Vasoflux, a New Anticoagulant With a Novel Mechanism of Action

Jeffrey I. Weitz, MD; Edward Young, PhD; Marilyn Johnston, ART; Alan R. Stafford, MSc; James C. Fredenburgh, PhD; Jack Hirsh, MD

Background—Heparin and direct thrombin inhibitors, such as hirudin, have limitations in the treatment of acute coronary syndromes. Heparin does not inactivate fibrin-bound thrombin, whereas hirudin fails to block thrombin generation. In contrast, Vasoflux is a novel anticoagulant that inactivates fibrin-bound thrombin and attenuates factor Xa generation.

Methods and Results—Vasoflux is prepared by depolymerization of heparin, restricting molecular size to between 3000 and 8000 Da, and reducing antithrombin affinity by periodate oxidation. Vasoflux catalyzes fibrin-bound thrombin inactivation by heparin cofactor II (HCII) and inhibits factor IXa activation of factor X independently of antithrombin and HCII. Compared with other anticoagulants in a thrombogenic extracorporeal circuit, Vasoflux maintains filter patency at concentrations that produce an activated clotting time (ACT) of 220 seconds. In contrast, to maintain filter patency, heparin, low-molecular-weight heparin (LMWH), and hirudin require concentrations that produced an ACT of 720, 415, and 1500 seconds, respectively, whereas dermatan sulfate was ineffective at concentrations that produced an ACT of 360 seconds.

Conclusions—Vasoflux is more effective than heparin and LMWH because it inactivates fibrin-bound thrombin and is superior to hirudin and dermatan sulfate because it also blocks factor Xa generation. (Circulation. 1999;99:682-689.)

Key Words: anticoagulants ■ heparin ■ coagulation

Acute coronary syndromes are triggered by rupture of atherosclerotic plaque and subsequent thrombosis. Given the central role of thrombosis in the pathogenesis of these syndromes, heparin has been a cornerstone of treatment. Heparin, however, has limitations in this setting. There is a clustering of recurrent ischemic events when heparin is stopped in patients with unstable angina,1 a phenomenon associated with reactivation of the coagulation system.2 Adjunctive heparin therapy also is of uncertain benefit in patients undergoing coronary thrombolysis. Ongoing thrombin generation occurs despite heparin therapy in these patients3 and may be a predisposing factor for reocclusion.4

The relative ineffectiveness of heparin in the treatment of arterial thrombosis has been attributed to the inability of the heparin/antithrombin complex to inhibit activated clotting factors bound to various components of the thrombus. Platelet-bound factor Xa within the thrombus is protected from inhibition by the heparin/antithrombin complex5 and can trigger local thrombin generation.6 Thrombin bound to fibrin also is protected7,8 and can amplify its own generation by activating platelets9 and factors V and VIII.10

Unlike heparin, direct thrombin inhibitors (such as hirudin) inactivate fibrin-bound thrombin.8 However, these agents have no effect on factor Xa, a concept supported by the observation that hirudin reduces fibrinopeptide A values but not the levels of prothrombin fragment 1.2, a marker of thrombin generation.11 Consequently, direct thrombin inhibitors must be given in high concentrations to block thrombin generated by platelet-bound factor Xa. Once treatment with these agents is stopped, ongoing thrombin generation can trigger reactivation of coagulation.12 This may explain the disappointing results with hirudin therapy, in which early benefits of hirudin over heparin were lost once treatment stopped.13,14

To overcome these limitations, we developed Vasoflux, a low-molecular-weight heparin (LMWH) derivative that has been chemically modified to reduce its affinity for antithrombin. Vasoflux acts as an anticoagulant by (1) catalyzing the inactivation of fibrin-bound thrombin by heparin cofactor II (HCII) and (2) blocking factor X activation by factor IXa independently of antithrombin and HCII. To examine the importance of inhibition of fibrin-bound thrombin, we compared Vasoflux with hirudin, a direct thrombin inhibitor that inactivates fibrin-bound thrombin,8 and dermatan sulfate, a selective HCII catalyst15 that also inactivates fibrin-bound thrombin,16,17 in a heparin-resistant extracorporeal circuit.
Methods

