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# Ability of Recombinant Factor VIIa to Reverse the Anticoagulant Effect of the Pentasaccharide Fondaparinux in Healthy Volunteers

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**Background**—The novel anticoagulant fondaparinux proved to be effective and safe in the postoperative prevention of venous thrombosis. Current phase III trials with this synthetic selective factor Xa inhibitor focus on its use in the treatment of patients with venous and arterial thrombosis. As with any anticoagulant therapy, there is a risk of bleeding complications; hence, a strategy to reverse the effects of fondaparinux is desirable. The aim of this study was to investigate whether recombinant factor VIIa (rFVIIa) could neutralize the anticoagulant effects of subcutaneously administered fondaparinux.

**Methods and Results**—In a randomized, placebo-controlled design, 16 healthy male subjects received either a single subcutaneous dose of fondaparinux (10 mg) and a single intravenous bolus of rFVIIa (90  $\mu\text{g}/\text{kg}$ ;  $n=8$ ), fondaparinux and placebo ( $n=4$ ), or placebo and rFVIIa ( $n=4$ ). Fondaparinux (or placebo) was administered 2 hours before rFVIIa (or placebo). Injection of rFVIIa after fondaparinux normalized the prolonged activated partial thromboplastin and prothrombin times and reversed the decrease in prothrombin activation fragments 1+2 ( $F_{1+2}$ ), as observed with fondaparinux alone. Thrombin-generation time and endogenous thrombin potential, which were inhibited by fondaparinux, normalized up to 6 hours after rFVIIa injection.

**Conclusions**—rFVIIa is capable of normalizing coagulation times and thrombin generation during fondaparinux treatment. The duration of this effect ranged from 2 to 6 hours after rFVIIa injection. These results suggest that rFVIIa may be useful to reverse the anticoagulant effect of fondaparinux in case of serious bleeding complications or need for acute surgery during treatment with fondaparinux. (*Circulation*. 2002;106:2550-2554.)

**Key Words:** anticoagulants ■ pharmacokinetics ■ pharmacology ■ hemorrhage ■ thrombosis

Novel anticoagulant agents aim at improved efficacy and safety by a more selective inhibition of the coagulation cascade, for example, targeting thrombin, as achieved by hirudin (-analogues),<sup>1,2</sup> or directing their activity at the factor VIIa-tissue factor complex by recombinant nematode anticoagulant protein c2 (NAPc2) or recombinant tissue factor pathway inhibitor.<sup>3-5</sup> Recently, fondaparinux, a novel selective factor Xa inhibitor, has been evaluated for the prevention and treatment of venous and arterial thrombosis.<sup>6-10</sup> Fondaparinux is a synthetic pentasaccharide that binds exclusively to the activation site of antithrombin, thereby increasing its activity toward factor Xa inactivation 300-fold.<sup>11,12</sup> In contrast to the other antithrombin-dependent anticoagulants, ie, unfractionated heparin and low-molecular-weight heparin, fondaparinux selectively inactivates factor Xa without thrombin inhibition. Fondaparinux was superior to low-molecular-

weight heparin in the prevention of venous thrombosis after elective major knee surgery and hip-fracture surgery, reducing the incidence of this complication by an average of 56%.<sup>7,8</sup> Currently, phase III trials explore the use of fondaparinux in the treatment of venous thromboembolism and acute coronary syndromes.<sup>9,10</sup>

The potential drawback of any anticoagulant agent is the risk of bleeding complications. Fondaparinux has a biological half-life of  $\approx 17$  hours, and a strategy to reverse the anticoagulant state in case of life-threatening bleeding or for acute surgery appears desirable. A candidate for reversal of the anticoagulant effect of fondaparinux is recombinant factor VIIa (rFVIIa), which has the ability to normalize prothrombin times in subjects taking warfarin<sup>13</sup> and to restore thrombin generation during inhibition of the tissue factor-factor VIIa complex.<sup>14</sup> Thus, we investigated in healthy male volunteers

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whether rFVIIa is able to neutralize the anticoagulant effect of 10 mg of fondaparinux, which is 4 times the dose used in prophylactic treatment of venous thromboembolism.

