Tissue-Type Plasminogen Activator Antigen and Plasminogen Activator Inhibitor in Diabetes Mellitus

Johan Auwerx, Roger Bouillon, Désiré Collen, and Jef Geboers

Parameters of fibrinolysis, including euglobulin fibrinolytic activity, tissue-type plasminogen activator (t-PA) antigen, plasminogen activator inhibitor (PA-inhibitor) activity, and plasmin-α2-antiplasmin complex (PAP) were studied in 62 patients (35 women and 27 men; ages 53 ± 16 years) with either insulin-dependent (IDDM) or noninsulin-dependent (NIDDM) diabetes mellitus. Compared to a control group of similar age (n = 57), the diabetic patients had a significantly lower mean euglobulin fibrinolytic activity (1.2 ± 0.7 vs 1.7 ± 1.1 ng/ml, p<0.01) but significantly higher mean t-PA antigen (15.7 ± 8.4 vs 6.6 ± 2.9 ng/ml, p<0.001) and PA-inhibitor activity (2.8 ± 1.3 vs 1.5 ± 0.7 IU/ml, p<0.001) levels. Significant univariate correlations were observed between PA-inhibitor activity and age (r = 0.32, p<0.05), diastolic blood pressure (r = 0.42, p<0.01) and euglobulin fibrinolytic activity (r = -0.40, p<0.01). In multivariate analysis, only body mass index (positively) and euglobulin fibrinolytic activity (negatively) remained significantly related to PA-inhibitor activity in the total diabetic population as well as in the NIDDM group. The only parameter in the IDDM group significantly related to PA-inhibitor activity was diastolic blood pressure. These results suggest that PA-inhibitor plays a role in the regulation of fibrinolysis in diabetes patients and that factors like obesity and hypertension may be related to reduced fibrinolysis via PA-inhibitor levels. (Arteriosclerosis 8:68-72, January/February 1988)

Diabetes patients are predisposed to cardiovascular complications, which occur earlier and more frequently than in comparable nondiabetic patients. Abnormalities in both lipid metabolism and hemostasis contribute to the development of vascular damage.1 In diabetes patients, spontaneous fibrinolytic activity is reported to be normal or low,2-6 while release of plasminogen activator from the vascular endothelium is abnormal in some cases.5,6 Juhan-Vague et al.7 recently demonstrated that elevated tissue-type plasminogen activator (t-PA) antigen levels in poorly controlled diabetics could be decreased by insulin administration. These authors argued that a decrease of t-PA antigen in hyperinsulinemic, insulin-treated diabetics could hamper the fibrinolytic capacity and thereby favor the development of atherosclerosis.

In the present study we have evaluated t-PA antigen and plasminogen activator inhibitor (PA-inhibitor) activity by both univariate and multivariate analysis in a well-defined group of moderately controlled diabetic patients, including both insulin-dependent and noninsulin-dependent subjects.

Methods

Sixty-two diabetic patients (35 women and 27 men) ages 16 to 79 years (53 ± 16 years, mean ± SD) were studied. The mean duration of diabetes was 10.8 ± 8.8 years. Of these, 29 were insulin-dependent (IDDM) and 33, noninsulin-dependent (NIDDM). All patients were on special diets. Except for three patients with NIDDM, all patients were treated with subcutaneous insulin injections (mean daily dosage, 30 ± 21 units). None received oral antidiabetic agents.

Blood samples were obtained from fasting patients at the diabetic outpatient clinic. The blood for determination of fibrinolytic parameters was collected on 1/3 volume of 0.1 M trisodium citrate and was cooled on ice. The t-PA antigen was measured by a two-site immunoradiometric assay by using rabbit antiserum against purified t-PA obtained from melanoma cell culture fluid.9 PA-inhibitor activity was estimated by the technique of Juhan-Vague et al.10 Plasmin-α2-antiplasmin (PAP) was measured with an enzyme-linked immunosorbent assay as previously described.11 Euglobulin fibrinolytic activity was measured on fibrin plates and was expressed in ng/ml by comparison with purified t-PA.9 Fibrinogen was estimated by a clotting rate assay.12 Glycosylated hemoglobin (HbA1) was measured by cation-exchange chromatography by using commercially available microcolumns and reagents (Isolab, Akron, Ohio).

