Endogenous Bradykinin Contributes to Increased Plasminogen Activator Inhibitor 1 Antigen following Hemodialysis

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ABSTRACT

Oxidative stress and inflammation predict cardiovascular events in chronic hemodialysis patients. Hemodialysis activates the kallikrein-kinin system, increasing bradykinin. Bradykinin promotes inflammation but also stimulates endothelial release of tissue-plasminogen activator and inhibits platelet aggregation. Understanding the detrimental and beneficial effects of endogenous bradykinin during hemodialysis has implications for the treatment of cardiovascular disease in the hemodialysis population. To test the hypothesis that bradykinin contributes to the inflammatory and fibrinolytic responses to dialysis, we conducted a double-blind, randomized, placebo-controlled crossover study comparing the effect of the bradykinin B2 receptor blocker HOE-140 with vehicle on markers of oxidative stress, inflammation, fibrinolysis, and coagulation in nine hemodialysis patients without coronary artery disease. Bradykinin receptor antagonism did not affect the mean arterial pressure or heart rate response to dialysis. Monocyte chemoattractant protein 1 (MCP-1) peaked postdialysis; HOE-140 blunted the increase in MCP-1 (5.9 ± 5.9 versus 25.6 ± 20.1 pg/ml, P = 0.01). HOE-140 also abolished the increase in plasminogen activator inhibitor 1 (PAI-1) antigen observed at the end of dialysis. In contrast, HOE-140 significantly accentuated the effect of dialysis on F2-isoprostanes and P-selectin. Taken together, these results suggest that endogenous bradykinin contributes to increases in MCP-1 and PAI-1 antigen after hemodialysis via its B2 receptor. Factors that increase the production of bradykinin or decrease its degradation may enhance the inflammatory response to hemodialysis.


ESRD patients face a 3.4-fold risk of cardiovascular events compared with the general population, and chronic hemodialysis (CHD) patients face a 100-fold higher risk of cardiovascular death compared with the general population in patients age 45 yr or younger. Traditional risk factors for atherosclerosis do not account for the increased incidence of cardiovascular events. On the other hand, biomarkers of oxidative stress and inflammation predict cardiovascular events in CHD patients.

During hemodialysis, contact of the blood with the dialyzer and dialysate activates the kallikrein-kinin system (KKS) and induces a systemic inflammatory response characterized by complement activation, leukocyte activation, and the generation of cytokines. For example, hemodialysis increases leukocyte expression of interleukin (IL)-1β, IL-8, and TNF-α, and circulating concentrations of IL-6. Increased inflammation may, in turn, contrib-
uctive to derangements of the fibrinolytic system in CHD pa-
tients. Cytokines such as TNF-α, IL-1β, and IL-6 stimulate
expression of plasminogen activator inhibitor-1 (PAI-1), the
major physiologic inhibitor of fibrinolysis in vivo.8 Tissue-type
plasminogen activator (t-PA) concentrations and activity in-
crease transiently during hemodialysis, whereas circulating
PAI-1 concentrations are increased in the CHD popula-
tion.9–11

Activation of the KKS during hemodialysis could have fa-
orable or deleterious effects on inflammation and the risk of
cardiovascular events. On the one hand, bradykinin causes va-
sodilation and stimulates the release of t-PA from the vascular
endothelium via bradykinin B2 receptor-dependent mecha-
nisms, and infused bradykinin inhibits platelet aggregation in
healthy subjects.12–14 On the other hand, bradykinin also stim-
ulates activation of nuclear factor κB and cytokine expression
via the B2 receptor.15,16 Understanding the contribution of en-
dogenous bradykinin to the inflammatory response to hemo-
dialysis has important implications for the prevention of
atherothrombotic events.

Specifically, angiotensin converting enzyme (ACE) inhibi-
tors and angiotensin receptor blockers (ARBs) decrease mor-
tality in patients at risk for cardiovascular events in the general
population,17,18 but studies in the CHD population provide
conflicting data and often do not discriminate between the
effects of ACE inhibitors and ARBs.19–22 The only prospective,
randomized clinical trial did not show a beneficial effect of
ACE inhibition in patients undergoing hemodialysis.23

Whereas ACE inhibitors and ARBs decrease angiotensin II for-
mation or action, ACE inhibitors also potentiate the effects of
bradykinin.24–26 Indeed, during hemodialysis with a polysul-
fone membrane, plasma bradykinin concentrations are higher
during treatment with an ACE inhibitor compared with during
treatment with an ARB.27 In light of differences in mechanism
of action between ACE inhibitors and ARBs, understanding
the contribution of endogenous bradykinin to the proinflam-
matory effects of hemodialysis could affect the use of
these agents in the CHD population.

