Impaired Endothelial Release of Tissue-Type Plasminogen Activator in Patients With Chronic Kidney Disease and Hypertension

Thórdís Hrafnskelsdóttir, Pia Ottosson, Thorarinn Gudnason, Ola Samuelsson, Sverker Jern

Abstract—We have shown that the capacity for local release of tissue-type plasminogen activator (tPA) from the vascular endothelium is impaired in patients with primary hypertension. Because this response is an important protective mechanism against intravascular clotting, we investigated whether this system is also defective in patients with advanced chronic kidney disease and hypertension. Nine nondiabetic nonsmoking men with chronic kidney disease (glomerular filtration rate 11 to 28 mL/min×1.73 m²; aged 33 to 75 years) were compared with age-matched healthy controls. Intravascular infusions of desmopressin, methacholine, and sodium nitroprusside were given locally in the brachial artery. Forearm blood flow was measured by venous occlusion plethysmography and blood collected repeatedly during the desmopressin infusion for determination of stimulated net and total cumulated release of tPA. The maximal release rate of active tPA (P<0.05) and the capacity for acute tPA release were markedly impaired in the renal patients as compared with healthy subjects (ANOVA, P=0.013). Accordingly, the accumulated release of tPA was 1905 (SEM 366) and 3387 (718) ng/L tissue, respectively (P<0.05). However, there were no significant differences in vasodilator responses between the groups. Thus, patients with advanced chronic kidney disease and hypertension have a markedly impaired capacity for acute release of tissue plasminogen activator, despite preserved endothelium-dependent vasodilation. This defect may contribute to a defective local defense against arterial thrombosis. (Hypertension. 2004;44:300-304.)

Key Words: kidney failure • endothelium • hypertension, essential • tissue-type plasminogen activator • hypertension, renal

In the healthy blood vessel, activation of platelets or the coagulation cascade induces a massive release of the key enzyme of the fibrinolytic system, tissue-type plasminogen activator (tPA) from the endothelium.1,2 This acute tPA response probably constitutes a counter-regulatory mechanism to prevent a clotting process (eg, when initiated by a plaque rupture) from progression into an occlusive thrombus. If the capacity for acute tPA release is defective, the likelihood of timely spontaneous thrombolysis is reduced. We recently showed that the capacity for tPA release is markedly impaired in patients with untreated primary hypertension,3 and further experimental ex vivo studies indicated that this defect could be because of the elevated intraluminal pressure per se.4 Chronic kidney disease (CKD) is frequently accompanied by hypertension and also with a markedly increased risk of atherothrombotic complications.5 The association of CKD with thrombotic events is somewhat puzzling because renal disease is typically associated with increased bleeding tendency due to platelet dysfunction and disturbed plasma coagulation.6 However, recent studies have indicated that chronic kidney disease is associated with an impaired function of the vascular endothelium similar to that observed in hypertension.7,8 If such a defect also involves the endothelial fibrinolytic system, it may provide a potential mechanism of reduced thromboresistance. Indeed, some early studies have shown that CKD is associated with an impaired fibrinolysis as assessed by the global clot lysis time test.9,10 More specifically, stimulation of V₂-receptor mediated tPA release by systemic administration of desmopressin has been found to induce a lesser increase in venous tPA levels in patients with renal failure than in healthy control subjects.11–13

However, the capacity to respond to a local thrombotic process by acute tPA release cannot be predicted from these studies because mixed venous plasma concentrations do not reflect regional secretion patterns. Furthermore, the very short half-life of tPA in plasma (3 to 5 minutes) makes plasma levels very sensitive to changes in hepatic clearance rates.14 In the present study we instead used a regional in vivo model developed by our group to directly quantify the capacity for stimulated tPA release in patients with advanced kidney disease. We demonstrate for the first time that chronic

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were carefully explained to each subject before informed consent by our institution. The nature, purpose, and potential risks of the study were excluded. None of the patients had cardiovascular complications.