All results are expressed as means ± SD. Statistical analysis was performed by calculating the Pearson product-moment correlation coefficients and by the unpaired Student's t test. Simple linear regression analysis was used to evaluate the univariate relationships between PA-inhibitor activity and age, body mass index (BMI), systolic blood pressure (SBP), and diastolic blood pressure (DBP), respectively. Backward multiple regression analysis was
performed to evaluate the simultaneous independent effects of several factors on PA-inhibitor activity.

Results

Table 1 shows the means and standard deviations of various parameters in diabetic and normal populations. No significant differences between men and women were observed. As expected, NIDDM subjects were significantly older, more obese, and more hypertensive than IDDM subjects. The serum concentration of fibrinogen, t-PA antigen, and PA-inhibitor activity were greater in both NIDDM and IDDM patients, but the t-PA antigen and PA-inhibitor concentrations were higher in NIDDM than in IDDM patients.

Euglobulin fibrinolytic activity was, however, lower in NIDDM patients (p < 0.01) than in IDDM patients. Plasmin-α2-antiplasmin complexes were not significantly different from values in the control population.

Pearson product-moment correlation coefficients between the various parameters studied in the two diabetic subgroups separately as well as combined are presented in Table 2. HbA1c, as a parameter of diabetes control was not significantly correlated with any of the fibrinolytic parameters.

Euglobulin fibrinolytic activity was negatively correlated with both PA-inhibitor activity (r = -0.48, p < 0.001) and t-PA antigen (r = -0.40, p < 0.01), and also correlated with BMI (r = -0.26, p < 0.05) and DBP (r = -0.30, p < 0.05).

Age was positively correlated with t-PA antigen (r = 0.53, p < 0.001) and weakly with PA-inhibitor activity (r = 0.32, p < 0.05; Figure 1). The influence of age on t-PA antigen reflected the correlation between both parameters (r = 0.43, p < 0.05), whereas BMI correlated significantly with PA-inhibitor activity only in the IDDM group (r = 0.55, p < 0.01).

Systolic blood pressure correlated significantly with both t-PA antigen (r = 0.42, p < 0.01) and with PA-inhibitor activity (r = 0.29, p < 0.05; Figure 1), whereas only the latter fibrinolytic parameter correlated also with diastolic blood pressure (r = 0.42, p < 0.01). In Figure 1 we show the univariate correlations between PA-inhibitor activity and age, BMI, SBP, and DBP, respectively.

Table 3 gives the results of a multivariate analysis (stepdown multiple regression analysis) of PA-inhibitor activity with age, BMI, SBP, DBP, HbA1c, fibrinogen, PAP, fibrin plate assay, duration of diabetes, and insulin dose. The final regression model indicated that only BMI and euglobulin fibrinolytic activity yielded significant and independent contributions to the explained variation of PA-inhibitor activity (49% in the NIDDM group and 50% in the combined IDDM + NIDDM group). The only parameter significantly related to PA-inhibitor activity in the IDDM group was DBP. The relationships between blood pressure and PA-inhibitor activity disappeared in multivariate analysis in both the NIDDM and the total group.