Materials
Unfractionated heparin from porcine intestinal mucosa (grade II; 166.9 USP units/mg) and Atroxin (the thrombin-like venom from Bothrops atrox) were purchased from Sigma Chemical Co. Human factor IX, factor IXa, α-thrombin, and plasminogen-free fibrinogen were obtained from Enzyme Research Laboratories. Heparin fractions of defined molecular weight (3000 and 5000 Da) were isolated from unfractionated heparin by gel filtration on a TSK G2000 SWXL column (30 cm × 7.8 mm, Supelco) as previously described. The LMWH used in these studies was enoxaparin (Rhône-Poulenc Rorer). Antithrombin and HCII, isolated from human plasma by affinity chromatography as described, migrated as single bands on SDS-PAGE analysis with apparent molecular weights of 58 000 and 70 000, respectively. When titrated against thrombin by the method of Olson et al, both inhibitors completely inactivated thrombin when inhibitor and enzyme were added in equimolar amounts. Phosphatidylcholine (75%)/phosphatidylserine (25%) vesicles were prepared as described.

Effect of Fibrin on Rates of Thrombin Inactivation by Antithrombin and HCII
To examine the effect of fibrin on the rates of thrombin inactivation by antithrombin or HCII, fibrin clots were formed in wells of a 96-well plate containing hydrophilic membranes (Millipore) by clotting 6 μmol/L fibrinogen (suspended in 20 μL of 20 mmol/L Tris-HCl, pH 7.4, 150 mmol/L NaCl (TBS) with 10 μL each of α-thrombin and CaCl₂ (final concentrations, 10 nmol/L and 1 mmol/L, respectively). After incubation for 30 minutes at 23°C, 40 μL of TBS containing 400 nmol/L HCII or antithrombin was added to consecutive clots at 5-minute intervals. Buffer alone was added to control clots. Five minutes after the last addition, 200 μL TBS containing 200 μmol/L of thrombin-directed substrate, tosyl-Gly-Pro-Arg-p-nitroanilide (Sigma), was added. After incubation for 60 minutes at 23°C, 75 μL of clot supernatant was removed and added to the wells of a 96-well plate prefilled with 75 μL of 0.2N acetic acid. Residual thrombin activity, determined by measuring absorbance at 405 nm, was plotted as a function of time. The slope of this line yielded the first-order rate constant. The second-order rate constant was calculated by dividing this value by the inhibitor concentration. Second-order rate constants for thrombin inhibition by antithrombin or HCII in the presence of fibrin were compared with those obtained in its absence. As controls, the rates of factor Xa inhibition in the absence and presence of fibrin were measured in a reaction with those obtained in its absence. As controls, the rates of factor Xa inhibition in the absence and presence of fibrin were measured in a reaction with those obtained in its absence.

Fluorescence Labeling of HCII
To label HCII with anilinonaphthalene-6-sulfonic acid (ANS), 10 μmol/L protein was dialyzed against 20 mmol/L HEPES, 100 mmol/L NaCl, 1 mmol/L EDTA, pH 7.0, for 18 hours at 4°C. After the protein had been recovered, 5 μL of a 10 mmol/L solution of 2-14-(iodoacetamide)anilino]naphthalene-6-sulfonic acid (Molecular Probes, Inc), dissolved in dimethyl sulfoxide, was added, and the reaction mixture was incubated for 60 minutes at 4°C in the dark. Unreacted fluorophore was removed by dialysis against 500 mL of TBS at 4°C with 4 changes of buffer over 18 hours. After dialysis, the sample was recovered, and protein concentration was determined by measurement of absorbance at 280 nm with an extinction coefficient of 1.1 (mg/mL)⁻¹ · cm⁻¹ and correction for light scattering and ANS absorption (ε₂₅ₒ = 26) as described. In the presence of heparin, ANS-labeled HCII complexed thrombin to the same extent as unlabeled protein, as assessed by SDS-PAGE (data not shown). The heparin-catalyzed rate of thrombin inhibition by ANS-labeled HCII was similar to that of unlabeled HCII [second-order rate constants of 2.0 · 10⁻⁸ and 3.7 · 10⁻⁸ (mol/L)⁻¹ · min⁻¹, respectively].

