

RECOMBINANT FACTOR VIIa INCREASES THE PRESSURE AT WHICH REBLEEDING OCCURS IN PORCINE UNCONTROLLED AORTIC HEMORRHAGE MODEL

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ABSTRACT—In trauma patients, resuscitation to endpoints below normal blood pressure (BP) levels may reduce further blood loss due to the rebleeding often caused by more aggressive resuscitation. However, patients whose BP is maintained at lower levels for extended periods are at increased risk for organ failure. The purpose of this study was to determine whether recombinant activated factor VII (rFVIIa) raises the BP level at which rebleeding occurs in a prospective, randomized, blinded study using a porcine model of uncontrolled hemorrhage and resuscitation. Thirty anesthetized 40-kg pigs were assigned to three groups ($n = 10/\text{group}$): control, low-dose rFVIIa (180 $\mu\text{g}/\text{kg}$), or high-dose (720 $\mu\text{g}/\text{kg}$). Vehicle or drug was infused 5 min before creating a 2.0-mm infrarenal aortotomy. Ten minutes later, resuscitation with lactated Ringer's (LR) solution at 100 mL/min was begun. Hemorrhage and LR volumes and BP were recorded continuously. We found that pretreatment with rFVIIa increased the mean arterial pressure at which rebleeding occurred during resuscitation (45 ± 3 , 69 ± 5 , and 66 ± 6 mmHg in the control, low-dose, and high-dose groups, respectively, $P = 0.003$). Rebleed hemorrhage volume was reduced with rFVIIa (39 ± 9 , 22 ± 7 , and 26 ± 5 mL/kg for control, low and high dose, respectively; $P = 0.055$). This is the first study to show that rFVIIa increases the BP at which rebleeding occurs during resuscitation in an injury to a major artery, suggesting the formation of a tight, stronger fibrin plug in the presence of high concentrations of rFVIIa.

KEYWORDS—Hemorrhagic shock, resuscitation, hemostasis, trauma, swine

INTRODUCTION

Researchers have found an association between aggressive resuscitation and poor outcome (1) and evidence that hypotensive resuscitation can reduce blood loss in hemorrhagic shock (2–5). However, patients, such as soldiers wounded in combat who frequently experience delayed evacuation and whose mean arterial pressure (MAP) may be kept below normal for prolonged periods, may also be at risk for increased organ failure (6). In a previous study using an uncontrolled hemorrhage aortotomy model, we identified a reproducible MAP for rebleeding during resuscitation, thus establishing a fixed endpoint for hypotensive resuscitation in pigs (7). The purpose of the current study was to determine whether administration of recombinant activated factor VII (rFVIIa) in pigs—with normal coagulation function, aortic injury, and uncontrolled hemorrhage—would increase rebleeding MAP in response to resuscitation, presumably by enhancing clot stability. As there has been no prior study of the effect of rFVIIa on severe arterial bleeding, recombinant FVIIa was administered before injury in this initial feasibility study to be able to detect an effect when it was given under ideal conditions of pretreatment, as may be relevant to elective surgery. The same doses (180 and 720 $\mu\text{g}/\text{kg}$) as used in previous studies of venous bleeding in a grade V liver injury model were chosen (8) for consistency.

Recombinant FVIIa is an FDA-approved drug developed primarily to treat hemophilia patients with inhibitors. In this population, rFVIIa has been found to induce effective hemostasis in conditions associated with serious bleeding of the central nervous system, gut, and muscle; in addition, rFVIIa has permitted these patients to undergo major surgical procedures previously contraindicated for them (9–11). Trauma studies have demonstrated the effectiveness of rFVIIa in decreasing blood loss in models of hypothermic coagulopathic swine with grade V liver injuries (8, 12), and a growing body of literature has documented its successful use in case reports of surgical and trauma patients with the acquired coagulopathy of trauma (13–17). In the current feasibility study, we administered rFVIIa to pigs preoperatively, using a previously described aortotomy model (7). Rebleeding MAP during resuscitation was increased, strongly indicating the increased strength of the hemostatic plug formed in the presence of rFVIIa.

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.

Surgical procedure

Thirty immature female 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 i.m.) and were maintained with isoflurane anesthesia in 50% oxygen on a ventilator. Ventilator settings were adjusted to maintain the animal's P_{CO_2} at 40 ± 2 torr during the

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baseline period, but were not adjusted after hemorrhage. Animal core body temperature was maintained at 37°C to 39°C.

An 18-gauge catheter was placed nonocclusively in the right carotid artery through which a continuous arterial blood gas monitoring catheter was advanced into the carotid artery. A thermofluor fiber optic catheter was placed occlusively into the left jugular vein and was advanced to the pulmonary artery for central venous O₂ saturation and continuous cardiac output measurements. A 0.050-inch polyvinyl catheter was placed occlusively in the left carotid artery for blood pressure monitoring. Catheters were placed occlusively in the femoral artery and vein for arterial blood sampling and intravenous infusion of the resuscitation solution. All catheter lumens were kept patent by the infusion of normal saline through a uniflow flush device. No heparin was used at any time during the study.

As pigs have a contractile spleen, the spleen was removed via a midline laparotomy. The spleen was immediately weighed and the animals were infused with warm lactated Ringer's (LR) solution at three times the splenic weight to replace the volume of blood contained in the removed spleen. Perforated sleeves were laid along the lateral dorsal abdominal walls bilaterally and suction tubes were placed in the sleeves. The abdominal organs were inspected for evidence of past or present disease processes, the presence of which precluded inclusion of the animal in the study.

