Effects of Angiotensin Converting Enzyme and Angiotensin II Receptor Inhibition on Impaired Fibrinolysis in Systemic Hypertension
Yunus Erdem, Celalettin Usalan, İbrahim C. Haznedaroğlu, Bülent Altun, Mustafa Arici, Ünal Yasavul, Çetin Turgan, and Sali Çağlar

Abnormalities in fibrinolysis have been reported in hypertension. Angiotensin converting enzyme (ACE) inhibitors have been shown to improve altered fibrinolytic balance in hypertensive patients. It has not been documented, however, whether this is due to a decrease in angiotensin II (Ang-II) generation or is a consequence of elevated local levels of bradykinin. Accordingly, the aim of this study was to determine the effects of an ACE inhibitor (perindopril) and an Ang-II receptor antagonist (losartan) on fibrinolytic kinetics.

We have examined the serum levels of the plasminogen activator inhibitor type-1 (PAI-1) antigen and activity, tissue plasminogen activator (t-PA) antigen and activity, soluble thrombomodulin (sTM), and tissue factor pathway inhibitor (TFPI) before and after reaching the target blood pressure (<140/90 mm Hg) in 13 hypertensive patients receiving perindopril (mean age 40 ± 11 years, 6 women, 7 men) and in 12 patients receiving losartan (mean age 38 ± 9 years, 6 women, 6 men). We also compared the baseline fibrinolytic activity of hypertensive patients with that of 12 normotensive control persons (mean age 40 ± 9 years, 6 women, 6 men). The mean basal plasma levels of PAI-1 antigen, PAI-1 activity, and sTM were significantly higher in the hypertensive patients than in normal controls (P < .005). The values of other analytes were similar in both groups. Increased plasma levels of PAI-1 antigen, PAI-1 activity, and sTM were reduced in patients after they were given perindopril and losartan (P < .005); the reductions in losartan-receiving group were more pronounced (P < .05). There were no significant effects on the plasma levels of t-PA antigen, t-PA activity, and TFPI in patients receiving the two therapeutic regimens (P > .05).

In conclusion, chronic hypertension is associated with hypofibrinolysis. The beneficial effect of ACE inhibitors on fibrinolysis seems to be related to the blockade of Ang-II, and increased kinin activity does not appear to play a major role. Am J Hypertens 1999;11:1071–1076 © 1999 American Journal of Hypertension, Ltd.

KEY WORDS: Hypertension, fibrinolysis, angiotensin converting enzyme inhibitor, angiotensin II receptor antagonist, angiotensin II, bradykinin.

Cardiovascular and cerebrovascular complications are the major cause of morbidity and mortality in systemic arterial hypertension.1 The increased risk of these complications due to arterial hypertension depends largely not only on blood pressure (BP), but may be also related to other factors that accelerate atherosclerosis.1 Previous studies suggest that thrombosis of cerebral and coronary arteries play an important role in the pathogenesis of stroke and myocardial infarction.2,3 Impaired fibrinolysis is also an established risk factor for these thrombotic events.3

Fibrinolytic activity is primarily determined by the delicate balance between the levels of tissue plasmin-
ogen activator (t-PA) and plasminogen activator inhibitor type-1 (PAI-1), both of which are synthesized in the vascular endothelium. Vascular endothelial injury, therefore, results in deviations from normal fibrinolytic kinetics. On the other hand, newly recognized vascular endothelial proteins such as thrombomodulin (TM) and tissue factor pathway inhibitor (TFPI) may also play significant roles in the regulation of fibrinolysis and coagulation. Several recent reports have shown that imbalance in these endothelial proteins associated with various vascular disease cause susceptibility to thrombotic disease.\(^4\)\(^5\)

