

# Recombinant activated factor VII effectively reverses the anticoagulant effects of heparin, enoxaparin, fondaparinux, argatroban, and bivalirudin *ex vivo* as measured using thromboelastography

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Bleeding is the major adverse reaction to anticoagulants, leading to significant morbidity and even mortality. Protamine is a specific antidote for heparin yet is only partially effective for enoxaparin, and the activated factor X inhibitor fondaparinux and the direct thrombin inhibitors argatroban and bivalirudin lack specific antidotes. We evaluated the ability of recombinant activated factor VII (rFVIIa), a general hemostatic agent, to reverse the anticoagulant effects of heparin, enoxaparin, fondaparinux, argatroban, and bivalirudin, as measured by thromboelastography. Whole-blood samples containing each test anticoagulant, with or without rFVIIa 1.5–4.5 µg/ml, were prepared *ex vivo* ( $n \geq 48$ , each anticoagulant) and analyzed by thromboelastography. The thromboelastography parameters of clot initiation, propagation, rigidity and elasticity were compared for the *ex-vivo* samples for each anticoagulant. The reversal ability of rFVIIa was also assessed using the standard clinical assay used to monitor each anticoagulant. Thromboelastography was performed on blood from eight stably anticoagulated patients, with and without exogenous rFVIIa. For each anticoagulant, rFVIIa significantly improved and, in some cases, completely normalized all thromboelastography parameters ( $P < 0.001$ ). rFVIIa significantly ( $P < 0.01$ ) decreased the activated partial

thromboplastin time for argatroban-containing, bivalirudin-containing, or heparin-containing blood yet did not affect the anti-activated factor X levels for enoxaparin-containing or fondaparinux-containing blood. By thromboelastography, rFVIIa exerted generally similar reversal effects on the anticoagulated patient samples as on the *ex-vivo* samples. In conclusion, rFVIIa effectively reverses the anticoagulant effects of heparin, enoxaparin, fondaparinux, argatroban, and bivalirudin, and should be considered for patients with excessive bleeding associated with these anticoagulants. *Blood Coagul Fibrinolysis* 18:547–553 © 2007 Lippincott Williams & Wilkins.

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## Introduction

The main adverse reaction to anticoagulants is bleeding, which can lead to significant complications including death [1]. The traditional anticoagulants, heparin and warfarin, have effective, specific antidotes in protamine and vitamin K, respectively, although the effect of vitamin K takes several hours. The more recently developed anticoagulants, however, including the direct thrombin inhibitors, argatroban and bivalirudin, and the antithrombin-dependent selective activated factor X (FXa) inhibitor fondaparinux, lack specific antidotes [1,2], and protamine has limited effectiveness as an antidote for low-molecular-weight heparin. The availability of a safe, effective agent with the ability to reverse the anticoagulant effects of these newer anticoagulants would reduce the likelihood of severe complications should bleeding occur.

Recombinant activated factor VII (rFVIIa) is an effective general hemostatic agent originally developed for the management of bleeding episodes in hemophilia patients with inhibitors [3]. Since its introduction, rFVIIa has been used for a wide variety of bleeding situations in both coagulopathic and noncoagulopathic patients [4]. Several reports have documented the ability of rFVIIa to reverse the effects of warfarin [5–7] in humans, and additional reports have demonstrated the ability of rFVIIa to reduce the bleeding effects of other anticoagulants including fondaparinux [8] and melagatran [9], a direct thrombin inhibitor. Only one study did not find a positive effect [10]; however, that study was conducted in rabbits, and it is known that human rFVIIa does not interact well with tissue factor from different animal species.

Thromboelastography assesses clot formation over time and measures several clot-related parameters including the time to clot initiation (reaction time), clot propagation (kinetic time and angle), peak clot rigidity (maximum amplitude) and clot elastic modulus [11]. These parameters allow for a much more complete characterization of the dynamics of clot formation than simple plasma-based assays such as the prothrombin time or activated partial thromboplastin time (aPTT). Thromboelastography is used clinically in the setting of cardiac and liver surgery and has been shown to be an effective way of assessing the effects of anticoagulants in these situations utilizing whole blood [12].