This study tested the hypothesis that activation of the KKS
and the formation of endogenous bradykinin contribute to
hypotension, increased inflammation, increased oxidative
stress, and increased fibrinolysis in response to hemodialysis.
To accomplish this, we compared the effect of continuous in-
fusion of the bradykinin B2 receptor-specific antagonist HOE-
140 versus vehicle on the acute responses to hemodialysis.

RESULTS

Baseline Patient Characteristics
Baseline characteristics appear in Table 1. Causes of ESRD
were hypertensive nephropathy (six of nine subjects), diabetic
nephropathy (one subject), focal segmental glomerulosclerosis
(one subject), and posterior urethral valves with chronic ob-
struction (one subject).

<table>
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<tr>
<td>ACE inhibitor use (yes:no)</td>
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<td>Calcium × phosphate product* (mg²/dl²)</td>
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<td>Parathyroid hormone level (mg/dl)</td>
<td>811.2 ± 188.3</td>
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<td>Heparin dose (units)</td>
<td>4611 ± 462</td>
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<tr>
<td>Erythropoietin dose (units)</td>
<td>5511 ± 2522</td>
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</table>

Table 1. Subject characteristics

Effects of Dialysis and Bradykinin Receptor Blockade on Hemodynamics

Mean arterial pressure (MAP) decreased during hemodialysis,
from 115.4 ± 7.0 to 90.1 ± 8.9 mmHg during placebo
and from 100.6 ± 5.7 to 89.2 ± 5.1 mmHg during HOE-140. Heart
rate increased significantly during hemodialysis (supplemental
data). Neither forearm blood flow (FBF) nor forearm vascular
resistance (FVR) changed after hemodialysis (supplemental
data). There was no effect of bradykinin receptor blockade on
baseline MAP, heart rate, FBF, and FVR or on the hemody-
namic response to hemodialysis (all P ≥ 0.05, supplemental
data).

Effects of Dialysis and Bradykinin Receptor Blockade on Oxidative Stress and Inflammatory Biomarkers

F₂-isoprostanes increased during hemodialysis during HOE-
140 (from 59.6 ± 7.8 to 80.0 ± 12.7 pg/ml at the end of dialysis
and 67.7 ± 8.6 pg/ml 2 h postdialysis, P = 0.007) but not
during vehicle (from 54.1 ± 6.1 to 58.5 ± 6.9 pg/ml at the end
of dialysis and 43.5 ± 3.4 pg/ml 2 h postdialysis, P = 0.49);
F₂-isoprostane concentrations were significantly higher post-
dialysis during administration of the bradykinin receptor an-
tagonist HOE-140 (P = 0.02 for effect of HOE-140) (sup-
plemental data). MCP-1 concentrations tended to decrease
during the first 30 min of hemodialysis but were increased at
the end of hemodialysis and continued to increase 2 h postdi-
alysis (Figure 1). HOE-140 significantly blunted the increase in

Figure 1. Effect of study drug and hemodialysis on MCP-1. *P <
0.05 versus T = 0 in post hoc analysis.
MCP-1 after hemodialysis (change from end dialysis of 5.9 ± 5.9 versus 25.6 ± 20.1 pg/ml, P = 0.01).

IL-6 (from 5.3 ± 1.1 to 6.9 ± 0.9 pg/ml at the end of dialysis during placebo, P = 0.045), IL-8 (from 17.7 ± 3.0 to 20.2 ± 3.7 pg/ml postdialysis, P = 0.08), and IL-10 (from 3.2 ± 0.7 to 3.7 ± 0.6 pg/ml at the end of dialysis, P = 0.02) all increased or tended to increase during and after hemodialysis (supplemental data). For both the proinflammatory cytokine IL-8 (P = 0.02 for HOE-140 by time interaction) and the anti-inflammatory cytokine IL-10 (P = 0.03), bradykinin receptor blockade enhanced this effect. There was no effect of HOE-140 on IL-6 concentrations. TNF-α, IL-1β, and IL-12p70 did not change during or after hemodialysis and there was no effect of HOE-140 on these cytokines (supplemental data).

