

RECOMBINANT FACTOR VIIA REDUCES REBLEED HEMORRHAGE VOLUME IN A SWINE AORTOTOMY MODEL: A RANDOMIZED DOUBLE-BLINDED STUDY

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ABSTRACT—Noncompressible hemorrhage requires hypotensive resuscitation until definitive measures can be taken to prevent rebleeding by sustaining blood pressure at subphysiological levels. Previous studies have demonstrated that a 180- or 720- $\mu\text{g kg}^{-1}$ dose of recombinant factor VIIa (rFVIIa) increases the MAP at which rebleeding occurs in a swine aortotomy model. The purpose of the current study was to determine the efficacy of a lower dose of 90 $\mu\text{g kg}^{-1}$ given prophylactically to prevent or reduce rebleeding in a prospective, randomized, blinded study using a porcine model of uncontrolled hemorrhage and resuscitation. Fourteen female 40-kg Yorkshire-cross pigs were splenectomized and instrumented with venous and arterial catheters. The infrarenal aorta was exposed, and suction catheters were placed along the right and left paracolic gutters. After a 10-min baseline, 90 $\mu\text{g kg}^{-1}$ (i.v.) of either rFVIIa ($n = 6$) or vehicle ($n = 8$) was administered. Five minutes later, an aortotomy was created using a 2.5-mm biopsy punch. The weight of the shed blood was continuously recorded. Lactated Ringer's was given (100 mL $\text{kg}^{-1} \text{min}^{-1}$) 10 min after aortotomy until rebleeding occurred. The MAP at rebleed and the subsequent rebleed hemorrhage volume was recorded over the 2-h study period. After rebleed occurred, lactated Ringer's sufficient to maintain MAP at baseline levels was given. Initial hemorrhage volume and rebleed MAP ($P = 0.31$) did not differ significantly between groups. Rebleed hemorrhage volume was reduced by 54% in the rFVIIa group from 79 ± 4 mL kg^{-1} in the vehicle group to 43 ± 6 mL kg^{-1} in the rFVIIa group (mean \pm SEM; $P < 0.005$). The MAP at which rebleed occurred was not different between the groups, 71 ± 4 mmHg in the rFVIIa group versus 59 ± 5 in the vehicle group. Prophylactic administration of rFVIIa at 90 $\mu\text{g kg}^{-1}$, a dose similar to the recommended dose in hemophilia patients with inhibitors, reduced rebleed hemorrhage volume, suggesting that this dose is effective in this swine aortotomy model.

KEYWORDS—Low dose, hypotensive, resuscitation, prophylaxis, splenectomized

ABBREVIATIONS—H and E—hematoxylin and eosin; LR—lactated Ringer; PT—prothrombin time; PTAH—phosphotungstic acid hematoxylin; PTT—partial thromboplastin time; rFVIIa—recombinant factor VIIa; TEG—thrombelastograph

INTRODUCTION

Hemorrhage is considered noncompressible if it stems from an anatomic location not amenable to placement of a tourniquet or to direct pressure. An example of a non-compressible wound would be an injury above the level of the inguinal ligament or proximal to the axilla. Such wounds present a significant challenge to health care providers who do not have the capability to provide definitive care. Currently, hypotensive resuscitation, a technique described in the most recent edition of the *Prehospital Trauma Life Support* manual (1), is the only tool that can mitigate hemorrhage from a noncompressible source. In this technique, additional hemorrhage, or the “rebleed” phenomenon, is avoided by sustaining blood pressure at a subphysiological level. Rebleeding happens when the patient's blood pressure is elevated to the point where the blood-clotting thrombus in the wound fails.

Recombinant factor VIIa (rFVIIa) given in doses of 180 or 720 $\mu\text{g kg}^{-1}$ in swine was found to increase the MAP at

which rebleeding occurs (2). Such an intervention in humans would theoretically permit resuscitation to a higher MAP while awaiting definitive medical care. Recombinant factor VIIa can be administered intravenously and acts at the site of injury. If effective, it would be of particular interest as a hemostatic adjunct in hypotensive resuscitation. Our study investigated the efficacy of a lower dose of 90 $\mu\text{g kg}^{-1}$ in decreasing hemorrhage and increasing the MAP level at which rebleeding occurred. Recombinant factor VIIa was administered before injury and reflects ideal conditions that also makes this study relevant to the prophylactic administration of rFVIIa in elective surgery.

MATERIALS AND METHODS

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 U.S. Army Institute of Surgical Research. All animals received care in strict compliance with the 1996 *Guide for the Care and Use of Laboratory Animals* by the National Research Council.

Fourteen immature Yorkshire cross pigs weighing 39 ± 1 kg were obtained from a local class-A dealer and were quarantined for 1 week before the experiment. The pigs were fasted overnight before surgery, with water available *ad libitum*. Animals were premedicated with tiletamine-zolazepam (2 mg kg^{-1} , i.m.) and were maintained with isoflurane anesthesia with an FiO_2 of 0.5 on a ventilator. Ventilator settings were adjusted to maintain Pco_2 at 40 ± 2 torr during the baseline period but were not adjusted after hemorrhage was initiated. Animal core body temperature was maintained at 37°C to 39°C .