In our study, we evaluated the hypothesis that rFVIIa is effective at reversing the anticoagulant effects of heparin, enoxaparin, fondaparinux, argatroban, and bivalirudin, as measured using thromboelastography.

## Materials and methods

### Materials

Thromboelastography was conducted using the TEG 5000 Thromboelastograph Hemostasis Analyzer with TEG Analytical Software Versions 3 (initially) and 4 (Haemoscope, Niles, Illinois, USA). The upgraded software in no way affects the test results. Thromboelastography disposable cups and pins were purchased from Haemoscope. Innovin (tissue factor) was purchased from Dade Behring (Deerfield, Illinois, USA). Argatroban (Argatroban; GlaxoSmithKline, Philadelphia, Pennsylvania, USA) and bivalirudin (Angiomax; The Medicines Company, Parsippany, New Jersey, USA) were provided by their manufacturers; fondaparinux (Arixtra; GlaxoSmithKline) and enoxaparin (Lovenox; Aventis, Philadelphia, Pennsylvania, USA) were purchased. Unfractionated, porcine heparin (referred to as 'heparin' herein) and protamine sulfate (Abraxis Pharmaceutical Products, Schaumburg, Illinois, USA) were provided by our hospital pharmacy. rFVIIa (Novoseven; Novo Nordisk, Bagsvaerd, Denmark) and the placebo (rFVIIa vehicle) were provided by the manufacturer.

### Thromboelastography methods

#### Ex-vivo samples

After giving informed consent for participation in an Institutional Review Board-approved study, healthy adult volunteers who were on no medications and had no bleeding disorder provided the whole blood for thromboelastography. The blood was obtained via atraumatic venipuncture using a two-syringe technique; the first 3 ml was discarded, and the blood was then drawn into a plain plastic syringe. In preliminary experiments, samples were split and run in duplicate in both channels of the two thromboelastography devices used throughout the study. The duplicate results were always nearly identical with a coefficient of variation less than 1%. This led to the schema whereby each sample was split four ways as detailed below

**Table 1 Concentrations of medications used in all experiments<sup>a</sup>**

Medication	Final concentration
Heparin	0.11 IU/ml
Enoxaparin	1.6 µg/ml
Fondaparinux	1.25 µg/ml
Argatroban	0.45 µg/ml
Bivalirudin	4 and 12 µg/ml
Protamine	67 µg/ml
Recombinant activated factor VII	1.5, 3, and 4.5 µg/ml

<sup>a</sup>The concentrations for each anticoagulant are in the range seen for their most commonly used licensed indications. For bivalirudin, a lower concentration (4 µg/ml) than for its licensed indication was also employed in order to provide a better comparison with heparin and argatroban. Of note, heparin does not have a licensed indication but the concentration presented is one that would lead to therapeutic anticoagulation. See the prescribing information for each agent for details [13–18].

without running duplicates, allowing us to control for variation between each individual venipuncture. The data from the preliminary experiments are not included in the final data set.

From each venipuncture, 4 ml blood was aliquoted, with 1 ml placed into each of four polypropylene tubes. A test anticoagulant was added to three of the four tubes (one tube was native blood without anticoagulant) to the target final concentration (Table 1 [13–18]). After gentle mixing, dilute tissue factor (Innovin) at a final dilution of 1 : 17 000 was added to each tube, and the tubes gently mixed again. Finally, rFVIIa at a final concentration of 1.5, 3, or 4.5 µg/ml was added to one tube and an equal volume of placebo to another tube, followed by gentle mixing again. For experiments with heparin, we also assessed the effect of protamine 67 µg/ml (an appropriate antidote concentration) as a positive control in an identical manner to rFVIIa. The samples were placed into the thromboelastograph at 37°C within 4 min of venipuncture. Thus, each experiment consisted of four arms: no additions (native blood), anticoagulant, anticoagulant plus rFVIIa, and anticoagulant plus placebo (control). The thromboelastograph was run until maximum amplitude was achieved, and both the clot formation curve and the results for the parameters (reaction time, kinetic time, angle, maximum amplitude, and clot elastic modulus) were collected and recorded.

