Recombinant activated factor VII decreases bleeding without increasing arterial thrombosis in rabbits

[Le facteur VII recombinant activé diminue le saignement sans augmenter la thrombose artérielle chez le lapin]

Maurizio Fattorutto MD,* Sandrine Tourreau-Pham MD,* Elisabeth Mazoyer MD,† Philippe Bonnin MD PhD,§ Martine Raphaël MD PhD,† Françoise Morin MD,‡ Michel Cupa MD,* Charles-Marc Samama MD PhD*

Purpose: To compare the effects of recombinant activated factor VII (rFVIIa) and platelet-rich plasma (PRP) in an experimental model of bleeding and arterial thrombosis.

Methods: The Folts model was used in 60 rabbits. After anesthesia, the carotid artery was exposed and a 75% stenosis was induced. A compression injury of the artery triggered a series of cyclic flow reductions (CFRs). After counting baseline CFRs, animals were assigned randomly to one of four groups ($n = 15$ in each): control, PRP, rFVIIa and placebo. Control animals received 10 mL·kg⁻¹ of saline while 10 mL·kg⁻¹ of a hydroxyethyl starch solution (200,000/6%/0.5) were infused in the three other groups. CFRs were measured again, followed by treatment with PRP, rFVIIa or placebo and by a final measurement of CFRs. At the end of each observation period, an ear immersion bleeding time (BT) was measured and a blood sample was drawn for the evaluation of hematological variables. Microvascular bleeding was evaluated at the end of the experiment in grams of blood shed from liver and spleen sections. Results are presented as median (range).

Results: rFVIIa shortened the BT and decreased microvascular bleeding as compared with placebo [60 (35–100) sec vs 110 (50–140) sec, $P = 0.0019$ and 9 (4–24) g vs 17 (5–28) g, $P = 0.002$, respectively]. rFVIIa did not increase CFRs [3(0–9) vs 1(0–5), $P = 0.11$].

Conclusion: rFVIIa led to a decrease in BT and microvascular bleeding but did not significantly affect arterial thrombosis in rabbits.

Objectif : Comparer les effets du facteur VII recombinant activé (rFVIIa) et du plasma riche en plaquettes (PRP) avec un modèle expérimental de saignement et de thrombose artérielle.

Méthode : Le modèle de Folts a été utilisé chez 60 lapins. Après l'anesthésie, l'artère carotide a été exposée et une sténose à 75 % a été induite. Une lésion postcompression a déclenché une série de réductions cycliques du flux (RCF). Après avoir fait la numération de base des RCF, les animaux ont été assignés aléatoirement à l'un des quatre groupes ($n = 15$ chacun) : témoin, PRP, rFVIIa et placebo. Les témoins ont reçu 10 mL·kg⁻¹ de solution saline et 10 mL·kg⁻¹ d'une solution d'amidon hydroxyéthylée (200 000/6 %/0,5) ont été perfusés chez les animaux des trois autres groupes. De nouvelles mesures des RCF ont été faites, suivies du traitement avec PRP, rFVIIa ou placebo et par une mesure finale des RCF. À la fin de chaque période d'observation, le temps de saignement (TS) de l'immersion de l'oreille a été mesuré et un échantillon de sang prélevé pour l'évaluation du bilan hématologique. À la fin de l'expérimentation, le saignement microvasculaire a été évalué en grammes de sang provenant de la coupe du foie et de la rate. Les résultats sont présentés par la médiane (valeurs extrêmes).

Résultats : Le rFVIIa a raccourci le TS et diminué le saignement microvasculaire, comparativement au placebo [60 (35–100) s vs 110 (50–140) s, $P = 0,0019$ et 9 (4–24) g vs 17 (5–28) g, $P = 0,002$, respectivement]. Le rFVIIa n'a pas augmenté les RCF [3(0–9) vs 1(0–5), $P = 0,11$].

Conclusion : Le rFVIIa a réduit le TS et le saignement microvasculaire, mais n'a pas affecté significativement la thrombose artérielle chez des lapins.

From the Department of Anesthesiology and Intensive care,* the Laboratory of Hematology,† and the Blood Transfusion Unit,‡ (Etablissement Français du Sang, site Avicenne), Hôpital Avicenne, Bobigny; and the Department of Functional Investigations,§ Hôpital Lariboisière, Paris, France.