### Materials and Methods

**Subjects**

Nine nonsmoking men with advanced CKD and ongoing blood pressure lowering treatment (CKD group) were recruited from the outpatient Nephrology Department at the Sahlgrenska University Hospital and compared with 9 age-matched nonsmoking healthy male subjects (NC group). The glomerular filtration rate in the outpatient Nephrology Department at the Sahlgrenska University Hospital was obtained for 15 minutes. A free interval of 30 minutes was allowed between the different substances. Two of the healthy control subjects did not receive a Mch infusion.

Blood sampling at baseline and during the DDAVP infusion. FBF after the end of DDAVP infusion. FBF was measured after each blood sampling at baseline and during the DDAVP infusion. FBF measurements during the SNP and Mch infusions followed the same protocol as for DDAVP.

### Blood Sampling and Biochemical Analyses

Blood was collected in tubes containing 1/10 vol 0.45 mol/L sodium citrate buffer, pH 4.3 (Stabilyte, Biopool International) and kept on ice until plasma was isolated by centrifugation at 4°C and 2000g for 20 minutes. Plasma aliquots were then immediately frozen and stored at <−70°C until assay.

Plasma concentrations of tPA and plasminogen activator inhibitor-1 (PAI-1) antigens were determined by ELISA (TinElize tPA, Biopool International, and COALIZE PAI-1, Chromogenix, Hemochrom Diagnostica AB). tPA and PAI-1 activities were assessed by biofunctional immunoassortment assays (Chromolize t-PA and Chromolize PAI-1, Biopool International). All samples were assayed in duplicate on the same test plate. Intraassay variation coefficients were <5%.

### Calculations

Forearm plasma flow (FFP) was determined by FBF and hematocrit. Total cumulative tPA release in response to DDAVP was estimated for each individual as area under the curve (AUC) from baseline until 20 minutes after terminating the infusion.

Forearm vascular resistance (FVR) was calculated as the ratio of MAP to FBF, and the vascular responses to the infusions evaluated as percent change in FVR from baseline.

### Statistical Analysis

Standard statistical methods were used. Unless otherwise stated, values are presented as mean and SEM. Between-group comparisons were performed by Student unpaired t test. Statistical evaluation of baseline fibrinolytic variables was done after logarithmic transformation. Wilcoxon signed rank sum test was used to evaluate the probability that net release/uptake indexes were different from 0. Responses to infusions were evaluated by 1-way (infusion) and 2-way (group and infusion) repeated-measures ANOVA. Correlations of fibrinolytic responses and clinical characteristics were evaluated by Pearson correlation. Findings were considered significant at P<0.05 (2-tailed tests).

### Results

As shown in Table 2, plasma concentrations and forearm release rates of the fibrinolytic factors at baseline were similar in the 2 groups (P=NS). At baseline there was a significant net release of active tPA in both groups, whereas the net release of tPA antigen only reached significance in the NC group (Table 2). There was no significant forearm release of either total or active PAI-1 in either group.

### Fibrinolytic Responses to Stimulation

DDAVP infusion induced a significant, dose-dependent increment in tPA release (antigen and active tPA) across the forearm in fixed order at a constant rate of 1 mL/min. The 2 lowest dose-steps were continued for 5 minutes, and the highest dose was administered for 15 minutes. A free interval of 30 minutes was allowed between the different substances. Two of the healthy control subjects did not receive a Mch infusion.

Baseline blood samples were collected simultaneously from the artery and vein 5 and 10 minutes before starting the SNP and DDAVP infusions, respectively. During the DDAVP infusion, venous blood samples were obtained at 2 and 4 minutes of each dose-step and, on the highest dose-step, also at 7 and 13 minutes. Arterial and venous blood was collected at 2, 10, and 20 minutes after the end of DDAVP infusion. FBF was measured after each blood sampling at baseline and during the DDAVP infusion. FBF measurements during the SNP and Mch infusions followed the same protocol as for DDAVP.

### Experimental Procedures

The perfused-forearm model has previously been described in detail. In brief, the subjects attended the laboratory in the morning after an overnight fast. Patients withheld all medical treatment on the study day.