**Methods**

**Subject Selection**

Healthy male subjects aged 18 to 45 years with a body mass index between 18 and 30 kg/m<sup>2</sup> and a maximum weight of 100 kg were eligible for the study. Subjects with a personal or family history of thrombosis or bleeding disorders were excluded. All subjects gave written informed consent. The study was approved by the Medical Ethics Committee of the Academic Medical Center, Amsterdam, the Netherlands. The study protocol was conducted in accordance with the International Conference on Harmonization of Good Clinical Practice Guidelines.<sup>15</sup>

**Study Drugs**

Subjects were randomized to 1 of 3 treatment strategies: (1) fondaparinux and placebo (n=4), (2) fondaparinux and rFVIIa (n=8), or (3) placebo and rFVIIa (n=4). Randomization was single blind (for subjects) for fondaparinux and double blind for rFVIIa. Fondaparinux (10 mg; Arixtra, Organon/Sanofi-Synthelabo) or placebo was administered as a single subcutaneous dose of 0.8 mL. Two hours after study drug administration, rFVIIa (Novo Seven, Novo Nordisk) 90 µg/kg or an equal amount of placebo was given as an intravenous bolus injection.

**Blood Sampling**

Blood samples were collected before fondaparinux or placebo administration (t=0) and 1.5, 2 (just before rFVIIa administration), 2.5, 3, 3.5, 4, 5, 6, 8, and 24 hours thereafter. At each sampling, the first 5 mL of blood was discarded, after which 9 mL was collected in tubes containing 1 mL of citrate (final concentration 0.32%), and

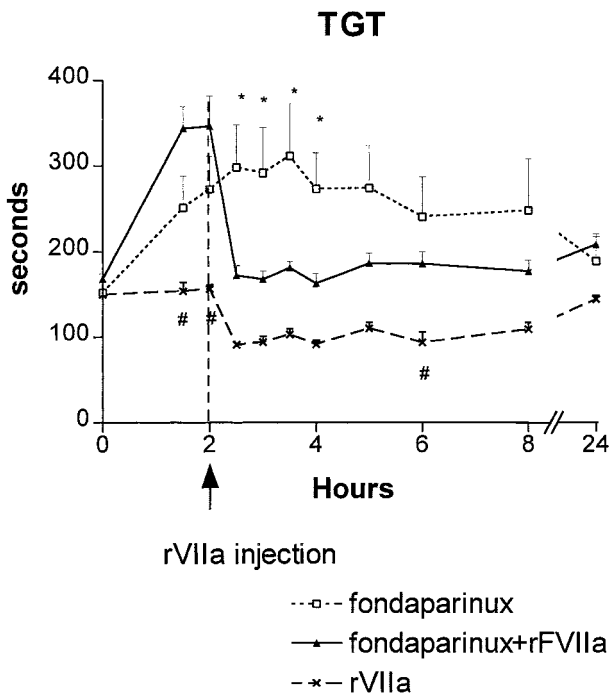
5 mL was collected in K3 EDTA evacuated container tubes. Blood was centrifuged at 2200g for 20 minutes at 18°C. Plasma was separated, pooled, and filled out in cryocups and frozen at -80°C until analysis was performed. These procedures were completed within 1 hour after blood sampling.