Affinities of Glycosaminoglycans for Antithrombin, HCII, and Factor IXa
To measure the affinities of Vasoflux, LMWH, and unfractionated heparin for antithrombin or factor IXa, a 1×1-cm quartz cuvette containing 100 nmol/L antithrombin or factor IXa in 2 mL TBS was excited at 280 nm (6-nm slit width), and intrinsic fluorescence was continuously monitored in time drive at 340 nm (6-nm slit width) with a Perkin-Elmer LS50B luminescence spectrometer. The contents of the cuvette were stirred with a micro stir-bar and maintained at 25°C with a recirculating water bath. Intrinsic fluorescence intensity was measured before (I₀) and after (I) addition of 5 to 10 μL of 10-mg/mL solutions of the various glycosaminoglycans. Titrations were continued until there was no change in I. After the experiment, I values were read from the time drive profile, and I/I₀ values were calculated and plotted versus glycosaminoglycan concentration. The data were analyzed as described below. The affinity of Vasoflux, LMWH, and unfractionated heparin for ANS-labeled HCII were determined similarly. A 1×1-cm quartz cuvette containing 50 nmol/L ANS-labeled HCII in 2 mL TBS was excited at 328 nm (10-nm slit width) and continuously monitored in time drive at 437 nm (10-nm slit width). I was measured before and after the addition of 5- to 10-μL aliquots of 60-mg/mL solutions of the various glycosaminoglycans. Intensity values read from time-drive profiles were corrected for the I obtained when equivalent concentrations of each glycosaminoglycan were titrated in the absence of ANS-labeled HCII. I/I₀ values were calculated and plotted versus the glycosaminoglycan concentration. Binding parameters were calculated by nonlinear regression of the binding isotherm equation using T buld Curve (Jandel) to solve for α, the maximum

Preparation of Vasoflux
Unfractionated heparin was depolymerized with nitrous acid to generate an LMWH fraction with a mean molecular weight of 5000. Unfractionated heparin, dissolved in distilled water, was mixed with sodium nitrite in a ratio of 33 to 1 (wt/wt). Concentrated HCl was added to lower the pH to 3.0. After 2 hours of incubation at 23°C, the pH was increased to 6.5 by addition of 5% NaOH. The resultant LMWH was then subjected to ultrafiltration with a 3000-Da-cutoff cellulose membrane fitted to an ultrafiltration unit (Millipore) and lyophilized. To reduce antithrombin affinity, the heparin fraction was oxidized with sodium periodate. Lyophilized LMWH was dissolved in 25 mmol/L sodium phosphate buffer, pH 7.0. Sodium periodate, dissolved in distilled water, was added to 20 mmol/L; after incubation in the dark for 24 hours at 23°C, 50% NaOH was added to raise the pH to 7.4, and the sample was again subjected to ultrafiltration with a 3000-Da-cutoff membrane. Aldehydes formed during the oxidation reaction were reduced with sodium borohydride to yield primary alcohols. After a final ultrafiltration step, the material was lyophilized.
Effect of Vasoflux and Heparin on Factor IXa-Induced Factor Xa Generation