Address correspondence to: Pr. Charles Marc Samama, Département d'Anesthésie-Réanimation, Hôpital Avicenne, 125, route de Stalingrad, 93009 Bobigny cedex, France. Phone: 33 1 48 95 55 91; Fax: 33 1 48 95 55 89; E-mail: cmsamama@invivo.edu

This work was supported by an unrestricted grant from Novo-Nordisk, Denmark.

This work was presented in part during the 10th annual meeting of the European Society of Anesthesiologists, Nice, April 2002.

Accepted for publication November 11, 2003.

Revision accepted April 12, 2004.

RECOMBINANT activated factor VII (rFVIIa) was first developed for the treatment of spontaneous or surgical bleeding in patients with congenital or acquired hemophilia with factors VIII or IX inhibitors.¹ FVIIa initiates the first stage of coagulation: the key to the initiation of hemostasis is the formation of tissue factor (TF)-activated factor VII complex at the site of a vascular lesion. The TF/FVIIa complex then activates factor X, resulting in the production of small amounts of thrombin in cells expressing TF.² Recently, rFVIIa has shown its potential usefulness in situations of severe bleeding with major coagulopathies when other types of treatment have failed.^{3,4} Massive transfusion may impair hemostasis.^{5,6} The limited availability of human blood transfusion products (platelet concentrates, plasma) and their potential risks (viral, bacterial, immunologic) has led to focus on rFVIIa as a potential substitute for platelet concentrates and/or plasma. A recent compassionate study including trauma patients has shown a high level of efficacy for this new potent hemostatic agent.³ However several concerns have been expressed on the potential thrombogenicity of this potent drug.⁷

In 1976, John Folts developed a canine model of coronary stenosis with intimal injury in order to reproduce experimentally the situation in patients with coronary artery disease.⁸

In this model, a vascular wall injury associated with stenosis of the artery induces cyclic flow reductions (CFRs) characterized by gradual decreases of flow to almost zero values followed by spontaneous restoration of flow. The endothelial damage induces the formation of a platelet plug, which embolizes and forms again in a cyclic fashion. Histologically, the thrombus is platelet-rich with some red cells but little fibrin. This model has been described as representing events occurring in patients with unstable angina.⁹ We, as others, have adapted Folts' model in another animal species, the rabbit, and we have chosen to study another vascular territory, the carotid artery.¹⁰⁻¹⁵ A model of bleeding has been developed and validated concomitantly.^{14,15} Bleeding is evaluated by the measurement of ear immersion bleeding time and microvascular bleeding after an hepato-splenic section is performed.

The availability of this double model of arterial thrombosis and bleeding has allowed our group to study different drugs interfering with thrombosis and/or hemostasis.^{11,14} This validated experimental model allows us to study the efficacy and the thrombogenicity of new hemostatic agents. The objective of the present study was to compare the effects of treat-

ment with rFVIIa and platelet-rich plasma (PRP) in this model. We choose to increase the sensitivity of the model by infusing a hydroxyethyl starch solution which is known to induce hemodilution and impair primary hemostasis while decreasing the level of von Willebrand factor.¹⁶⁻¹⁸ We aimed to demonstrate the efficacy of rFVIIa in decreasing bleeding in such conditions and calculated the sample size in this respect. Concomitantly, we decided to observe thrombosis, but no hypothesis was prioritized at any time.

Material and methods

The animals were treated in accordance with the ethical rules of the Institut National de la Santé et de la Recherche Médicale and the Institut National de la Recherche Agronomique.

The study involved two parts: the application of the Folts model to the carotid arteries of the rabbit and a bleeding model involving liver and spleen sections and ear immersion bleeding time.

Surgical procedure

This prospective study included 60 male New Zealand rabbits that weighed 2.8 ± 0.2 kg (Elevage des Dombes, Romans, France). All were males with the same blood group. Anesthesia was induced using *iv* sodium pentobarbital ($30 \text{ mg}\cdot\text{kg}^{-1}$; Sanofi-Santé Vétérinaire, Libourne, France) and was maintained with sodium pentobarbital ($10 \text{ mg}\cdot\text{kg}^{-1}$), as required. Tracheotomy and mechanical ventilation (Harvard Apparatus; Harvard Instruments, Boston, MA, USA) were performed (respiratory rate, $40 \text{ cycles}\cdot\text{min}^{-1}$; tidal volume, 15 mL). Body temperature was recorded continuously using a rectal probe and maintained at approximately 38°C using an electric blanket (Homeothermic Blanket Control Unit; Harvard Apparatus, Boston, MA, USA) and a warming table (Scientific Research Instruments, Edenbridge, Kent, UK). A femoral artery catheter (22 gauge, Becton Dickinson-France, Le Pont de Claix, France) was placed and arterial blood pressure recorded continuously. The electric activity of the heart was recorded using three hypodermic electrodes.