A possible link between the renin angiotensin system (RAS) and fibrinolysis in human beings has recently been suggested.\(^6\) The systemic infusion of angiotensin II (Ang-II) results in a rapid and significant dose-dependent increase in plasma PAI-1 levels in normotensive and hypertensive persons.\(^7\) Moreover, angiotensin converting enzyme (ACE) is responsible for the degradation of bradykinin, the most potent stimulus for t-PA secretion.\(^8\) On the other hand, recent data indicate that the use of ACE inhibitors results in reduced rates of coronary thrombosis and recurrent myocardial infarction in patients with left ventricular dysfunction.\(^9\)\(^10\) However, the mechanism of this benefit cannot be completely explained by the antihypertensive and antithrombotic effects of ACE inhibitors.\(^9\)\(^10\) It may be related to the known property of Ang-II to increase PAI-1 levels; conceivably, ACE inhibitors would depress PAI-1 production. Alternatively, ACE inhibitors increase kinin concentration via inhibition of bradykinin degradation, which may lead to an increase in t-PA production. Both pathways may improve fibrinolytic balance and be accompanied by reduced coronary artery thrombosis. However, the relative roles of the two mechanisms on the fibrinolytic activity conferred by ACE inhibitors has not yet been established in a clinical study.

Accordingly, we designed this study to evaluate the fibrinolytic kinetics in chronic systemic hypertension. To determine the exact mechanism of the beneficial effect of ACE inhibitors on fibrinolysis, we examined the effects of an ACE inhibitor (perindopril) and an Ang-II receptor antagonist (losartan) on plasma levels of fibrinolytic activity profiles in hypertensive patients. We aimed to indirectly evaluate the relative roles of the Ang-II and the kinin system in this beneficial effect.

**MATERIALS AND METHODS**

**Patients** Thirty-four outpatients (17 men and 17 women) referred to our hypertension unit participated in this study. Each patient had a well-established history of chronically elevated BP without any underlying cause and had been treated for at least 3 years with one or more antihypertensive agents. Inclusion criteria consisted of the following: (1) adults aged 18 to 65 years; and (2) the presence of mild to moderate essential hypertension with an average sitting systolic BP (SBP) between 160 and 210 mm Hg, a sitting diastolic BP (DBP) between 95 and 115 mm Hg, or both, on three consecutive visits. Exclusion criteria consisted of the presence of the following: (1) secondary hypertension including renovascular hypertension; (2) severe hypertension defined by sitting SBP >210 mm Hg and sitting DBP >115 mm Hg at any time during the run-in period; (3) evidence of clinical cardiovascular disease (including stroke or myocardial infarction in the previous 6 months, angina, clinically significant arrhythmia or heart block, or congestive heart failure), or renal failure (serum creatinine > 1.5 mg/dL); (4) life-threatening medical conditions (cancer, and so forth); (5) hyperlipidemia and diabetes; (6) a history of smoking; (7) a history of alcoholism or other drug abuse; or (8) the use of sedatives, tranquilizers, or oral contraceptives.

After a 4-week washout period, patients who still met the inclusion criteria were randomized to receive active treatment with either 2 mg perindopril (15 patients) or 50 mg losartan (15 patients). During the study period, all patients continued on their usual diet and activities. Drugs were administered in the morning at approximately 9 AM and after breakfast. These patients were followed at 4-week intervals for 6 months at the outpatient clinic. Dosage of medication was increased gradually to 8 mg/day in the perindopril group and to 100 mg/day in the losartan group, when necessary.

An additional 12 normotensive healthy volunteers (6 men, 6 women) matched with the patients for approximate age, was selected as a control group. Control persons had no evidence of present or past hypertension, cardiovascular disease, hyperlipidemia, or any other systemic condition and they were taking no medications. The normotensive subjects had a SBP < 130 mm Hg and a DBP <85 mm Hg. Table 1 shows the characteristics of the study subjects.