**Assays**

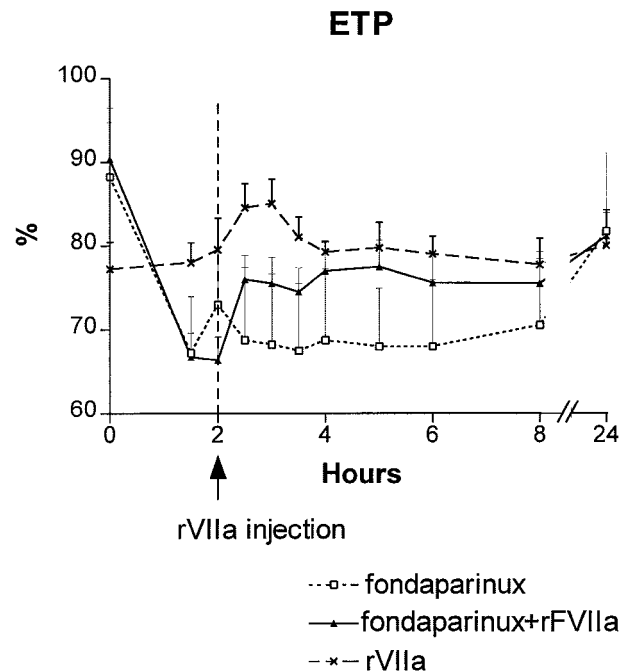
Thrombin-generation time was measured spectrophotometrically by the fibrin polymerization method. Thrombin generation was initiated by the addition of calcium and recombinant tissue factor (5000× diluted prothrombin time concentration), and results were expressed as T 1/2 (time to reach the midpoint of clear to maximal turbid density). The endogenous thrombin potential was determined as described previously.<sup>16</sup> In short, thrombin potential was determined amidolytically at 37°C in defibrinated plasma containing phospholipids, tissue factor, and calcium. Results were expressed as a percentage of standard pooled plasma. Plasma concentration of prothrombin fragment 1+2 (F<sub>1+2</sub>) was measured by sandwich-type ELISA assay (Dade-Behring). Activated partial thromboplastin time (aPTT) and prothrombin time (PT) were determined according to standard methods. Plasma levels of factor VII antigen were determined with the Asserachrom VII:Ag assay (Diagnostica Stago). Fondaparinux plasma concentrations were measured by an amidolytic photometric assay method based on the anti-Xa activity of the antithrombin-fondaparinux complex. During sample preparation, factor Xa and the chromogenic substrate S-2222 were added to the samples, after which the amount of hydrolyzed substrate was measured by a spectrophotometer.

**Statistical Analysis**

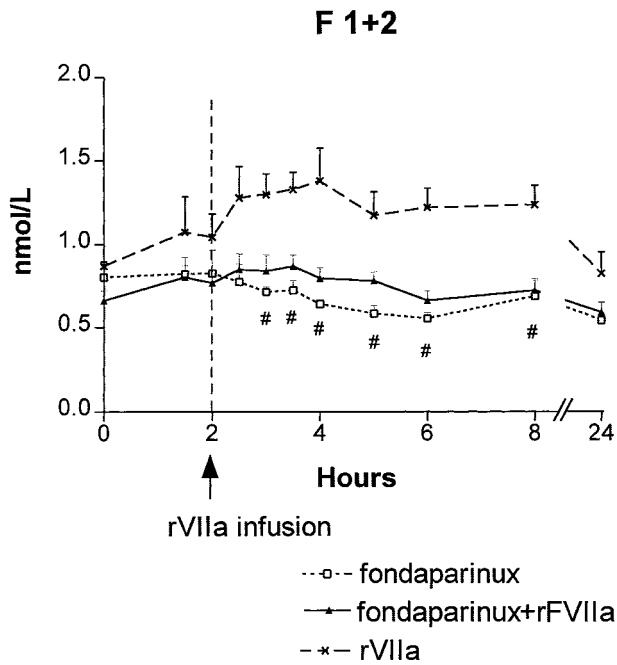
Differences between treatment groups were compared with an ANCOVA on the log-transformed area under the curve measured between the time points 2 and 8 hours after fondaparinux (or placebo) administration, with the log-transformed baseline values as covariate. Additionally, pairwise comparisons with ANOVA and post hoc Scheffé adjustment were performed to identify differences between groups per time point. To detect differences in mean



**Figure 1.** Effect of fondaparinux and/or rFVIIa administration on thrombin-generation time (TGT). Subcutaneous administration of fondaparinux or placebo at t=0. After 2 hours, rFVIIa or placebo was injected intravenously. \*P<0.05 (post hoc Scheffé test) per time point, fondaparinux plus rFVIIa vs fondaparinux alone; #P<0.05, fondaparinux plus rFVIIa vs rFVIIa alone.



**Figure 2.** Effect of fondaparinux and/or rFVIIa administration on endogenous thrombin potential (ETP). Subcutaneous administration of fondaparinux or placebo at t=0. After 2 hours, rFVIIa or placebo was injected intravenously. No significant differences were observed between groups at any time point.



