

Intravenous rFVIIa Administered for Hemorrhage Control in Hypothermic Coagulopathic Swine with Grade V Liver Injuries

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Background: Intravenous administration of recombinant activated human clotting factor VII (rFVIIa) has been used successfully to prevent bleeding in hemophilia patients undergoing elective surgery, but not in previously normal trauma patients. This study was conducted to determine whether rFVIIa was a useful adjunct to gauze packing for decreasing blood loss from grade V liver injuries in hypothermic and coagulopathic swine.

Methods: All animals ($n = 10, 35 \pm 2$ kg) underwent a 60% isovolemic exchange transfusion with 6% hydroxyethyl starch and were cooled to 33°C core temperature. The swine then received a grade V liver injury and 30 seconds later, either 180 µg/kg rFVIIa, or saline control. All animals were gauze packed 30 seconds after injury and resuscitated 5.5 minutes after injury with lactated Ringer's solu-

tion to their preinjury mean arterial pressure. Posttreatment blood loss, mean arterial pressure, resuscitation volume, and clotting studies were monitored for 1 hour. Histology of lung, kidney, and small bowel were obtained to evaluate for the presence of microvascular thrombi.

Results: At the time of injury, core temperature was $33.3^\circ \pm 0.4^\circ\text{C}$, hemoglobin was 6 ± 0.7 g/dL, prothrombin time was 19.1 ± 1.0 seconds, activated partial thromboplastin time was 29.0 ± 4.8 seconds, fibrinogen was 91 ± 20 mg/dL, and platelets were $221 \pm 57 \times 10^5/\text{mL}$, with no differences between groups ($p > 0.05$). Clotting factor levels confirmed a coagulopathy at the preinjury point. The posttreatment blood loss was less ($p < 0.05$) in group 1 (527 ± 323 mL), than in group 2 (976 ± 573 mL). The resuscitation volume was not different ($p > 0.05$). One-hour

survival in both groups was 100%. Compared with the control group, rFVIIa increased the circulating levels of VIIa and, despite hypothermia, shortened the prothrombin time 5 minutes after injection ($p < 0.05$). Laboratory evaluation revealed no systemic activation of the clotting cascade. Postmortem evaluation revealed no evidence of large clots in the hepatic veins or inferior vena cava, or microscopic thrombi in lung, kidney, or small intestine.

Conclusion: rFVIIa reduced blood loss and restored abnormal coagulation function when used in conjunction with liver packing in hypothermic and coagulopathic swine. No adverse effects were identified.

Key Words: Hypothermia, Coagulopathy, Coagulation factor VII, rFVIIa, Hemorrhage, Animals, Liver, Injury.

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Trauma is the most frequent cause of death in Americans under the age of 34 years, with up to 80% of all early trauma deaths from uncontrolled hemorrhage.^{1–7} As many as 99% of these hemorrhagic deaths are from truncal injuries that are not amenable to manual compression. With certain injuries, such as American Association for the Surgery of Trauma (AAST) grade V liver injury,⁸ even excellent care in Level I trauma centers still results in greater than 50% mortality.⁹ Rapid initiation of damage control techniques such as liver packing appears to be helpful, but the mortality

remains high even in expert hands. The need for better methods of hemorrhage control in trauma surgery has been recognized and has been the subject of intensive study.^{10–29}

The use of site-specific procoagulant intravenous adjuncts for hemorrhage control has been relatively ignored in the trauma patient. Several drugs have been used to decrease bleeding during elective surgery.^{30–37} Recombinant activated human blood clotting factor VII (rFVIIa), originally isolated and cloned to treat hemophilia patients with inhibitors to factors VIII and IX during critical bleeding episodes or major surgery,^{38,39} has recently been used to reverse the acquired coagulopathy of hemorrhage and resuscitation in previously normal patients.^{40–45} rFVIIa has been used successfully in a variety of difficult clinical situations: a single gunshot wound victim who underwent damage control maneuvers,⁴⁰ orthotopic liver transplantation,⁴¹ postsurgical intra-abdominal hemorrhage,⁴² massive gastrointestinal bleeding,⁴³ retropubic prostatectomy,⁴⁴ and heart valve replacement.⁴⁵ It has also been used to rapidly reverse the effect of Coumadin anticoagulation in healthy volunteers,⁴⁶ and correct prothrombin times in cirrhotic patients.⁴⁷

rFVIIa is an attractive candidate therapy for acquired forms of coagulopathy because it is only active in the pres-

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ence of exposed tissue factor (TF), and has a rapid onset of action. Tissue factor is not normally exposed to the circulating blood, as it resides in the subendothelial media, and becomes exposed to circulating FVIIa only on vessel injury.^{48,49} When bound to exposed TF, the FVIIa that normally circulates in plasma initiates activation of the extrinsic clotting system at the site of injury without causing systemic activation of the coagulation system.

Because of these data, we performed a prospective, blinded trial that evaluated the ability of rFVIIa to reduce blood loss after liver packing in a hypothermic, coagulopathic animal model of AAST grade V liver injury. We hypothesized that adjunctive treatment with rFVIIa after gauze packing would decrease blood loss when compared with saline. Such treatment appears to be an effective hemostatic adjunct and without complications in this trauma model.

MATERIALS AND METHODS

Ten crossbred commercial swine weighing 35 ± 2 kg were used in this study. Animals were housed indoors. A complete corn-soybean meal-based ration was fed at 2.0 kg/pig/day and water was available ad libitum. All animals were maintained in an Association for Assessment and Accreditation of Laboratory Animal Care International accredited facility. The protocol was approved by the Animal Care and Use Committee of the Chaim Sheba Medical Center, Tel Hashomer, Israel. All animals received care in strict compliance with the *Guide for the Care and Use of Laboratory Animals*.⁵⁰

Animals were fasted 18 to 24 hours before the surgical procedure, with water allowed ad libitum. After premedication with glycopyrrolate (0.01 mg/kg) and zolazepam (Tela-zol) (4 mg/kg), anesthesia was induced with thiopental sodium (5 mg/kg) and maintained with isoflurane (1–2%) and nitrous oxide/oxygen (50%/50%). All animals were intubated and mechanically ventilated. Carotid arterial, jugular venous, and femoral lines were placed via cutdown. Retrohepatic central venous core temperature was monitored continuously with a temperature probe placed directly posterior to the liver through the femoral venous sheath. Splenectomy and urinary bladder catheter placement were performed after laparotomy. The abdominal cavity was left open to facilitate heat loss. Stable mean arterial pressure (MAP) for 15 minutes was required before further experimental procedures.

This dilution and injury procedure has been described in detail elsewhere.^{24,25} The animals underwent a 60% of estimated blood volume, isovolemic, hypothermic exchange transfusion with 6% hydroxyethyl starch (M_r 200,000; HAES-sterile, Fresenius, Germany) cooled to 33°C. Blood volume was estimated using the following equation: blood volume (mL/kg) = $161.4751 (\text{body weight}^{-0.2197})$. This equation was derived from data from Bush et al.⁵¹ and yielded an R^2 value of 0.9918. Briefly, the exchange transfusion was accomplished over three 10-minute intervals. Each interval removed 20% of the original estimated blood volume, and

simultaneously, this volume was replaced with the 33°C HAES-sterile (2 mL/kg/min) through the cervical jugular line. If an animal's core temperature was above 33°C at the conclusion of the controlled hemorrhage cycle, the abdominal cavity was irrigated with 4°C lactated Ringer's solution until the desired core temperature was reached. This isovolemic hemodilution technique reproducibly yielded a hypothermic, coagulopathic animal in hemodynamically stable condition.