Catheters were then inserted into the brachial artery and a deep antecubital vein in the dominant arm. Forearm blood flow (FFB) was assessed by computerized venous occlusion plethysmography. Mean intraarterial pressure (MAP) was monitored throughout the infusion. Heart rate, per minute was assessed by computerized venous occlusion plethysmography. Mean arterial blood pressure,† mm Hg was measured intraarterially.

### TABLE 1. Clinical and Hemodynamic Characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>CKD Group</th>
<th>NC Group</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>56.4 (4.3)</td>
<td>53.7 (5.1)</td>
<td>NS</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>26.9 (0.7)</td>
<td>24.1 (1.0)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Waist/hip ratio</td>
<td>0.95 (0.02)</td>
<td>0.89 (0.02)</td>
<td>NS</td>
</tr>
<tr>
<td>Serum cholesterol, mmol/L</td>
<td>6.1 (0.4)</td>
<td>5.7 (0.3)</td>
<td>NS</td>
</tr>
<tr>
<td>Serum triglycerides, mmol/L</td>
<td>2.1 (0.3)</td>
<td>1.2 (0.3)</td>
<td>NS</td>
</tr>
<tr>
<td>Systolic blood pressure,† mm Hg</td>
<td>147 (5.5)</td>
<td>123 (4.5)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Diastolic blood pressure,† mm Hg</td>
<td>77 (2.3)</td>
<td>65 (2.4)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Mean arterial blood pressure,† mm Hg</td>
<td>104.1 (3.0)</td>
<td>88.3 (2.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Heart rate, per minute</td>
<td>59.9 (2.3)</td>
<td>54.1 (2.1)</td>
<td>NS</td>
</tr>
<tr>
<td>Forearm blood flow, mL/min×L tissue</td>
<td>41.8 (5.0)</td>
<td>35.6 (3.6)</td>
<td>NS</td>
</tr>
<tr>
<td>Forearm vascular resistance, AU‡</td>
<td>2.73 (0.24)</td>
<td>2.74 (0.30)</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are mean (SEM).

*Student 2-sample t test.
†Measured intraarterially.
‡Arbitrate units

Kidney disease with concomitant hypertension is characterized by a markedly impaired capacity for stimulated local tPA release. This defect may contribute to a reduced chance of timely spontaneous thrombolysis in case of an atherothrombotic event.
TABLE 2. Fibrinolytic System at Baseline

<table>
<thead>
<tr>
<th>Fibrinolytic Factor</th>
<th>CKD Group</th>
<th>NC Group</th>
<th><em>P</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>tPA antigen</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arterial concentration, ng/mL</td>
<td>9.7 (1.3)</td>
<td>6.61 (2.1)</td>
<td>0.06</td>
</tr>
<tr>
<td>Net-release rate, ng/min×L tissue</td>
<td>10.9 (7.9)</td>
<td>9.65 (2.4)†</td>
<td>NS</td>
</tr>
<tr>
<td>Active tPA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arterial activity, ng/mL</td>
<td>0.8 (0.1)</td>
<td>1.0 (0.2) NS</td>
<td></td>
</tr>
<tr>
<td>Net release rate, ng/min×L tissue</td>
<td>4.4 (1.6)†</td>
<td>2.1 (0.8)†</td>
<td>NS</td>
</tr>
<tr>
<td>PAI-1 antigen</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arterial concentration, ng/mL</td>
<td>33.3 (5.5)</td>
<td>31.2 (6.6) NS</td>
<td></td>
</tr>
<tr>
<td>Net release rate, ng/min×L tissue</td>
<td>17 (46)</td>
<td>-23 (37) NS</td>
<td></td>
</tr>
<tr>
<td>Active PAI-1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arterial activity, ng/mL</td>
<td>9.3 (2.8)</td>
<td>7.8 (3.2) NS</td>
<td></td>
</tr>
<tr>
<td>Net release rate, ng/min×L tissue</td>
<td>2.1 (7.7)</td>
<td>15.7 (14.3) NS</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean and (SEM).

