The Effect of Recombinant Factor VIIa on Noncoagulopathic Pigs with Grade V Liver Injuries

Martin A Schreiber, MD, FACS, John B Holcomb, MD, FACS, Ulla Hedner, MD, Susan I Brundage, MD, FACS, Joseph M Macaitis, BS, Nori Aoki, MD, Zhi Hong Meng, PhD, David J T weardy, MD, Keith Hoots, MD

BACKGROUND: Recombinant Factor VIIa (rFVIIa) has been used to decrease bleeding in a number of settings, including hemophilia, liver transplantation, intractable bleeding, and cirrhosis. It has also been shown to reduce bleeding in coagulopathic pigs with Grade V liver injuries when used as an adjunct to packing. This study was performed to determine if rFVIIa would reduce blood loss after a Grade V liver injury in noncoagulopathic pigs when used as sole therapy.

STUDY DESIGN: Thirty normothermic animals were randomized to receive either 150 μg/kg of rFVIIa or normal saline intravenously. After laparotomy and splenectomy, a standardized Grade V liver injury was made with a liver clamp. Thirty seconds after injury, blinded therapy was given. Blood loss was measured 15 minutes after injury and the abdomen was closed. Animals were resuscitated to their baseline blood pressure and the study was continued for 2 hours. Serial coagulation parameters were obtained. Following the study period, blood loss was measured and an autopsy was performed. Grossly normal areas of lung were examined for evidence of intravascular thrombosis.

RESULTS: Mean Factor VII:C levels increased 155-fold in the treatment group after infusion of rFVIIa. The mean prothrombin time in the treatment group decreased from 9.8 ± 0.4 seconds to 7.3 ± 0.2 seconds and remained significantly different from the control group throughout the study (p < 0.01). There were no differences in other coagulation parameters. Mean initial blood loss was 822 ± 266 mL in the treatment group and 768 ± 215 mL in the control group (p = 0.6). Rebleeding blood volume was 397 ± 191 mL in the treatment group and 437 ± 274 mL (p = 0.6) in the control group. Lung histology revealed no evidence of abnormal microvascular thrombosis.

CONCLUSIONS: rFVIIa does not reduce blood loss after Grade V liver injury when it is used as sole therapy in warm noncoagulopathic pigs. (J Am Coll Surg 2003;196:691–697. © 2003 by the American College of Surgeons)

Trauma is the leading cause of death for people between the ages of 1 and 44 in the United States. The majority of deaths that occur during the first 48 hours after a traumatic event are the result of hemorrhage. The use of an intravenous agent that reduces blood loss could potentially save lives and conserve the decreasing supply of blood products.

Recombinant Factor VIIa (rFVIIa) has been described as a universal hemostatic agent. It was originally designed to treat hemophiliacs with inhibitors to Factor VIII and Factor IX and it is approved by the Food and Drug Administration for that indication. Successful use of the drug has also been reported in patients with platelet disorders, cirrhosis, intractable bleeding from various etiologies, liver transplantation, and cardiac surgery.

The published uses of rFVIIa in the clinical trauma setting are limited to case reports and a case series. Although these reports are promising, the use of rFVIIa in trauma patients has not been studied prospectively. The drug has been shown to be effective in a swine liver model.
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**Authors:**

**Performing Organization:**
United States Army Institute of Surgical Research, JBSA Fort Sam Houston, TX 78232

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The animals were cold and dilutionally coagulopathic and rFVIIa was used as an adjunct to gauze packing. Treated animals demonstrated a marked reduction in blood loss compared with control animals. In light of the increasing body of data suggesting the efficacy of rFVIIa in acquired coagulopathies and to test the hemostatic envelope of the drug, we conducted this study to determine if rFVIIa would reduce blood loss in warm, noncoagulopathic animals when used as sole therapy. We also evaluated the safety of the drug in animals without a preexisting coagulopathy.

METHODS

Three-month-old Yorkshire crossbred swine weighing 40 kg were used. All animals were free of disease and in apparent excellent health. Animals were allowed free access to water and to a commercial laboratory swine food. Food was withheld the night before the study. All animals were maintained in an Association for Assessment and Accreditation of Laboratory Animal Care International–accredited facility, and all experimental manipulations were performed in accordance with the National Research Council’s Guide for the Care and Use of Laboratory Animals. The protocol was approved by the Institutional Animal Care and Use Committee at Baylor College of Medicine.

The swine were anesthetized with an intramuscular injection of 4.4 mg/kg of Telazol (Fort Dodge Animal Health). The animals were also given 0.25 mg/kg of glycopyrrolate intramuscularly. They were then intubated with a 7-mm Mallinckrodt endotracheal tube and placed on mechanical ventilation with settings of 10 mL/kg tidal volume, a rate of 12 to 14 breaths per minute, and 100% oxygen. Anesthesia was maintained using 2% isoflurane and an esophageal temperature probe was placed.

Once the animals were fully anesthetized, cutdowns were performed and polyethylene tubing was placed in the left external jugular vein and left common carotid artery. The venous line was used for drug infusion and fluid resuscitation. The arterial line was used for continuous blood pressure monitoring and blood sampling. Mean arterial pressure, systolic pressure, diastolic pressure, and heart rate were recorded and averaged every 10 seconds using a digital data collection system with a blood pressure analyzer.

The animals underwent midline laparotomy, Foley catheter placement, and splenectomy. The spleen was weighed and lactated Ringer’s solution at 40°C was infused at 100 mL/min to replace three times the spleen weight. Splenectomies are performed in swine hemorrhage models because pigs have distensible spleens that can contain a highly variable amount of blood. The animals were warmed to a standardized esophageal temperature of 38°C, using a Bair Hugger warming system (Augustine Medical Inc). The abdomen was then closed with towel clamps.

