levels of PAI-1 activity (25.3±6.7 versus 20.1±7.1 AU/mL, p=0.004) and t-PA antigen (10.6±4.3 versus 8.4±3.3 ng/mL, p=0.03) than the nondiabetic survivors. No difference in PAI-1 antigen levels was found between the diabetic subjects and either the nondiabetic control subjects or survivors of myocardial infarction. After venous occlusion in control subjects, there was a significant increase in PAI-1 antigen (mean 26.7%, range 14.1–58.1% in nondiabetics and mean 25.2%, range 6.2–39.7% in diabetics) and t-PA antigen (mean 78.3%, range 13.6–186.2% for nondiabetic and mean 40.7%, range 17.5–76.2% for diabetic subjects), but in the survivors of myocardial infarction, no significant effect of venous occlusion was observed. These results suggest that diabetic subjects have higher PAI-1 activity levels than nondiabetic subjects both after acute myocardial infarction and in the absence of ischemic heart disease. The similar levels of PAI-1 antigen between the diabetic and nondiabetic subjects suggest that increased levels of t-PA-PAI-1 complexes cannot explain the increase in t-PA antigen in diabetic subjects. (Arteriosclerosis and Thrombosis 1993;13:415–420)

KEY WORDS • plasminogen activator inhibitor-1 • diabetes • myocardial infarction

The cause of the high morbidity and mortality from ischemic heart disease and myocardial infarction in diabetic subjects remains unclear. The recent development of assays for specific components of the fibrinolytic system has led to renewed interest in the potential role of the fibrinolytic system in coronary artery disease and myocardial infarction. Recent studies have shown reduced fibrinolytic activity, largely due to elevated plasminogen activator inhibitor (PAI-1) activity, in various subgroups with an increased risk of coronary artery disease, including obese subjects, patients with hypertension, patients with angina pectoris, and diabetic subjects without coronary artery disease. Elevated PAI-1 activity levels have also been demonstrated in young survivors of myocardial infarction and have been shown to predispose to reinfection in this group. Clinical manifestations of ischemic heart disease result from the progressive development of an atherosclerotic plaque and subsequent thrombus formation. The role of fibrin deposition in the obstruction of coronary arteries that leads to acute myocardial infarction is obvious, but fibrin deposition on the vessel wall may also have a more subtle role by initiating endothelial cell injury and stimulating cell proliferation. Therefore, hypofibrinolysis may be an important risk factor for ischemic heart disease.

Because abnormalities of fibrinolysis have previously been described in relation to diabetes and also in nondiabetic survivors of myocardial infarction, we wished to test the hypothesis that diabetic survivors of myocardial infarction have reduced fibrinolytic activity due to elevated PAI-1 activity compared with subjects without ischemic heart disease and nondiabetic survivors of myocardial infarction. We also wished to determine whether the elevated PAI-1 activity was due to increased PAI-1 antigen or decreased tissue-type plasminogen activator (t-PA) antigen levels.

Methods

Patients

Seventy-four male subjects who had a confirmed myocardial infarction (World Health Organization criteria) between 6 and 24 months previously were
studied. There were 24 diabetic subjects, of whom 22 were previously known diabetics with a median duration of diabetes of 3.5 years (range, 9 months–15 years) and two who were diagnosed at the time of study on the basis of fasting hyperglycemia (plasma glucose ≥7.0 mmol/L) and an elevated glycated hemoglobin16 (>8.5%). All of the subjects were non-insulin-dependent diabetics, 12 of whom were controlled by diet alone and 10 who were taking oral hypoglycemic agents (nine a sulfonylurea and one a sulfonylurea plus metformin). Thirteen of the 50 nondiabetic subjects had impaired glucose tolerance (2-hour plasma glucose ≥7.8 and ≤11.1 mmol/L).17