In preliminary studies, we used several concentrations of each anticoagulant in order to determine a concentration that resulted in a significant change from baseline of all the thromboelastography parameters indicating a marked anticoagulant effect. We then selected the concentration closest to the therapeutic concentration for the reversal experiments (Table 1). The concentrations used in the final experiments were in the therapeutic range for all the anticoagulants for their licensed indications (Table 1) [13–17]. We also employed a lower concentration of bivalirudin, which allowed for a more meaningful comparison with heparin and argatroban. The concentrations of rFVIIa tested were representative of that expected with doses of 90 to 270 µg/kg.

### Plasma assay methods

In addition to performing thromboelastography, we performed the same experiments as above using the laboratory test, when needed, to monitor the anticoagulant (aPTT for heparin, argatroban, and bivalirudin, and anti-FXa assays for enoxaparin and fondaparinux). For these experiments, blood was collected as described above and was placed into four 3.2% buffered sodium citrate glass tubes at a 1:9 ratio of citrate to whole blood. To each tube of whole blood, we added nothing, added anticoagulant, added anticoagulant plus rFVIIa 1.5  $\mu\text{g/ml}$ , or added anticoagulant plus placebo as described above. After gentle mixing, the plasma was separated, placed into polypropylene tubes, snap-frozen and kept at  $-70^{\circ}\text{C}$  until shipping. Once all the samples were prepared, they were collectively shipped to the Blood Center of Wisconsin's hemostasis reference laboratory on dry ice where these assays were performed as described below. All samples arrived frozen to the reference laboratory.

The aPTTs were performed using Platelin LS thromboplastin on a Dade Behring BCS coagulation analyzer. The anti-FXa assays were performed utilizing a method of analysis of accelerating drugs of antithrombin with the addition of standard amounts of antithrombin and FXa and patient plasma as the source of the drug followed by a chromogenic assay for residual FXa activity. The residual activity is inversely proportional to the drug level in patient plasma. Enoxaparin or fondaparinux as appropriate are used to generate the standard curves. Results for enoxaparin are expressed as anti-FXa units while results for fondaparinux were expressed as milligrams per liter.

### In-vivo samples

The present portion of the study was separately approved by the Institutional Review Board, and patients/parents gave informed consent before blood sampling. A venous blood sample was drawn in the same manner as described above from patients being treated for deep vein thrombosis on anticoagulation with argatroban, bivalirudin, enoxaparin, or fondaparinux (of note, the use of argatroban and bivalirudin for treatment of deep vein thrombosis is not a licensed indication). The aPTTs or anti-FXa levels were in the therapeutic range at the time the sample was drawn. The sample was divided into three aliquots in order to perform the same experiments with tissue factor activation as described above for the ex-vivo samples. To each of the three aliquots, we added either nothing (native blood, which in this case was already anticoagulated), rFVIIa 1.5  $\mu\text{g/ml}$ , or placebo at an equal volume to the rFVIIa. TEG was run within 4 min of sampling as described in the Thromboelastography methods section.

### Statistical analysis

Statistical analyses were performed using STATA statistical software version 9 (College Station, Texas, USA).

Statistical tests were two-sided and conducted at the 0.05 level of significance. The analysis compared the thromboelastography parameters of the native blood with the anticoagulant, the anticoagulant plus rFVIIa, or the anticoagulant plus placebo. Since no concentration-response effect of rFVIIa could be demonstrated, we combined the results of all the rFVIIa concentrations for the analysis.

Each experiment included the values for the outcome variables reaction time, kinetic time, angle, maximum amplitude, and clot elastic modulus for native blood, for anticoagulant, for anticoagulant plus rFVIIa, and for anticoagulant plus placebo (control). The results for each anticoagulant and each outcome variable were analyzed separately. The primary analysis method was a main effects mixed linear model. In this analysis, each experiment was a random effect and anticoagulant, anticoagulant plus rFVIIa, and anticoagulant plus placebo were the three fixed effects. The baseline was used as the reference value. Multiple testing was performed between the three fixed effects using the Wald test.