**Figure 3.** Effect of fondaparinux and/or rFVIIa administration on  $F_{1+2}$ . Subcutaneous administration of fondaparinux or placebo at  $t=0$ . After 2 hours, rFVIIa or placebo was injected intravenously. # $P<0.05$  (post hoc Scheffé test) per time point, fondaparinux plus rFVIIa vs rFVIIa alone.

parameter values within a group, paired  $t$  tests were used. A probability value of  $<0.05$  was considered statistically significant. Figures present mean values plus SDs per group for each parameter.

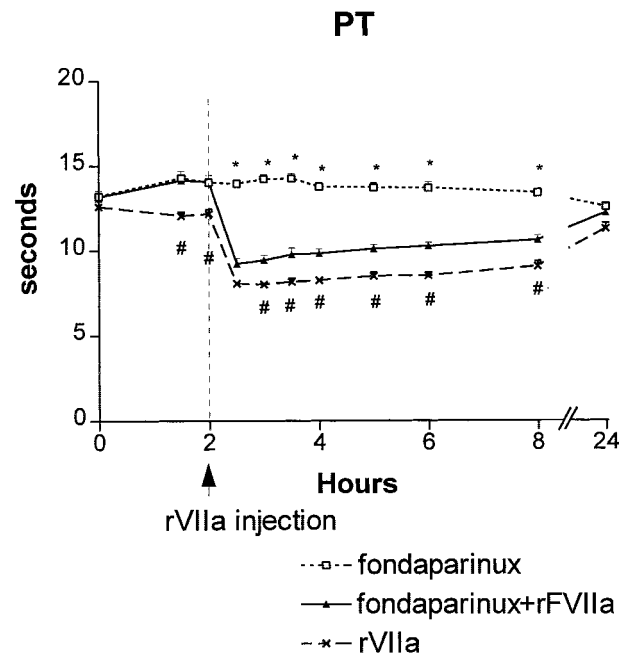
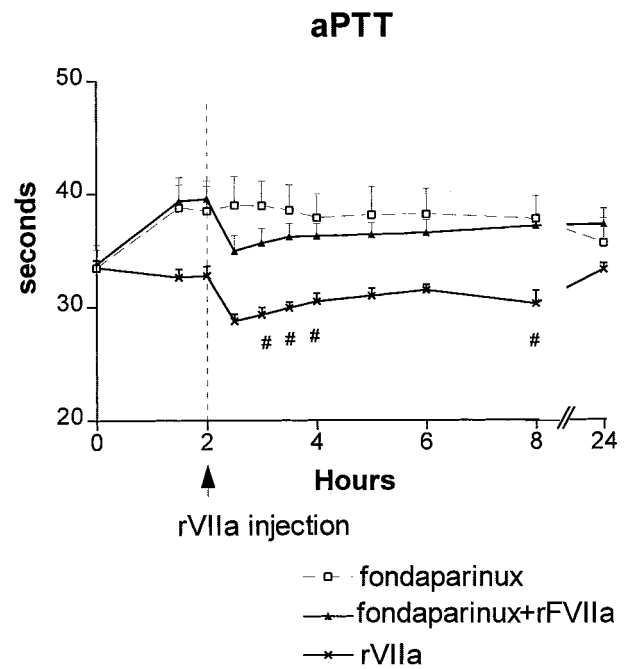
### Results

#### Thrombin Generation and Thrombin Activity

Fondaparinux doubled the thrombin-generation time, which remained elevated up to 8 hours after administration (Figure 1). Rapid normalization occurred after administration of rFVIIa, persisting at least 6 hours after injection. This marked reduction was reflected in the significantly lower area under the curve between time points 2 and 8 in the fondaparinux plus rFVIIa group than in the fondaparinux-alone group ( $P<0.001$ ). rFVIIa alone resulted in a similar proportional decrease in the thrombin-generation time as observed in the fondaparinux plus rFVIIa group.

The endogenous thrombin potential decreased by 24% after fondaparinux administration ( $P=0.001$ ; Figure 2). This reduction persisted up to 8 hours after administration. A rapid increase, although not to baseline levels, was obtained by rFVIIa injection. The endogenous thrombin potential between time points 2 and 8 hours was 9% higher in the fondaparinux plus rFVIIa group than in the fondaparinux-alone group ( $P=0.056$ ). Injection of rFVIIa alone resulted in a temporary increase, with a maximum of 10% at 0.5 and 1 hour after injection and a return to baseline values 2 hours after injection.