A 0.05-in. polyvinyl catheter was placed occlusively in the right carotid artery for blood pressure monitoring. Catheters were placed into the right femoral artery and vein for arterial blood sampling and intravenous infusion of lactated Ringer's

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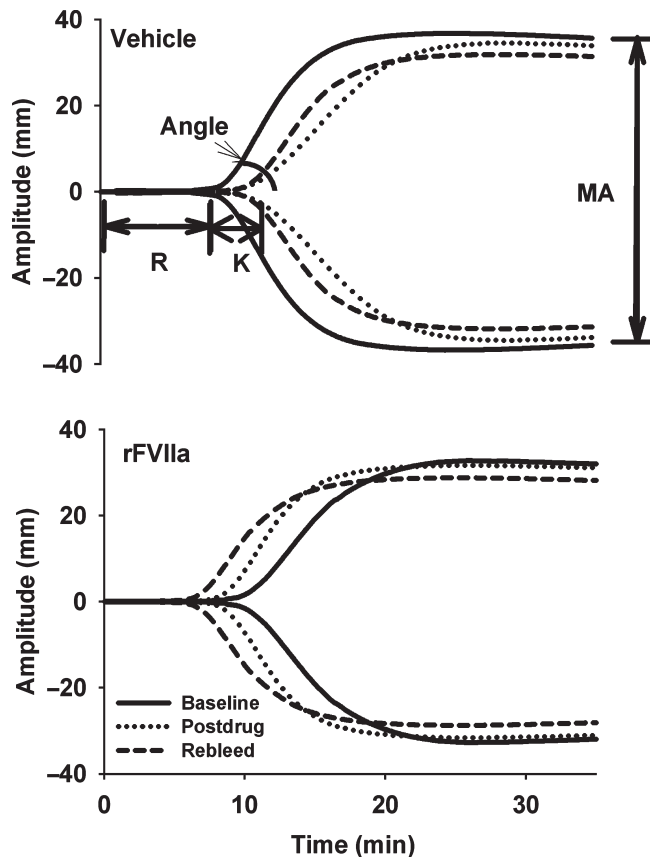


FIG. 1. Representative example of the TEG tracings from the baseline, 5-minute-post drug or vehicle, and rebleed samples in a vehicle control animal (top) and a rFVIIa animal (bottom). The R is the reaction time of clot initiation and is dependent on factor concentration. The K is a measurement of clot kinetics and is the time from R to where the amplitude reaches 20 mm. The angle is measured between the tangent of the upward slope and a horizontal line. It is a function of the rate of polymerization of fibrin (clot strengthening) and is affected by the fibrinogen level. The MA is a measurement of the maximum clot strength and is affected by platelet function and aggregation, as well as fibrinogen levels.

(LR) solution through a uniflow flush device. No heparin was used at any time during the study.

Because pigs have a contractile spleen, the spleen was removed via midline laparotomy. The spleen was immediately weighed (spleen weight ranged from 132 to 345 g; the spleen weight in the vehicle group averaged 233 ± 25 g; rFVIIa averaged 217 ± 27 g; $P = 0.7$), and the animals were infused with warm LR solution at three times the weight of the spleen (LR volume administered ranged from 423 to 1,035 mL; the volume administered to the vehicle group averaged 667 ± 93 mL; rFVIIa averaged 711 ± 68 mL; $P = 0.7$) to replace the volume of blood contained in the removed spleen. Perforated sleeves were placed along the lateral dorsal abdominal walls bilaterally, and suction tubes were placed in the sleeves. The abdominal organs were inspected for past or present disease processes, the presence of which would exclude an animal from the study.

After instrumentation and a stable MAP was obtained, a 10-min baseline period was initiated, and hemodynamic measurements were made. After the baseline sample was drawn, the rFVIIa or vehicle was injected into the femoral vein catheter, followed by a 10-mL saline flush. The syringe was prepared with either vehicle or $90 \mu\text{g kg}^{-1}$ of rFVIIa from a vial that was coded with a number and contained no indication of the contents. The vehicle consisted of a solution of 10 mM CaCl_2 , 10 mM NaCl, and 10 mM histidine, pH 6.0. The same size and volume vials (10 mL) were used for vehicle and rFVIIa-treated animals. These vials were prepared by Novo Nordisk and sent to our institution. The concentration of the vial ($405 \mu\text{g mL}^{-1}$ in 10 mL of vehicle) provided the $90 \mu\text{g kg}^{-1}$ dose of the maximum sized animal allowed in our study (45 kg). For animals that weighed less, only the volume required was withdrawn to provide the required dose. The volume of the drug given ranged from 8 to 10 mL, with the average volume in the vehicle group equal to 9.3 ± 0.9 mL, and the volume given to the rFVIIa group equal to 8.8 ± 0.3 mL ($P = 0.2$). The decoded information was not provided until completion of the study. The intestines were retracted and the infrarenal aorta exposed approximately 3 cm rostral to the bifurcation of the aorta 5 min after the bolus was completed. A