The right carotid artery was exposed and carefully isolated over approximately 2 cm. A 1.5-mm diameter precalibrated electromagnetic circular flow probe (Skalar Instruments, Delft, The Netherlands) was placed around the right common carotid artery on the distal part of the exposed segment and connected to a flowmeter (model MDL 1401; Skalar Instruments). Zero calibration was obtained directly by occluding the artery with a cotton-tipped swab. Hemodynamic and electrocardiographic variables were recorded con-

FIGURE After CFR 1, the control group received saline and the PRP, rVIIa and placebo groups received hydroxyethyl starch. After CFR 2, the placebo group received no further treatment; PRP, rVIIa or placebo was infused in the other animals. HS = hepatosplenic; BT = bleeding time; CFR = cyclic flow reduction; PRP = platelet-rich plasma. rFVIIa = recombinant activated factor VII.

tinuously using a monitoring system (Biopac®, Santa Barbara, CA, USA) connected to a computer system (IMac®, Apple Computer Inc., USA). All layouts were interpreted by an independent observer blinded to group assignment.

Thrombosis

After a ten-minute stabilization period (baseline flow), a 75% stenosis of the common carotid artery was produced by placing a vascular clamp (Harvard Instruments) around the artery proximally to the 2-cm exposed segment. This 75% stenosis led to a 10% reduction in baseline carotid flow. The stenosis was released after ten minutes. An arterial injury with de-endothelialization was induced by cross-clamping the middle of the exposed segment of the artery three times within ten seconds. This was accomplished with a Mayo-Hegar needle holder forceps (Harvard Instruments) closed at the third ratchet position. The 75% stenosis was reinstituted at the level of the injured carotid. This triggered a series of CFRs characterized by repetitive decreases in blood flow, followed by an abrupt spontaneous return of flow to its original level. Beginning with the first CFR, the process was observed for 20 min (baseline, CFR 1). The number of CFRs during this 20-min observation period was noted. If no CFR was observed during this period, the arterial injury was repeated at the same site, and a new 20-min period of observation was allowed. If no CFR

occurred during this period, the injury was induced on the contralateral carotid artery. This first period (CFR 1) was common to all animals.

A randomization table was prepared and used. The subsequent protocol depended upon the randomization scheme (Figure):

- in the Control group, after administration of 10 mL·kg⁻¹ of saline solution, a second 20-min period of CFR recording was started (CFR 2). After ten minutes, a third period was started (CFR 3).

- in the PRP group, after infusion of 10 mL·kg⁻¹ of hydroxyethyl starch (HES; Haesteril® 200,000/6%/0.5, Fresenius Kabi, Sèvres, France), a second CFR period was started (CFR 2). After a ten-minute period, a third 20-min recording period was started after infusion of 10 mL·kg⁻¹ of PRP (CFR 3). Homologous blood was obtained from other rabbits by blood letting. The blood collected was centrifuged (600 rpm for 30 min), enabling the fractionation of red blood cells and PRP.

- in the rFVIIa and placebo groups, a second observation period started after the infusion of 10 mL·kg⁻¹ of HES (CFR 2). After a ten-minute waiting period, the animals were given in a blinded fashion either 100 µg·kg⁻¹ of rFVIIa (1 mL) *iv* (Novoseven®, Novo Nordisk, Bagsvoerd, Denmark) or placebo (saline solution, 1 mL) *iv*.

A third observation period was initiated ten minutes after this injection (CFR 3).

The carotid artery stenosis was completely released and re-established after each observation period in order to restore basal carotid flow.

Bleeding

The ear immersion bleeding time was measured after each CFR period. The ear was cleaned and shaved on its external side. An automated incision (5 mm) was made with a bleeding time device (Simplate®; Organon technika, Fresnes, France) and the ear placed in a beaker containing 20 mL saline solution maintained at 38°C. Duration of bleeding time was measured until the trickle caused by the incision stopped, as reported previously.^{14,15}

At the end of the experiment, immediately after the third CFR period, a laparotomy was performed. The spleen and liver were isolated and incised in a standardized fashion. The spleen was transected at its free border from the lower pole to the mid level. For the liver, a 2-cm section parallel to the plane of the ligament falciform was made between the right and left lobes. Bleeding was recorded until it stopped. Swabs, placed close to the spleen and the liver before the transection, were weighed in order to estimate blood loss.