## Results

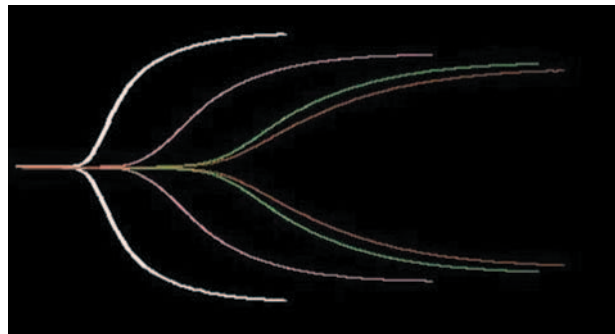
### Ex-vivo samples

A total of 11 volunteers provided blood samples for the ex-vivo experiments. The following number of experiments was run for each anticoagulant (an experiment is defined as one venipuncture with each of the four study arms): heparin, 48 experiments; enoxaparin, 48 experiments; fondaparinux, 48 experiments; argatroban, 112 experiments; bivalirudin (standard concentration), 80 experiments; and bivalirudin (low concentration), 64.

Each anticoagulant led to a significant prolongation of the reaction time and the kinetic time while the antithrombin-dependent agents (heparin, enoxaparin, and fondaparinux) affected all the thromboelastography parameters, as has been described elsewhere [19]. Reversal of the anticoagulant effect was assessed qualitatively by noting the restoration (or near restoration) of a normal clot formation curve; that is, the clot signature curve of the anticoagulant plus rFVIIa was similar to that of native blood. The control curve (with the rFVIIa placebo) was similar to the curve with the anticoagulant alone, as expected (Figs 1–5). The reversal effect was assessed quantitatively by analyzing the five thromboelastography parameters and the appropriate plasma assays with each of the four arms of the experiments (Table 2). There was no difference in the results (curves or parameters) as the rFVIIa concentration was increased from 1.5 to 4.5  $\mu\text{g/ml}$ .

rFVIIa reversed the effects of each test anticoagulant; however some differences were noted among the responses of individual anticoagulants (Figs 1–5 and Table 2). With respect to the antithrombin-dependent agents, reversal of the effects of enoxaparin and

**Fig. 1**

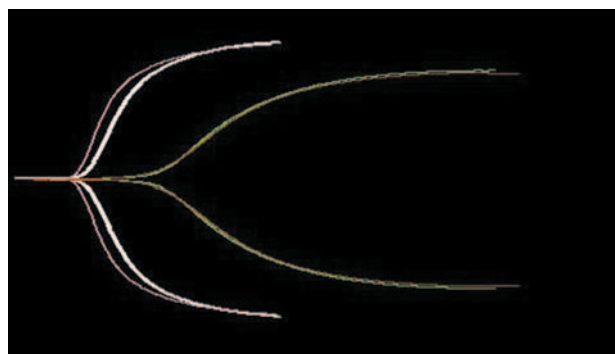


Representative clot signature for experiment with heparin. White, baseline (native blood); green, heparin; pink, heparin plus recombinant activated factor VII; red, heparin plus placebo.

fondaparinux was complete; that is, the curve with anti-coagulant plus rFVIIa was identical to that of the native blood, whereas the reversal of heparin was partial. Of note, protamine completely reversed the heparin effect (data not shown). For the direct thrombin inhibitors, rFVIIa statistically significantly reversed the anticoagulant effects of argatroban and both concentrations of bivalirudin, although the reversal was qualitatively less profound for the standard concentration of bivalirudin (see Figs 4–6). Our experiments did not allow for a direct comparison of the reversal effect of rFVIIa for any one anticoagulant versus another.

The results for the plasma assays (Table 2) demonstrated that rFVIIa was effective at partially correcting the effects of heparin, argatroban, and both concentrations of bivalirudin as measured by the aPTT. No effect, however, could be demonstrated for enoxaparin or fondaparinux as assessed by the anti-FXa assay.