Plasma levels of prothrombin activation peptides  $F_{1+2}$  (which indicate prothrombin to thrombin conversion) were reduced after administration of fondaparinux (from  $0.80\pm 0.14$  nmol/L at baseline to a minimum of  $0.54\pm 0.07$



**Figure 4.** Effect of fondaparinux and/or rFVIIa administration on aPTT and PT. Subcutaneous administration of fondaparinux or placebo at  $t=0$ . After 2 hours, rFVIIa or placebo was injected intravenously. \* $P<0.05$  (post hoc Scheffé test) per time point, fondaparinux plus rFVIIa vs fondaparinux alone; # $P<0.05$ , fondaparinux plus rFVIIa vs rFVIIa alone.

nmol/L at 24 hours;  $P=0.045$ ; Figure 3). Administration of rFVIIa prevented this decrease up to 3 hours after injection. Prothrombin activation between time points 2 and 8 hours was increased by 34% in the fondaparinux plus rFVIIa group compared with the fondaparinux-alone group ( $P=0.022$ ).

#### Clotting Times

Administration of fondaparinux resulted in a mean increase of the aPTT from  $33.5\pm 4.1$  to  $38.8\pm 5.3$  seconds 1.5 hours

after administration ( $P=0.004$ ). The aPTT remained elevated at least 8 hours after administration (Figure 4, top). Immediately after rFVIIa injection, the aPTT decreased to values similar to baseline, whereas it remained prolonged after placebo injection. After this initial correction, the aPTT gradually increased again, and 6 hours after injection, the effect of rFVIIa was no longer detectable. The area-under-the-curve analysis between time points 2 and 8 hours revealed a significant reduction in aPTT in the fondaparinux plus rFVIIa group compared with the fondaparinux-alone group ( $P=0.015$ ). Administration of rFVIIa alone produced a similar relative decrease and duration of the aPTT as observed in the fondaparinux plus rFVIIa group.

The PT increased slightly after fondaparinux administration, from  $13.2\pm 0.6$  to  $14.3\pm 0.9$  seconds at 1.5 hours (Figure 4, bottom). Subsequent administration of rFVIIa resulted in a marked shortening of the PT to  $9.2\pm 0.9$  seconds ( $P<0.0001$ ). Between time points 2 and 8 hours, rFVIIa injection resulted in a 26% reduction of the PT after fondaparinux compared with fondaparinux alone ( $P<0.001$ ). In the group receiving rFVIIa alone, the PT fell to  $8.0\pm 0.3$  seconds and remained lower up to 24 hours.

### Factor VII and Fondaparinux Plasma Levels

Injection of rFVIIa resulted in a sharp increase of factor VII plasma levels, from 64% to 251% at 30 minutes after injection. Thereafter, factor VII levels decreased with an estimated plasma half-life of 1.25 hours and reached virtually normal levels at 24 hours after injection (data not shown). Subjects treated with fondaparinux and rFVIIa had somewhat lower maximal peak factor VII levels (186% at 30 minutes), with a similar plasma half-life as the rFVIIa-alone group.

Injection of rFVIIa had no effect on the pharmacokinetic profile of fondaparinux. Maximum plasma levels of fondaparinux were reached at 1.5 to 2.5 hours after administration ( $\pm 1.1$  mg/L), with a half-life of 16 hours, and plasma levels at 24 hours of  $\approx 0.3$  mg/L (data not shown).

### Discussion

The central role of factor Xa in coagulation makes this protease a desirable target for antithrombotic therapy. Heparin and low-molecular-weight heparins are inhibitors of factor Xa, but their lack of specificity (due to simultaneous inhibition of thrombin and other activated coagulation factors) may contribute to a relatively small therapeutic window and to the risk of bleeding. Pentasaccharides are synthetic agents capable of highly selective factor Xa inhibition. Fondaparinux 2.5 mg SC was shown to effectively and safely prevent venous thromboembolism after orthopedic surgery.<sup>6–8</sup> Fondaparinux has a relatively long elimination half-life, and higher dosages are now being evaluated for the treatment of venous and arterial thrombotic disease. For patients who experience bleeding complications or require acute surgical intervention, reversal of the anticoagulant effect of fondaparinux may be desirable. Our results demonstrate that administration of rFVIIa is able to overcome the inhibition of thrombin generation in healthy subjects treated with fondaparinux 10 mg SC. We observed a normalization of the fondaparinux-induced prolongation of aPTT and PT by administration of