hole was made in the aorta using a 2.5-mm disposable skin biopsy punch. With the punch plugging the aorta, the bowel was returned to its correct anatomical position over the site of injury. The punch was removed, which initiated bleeding. The weight of the suctioned blood was continuously recorded throughout the hemorrhage and monitoring period. The hemorrhage volume per animal weight was calculated as total blood loss / animal weight (grams per kilogram). The specific gravity of the blood was rounded to 1 mL g^{-1} for this study, and all weights of blood were converted on a 1-g-to-1-mL basis. After the cessation of bleeding from the aortotomy (referred to as the initial hemorrhage), the scales were set to zero so that any rebleeding could be quantified (referred to as the rebleed hemorrhage volume). At the end of the study (death or 2 h), the abdomen was observed for any residual blood. Minimal fluid was found, indicating that the blood suctioned into the canisters was complete.

Resuscitation at 100 mL min^{-1} with LR at 37°C was begun 10 min after the hole was made. Rebleed pressure was defined as the blood pressure at the time blood began to appear in the suction canisters. After the rebleed MAP was determined, animals were resuscitated to within ± 5 mmHg of baseline MAP by turning the pump on and off. Total volume of LR administered and rebleed hemorrhage volume were recorded. Survival times were recorded up to 2 h. At the end of 2 h, the surviving animals were euthanized with pentobarbital at 90 mg kg^{-1} (i.v.).

Arterial blood samples for coagulation status, including complete blood count, prothrombin time (PT), activated partial thromboplastin time (PTT), fibrinogen, and thrombelastography (TEG) were collected at five events: baseline, 5 min after drug infusion, after 12.5 mL kg^{-1} LR infusion, at rebleed, or at 120 min or death. Arterial samples for blood gases were collected at baseline and at five specific times after the initiation of hemorrhage: 10, 30, 60, 90, and 120 min, or at death. Thrombelastography was measured as follows: arterial blood (3.2% sodium citrate vacutainer; BD, Franklin Lakes, NJ; 4.5 mL draw) was incubated at room temperature for 15 min. Twenty microliters of calcium chloride (0.2 M;

TABLE 1. Comparison of selected variables between the vehicle control animals treated with buffer vehicle vs. isotonic sodium chloride solution

Variable	Buffer	Isotonic sodium chloride solution
BL MAP (mmHg)	80 ± 5	88 ± 10
RBLD MAP (mmHg)	64 ± 3	59 ± 10
Initial HV (mL/kg)	20 ± 2	21 ± 2
RBLD HV (mL/kg)	72 ± 6	80 ± 2
RV (mL/kg)	238 ± 13	250 ± 18
BL platelets ($\times 10^3 \text{ mL}^{-1}$)	408 ± 45	$227 \pm 44^*$
RBLD platelets ($\times 10^3 \text{ mL}^{-1}$)	303 ± 19	$198 \pm 36^*$
BL PT (s)	12.7 ± 0.6	12.0 ± 0.2
RBLD PT (s)	15.0 ± 0.6	14.3 ± 0.6
BL activated PTT (s)	$25 \pm$	27 ± 4
RBLD activated PTT (s)	24 ± 2	25 ± 6
BL fibrinogen (mg mL^{-1})	138 ± 7	141 ± 17
RBLD fibrinogen (mg mL^{-1})	84 ± 23	106 ± 17
BL hematocrit (% RBC)	35 ± 2	36 ± 1
RBLD hematocrit (% RBC)	20 ± 2	23 ± 2
BL TEG-R (min)	11.8 ± 1.8	11.8 ± 2.2
RBLD TEG-R (min)	9.9 ± 1.0	12.1 ± 2.0
BL TEG-K (min)	4.8 ± 1.0	5.2 ± 1.4
RBLD TEG-K (min)	3.4 ± 0.3	4.9 ± 1.4
BL TEG angle (degrees)	45 ± 5	41 ± 7
RBLD TEG angle (degrees)	49 ± 3	42 ± 6
BL TEG MA (mm)	61 ± 4	57 ± 3
RBLD TEG MA (mm)	53 ± 1	54 ± 2

Values indicate mean \pm SEM.

* $P < 0.05$ different from buffer.

BL indicates baseline; HV, hemorrhage volume; RBLD, rebleed; RV, resuscitation volume.