Experimental procedure

After the instrumentation was completed and a stable MAP was obtained, a 10-min baseline period was begun and hemodynamic measurements were made. After the baseline sample was drawn, the appropriate treatment was infused into the femoral vein catheter at 5 mL/min for 5 min, followed by a 10-mL saline flush. The syringe was prepared with the vehicle or 180 or 720 µg/kg of rFVIIa (*n* = 10 per group) by a technician in another laboratory so that the experimenters were completely blinded to the treatment given. The saline vehicle or dose of rFVIIa was diluted with saline so that the volume in each syringe was a constant 50 mL. Furthermore, the syringe was completely covered with black plastic tape because there was a visibly increased viscosity of the rFVIIa solution compared with the saline vehicle. Five minutes after the infusion was completed, the intestines were retracted and the infrarenal aorta was exposed approximately 3 cm rostral to the bifurcation of the aorta. A hole was then made in the aorta with a 2.0-mm disposable skin biopsy punch. With the punch plugging the hole, the intestines were then restored to their natural position over the injury site. The punch was removed, which initiated bleeding. The weight of 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 blood was rounded to 1 mL/g 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 volume), the suction canisters were changed to empty canisters and the balances were zeroed so that any rebleeding could be quantitated (referred to as the rebleed hemorrhage volume). At the end of the experiment (death of the animal or 2 h), the abdomen was observed for any residual blood; minimal fluid was found, indicating that the blood suctioned into the canisters was greater than 95% complete.

Ten minutes after the hole was made, resuscitation at 100 mL/min with LR at 37°C was begun. Rebleed pressure was determined by noting the blood pressure at the time blood appeared in the suction canisters. If the MAP reached a plateau after 4 L of fluid was given without causing rebleeding, the LR pump was stopped and an infusion of epinephrine at 1.0 µg/kg/min at a rate of 3 mL/min or higher, as needed, was given to raise MAP to as high as 200 mmHg. If no rebleeding occurred with this treatment, the animal was recorded as a non-rebleeder. Resuscitation was given as long as the MAP was less than the baseline MAP, and was turned on and off ±5 mmHg of baseline MAP once rebleeding occurred. Total volume of LR administered and the rebleed hemorrhage volume were recorded. Survival time up to 2 h was recorded. At the end of 2 h, the surviving animals were euthanized.

Samples

Arterial blood samples (20 mL) were collected at baseline (–10 min), nadir of the MAP, and the time of rebleeding for lactate, complete blood count, spun hematocrit, and plasma protein concentration. Carotid artery pressure was recorded using a polygraph. All analog data was continuously acquired on a data acquisition system.

Samples from heart, lung, liver, kidney, skeletal muscle, and duodenum were harvested within 10 min of death and were placed in neutral buffered 10% formalin. Thin sections (no greater than 5 mm) of tissue were taken at necropsy to enhance formalin fixation of tissue. In all animals, tissue sections were taken from the same location in each organ for consistency. Formalin-fixed, paraffin-embedded sections of each of the tissues were stained with hematoxylin and eosin, phosphotungstic acid hematoxylin (PTAH, elastin), and Masson's trichrome (MTS, collagen). Collagen exhibits eosinophilic staining on routine hematoxylin and eosin, so the combined use of PTAH and MTS staining of replicate sections allowed the pathologist to differentiate between fibrin and collagen. Fibrin stains purple on PTAH, and collagen stains bright blue on MTS (18). Each tissue was evaluated for the presence of microthrombi in the microvasculature. The pathologist was blinded to treatment.

Coagulation assays

Factor VII concentration was measured with a one-stage clotting assay using an ACL Futura Coagulation System (Instrumentation Laboratory, Lexington, MA). For the assay, human standards and controls provided by the manufacturer were used. The ACL Futura methodology reports the results in percentage of activity. These percentages were divided by 100 to convert the units from percentage of activity to units per milliliter. Pooled pig plasma was used as an additional control. Mean FVII was 1.055 ± 0.073 U/mL for pooled normal pig plasma, with intra-assay and interassay coefficients of variation (CV) of 2.7% and 4.9%, respectively. Standard prothrombin time (PT, using commercial rabbit brain reagent), thrombin time (TT), and fibrinogen concentrations were determined at 37°C using the ACL Futura Coagulation System according to manufacturer instructions. Mean clotting time for the standard PT assay using pooled normal pig plasma was 12.87 ± 0.15 s, with intra-assay and interassay CV of 1.22% and 1.16%, respectively. Mean TT was 18.85 ± 0.59 s for pooled normal pig plasma, with intra-assay and interassay CV of 2.38% and 3.14%, respectively. Fibrinogen concentration for pooled normal pig plasma was 118.9 ± 13.6 mg/dL, and intra-assay and interassay CV were 6.41% and 11.41%, respectively.

Thrombin-antithrombin III complex (TAT) concentrations were quantitated using the Enzygnost TAT microenzyme immunoassay (Dade Behring, Marburg, Germany), which has previously been demonstrated to crossreact with porcine TAT (19).