TABLE I Weight, temperature and hemodynamic variables

	Control (n = 15)	PRP (n = 15)	rFVIIa (n = 15)	Placebo (n = 15)
Weight (g)	2.760 ± 220	2.690 ± 190	2.630 ± 200	2.790 ± 210
Temperature (°C)	37.8 ± 0.6	38.1 ± 0.6	37.6 ± 0.8	38.3 ± 0.6
Mean arterial pressure (mmHg)	75 ± 10	80 ± 13	74 ± 8	99 ± 10
Heart rate (beats·min ⁻¹)	222 ± 34	224 ± 16	227 ± 17	223 ± 14

Values expressed as means ± SD. No significant differences between groups. rFVIIa = recombinant activated factor VII; PRP = platelet-rich plasma.

The total amount of blood lost (spleen and liver) was estimated ten minutes after the beginning of the laparotomy, as reported previously.^{13,14}

Hematological variables

After each CFR recording period, an arterial blood sample was drawn into an EDTA tube (Vacutainer®, Becton Dickinson, Le Pont de Claix, France). Variables measured using an automated device (Advisa Hematology System 120®, Bayer, Germany) were: hematocrit, hemoglobin level and platelet count. Samples were also drawn in order to study hemostasis. Blood samples were collected in 3.8% trisodium citrate (9:1 v/v) (Vacutainer®, Becton Dickinson). Variables were determined automatically (STA Compact®, Diagnostica Stago, Asnières, France) in platelet-poor plasma obtained by centrifugation at 4,000 rpm for ten minutes: prothrombin time (PT; Neoplastine®, Diagnostica Stago), activated partial thromboplastin time (aPTT; STA®-PTT automate 5, Diagnostica Stago), fibrinogen level (STA-Fibrinogen®, Diagnostica Stago), and clotting factor VII assay (STA-Déficient VII®, Diagnostica Stago).

Platelet aggregation

Ex vivo platelet aggregation (Pack 4 - Platelet aggregation chromogenic kinetic system; Helena Laboratories, Beaumont, TX, USA) was analyzed on PRP and calibrated with platelet-poor plasma. PRP was obtained by centrifuging whole blood at 200 g for ten minutes at 37°C. Platelet-poor plasma was prepared from the same blood sample by centrifuging blood at 1500 g for 20 min. Platelets were counted in PRP to check the homogeneity of the samples. Platelet aggregation was induced by 5 mg·mL⁻¹ arachidonic acid (Helena-France, St. Leu, France). The increase in light transmission was recorded for four minutes after the agonist was added. Aggregation induced by the agonist in PRP was evaluated by measuring light transmission in stimulated PRP, assuming

TABLE II Cyclic flow reductions

	Control (n = 15)	PRP (n = 15)	rFVIIa (n = 15)	Placebo (n = 15)
CFR 1	6 (3 - 10)	5 (3 - 15)	6 (3 - 10)	6 (3 - 9)
CFR 2	3 (0 - 7)	1 (0 - 6) *	1 (0 - 6) *	1 (0 - 6) *
CFR 3	4 (0 - 7)	1 (0 - 7)	3 (0 - 9) †	1 (0 - 5)

Values are expressed as median (range). CFR 1 = baseline CFRs; CFR 2 = CFRs after infusion of normal saline in the control group and dilution by hydroxy-ethyl-starch in other groups; CFR 3 = CFRs after treatment with rFVIIa, placebo or platelet-rich plasma. **P* < 0.05 *vs* CFR 1 within each group. †*P* < 0.05 *vs* placebo within each observation period. CFR = cyclic flow reductions; PRP = platelet-rich plasma; rFVIIa = recombinant activated factor VII.

that light transmission was 100% in platelet-poor plasma and 0% in non stimulated PRP. The maximal intensity of platelet aggregation was defined as the maximal increase in light transmission, and the velocity of platelet aggregation (slope of the curve) was defined as the speed of the increase in light transmission, after the aggregating agent was added.