*Comparison of the groups by unpaired t test, after logarithmic transformation of arterial concentrations.
†Calculated net release/uptake was significantly different from 0.

1). However, the tPA release response was markedly attenuated in CKD patients as compared with control subjects (Figure 1; 2-way ANOVA, *P*=0.013). The cumulative amount of tPA antigen (AUC) secreted during stimulation was also significantly reduced to 1905 (366) ng/L forearm tissue in the CKD group as compared with 3387 (718) ng/L tissue in the NC group (*P*<0.05; *t* test). The stimulated increment in active tPA was also greatly attenuated in the CKD as compared with the NC group (*P*=0.0001; 2-way ANOVA, Figure 1). This was further illustrated as the release rate of active tPA increased at most only 8-fold in the CKD group during the DDAVP infusion, or from 7.0 (2.0) to 56.7 (9.5) ng/min×L tissue, as compared with a 20-fold increase in the NC group, from 5.6 (0.9) at baseline to 111.4 (22.0) ng/min×L tissue at maximum (*P*<0.05; *t* test). Furthermore, the AUC for active tPA was significantly less in the CKD group compared with the NC group (1072 [190] versus 2491 [599] ng/L tissue, respectively; *P*<0.05). For the whole group, the maximal tPA release rate was negatively correlated to MAP (*r*= −0.514, *P*<0.05) but not to body mass index or hematocrit (data not shown).

There were no significant changes in the release of PAI-1 antigen over the forearm during DDAVP infusion (*P*=0.53; 1-way ANOVA), and the responses were similar in the 2 groups (*P*=0.87; 2-way ANOVA).

Vasodilator Responses to Stimulation

Forearm blood flow responses and relative changes in forearm vascular resistance during the infusions are shown in Figure 2. In both groups, all 3 infusions induced significant and dose-dependent increases in FBF and concomitant decreases in FVR (*P*=0.0001 throughout; 1-way ANOVAs). Vasodilator responses during the infusions were superimposable in the 2 groups when expressed as relative change in forearm vascular resistance (NS throughout; 2-way ANOVAs). However, because of the higher blood pressure, the absolute increase in FBF during Mch infusion was greater in the CKD group than in the NC group (*P*<0.001; 2-way ANOVA; Figure 2).

Discussion

This is the first study to demonstrate that patients with chronic kidney disease and hypertension have a markedly impaired capacity for acute tPA release from the vascular endothelium. On the average, the total amount of tPA released during stimulation was about half of that observed in healthy subjects. The capacity to increase active tPA in the CKD group was even more markedly reduced and reached only ~43% compared with that of the NC group. Also, there was a substantial difference in the maximal increase of the active fraction of tPA, which was twice as great in healthy controls as in patients with CKD. Interestingly, the disturbance in the endothelial function in CKD was confined to the release of tPA because there was no significant reduction in the capacity for endothelium-dependent vasodilatation.

Theoretically, the mechanism behind the defective tPA response could be located either at the receptor level (or its signaling pathways) or involve some disturbance of the synthetic/secretory machinery. However, because vasodilator and fibrinolytic responses to DDAVP are considered to be mediated by the same V2 receptor,19 the preserved vasorelaxant response to the agonist argues against a defective receptor-signaling mechanism. Instead, it appears more likely...
that the amount of tPA available for regulated release is somehow reduced, either by suppressed synthesis or depletion of the endothelial intracellular stores. Whether this is an effect of the chronic kidney disease itself or because of some other associated condition cannot be determined from the present data.

The impaired tPA release during DDAVP stimulation is similar to what we previously have observed in patients with untreated primary hypertension. However, the average mean arterial blood pressure of the group with primary hypertension was somewhat higher than that of the present CKD group (114 versus 104 mm Hg, respectively). The corresponding accumulated tPA release in hypertensive and CKD patients was 37% and 55% of their respective control groups. Thus, it is possible that the reduced capacity for tPA release observed in both patient groups is because of the raised blood pressure. To test this hypothesis, one would either have to stop medication—which was considered unethical in this group of patients—or intensify antihypertensive treatment to reach strictly normotensive blood pressure levels. Because the majority of the patients were already on triplet-drug therapy, one would expect additional treatment only to produce a marginal further blood pressure reduction. Hence, to clarify the effect of the blood pressure per se, invasive studies in a much larger sample of subjects, or other experimental approaches, would be required.