Data analysis

Data were analyzed by one-, two-, or three-factor analysis of variance with repeated measures on the time factor when appropriate. Student Newman-Keuls tests were used for *post hoc* comparisons. The rebleed hemorrhage volume data was analyzed using orthogonal contrasts. Survival trend data were analyzed using the Cochran-Armitage trend exact test. Differences were considered significant at *P* < 0.05. Data are expressed as means ± SEM except as noted.

RESULTS

Three basic patterns emerged in response to three kinds of resuscitation: continuous rebleeding during the entire period of resuscitation (control); starting and stopping of the rebleeding for several cycles despite continuing resuscitation (low dose); and single incidents of brief rebleeding despite continued resuscitation (high dose). Figure 1 shows examples from each of the three groups. More animals required a vasoconstrictor to elevate the blood pressure to the rebleeding point in the high-dose group compared with the other groups, and two of the four animals in the high-dose group did not rebleed at all (although this did not reach statistical significance). The presence of rFVIIa changed the bleeding pattern from a continuous to an intermittent bleeding pattern in those that rebled. The incidence of each of the patterns with respect to the treatment group is given in Table 1. Pretreatment with rFVIIa significantly increased the MAP at which rebleeding occurred during resuscitation of an uncontrolled hemorrhage, from 45 ± 3 mmHg in the control group to 69 ± 5 mmHg in the low-dose group and 66 ± 6 mmHg in the high-dose (*P* = 0.003; Fig. 2). In the high-dose group, four out of 10 animals required epinephrine to increase the MAP to higher levels, and two of these animals reached a maximum MAP with no rebleeding (Fig. 2). In the control group, nine out of 10 animals rebled at MAPs below their baseline MAP (71 ± 2 mmHg). In the two treatment groups, only two animals from each group rebled at MAPs below their baseline MAP (69 ± 3 and 70 ± 2 mmHg, respectively for the low- and high-dose groups). These observations included those animals that received epinephrine. The rebleed MAPs, excluding animals that received epinephrine, was averaged for each group (*P* < 0.05; Fig. 2).

Recombinant FVIIa-treated animals received more resuscitation fluid volume before rebleeding occurred (55 ± 12 mL/kg, high dose vs. 20 ± 9 mL/kg control, *P* = 0.006; Fig. 3). Similarly, resuscitation extended over a longer period of time

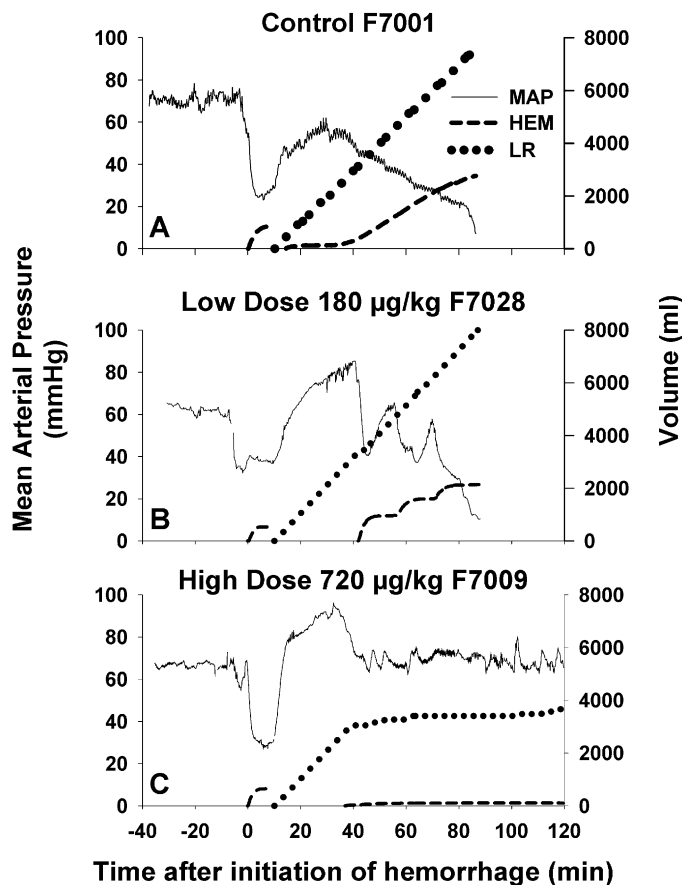


FIG. 1. Individual animal representative results. Solid line, Arterial blood pressure (MAP); dashed line, hemorrhage and rebleed cumulative volume (HEM); dotted line, cumulative lactated Ringer's resuscitation volume (LR) with either saline vehicle control, and low or high dose of rFVIIa. Baseline period precedes aortotomy at time zero and spontaneous clot (first HEM line). (A) At 10 min, resuscitation with LR was given at 100 mL/min until rebleeding occurred (second HEM line). Next, LR was given to maintain baseline MAP. Control (top) bled continuously as long as LR was given. (B) Low-dose (middle) bled intermittently. (C) High-dose (bottom) rebled slightly, stopped, and the animal's MAP was stable.

before rebleeding was induced (21 ± 5 min, high dose vs. 8 ± 4 min, control, $P = 0.005$; Fig. 3). There was a trend toward a reduced rebleed hemorrhage volume with rFVIIa, but it did not reach statistical significance ($P = 0.055$; Fig. 3). There was no reduction in the initial hemorrhage volume between the groups, despite high levels of circulating rFVIIa (Fig. 3).

