Impaired Fibrinolysis in Hypertension and Obesity Due to High Plasminogen Activator Inhibitor-1 Level in Plasma

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Abstract In order to elucidate the influence of the risk factors of coronary heart disease on the fibrinolytic activity, relationships between blood pressure, body mass index (BMI), plasma lipoprotein (a) (Lp(a)) level and the plasma levels of tissue plasminogen activator (tPA) and plasminogen activator inhibitor-1 (PAI-1) were analyzed in the subjects with mild hypertension. Systolic blood pressure showed a positive correlation with total PAI-1 and free PAI-1. Diastolic blood pressure showed no correlation with these proteins involved in the fibrinolytic system. BMI had a positive correlation with total PAI-1, free PAI-1 and euglobulin clot lysis time (ECLT). Plasma Lp(a) level showed correlation with neither blood pressure nor fibrinolytic parameters, but it showed weak negative correlation with body mass index (BMI). These results suggest that high blood pressure and obesity tend to increase free PAI-1 which reduces fibrinolytic activity. Lp(a), however, seems not to influence directly the fibrinolytic system but may work to decrease fibrinolytic activity only in conjunction with other risk factors. The effects of daily drinking of alcohol and smoking on the fibrinolytic system were also investigated in the present study and we obtained the results that habitual drinking increased plasma levels of both tPA and PAI-1 whereas smoking did not affect fibrinolytic activity. These results suggest that risk factors for coronary heart disease such as hypertension and obesity are closely related to the impaired fibrinolysis.

Key words: hypertension, fibrinolysis, PAI-1, Lp(a).

Received on October 1, 1992; Accepted on February 18, 1993

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It is established that atherosclerosis and thrombus formation are the primary factors to develop the occlusive vascular disease [1, 2] and that impaired fibrinolysis is strongly related to these factors. Plasma levels of tissue plasminogen activator (tPA) and its fast-acting inhibitor, plasminogen activator inhibitor 1 (PAI-1), are considered to be important in controlling the fibrinolytic activity, since total fibrinolytic activity is fine-tuned by the balance of these proteins [3, 4]. The physiological relevance of PAI-1 is proved by a bleeding tendency in its congenitally deficient patient [5], and by a thrombotic tendency of transgenic mice which have higher expression of PAI-1 [6]. An intimate correlation between plasma PAI-1 levels and the occurrence of coronary heart disease is also reported [7]. The control of the production and the release of these proteins, therefore, seem to be important in maintaining the normal fibrinolytic activity. Recently much attention has been paid to the analysis of the influence of the risk factors for coronary heart disease on the plasma concentrations of tPA and PAI-1 [8–11]. Among them body mass index (BMI) and plasma levels of lipids including triglyceride and low density lipoprotein (LDL) are directly related to impaired fibrinolysis by increasing plasma concentration of PAI-1 [8, 10, 11]. Hypertension is considered to be another important risk factor which initiates atherosclerosis and thrombogenic state. In the present study we therefore focused our studies on the factors which are closely related to the blood pressure and analyzed their contributions to the expression of the fibrinolytic activity.

MATERIALS AND METHODS

Among 2,388 male employees who were screened for hypertension at the periodical medical checkup in a factory, 96 (34.3 ± 8.0, range 20–55 years old) who showed mild hypertension (either systolic pressure was higher than 140 mmHg or diastolic pressure was higher than 90 mmHg) were subject to this study. All the subjects were recalled at 9 o’clock a.m. on a day about 1 month after the first screening test and body weight, body height and blood pressure were measured, followed by the blood collection. Persons who had taken any kind of drugs at least 3 d before this study were excluded. Persons who had a history of cardiovascular disease or had previously been treated for hypertension or diabetes mellitus were also excluded. Nineteen out of the 96 were excluded because of the above reasons; the remaining 77 subjects were analyzed in this study. All the subjects were informed about the purpose of the study, and gave their consent.

Blood pressure measurements. Blood pressure was measured in a sitting position twice after a minimum resting period of 5 min in the morning (from 9:00 to 10:00), and the lower value of the two measurements was taken as blood pressure.

Blood sampling. After the measurement of blood pressure, blood samples were withdrawn from the antecubital vein with a minimum venous stasis to siliconized evacuated tubes containing 1/10 volume of 3.8% sodium citrate. After
centrifugation at about 2,000 × g at 4°C for 20 min, platelet-poor plasma was obtained. Plasma samples were stored at -70°C until analyses.

*Plasma euglobulin fraction.* The plasma euglobulin fraction was obtained by 20 times dilution of citrated plasma and acidification at pH 5.2. After leaving for 1 h at 4°C followed by centrifugation (approximately 2,000 × g) for 10 min at 4°C, the precipitate was dissolved in 0.5 ml of 0.1 M Tris HCl buffer pH 7.4. All the samples were used for ECLT assay immediately after the preparation.

*ECLT assay.* The clot lysis of the euglobulin was measured using an automatic microtiter plate reader as described before [12, 13].