**Fig. 2**



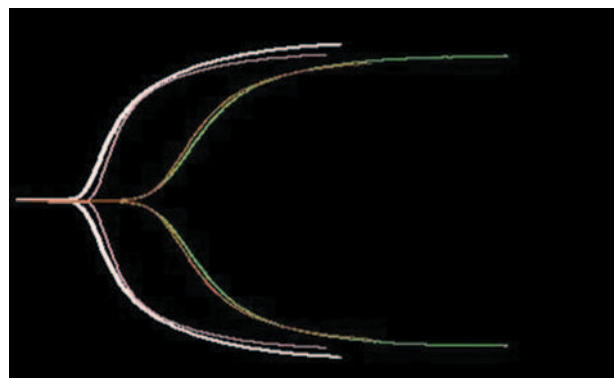
Representative clot signature for experiment with enoxaparin. White, baseline (native blood); green, enoxaparin; pink, enoxaparin plus recombinant activated factor VII; red, enoxaparin plus placebo.

**Fig. 3**



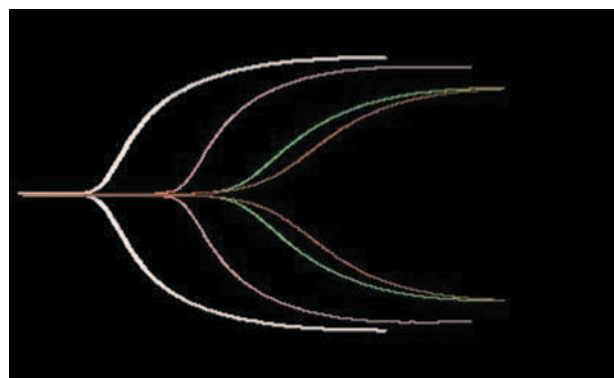
Representative clot signature for experiment with fondaparinux. White, baseline (native blood); green, fondaparinux; pink, fondaparinux plus recombinant activated factor VII; red, fondaparinux plus placebo.

**Fig. 4**



Representative clot signature for experiment with argatroban. White, baseline (native blood); green, argatroban; pink, argatroban plus recombinant activated factor VII; red, argatroban plus placebo.

**Fig. 5**



Representative clot signature for experiment with bivalirudin—standard concentration. White, baseline (native blood); green, bivalirudin; pink, bivalirudin plus recombinant activated factor VII; red, bivalirudin plus placebo.