All subjects attended the Clinical Investigation Unit of the Academic Unit of Diabetes and Endocrinology at the Whittington Hospital after an overnight fast. Venous blood samples for estimation of fasting plasma glucose, glycated hemoglobin, serum lipids, PAI-1 activity, PAI-1 antigen, and t-PA antigen were collected from an antecubital vein using a 21G butterfly needle. After 10 minutes of venous occlusion (using a sphygmomanometer cuff midway between the systolic and diastolic blood pressure), a second blood sample was collected for estimation of PAI-1 activity and antigen and t-PA antigen levels. Blood samples for measurement of fibrinolytic parameters were collected into tubes containing sodium citrate (nine parts of blood mixed with one part of 3.8% sodium citrate) and centrifuged for 15 minutes at 3,000 g and 4°C, and the plasma was then stored at −70°C until analyzed. An oral glucose tolerance test was performed (75 g glucose), and plasma glucose level was measured at 30-minute intervals for 2 hours. Blood pressure was measured with a mercury sphygmomanometer after 10 minutes of rest. Body mass index (weight in kilograms divided by height in meters squared) and waist-to-hip ratios (ratio of the circumference of the waist at the level of the umbilicus and that of the hips measured with a steel tape at the level of the greater trochanters and the symphysis pubis) were calculated.

Control Subjects

Fibrinolytic parameters were also measured in 35 healthy male subjects with no clinical or electrocardiographic evidence of ischemic heart disease or hypertension. Twenty were nondiabetic and 15 were non–insulin-dependent diabetics. The median duration of diabetes was 3 years (range, 1–15). Eight were treated by diet alone, five were receiving oral hypoglycemic agents (one a sulfonylurea, two metformin, and two a combination of a sulfonylurea plus metformin), and two were receiving insulin because of failure of the oral hypoglycemic agents to control their disease. The control subjects were recruited from routine admissions for minor surgical procedures over a 1-month period, and the blood samples were taken before any other intervention. The diabetic subjects were recruited from the routine diabetic outpatient clinic over a 4-week period.

The study was approved by the hospital’s Ethics Committee, and the subjects gave informed consent.

Assays

Plasma glucose level was measured by a glucose oxidase method (Beckman, Brea, Calif.). Glycated hemoglobin was measured by agar gel electrophoresis (Corning Medical, Halstead, UK) and serum lipids by enzymatic colorimetric methods (total cholesterol, Boehringer Mannheim, Meylan, France; triglycerides, Roche Diagnostica, Herts, UK). High density lipoprotein cholesterol was measured by the same method after the low density lipoproteins were quantitatively precipitated out by the addition of phosphotungstic acid in the presence of magnesium ions. Low density lipoprotein cholesterol was estimated using the Friedewald equation.18 PAI-1 activity was measured using a chromogenic assay (Kabi Diagnostica, Molndal, Sweden). In this assay a fixed amount of t-PA is added in excess to undiluted plasma and forms an inactive complex with PAI-1. Plasminogen is then activated to plasmin by residual t-PA in the presence of a stimulator. The amount of plasmin is directly proportional to the residual t-PA activity and hence inversely proportional to the PAI-1 activity of the sample. The amount of plasmin is determined by measuring the amidolytic activity of plasmin on the chromogenic substrate S-2403. The intra-assay coefficient of variation (CV) was 4.5%, and the interassay CV was 8%. Both PAI-1 antigen and t-PA antigen levels were measured using enzyme-linked immunosorbent assays (Biopool AB, Umeå, Sweden). The Biopool TintElize PAI-1 antigen assay quantifies free, complexed, and latent human endothelial type PAI-1. The t-PA–PAI-1 and urokinase-type plasminogen activator–PAI-1 complexes are recovered with about the same efficiency as free PAI-1. The within-assay CV was 4.5% and the between-assay CV 7.5%. The Biopool TintElize t-PA antigen assay quantifies human single-chain and two-chain t-PA antigen. The immunoreactivity of single-chain and two-chain t-PA in complex with PAI-1, α2-antiplasmin, and PAI-2 is approximately 90%. The within-assay CV was 6% and the between-assay CV 10%.