Effects of Dialysis and Bradykinin Receptor Blockade on Markers of Fibrinolysis and Coagulation

PAI-1 antigen increased significantly during (from 6.1 ± 1.1 to 10.4 ± 2.1 ng/ml at the end of hemodialysis, P = 0.04) and after hemodialysis (14.6 ± 6.2 ng/ml 2 h postdialysis, P = 0.01 versus baseline) during placebo (Figure 2). The bradykinin receptor antagonist HOE-140 prevented this effect (PAI-1 2 h post-
dialysis 6.9 ± 1.5 ng/ml HOE-140 arm). When the two patients taking an ACE inhibitor were excluded from the analysis, PAI-1 antigen increased from 5.9 ± 1.5 ng/ml predialysis to 16.9 ± 9.2 ng/ml 2 h post dialysis (P = 0.03) during placebo but not during HOE-140 (7.5 ± 2.1 to 7.7 ± 1.8 ng/ml). PAI-1 activity decreased during and after hemodialysis, whereas t-PA antigen and activity increased (Table 2). There was no effect of HOE-140 on PAI-1 activity or t-PA antigen or activity (Table 2). However, in post hoc analysis, PAI-1 activity decreased significantly during hemodialysis during HOE-140 and not during placebo. d-dimer concentrations increased during hemodialysis, and there was a significant interactive effect of hemodialysis and HOE-140 on d-dimer concentrations (Table 2). P-selectin, a marker of platelet activation, increased during and after hemodialysis, an effect that occurred earlier during bradykinin receptor antagonism (Figure 3).

**DISCUSSION**

Using the bradykinin B2 receptor blocker HOE-140 to dissect the role of endogenous bradykinin, this study tested the hypothesis that activation of the KKS and the formation of endogenous bradykinin contribute to the hypotensive, proinflammatory, profibrinolytic, and anti-coagulant responses to hemodialysis. The major finding is that endogenous bradykinin contributes to increased MCP-1 and PAI-1 concentrations after hemodialysis via a B2-receptor dependent mechanism. Given that PAI-1 antigen and activity independently predict cardiovascular mortality and thrombotic events in CHD patients, the data suggest that factors that increase the formation of bradykinin or decrease its degradation would increase cardiovascular risk in the CHD population.

CHD patients exhibit abnormalities of platelet function, fibrinolysis, and coagulation. Fibrinolytic activity increases acutely during hemodialysis, largely related to an increase in t-PA, but falls precipitously after hemodialysis. Most investi-

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**Table 2. Effect of treatment on biomarkers of fibrinolysis**

<table>
<thead>
<tr>
<th></th>
<th>T = 0</th>
<th>T = 15 min</th>
<th>T = 30 min</th>
<th>T = 4 h</th>
<th>T = 6 h</th>
<th>P Value Drug</th>
<th>P Value Time</th>
<th>P Value Drug x Time</th>
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<tr>
<td>PAI-1 activity (IU/ml)</td>
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<td>vehicle</td>
<td>4.12 ± 1.06</td>
<td>3.67 ± 0.84</td>
<td>3.52 ± 0.78</td>
<td>2.24 ± 0.24</td>
<td>2.71 ± 0.53</td>
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<td>HOE-140</td>
<td>5.34 ± 1.89</td>
<td>4.74 ± 1.65</td>
<td>4.47 ± 1.59a</td>
<td>3.13 ± 0.91a</td>
<td>3.26 ± 0.73</td>
<td>0.02</td>
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<tr>
<td>t-PA antigen (ng/ml)</td>
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<tr>
<td>vehicle</td>
<td>3.79 ± 0.83</td>
<td>4.16 ± 0.84</td>
<td>4.43 ± 0.65</td>
<td>7.67 ± 1.18a</td>
<td>6.45 ± 1.12</td>
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<td>HOE-140</td>
<td>4.60 ± 0.39</td>
<td>5.94 ± 0.93</td>
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<td>t-PA activity (IU/ml)</td>
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<td>vehicle</td>
<td>0.28 ± 0.05</td>
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<td>0.41 ± 0.03a</td>
<td>0.79 ± 0.09a</td>
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<td>HOE-140</td>
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<td>131.19 ± 27.73</td>
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<td>HOE-140</td>
<td>134.82 ± 29.67</td>
<td>126.66 ± 19.90</td>
<td>145.38 ± 32.39</td>
<td>189.15 ± 35.46a</td>
<td>162.35 ± 32.09</td>
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Mean ± SEM.