**Study Protocol** All patients gave informed consent to participation according to the ethical principles for human investigations as outlined in the 2nd Declaration of Helsinki. All patients were evaluated in an outpatient setting. Medical history and physical examination were recorded for each patient, and each underwent tests for routine biochemical and hematological monitoring. BP was measured with a mercury sphygmomanometer and cuffs were adapted to the arm circumference after 15 min in the recumbent position. SBP was taken as the appearance of Korotkoff sounds and DBP as the point of their disappearance (phase V). Secondary causes of hypertension, metabolic abnormalities, and evidence of damage to end organs were sought by studying each patient’s history;
TABLE 1. CLINICAL CHARACTERISTICS OF THE HYPERTENSIVE PATIENTS AND CONTROLS

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control Group (n = 12)</th>
<th>Perindopril Group (n = 13)</th>
<th>Losartan Group (n = 12)</th>
<th>All Patients (n = 25)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age (years)</td>
<td>40 ± 9</td>
<td>40 ± 11</td>
<td>38 ± 9</td>
<td>39 ± 9</td>
</tr>
<tr>
<td>Gender (women/men)</td>
<td>6/6</td>
<td>6/7</td>
<td>6/6</td>
<td>13/12</td>
</tr>
<tr>
<td>SBP (mm Hg)</td>
<td>126 ± 1.2</td>
<td>170 ± 3.7</td>
<td>165 ± 3.9</td>
<td>168 ± 3.7*</td>
</tr>
<tr>
<td>DBP (mm Hg)</td>
<td>84 ± 0.8</td>
<td>100 ± 0.8</td>
<td>101 ± 1.2</td>
<td>101 ± 1.1*</td>
</tr>
<tr>
<td>Fasting plasma cholesterol (mg/dL)</td>
<td>182 ± 6</td>
<td>186 ± 8</td>
<td>183 ± 8</td>
<td>185 ± 7</td>
</tr>
<tr>
<td>Fasting plasma glucose (mg/dL)</td>
<td>84 ± 4</td>
<td>87 ± 5</td>
<td>88 ± 5</td>
<td>87 ± 6</td>
</tr>
<tr>
<td>Plasma creatinine (mg/dL)</td>
<td>0.92 ± 0.16</td>
<td>0.96 ± 0.23</td>
<td>0.93 ± 0.25</td>
<td>0.95 ± 0.25</td>
</tr>
</tbody>
</table>

Values are means ± SEM.

*P < .05 hypertensive patients vs controls.

performing a physical examination; and conducting echocardiography, renal imaging, and laboratory tests that included plasma renin activity (PRA), urinalysis, creatinine and electrolyte levels in plasma, and, when clinically indicated, vanilmandelic acid excretion in urine. A history was obtained for all healthy control subjects; all received a physical examination and underwent a urinalysis test. Blood samples for PAI-1 antigen and activity, t-PA antigen and activity, TFPI level, and soluble thrombomodulin (sTM) level were obtained at baseline in all patients. After the SBP had remained below 140 mm Hg and DBP had remained below 90 mm Hg for 12 weeks, we obtained serum (patient fasting) for these analytes. Hypertensive patients did not discontinue oral antihypertensive medication.

**Blood Samples** The study was a cross-sectional determination of t-PA antigen levels, t-PA activities, PAI-1 antigen levels, PAI-1 activities, TFPI levels, and TM levels in plasma samples taken from hypertensive patients and healthy volunteer controls. All samples were taken from patients in the fasting state and during the morning hours to avoid the effect of diurnal variation of the hemostatic system. After a 30-min rest in the sitting position, blood samples were drawn from the large antecubital veins of patients. All venipunctures were carried out without interruption of venous flow and with a 19-G butterfly needle connected to a plastic syringe. Fifteen milliliters of blood were drawn and the first few milliliters were discarded; 4.5 mL was transferred immediately to Stabilyte tubes (Biopool, Sweden) for determination of t-PA and PAI-1 activity. Nine milliliters were transferred to polypropylene tubes containing 1 mL trisodium citrate (0.109 mol/L) to determine the levels of other analytes. The tubes were then centrifuged at 3000 rpm for 15 min at 10 to 18°C. The supernatant plasma samples were stored in plastic tubes at −30°C until assayed.