Statistics

Results are expressed as mean and SD for normally distributed data and median and range for skewed data. Differences between groups were tested with unpaired Student’s t tests for normally distributed data and the Mann-Whitney U tests for skewed data. Relations between variables were assessed using Pearson’s correlation for normally distributed data and Spearman’s rank correlation for skewed data. The homogeneity of correlations was assessed using Fisher’s tanh−1 transformation and the test for the homogeneity of a set of correlation coefficients.19

Results

The characteristics of the subjects studied are shown in Table 1. There were no differences in age, body mass index, waist-to-hip ratio, blood pressure, or total cholesterol between the two groups. As expected, diabetic subjects had significantly higher fasting plasma glucose, glycated hemoglobin, and triglyceride and lower high density lipoprotein cholesterol levels than nondiabetic subjects. Apart from plasma glucose and glycated hemoglobin, there were no significant differences in the other measurements between those subjects with impaired glucose tolerance (n=13) and those with either normal glucose tolerance (n=37) or diabetes mellitus (n=24) (data not shown). The 15 diabetic and 20 nondiabetic control subjects were not significantly dif-
PAI-1 activity levels was observed. Antigen levels after venous occlusion, but a slight fall in there was no significant change in PAI-1 antigen or t-PA activity after venous occlusion. The median percent increase in t-PA antigen was greater in the nondiabetic control subjects than in the diabetic control subjects (mean 78.3%, range 13.6-186.2% versus mean 40.4%, range 17.5-76.2%). In the survivors of myocardial infarction, there was no significant change in PAI-1 antigen or t-PA antigen levels after venous occlusion, but a slight fall in PAI-1 activity levels was observed.

Table 1. Characteristics of Postmyocardial Infarction Diabetic and Nondiabetic Subjects

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Diabetic (n=24)</th>
<th>Nondiabetic (n=50)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>59.5±10.6</td>
<td>58.0±8.5</td>
<td>NS</td>
</tr>
<tr>
<td>Duration of diabetes (years)</td>
<td>3.5 (0.75-15)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment for diabetes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diet</td>
<td>14 (58.3%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oral hypoglycemic agent</td>
<td>10 (41.7%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time from AMI (months)</td>
<td>9.5 (6-24)</td>
<td>9.5 (6-24)</td>
<td>NS</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>28.1 (22.2-32.7)</td>
<td>25.4 (20.4-37.5)</td>
<td>NS</td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
<td>0.97 (0.88-1.1)</td>
<td>0.95 (0.83-1.0)</td>
<td>0.02</td>
</tr>
<tr>
<td>Fasting plasma glucose (mmol/L)</td>
<td>9.6±2.9</td>
<td>5.5±0.5</td>
<td>0.0001</td>
</tr>
<tr>
<td>Plasma glucose, 30 minutes (mmol/L)</td>
<td>13.5±3.4</td>
<td>9.0±1.6</td>
<td>0.0001</td>
</tr>
<tr>
<td>Plasma glucose, 120 minutes (mmol/L)</td>
<td>17.0±4.7</td>
<td>6.5±2.1</td>
<td>0.0001</td>
</tr>
<tr>
<td>Glycated hemoglobin (%)</td>
<td>9.9±2.1</td>
<td>7.2±0.7</td>
<td>0.0001</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>2.9 (1.2-8.1)</td>
<td>1.9 (0.6-6.5)</td>
<td>0.01</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>6.8±1.4</td>
<td>6.7±1.2</td>
<td>NS</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/L)</td>
<td>0.9 (0.6-1.6)</td>
<td>1.0 (0.5-1.7)</td>
<td>NS</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td>4.65±1.1</td>
<td>4.7±1.0</td>
<td>NS</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>140±20</td>
<td>135±20</td>
<td>NS</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>89.5±8.3</td>
<td>82.9±8.1</td>
<td>0.002</td>
</tr>
</tbody>
</table>

AMI, acute myocardial infarction; HDL, high density lipoprotein; LDL, low density lipoprotein. Values are mean±SD for normally distributed data and median and (range) for skewed data.

Different from the respective myocardial infarction patients in age (60.9±9.1 and 61.4±11.1 years; NS). The median time from acute myocardial infarction to study was identical in the diabetic and nondiabetic groups.