*P < 0.05 versus T = 0 in post hoc analysis.
Bradykinin may be predicted to increase PAI-1 expression through its proinflammatory effects or to decrease PAI-1 expression by increasing the formation of nitric oxide (NO), which downregulates PAI-1 expression through a cGMP-dependent mechanism. Studies in cultured cells provide conflicting information regarding the role of bradykinin in the regulation of PAI-1 expression. Kimura et al. demonstrated that bradykinin increases PAI-1 mRNA and protein expression in rat vascular endothelial cells and that the effect is negatively affected by NO. In contrast, Okada et al. reported that bradykinin decreases PAI-1 expression in murine renal tubulointerstitial cells during ACE inhibition. The finding that bradykinin B2 receptor blockade abolished the rise in PAI-1 after hemodialysis in the study presented here suggests that in CHD patients that exhibit endothelial dysfunction, the proinflammatory effects of bradykinin predominate in regulating PAI-1, at least during hemodialysis. PAI-1 antigen is both an acute-phase reactant and an inhibitor of fibrinolysis. During hemodialysis, increased t-PA antigen would complex with PAI-1 antigen to decrease its activity; however, in post hoc analysis PAI-1 activity decreased only during HOE-140. Taken together with the observation that d-dimer concentrations were higher during and after HOE-140, these data suggest that endogenous bradykinin receptor blockade attenuates fibrinolysis.

As reported previously, t-PA antigen and activity increased during and after hemodialysis in this study. Although bradykinin stimulates the release of t-PA from the vascular endothelium through a B2-receptor-dependent mechanism, our data do not support a significant contribution of bradykinin to increased t-PA during hemodialysis. Bradykinin-stimulated t-PA release is attenuated in conditions associated with endothelial dysfunction such as smoking, obesity, and hypertension, and ESRD is characterized by endothelial dysfunction. During hemodialysis, factors other than bradykinin (e.g., direct endothelial damage from high shear stress or from the effects of extracorporeal circulation) may contribute to increased t-PA release.

Concentrations of the proinflammatory cytokines IL-6 and IL-8, the anti-inflammatory cytokine IL-10, and chemotactic factors such as MCP-1 are chronically elevated in CHD patients. We found that hemodialysis increased the concentrations of these cytokines and chemokines, consistent with previously published reports by our laboratory and others. That bradykinin B2 receptor blockade attenuated the rise in MCP-1 after hemodialysis concurs with data from cell culture studies. Thus, bradykinin stimulates lung fibroblasts, type II alveolar cells, macrophages, and bronchial epithelial cells to release inflammatory cytokines and chemotactic factors such as MCP-1, whereas B2 receptor antagonism decreases cytokine-induced neutrophil migration. On the basis of these data, ACE inhibitors, which potentiate bradykinin, could have a proinflammatory effect during hemodialysis.

Whereas bradykinin B2 receptor blockade decreased MCP-1 and PAI-1 after hemodialysis, bradykinin receptor blockade was associated with an increase in F2-isoprostanes postdialysis, suggesting that endogenous bradykinin decreases oxidative stress in patients undergoing hemodialysis. This concurs with recent in vitro and animal model data suggesting that bradykinin reduces oxidative stress via its B2 receptor but conflicts with other studies showing that bradykinin augments production of reactive oxygen species after ischemia-reperfusion injury.

In addition, we found that as measured by circulating P-selectin concentrations, platelet aggregation increased earlier during dialysis when the bradykinin B2 receptor was blocked. Endogenous bradykinin stimulates the release of two potent inhibitors of platelet aggregation via its B2 receptor—prostacyclin and NO. Both of these endothelial-derived factors modulate platelet-vessel wall interactions, inhibit aggregation, and promote disaggregation in vitro and in animal models. Studies in bradykinin B2 receptor knockout mice indicate that the anti-aggregatory effects of bradykinin occur via the B2 receptor, although bradykinin and its metabolite BK1–5 can also inhibit thrombin-induced platelet aggregation via interactions with protease-activated receptor 1.