The effect of Vasoflux on factor IXa-induced factor Xa generation was compared with that of unfractionated heparin in plasma immunodepleted of antithrombin and prothrombin. Factor IXa (85 nmol/L), 4 μmol/L PCPS, and 7.5 mmol/L CaCl₂ suspended in 400 μL of 10 mmol/L HEPES, pH 6.8, and 150 mmol/L NaCl containing 0.1% BSA were incubated at 37°C with 200 μL of immunodepleted plasma. At 20-second intervals, 20 μL of the reaction mixture was added to wells of a 96-well plate prefilled with 80 μL of 10 mmol/L EDTA to stop the reaction. This mixture (50 μL) was added to wells containing 150 μL of 200 μmol/L factor Xa–directed substrate, benzoxycarbonyl-Ile-Glu-(OR)-Gly-Arg p-nitroanilide (S2222; Chromogenix, Helena Laboratories), and absorbance was monitored at 405 nm.

Coagulation Assays

Activated clotting times (ACTs) were measured with a Hemochron whole-blood coagulation system (International Technologic Corp). Activated partial thromboplastin times (aPTTs) were measured with Thrombostil (Ortho), a commercial reagent containing rabbit brain phospholipid extract with a micronized silica activator. Dilute prothrombin times were determined with Thromborel (Behringwerke AG), a human plasma–derived thromboplastin with an international sensitivity index of 1.16, diluted to obtain a baseline prothrombin time of 60 seconds. With this Thromborel concentration, the prothrombin time in factor IX–deficient plasma was identical to that in factor IX–sufficient plasma, consistent with factor IX–independent activation of factor X by the factor VIIa/tissue factor complex. Thrombin clotting times were performed by diluting 50 μL of plasma in 150 μL of calcium-containing Seeger’s buffer and initiating clotting by addition of 5 U thrombin diluted in 50 μL of 10 mmol/L HEPES, pH 6.8, and 150 mmol/L NaCl. Clotting reagents were added to plasma in the absence or presence of Vasoflux, LMWH (4 to 125 mg/mL), or unfractionated heparin (0.1 to 0.5 U/mL). A modified aPTT also was performed, for which Thrombostil was preincubated with plasma for 5 minutes before addition of CaCl₂ and various glycosaminoglycans. The effect of the glycosaminoglycans on factor IXa-initiated clotting reactions could thus be determined. For all clotting assays, an ST4 coagulation analyzer (Diagnostica Stago) was used.

Anti–Factor Xa Assays

Vasoflux or LMWH (in concentrations ranging from 4 to 125 μg/mL) was added to plasma, and anti–factor Xa activity was measured on the ACL 3000 (Instrument Laboratory SpA) with a commercial factor Xa heparin kit (Diagnostica Stago).

Extracorporeal Circuit

With a 21-gauge butterfly needle, 3 mL of blood was collected from the antecubital veins of healthy volunteers for baseline ACT determination. With the same needle, 27 mL of blood was then collected into each of eight 30-mL plastic syringes prefilled with 3 mL of 3.8% trisodium citrate. Blood from each volunteer was pooled and spiked with 1511-labeled fibrinogen (~75 000 cpm/mL). Vasoflux (75 to 175 μg/mL), heparin (0.5 to 2.0 U/mL), LMWH (2.0 to 8.0 U/mL), hirudin (2 to 8 μg/mL), or dermatan sulfate (1.0 to 4.0 mg/mL) was added to 50-mL aliquots of blood incubated at 37°C in a recirculating water bath. With a Master-flex roller pump system (Cole-Parmer Instrument Co) and R3603 Tygon tubing (ID, 3⁄16 in; OD, 5⁄16 in; and wall, 3⁄32 in; Fisher Scientific Co), blood was pumped through a 40-μm blood transfusion filter (Pall Biomedical Inc). Pressure in the filter was monitored with a pressure transducer (Sorenson TransPac; Abbott Critical Care Systems) and Hewlett-Packard model 78205 D pressure monitor attached proximal to the filter via a connector and 3-way stopcocks. Anticoagulated blood was circulated through the system for 5 minutes before recalcification by addition of 800 μL of 1 mol/L CaCl₂. After an additional 5 minutes, 3 mL of blood was withdrawn for ACT determination. Over the subsequent 90-minute period, pressure across the filter was monitored continuously. At the end of this interval, or when flow across the filter was impaired, the experiment was terminated and consumption of 1511-labeled fibrinogen was determined by expressing residual blood radioactivity as a percentage of original radioactivity.