*tPA.* tPA antigen in plasma was determined by enzyme-immuno assay (EIA) [14], which measures both free tPA and tPA–PAI-1 complex.

*Total PAI-1, free PAI-1 and tPA–PAI-1 complex.* tPA–PAI-1 complex and total PAI-1 in plasma were measured by EIA [13, 15]. The concentration of free PAI-1 was calculated by subtracting the concentration of tPA–PAI-1 complex from that of total PAI-1 [13, 15].

*Lipoprotein (a) (Lp(a)).* Lp(a) was assayed by ELISA kit (Terumo Medical Co., MD, USA).

*Statistical analysis.* Goodness-of-fit X² was used to test whether individual data had normal distribution or not. In parameters not normally distributed, logarithmic transformation was performed: In such cases, the mean value, the 10th and the 90th percentile were calculated on the logarithmic values and then the anti-log was calculated. Unpaired Student’s t-test was used to compare values of different groups.

**RESULTS**

*Blood pressure and fibrinolytic parameters*

The precise measurement of the blood pressure (two times measurements after 5 min rest) revealed that 19 out of the 77 subjects showed high systolic blood pressure (>140 mmHg) and that 35 subjects showed high diastolic pressure (>90 mmHg). Eleven subjects showed both systolic and diastolic high blood pressure. Plasma levels of fibrinolytic parameters of the 77 subjects are summarized in Table 1.

Systolic pressure showed a weak positive correlation with total PAI-1 level ($r=0.238, p=0.037$) and free PAI-1 level ($r=0.253, p=0.027$), whereas it did not show any significant correlation with ECLT or the levels of tPA and tPA–PAI-1 complex (Table 2). Systolic pressure did not show a significant correlation with age and BMI. Diastolic pressure showed a positive correlation with tPA ($r=0.224, p=0.050$). However, when we avoid the influence of age which had positive correlation with both diastolic pressure ($r=0.360, p=0.001$) and tPA ($r=0.372, p=0.001$), calculated partial correlation coefficient between these parameters ($r=0.104$) was not significant.
Table 1. Distribution of the fibrinolytic parameters in the subjects.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>ECLT (h)</td>
<td>7.9</td>
<td>±2.0*</td>
<td></td>
</tr>
<tr>
<td>tPA (ng/ml)</td>
<td>9.1</td>
<td>(4.9 to 15.4)</td>
<td></td>
</tr>
<tr>
<td>Total PAI-1 (ng/ml)</td>
<td>13.3</td>
<td>(6.3 to 28.5)</td>
<td></td>
</tr>
<tr>
<td>tPA-PAI-1 (ng/ml)</td>
<td>4.3</td>
<td>(2.2 to 8.1)</td>
<td></td>
</tr>
<tr>
<td>Free PAI-1 (ng/ml)</td>
<td>8.3</td>
<td>(2.6 to 22.4)</td>
<td></td>
</tr>
<tr>
<td>Lp(a) (mg/dl)</td>
<td>9.1</td>
<td>(4.4 to 30.9)</td>
<td></td>
</tr>
</tbody>
</table>

*Mean±SD (the 10th to the 90th percentile). †The concentration is expressed by the order of ng/ml of PAI-1.

Table 2. Correlations between blood pressure, BMI, age and the plasma levels of fibrinolytic parameters.

<table>
<thead>
<tr>
<th></th>
<th>ECLT</th>
<th>Total PAI-1</th>
<th>t-PA-PAI-1 complex</th>
<th>Free PAI-1</th>
<th>tPA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic BP</td>
<td>NS</td>
<td>0.238*</td>
<td>NS</td>
<td>0.253*</td>
<td>NS</td>
</tr>
<tr>
<td>Diastolic BP</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>0.224*†</td>
</tr>
<tr>
<td>BMI</td>
<td>0.306**</td>
<td>0.392***</td>
<td>0.314***</td>
<td>0.351****</td>
<td>0.444***</td>
</tr>
<tr>
<td>Age</td>
<td>NS</td>
<td>NS</td>
<td>0.381***</td>
<td>NS</td>
<td>0.372***</td>
</tr>
</tbody>
</table>

*p < 0.05, **p < 0.01, ***p < 0.001. NS, not significant. †Significance disappeared when the influences of age on tPA and diastolic pressure were avoided.

BMI and fibrinolytic parameters

BMI showed a relatively strong correlation with tPA (r = -0.444, p < 0.001) and total PAI-1 (r = -0.392, p < 0.001). It also showed a positive correlation with free PAI-1 level (r = 0.351, p < 0.005) and ECLT (r = 0.306, p = 0.009).

Lp(a)

Lp(a) is a risk factor for coronary heart disease [16] and a competitive inhibitor for Glu-plasminogen to bind to fibrin [17] due to its high homology in their structure [18]. We analyzed the contribution of Lp(a) to blood pressure and fibrinolysis. Since values of Lp(a) were not normally distributed, log(Lp(a) - 3) was used for statistical analysis. Lp(a) was negatively correlated to BMI (r = -0.347, p = 0.020). Weak negative correlations of Lp(a) with ECLT (r = -0.258, p = 0.030) and free PAI-1 (r = -0.227, p = 0.047) were observed but the correlations were not significant after the calculation of the partial correlation coefficient in order to avoid the influence of BMI.