The plasma activity of rFVIIa showed a dose-related increase in the two doses administered (Fig. 4). PT was measured to verify that the exogenously administered rFVIIa resulted in a functional enhancement of the extrinsic pathway. The PT, either normal or species specific, was shortened compared with normal by the lower dose and was not further affected by the higher dose at the time the rFVIIa concentration

was the highest (Fig. 4). The PT gradually returned to normal as the rFVIIa levels fell (Fig. 4). The TT and the TAT were measured to determine if a systemic activation of the coagulation system was induced by the rFVIIa. There were no significant changes, among groups or with time, in either of the variables (Fig. 4).

The coagulation parameters (PT, Fig. 4, and fibrinogen, Fig. 5) formed elements (platelets and hemoglobin, Fig. 5), and indices of plasma dilution (protein, Fig. 5) showed that all groups were similarly diluted by this aggressive resuscitation paradigm. Each group received similar total volumes of LR during the 2-h experiment (Fig. 6). Therefore, the difference in the rebleed MAP was not related to the differences induced by dilution of critical factors, but was likely due to the presence of rFVIIa.

Although the reduction of rebleed hemorrhage volume with rFVIIa treatment did not reach statistical significance ($P = 0.055$; Figs. 1 and 6), there was a significant metabolic consequence from the increased blood loss in the control group, leading to an elevated plasma lactate concentration compared with the 180 and 720 $\mu\text{g/kg}$ groups (14.2 ± 1.4 , 9.1 ± 1.1 , and 9.9 ± 1.3 mmol/L, respectively, $P = 0.004$; Fig. 5) and a trend toward a more negative base excess in the control group compared with the 180 and 720 $\mu\text{g/kg}$ groups (-8.2 ± 1.6 , -2.9 ± 2.0 , and -3.7 ± 1.4 mmol/L, respectively, $P = 0.082$; Fig. 6). The change in the arterial base excess was not due to changes in the ventilation because the animals were on a ventilator. Because we continued to resuscitate animals as long as the MAP remained below baseline, we were able to observe at what point the animals rebled and we recorded the total hemorrhage volume. However, this meant that we were unable to observe any appreciable difference in survival times. Although not significant, survival time in the control was shorter than that in the low- and high-dose FVII groups (73 ± 11 , 87 ± 11 , and 95 ± 11 min, respectively, $P = 0.238$). In addition, the number of survivors at 2 h increased ($P = 0.0242$) with dose: from two out of 10 in the control group, to five out of 10 in the low-dose group, and seven out of 10 in the high-dose group.

Tissues from the left ventricle, lung, liver, kidney, duodenum, and skeletal muscle were examined for presence of microthrombi. Great care was taken to harvest the tissues within 10 min of death and to put them immediately into formalin. Three different stains were used to comprehensively look for microthrombi. There was no evidence of intravascular clots associated with rFVIIa treatment in any of the tissues.

DISCUSSION

This study lends further credence to the theory that inclusion of rFVIIa in resuscitation regimens may permit resuscitation at

TABLE 1. Incidence of bleeding patterns with respect to rFVIIa dose

Treatment group	Epinephrine (number required/total)	Started then stopped rebleeding with resuscitation/number that rebled	Continuous rebleeding with resuscitation/number that rebled	No rebleeding even with MAP = 200 mm Hg/total
Vehicle	1/10	3/10	7/10	0
180 $\mu\text{g/kg}$	1/10	10/10 [†]	0/10 [†]	0
720 $\mu\text{g/kg}$	4/10	7/8	1/8 [†]	2/10

[†] $P < 0.01$ different from vehicle.

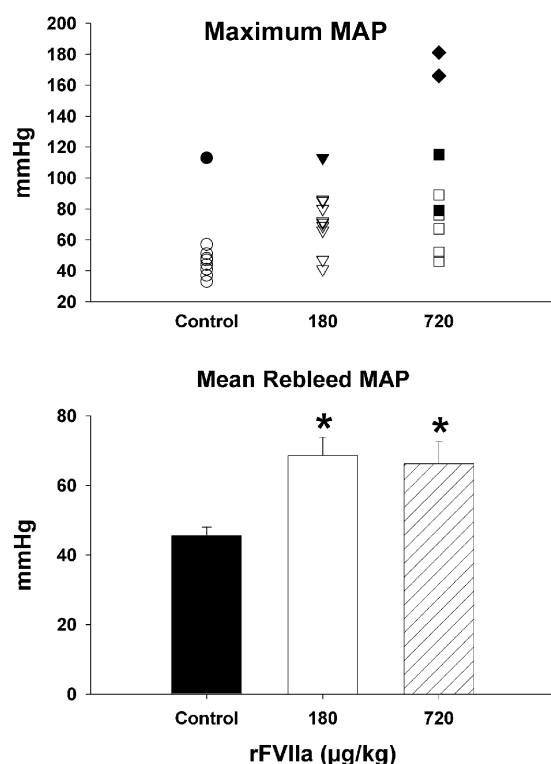


FIG. 2. Individual rebleed MAPs within each group (top) and mean rebleed MAP per group (bottom). Open symbols represent no epinephrine to induce rebleeding. Closed symbols represent epinephrine to induce rebleeding. Closed diamonds represent high-dose animals that did not rebleed at maximum dose of epinephrine.

higher blood pressures, increase the stability of clots, and reduce the risk of rebleeding and hemorrhage. In addition to increasing the pressure at which rebleeding occurred, treatment with rFVIIa reduced the rebleed hemorrhage volume ($P = 0.055$). A large variation in the volume of initial and rebleed hemorrhage in all groups resulted in a lack of statistical significance in comparisons of hemorrhage volumes. However, increased blood loss in the control group had negative metabolic consequences, resulting in a lower base excess as well as a lower central venous oxygen saturation.