Arterial blood samples were collected before hemodilution; at the end of exchange transfusion and before injury; and at 5, 15, 30, and 60 minutes after injury. Blood was analyzed to determine hemoglobin concentration, platelet count, prothrombin time (PT), activated partial thromboplastin time (aPTT), fibrinogen concentration, thrombin-antithrombin complex (TAT), thrombin time (TT), platelet adhesion to extracellular matrix (CPA), and FVII:C. In four animals in each group, the thrombelastographic (TEG) pattern was recorded. PT was performed with the thromboplastin C (Dade Behring, Deerfield, IL) reagent that gives longer clotting times. The test was performed on a Coabs Fibro (Roche, Nutley, NJ) coagulometer, since the standard automated device (Medical Laboratory MLA Electra 1000, Rockville, MD) was unable to read the short clotting times after rFVIIa administration and resulted in levels below its detection threshold. PTT was performed with PTT-LA (Diagnostics Stago, Asnières, France) that is used for detection of lupus anticoagulant, since this reagent gives longer clotting times and theoretically is more sensitive to changes in coagulation state. The PT, aPTT, and TT were assayed at the standard 37.8°C and at 33°C. Thrombin-antithrombin III complexes were assayed with Enzygnost TAT micro (Dade Behring). FVII:C level was determined with FVII deficient plasma (Dade Behring) and hemostasis reference plasma (Biopool international, Ventura, CA) using Sysmex CA-1500 (Long Grove, IL). Platelet adhesion to extracellular matrix was determined by the percentage of total area covered with adhered platelets.^{52–56} Thromboelastography was performed with the Thromboelastograph Analyzer model 5000 (Haemoscope Corporation, Niles, IL).

After the coagulopathy had been induced, the injury was created. The liver trauma model was designed to replicate human AAST grade V injuries.⁸ Using a ring clamp modified with two cutting blades that seat into a base plate, the injury was produced by double clamping of the instrument through the midportion of the liver parenchyma. The blood lost from the injury during this 30-second time period (pretreatment blood loss) was removed by a combination of continuous suctioning in the deep subdiaphragmatic space and collection by three preweighed sponges (removed before treatment).

Animals were assigned randomly to treatment groups and were designated as follows: 180 µg/kg rFVIIa, or saline control. Packing was used in both groups, as previous published experiments documented a 100% mortality in the no-treatment group.²⁴

All investigators involved with animal preparation, liver injury, gauze packing, blood collection and laboratory tests were blinded to the treatment groups.

Ten milliliters of the appropriate solution was injected into the jugular line 30 seconds after injury. Resuscitation was initiated 5.5 minutes after injury with (40°C) lactated Ringer's solution. The goal of resuscitation was a return to and maintenance of preinjury MAP. Fluid was administered at 250 mL/min with a pressure bag. This resuscitation regimen was continued until the goal was reached and reinitiated if MAP decreased during the 60-minute study period. Simultaneously with initiation of resuscitation, gauze packing around the liver was initiated. Hemostasis was evaluated, and if incomplete, more packs were placed. This packing technique is similar to the procedure used in previous studies.^{24,25} Unlike our previous work with this trauma model where resuscitation was initiated 30 seconds after injury, in this experiment resuscitation with 40°C lactated Ringer's solution was delayed until 5.5 minutes after injury to increase the opportunity for local procoagulant effect.^{24,25}

The abdomen was closed and the animal monitored for 60 minutes after injury or until death, whichever came first. Mean arterial, systolic, and diastolic blood pressures, and heart rate, were recorded at 10-second intervals throughout the study period using a continuous data collection system (Micro-Med, Louisville, KY). Death before 60 minutes was defined as a heart rate of zero. At 60 minutes, surviving animals were killed by an overdose of sodium pentobarbital.

At the end of the study period, each abdomen was opened and the intra-peritoneal blood was suctioned and measured. The total blood loss was divided into the pretreatment model-dependent portion (during the first 30 seconds after injury), and posttreatment blood loss, which included blood shed during gauze packing, free blood in the peritoneal cavity at study completion, and the difference in wet and dry laparotomy pad weights. Total resuscitation fluid use was recorded for each animal. Further documentation of the liver injury was achieved by excision and inspection of each liver and inferior vena cava at the conclusion of the experimental period. Representative samples of small bowel, lung, and kidney were taken from each animal at the time of death for histologic analysis.

Data were maintained in a microcomputer spreadsheet program (Excel, Microsoft, Redmond, WA). Means and standard deviations were calculated using the intrinsic Excel functions. Comparisons between treatment groups at individual time points and within treatment groups at different times were performed using the Excel TTEST function. The Tukey box plot was used to examine the data for nonnormal distribution, using the box plot function of SyStat 6 (SPSS, Inc., Chicago, IL). When nonnormality was detected, the Wilcoxon rank sum test was performed using the method and tables of Seigel.²⁶ Arithmetic means and standard deviations are reported.

RESULTS

Ten swine survived anesthesia and exchange transfusion and received the correct injury. All further comments refer to these five treated and five control animals.

After the 60% exchange transfusion of cold 6% hydroxyethyl starch solution (M_r 200,000), the animals' core temperature at the preinjury time point was $33.3^\circ \pm 0.4^\circ\text{C}$, with no significant differences between groups. Hemoglobin, fibrinogen, and platelet counts were decreased (Table 1), and the PT lengthened compared with baseline values (Table 2). The aPTT and lactate were not different between groups. The studies of systemic activation of the clotting system (TT, TAT, CPA, TEG) did not reveal any differences. In the hemodilution phase of the study, the FVII:C decreased 52% in both groups ($p < 0.05$). There were no significant differences between the rFVIIa-treated and control groups after the exchange transfusion.

All animals received a grade V liver injury with a large $8 \times 4 \times 4$ -cm parenchymal defect and laceration of one to three major central hepatic veins as demonstrated by probing each of the major vessels in the liver at the end of the experiment. In the treated group a mean of 1.2 hepatic veins were injured, whereas in the saline group there were two ($p < 0.05$). Despite this anatomic difference, the physiologic response to injury was the same. Mean blood loss during the 30 seconds after injury was not different, 286 ± 97 mL in the rFVIIa group and 284 ± 117 mL in the control group ($p > 0.05$). Likewise, the mean percentage drop in blood pressure from baseline 30 seconds after injury in the rFVIIa group was $21.3 \pm 13.6\%$, and for the control group was $16.2 \pm 6.8\%$, which were not different ($p > 0.05$).

Five minutes after drug injection, all animals were successfully gauze packed and received resuscitation fluids that restored their mean blood pressure to pretreatment levels. All animals survived the hour of postinjury observation. The amount of resuscitation fluid administered to restore preinjury mean blood pressure was not different between groups, $2,070 \pm 1,217$ mL in the rFVIIa group and $2,792 \pm 2,463$ mL in the control group ($p > 0.05$). The posttreatment blood loss measured in the abdomen at the end of the study hour was 527 ± 323 mL in the treated animals and 976 ± 573 mL in the control group (Fig. 1). rFVIIa reduced the posttreatment blood loss by 46%. Tukey box plot analysis revealed that posttreatment blood loss data were not normally distributed. Therefore, these data were analyzed by the Wilcoxon rank sum test, which revealed a reduced blood loss in the rFVIIa-treated pigs ($p < 0.05$).