rFVIIa. Sensitive thrombin-generation assays demonstrated the efficacy of rFVIIa in restoring impaired thrombin formation after fondaparinux administration. These in vivo results add to in vitro data that show that rFVIIa reverses not only the anticoagulant effect of fondaparinux but also the profibrinolytic effects of this agent,<sup>17</sup> probably through activation of thrombin-activatable fibrinolysis inhibitor (TAFI) by rFVIIa.<sup>18</sup> This effect appears not to be specific for fondaparinux, because rFVIIa is able to reverse the effect of other anticoagulants, such as the tissue factor inhibitor rNAPC2,<sup>14</sup> which indicates that infusion of high levels of factor VIIa activates sufficient amounts of non-fondaparinux-inhibited factor X to achieve normal thrombin generation.

Fondaparinux administration resulted in both an aPTT and PT increase up to 5.6 and 1.1 seconds, respectively. Although these postfondaparinux clotting times were significantly higher than baseline, these measurements are difficult to use in a clinical setting on individual patients to determine the anticoagulant effect of fondaparinux, because the effects are relatively small and are not always consistent with changes in anti-Xa levels. Although the observed normalization of coagulation in vivo strongly suggests clinical efficacy, it is unknown whether these results imply that rFVIIa is effective in the treatment of clinically significant hemorrhages during fondaparinux therapy, even though rFVIIa demonstrated efficacy in other clinical bleeding conditions.<sup>14,19–25</sup>

The duration of the effect of a single 90- $\mu$ g/kg IV dose of rFVIIa differed between parameters and varied from 2 to 6 hours. Continued activation up to 6 hours as seen with the prothrombin and thrombin-generation assays indicated a sustained effect of rFVIIa, which overcame the inhibitory effect of fondaparinux. These data support previous observations in which rFVIIa was capable of inducing thrombin generation in the absence of tissue factor,<sup>14,26,27</sup> possibly because of the high supraphysiological plasma levels of factor VIIa obtained (251%). In patients with active bleeding sites, the complex of exposed tissue factor and factor VIIa has an even greater potential of factor X activation than with rFVIIa alone, and therefore the effect of rFVIIa is likely to be more pronounced in these cases.

The safety of rFVIIa administration needs consideration. The number of reports of thrombotic complications after rFVIIa therapy is relatively low,<sup>28–30</sup> and its application is extending rapidly for various indications.<sup>19–25,31–35</sup> However, the risk of thrombotic complications is greater in patients treated with anticoagulant therapy because of recently diagnosed venous or arterial thrombosis, especially in patients treated for acute coronary syndromes because of increased tissue factor expression at the culprit coronary lesion. Therefore, until more evidence becomes available, rFVIIa should be used prudently and only if conventional treatments fail. The dosage of rFVIIa used in the present study did not result in an overshoot of coagulation, with none of the parameters showing possible procoagulant values.

Current data in more than 2000 patients receiving the prophylactic 2.5-mg dose of fondaparinux<sup>6–8</sup> reveal a low incidence of serious bleeding complications, with no bleeding in critical organs and 0.4% of bleeding cases requiring reoperation. Higher dosages (up to 12 mg) used in the arterial

thrombosis trials (unstable angina) had an incidence of major bleeding that varied between 0% and 1.8%, without a clear dose-response relationship,<sup>10</sup> although combination with a thrombolytic agent (alteplase) increased the incidence of major bleeding between 4.9% and 7.8%, rates similar to those for patients receiving alteplase and unfractionated.<sup>9</sup> However, clinical experience is limited, and in case of bleeding complications in vital organs (eg, intracranial) or life-threatening bleeding, the use of an antidote may be desirable.

We conclude that rFVIIa is capable of normalizing thrombin generation after subcutaneous administration of 10 mg of fondaparinux in healthy male subjects and may be a suitable antidote in case of serious bleeding complications in patients treated with fondaparinux.