Figure 1 shows PAI-1 activity levels in the control subjects and in the survivors of myocardial infarction. In the control group, PAI-1 activity levels were significantly higher in the diabetic compared with the nondiabetic subjects. In the survivors of myocardial infarction, PAI-1 activity levels were significantly higher in the diabetic compared with the nondiabetic subjects in both groups of subjects compared with control subjects. PAI-1 antigen levels were significantly higher in the survivors of myocardial infarction than in the control subjects without ischemic heart disease but were not significantly different in diabetic compared with nondiabetic subjects. T-PA antigen levels were significantly higher in the survivors of myocardial infarction than in the control subjects and in both groups were significantly higher in diabetic compared with the nondiabetic subjects. There were no significant differences in the fibrinolytic variables measured between subjects with impaired glucose tolerance and those with normal glucose tolerance (data not shown).

The results of venous occlusion are shown in Table 2. In both the diabetic and nondiabetic control subjects, there was a significant increase in t-PA antigen and PAI-1 antigen but no significant change in PAI-1 activity after venous occlusion. The median percent increase in t-PA antigen was greater in the nondiabetic control subjects than in the diabetic control subjects (mean 78.3%, range 13.6-186.2% versus mean 40.4%, range 17.5-76.2%). In the survivors of myocardial infarction, there was no significant change in PAI-1 antigen or t-PA antigen levels after venous occlusion, but a slight fall in PAI-1 activity levels was observed.

There were positive correlations between PAI-1 activity and both PAI-1 antigen 
(\( r = 0.50, p = 0.0001 \)) and t-PA antigen 
(\( r = 0.41, p < 0.01 \)) and between PAI-1 antigen and t-PA antigen 
(\( r = 0.43, p = 0.0001 \)). The correlation between PAI-1 activity and antigen in the diabetic subjects was \( r = 0.76, p = 0.0001 \) compared with 
(\( r = 0.44, p = 0.0001 \) in the nondiabetic subjects \( \chi^2 = 3.98, df = 1, 0.02 < p < 0.05 \)).

Discussion

There is an emerging consensus that PAI-1 activity is increased in non-insulin-dependent diabetic subjects. In this study, we found that diabetic subjects without clinical evidence of ischemic heart disease had significantly higher PAI-1 activity and t-PA antigen levels compared with nondiabetic subjects and that diabetic survivors of myocardial infarction had higher PAI-1 activity and t-PA antigen levels than both control subjects and nondiabetic survivors of myocardial infarction.

Whether the elevated PAI-1 after myocardial infarction is a cause or a consequence of the acute myocardial infarction is not clear. Acute myocardial infarction has been shown to suppress fibrinolytic function, but the duration of this effect is unknown. Therefore, we studied the patients at least 6 months after the acute infarction, as metabolic changes associated with myocardial infarction and in particular an acute-phase reactant response persisting 6 months after infarction would be unusual. Moreover, our finding that elevated PAI-1 activity levels on admission with acute myocardial infarction persist at follow-up 6-24 months later suggests that these levels may reflect preinfarction levels.

In any case, it is likely that elevated PAI-1 activity levels, however they arise in postmyocardial infarction patients, represent an increased risk for recurrent myocardial infarction.
The cause of the elevated PAI-1 activity is not clear from this study. To our surprise we found no elevation of PAI-1 antigen levels in diabetic compared with the nondiabetic subjects, which might suggest that the increased PAI-1 activity is not related to an increased number of molecules but increased specific activity. One possible explanation for the finding of increased PAI-1 activity without a concomitant increase in PAI-1 antigen levels is the presence of an inhibitor(s) other than PAI-1. The PAI-1 activity assay measures the inhibitory capacity of the plasma against t-PA, and although PAI-1 is the principal inhibitor of t-PA in human plasma, other inhibitors such as α2-antiplasmin and C1 esterase inhibitor or an as-yet-unidentified inhibitor may also contribute. However, to date there is no evidence that these other inhibitors of t-PA are increased in diabetic subjects.