There was a significant trend toward increased number of survivors in the groups that received rFVIIa, although not in survival time. The design of this experiment required that only LR was given, therefore, most of those animals that died did so because of extremely low hemoglobin levels (<3 g/dL) due to hemodilution. Because the animals that received rFVIIa received more LR before they rebled, they were significantly hemodiluted even though they tended to bleed less. This study was not designed to mimic the clinical scenario after injury, but to answer the question of what effect rFVIIa had on the rebleeding MAP and bleeding response to aggressive resuscitation. Because the primary goal of this study was to determine the rebleeding MAP, epinephrine was used to force the blood pressure to the rebleeding point in animals in which resuscitation alone could not raise blood pressure high enough to induce rebleeding. The determination of long-term survival was not an appropriate end-point for this type of study because this study was designed to be an *in vivo* bioassay for determining the strength of the clot in a vascular injury: a 2-h study period was

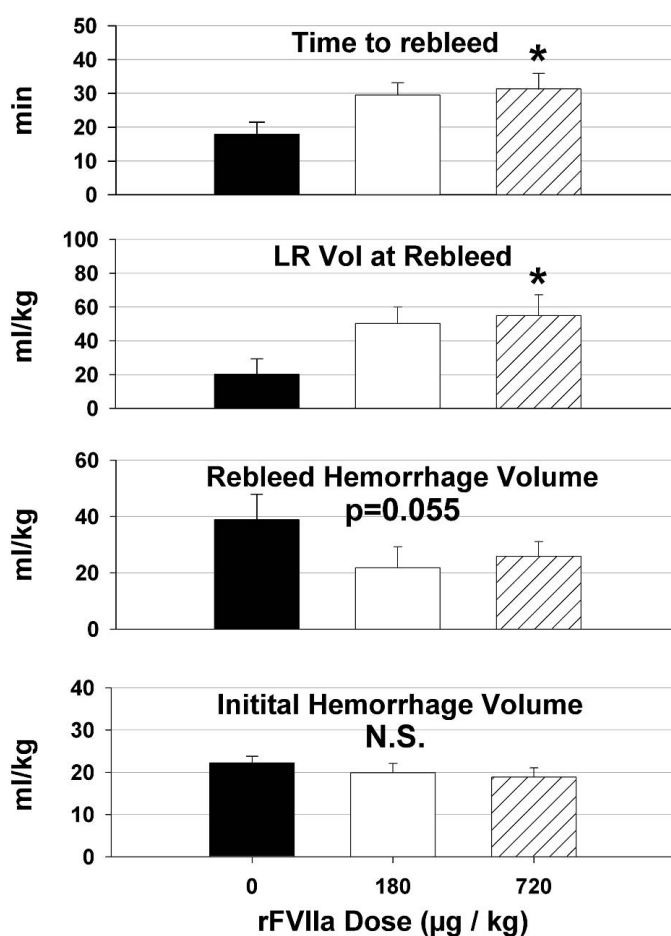


FIG. 3. Rebleed time in minutes and volumes in milliliters per kilogram of body weight. N.S., No significance. * $P < 0.05$ for difference between high-dose and control groups.

determined to be a sufficient time to adequately determine the rebleed MAP and rebleed hemorrhage volume.

In our previous study that determined the rebleed MAP, there was no effect of a delay in onset of resuscitation between 5, 15, and 30 min after the end of the initial hemorrhage on the rebleed point. Therefore, we chose to start resuscitation 10 min after the initiation of hemorrhage, which corresponded to the 5-min delay after the end of the initial hemorrhage in the previous study. This was not designed to mimic a combat or urban rescue scenario, but to determine the strength of the clot that formed from a vascular injury.

The coagulation parameters, formed elements, and indices of plasma dilution showed that all groups were similarly diluted by this aggressive resuscitation paradigm. Each group received similar total volumes of LR, therefore, the difference in the rebleed MAP could not be due to the differences induced by dilution of critical factors. Throughout the 2-h observation period, a significant shortening of the PT was observed, indicating the presence of substantial amounts of rFVIIa in the circulating blood. High levels of rFVIIa has been shown to enhance thrombin generation on the surface of thrombin-activated platelets, thereby inducing the formation of hemostatic plugs characterized by a tight and firm fibrin structure (20). Such hemostatic plugs are more resistant to premature lysis and are therefore more prone to maintain hemostasis (21).