Statistical analysis

Sample size was calculated assuming that rVIIa would decrease bleeding by 50%. A 20% beta risk and a 5% alpha risk were retained and, therefore, according to these experimental conditions, 14 rabbits per group had to be included. No additional calculation was performed with regard to the thrombotic risk because no hypothesis could be prioritized.

Data are expressed as mean ± standard deviation, except for discrete variables (CFRs) and variables with non-Gaussian distribution (bleeding time, spleen and liver bleeding), which are expressed as medians with ranges. Several mean values were compared using analysis of variance, followed by the Scheffé test. Medians were compared using the Kruskal-Wallis test for independent measures, followed, when significant,

by a Mann–Whitney U test with Bonferroni correction, and by the Friedman test for repeated measure and, when significant, by the Wilcoxon rank sum test with Bonferroni correction. Probability values α less than 0.05 were required to reject the null hypothesis. Statistical analysis was performed using Statview SE Graphics (Abacus Concepts, Berkeley, CA, USA).

Results

Seventy-two rabbits were randomized; 12 rabbits failed to develop CFRs and were excluded. There were no significant differences between the four groups for mean body weight, temperature, mean arterial blood pressure and heart rate (Table I).

TABLE III Ear bleeding time and hepato-splenic bleeding

	Control (n = 15)	PRP (n = 15)	rFVIIa (n = 15)	Placebo (n = 15)
<i>Bleeding time (sec)</i>				
CFR 1	60 (20–80)	60 (40–70)	60 (35–75)	60 (40–70)
CFR 2	80 (35–110)	85 (60–130)*	95 (50–125)*	100 (30–150)*
CFR 3	80 (35–111)	95 (35–120)	60 (35–100)†	110 (50–140)
Hepato-splenic bleeding (g)	13 (5–28)	18 (8–25)	9 (4–24)†	17 (5–28)

Values expressed as medians (range). CFR 1 = baseline CFRs; CFR 2 = CFRs after infusion of normal saline in the control group and dilution by hydroxyethyl starch in other groups; CFR 3 = CFRs after treatment with rFVIIa, placebo or platelet-rich plasma. * $P < 0.05$ vs CFR 1 within each group † $P < 0.05$ vs placebo within each observation period. CFR = cyclic flow reductions; PRP = platelet-rich plasma; rFVIIa = recombinant activated factor VII.

TABLE IV Hematological variables

	Control (n = 15)	PRP (n = 15)	rFVIIa (n = 15)	Placebo (n = 15)
<i>Ht (%)</i>				
CFR 1	39.5 ± 2.7	37.8 ± 3.4	37.8 ± 2.5	35.8 ± 2.7
CFR 2	36.4 ± 2.7	31.6 ± 4.2*	31.3 ± 3.7*	30.9 ± 3.2*
CFR 3	35.5 ± 2.6	28.2 ± 4.2	30.8 ± 3.4	30.6 ± 3.5
<i>Platelets (x 1000-μL⁻¹)</i>				
CFR 1	334 ± 126	334 ± 92	309 ± 89	358 ± 87
CFR 2	298 ± 98	251 ± 92*	199 ± 77*	250 ± 54*
CFR 3	268 ± 89	241 ± 86	189 ± 61	258 ± 69
<i>PT (sec)</i>				
CFR 1	8.6 ± 0.4	8.8 ± 0.6	8.8 ± 0.4	8.5 ± 0.2
CFR 2	8.8 ± 0.5	9.2 ± 0.9	9.4 ± 0.5	9.2 ± 0.4
CFR 3	8.8 ± 0.5	9.0 ± 0.8	7.5 ± 0.5	9.1 ± 0.5
<i>aPTT (sec)</i>				
CFR 1	99 ± 21	103 ± 35	96 ± 16	93 ± 25
CFR 2	92 ± 22	99 ± 29	93 ± 28	104 ± 34
CFR 3	102 ± 17	106 ± 34	60 ± 12	101 ± 47
<i>Fg (g·L⁻¹)</i>				
CFR 1	2.4 ± 0.3	2.8 ± 0.8	2.4 ± 0.3	2.8 ± 0.3
CFR 2	1.8 ± 0.5	2.3 ± 0.6	1.8 ± 0.2	2.1 ± 0.2
CFR 3	1.9 ± 0.4	2.2 ± 0.6	1.7 ± 0.2	2.0 ± 0.2
<i>AA (%)</i>				
CFR 1	68 ± 12	68 ± 16	67 ± 13	62 ± 9
CFR 2	70 ± 17	53 ± 24*	55 ± 16 *	53 ± 8 *
CFR 3	52 ± 12	49 ± 24	52 ± 18	49 ± 14