Interestingly, however, there are some experimental data to support the notion that high pressure impairs endothelial tPA production. We recently demonstrated in an ex vivo perfusion model that both tPA synthesis and release from endothelial cells in isolated human conduit vessels was markedly suppressed when intraluminal pressure was elevated to the high physiological range. The intracellular signal transduction pathways mediating the effect of intravascular pressure are, as yet, not fully elucidated, but this observation offers a potential explanation for the observed defective tPA secretory response in patients with high blood pressure. Alternatively, the diminished tPA response in CKD may be related to an underlying atherosclerotic vasculopathy and/or an increased chronic inflammatory burden associated therewith. Cytokines, such as interleukin 1-β and tumor necrosis factor-α, have been shown to reduce fibrinolytic activity by suppressing the synthesis of tPA in cultured endothelial cells. Atherosclerotic changes in arteries may also by themselves contribute to a decreased regulated tPA release. In line with this, a recent study showed that the capacity for tPA release in the left anterior descending coronary artery varied inversely with plaque burden of the artery. Although the patients in the present study did not have clinical signs of atherosclerotic disease, they are still likely to have a higher prevalence of atherosclerotic changes than the age-matched healthy control subjects.

In the present study, impaired fibrinolytic capacity in the patients with CKD was not associated with reduced endothelium-dependent vasodilatation. This observation indicates 2 things. First, that a defective endothelium-mediated vasodilator response is not an obligatory finding in this condition, and, second, that changes in the fibrinolytic and vasomotor functions of the endothelium do not necessarily go hand in hand. Actually, we recently demonstrated that the capacity for tPA release increased with age, whereas endothelial-dependent vasodilatation was unaffected.

Previous studies on the vasomotor responses in CKD have produced somewhat conflicting results. Most studies reporting an impaired vasodilator response have included patients in chronic dialysis, which may itself induce changes in endothelial function. Accordingly, endothelium-dependent responses of the microcirculation were impaired in CKD patients treated with hemodialysis but not in conservatively treated patients. Furthermore, Thuraisingham et al could not demonstrate an impaired endothelium-dependent vasorelaxation in an experimental ex vivo animal study of advanced renal insufficiency, whereas a large study on patients in the predialysis range reported attenuation in methacholine-induced vasodilatation. Moreover, it is possible that this defect is selective for muscarinic receptor agonist as was found in a recent study on the age-dependent vasodilation capacity. Thus, the issue to which extent renal impairment...
per se impairs endothelium-dependent vasodilatation remains to be further clarified.

Our previous in vivo studies have shown that in a healthy man the tPA release response is very dynamic and the release rate of active tPA can increase at least 25-fold within a few minutes. The importance of tPA-mediated plasminogen activation for maintenance of vascular patency has been confirmed in gene targeting and gene transfer studies. Hence, the defective capacity for tPA release in CKD patients may reduce the potential for timely spontaneous fibrinolysis in cases of an atherothrombotic event. In support of this hypothesis, we recently showed in a prospective epidemiological study that a genetic variation at the tPA locus, which is associated with low local tPA secretion rate, is a predictor of a first myocardial infarction.

Perspectives

In conclusion, we found that chronic kidney disease with accompanied hypertension was associated with a markedly impaired capacity for acute tPA release. This defective capacity for rapid activation of the fibrinolytic system in response to intravascular clotting may be a hitherto unrecognized mechanism for increased risk of atherothrombotic events in patients with renal failure. The impaired endothelial fibrinolytic response may be related to the elevated blood pressure or some other mechanism associated with the impaired renal function. It remains, however, to be evaluated whether this defect can be reversed by an intensive antihypertensive or other vasculoprotective treatment.

Acknowledgments

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References

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