Table 2 Quantitative results for thromboelastography analyses

Parameter	Native blood	Anticoagulant	Anticoagulant + rFVIIa	Anticoagulant + control
<b>Heparin</b>				
<i>R</i> (min)*	7.2 (0.9)	23.1 (4.2)	11.6 (1.3)	25.7 (2.2)
<i>K</i> (min)*	2.3 (0.3)	10.6 (2.6)	3.1 (2.6)	11.7 (2.3)
Angle (deg)*	57.1 (5.5)	21.1 (3.8)	48.9 (15.1)	20.4 (2.9)
<i>MA</i> (mm)*	60.5 (3.6)	45.8 (5.6)	58.2 (2)	43.8 (2.6)
<i>G</i> (dynes/cm <sup>2</sup> )*	8.4 (0.8)	4 (0.4)	7.1 (0.6)	3.7 (0.3)
aPTT (s) <sup>†</sup>	30.2 (2.1)	79.5 (11.2)	49.4 (5.9)	78.5 (10.2)
<b>Enoxaparin</b>				
<i>R</i> (min)*	9 (0.7)	21.5 (2)	8.5 (0.5)	20.8 (1.6)
<i>K</i> (min)*	3.3 (0.2)	9.9 (1.1)	3.1 (0.8)	9.2 (0.3)
Angle (deg)*	55.2 (2)	20.1 (1.8)	55.4 (3)	20.8 (1.8)
<i>MA</i> (mm)*	58.7 (2.2)	29.5 (5.6)	56.1 (2.7)	28 (2.7)
<i>G</i> (dynes/cm <sup>2</sup> )*	7.8 (0.3)	2.5 (0.7)	7.7 (0.4)	2.6 (0.4)
Anti-activated factor X (IU/ml) <sup>‡</sup>	<0.1	0.48 (0.08)	0.48 (0.07)	0.47 (0.08)
<b>Fondaparinux</b>				
<i>R</i> (min)*	7.6 (1)	28.5 (3.4)	8.2 (1.2)	28.2 (2.2)
<i>K</i> (min)*	2.8 (0.4)	18.3 (5)	3.8 (0.4)	20 (3.1)
Angle (deg)*	54.4 (2)	12.7 (3.3)	54 (2.7)	13 (3.3)
<i>MA</i> (mm)*	60.4 (2.5)	25.1 (5.3)	59.1 (2.6)	25.3 (3)
<i>G</i> (dynes/cm <sup>2</sup> )*	8.1 (0.5)	1.9 (0.4)	7.9 (0.4)	1.9 (0.4)
Fondaparinux (mg/l) <sup>‡</sup>	<0.1	2.48 (0.3)	2.42 (0.3)	2.45 (0.3)
<b>Argatroban</b>				
<i>R</i> (min)*	7.8 (1.2)	16.4 (1.6)	9.3 (0.9)	16.3 (1.8)
<i>K</i> (min)*	2.8 (0.4)	5 (0.6)	2.8 (0.5)	4.9 (0.6)
Angle (deg)*	54.6 (2.7)	39 (3.1)	56 (5.2)	39.5 (4.4)
<i>MA</i> (mm)*	63.2 (2.2)	56.1 (2.3)	59.8 (1.9)	55.2 (1.8)
<i>G</i> (dynes/cm <sup>2</sup> )*	8.7 (0.8)	6.4 (0.4)	8 (0.7)	6.4 (0.4)
aPTT (s) <sup>†</sup>	30.2 (2.1)	77.7 (9.4)	66.3 (6.8)	77.1 (8.8)
<b>Bivalirudin, standard concentration</b>				
<i>R</i> (min)*	8.5 (1.3)	34.7 (4)	19.3 (2.3)	32.7 (4.1)
<i>K</i> (min)*	3.1 (0.7)	9.1 (1.5)	4 (0.8)	8.7 (1)
Angle (deg)*	51.2 (5.8)	24.9 (5.7)	43.8 (7.2)	26.9 (5.4)
<i>MA</i> (mm)*	60.9 (6.3)	44.6 (5.2)	53.3 (3.7)	43.5 (4.1)
<i>G</i> (dynes/cm <sup>2</sup> )*	8.4 (0.8)	3.8 (0.7)	6.7 (0.8)	4.1 (0.6)
aPTT (s) <sup>†</sup>	30.2 (2.1)	175.4 (18.6)	160.4 (16.3)	179.8 (17.8)
<b>Bivalirudin, low concentration</b>				
<i>R</i> (min)*	8.4 (1.4)	15.7 (1.5)	8.6 (1.1)	16.1 (1.7)
<i>K</i> (min)*	3.1 (0.5)	5.2 (0.5)	2.9 (0.7)	5 (0.4)
Angle (deg)*	53.2 (2.9)	44 (2.9)	56.8 (4.8)	42.2 (3.8)
<i>MA</i> (mm)*	62.2 (2.4)	58.4 (2.5)	60.3 (2.1)	57.8 (1.9)
<i>G</i> (dynes/cm <sup>2</sup> )*	8.7 (0.8)	6.5 (0.4)	8.2 (0.7)	6.6 (0.8)
aPTT (s) <sup>†</sup>	31.8 (2.4)	74.5 (8.7)	65.7 (6.5)	75.9 (8.3)

Data presented as the mean (SD). rFVIIa, recombinant activated factor VII; *R* (reaction time), time to clot initiation; *K* (kinetic time) and angle, clot propagation; *MA* (maximum amplitude), peak clot rigidity; *G*, clot elastic modulus; aPTT, activated partial thromboplastin time.

\*For each thromboelastography parameter,  $P < 0.001$  for native blood versus anticoagulant and for anticoagulant versus anticoagulant + rFVIIa, and  $P > 0.05$  for native blood versus anticoagulant + rFVIIa and for anticoagulant versus anticoagulant + control.