Haemoscope Corp., Niles, Ill) and 20 μL of 1:2,500 Innovin ($\sim 5 \text{ pg mL}^{-1}$; lyophilized recombinant human tissue factor from Dade Behring, B4212-50, diluted with 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid/bovine serum albumin buffer [20 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, 0.14 M sodium chloride, 2% bovine serum albumin, pH 7.4]) was placed into the plastic cups (Haemoscope Corp.) set to the animal's body temperature, and 300 μL of blood was added. The TEG, with recombinant human tissue factor as an accelerator mimicking conditions of the extrinsic clotting pathway, measures the viscoelastic properties of blood as it clots under a low-shear environment resembling slow venous flow. The patterns of changes in shear elasticity enable the determination of the kinetics of clot formation and the strength of the formed clot. The strength of the clot provides information about the ability of the clot to perform the work of hemostasis, whereas the kinetics determine the adequacy of quantitative factors available to clot formation. Figure 1 shows representative curves from the baseline, postinjection, and rebled time points in the vehicle and rFVIIa groups. The R, K, angle, and maximal amplitude (MA) are shown on the figure. Carotid artery pressure was recorded continuously using a polygraph. All analog data were continuously acquired on a data acquisition system.

Samples from the heart, lung, brain, kidney, and adrenal glands were harvested within 10 min of death and were placed in neutral-buffered 10% formalin. In all animals, tissue sections were taken from the same location in each organ. Formalin-fixed, paraffin-embedded sections of each of the tissues were stained with hematoxylin and eosin (H and E) and phosphotungstic acid hematoxylin (PTAH). Collagen exhibits eosinophilic staining on routine H and E, whereas fibrin stains purple on PTAH. The combined use of PTAH and H and E staining of replicate sections allowed the pathologist (blinded to treatment) to differentiate between fibrin and collagen. Each tissue was evaluated for the presence of microthrombi in the microvasculature.

We had originally planned $n = 6$ per group, but one of the vehicle animals met exclusion criteria of a preexisting disease (septal defect resulting in arterialized venous blood gases) and could not be used. We had performed three pilot experiments using saline instead of coded tubes. Because they were indistinguishable from the vehicle animals in their responses, we included them. Rather than arbitrarily selecting one of the three, we included all three, thus, the difference in number of animals between the groups. The baseline and rebled responses to selected variables between the buffer vehicle and the isotonic sodium chloride solution animals are shown in Table 1. There were no significant differences between the two groups except for the platelet numbers. These differences, although statistically significant, would not be expected to be physiologically significant because the values were within the ranges of normal values for pigs (personal observation: platelets range from 126 to 507; $n = 376$; 314 ± 82 , mean \pm SD).

Statistical analysis

Data analyses were performed using SAS, version 8.1 (SAS Institute, Inc., Cary, NC) and SPSS version 14.0. Data were analyzed by one- or two-factor ANOVA corrected for repeated measures on the time factor, followed by Student-Newman-Keuls method for post hoc comparisons. A $P \leq 0.05$ was considered statistically significant. Categorical data such as rebleeding (yes/no) were analyzed by chi-square to determine treatment effects. Data were expressed as mean \pm SEM.

RESULTS

A significantly lower rebled hemorrhage volume ($43 \pm 6 \text{ mL kg}^{-1}$) was observed in animals that received $90 \mu\text{g kg}^{-1}$ pretreatment of rFVIIa ($P < 0.001$, Fig. 2) compared with $79 \pm 4 \text{ mL kg}^{-1}$ in control animals. The MAP at which rebleeding occurred showed a trend toward an increase in the treatment group ($59 \pm 5 \text{ mmHg}$ in control animals vs. $71 \pm 4 \text{ mmHg}$; Fig. 2) but did not achieve statistical significance ($P = 0.09$). The resuscitation volumes received before rebleeding were similar in the treatment and vehicle groups (37 ± 13 vs. $38 \pm 5 \text{ mL kg}^{-1}$, respectively), indicating that the amount of dilution of coagulation factors was similar between the two groups at the time of rebleeding. The final resuscitation volumes were less in the treatment group compared with the vehicle group (177 ± 27 vs. $244 \pm 11 \text{ mL kg}^{-1}$, respectively; $P = 0.027$; Fig. 2).

After treatment with rFVIIa, PT in the treatment group decreased significantly from a pretreatment mean of 12.1 ± 0.1 to $8.0 \pm 0.5 \text{ s}$ ($P < 0.001$), whereas no difference was seen

in the vehicle group (Fig. 3). There was also a significant difference in PT at rebled from 14.6 ± 0.4 in the vehicle group versus $10.7 \pm 0.5 \text{ s}$ in the treatment group ($P < 0.001$). There was no significant difference in PTT in response to the treatment (Fig. 3). After resuscitation, platelets were significantly diluted from 250 ± 27 at rebled to $47 \pm 10 \times 10^3 \text{ mL}^{-1}$ final value in the vehicle group and from 261 ± 38 at rebled to $107 \pm 33 \times 10^3 \text{ mL}^{-1}$ final value in the treatment group. Fibrinogen levels also fell from 95 ± 14 at rebled to $35 \pm 15 \text{ mg mL}^{-1}$ final value in the vehicle group and from 125 ± 20 to $55 \pm 10 \text{ mg mL}^{-1}$ in the treatment group. Hematocrit fell during the course of the study, reflecting the significant hemodilution that occurred, but there was no difference between the two groups.