Bradykinin causes vasodilation via the B2 receptor-mediated release of prostacynin, NO, and endothelium-derived hyperpolarizing factor; however, the lack of effect of HOE-140 on the hypotensive response to hemodialysis suggests that endogenous bradykinin does not substantially contribute to the hemodynamic response to hemodialysis. Blood pressure (BP) is volume-dependent in patients with ESRD, and BP decreases during hemodialysis because of decreased intravascular plasma volume, whereas heart rate rises because of activation of the baroreflex.
subjects with evidence of coronary artery disease because we were studying an investigational drug that had the potential to increase BP or cause coronary vasoconstriction. Thus, in our subjects, concentrations of inflammatory biomarkers were lower than have been reported previously. Second, our small study included subjects who tended to be young, obese, predominantly African American, and male. Therefore, the results may not be readily generalizable to a large population of CHD patients.

In conclusion, despite a favorable effect on oxidative stress, endogenous bradykinin contributes to increases in MCP-1 and PAI-1 antigen during and after hemodialysis via a B2-receptor dependent mechanism. These data support a net proinflammatory role of endogenous bradykinin during hemodialysis and suggest that ACE inhibitors, which prevent the degradation of bradykinin, could have a proinflammatory effect in CHD patients. The results of our study provide a plausible biologic mechanism for the lack of a beneficial effect of ACE inhibitors in patients with ESRD and left ventricular hypertrophy in trials such as FOSIDIAL.

CONCISE METHODS

Study Population

Nine CHD patients without evidence of CAD or congestive heart failure completed the study. The study was performed according to the Declaration of Helsinki after being approved by the Vanderbilt University Institutional Review Board, and all patients gave written informed consent. The subjects were on a three-times-weekly hemodialysis schedule with polysulphone membrane (Optiflux 160) for at least 6 mo before the study. Dialysis adequacy was determined based on the total clearance (Kt) to total body water or urea distribution space (V) ratio (Kt/V) of 1.2 or greater. Hemodialysis was carried out for 4 h on each study day with the same flow rates (400 ml/min blood flow rate and 800 ml/min dialysis flow rate) and ultrafiltration (varied for each subject) on the two study days for each subject. Subjects received heparin (4611 ± 262 U) as a bolus per typical hemodialysis protocol at the beginning of each hemodialysis session with no requirement for continuous infusion (Table 1). Subjects also received erythropoietin (5511 ± 2522 U) three times weekly per typical hemodialysis protocol (Table 1). The study drug constituted the only varied condition for each subject between the two study days. Antihypertensive medications in use at the time of the study and the numbers of subjects taking each one included beta blockers (metoprolol: one, labetalol: one); ACE inhibitors (lisinopril: one, enalapril: one); calcium channel blockers (nifedipine: four); and α2-adrenergic agonists (clonidine: two).

Subjects with documented coronary artery disease were excluded, and all subjects had a negative dobutamine echo before study. Individuals with a body mass index (BMI) greater than 35 mg/kg·m² history of kidney transplant less than 6 mo before study; use of anti-inflammatory medications other than aspirin less than 325 mg per day; use of immunosuppressive drugs within 1 mo before study; history of active connective tissue disease; history of acute infectious disease within 1 mo before study; AIDS; history of myocardial infarction or cerebrovascular event within 3 mo; advanced liver disease; gastrointestinal dysfunction requiring parenteral nutrition; active malignancy excluding basal cell carcinoma of the skin; anticipated live donor kidney transplants; use of vitamin E greater than 60 IU per day or vitamin C greater than 500 mg per day; pregnancy, breastfeeding, or childbearing potential; history of poor adherence to hemodialysis or medical regimen; or inability to provide consent were excluded.