Statistical Analyses

Where appropriate, means and 95% CIs were calculated. Significance of differences was determined by 2-way ANOVA or linear regression analysis. A value of P<0.05 was considered statistically significant.

Results

Effect of Fibrin on Rates of Thrombin Inhibition by Antithrombin and HCII

The rate of thrombin inhibition by HCII in the presence and absence of fibrin is similar [second-order rate constants of 3.2×10⁴ and 4.0×10⁴ (mol/L)⁻¹ · min⁻¹, respectively]. In contrast, fibrin reduces the rate of thrombin inactivation by antithrombin 9-fold [from a second-order rate constant of 4.0×10⁴ to 4.4×10³ (mol/L)⁻¹ · min⁻¹]. This is unlikely to reflect rate-limited diffusion of antithrombin into the fibrin clot, because the rate of factor Xa inhibition by antithrombin is unaffected by fibrin [second-order rate constants of 1.5×10⁴ and 1.4×10⁴ (mol/L)⁻¹ · min⁻¹, respectively] and the molecular weights of antithrombin and HCII are similar.

Inhibition of Fibrin-Bound Thrombin by Heparin Fractions of Various Molecular Weights

At concentrations of ≥30 μg/mL (Figure 1), a 5000 Da heparin fraction inhibits fibrin-bound thrombin to almost the same extent as equivalent concentrations of unfractionated heparin (P=NS by 2-way ANOVA), whereas a 3000 Da heparin fraction has significantly lower activity (P<0.001).

We prepared a size-restricted 5000-Da heparin fraction by first depolymerizing unfractionated heparin with nitrous acid. On gel filtration analysis, its molecular weight ranged from 1000 to 8000 Da (mean, 5000 Da). The nitrous acid concen-
tration was chosen to produce sufficient depolymerization such that, by size-exclusion chromatography, <3% of the material was ≥8000 Da. Heparin chains <3000 Da were removed by ultrafiltration.

Effect of Periodate Oxidation Followed by Borohydride Reduction on the Affinity of a Size-Restricted Heparin Fraction for Antithrombin and HCII

We chemically modified the size-restricted LMWH fraction to reduce its affinity for antithrombin 1500-fold (from a \( K_d \) value of 24.7 nmol/L to 43.7 μmol/L) so as to target the agent to HCII and allow its use in high concentrations. Such concentrations are needed for inactivation of fibrin-bound thrombin because Vasoflux has low affinity for HCII: \( K_d \) = 60.5 μmol/L (Figure 2), an affinity similar to those of LMWH and dermatan sulfate (\( K_d \) values of 62.6 and 71 μmol/L, respectively), but lower than that of unfractionated heparin (\( K_d \) = 13.1 μmol/L).

Effect of Vasoflux on Coagulation Assays

Vasoflux has minimal effects on the thrombin clotting time (Figure 3) in control plasma because it has low affinity for antithrombin, and the heparin chains are too short to bridge HCII to thrombin. At clinically relevant concentrations (Table 1), LMWH prolongs the thrombin clotting time to a greater extent than Vasoflux because, unlike Vasoflux, its pentasaccharide sequence is intact. Consequently, longer heparin chains within the preparation bridge antithrombin to thrombin. This concept is supported by the observation that like Vasoflux, LMWH also has a minimal effect on the thrombin clotting time in antithrombin-depleted plasma. Clinically relevant doses of unfractionated heparin (concentrations up to 6 μg/mL, or 1.0 U/mL) have the most marked effects on the thrombin clotting time (Figure 3). This reflects the capacity of longer heparin chains to bridge antithrombin and HCII to thrombin because unfractionated heparin still prolongs the thrombin clotting time in antithrombin-depleted plasma, albeit to a lesser extent.