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### References

- Greinacher A, Lubenow N. Recombinant hirudin in clinical practice: focus on lepirudin. *Circulation*. 2001;103:1479–1484.
- Weitz JI, Buller HR. Direct thrombin inhibitors in acute coronary syndromes. *Circulation*. 2002;105:1004–1011.
- Lee A, Agnelli G, Buller H, et al. Dose-response study of recombinant factor VIIa/tissue factor inhibitor recombinant nematode anticoagulant protein c2 in prevention of postoperative venous thromboembolism in patients undergoing total knee replacement. *Circulation*. 2001;104:74–78.
- de Jonge E, Dekkers PE, Creasey AA, et al. Tissue factor pathway inhibitor dose-dependently inhibits coagulation activation without influencing the fibrinolytic and cytokine response during human endotoxemia. *Blood*. 2000;95:1124–1129.
- Abraham E, Reinhart K, Svoboda P, et al. Assessment of the safety of recombinant tissue factor pathway inhibitor in patients with severe sepsis: a multicenter, randomized, placebo-controlled, single-blind, dose escalation study. *Crit Care Med*. 2001;29:2081–2089.
- Turpie AG, Gallus AS, Hoek JA, et al. A synthetic pentasaccharide for the prevention of deep-vein thrombosis after total hip replacement. *N Engl J Med*. 2001;344:619–625.
- Eriksson BI, Bauer KA, Lassen MR, et al. Fondaparinux compared with enoxaparin for the prevention of venous thromboembolism after hip-fracture surgery. *N Engl J Med*. 2001;345:1298–1304.
- Bauer K, Eriksson B, Lassen MR, et al. Fondaparinux compared with enoxaparin for the prevention of venous thromboembolism after elective major knee surgery. *N Engl J Med*. 2001;345:1305–1310.
- Coussement PK, Bassand JP, Convens C, et al. A synthetic factor-Xa inhibitor (ORG31540/SR9017A) as an adjunct to fibrinolysis in acute myocardial infarction: the PENTALYSE study. *Eur Heart J*. 2001;22:1716–1724.
- Simoons M, for the PENTUA study group. PENTAsaccharide in patients with Unstable Angina: PENTUA study. Presented at: Plenary X: Late Breaking Clinical Trials, Scientific Sessions, American Heart Association, AHA Congress; November 14, 2001; Anaheim, Calif.
- Olson ST, Björk I, Sheffer R, et al. Role of the antithrombin-binding pentasaccharide in heparin acceleration of antithrombin-proteinase reactions: resolution of the antithrombin conformational change contribution to heparin rate enhancement. *J Biol Chem*. 1992;267:12528–12538.
- Lormeau JC, Hérault JP. The effect of the synthetic pentasaccharide SR 90107/ORG 31540 on thrombin generation ex vivo is uniquely due to ATIII-mediated neutralization of factor Xa. *Thromb Haemost*. 1995;74:1474–1477.
- Erhardtson E, Nony P, Dechavanne M, et al. The effect of recombinant factor VIIa (NovoSeven) in healthy volunteers receiving acenocoumarol to an International Normalized Ratio above 2.0. *Blood Coagul Fibrinolysis*. 1998;9:741–748.
- Friederich PW, Levi M, Bauer KA, et al. Ability of recombinant factor VIIa to generate thrombin during inhibition of tissue factor in human subjects. *Circulation*. 2001;103:2555–2559.
- International Conference on Harmonisation. Good clinical practice: consolidated guideline. *Federal Register*. 1997;62:25691–25709.
- Rosing J, Tans G, Nicolaes GA, et al. Oral contraceptives and venous thrombosis: different sensitivities to activated protein C in women using second- and third-generation oral contraceptives. *Br J Haematol*. 1997;97:233–238.
- Lisman T, Adelmeijer J, Nieuwenhuis HK, et al. Recombinant factor VIIa reverses the anticoagulant and profibrinolytic effect of synthetic pentasaccharide ORG 31540/SR90107A in vitro. *Blood*. 2001;98:75b. Abstract.
- Lisman T, Mosnier LO, Lambert T, et al. Inhibition of fibrinolysis by recombinant factor VIIa in plasma from patients with severe hemophilia A. *Blood*. 2002;99:175–179.