Within 5 minutes of treatment with rFVIIa, the PT shortened significantly compared with the pretreatment values, whereas the aPTT did not change (Table 2). These differences were maintained whether they were measured at 33°C or 37.8°C . Although numerically the hemoglobin concentration, fibrinogen concentration, and platelet count values were lower in the control group at the study conclusion, the differences did not reach significance. There was no effect of the

Table 1 Serial Laboratory Values in Hypothermic and Coagulopathic Swine with Grade V Liver Injury and Gauze Packing Followed By Adjuvant rFVIIa or Placebo. Mean $\pm$ SD are shown.

	T-1	T-2	T-3	T-4	T-5	T-6
Hgb (g/dL)						
Control	10.5 $\pm$ 0.6 ^a	5.8 $\pm$ 0.5 ^b	5.7 $\pm$ 0.8 ^b	4.7 $\pm$ 1.6 ^b	4.5 $\pm$ 1.4 ^b	4.4 $\pm$ 1.8 ^b
rFVIIa	11.3 $\pm$ 1.4 ^a	6.2 $\pm$ 0.8 ^b	6.4 $\pm$ 0.7 ^b	5.5 $\pm$ 1.0 ^b	5.7 $\pm$ 1.0 ^b	6.4 $\pm$ 1.2 ^b
Plt (10 ⁶)						
Control	475 $\pm$ 89 ^a	231 $\pm$ 58 ^b	256 $\pm$ 59 ^b	233 $\pm$ 74 ^b	224 $\pm$ 67 ^b	220 $\pm$ 74 ^b
rFVIIa	433 $\pm$ 94 ^a	212 $\pm$ 61 ^b	250 $\pm$ 57 ^b	232 $\pm$ 69 ^b	229 $\pm$ 62 ^b	272 $\pm$ 63 ^b
Fib (mg/dL)						
Control	174 $\pm$ 7 ^a	80 $\pm$ 13 ^b	76 $\pm$ 11 ^b	64 $\pm$ 27 ^b	58 $\pm$ 23 ^b	57 $\pm$ 24 ^b
rFVIIa	227 $\pm$ 38 ^a	103 $\pm$ 21 ^b	107 $\pm$ 18 ^b	87 $\pm$ 23 ^b	79 $\pm$ 45 ^b	85 $\pm$ 30 ^b
Lactate (mmol/L)						
Control	12.6 $\pm$ 7.7 ^a	11.1 $\pm$ 4.6 ^a	14.4 $\pm$ 3.8 ^a	20.5 $\pm$ 8.1 ^a	23.0 $\pm$ 7.6 ^a	30.3 $\pm$ 13.6 ^b
rFVIIa	10.4 $\pm$ 2.2 ^a	11.8 $\pm$ 5.8 ^a	19.0 $\pm$ 7.6 ^a	26.0 $\pm$ 11.4 ^a	27.2 $\pm$ 9.4 ^a	31.4 $\pm$ 6.5 ^b

T-1 = baseline, T-2 = postdilution and preinjury, T-3 = 5 minutes after injury and treatment, T-4 = 15 minutes after injury, T-5 = 30 minutes after injury, T-6 = 60 minutes after injury.

^{a,b} Time-dependent differences ($p < 0.05$) for each variable, within treatment group. Differences within time groups are discussed in the Results section.

Table 2 Serial Coagulation Values Measured at 33°C or 37.8°C in Hypothermic and Coagulopathic Swine with Grade V Liver Injury Treated with Gauze Packing and Adjuvant rFVIIa or Placebo. Mean $\pm$ SD are shown.

		T-1	T-2	T-3	T-4	T-5	T-6
PT (sec)	Temp.						
Control	33°C	18 $\pm$ 1.6 ^a	19 $\pm$ 1.4 ^a	20 $\pm$ 0.7 ^a	19 $\pm$ 0.8 ^a	19 $\pm$ 0.8 ^a	21 $\pm$ 3.3 ^a
	37.8°C	13 $\pm$ 0.3 ^a	13 $\pm$ 1.2 ^a	13 $\pm$ 0.5 ^a	13 $\pm$ 1.0 ^a	13 $\pm$ 0.2 ^a	14 $\pm$ 1.0 ^a
rFVIIa	33°C	18 $\pm$ 0.9 ^a	19 $\pm$ 0.7 ^a	11 $\pm$ 1.1 ^b	11 $\pm$ 0.5 ^b	12 $\pm$ 0.4 ^b	12 $\pm$ 1.0 ^b
	37.8°C	13 $\pm$ 0.8 ^a	13 $\pm$ 0.9 ^a	8 $\pm$ 0.4 ^b	8 $\pm$ 0.5 ^b	8 $\pm$ 0.6 ^b	8 $\pm$ 0.8 ^b
PTT (sec)	Temp.						
Control	33°C	50 $\pm$ 8 ^a	29 $\pm$ 6 ^b	26 $\pm$ 1 ^b	28 $\pm$ 3 ^b	28 $\pm$ 2 ^b	28 $\pm$ 5 ^b
	37.8°C	30 $\pm$ 5 ^a	23 $\pm$ 4 ^a	23 $\pm$ 3 ^a	21 $\pm$ 5 ^a	23 $\pm$ 6 ^a	23 $\pm$ 6 ^a
rFVIIa	33°C	30 $\pm$ 6 ^a	29 $\pm$ 4 ^a	28 $\pm$ 3 ^a	29 $\pm$ 4 ^a	27 $\pm$ 3 ^a	28 $\pm$ 3 ^a
	37.8°C	45 $\pm$ 16 ^a	27 $\pm$ 6 ^a	23 $\pm$ 6 ^a	20 $\pm$ 3 ^b	23 $\pm$ 6 ^a	23 $\pm$ 6 ^a
TT (sec)	Temp.						
Control	33°C	27.1 $\pm$ 3.4 ^a	22.4 $\pm$ 1.7 ^a	22.5 $\pm$ 0.6 ^a	25.0 $\pm$ 3.4 ^a	25.0 $\pm$ 1.8 ^a	27.6 $\pm$ 2.8 ^a
	37.8°C	25.1 $\pm$ 0.3 ^a	15.6 $\pm$ 0.8 ^b	16.4 $\pm$ 1.1 ^b	19.1 $\pm$ 1.9 ^b	20.6 $\pm$ 3.5 ^b	20.1 $\pm$ 2.6 ^b
rFVIIa	33°C	26.6 $\pm$ 3.4 ^a	23.4 $\pm$ 3.2 ^a	23.6 $\pm$ 3.0 ^a	24.5 $\pm$ 2.9 ^a	25.0 $\pm$ 3.0 ^a	28.4 $\pm$ 3.8 ^a
	37.8°C	25.8 $\pm$ 2.9 ^a	15.6 $\pm$ 1.6 ^b	16.4 $\pm$ 1.6 ^b	18.1 $\pm$ 2.8 ^b	18.3 $\pm$ 3.2 ^b	19.7 $\pm$ 3.6 ^b
TAT							
Control		27 $\pm$ 10.4 ^a	73 $\pm$ 37.4 ^a	68 $\pm$ 33.5 ^a	78 $\pm$ 39.5 ^a	86 $\pm$ 32.9 ^a	89 $\pm$ 34.4 ^a
rFVIIa		17 $\pm$ 7.6 ^a	23 $\pm$ 6.9 ^a	32 $\pm$ 18.2 ^a	40 $\pm$ 12.2 ^a	66 $\pm$ 34.4 ^a	99 $\pm$ 58.8 ^a
CPA							
Control		36.0 $\pm$ 10.4 ^a	16.3 $\pm$ 10.9 ^a	12.5 $\pm$ 4.9 ^b	11.5 $\pm$ 6.8 ^b	8.7 $\pm$ 5.1 ^b	12.1 $\pm$ 9.3 ^b
rFVIIa		36.4 $\pm$ 8.7 ^a	15.3 $\pm$ 6.1 ^b	15.4 $\pm$ 8.8 ^b	15.6 $\pm$ 10.5 ^b	16.7 $\pm$ 10.6 ^b	22.4 $\pm$ 15.9 ^c
FVII:C (IU/ml)							
Control		0.55 $\pm$ 0.1 ^a	0.33 $\pm$ 0.17 ^b	0.27 $\pm$ 0.09 ^b	0.23 $\pm$ 0.1 ^b	0.24 $\pm$ 0.08 ^b	0.23 $\pm$ 0.09 ^b
rFVIIa		0.57 $\pm$ 0.1 ^a	0.22 $\pm$ 0.03 ^b	35.4 $\pm$ 9.5 ^c	28.2 $\pm$ 7.1 ^c	23.2 $\pm$ 4.3 ^c	21.4 $\pm$ 4.3 ^c