Vasoflux has no effect on the dilute prothrombin time (Figure 4). In contrast, LMWH produces concentration-dependent prolongation of the dilute prothrombin time. This reflects its ability to catalyze factor Xa and thrombin inactivation by antithrombin, because LMWH has no effect on the dilute prothrombin time in antithrombin-depleted plasma.
TABLE 1. Comparison of Therapeutic Concentrations, and Doses Needed to Achieve These Concentrations, for Heparin, LMWH, Vasoflux, Hirudin, and Dermatan Sulfate

<table>
<thead>
<tr>
<th>Anticoagulant</th>
<th>Therapeutic Concentration, μg/mL</th>
<th>Dose to Achieve Therapeutic Concentration, mg · kg⁻¹ · h⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heparin</td>
<td>1.2–3.0</td>
<td>0.10–0.25</td>
</tr>
<tr>
<td>LMWH</td>
<td>5–15</td>
<td>0.06–0.18</td>
</tr>
<tr>
<td>Vasoflux*</td>
<td>40–80</td>
<td>4–8</td>
</tr>
<tr>
<td>Hirudin†</td>
<td>0.5–1.0</td>
<td>0.1–0.2</td>
</tr>
<tr>
<td>Dermatan sulfate‡</td>
<td>3–8</td>
<td>0.12–0.36</td>
</tr>
</tbody>
</table>

*Therapeutic concentrations of Vasoflux have yet to be established in humans. Vasoflux concentrations ≥40 μg/mL are effective in vitro and in rabbit arterial thrombosis models.†Hirudin doses of 0.1 to 0.2 mg · kg⁻¹ · h⁻¹ produce a 2- to 3-fold prolongation of the APTT.‡Dermatan sulfate doses of 2.9 or 8.6 mg · kg⁻¹ · h⁻¹ (which produced plasma levels of 3 to 8 μg/mL) were significantly better than placebo at reducing postoperative deep vein thrombosis in hip surgery patients.

(data not shown). Clinically relevant concentrations of heparin prolong the dilute prothrombin time to a greater extent than LMWH (data not shown), suggesting that inactivation of thrombin is more important for this activity than factor Xa inhibition.

Vasoflux has almost no anti–factor Xa activity, consistent with its low affinity for antithrombin (Figure 5). In contrast, LMWH addition to plasma produces a concentration-dependent increase in anti–factor Xa activity. These data are supported by the observation that the specific anti–factor Xa activities of Vasoflux and LMWH are 0.9 and 96 U/mg, respectively.

In control plasma (Figure 4) and plasma immunodepleted of HCII and antithrombin (Figure 6), Vasoflux prolongs the aPTT in a concentration-dependent fashion. LMWH prolongs the aPTT to a greater extent than Vasoflux (Figure 4), reflecting its ability to catalyze factor Xa. However, thrombin inactivation by antithrombin because LMWH has less effect on the aPTT, regardless of when it is added (Figure 4), consistent with the concept that it prolongs the aPTT by inhibiting factor Xa and thrombin.

**Figure 4.** Effects of Vasoflux and LMWH on dilute prothrombin time (▲), aPTT (●), and modified aPTT (■) ratios. Points represent means of 2 experiments, each done in duplicate; bars reflect 95% CI. Linear regression analysis shows significant (P<0.001) differences among slopes of all lines.

**Figure 6.** Comparison of effects of Vasoflux and LMWH on aPTT ratio in control plasma (●) and plasma immunodepleted of antithrombin and HCII (■). Points represent means of 2 experiments, each done in duplicate; bars reflect 95% CI. Linear regression analysis shows significant (P<0.001) differences among slopes of all lines.