Values expressed as means ± SD. Ht = hematocrit; PT = prothrombin time; aPTT = activated partial thromboplastin time; Fg = fibrinogen level; AA = arachidonic acid-induced platelet aggregation; CFR 1 = baseline CFRs; CFR 2 = CFRs after infusion of normal saline in the control group and dilution by hydroxyethyl starch in the other groups; CFR 3 = CFRs after treatment with rFVIIa, placebo or platelet-rich plasma. * $P < 0.05$ vs CFR 1 within each group. CFR = cyclic flow reductions; PRP = platelet-rich plasma; rFVIIa = recombinant activated factor VII.

Thrombosis

The number of CFRs, at baseline, was similar between the four groups. The number of CFRs decreased after hemodilution with HES in the PRP, rFVIIa and placebo groups but no significant variations were seen after the infusion of normal saline in the control group. CFRs did not increase significantly after the administration of rFVIIa ($P = 0.11$; Table II).

Bleeding

Ear bleeding time, at baseline, was similar between the four groups. It increased in all animals hemodiluted with HES, but not in those hemodiluted with normal saline (Table III). The transfusion of PRP did not correct the bleeding time. In contrast, the infusion of rFVIIa resulted in a significant shortening of the bleeding time as compared to placebo. Hepato-splenic bleeding showed no significant decrease in the PRP group in comparison with placebo. Infusion of rFVIIa significantly decreased hepato-splenic bleeding when compared to placebo (Table III).

Hematological variables

Hemoglobin level, hematocrit, platelet aggregation, fibrinogen level, platelet count, PT or aPTT were similar in the four study groups at baseline (Table IV).

Hemoglobin level, hematocrit, platelet count and platelet aggregation decreased after hemodilution with HES. The decrease was not significant in the control group.

After treatment with rFVIIa, the PT and aPTT decreased in comparison with placebo (Table IV). The level of clotting factor VII increased in the rFVIIa group: $1,784 \pm 345\%$ after treatment *vs* $548 \pm 231\%$ after the first CFR period ($P = 0.001$).

Discussion

We have studied the hemostatic and potential pro-thrombotic effects of rFVIIa in a double model of arterial thrombosis and bleeding in the rabbit. Our results show a decrease in bleeding time and hepato-splenic bleeding in animals treated with rFVIIa, in comparison with placebo and PRP, with no increase in arterial thrombosis. Recombinant factor VIIa was more effective than PRP in controlling bleeding.

Recombinant factor VIIa has been tested in various animal models of bleeding which have shown the normalization of bleeding.^{19,20} Clinically, rFVIIa has enabled the control of life-threatening bleeding in patients when other measures, including platelet transfusion, had failed.^{21,22} Between 1988 and 2001, more than 6,500 patients with hemophilia or other bleeding disorders have been treated and more than 180,000

standard doses have been administered; efficacy rates were more than 80%.^{23,24}

The effect of rFVIIa on the bleeding time has also been evaluated in humans and animals with an effect similar to that found in our study, i.e., shortening of the bleeding time.^{25,26} The beneficial effects of rFVIIa on hemostasis have been explained by its unique mechanism of action. *In vitro*, Monroe *et al.* have shown that, beyond the phase of initiation of coagulation, large amounts of rFVIIa bind to the membrane of activated platelets and directly activate factor X enabling the production of large amounts of thrombin. The efficacy of rVIIa can be explained by the localized production of thrombin on activated platelets, independently of TF.²⁷ Hence, rFVIIa does not appear to bind to non-activated platelets. This would enable it to exert its effects locally, at the site of the initiation of coagulation.

The absence of any significant increase in arterial thrombosis in the form of cyclic flow reductions in the group treated with rFVIIa could confirm the non-thrombogenic potential of the drug. This result is in agreement with previous studies showing that rFVIIa is unable to induce the activation of coagulation in rabbits.^{28,29} However, while rVIIa did not increase the incidence of CFRs, the results are still debatable, specially because of the relatively small number of animals. As mentioned previously, no sample size could be calculated due to the lack of any hypothesis concerning the increase or the decrease of the thrombotic risk in this model. Nevertheless a type II error cannot be excluded even if CFRs remained approximately 50% less frequent than at baseline.