**Assays** PRA was estimated radioimmunologically (Radioimmunoassay, Sorin, Rencz, CisBio Int., France), the reference (supine) range with normal salt intake being 0.2 to 5.7 ng/mL/h. PAI-1 and t-PA antigen levels were quantified with ELISA Tintelize kit (Biopool, Sweden). PAI-1 and t-PA activities were measured by Chromogenic Assay (Biopool, Sweden). The intra- and interassay coefficients of variation ranged from 5.2% to 8.7%, and from 6.5% to 9.4%, respectively. Plasma TFPI levels were measured with the IMUBIND TFPI ELISA kit (American Diagnostica Inc., Greenwich, CT) that uses a murine anti-TFPI monoclonal antibody as the capture antibody. The results were expressed as ng/mL. The mean and median TFPI values, as well as the interquartile ratios, were determined for each group. According to American Diagnostica Inc., the reference range for plasma TFPI concentration was 75 to 120 ng/mL; this was verified by measuring the TFPI concentration in the plasmas of 40 healthy persons and excluding the heparin-associated fraction. Plasma TM levels were determined with a two-site ELISA with two monoclonal antihuman TM antibodies (ELISA, Asserachrom Thrombomodulin, Diagnostica Stago, France).

**Statistical Analysis** The differences in baseline t-PA antigen and activity, PAI-1 antigen and activity, TFPI level, and TM level between hypertensive patients and healthy volunteers were evaluated by applying the nonparametric Mann-Whitney U-Wilcoxon rank sum W test. Changes in the levels of these analytes before and after control of hypertension (<140/90) was achieved by both the perindopril group and losartan group were assessed by Wilcoxon matched-pairs signed-rank test. The differences between pretreatment and posttreatment values for all analytes in the perindopril and losartan groups were also compared by applying the Mann-Whitney U-Wilcoxon rank sum W test. The data were analyzed with the SPSS (V6.0) for Windows (SPSS Inc.) and expressed as mean ± SD. P < .05 was considered significant.

**RESULTS** The values of the demographic and clinical variables were comparable in the perindopril and losartan groups.
No significant differences between two groups were observed in the levels of routine biochemical and hematological analytes before and after the antihypertensive treatment (Table 1).

In spite of a maximal antihypertensive dose at the beginning of the twelfth week of the study, the mean SBP remained >160 mm Hg or DBP >95 mm Hg (or both) in 2 of the 15 patients in the perindopril group and 3 of the 15 patients in the losartan group. These subjects were excluded from this study. Both perindopril and losartan administration resulted in normalization of BP in all other patients within the first 3 months of therapy. Indeed, the mean BP in the sitting position fell from 170 ± 3.7/100 ± 0.8 to 137 ± 4.0/85 ± 1.1 in patients given perindopril and from 165 ± 3.9/101 ± 1.2 to 139 ± 3.3/86 ± 1.2 in those given losartan. Both drugs were effective in lowering BP and their antihypertensive effects were not different.

The mean basal plasma levels of PAI-1 antigen, PAI-1 activity, and sTM of hypertensive patients were higher than those of normotensive healthy volunteers ($P < .005$) (Table 2, Figure 1). The mean basal plasma levels of t-PA antigen, t-PA activity, and TFPI were similar in the hypertensive and control subjects ($P > .05$) (Table 2). There were also no significant differences in the values of all these analytes between the perindopril and losartan groups at the baseline state ($P > .05$). The mean basal PRA were similar in the perindopril and losartan group. After antihypertensive therapy, the mean PRA increased significantly in both groups ($P < .005$), and the increments did not differ statistically in the two groups ($P > .05$) (Table 3).

Increased mean plasma levels of PAI-1 antigen, PAI-1 activity, and sTM were reduced in both treatment groups ($P < .005$). However, the reductions in the losartan group were more pronounced ($P < .05$) (Table 3, Figure 2). There were no significant effects on the plasma levels of t-PA antigen, t-PA activity and TFPI by the two therapeutic regimens ($P > .05$) (Table 3).