Although there was a significant correlation between PAI-1 activity and antigen, the relation was weak, and it may differ in diabetic compared with nondiabetic subjects. In addition to the complexed and free forms, PAI-1 also exists in a latent form in plasma. This molecule is immunologically similar to the active form but has no antiafactor activity in its native state, although it can be activated by negatively charged phospholipids and chemical denaturants. One possible explanation for the finding of increased PAI-1 activity but not antigen levels in diabetic compared with nondiabetic subjects is that a greater proportion of latent PAI-1 is in an active form in diabetic subjects. This could result from increased conversion of the latent to the active form, or alternatively, as PAI-1 is released from endothelial cells in the active form and then becomes inactivated, less of the active form is converted into the latent form in diabetic subjects. The increased correlation between activity and antigen in diabetic compared with nondiabetic subjects might sug-
gest a greater ratio of active to inactive PAI-1. However, further study would be necessary to confirm this finding and also to determine the factors that influence the conversion between active and latent PAI-1 in vivo.

The finding of elevated PAI-1 activity but not antigen does, however, have several other possible explanations. First, there is the possibility of a statistical error: because of the wide standard deviation in the PAI-1 antigen results, the power of the study to detect a difference is reduced. Second, the findings may be a consequence of PAI-1 antigen assay imprecision or a reduced ability of the assay to detect PAI-1–t-PA complexes; the former is unlikely, however, as we found a significant correlation between both PAI-1 activity and antigen levels and good within- and between-assay CVs. A third possible explanation is that less PAI-1 antigen is neutralized by complex formation with t-PA antigen or other plasma inhibitors in diabetic subjects. However, we also found that t-PA antigen levels were increased in the diabetic subjects, and because the specific activity of bound and free t-PA is identical in the antigen assay, this implies that if less of the PAI-1 is in complexed form, this is not because of a reduction in t-PA antigen levels. One final alternative explanation is that as a result of long-standing hyperglycemia, glycation of t-PA occurs, so that its activity as a plasminogen activator is partially impaired or lost and it is no longer available to bind PAI-1.31

The endothelial cell is an important source of PAI-1 as well as of t-PA, and the venous occlusion test is regarded as a test of endothelial cell function, stimulating the release of PAI-1 antigen and t-PA antigen.32–34 Unlike in the control subjects in this study, levels of PAI-1 antigen and t-PA antigen did not increase after venous occlusion in survivors of myocardial infarction. It is possible that in patients with vascular disease, there is a slow, continuous release of t-PA and PAI-1 from endothelial cells, as indicated by the elevated resting levels, resulting in reduced stores in endothelial cells and a consequent reduced release with venous occlusion. Our findings suggest that endothelial cell release of t-PA and PAI-1 is increased in diabetic subjects even in the absence of clinical evidence of macrovascular disease, which may be a reflection of subclinical vascular disease. This process is exaggerated in subjects with evidence of macrovascular disease (i.e., survivors of myocardial infarction), and in this group, venous occlusion no longer results in increased levels of t-PA antigen or PAI-1 antigen. The finding that even in the survivors of myocardial infarction that diabetic subjects had higher levels than nondiabetic subjects suggests that diabetes may have an independent effect on the vasculature.

In conclusion, we found that non–insulin-dependent diabetic subjects had higher PAI-1 activity than nondiabetic subjects both after myocardial infarction and in the absence of clinical evidence of coronary artery disease. Whether the abnormalities of fibrinolysis in diabetes and ischemic heart disease are a cause or effect of coronary artery disease remains unknown, but in any case, there is increasing evidence that abnormalities of fibrinolysis and in particular elevated PAI-1 activity levels play an important role in the development of vascular disease and may therefore contribute to the increased risk of coronary artery disease in diabetic subjects. However, contrary to our hypothesis, we found no evidence that elevated PAI-1 activity was due to increased PAI-1 antigen levels or reduced t-PA antigen levels. The mechanisms for this will require further study. To elucidate this problem, we propose to investigate latent/inactive and active forms of PAI-1 and their mechanisms of activation in diabetic subjects. It may also be helpful to look at markers of platelet and vessel wall function and their correlation with PAI-1 to determine the origin of PAI-1 in these subjects.

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