<sup>†</sup> $P < 0.01$  for anticoagulant versus anticoagulant + rFVIIa. <sup>‡</sup> $P < 0.001$  for native blood versus the other three groups;  $P > 0.05$  for comparisons among the three anticoagulant-containing groups.

### In-vivo samples

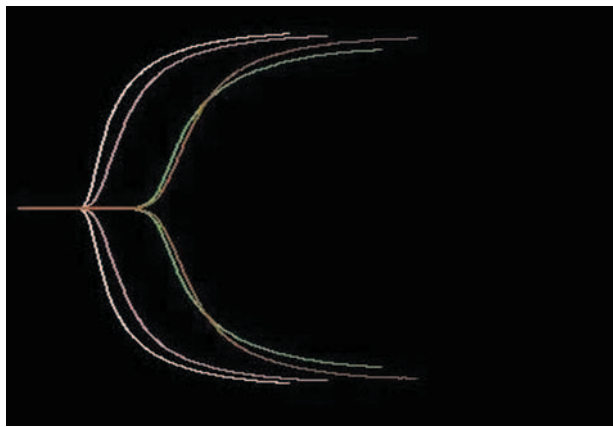
We performed thromboelastography on the blood of nine patients stably anticoagulated on heparin ( $n = 2$ ), bivalirudin ( $n = 3$ ), argatroban ( $n = 2$ ), enoxaparin ( $n = 1$ ) or fondaparinux ( $n = 1$ ), with and without ex-vivo addition of rFVIIa or placebo. The thromboelastography results were largely similar to those of the ex-vivo experiments (data not shown). In these experiments, there was complete restoration of the normal clot curve for all the samples from patients on bivalirudin, argatroban, enoxaparin, and fondaparinux, and a near complete restoration for heparin. The complete correction of the thromboelastography clot curve for the in-vivo bivalirudin samples suggests that the concentration of medication in these patients correlates with the low concentration used in the ex-vivo studies (serum bivalirudin levels were not

done). This can be explained by the fact that these patients were receiving bivalirudin for treatment of deep vein thrombosis and had aPTTs in the range of 56–75 s, which is similar to the aPTT results for the low concentration of bivalirudin. Although there were too few samples to perform a meaningful statistical analysis, when taken together, these in-vivo data support the ex-vivo data and suggest a potential utility for rFVIIa to be an effective nonspecific antidote for patients stably anticoagulated with the above agents.

### Discussion

Several new anticoagulants have recently been introduced for a variety of indications, and these medications have improved pharmacokinetic properties over heparin and warfarin, and thus their use is increasing. A major

Fig. 6



Representative clot signature for experiment with bivalirudin—low concentration. White, baseline (native blood); green, bivalirudin; pink, bivalirudin plus recombinant activated factor VII; red, bivalirudin plus placebo.

limitation of these agents as compared with heparin and warfarin is the lack of an effective, specific antidote or reversal agent. If significant bleeding occurs while patients are receiving these agents, therefore, therapeutic options to eliminate these agents or reverse their anticoagulant effect are limited. The availability of an agent that can safely, effectively, and rapidly reverse the anticoagulant effect would reduce the likelihood of severe complications, even death, if bleeding occurs [20].

In the present study, we demonstrated the ability of rFVIIa to reverse the effects, as measured by thromboelastography, of heparin, enoxaparin, argatroban, bivalirudin, and fondaparinux on human whole blood. Thromboelastography demonstrated that all clot characteristics (clot initiation and propagation, clot rigidity, and clot elasticity) revert to normal or are significantly improved following addition of rFVIIa to anticoagulated blood. Although rFVIIa improved the clot characteristics of heparinized blood, it was not as effective as protamine, which led to a complete reversal. The reversal of the effects of the standard concentration of bivalirudin (the typical concentration during percutaneous coronary interventions) was less complete than for the lower concentration of bivalirudin or for argatroban (Table 2 and Figs 4–6), although direct statistical comparisons were not made. This is probably due to the higher intensity of anticoagulation achieved with this concentration of bivalirudin (note the differences in the aPTTs). We employed the higher concentration in this study in an attempt to mimic the clinical situation in which these agents are indicated (treatment of heparin-induced thrombocytopenia for argatroban and percutaneous coronary interventions for bivalirudin). Of note, the in-vivo samples of bivalirudin had an aPTT similar to the aPTT of the ex-vivo low