**Table 2. Fibrinolytic System Activity Profiles of Hypertensive Patients and Controls**

<table>
<thead>
<tr>
<th>Analyte and Level</th>
<th>Hypertensive Patients (n = 25)</th>
<th>Control Group (n = 12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAI-1 antigen (IU/mL)</td>
<td>$134.48 ± 136.56^\ast$</td>
<td>$37.92 ± 9.91$</td>
</tr>
<tr>
<td>PAI-1 activity (IU/mL)</td>
<td>$14.54 ± 6.96^\ast$</td>
<td>$8.28 ± 1.81$</td>
</tr>
<tr>
<td>t-PA antigen (ng/mL)</td>
<td>$4.94 ± 1.38$</td>
<td>$4.74 ± 1.48$</td>
</tr>
<tr>
<td>t-PA activity (ng/mL)</td>
<td>$0.63 ± 0.34$</td>
<td>$0.69 ± 0.38$</td>
</tr>
<tr>
<td>TFPI (ng/mL)</td>
<td>$126.76 ± 64.53$</td>
<td>$128.00 ± 46.63$</td>
</tr>
<tr>
<td>sTM (ng/mL)</td>
<td>$61.52 ± 32.64^\ast$</td>
<td>$37.18 ± 6.18$</td>
</tr>
</tbody>
</table>

*Values are means ± SEM.*

* $P < .005$ hypertensive patients vs control.

It is now clear that thrombosis is the result of a progressive alteration of the vascular system. Disruption of normal, nontrombogenic vascular endothelial results in the synthesis and release of procoagulant and anticoagulant substances. Increased plasma levels of substances associated with vascular endothelial injury may reflect preclinical thrombosis. TM expressed on endothelial cells binds thrombin and initiates anticoagulant pathway activity. Therefore, TM plays a crucial role in the regulation of blood coagulation and fibrinolysis. Soluble functional proteolytic fragments of TM (sTM) are also present in circulating plasma. The plasma level of sTM, considered to be a marker of vascular endothelial injury, is increased in various vascular disorders. This study shows that plasma levels of sTM were significantly increased in hypertensive patients compared to normotensive controls. This finding may indicate increased endothelial damage in the vascular bed in chronic hypertension. On the other hand, it was recently reported that sTM accelerated the activation of thrombin-activated fibrinolysis inhibitor in plasma and may inhibit fibrinolysis via this way. Increased plasma levels of sTM therefore may reflect impaired fibrinolysis and susceptibility to thrombosis. That increased PAI-1 activity is a marker of impaired fibrinolysis was also shown in this study. Increased levels of both of these endothelial substances verified that chronic hypertension is associated with impaired fibrinolysis and the prothrombotic state.

Several recent reports have shown that PAI-1 activity is elevated and t-PA activity is depressed in patients with systemic hypertension. Our findings are consistent with those of previous studies; our results demonstrate that patients with hypertension are hypofibrinolytic (ie, the functional levels of PAI-1 antigen and activity are significantly increased in hypertensive patients compared to normotensive persons). Various pathophysiological factors including insulin resistance with hyperin-
sulinemia, atherosclerosis, disorders of glucose and lipid metabolism, and endothelial cell injury are associated with impaired fibrinolysis in hypertension. Most previous studies evaluating abnormal fibrinolysis in hypertension have focused on disorders of glucose and lipid metabolism, and a close relationship has been demonstrated between these conditions and alterations of fibrinolysis. Furthermore, it has recently been reported that hypertension itself may be associated with impaired fibrinolysis. This observation was confirmed in our study, which showed that a group of hypertensive patients without glucose intolerance or dyslipidemia still had higher PAI-1 activity, reflecting impaired fibrinolysis. These findings may be ascribed to endothelial cell injury associated with chronic hypertension. In contrast to the results of previous studies, plasma levels of t-PA antigen and activity in hypertensive patients did not differ from those of normotensive subjects in our study.

The second aspect of our study was that blockade of the RAS influenced the altered fibrinolytic kinetics in systemic hypertension, because both perindopril and losartan attenuated the increase in PAI-1 and sTM levels. That blockade of Ang-II action with such drugs resulted in a reduction of the increased plasma levels of PAI-1 and sTM suggested that Ang-II may contribute to the release of these substances from injured endothelium. Several studies indicated that the RAS plays a role in the regulation of fibrinolytic balance, and this relationship may contribute to an “antithrombotic” effect of ACE inhibitors. The systemic infusion of Ang-II has been reported to increase circulating levels of PAI-1 in experimental and clinical studies. In addition, clinical studies have shown that ACE inhibition reduces the rate of recurrent myocardial infarction in patients with left ventricular dysfunction. Although these studies suggest that Ang-II may inhibit fibrinolysis in the vasculature, the exact role of RAS in fibrinolysis as well as on PAI-1 levels has not been reported in a clinical study.