Thromboelastograph analysis of coagulation function demonstrated a significant difference in the R time between treatment and control groups (Fig. 4). These significant differences were evident after injection of rFVIIa, after resuscitation,

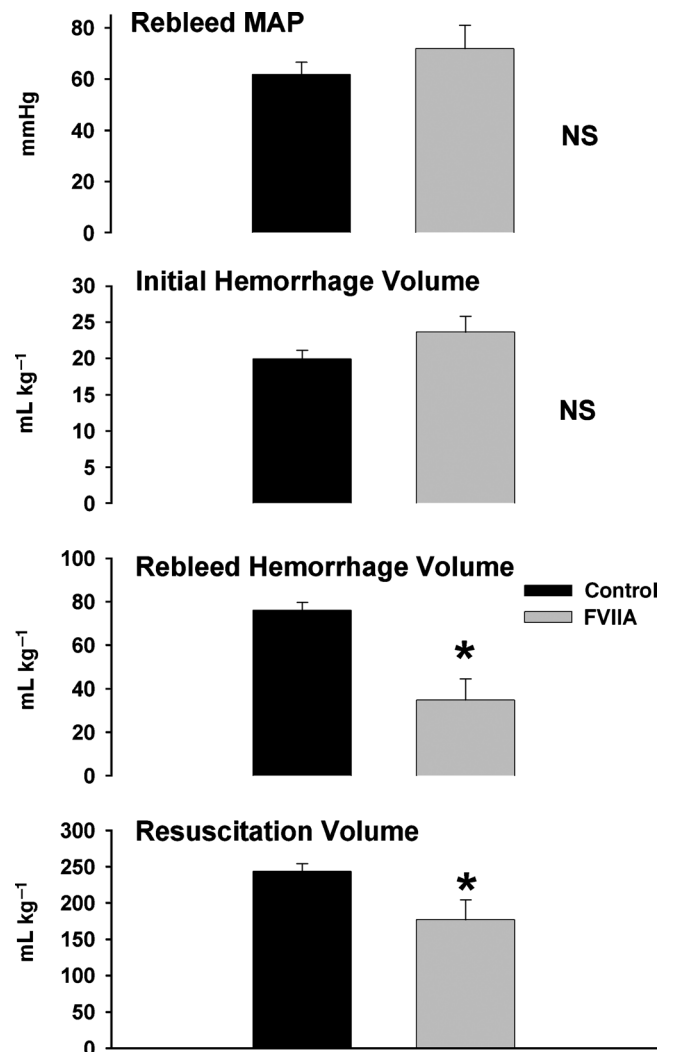


Fig. 2. MAP and hemorrhage volumes. MAP at which rebled occurred, the volume of initial hemorrhage, the volume of hemorrhage after rebled occurred, and the total volume of resuscitation for the control ($n = 8$) and treatment ($n = 6$) arms of this study. The rebled hemorrhage ($P < 0.001$) and resuscitation ($P < 0.027$) volume was reduced in the treatment group compared with the vehicle group. Data expressed as mean \pm SEM (* indicates difference between groups; NS, no significance).

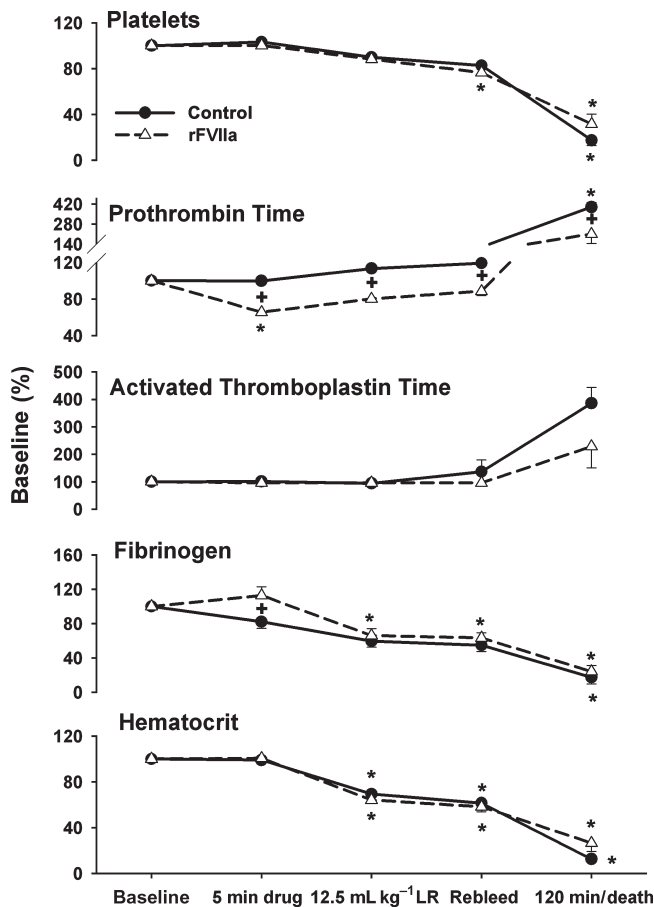


FIG. 3. Coagulation factor analysis. The data are expressed as percent of baseline. Table 1 shows the baseline values. After the administration of rFVIIa, PT decreased significantly. At the point at which rebleeding occurred, there was still a significant difference between groups. The graphs that depict the platelet and fibrinogen concentrations over time show significant dilution from the point at which resuscitation was initiated and at the end of the experiment (+ indicates $P < 0.05$ between groups at time point; * $P < 0.05$ from baseline value within each group).