The observation that Vasoflux prolongs the aPTT but not the prothrombin time suggests that it targets the intrinsic pathway, a concept supported by its lack of anti–factor Xa activity and its minimal effect on the thrombin clotting time. We therefore performed a modified aPTT in which micronized silica and phospholipid are incubated in plasma for 5 minutes before recalcification in the absence or presence of Vasoflux. Preincubation results in activation of the contact system and generation of factor Xa, which triggers clotting on addition of calcium. When Vasoflux is added after preincubation, it has less effect on the aPTT than when it is present throughout (Figure 4). This suggests that a small part of its effect is to inhibit contact activation. LMWH has almost the same effect on the aPTT, regardless of when it is added (Figure 4), consistent with the concept that it prolongs the aPTT by inhibiting factor Xa and thrombin.

**Effect of Vasoflux on Factor Xa Generation**

To explore the antithrombin- and HCII-independent mechanism by which Vasoflux prolongs the aPTT, we examined its capacity to inhibit factor IXa–mediated factor Xa generation in plasma immunodepleted of antithrombin and prothrombin. Vasoflux blocks factor Xa generation (Figure 8), whereas clinically relevant concentrations of unfractionated heparin (0.5 or 1.0 U/mL; ie, 3 to 6 μg/mL) or LMWH (5 to 10 μg/mL; data not shown) have only minimal effects on factor Xa generation in the absence of antithrombin. The sites of
Vasoflux action are compared with those of unfractionated heparin and LMWH in Figure 9.

**Affinity of Vasoflux for Factor IXa/IXa**

On the basis of intrinsic fluorescence measurements, Vasoflux binds to factor IX and factor IXa with $K_d$ values of 8.2 and 7.2 $\mu$mol/L, respectively (data not shown), values similar to those for unfractionated heparin ($K_d$ values of 4.2 and 4.0 $\mu$mol/L, respectively) and LMWH ($K_d$ values of 5.9 and 6.4 $\mu$mol/L, respectively).

**Comparison of Vasoflux With Other Anticoagulants in an Extracorporeal Circuit**

When human blood is circulated through a thrombogenic 40-$\mu$m blood filter (Figure 10), unfractionated heparin and LMWH must be used in concentrations of 2.0 and 8.0 U/mL, respectively, to maintain filter patency and prevent fibrinogen consumption, concentrations that produce a starting ACT of 720 and 415 seconds, respectively (Table 2). Vasoflux also prolongs the time to filter failure and prevents fibrinogen consumption, and at 125 $\mu$g/mL, a concentration that produces a starting ACT of 220 seconds, Vasoflux maintains filter patency and reduces fibrinogen consumption to background levels.

To maintain filter patency and to prevent fibrinogen consumption, 8 $\mu$g/mL hirudin is required, a concentration that produces an unmeasurable ACT. Dermatan sulfate is ineffective, even at concentrations that produce a starting ACT of 360 seconds. These observations indicate that inactivation of free and fibrin-bound thrombin is insufficient to prevent clotting in this thrombogenic circuit.

When a neutralizing antibody against HCII is added (Table 3), the Vasoflux concentration needed to maintain filter patency is increased from 125 to 360 $\mu$g/mL, although a concentration of 300 $\mu$g/mL is partially effective. However, even with these high Vasoflux concentrations, fibrinogen consumption is not totally blocked. These findings indicate that the HCII-dependent and -independent activities of Vasoflux are both important determinants of its antithrombotic effect.