T-1 = baseline, T-2 = postdilution and preinjury, T-3 = 5 minutes after injury and treatment, T-4 = 15 minutes after injury, T-5 = 30 minutes after injury, T-6 = 60 minutes after injury.

^{a-c} Time-dependent differences ($p < 0.05$) for each variable, within treatment group. Differences within time groups are discussed in the Results section.

rFVIIa administration on CPA, TT, TAT, or any component of the TEG (Table 2). Serum lactate levels were increased in both groups, but were not different between groups at the study conclusion. As expected, after treatment with rFVIIa, the measured levels of FVII:C significantly increased (160-fold). The PT shortened from 10.4 $\pm$ 0.3 seconds to 7 $\pm$ 0.5 seconds in the treated group. This FVII:C increase was asso-

ciated with a concomitant shortening of PT measurements that was independent of temperature.

Examination of the abdominal vena cava after removal of the liver at the end of the study showed no evidence of blood clots. Micromorphologic examination of samples of lung, liver, and kidney showed no evidence of microvascular thrombi.

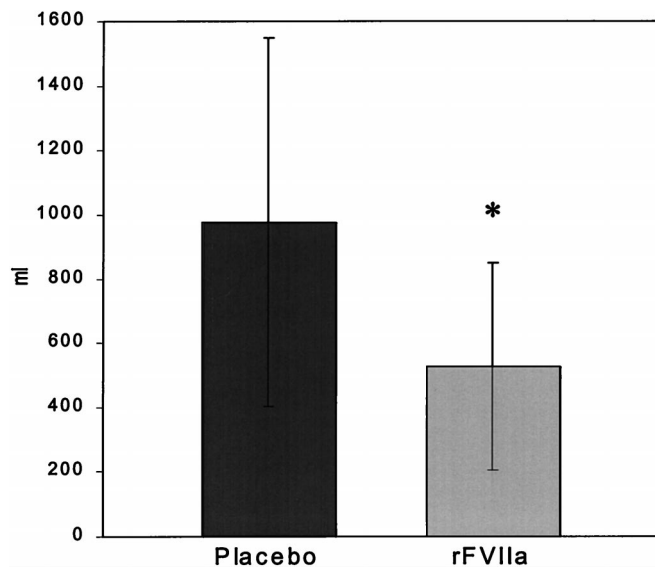


Fig. 1. Posttreatment blood loss in swine with an induced hypothermic and dilutional coagulopathy followed by grade V liver injury and gauze packing, and adjunctively treated with either rFVIIa or placebo. Means $\pm$ SD are shown. * $p < 0.05$.

DISCUSSION

Control of hemorrhage is critical to the survival of military and civilian trauma patients, in both prehospital and inpatient care. Mortality data from the Vietnam War indicate that more than half of combat deaths were because of uncontrolled truncal hemorrhage.^{57–60} At least 10% of the soldiers that exsanguinated did so after receiving hospital-based care.⁵⁷ Although improved transit times mean that only 40% of civilian trauma patients die before reaching the hospital, the consequences of continued hemorrhage are such that 82% of all early inpatient civilian trauma deaths were caused by uncontrolled hemorrhage in one study, and 50% were the result of severe liver injuries.² Truncal hemorrhage is the most important cause of civilian hemorrhagic death, with 99% of all sites of fatal hemorrhage occurring proximal to the axillary and groin creases. These data suggest that a substantial decrease in combat and civilian mortality can be effected by the development of a simple, rapid, and effective method of truncal hemorrhage control.

To address this need, we studied the administration of rFVIIa used as an adjunct for hemorrhage control in an animal model of grade V liver injury. This models a clinical situation that is associated with a greater than 50% mortality in humans.⁹ As shown previously, this injury model consistently yields a large parenchymal liver defect and lacerates major central hepatic veins.^{24,25} With the addition of a hypothermic hemodilution, all animals sustained an equivalent degree of dilutional and hypothermic coagulopathy. The injury caused an equivalent volume of pretreatment blood loss and drop in MAP in treatment and control groups. Treatment with 180 μ g/kg of rFVIIa was associated with a 46% reduction in blood loss ($p < 0.05$). We feel these data demonstrate

that physiologically the two groups were equivalent, and that the differences between the two groups were related to the activity of the rFVIIa.

Normally, only 1% of circulating FVII is in the active form. The addition of 180 μ g/kg of rFVIIa would be expected to increase the concentration of the active form of FVII by 450% and ensure that most exposed TF would bind FVIIa. High concentrations of thrombin produced by the TF-FVIIa complex can directly activate platelets, bypassing the exquisitely temperature-sensitive mechanism of normal platelet activation involving von Willebrand factor; platelet glycoproteins Ib, IX, and V; and thromboxane. Active platelets in turn provide a surface that amplifies the activity of the X-ase and prothrombinase plasma coagulation enzyme complexes 10,000-fold. The combination of these two effects can lead to a 1 million-fold amplification of coagulation localized at the site of injury and that is not highly temperature dependent.

Laboratory measures of blood cells and coagulation proteins and functions (Tables 1–3) reflect the changes from hypothermic dilution, rFVIIa delivery, and partially controlled hemorrhage with resuscitation. In studies examining coagulation defects associated with hypothermia, it is more reflective of the *in vivo* state to perform coagulation tests at the temperature of the animal from which the samples were collected, in addition to the standard temperature of 37°C.^{61–67} Prolonged PT and aPTT have been previously reported in swine, as well as humans, when assayed at temperatures below 37.8°C.^{62,63} Additionally, Gubler et al.⁶¹ reported that hypothermia and dilutional coagulopathy combined to produce PT and aPTT prolongations greater than hypothermia alone. In the current study, preinjury hemodilution and hypothermia resulted in groups that were equally coagulopathic.