Effect of daily alcohol drinking and smoking on fibrinolytic activity

There was no significant difference between daily drinkers (45 subjects, who drink more than 350 ml of beer every day) and non-drinkers (32 subjects) in age,
BMI and blood pressure. In daily drinkers, both tPA level and total PAI-1 level were higher than those in non-drinkers (Fig. 1). The level of tPA–PAI-1 complex were also higher in daily drinkers but there was not significant difference between these two groups in ECLT and free PAI-1 level. Lp(a) level was also higher in daily drinkers (mean 10.6, the 10th–90th percentile 5.5–31.1 mg/dl) than that of non-drinkers (mean 7.6, the 10th–90th percentile 1.2–26.6 mg/dl) ($p = 0.0369$).

There was no significant difference between smokers (36 subjects) and non-smokers (41 subjects) in the parameters analyzed in the present study.

**DISCUSSION**

In this study we tried to elucidate the influence of blood pressure on the fibrinolytic system. Systolic pressure showed a significant positive correlation with the concentrations of both total PAI-1 and free PAI-1. The result confirms previous reports which showed that systolic pressure had a positive correlation with either PAI antigen level [11] or PAI activity [10] in patients with mild hypertension or angina pectoris, respectively. Since free PAI-1 mainly controls the overall fibrinolytic activity [13], high systolic blood pressure tends to decrease fibrinolytic activity. The present study may suggest that not only severe hypertension but even mild hypertension decreases fibrinolytic activity and then develops into atheroscle-
rosis. The proper treatment of hypertension even in mild state, therefore, may be important in decreasing the risk of atherosclerosis. ECLT, which shows total fibrinolytic activity, however, did not show a meaningful correlation with blood pressure. The influences of many other factors to regulate concentrations of PAI-1 and tPA in plasma (see Ref. [19]), therefore, must be involved in the control of systemic fibrinolytic activity. Nevertheless, increase in plasma PAI-1 level accompanied with increase in systolic blood pressure may play an important role for fibrin deposition on endothelial cells and the development of atherosclerosis in vivo together with other risk factors.

BMI and the plasma level of triglyceride are reported to have a close correlation with plasma PAI-1 concentration [10,11]. We also showed a positive correlation of BMI not only with total PAI-1 but also with tPA–PAI-1 complex and free PAI-1 in plasma. Higher levels of free PAI-1 in subjects with higher BMI suggest that the balance of tPA and PAI-1 is directed to the thrombogenic condition, as suggested by their longer ECLT. Therefore obesity could be a risk factor for thrombotic disease. Insulin resistance is reported to be deeply related to impaired fibrinolysis by increasing plasma PAI-1 [20] and to hypertension [21]. Attempts, therefore, to decrease insulin resistance such as weight control must be recommended from a fibrinolytic point of view.

Lp(a) is another lipoprotein which may play an important role in fibrinolysis because of its structural homology to plasminogen molecule [18]. It seems to behave as a competitive inhibitor for plasminogen to bind to fibrin [17] and to inhibit fibrinolysis [22]. We assayed ECLT to clarify the possible role of Lp(a) in clot lysis. ECLT, however, did not show a correlation with Lp(a) level although large amounts of Lp(a) were contained in the euglobulin fraction (78.6±15.9% of plasma concentration, n = 4). The significant contribution of Lp(a) to clot lysis, therefore, may be questionable. Another possible role for Lp(a) to enhance the synthesis of PAI-1 in cultured endothelial cells and to regulate the concentration of PAI-1 [23] was also questionable because of the fact that Lp(a) did not have any relationship with fibrinolytic parameters. These results support the hypothesis that high levels of Lp(a) may bind to fibrin, leading to the deposit of lipid in the vessel walls, thus being a potential marker for coronary heart disease and its risk may be enhanced with the association of other risk factors like hyperlipidemia or high blood pressure [24].

Daily drinking of alcohol and smoking are considered to be risk factors for coronary heart disease. We analyzed the effects of these factors on fibrinolysis. Daily drinking of alcohol increased plasma levels of both tPA and PAI-1 mainly as a form of tPA–PAI-1 complex. Free PAI-1 level and total fibrinolytic activity, however, was not significantly influenced by alcohol drinking. Recently, we showed that alcohol drinking immediately increased plasma levels of PAI-1 [25]. In the present study we show that chronic daily drinking also influenced PAI-1 level.

The present results suggest that most of risk factors for coronary heart disease such as hypertension and obesity are closely related to impaired fibrinolysis mainly
by increasing plasma PAI-1. However, Lp(a), a risk factor to develop coronary heart disease [16] by inhibiting fibrinolysis, did not affect fibrinolysis in the present study. The collaboration of these risk factors together with other risk factors may strongly depress fibrinolytic activity and then develop into atherosclerosis.

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