**Discussion**

Vasoflux, a derivative of heparin, is a new antithrombotic agent with novel properties. Whereas heparin and LMWH...
Hirudin, m
Heparin, U/mL
Dermatan sulfate, m

tion of factor Xa and thrombin by antithrombin, 29,30 Vasoflux
exert their anticoagulant activity by catalyzing the inactiva-

TABLE 2. Effectiveness of Vasoflux, Heparin, LMWH, Hirudin, or Dermatan Sulfate in an Extracorporeal Circuit

<table>
<thead>
<tr>
<th>Addition</th>
<th>Starting ACT, s</th>
<th>Time to Filter Failure, min</th>
<th>Fibrinogen Consumption, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vasoflux, μg/mL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>75</td>
<td>200</td>
<td>18</td>
<td>85</td>
</tr>
<tr>
<td>125</td>
<td>220</td>
<td>&gt;90</td>
<td>10</td>
</tr>
<tr>
<td>175</td>
<td>240</td>
<td>&gt;90</td>
<td>10</td>
</tr>
<tr>
<td>Heparin, U/mL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td>225</td>
<td>12</td>
<td>&gt;90</td>
</tr>
<tr>
<td>1.0</td>
<td>300</td>
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<tr>
<td>1.5</td>
<td>480</td>
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<tr>
<td>2.0</td>
<td>720</td>
<td>&gt;90</td>
<td>31</td>
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<tr>
<td>LMWH, U/mL</td>
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<tr>
<td>2.0</td>
<td>200</td>
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<td>&gt;90</td>
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<tr>
<td>4.0</td>
<td>254</td>
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<td>&gt;90</td>
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<tr>
<td>6.0</td>
<td>402</td>
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<td>86</td>
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<tr>
<td>8.0</td>
<td>415</td>
<td>&gt;90</td>
<td>24</td>
</tr>
<tr>
<td>Hirudin, μg/mL</td>
<td></td>
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</tr>
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<td>2</td>
<td>300</td>
<td>30</td>
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<td>4</td>
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<tr>
<td>8</td>
<td>&gt;1500</td>
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<tr>
<td>Dermatan sulfate, μg/mL</td>
<td></td>
<td></td>
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</tr>
<tr>
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<td>&gt;90</td>
</tr>
<tr>
<td>2000</td>
<td>320</td>
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</tr>
<tr>
<td>4000</td>
<td>360</td>
<td>30</td>
<td>80</td>
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exert their anticoagulant activity by catalyzing the inactiva-

TABLE 3. Effect of an Inhibitory IgG Against HCII (+) Compared With Control IgG (−) on the Effectiveness of Vasoflux in an Extracorporeal Circuit

<table>
<thead>
<tr>
<th>Vasoflux Concentration, μg/mL</th>
<th>Starting ACT, s</th>
<th>Time to Filter Failure, min</th>
<th>Fibrinogen Consumption, %</th>
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</thead>
<tbody>
<tr>
<td>0</td>
<td>138</td>
<td>-</td>
<td>-</td>
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<tr>
<td>240</td>
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<td>246</td>
<td>-</td>
</tr>
<tr>
<td>300</td>
<td>334</td>
<td>279</td>
<td>-</td>
</tr>
<tr>
<td>360</td>
<td>357</td>
<td>300</td>
<td>-</td>
</tr>
</tbody>
</table>

Vasoflux, a New Anticoagulant
thrombin but also blocks factor Xa generation. Vasoflux is better than heparin and LMWH because it inactivates fibrin-bound thrombin. With minimal effects on fluid-phase thrombin and little anti-factor Xa activity, Vasoflux is less likely than heparin or LMWH to interfere with the burst of thrombin generated when blood is exposed to high concentrations of tissue factor found in the adventitia of severed vessels. Because Vasoflux inhibits fibrin-bound thrombin to a greater extent than fluid-phase thrombin, Vasoflux is likely to cause less bleeding than hirudin, a concept supported by preliminary studies comparing the relative efficacy and safety of these agents in a rabbit arterial thrombosis model.26

Vasoflux is currently being compared with heparin as an adjunct to streptokinase in patients with acute myocardial infarction, with 90-minute infarct-related artery patency used as the efficacy end point. Once the effective dose of Vasoflux is established, a large phase III trial will be needed to determine whether Vasoflux will improve clinical outcome when used as an adjunct to thrombolytic therapy.

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References
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