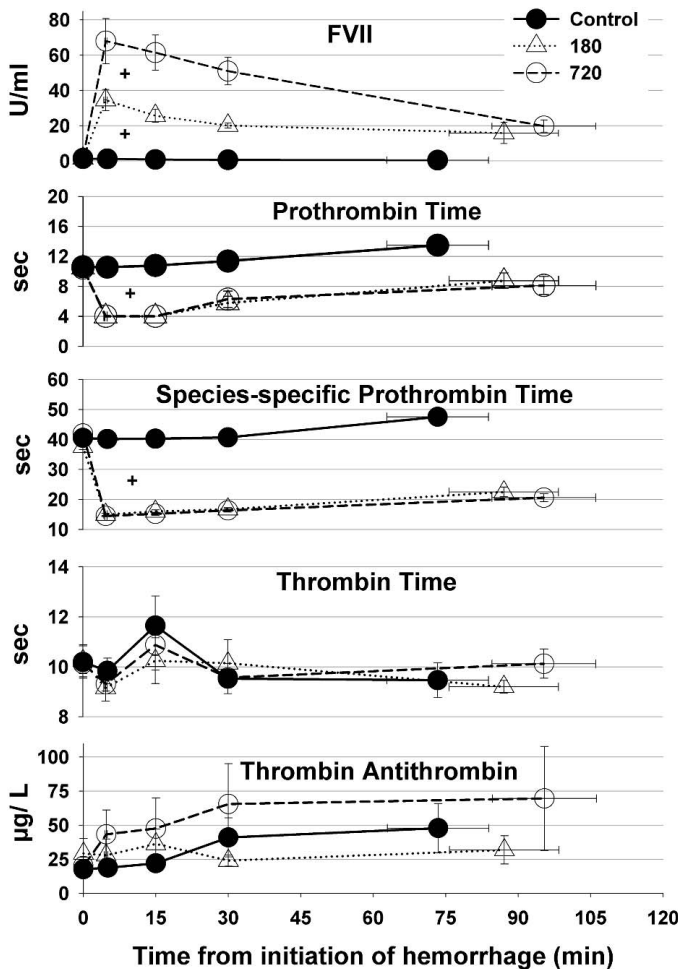


FIG. 4. rFVIIa concentration and coagulation parameters. The time points include those up to the 30-min time point (which was the last regularly scheduled time for which all animals were alive) and just before the animal's death. The values at death are graphed at the survival time, averaged per group. Therefore, SE bars are included for both the x and y axis for the death sample. $+P < 0.05$ for differences between high-dose and control groups and low-dose groups and control groups.

The current study shows that hemostatic plugs formed in the presence of high concentrations of rFVIIa also are more resistant against an increased MAP, which may be of significant importance in a trauma situation with high pressure arterial bleeding. Previous studies also showed that rFVIIa decreased the uncontrolled bleeding in a grade V liver injury model by 46% (12). In this model, rFVIIa was administered after a dilutional coagulopathy was induced. However, one single injection of rFVIIa given before the enucleation of the prostate gland decreased the requirement of transfusion significantly, indicating that extra rFVIIa given prophylactically in individuals with a normal coagulation pattern did induce hemostatic plugs, effectively preventing an excess of bleeding after trauma (22).

TT and TAT were not different among the groups. Thus, there was no laboratory evidence of a systemic activation of the clotting system. Furthermore, histopathological examination of tissues did not reveal systemic coagulation. Most microthrombi undergo fibrinolysis within 3 h of death (18), therefore, tissues were collected within 10 min of death to facilitate microthrombi preservation. The absence of microthrombi suggests that the activity of the exogenous administration of rFVIIa was

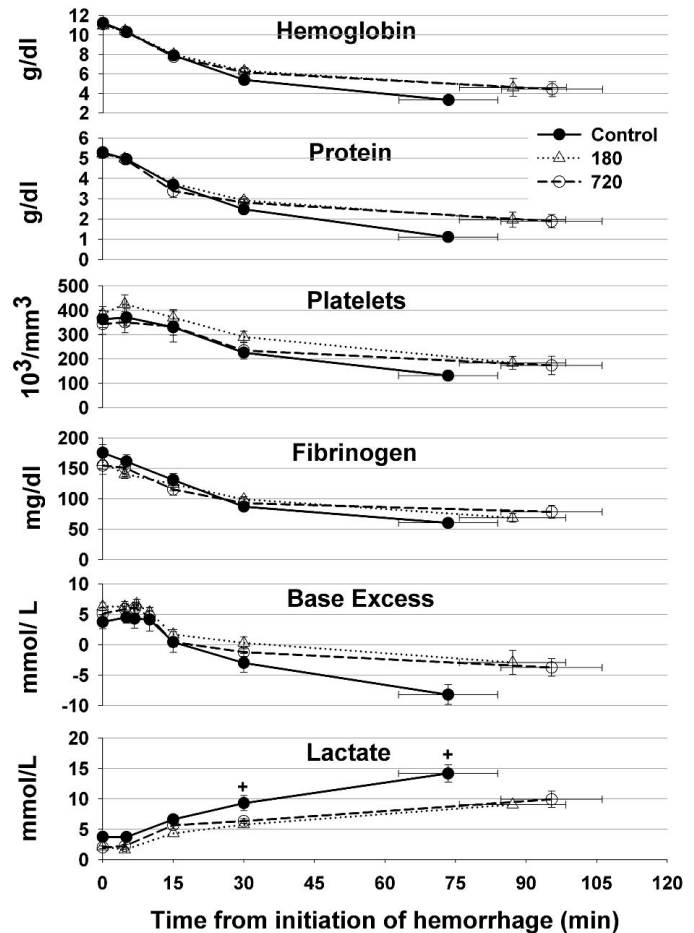


FIG. 5. Fibrinogen, formed elements, and indices of plasma dilution. Compared with baseline values, all displayed variables exhibited significant changes over time. Only arterial lactate concentration was significantly higher in the control group by 30 min compared with rFVIIa groups ($+P < 0.05$).

restricted to the local site of vascular injury without producing a pathologic activation of the extrinsic and intrinsic pathways of coagulation.