- Elg M, Carlsson S, Gustafsson D. Effect of activated prothrombin complex concentrate or recombinant factor VIIa on the bleeding time and thrombus formation during anticoagulation with a direct thrombin inhibitor. *Thromb Res*. 2001;101:145–157.
- Meijer K, de Graaff WE, Daenen SM, et al. Successful treatment of massive hemoptysis in acute leukemia with recombinant factor VIIa. *Arch Intern Med*. 2000;160:2216–2217.
- Arkin S, Blei F, Fettes J, et al. Human coagulation factor FVIIa (recombinant) in the management of limb-threatening bleeds unresponsive to alternative therapies: results from the NovoSeven emergency-use programme in patients with severe haemophilia or with acquired inhibitors. *Blood Coagul Fibrinolysis*. 2000;11:255–259.
- Holm M, Andreassen R, Ingerslev J. Management of bleeding using recombinant factor VIIa in a patient suffering from bleeding tendency due to a lupus anticoagulant-hypoprothrombinemia syndrome. *Thromb Haemost*. 1999;82:1776–1778.
- Smith OP, Hann IM. rVIIa therapy to secure haemostasis during central line insertion in children with high-responding FVIII inhibitors. *Br J Haematol*. 1996;92:1002–1004.
- Majumdar G, Savidge GF. Recombinant factor VIIa for intracranial haemorrhage in a Jehovah's Witness with severe haemophilia A and factor VIII inhibitors. *Blood Coagul Fibrinolysis*. 1993;4:1031–1033.
- Tarantino MD, Aberle R. Recombinant factor VIIa (NovoSeven) for post-prostatectomy hemorrhage in a patient with type I von Willebrand disease. *Am J Hematol*. 2001;68:62–63.
- Monroe DM, Hoffman M, Oliver JA, et al. A possible mechanism of action of activated factor VII independent of tissue factor. *Blood Coagul Fibrinolysis*. 1998;9(suppl 1):S15–S20.
- Hoffman M, Monroe DM III, Roberts HR. Activated factor VII activates factors IX and X on the surface of activated platelets: thoughts on the mechanism of action of high-dose activated factor VII. *Blood Coagul Fibrinolysis*. 1998;9(suppl 1):S61–S65.
- Peerlinck K, Vermeylen J. Acute myocardial infarction following administration of recombinant activated factor VII (Novo Seven) in a patient with haemophilia A and inhibitor. *Thromb Haemost*. 1999;82:1775–1776.
- Roberts HR. Clinical experience with activated factor VII: focus on safety aspects. *Blood Coagul Fibrinolysis*. 1998;9(suppl 1):S115–S118.
- Hay CR, Negrier C, Ludlam CA. The treatment of bleeding in acquired haemophilia with recombinant factor VIIa: a multicentre study. *Thromb Haemost*. 1997;78:1463–1467.
- Bernstein D. Effectiveness of the recombinant factor VIIa in patients with the coagulopathy of advanced child's B and C cirrhosis. *Semin Thromb Hemost*. 2000;26:437–438.
- Friederich PW, Geerdink MG, Spataro M, et al. The effect of the administration of recombinant activated factor VII (NovoSeven) on perioperative blood loss in patients undergoing transabdominal retroperitoneal prostatectomy: the PROSE study. *Blood Coagul Fibrinolysis*. 2000;11(suppl 1):S129–S132.
- Pickard JD, Kirkpatrick PJ, Melsen T, et al. Potential role of NovoSeven in the prevention of rebleeding following aneurysmal subarachnoid haemorrhage. *Blood Coagul Fibrinolysis*. 2000;11(suppl 1):S117–S120.
- d'Oiron R, Menart C, Trzeciak MC, et al. Use of recombinant factor VIIa in 3 patients with inherited type I Glanzmann's thrombasthenia undergoing invasive procedures. *Thromb Haemost*. 2000;83:644–647.
- White B, Martin M, Kelleher S, et al. Successful use of recombinant FVIIa (Novoseven) in the management of pulmonary haemorrhage secondary to Aspergillus infection in a patient with leukaemia and acquired FVII deficiency. *Br J Haematol*. 1999;106:254–255.