and at the point at which rebleeding was observed. The R time of the rFVIIa group at rebled was 8.4 ± 1.1 compared with 11.1 ± 0.8 min ($P < 0.05$) observed in the control group. There were no other statistically significant differences between the two groups observed in α angle, K , or MA.

The two groups started from similar values in all parameters except the hematocrit and TEG-MA (Table 2). These differences, although statistically significant, would not be expected to be physiologically significant because the values were within the ranges of normal values for pigs (personal observation: hematocrits range from 26.7 to 38.8; $n = 665$; 32.7 ± 2.7 ; TEG-MA range from 50.5 to 76.5; $n = 72$; 62.6 ± 5.4 , mean \pm SD).

There was no significant difference between the groups in arterial pH (7.4 ± 0.1), and the pH did not change significantly during the course of the study (data not shown).

Three patterns of hemorrhage, continuous, intermittent, and isolated rebleed, emerged in this model of uncontrolled hemorrhage and rebleed. A representative example of the three patterns is shown in Figure 5. Continuous hemorrhage was seen in all eight control animals (top, Fig. 5). In these animals, once rebleeding began, it persisted during the resuscitation to maintain blood pressure. Intermittent hemor-

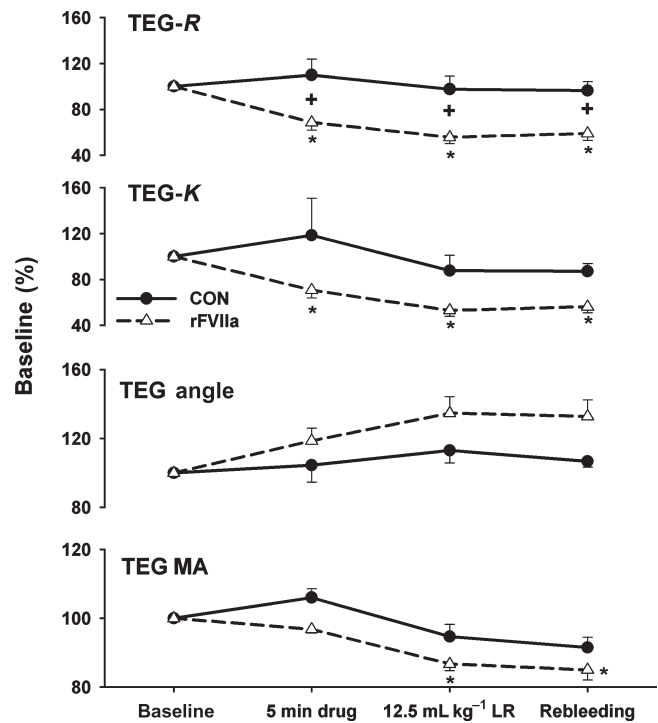


FIG. 4. Thromboelastography. The data are expressed as percent of baseline. Table 1 shows the baseline values. Thromboelastograph (TEG) results as percentage change from baseline. A significant change was observed in the R time. Although there seems to be some difference in the angle and maximal amplitude, these were not statistically significant (+ indicates $P < 0.05$ between groups at time point; * $P < 0.05$ from baseline value within each group).

rhage was observed in four of the six animals that received rFVIIa (middle, Fig. 5). Several brief episodes of rebleed were observed, and the MAP dropped below the trigger criteria for crystalloid resuscitation. A subsequent response to the crystalloid infusion was an increase in blood pressure, followed by a second episode of rebleeding. Isolated rebleeding was observed in two animals that received rFVIIa (bottom, Fig. 5). This pattern was characterized by an episode of rebleeding, followed by hemostasis and return of MAP to baseline during volume replacement. The blood pressure

TABLE 2. Baseline values of variables

Variable	Control	rFVIIa
MAP (mmHg)	84 \pm 5	88 \pm 4
Platelets ($\times 10^3$ mL ⁻¹)	317 \pm 45	339 \pm 33
PT (s)	12.3 \pm 0.3	12.1 \pm 0.1
Activated PTT (s)	26 \pm 2	24 \pm 1
Fibrinogen (mg mL ⁻¹)	181 \pm 22	201 \pm 36
Hematocrit (% RBC)	35 \pm 0.9	31 \pm 0.5*
TEG-SP (min)	9.6 \pm 1.1	10.7 \pm 0.6
TEG-R (min)	11.8 \pm 1.3	14.2 \pm 1.1
TEG-K (min)	5.0 \pm 0.8	5.4 \pm 0.9
TEG angle (degrees)	43 \pm 4	42 \pm 4
TEG MA (mm)	59 \pm 3	72 \pm 2*

Values indicate mean \pm SEM. * $P < 0.05$ different from control.