For suspected noncompressible bleeding, an intravenous resuscitation solution containing a drug that would enhance clotting or clot stability only at the bleeding sites would be an important advance in civilian trauma and combat casualty prehospital care (23). Recombinant FVIIa may be the drug of choice in such a setting because it is active principally at the site of injury. Thus far, experience indicates that the administration of rFVIIa induces hemostasis by increasing the generation of thrombin in individuals with impaired thrombin generation, (due to hemophilia, platelet defects, hemodilution in trauma, etc.), and thereby initiates the formation of a hemostatic plug with a tight fibrin structure more able to initiate and to maintain hemostasis (12, 24, 25). Hemostatic plugs formed in the presence of high concentrations of rFVIIa have been found to develop a tighter fibrin structure making them less permeable and thus less prone to premature lysis (20, 21).

Recombinant FVIIa has been safely used in a variety of clinical settings, including treatment of hemophiliacs (26), trauma patients (16, 27), and elective surgery (22). Previous swine studies have shown that rFVIIa reduces uncontrolled hemorrhage by 46% compared with vehicle-treated animals in

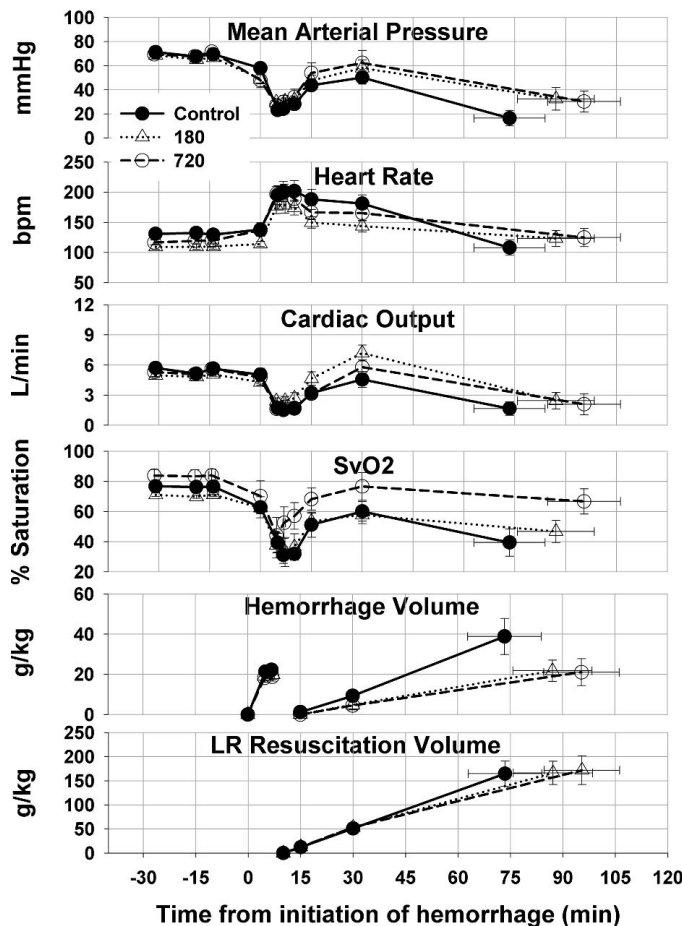


FIG. 6. Time course of hemodynamic responses to the hemorrhage and resuscitation. Changes were significant in all variables for time (asterisks not shown for clarity), but not among groups.

a grade V liver injury model when rFVIIa is used as an adjunct to standard gauze packing with cold-induced, dilutional coagulopathy (8, 12). However, few studies have explored the effect of rFVIIa in noncoagulopathic subjects. These data suggest that the administration of rFVIIa before operative intervention may be effective in reducing the risk of arterial as well as venous bleeding.

In conclusion, this is the first study to demonstrate that during resuscitation of an arterial injury, rFVIIa can increase the level at which MAP becomes vulnerable to rebleeding and may also reduce hemorrhage volume. These results, along with existing case reports of the successful use of rFVIIa to reduce intractable bleeding in trauma, further our understanding of the role of rFVIIa in hemostasis and lay the groundwork for testing the efficacy of rFVIIa during standard resuscitation treatment after blood loss in studies that will more closely reflect clinical practice after injury.

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REFERENCES

1. Dries DJ: Hypotensive resuscitation. *Shock* 6:311–316, 1996.
2. Stern SA, Wang X, Mertz M, Chowanski ZP, Remick DG, Kim HM, Dronen