Clinically, the thrombogenic potential of the drug remains controversial. In a phase I study involving 15 hemophilia patients treated with rFVIIa, no evidence was found of any significant change in levels of fibrinogen, thrombin-antithrombin complexes and D-dimers.³⁰ Nevertheless, rare cases of arterial thrombosis have been reported in the literature.^{7,31,32}

In order to increase the sensitivity of our model, we administered HES which interferes with hemostasis by decreasing the hematocrit, diluting the platelet pool and decreasing factor VIII/von Willebrand factor complex concentration and, therefore, increases bleeding.¹⁶⁻¹⁸ In effect, hemodilution with HES resulted in an increase in bleeding time but microvascular bleeding was only slightly increased. The increase in bleeding time and the decrease in CFRs after blood volume expansion with HES can be explained by the drop in platelet count, hematocrit, platelet aggregation and the specific effect of HES on von Willebrand factor. Marret *et al.*, using Folts' arte-

rial thrombosis model in the rabbit, found a significant reduction in CFRs after hemodilution with a gelatin solution.³³ In humans, only dextrans have been shown to develop antithrombotic properties.³⁴ While HES increased the sensitivity to bleeding in this model, it probably decreased the sensitivity to thrombosis. The administration of rVIIa to normal rabbits (not having received HES) might have resulted in a less favourable effect on CFRs. In addition, some may argue that a real control group was absent because all treated animals underwent hemodilution with HES. On the other hand, hemodilution with HES is more likely to represent the clinical situation of the bleeding patient.

The bleeding model showed that treatment with rFVIIa effectively decreased the bleeding time and spleen and liver bleeding in rabbits pretreated with HES. In contrast, in this platelet-dependent model of arterial thrombosis, treatment with rFVIIa did not significantly increase cyclic flow reductions (thrombosis) the occurrence of which was impaired by the administration of HES. By virtue of these properties, rFVIIa is a promising hemostatic adjuvant for the treatment of bleeding.

References

- 1 Hay CR, Negrier C, Ludlam CA. The treatment of bleeding in acquired haemophilia with recombinant factor VIIa: a multicentre study. *Thromb Haemost* 1997; 78: 1463-7.
- 2 Kristensen J, Killander A, Hippe E, et al. Clinical experience with recombinant factor VIIa in patients with thrombocytopenia. *Haemostasis* 1996; 26(Suppl 1): 159-64.
- 3 Martinowitz U, Kenet G, Segal E, et al. Recombinant activated factor VII for adjunctive hemorrhage control in trauma. *J Trauma* 2001; 51: 431-9.
- 4 Weiskopf RB. Intraoperative use of recombinant activated coagulation factor VII. *Anesthesiology* 2002; 96: 1287-9.
- 5 Cinat ME, Wallace WC, Nastanski F, et al. Improved survival following massive transfusion in patients who have undergone trauma. *Arch Surg* 1999; 134: 964-70.
- 6 Hiippala S. Replacement of massive blood loss. *Vox Sang* 1998; 74: 399-407.
- 7 Aledort LM. rFVIIa-its thrombogenicity (Letter). *Thromb Haemost* 2000; 84: 522-3.
- 8 Folts JD, Crowell EB Jr, Rowe GG. Platelet aggregation in partially obstructed vessels and its elimination with aspirin. *Circulation* 1976; 54: 365-70.
- 9 Eichhorn EJ, Grayburn PA, Willard JE, et al. Spontaneous alterations in coronary blood flow velocity before and after coronary angioplasty in patients with severe angina. *J Am Coll Cardiol* 1991; 17: 43-52.
- 10 Collier BS, Folts JD, Scudder LE, Smith SR. Antithrombotic effect of a monoclonal antibody to the platelet glycoprotein IIb/IIIa receptor in an experimental animal model. *Blood* 1986; 68: 783-6.
- 11 Samama CM, Bonnin P, Bonneau M, et al. Comparative arterial antithrombotic activity of clopidogrel and acetylsalicylic acid in the pig. *Thromb Haemost* 1992; 68: 500-5.
- 12 Golino P, Ragni M, Cirillo P, et al. Antithrombotic effects of recombinant human, active site-blocked factor VIIa in a rabbit model of recurrent arterial thrombosis. *Circ Res* 1998; 82: 39-46.
- 13 Golino P, Ragni M, Cirillo P, et al. Effects of recombinant active site-blocked activated factor VII in rabbit models of carotid stenosis and myocardial infarction. *Blood Coagul Fibrinolysis* 2000; 11(Suppl 1): S149-58.
- 14 Delaporte-Cerceau S, Samama CM, Riou B, Bonnin P, Guillochon JJ, Coriat P. Ketorolac and enoxaparin affect arterial thrombosis and bleeding in the rabbit. *Anesthesiology* 1998; 88: 1310-7.
- 15 Ouaknine-Orlando B, Samama CM, Riou B, et al. Role of the hematocrit in a rabbit model of arterial thrombosis and bleeding. *Anesthesiology* 1999; 90: 1454-61.
- 16 Treib J, Haass A, Pindur G. Coagulation disorders caused by hydroxyethyl starch. *Thromb Haemost* 1997; 78: 974-83.
- 17 Boldt J, Knothe C, Zickmann B, Andres P, Dapper F, Hempelmann G. Influence of different intravascular volume therapies on platelet function in patients undergoing cardiopulmonary bypass. *Anesth Analg* 1993; 76: 1185-90.
- 18 Vogt NH, Bothner U, Lerch G, Lindner KH, Georgieff M. Large-dose administration of 6% hydroxyethyl starch 200/0.5 for total hip arthroplasty: plasma homeostasis, hemostasis, and renal function compared to use of 5% human albumin. *Anesth Analg* 1996; 83: 262-8.
- 19 Dinness V, Lund-Hansen T, Hedner U. Effect of recombinant human FVIIa on warfarin-induced bleeding in rats. *Thromb Res* 1990; 59: 921-9.
- 20 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: 145-57.
- 21 Vlot AJ, Ton E, Mackaay AJ, Kramer MH, Gaillard CA. Treatment of a severely bleeding patient without preexisting coagulopathy with activated recombinant factor VII. *Am J Med* 2000; 108: 421-2.