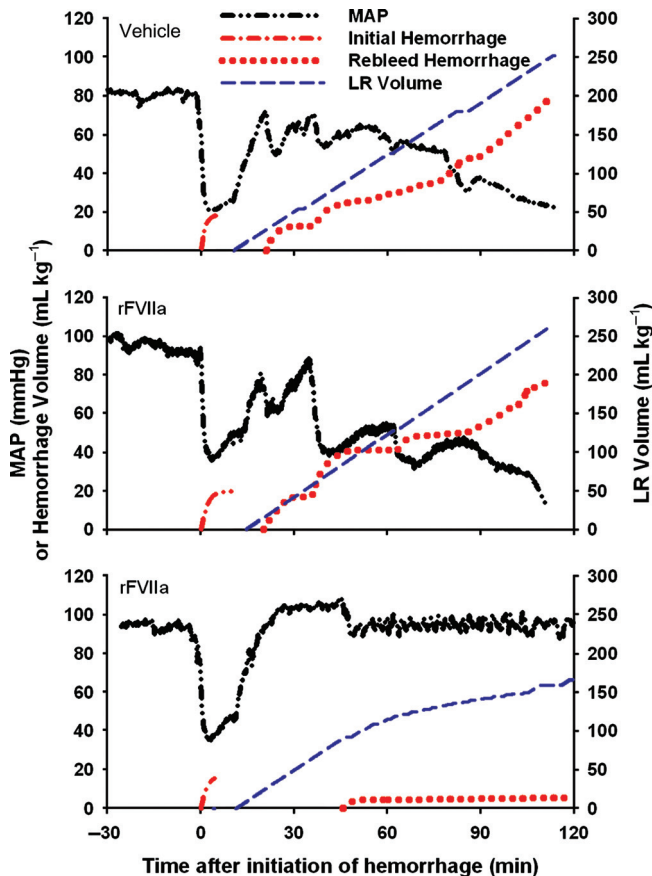


FIG. 5. Representative bleeding patterns. Hemodynamic data of continuous rebleeding (top). Hemodynamic data of intermittent rebleeding (middle). Hemodynamic data of isolated rebleeding (bottom).

stabilized and was maintained at baseline with minimal further crystalloid requirements.

Samples (7 slides for each animal, total of 98 slides for the 14 animals) from the brain (three slides from each animal, two from cerebrum, one from cerebellum/brain stem), one slide each from left ventricle, lung, kidney, and adrenal glands were examined for the presence of microthrombi. All tissues were removed within 10 min of death of the animal and immediately preserved in formalin. There was no microscopic evidence of intravascular thrombosis associated with rFVIIa treatment in any of the tissues (data not shown). Lung tissues from two animals in the vehicle group and three animals in the rFVIIa groups showed evidence of chronic background lesions (e.g., multifocal neutrophilic and histiocytic infiltrates and lymphoid proliferation and subacute pleuritis) consistent with enzootic disease that is common in commercial swine herds and were not considered to be treatment effects.

Two of the six animals in the treatment group survived the 120 min, with an average survival time of 89 ± 13 min. Only one of eight animals in the vehicle group survived the 120 min, with an average survival time of 107 ± 3 min. There was no significant difference in the survival time between the two groups.

DISCUSSION

This study adds to the growing body of literature of both human and animal studies suggesting that rFVIIa is effective

and safe when used as a hemostatic adjunct (3–8). Previous studies using the same hemorrhage model showed that rFVIIa increased the strength of the formed blood clot, resulting in a higher rebleed MAP (2) and an almost 50% reduction in the hemorrhage volume, which, however, did not quite reach significance ($P = 0.055$). However, this was at doses of at least $180 \mu\text{g kg}^{-1}$, twice the dose used in the current study. In this lower dose study, no effect on the rebleed pressure was seen, probably due to the lower dose, but the reduction in hemorrhage volume was significant. This reduction in blood loss is consistent with what has been seen in clinical observations.