- SC: Under-resuscitation of near-lethal uncontrolled hemorrhage: effects on mortality and end-organ function at 72 hours. *Shock* 15:16–23, 2001.
3. Kowalenko T, Stern S, Dronen S, Wang X: Improved outcome with hypotensive resuscitation of uncontrolled hemorrhagic shock in a swine model. *J Trauma* 33:349–353, 1992.
4. Nan X, Xi-Chun W, You-Fang D, Ren L, Kun-Lun T: Effect of initial fluid resuscitation on subsequent treatment in uncontrolled hemorrhagic shock in rats. *Shock* 21:276–280, 2004.
5. Riddez L, Drobin D, Sjostrand F, Svensen C, Hahn RG: Lower dose of hypertonic saline Dextran reduces the risk of lethal rebleeding in uncontrolled hemorrhage. *Shock* 17:377–382, 2002.
6. Varela JE, Cohn SM, Diaz I, Giannotti GD, Proctor KG: Splanchnic perfusion during delayed, hypotensive, or aggressive fluid resuscitation from uncontrolled hemorrhage. *Shock* 20:476–480, 2003.
7. Sondeen JL, Coppes VG, Holcomb JB: Blood pressure at which rebleeding occurs after resuscitation in swine with aortic injury. *J Trauma* 54:S110–S115, 2003.
8. Schreiber M, Holcomb J, Hedner U, Brundage S, Macaitis J, Aoki N, Meng ZH, Twardy DJ, Hoots K: The effect of recombinant factor VIIa on non-coagulopathic pigs with grade V liver injuries. *J Am Coll Surg* 196:691–697, 2003.
9. Hedner U, Ingerslev J: Clinical use of recombinant FVIIa (rFVIIa). *Transfus Sci* 19:163–176, 1998.
10. Hedner U, Erhardtsen E: Future possibilities in the regulation of the extrinsic pathway: rFVIIa and TFPI. *Ann Med* 32:68–72, 2000.
11. Shapiro AD, Gilchrist GS, Hoots WK, Cooper HA, Gastineau DA: Prospective, randomised trial of two doses of rFVIIa (NovoSeven) in haemophilia patients with inhibitors undergoing surgery. *Thromb Haemost* 80:773–778, 1998.
12. Martinowitz U, Holcomb JB, Pusateri AE, Stein M, Onaca N, Freidman M, Macaitis JM, Castel D, Hedner U, Hess JR: Intravenous rFVIIa administered for hemorrhage control in hypothermic coagulopathic swine with grade V liver injuries. *J Trauma* 50:721–729, 2001.
13. Al Douri M, Shafi T, Al Khudairi D, Al Bokhari E, Black L, Akinwale N, Osman Musa M, Al Homaidhi A, Al Fagih M, Borum Andreasen R: 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* 11:S121–S127, 2000.
14. Dutton RP, Hess JR, Scales TM: Recombinant factor VIIa for control of hemorrhage: early experience in critically ill trauma patients. *J Clin Anesth* 15:184–188, 2003.
15. Holcomb J, Neville L, Fischer C, Hoots K: Use of recombinant FVIIa for intra-peritoneal coagulopathic bleeding in a septic patient. *Curr Surg* 60:423–427, 2003.
16. Martinowitz U, Kenet G, Segal E, Luboshitz J, Lubetsky A, Ingerslev J, Lynn M: Recombinant activated factor VII for adjunctive hemorrhage control in trauma. *J Trauma* 51:431–438, 2001.
17. O'Neill PA, Bluth M, Gloster ES, Wali D, Priovolos S, DiMaio TM, Essex DW, Catanese CA, Strauss RA: Successful use of recombinant activated factor VII for trauma-associated hemorrhage in a patient without preexisting coagulopathy. *J Trauma* 52:400–405, 2002.
18. Robinson WF, Maxie MG: The cardiovascular system. In Jubb KVF, Kennedy PC, Palmer N (eds.): *Pathology of Domestic Animals*, ed 4. San Diego, CA: Academic Press, 1993, pp 62–64.
19. Ravanat C, Freund M, Dol F, Cadroy Y, Roussi J, Incardona F, Maffrand JP, Boneu B, Drouet L, Legrand C, Herbert J-M, Cazenave J-P: Cross-reactivity of human molecular markers for detection of prethrombotic states in various animal species. *Blood Coagul Fibrinolysis* 6:446–455, 1995.
20. He S, Blomback M, Jacobsson Ekman G, Hedner U: The role of recombinant factor VIIa (FVIIa) in fibrin structure in the absence of FVIII/FIX. *J Thromb Haemost* 1:1215–1219, 2003.
21. Weisel JW, Veklich Y, Collet JP, Francis CW: Structural studies of fibrinolysis by electron and light microscopy. *Thromb Haemost* 82:277–282, 1999.
22. Friederich PW, Henny CP, Messelink EJ, Geerdink MG, Keller T, Kurth KH, Buller HR, Levi M: Effect of recombinant activated factor VII on perioperative blood loss in patients undergoing retropubic prostatectomy: a double-blind placebo-controlled randomised trial. *Lancet* 361:201–205, 2003.
23. Sapsford W: A role for recombinant activated factor VII in trauma? *Trauma* 4:117–123, 2002.
24. Blomback B, Carlsson K, Fatah K, Hessel B, Procyk R: Fibrin in human plasma: gel architectures governed by rate and nature of fibrinogen activation. *Thromb Res* 75:521–538, 1994.
25. Hedner U: Recombinant activated factor VII as a universal haemostatic agent. *Blood Coag Fibrinolysis* 9:S147–S152, 1998.
26. Hedner U, Erhardtsen E: Potential role for rFVIIa in transfusion medicine. *Transfusion* 42:114–124, 2002.
27. Lynn M, Jeroukhimov I, Klein Y, Martinowitz U: Updates in the management of severe coagulopathy in trauma patients. *Intensive Care Med* 28:S241–S247, 2002.