concentration of bivalirudin and argatroban experiments, and in both of these situations there was nearly complete reversal of the thromboelastography curves with rFVIIa. It is therefore reasonable to conclude that the ability of rFVIIa to reverse the anticoagulant effects of argatroban and bivalirudin as measured by thromboelastography are similar as long as the intensity of anticoagulation is in the same range.

We also performed plasma-based coagulation assays as appropriate for each anticoagulant (aPTT or anti-FXa activity) utilizing the same schema as for the thromboelastography. There was a statistically significant, albeit partial, correction of the aPTT for heparin, argatroban, and both concentrations of bivalirudin with rFVIIa, but no effect on the anti-FXa assays for enoxaparin and fondaparinux. Interestingly, thromboelastography demonstrated complete reversal of the anticoagulant effect of enoxaparin and fondaparinux in all experiments. This suggests that the aPTT and anti-FXa assays are not sensitive to the effects of rFVIIa as a reversal agent for anticoagulant effects. This is probably secondary to the mechanism of action of rFVIIa, which functions best in the presence of tissue factor and platelets, both of which were present in the thromboelastography experiments but absent in the plasma-based assays. We thus believe that, relative to the aPTT and anti-FXa assay, thromboelastography is a more sensitive way to measure the effects of rFVIIa as a reversal agent for anticoagulants.

Several previous studies have assessed the ability of rFVIIa to reduce the bleeding effects of a variety of anticoagulants using several different methods [21]. There are several reports [5–7] demonstrating the ability of rFVIIa to reverse the anticoagulant effect of vitamin K antagonists, a drug class that we did not evaluate. Clinical trials have demonstrated the efficacy of rFVIIa to reverse the effect of fondaparinux as assessed by the thrombin generation time, endogenous thrombin potential, aPTT, and prothrombin time [8]. The only study assessing rFVIIa as an antidote to low-molecular-weight heparin was an animal study that did not demonstrate a reversal effect [10], which was probably due to the fact that human rFVIIa does not interact well with animal tissue factor. Regarding direct thrombin inhibitors, the only clinical study involved the use of melagatran as the anticoagulant [9], and rFVIIa at a dose of 90  $\mu\text{g}/\text{kg}$  did not reverse various measures of anticoagulation including the endogenous thrombin potential, prothrombin time, and aPTT. A single case report describes the use of rFVIIa to control excessive bleeding in a child on argatroban and did not suggest a rapid reversal of the anticoagulant effect of argatroban at a rFVIIa dose of 90  $\mu\text{g}/\text{kg}$  [22]. There are no formal studies involving rFVIIa with argatroban, bivalirudin or heparin. Other studies have demonstrated the ability of another hemostatic agent used in hemophilia patients with inhibitors (Factor Eight Inhibitor Bypass Activity; Baxter

Bioscience, Westlake Village, California, USA) to reduce the bleeding time in animals injected with melagatran [23].

The limitations of this study include the fact that we had few in-vivo samples to test, although the results from those samples were mostly consistent with the ex-vivo spiking experiments. It would be informative to determine whether the same results could be obtained from a larger number of samples and, more importantly, with in-vivo administration of rFVIIa. Second, while thromboelastography is an effective assay for assessing clot formation, the assay is performed in static conditions (i.e. without shear). Whether the results are applicable to flowing blood remains to be determined.

In conclusion, rFVIIa reversed the anticoagulant effect of all anticoagulants tested as assessed by thromboelastography. It also reversed the effect of heparin, argatroban, and bivalirudin as assessed by the aPTT. rFVIIa should be considered for the management of anticoagulation-related bleeding due to enoxaparin (if protamine fails), fondaparinux, argatroban, and bivalirudin.

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