Study Protocol

Subjects participated in a prospective, randomized, double-blind, placebo-controlled, crossover trial of the effects of placebo versus HOE-140, a bradykinin B2 receptor antagonist, during hemodialysis. Subjects were randomized to treatment with vehicle or HOE-140 on each of two study days separated by 1 wk. Subjects reported to the Vanderbilt General Clinical Research Center for study drug administration, hemodialysis, hemodynamic monitoring, and plasma sampling (Figure 4). HOE-140 (or the equivalent volume of vehicle) was infused intravenously at 100 µg/kg over 1 h before dialysis and at 50 µg/kg per h for the duration of dialysis. This dose has been shown to significantly blunt the vasodilatory response to intra-arterial bradykinin. BP was determined using an automated oscillometric recording device every 5 min during the study (Dinamap, Critikon, Carlsbad, CA), using the average of three readings at each time point. Blood for biochemical analysis was collected 1 h before dialysis (T = −60), at initiation of dialysis (T = 0), 15 and 30 min after initiation of dialysis (T = 15 and 30 min), at termination of dialysis (T = 4 h), and 2 h after termination of dialysis (T = 6 h). In the first two subjects studied, blood was also obtained at 48 h, but this collection was discontinued because all parameters had returned to baseline at this time point. FBF was measured using strain-gauge venous occlusion plethysmography (D.E. Hokanson, Bellevue, WA), as described previously, at each of the six timepoints outlined above. FBF was measured in milliliters per 100 ml of volume tissue per minute. FVR was calculated as the ratio of MAP to FBF and expressed as arbitrary units (AU).
Laboratory Procedures
Blood was centrifuged immediately after collection and stored at −80°C until assay. TNF-α, IL-1β, IL-6, IL-8, IL-10, IL-12p70, and MCP-1 were measured using Luminex immunoassay technology and commercially available kits (Linco Research, St. Charles, MO). Coefficients of variation (interassay and intra-assay, respectively) and limits of detection for the inflammatory markers are as follows: 0.037, 0.020, and 0.7 pg/ml for TNF-α; 0.083, 0.030, and 2.3 pg/ml for IL-1β; 0.067, 0.027, and 1.6 pg/ml for IL-6; 0.037, 0.023, and 1.2 pg/ml for IL-8; 0.090, 0.037, and 0.13 mg/ml for IL-10; 0.030, 0.033, and 0.6 pg/ml for IL-12p70; and 0.080, 0.037, and 1.3 pg/ml for MCP-1. Blood for F$_2$-isoprostanes was collected in chilled tubes containing EDTA, and F$_2$-isoprostane levels were measured using negative-ion gas-chromatography mass spectrometry. Blood for PAI-1, t-PA, and d-dimer assays was collected in vacutainer tubes containing acidified 0.105 M sodium citrate (Biopool, Umea, Sweden). PAI-1 and t-PA antigen were determined using two-site ELISAs (Elisa, Immulysse, and TintElize). PAI-1 and t-PA activity were measured using chromogenic substrates and standardized commercial kits (Chromolize, Biopool), with results expressed as units per milliliter (IU/ml). d-dimer was measured using a standardized commercial kit (TintElize, Biopool), with results expressed as nanograms per milliliter. P-selectin was measured using a commercially available ELISA kit (R&D, Minneapolis, MN). Coefficients of variation (interassay and intra-assay, respectively) and limits of detection for the coagulation and fibrinolysis markers are as follows: 0.019, 0.024, and 20 ng/ml for PAI-1; 0.055, 0.035, and 6 ng/ml for t-PA; 0.040, 0.040, and 40 ng/ml for d-dimer; and 0.049, 0.088, and 0.5 ng/ml for P-selectin.

Statistical Methods
Data are presented as mean ± SEM. Missing variables in the data were replaced using series means. Baseline characteristics on the two study days were compared using a paired t test or Wilcoxon signed-rank test. General linear model repeated measure ANOVA was used to evaluate the effect of hemodialysis and drug (HOE-140 placebo) over time. Gender or race was included as a between-subject variable if there was evidence for a direct or interactive effect of these variables. A two-sided P value less than 0.05 was considered significant. Statistical analyses were performed using SPSS software v.15.0 (SPSS, Inc., Chicago, IL).

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DISCLOSURES
Dr. Brown has consulted for Jerini AG and Baxter in the past year but received neither financial support for nor intellectual input in the study presented here. None of the other authors have any relationships to report.

REFERENCES