Because the rFVIIa binding to exposed TF is the initial step in a series of enzymatic reactions culminating in fibrinogen conversion to fibrin and resulting in clot, it was theoretically possible that the performance of the rFVIIa could have been affected by temperature. However, the high concentrations of circulating rFVIIa completely reversed the prolonged PT within 5 minutes, despite the deleterious effects of hypothermia and dilution posed by this model. Since the PT mainly reflects the content of active FVII/FVIIa present in the plasma sample, normalization of the PT means that the FVIIa was enzymatically active in the presence of TF normally present in the reagent. The expected phenomenon of PT shortening to less than normal values after administration of the drug occurs because the test reagents include TF that forms a complex with VIIa, and initiates *in vitro* coagulation in the test tube. This effect was demonstrated at both 33° and 37.8°C. This in fact reflects the effect of rFVIIa at the site of injury, rather than a systemic hypercoagulable effect. Previous prospective demonstrations of the effect of rFVIIa in normalizing a prolonged PT have been performed in cirrhotic patients or in healthy patients after Coumadin administration, without the complicating factor of hypothermia.^{47,48} Kenet et

Table 3 Serial Components of the Thromboelastogram Measured at 37.8°C in Hypothermic and Coagulopathic Swine with Grade V Liver Injury Treated with Gauze Packing and Adjuvant rFVIIa or Placebo. Mean $\pm$ SD are shown.

	T-1	T-2	T-3	T-4	T-5	T-6
SP						
Control	131 $\pm$ 15	91 $\pm$ 26	87 $\pm$ 19	86.5 $\pm$ 34.6	96.3 $\pm$ 19.9	106.2 $\pm$ 37.6
rFVIIa	137 $\pm$ 56	130 $\pm$ 53	103 $\pm$ 31	122.8 $\pm$ 64.6	111.5 $\pm$ 32.7	145.0 $\pm$ 53.0
R						
Control	141.5 $\pm$ 11.4	107.3 $\pm$ 27.3	101.3 $\pm$ 21.9	103.5 $\pm$ 36.4	113.0 $\pm$ 21.2	122.2 $\pm$ 36.7
rFVIIa	150 $\pm$ 61.0	149.5 $\pm$ 60.5	112.3 $\pm$ 32.9	139.5 $\pm$ 71.3	125.8 $\pm$ 40.8	139.0 $\pm$ 69.3
K						
Control	45.8 $\pm$.5	58.5 $\pm$ 12.3	48.8 $\pm$ 4.1	61.5 $\pm$ 26.8	72.5 $\pm$ 49.7	63.5 $\pm$ 28.4
rFVIIa	46.0 $\pm$.82	67.3 $\pm$ 16.2	55.3 $\pm$ 9.9	62.0 $\pm$ 15.8	55.0 $\pm$ 10.4	64.0 $\pm$ 18.0
ANG						
Control	83.0 $\pm$.4	74.4 $\pm$ 4.6	76.4 $\pm$ 4.3	73.4 $\pm$ 6.7	70.9 $\pm$ 10.1	72.9 $\pm$ 7.7
rFVIIa	81.9 $\pm$ 1.9	76.0 $\pm$ 3.2	77.4 $\pm$ 3.6	76.5 $\pm$ 4.0	74.8 $\pm$ 7.4	71.7 $\pm$ 8.6
MA						
Control	79.3 $\pm$ 1.2	64.2 $\pm$ 3.2	66.5 $\pm$ 2.4	63.6 $\pm$ 11.6	59.9 $\pm$ 13.9	61.6 $\pm$ 9.9
rFVIIa	71.4 $\pm$ 16.6	58.4 $\pm$ 13.7	61.1 $\pm$ 14.1	57.9 $\pm$ 12.9	60.0 $\pm$ 13.5	59.0 $\pm$ 13.2
TMA						
Control	637 $\pm$ 74	780.5 $\pm$ 212	816.8 $\pm$ 116.7	807.5 $\pm$ 129.5	948.3 $\pm$ 67.8	858.5 $\pm$ 134.8
rFVIIa	637 $\pm$ 73.2	783 $\pm$ 137.3	787 $\pm$ 136.4	739 $\pm$ 125.6	860 $\pm$ 191	766 $\pm$ 219.4

T-1 = baseline, T-2 = postdilution and preinjury, T-3 = 5 minutes after injury and treatment, T-4 = 15 minutes after injury, T-5 = 30 minutes after injury, T-6 = 60 minutes after injury. Differences are discussed in Results.

al. have demonstrated the effectiveness of rFVIIa in a case report after damage control in a hypothermic trauma patient.⁴¹ A series of case reports presented by Martinowitz et al. likewise demonstrate effectiveness at hypothermic temperatures.⁶⁸ To our knowledge, the data presented herein represent the first prospective, controlled results documenting that rFVIIa is active within 5 minutes of administration despite hypothermia and dilution. Taken together, these findings demonstrate that rFVIIa retains its enzymatic activity in the presence of TF, hypothermia, and dilution. This may be of importance in the reversal of the recognized coagulopathic complications from hypothermia in trauma patients, a key component of the bloody vicious cycle.⁶⁹ Thus, the case reports and this animal study describe a potential new rapid method of adjunctive hemorrhage control in the care of the hypothermic traumatized patient.

Importantly, the three laboratory tests that evaluate systemic activation of the clotting cascade, TAT, CPA, and TEG, were not different between the two groups (Tables 2 and 3). This demonstrates that in this hypothermic, diluted, and hypotensive trauma model, there was no identifiable laboratory evidence of systemic activation of the clotting cascade by rFVIIa.

In the current study, rFVIIa was used as an adjunct to the gauze liver packing for hemorrhage control. This is accomplished by simply injecting 10 mL of saline containing the dissolved recombinant protein. The drug should be stored at 4°C (package insert, Novo Nordisk, Bagsvaerd, Denmark), but can remain mixed at room temperature for up to 24 hours.⁷⁰ It requires no blood bank support and, because the drug is a recombinant product, should not transmit disease. The method described herein using rFVIIa is a simple adjunct

to rapid gauze packing and adds active intravascular hemostasis at the site of injury by modulating the coagulation cascade, and controlling hemorrhage from grade V injuries in this swine model.

Originally isolated and later cloned to treat hemophilia patients with inhibitors to factors VIII or IX, or inadequate levels of FVII, rFVIIa has dramatically improved the lives and reduced the mortality of these patients. It has become the treatment of choice for hemophilia A and B patients with inhibitors to missing coagulation factors. Two dose-finding studies in hemophilia patients document the speed, efficacy, and safety of rFVIIa.^{71,72}

rFVIIa may hold the most promise of the injectable hemorrhage control drugs because it is the initiating event in the extrinsic pathway, the so-called tissue trauma pathway, and provides site-specific thrombin generation by enhancing the TF:FVIIa assembly at the site of vessel injury. As demonstrated by Hedner and Kiesel in 1983, administration of rFVIIa accelerates thrombin generation and thus more effective hemostasis.³⁹ Normal hemostasis is initiated by the formation of a complex between TF exposed after injury to the vessel wall and already activated rFVIIa present in a concentration of around 1% of the total FVII.