- 22 Monroe DM, Hoffman M, Allen GA, Roberts HR. The factor VII–platelet interplay: effectiveness of recombinant factor VIIa in the treatment of bleeding in severe thrombocytopathia. *Semin Thromb Hemost* 2000; 26: 373–7.
- 23 Hedner U, Glazer S, Pingel K, *et al.* Successful use of recombinant factor VIIa in patient with severe haemophilia A during synovectomy (Letter). *Lancet* 1988; 2: 1193.
- 24 Roberts HR. Recombinant factor VIIa (Novoseven®) and the safety of treatment. *Semin Hematol* 2001; 38(Suppl 12): 48–50.
- 25 Hedner U. Recombinant activated factor VII as a universal haemostatic agent. *Blood Coagul Fibrinolysis* 1998; 9(Suppl 1): S147–52.
- 26 Elg M, Carlsson S, Gustafsson D. Effects of agents, used to treat bleeding disorders, on bleeding time prolonged by a very high dose of a direct thrombin inhibitor in anesthetized rats and rabbits. *Thromb Res* 2001; 101: 159–70.
- 27 Monroe DM, Hoffman M, Oliver JA, Roberts HR. Platelet activity of high-dose factor VIIa is independent of tissue factor. *Br J Haematol* 1997; 99: 542–7.
- 28 Hedner U, Ljungberg J, Lund-Hansen T. Comparison of the effect of plasma-derived recombinant human FVIIa in vitro and in a rabbit model. *Blood Coagul Fibrinolysis* 1990; 1: 145–51.
- 29 Dinness V, Bregengaard C, Erhardtsen E, Hedner U. Recombinant human factor VIIa (rRFVIIa) in a rabbit stasis model. *Thromb Res* 1992; 67: 233–41.
- 30 Macik BG, Lindley CM, Lusher J, *et al.* Safety and initial clinical efficacy of the dose levels of recombinant activated factor VII (rFVIIa): results of a phase I study. *Blood Coagul Fibrinolysis* 1993; 4: 521–7.
- 31 Green D. Complications associated with the treatment of haemophiliacs with inhibitors. *Haemophilia* 1999; 5(Suppl 3): 11–7.
- 32 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: 1775–6.
- 33 Marret E, Bonnin P, Mazoyer E, *et al.* Effects of a polymerized bovine-derived hemoglobin solution in a rabbit model of arterial thrombosis and bleeding. *Anesth Analg* 2004; 98: 604–10.
- 34 Imperiale TF, Speroff T. A meta-analysis of methods to prevent venous thromboembolism following total hip replacement. *JAMA* 1994; 271: 1780–5.