Discussion

The group of diabetes patients consisting of both typical insulin- and noninsulin-dependent subjects showed a decrease in blood fibrinolytic activity that agrees with previous reports. The reduced plasma fibrinolytic activity showed a strong negative correlation with PA-inhibitor activity levels, which is indicative of the importance of this inhibitor in the regulation of fibrinolysis. The elevated level of PA-inhibitor activity causes inhibition of t-PA, resulting in a decreased fibrinolytic capacity.
creased t-PA antigen levels in the presence of reduced fibrinolytic activity can be explained on the basis of the occurrence of inactive t-PA-PA-inhibitor complexes in blood resulting in reduced free t-PA levels.17 The abnormal fibrinolytic activity was found in both types of diabetes despite their difference in etiology and associated characteristics (for instance, age, BMI, insulin dosage). The alterations of the concentration of t-PA antigen and PA-inhibitor activity seem to be independent of diabetes control in these patients, since none of these measured parameters were correlated with the hemoglobin A1c level. Geiger and Binder18 have recently reported an additional abnormality in the fibrinolytic system of diabetic patients secondary to metabolic dysregulation. In agreement with the recent results of Vague et al.,19 we found a positive correlation of BMI with PA-inhibitor activity and a negative correlation between BMI and euglobulin fibrinolytic activity. This observation further supports the extensively documented decreased fibrinolytic activity in obesity.6,19-23 Although statistically highly significant for the whole diabetes group, this correlation was mostly due to the strong correlation of BMI with PA-inhibitor activity in NIDDM subjects. PA-inhibitor activity and t-PA antigen concentrations are influenced by age and obesity, and the effect of these two parameters on the PA-inhibitor activity level may contribute to the genesis of vascular complications in NIDDM. Elevated BMI is often associated with NIDDM which, in turn, predisposes to insulin resistance and hyperinsulinism rather than to insulin deficiency.24,25 This hyperinsulinemia may predispose to vascular disease.20 Our finding of a correlation of PA-inhibitor activity and BMI therefore strengthens the hypothesis recently formulated by Vague et al.,19 which states that the relative hyperinsulinism in obese individuals, through its effects on PA-inhibitor activity, results in lower fibrinolysis and thus enhanced development of vascular disease. In insulin-treated IDDM patients, who are also in a state of "therapeutic" hyperinsulinemia, the same mechanism might be operative as suggested by the correlation of insulin dose and PA-inhibitor activity (r = 0.4, p<0.05). In contrast to Juhun et al.,7 we could not find a statistically significant negative correlation between insulin administration and t-PA blood levels. Possibly this discrepancy is due to the better diabetes control in our patients which might obscure such a correlation. In IDDM patients, a significant correlation was also found between the systolic and diastolic blood pressure and the PA-inhibitor activity and the euglobulin fibrinolytic activity. This confirms earlier reports of decreased fibrinolysis in hypertension.27,28

The causal relationship between diabetes itself and increased PA-inhibitor activity cannot be derived from our cross-sectional study, and multiple correlation analysis can only suggest possible etiologic factors. Nevertheless, since diabetics, and especially noninsulin-dependent patients, present several factors (like obesity, hypertension, hyperinsulinism, and old age) which are associated with a decreased fibrinolytic capacity, probably mediated by an
increased PA-inhibitor activity, these subjects may be predisposed to the development of thrombotic and vascular disease because of disturbed fibrinolysis. It might be useful to further investigate and interfere therapeutically in this interrelationship between PA-inhibitor activity, fibrinolytic capacity, and vascular complications since it might explain the clustering of nonlipid atheromatosis risk factors in diabetes.29, 30

Table 3. Multiple Regression Analysis of PA-inhibitor Activity

<table>
<thead>
<tr>
<th></th>
<th>Total group (n = 62)</th>
<th>IDDM (n = 29)</th>
<th>NIDDM (n = 33)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>b</td>
<td>r_p</td>
<td>b</td>
</tr>
<tr>
<td>Constant</td>
<td>-1.487</td>
<td></td>
<td>-2.806</td>
</tr>
<tr>
<td>BMI</td>
<td>0.186</td>
<td>0.59*</td>
<td>-2.806</td>
</tr>
<tr>
<td>Fibrin plate assay</td>
<td>-6.109</td>
<td>-0.42*</td>
<td>-6.109</td>
</tr>
<tr>
<td>DBP</td>
<td>-1.850</td>
<td>0.055</td>
<td>-1.850</td>
</tr>
<tr>
<td>R²</td>
<td>0.50</td>
<td>0.35</td>
<td></td>
</tr>
</tbody>
</table>

The independent variables included in the regression model were: age, BMI, SBP, DBP, duration of diabetes, insulin dose, HbA1c, PAP, fibrinogen, and fibrin plate assay.

b = partial regression coefficient; r_p = partial correlation coefficient; R² = coefficient of determination (percent variance explained by model). See legend in Table 1 for abbreviations.

*p < 0.001; †p < 0.01 for significance of regression slopes.

References


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Index Terms: diabetes mellitus • fibrinolysis • plasminogen activator • plasminogen activator inhibitor • atherosclerosis
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