Although the main effect of rFVIIa is compartmentalized at the site of injury, there are some conditions where the theoretical risk of thromboembolic complications may be increased. Of concern, blunt trauma patients often have multiple areas of contusion, laceration, or fracture. These damaged sites all contain exposed tissue factor, and there exists the potential for unintended intravascular clotting from non-bleeding areas. Ongoing or planned animal and human studies are addressing these concerns. Because of these diffuse

thrombotic concerns, initial use of rFVIIa should be in those trauma patients with an anticipated high mortality.

During gram-negative sepsis, activated monocytes express TF on their surface which, together with high concentration of circulating rFVIIa, may lead to systemic activation of coagulation.⁷³ Two patients with disseminated intravascular coagulopathy caused by sepsis were treated with rFVIIa without thromboembolic complications, but a larger experience is required to evaluate the risk in these patients.⁷⁴ Atheromatous plaques also contain TF, which normally is encrypted but becomes exposed when the plaque is ruptured. Concomitant plaque rupture and administration of rFVIIa may lead to coronary ischemia and acute myocardial infarct (MI).⁷⁵

Several authors have reported their experience with thrombotic events (MI and stroke) with the systemic use of rFVIIa.^{75–79} In the 17 years rFVIIa has been clinically used, only six documented cases of MI have occurred, supporting the claim that a limited number of thromboembolic events have resulted from widespread use.^{78,79} The majority of these cases have occurred in patients age 60 or greater. Importantly, in this animal model of injury, hypotension, and reperfusion, there was no gross or microscopic evidence of posttreatment thrombosis, nor was there biochemical evidence of systemic activation of the clotting cascade. However, a study duration of only 60 minutes after injection prevents any definitive conclusions about long-term clinically significant thrombotic complications.

Published data of rFVIIa use in previously normal patients is increasing. In a prospective study of cirrhotic patients with abnormal PTs, intravenous injection of rFVIIa corrected the PT within 5 minutes, and at higher doses the PT remained normal for up to 24 hours, reflecting the presence of extra FVIIa in the patient's circulation.⁴⁸ This is understandable when one considers that the main hemostatic defect in cirrhosis is a deficiency of adequate levels of FVII. Furthermore, Erhardtsen et al. demonstrated that rFVIIa could reverse the effects of Coumadin for up to 24 hours in normal volunteers.⁴⁷ No complications were noted in either study. Successful use after gauze packing in a hypothermic trauma patient with a gunshot wound to the inferior vena cava has been reported.⁴¹ Patients with gastrointestinal bleeding have been reported, totaling three nonhemophiliac patients.^{43,44} Furthermore, in a series of six patients undergoing orthotopic liver transplantation, rFVIIa decreased transfusion requirements 75% when compared with historical controls.⁴² None of these reports document acute thrombosis or long-term complications, whereas all document hemostatic efficacy. All of the case reports in these very ill patients document rapid decrease in blood loss and transfusion requirements. In a patient that died after administration of rFVIIa, the autopsy revealed no evidence of systemic thrombosis.⁴³ The findings in the current animal study are consistent with the increasing clinical experience with rFVIIa in nonhemophiliac patients with an acquired hemostatic abnormality.

Despite the use of a well-documented surgical model and appropriate operator blinding, this study has several obvious weaknesses. One problem is the uneven distribution of injured central lobar veins between the two groups. The treatment group had fewer veins injured than the control group. Nevertheless, the blood loss and blood pressure changes were the same between groups before treatment. Moreover, in our experience with this model in over 300 individual experiments, the number of central lobar veins injured does not correlate with blood loss; rather, blood loss is associated with the effectiveness of hemorrhage control. The most important concern is the small size of the study, with only five animals in each treatment group. As a result, this study should be viewed as exploratory, and the data as suggestive.

A strength of the current study was the ability to completely blind the investigators to the rFVIIa treatment group. This is unusual in hemorrhage control studies, because at some point the different techniques under evaluation usually become obvious to the surgical investigators. None of the investigators involved in creating the injury, blood collection, gauze packing, or injury scoring were involved in drug selection or delivery. Therefore, we feel that the 46% decrease in blood loss in the rFVIIa group was because of improved hemostasis, rather than a bias in favor of the study group or a poor packing technique. Despite the differences in blood loss in the study, there were no differences in survival between the groups. This was because of the short duration of the study, the hemostatic efficacy of the gauze packing, and the relatively small amount of blood loss in the control group (33% of the estimated blood volume).

Because the majority of early civilian and military trauma deaths are from hemorrhage, we have initiated a series of animal studies evaluating new methods of hemostasis. These methods use manual compression,^{24,25,30} intracavitary administration,²⁸ and now intravascular injection. Some of these approaches will require many years to develop a usable product. We feel that a U.S. Food and Drug Administration–approved injectable drug, proven safe for even home use in hemophiliacs,⁸⁰ that circulates and accelerates clotting at the site of damaged endothelium is a logical and possibly useful addition to the rapidly expanding hemorrhage control techniques available to medics and surgeons.

REFERENCES

1. Committee on Injury Prevention, and Control, Institute of Medicine. In: Bonnie RJ, Fulco CE, Liverman CT, eds. *Reducing the Burden of Injury: Advancing Prevention and Treatment*. Washington, DC: National Academy Press; 1999.
2. Sauaia A, Moore FA, Moore EE, et al. Epidemiology of trauma deaths: reassessment. *J Trauma*. 1995;38:185–193.
3. Hoyt D, Bulger E, Knudson M, et al. Deaths in the operating room: an analysis of a multi-center experience. *J Trauma*. 1994;37:426–432.
4. Shackford SR, Mackersie RC, Davis JW, et al. Epidemiology and pathology of trauma deaths occurring in a level 1 trauma center in a regionalized system: the importance of secondary brain injury. *J Trauma*. 1989;29:1392–1397.