ACE inhibitors may improve fibrinolytic system activity. It has not been documented, however, whether this is due to a decrease in Ang-II generation (which increases PAI-1 levels) or to the ability of ACE inhibitors to regulate bradykinin (which can increase the release and production of t-PA). In this study, chronic administration of ACE inhibitor (perindopril) and Ang-II receptor antagonist (losartan) resulted in lowering of PAI-1 and sTM levels. The reductions in the losartan group were more pronounced. Moreover, there was not any difference in t-PA activity before and after treatment in these two groups. These data may indicate that the decrease in Ang-II generation is the major mechanism accounting for the beneficial effect of ACE inhibitors on fibrinolysis, and this seems to be independent of bradykinin. However, the beneficial effects of ACE inhibitors and Ang-II receptor antagonists on fibrinolysis may also be related to the BP-lowering property of these drugs. It therefore appears reasonable to compare the fibrinolytic effects of ACE inhibitors or Ang-II receptor antagonists with

### Table 3. Fibrinolytic System Activity Profiles of Hypertensive Patients Before and After Administration of Perindopril and Losartan

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Perindopril Group (n = 13)</th>
<th>Losartan Group (n = 12)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pretreatment</td>
<td>Posttreatment</td>
</tr>
<tr>
<td>PAI-I antigen (IU/mL)</td>
<td>141.54 ± 160.53</td>
<td>112.23 ± 108.08*</td>
</tr>
<tr>
<td>PAI-I activity (IU/mL)</td>
<td>13.72 ± 6.80</td>
<td>8.85 ± 6.22*</td>
</tr>
<tr>
<td>t-PA antigen (ng/mL)</td>
<td>5.18 ± 1.32</td>
<td>5.09 ± 1.14</td>
</tr>
<tr>
<td>t-PA activity (ng/mL)</td>
<td>0.57 ± 0.41</td>
<td>0.50 ± 0.36</td>
</tr>
<tr>
<td>TFPI (ng/mL)</td>
<td>109.00 ± 50.51</td>
<td>107.38 ± 49.17</td>
</tr>
<tr>
<td>sTM (ng/mL)</td>
<td>61.00 ± 31.21</td>
<td>46.02 ± 25.74*</td>
</tr>
</tbody>
</table>

Values are means ± SEM.

* P <.005 posttreatment vs pretreatment.

# P <.05 losartan vs perindopril.

![Figure 2](image-url)
those of other classes of antihypertensive drugs. Further study is necessary to identify the interaction of antihypertensive agents with the fibrinolytic system.

Finally, plasma levels of TFPI were similar in the hypertensive and control subjects in this study. TFPI is a factor Xa-dependent inhibitor for the factor VIIa tissue factor complex and regulates the extrinsic pathway of blood coagulation. It has been demonstrated that decreased levels of this endothelial substance result in susceptibility to thrombotic disorders. To verify our hypothesis that low plasma levels of TFPI may contribute to the alterations of fibrinolytic kinetics in hypertension, we determined plasma levels of TFPI in hypertensive and normotensive subjects and examined the effects of perindopril and losartan on plasma TFPI levels. However, we did not find any differences between the hypertensive patients and normotensive controls, and the two therapeutic regimens had no significant effects on the plasma levels of TFPI.

In conclusion, increased levels of PAI-1 and sTM suggest that chronic hypertension is associated with hypofibrinolysis. Impaired fibrinolysis may explain the high incidence of thrombotic complications in hypertension. ACE inhibitors have a beneficial effect on impaired fibrinolytic activity, and similar effects have been found with the use of Ang-II receptor antagonists. This observation suggests that the effects of ACE inhibitors are related to the blockade of Ang-II action rather than increased kinin activity. These findings may have important physiological significance for the rational use of the Ang-II receptor antagonists (as well as ACE inhibitors) in the treatment of vascular complications associated with hypertension.

REFERENCES