The largest prospective trial to date used a $200\text{-}\mu\text{g kg}^{-1}$ rFVIIa dose and followed by two $100\text{-}\mu\text{g kg}^{-1}$ doses (3). This treatment regimen demonstrated a reduction in the blood transfusion requirements in patients who sustained blunt trauma. Two recent retrospective reviews of low-dose ($40 \mu\text{g kg}^{-1}$), off-label rFVIIa use in patients with traumatic hemorrhage with nonsurgical, coagulopathic bleeding (9, 10) and refractory hemorrhage after cardiopulmonary bypass ($11 - 21 \mu\text{g kg}^{-1}$) (10) support the hypothesis that lower doses of rFVIIa are sufficient to augment the innate coagulation system and obtain hemostasis. In the article by Harrison et al. (9), a low dose of $40 \mu\text{g kg}^{-1}$ was used on patients with traumatic hemorrhage as an adjunct to their massive transfusion protocol. Twenty-nine patients were compared against case-matched controls and showed decreased blood product use, units of packed red blood cells, platelets, and cryoprecipitate (9). Romagnoli et al. (10) administered a slow 1.2-mg bolus (i.v.) of rFVIIa at the end of a transfusion protocol to 15 patients. When compared against case-matched controls, the patients who received rFVIIa demonstrated improved coagulation function profiles, decreased blood loss, and blood product requirements. These results parallel those found in the current study where hemorrhage volume was reduced. Although small and retrospective in nature, these studies highlight the potential for options of lower doses in hemorrhagic patients.

A retrospective study performed at our institution by the primary author demonstrated the potential relationship between the observed decrease in PT in response to the rFVIIa and patient outcome (11). The lower dose of $90 \mu\text{g kg}^{-1}$ was sufficient to lower the PT observed at rebleed in the treatment group compared with vehicle. Thrombelastograph data from our study, supported by this observation, suggest that in cases in which the initial clot “pops,” a dose of $90 \mu\text{g kg}^{-1}$ will produce serum concentrations of factor VII high enough to reduce hemorrhage by accelerating thrombin production and formation of a second clot.

In the current study and in the previous study using the same model (2), no decrease in initial hemorrhage volume occurred despite supraphysiological coagulation function. It seems that this was the result of the brisk, high-pressure hemorrhage seen in this model. Once the pressure head at the site of injury decreases to a low-enough level to allow the clotting factors adequate time to interact with the activated platelets and injured endothelium, the clot formed seems to be more effective at decreasing hemorrhage than the clot seen in the vehicle group. This was evidenced by the observed

patterns of rebleed and the overall rebleed hemorrhage volumes. The TEG assessment of coagulation function provides a hint as to the possible reason for this observation. There was a shortening of the *R* time, which represents the velocity of initial fibrin formation, whereas MA and angle did not change significantly. Once the pressure at the bleeding site decreased to a sufficiently low level, the treated animals were able to form an effective thrombus quicker and thus reduce the rebleed hemorrhage volume. When the pressure rose again above a critical level, subsequent rebleed episodes were observed in some animals. The thrombus then seemed to have reformed because the bleeding ceased again until the factors became too diluted, and continuous bleeding occurred with continued resuscitation. This aspect of the model—the continued use of crystalloid to assess the strength of the clot—eventually results in the dilution of the factors we are evaluating and is a weakness of the model because we are measuring coagulation effects under changing conditions.

Previous porcine studies of uncontrolled hemorrhage secondary to a grade V liver injury in cold, coagulopathic animals demonstrated a 46% to 50% reduction in hemorrhage volume when the animals were treated with either 180 or 720 $\mu\text{g kg}^{-1}$ of rFVIIa (7, 8). In contrast, two studies using a similar grade V liver injury swine did not show a reduction in hemorrhage volume (12, 13). Klemcke et al. (12) did not show a reduction in hemorrhage volume in cold, coagulopathic pigs that were treated with the same two doses (180 and 720 $\mu\text{g kg}^{-1}$) of rFVIIa previously reported as effective (7, 8). Schreiber et al. (8) hemodiluted with 5% albumin, and Martinowitz et al. (7) used a low molecular weight (200 kd) 6% hetastarch. In contrast, Boldt et al. (14) used high molecular weight (450 kd) 6% hetastarch to hemodilute the pigs, which has been found to cause coagulation deficits compared with low molecular weight hetastarch formulations. Schreiber et al. (13) had repeated their previous study (but in noncoagulopathic, normothermic pigs) and found no reduction in hemorrhage volume. However, in that study, there was no gauze packing (13) compared with their coagulopathic study (8), and it may be that support for clot formation may be required for rFVIIa to have efficacy.

We chose the 90- $\mu\text{g kg}^{-1}$ dose of rFVIIa as our low dose because it was the lowest dose that had been shown in pigs to be effective in a dose-escalation study (15). The dose we used was not as low as has been used in humans or that is recommended on its label because the tissue factor–FVIIa interaction is species-specific, and human FVII seems to have only between 5% and 50% activity when exposed to porcine tissue factor (15).

The results of these studies combined with our findings suggest that prophylactic use of rFVIIa may be ineffective in reducing initial hemorrhage in a model or injury with high pressure bleed and high shear force such as a large arterial injury. Recombinant factor VIIa may be ideally suited for large soft tissue injuries, solid organ injuries, and hemorrhage

associated with coagulopathy because these are typically low pressure in nature and do not generate the shear forces seen in arterial injuries. The results of this study, however, add to the growing pool of data from human and animal studies supporting the use of rFVIIa as a hemostatic adjunct in the treatment of life-threatening hemorrhage.

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