5. Acosta JA, Yang JC, Winchell RJ, et al. Lethal injuries and time to death in a level 1 trauma center. *J Am Coll Surg.* 1998;186:528–533.
6. Shackford SR, Mackersie RC, Holbrook TL, et al. The epidemiology of trauma. *Arch Surg.* 1993;128:571–575.
7. Baker CC. Epidemiology of trauma deaths. *Am J Surg.* 1980;140:144–150.
8. Moore E, Cogbill T, Jurkovich G, et al. Organ injury scaling: spleen and liver (1994 revision). *J Trauma.* 1995;38:323–324.
9. Pachter HL, Liang HG, Hofstetter SR. Liver and biliary tract trauma. In: Mattox KL, Feliciano DV, Moore EE, eds. *Trauma.* 4th ed. Stamford, CT: Appleton & Lange; 2000:633–682.
10. Kram HB, Reuben BI, Fleming AW, Shoemaker WC. Use of fibrin glue in hepatic trauma. *J Trauma.* 1988;28:1195–1201.
11. Ochsner MG, Maniscalco-Theberge ME, Champion HR. Fibrin glue as a hemostatic agent in hepatic and splenic trauma. *J Trauma.* 1990;30:884–887.
12. Kram HB, del Junco T, Clark SR, et al. Techniques of splenic preservation using fibrin glue. *J Trauma.* 1990;30:97–101.
13. Kram HB, Nathan RC, Stafford FJ, Fleming AW, Shoemaker WC. Fibrin glue achieves hemostasis in patients with coagulation disorders. *Arch Surg.* 1989;124:385–387.
14. Larson M, Bowersox JC, Lim RC Jr, Hess JR. Efficacy of a fibrin hemostatic bandage in controlling hemorrhage from experimental arterial injuries. *Arch Surg.* 1995;30:420–422.
15. Jackson MR, Friedman SA, Carter AJ, et al. Hemostatic efficacy of a fibrin sealant-based topical agent in a femoral artery injury model: a randomized, blinded, placebo-controlled study. *J Vasc Surg.* 1997;26:274–280.
16. Holcomb JB, Pusateri AE, Hess JR, et al. Implications of new dry fibrin sealant technology for trauma surgery. *Surg Clin North Am.* 1997;77:943–952.
17. Bayer V, Gallanger A, Urely P, et al. Renal hemorrhage model in the heparinized rat: a small animal model for the rapid assessment of topical hemostatic agents [abstract]. *Thromb Haemost.* 1997;77(suppl):S251. Abstract PS-1022.
18. Cornum RL, Gresham V, Bell J, et al. Evaluation of an absorbable fibrin adhesive dressing in canine prostatectomies [abstract]. *Thromb Haemost.* 1997;77(suppl):S196. Abstract OC-794.
19. Hess J, Holcomb J, MacPhee M. Hemorrhage control with a dry powdered thrombin and fibrinogen bandage [abstract]. *Thromb Haemost.* 1997;77(suppl):S196. Abstract OC-793.
20. Pusateri A, Holcomb J, Harris R, et al. Hemodynamic effect of intravenous bovine or human thrombin in swine [abstract]. *Thromb Haemost.* 1997;77(suppl):S251. Abstract PS-1026.
21. Holcomb JB, MacPhee MJ, Hetz SP, et al. Efficacy of a dry fibrin sealant dressing for hemorrhage control after ballistic injury. *Arch Surg.* 1998;133:32–35.
22. Tock B, Drohan W, Hess JR, et al. Haemophilia and advanced fibrin sealant technologies. *Hemophilia.* 1998;4:449–453.
23. Cohn SM, Cross JH, Ivy ME, Feinstein AJ, Samotowka MA. Fibrin glue terminates massive bleeding after complex hepatic injury. *J Trauma.* 1998;45:666–672.
24. Holcomb JB, Pusateri AE, Harris RA, et al. Effect of dry fibrin sealant dressings versus gauze packing on blood loss in grade V liver injuries in resuscitated swine. *J Trauma.* 1999;46:49–57.
25. Holcomb JB, Pusateri AE, Harris RA, et al. Dry fibrin sealant dressings reduce blood loss, resuscitation volume and improve survival in hypothermic coagulopathic swine with grade V liver injuries. *J Trauma.* 1999;47:233–242.
26. Siegal S. *Nonparametric Statistics for the Behavioral Sciences.* New York: McGraw-Hill; 1956.
27. Cornum R, Bell J, Gresham V, Brinkley W, Beall D, MacPhee M. Intraoperative use of the absorbable fibrin adhesive bandage: long term effects. *J Urol.* 1999;162:1817–1820.
28. Holcomb JB, McClain JM, Pusateri AE, et al. Fibrin sealant foam sprayed directly on liver injuries decreases blood loss in resuscitated rats. *J Trauma.* 2000;49:246–250.
29. Cornum RL, Morey AF, Harris R, et al. Does the absorbable fibrin adhesive bandage facilitate partial nephrectomy? *J Urol.* 2000;164(3 pt 1):864–867.
30. Sondeen JL, Holcomb JB, Pusateri AE, Coppes VG, Gaddy CE. Fibrin dressing stops bleeding from aortic injury with one four-minute application. Poster presentation at the 60th Annual Meeting of the American Association for the Surgery of Trauma; October 12, 2000; San Antonio, TX.
31. Terrell M, Walenga J, Koza M, Pifarre R. Efficacy of aprotinin with various anticoagulant agents in cardiopulmonary bypass. *Ann Thorac Surg.* 1996;62:506–511.
32. Lentschener C, Benhamou D, Mercier F, et al. Aprotinin reduces blood loss in patients undergoing elective liver resection. *Anesth Analg.* 1997;84:875–881.
33. Patrassi G, Viero M, Sartori M, et al. Aprotinin efficacy on intraoperative bleeding and transfusion requirements in orthotopic liver transplantation. *Transfusion.* 1994;34:507–511.
34. Murkin J, Shannon N, Bourne R, et al. Aprotinin decreases blood loss in patients undergoing revision or bilateral total hip arthroplasty. *Anesth Analg.* 1995;80:343–348.
35. Katsaros D, Petricevic M, Snow N, et al. Tranexamic acid reduces post-bypass blood use: a double-blinded, prospective, randomized study of 210 patients. *Ann Thorac Surg.* 1996;61:1131–1135.
36. Boylan J, Klinck J, Sandler A, et al. Tranexamic acid reduces blood loss, transfusion requirements, and coagulation factor use in primary orthotopic liver transplantation. *Anesthesiology.* 1992;85:1043–1048.
37. Hiipala S, Strid L, Wennerstrand M. Tranexamic acid radically decreases blood loss and transfusions associated with total knee arthroplasty. *Anesth Analg.* 1997;84:839–844.
38. Montesano R, Gustafson P, Palanzo D, et al. The effect of low-dose epsilon-aminocaproic acid on patients following coronary artery bypass surgery. *Perfusion.* 1996;11:53–56.
39. Hedner U, Kisel W. The use of human factor VIIa in the treatment of two hemophilia patients with high-titer inhibitors. *J Clin Invest.* 1983;71:1836–1844.
40. Hedner U, Ingerslev J. Clinical use of recombinant (rFVIIa). *Transfus Sci.* 1998;19:163–176.
41. Kenet G, Walden R, Eldad A, Martinowitz U. Treatment of traumatic bleeding with recombinant factor VIIa. *Lancet.* 1999;354:1879.
42. Hendriks H, Meijer K, Hagenaaers A, et al. Recombinant factor VIIa (rFVIIa, NovoSeven) decreases blood loss and blood product requirements during orthotopic liver transplantation. Oral presentation at the 5th Novo Nordisk Symposium on the treatment of bleeding and thrombotic disorders, May 7–8, 1999, Copenhagen, Denmark.
43. White B, McHale J, Ravi N, et al. Successful use of recombinant FVIIa (Novoseven) in the management of intractable post-surgical intra-abdominal haemorrhage. *Br J Haematol.* 1999;107:677–678.
44. Vlot AJ, Ton E, Mackaay AJC, Kramer MHH, Gaillard CAJM. Treatment of a severely bleeding patient without preexisting coagulopathy with activated recombinant factor VII. *Am J Med.* 2000;108:421–423.
45. 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 retropubic prostatectomy: the PROSE study. *Blood Coagul Fibrinolysis.* 2000;11(suppl 1):S129–S132.
46. Al Douri M, Shafi T, Al Khudairi D, et al. Effect of the administration of recombinant activated factor VII (rFVIIa; NovoSeven) in the management of severe uncontrolled bleeding in

- patients undergoing heart valve replacement surgery. *Blood Coagul Fibrinolysis*. 2000;11(suppl 1):S121–S127.
47. Erhardtsen E, Nony P, Dechavanne M, French P, Boissel JP, Hedner U. 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.
 48. Bernstein DE, Jeffers L, Erhardtsen E, et al. rFVIIa corrects prothrombin time in cirrhotic patients: a preliminary study. *Gastroenterology*. 1997;113:1930–1937.
 49. Rapaport SI, Rao LVM. Initiation and regulation of tissue factor-dependent blood coagulation. *Arterioscler Thromb*. 1992;12:1111–1121.
 50. Institute of Laboratory Animal Resources Commission on Life Sciences, National Research Council. *Guide for the Care and Use of Laboratory Animals*. Washington, DC: National Academy Press; 1996.
 51. Rapaport SI, Rao LVM. The tissue factor pathway: how it has become a “prima ballerina.” *Thromb Haemost*. 1995;74:7–17.
 52. Bush JA, Jensen WN, Cartwright GE, Wintrobe MM. Blood volume studies in normal and anemic swine. *Am J Physiol*. 1955;181:9–14.
 53. Gospodarowicz D, Vlodavsky I, Savion N. The role of fibroblast growth factor and the extra-cellular matrix in the control of proliferation and differentiation of corneal endothelial cells. *Vision Res*. 1981;21:87–103.
 54. Gospodarowicz D, Greenburg G, Foidart JM, Savion N. The production and localization of laminin in cultured vascular and corneal endothelial cells. *J Cell Physiol*. 1981;107:173–183.
 55. Varon D, Dardik R, Shenkman B, et al. A new method for quantitative analysis of whole blood platelet interaction with extracellular matrix under flow conditions. *Thromb Res*. 1997;85:283–294.
 56. Kenet G, Lubetsky A, Shenkman B, Tanarin I, Dardik R, Rechavi A. Cone and platelet analyzer (CPA): a new test for the prediction of bleeding among thrombocytopenic patients. *Br J Haematol*. 1998;101:255–259.
 57. Bellamy RF. The causes of death in conventional land warfare: implications for combat casualty research. *Mil Med*. 1984;149:55–62.
 58. Bellamy RF, Maningas PA, Vayer JS. Epidemiology of trauma: military experience. *Ann Emerg Med*. 1986;15:1384–1388.
 59. Carey M. Analysis of wounds incurred by U.S. Army Seventh Corps personnel treated in Corps hospitals during Operation Desert Storm, February 20 to March 10, 1991. *J Trauma*. 1996;40(3 suppl):S165–S169.
 60. Zajtchuck R, Sullivan GR. Battlefield trauma care: focus on advanced technology. *Mil Med*. 1995;160:1–7.
 61. Gubler KD, Gentilello LM, Hassantash A, Maier RV. The impact of hypothermia and dilutional coagulopathy. *J Trauma*. 1994;36:847–851.
 62. Rohrer MJ, Natale AM. Effect of hypothermia on the coagulation cascade. *Crit Care Med*. 1992;20:1402–1405.
 63. Staab DB, Sorenson VJ, Fath JJ, Raman SBK, Horst HM, Obeid FN. Coagulation defects resulting from ambient temperature-induced hypothermia. *J Trauma*. 1994;36:634–638.
 64. Gentilello LM, Jurkovich GJ, Stark MS, Hassantash SA, O’Keefe GE. Is hypothermia in the victim of major trauma protective or harmful? A randomized prospective study. *Ann Surg*. 1997;226:439–449.
 65. Watts DD, Trask A, Soeken K, Perdue P, Dols S, Kaufman K. Hypothermic coagulopathy in trauma: effect of varying levels of hypothermia on enzyme speed, platelet function, and fibrinolytic activity. *J Trauma*. 1998;44:846–854.
 66. Reed RL, Bracey AW, Hudson JD, Miller TA, Fischer RP. Hypothermia and blood coagulation: dissociation between enzyme activity and clotting factor levels. *Circ Shock*. 1990;32:141–152.
 67. Reed RL, Johnson TD, Hudson JD, Fischer RP. The disparity between hypothermic coagulopathy and clotting studies. *J Trauma*. 1992;33:465–470.
 68. Martinowitz U, Kenet G, Onaca N, Luboshitz J, Amit J, Heim M. New treatment of uncontrolled hemorrhage in trauma/surgical patients: induction of local hypercoagulation. Presented at the 60th Annual Meeting of the American Association for the Surgery of Trauma; October 14, 2000; San Antonio, TX.
 69. Cosgriff N, Moore E, Sauaia A, et al. Predicting life-threatening coagulopathy in the massively transfused trauma patient: hypothermia and acidosis revisited. *J Trauma*. 1997;42:857–862.
 70. Morado M, Villar A, Jimenez-Yuste V, et al. Study of the stability of recombinant factor VIIa in vitro [abstract]. *Thromb Haemost*. 1999;81(suppl):764. Abstract 2414.
 71. Shapiro AD, Gilchrist GS, Hoots WK, Cooper HA, Gastineau DA. Prospective, randomized trial of two doses of rFVIIa (Novoseven) in haemophilia patients with inhibitors undergoing surgery. *Thromb Haemost*. 1998;70:773–778.
 72. Key NS, Aledort LM, Beardsley D, et al. Home treatment of mild to moderate bleeding episodes using recombinant factor VIIa (Novoseven) in haemophiliacs with inhibitors. *Thromb Haemost*. 1998;80:912–918.
 73. Pernerstorfer T, Stohlawetz P, Hollenstein U, et al. Endotoxin-induced activation of the coagulation cascade in humans: effect of acetylsalicylic acid and acetaminophen. *Arterioscler Thromb Vasc Biol*. 1999;19:2517–2523.
 74. Chuansumrit A, Chantarojanasiri T, Isarangkura P, Teeraratkul S, Hongeng S, Hathirat P. Recombinant activated factor VII in children with acute bleeding resulting from liver failure and disseminated intravascular coagulation. *Blood Coagul Fibrinolysis*. 2000;11(suppl 1):S101–S105.
 75. Kaikita K, Takeya M, Ogawa H, Suefuji H, Yasue H, Takahashi K. Co-localization of tissue factor and tissue factor pathway inhibitor in coronary atherosclerosis. *J Pathol*. 1999;188:180–188.
 76. Roberts HR. Clinical experience with activated factor VII: focus on safety aspects. *Blood Coagul Fibrinolysis*. 1998;9(suppl 1):S115–S118.
 77. Hedner U. NovoSeven as a universal haemostatic agent. *Blood Coagul Fibrinolysis*. 2000;11(suppl 1):S107–S111.
 78. Peerlinck K, Vermeylen J. Acute myocardial infarction following administration of recombinant factor VII (Novoseven) in a patient with haemophilia A and inhibitor. *Thromb Haemost*. 1999;82:1775–1776.
 79. Hedner U. Reported serious adverse events during the use of rFVIIa. In: Scharrer I, Hedner U, Verlag W, eds. *Recombinant Factor VIIa. Update on Clinical Experiences*. International Workshop on Novoseven, April 24, 1998, Frankfurt, Germany. Wissenschafts Verlag Angela Weller, Peter-Schnellbach-Strasse 41/1, d-69151 Neckargemünd, Germany.
 80. Santagostino E, Gringeri A, Mannucci PM. Home treatment with recombinant activated factor VII in patients with factor VIII inhibitors: the advantages of early intervention. *Br J Haematol*. 1999;104:22–26.