After a 10-minute stabilization period, the abdomen was reopened and preweighed laparotomy pads were placed in both gutters and the pelvis to facilitate blood collection. A standardized Grade V liver injury was made with a specially designed liver clamp. For the purposes of this model, a Grade V injury is defined as an injury to a central hepatic vein. This is consistent with the definition of a Grade V injury as indicated by the American Association for the Surgery of Trauma Organ Injury Scaling system. The current animal model is based upon our experience in previous studies of hemorrhage control using the Grade V liver injury model. The clamp was positioned in the middle of the liver, placing the right hepatic vein, left hepatic vein, and portal vein at risk for injury. Blood loss was collected by suction. Thirty seconds after injury, blinded therapy consisting of either 150 μg/kg of rFVIIa or the equivalent amount of buffer solution (control hemorrhagic shock group) was infused. Bleeding was monitored for a maximum of 15 minutes after injury. The laparotomy pads were removed and the abdomen was closed either when bleeding ceased if less than 15 minutes or 15 minutes after injury. Blood loss was calculated as the sum of the volume of blood suctioned and the difference in weight of the laparotomy pads before and after bleeding.

Lactated Ringer’s resuscitation at 100 mL/min was begun 15 minutes after injury. Resuscitation was delayed 15 minutes to allow the animals to reach their nadir blood pressure. Animals were resuscitated back to their baseline mean arterial pressure (MAP) and the study was continued for 2 hours after injury. Lactated Ringer’s solution was infused as necessary to maintain the baseline MAP within 5 mmHg of the preinjury pressure. After completion of the 2-hour study period, the abdomen was reopened and blood loss was determined by adding the volume of blood to the weight of the clots. Following euthanasia with 10 mL of Euthasol, the left lung was removed and distended with formaldehyde for
histology. The liver was removed and the number of injured veins was recorded to ensure that injuries were comparable between groups.

Blood assays included prothrombin time, partial thromboplastin time (PTT), complete blood count, fibrinogen, Factor VII clotting activity (FVII:C), thrombin–antithrombin complexes (TATs), and arterial blood gases. Specimens were collected at baseline, 1 minute after study drug infusion, 15 minutes after injury, when the animal reached baseline MAP with resuscitation, and at the end of the 2-hour study period.

Lung histology was examined by an independent blinded pathologist. Hematoxylin and eosin staining and immunostaining for fibrin were performed. Immunostains were prepared using a two-layered method. After antigen retrieval using 1% protease, rabbit antihuman fibrinogen antibody (DAKO A0080) was applied as primary antibody and goat antirabbit antibody labeled with horseradish peroxidase (Jackson 111-035-003) as secondary antibody. Vector red served as chromogene. A positive control section was included. As a negative control, an adjacent section was stained as above without the addition of the primary antibody.

Statistical analysis
Student’s t-test was used to compare means of continuous variables. Dichotomous data were analyzed with the chi-square test. If the value of a categorical variable in any cell were less than five, Fisher’s exact test was used.

RESULTS
Fifteen animals were randomly allocated to each study group. One animal was prospectively excluded from the control group before injury or treatment because of the finding of a grossly abnormal liver at laparotomy. All animals survived the 2-hour study period. Figure 1 shows the FVII:C concentrations measured over the
course of the study. Infusion of rFVIIa resulted in a mean increase in FVII:C levels by 155-fold. Factor VII:C levels remained elevated throughout the study compared with controls (p < 0.01). Figure 2 shows serial prothrombin time and PTT measurements. Prothrombin time was notably reduced in the treatment group. This effect also persisted throughout the study (p < 0.01). There was no effect of rFVIIa on PTT.

Figure 3 shows MAP measured over the course of the study in the two groups. There was no significant difference. As Figure 4 shows, blood loss at 15 minutes and 2 hours was similar between the two groups. The resuscitation requirement to achieve and maintain the baseline MAP was 7,789 ± 1,680 mL in the treatment group and 7,579 ± 2,314 mL in the control group (p = 0.8). Injury severity was similar between the groups as determined by examination of the livers. Necropsies revealed a mean of 1.7 ± 0.8 vessels injured in the treatment group and 1.5 ± 0.5 vessels in the control group (p = 0.4).

Figure 5 shows serial TAT measurements. Thrombin-antithrombin complexes increased to an equal degree in both groups. Fibrin stains of the lung were positive in all animals with the exception of one animal in the treatment arm. There was no evidence of associated circulatory disturbances, including infarction, bleeding, or edema, in relation to the presence of fibrin-positive material. This indicates that the presence of fibrin represents agonal or postmortem clot formation and not thrombotic events that occurred during the study period. As Figure 6 shows, there was no decrease in oxygenation in the treatment group compared with the control group.

DISCUSSION

Factor VII is a normal component of the extrinsic clotting pathway. Following trauma, hemostasis is initiated by the complex formed by tissue factor (TF) and activated Factor VII (FVIIa). Tissue factor is only exposed
after injury and FVIIa makes up approximately 1% of the total Factor VII by mass. The TF-FVIIa complex, which is anchored at the site of injury, causes the conversion of Factor X into Factor Xa, which then converts prothrombin into thrombin and causes platelet activation. In addition, the TF-FVIIa complex catalyzes the activation of Factor IX to IXa, which also ultimately results in the conversion of prothrombin to thrombin.

There is additional evidence suggesting that rFVIIa can activate Factor X through a tissue factor-independent mechanism. This mechanism has been shown to result in increased thrombin production and more rapid activation of platelets. High concentrations of rFVIIa have also been found to activate FIX and FX on the surface of activated platelets. This results in platelet surface thrombin generation independent of tissue factor. Thrombin production is proportional to the concentration of rFVIIa.

Thrombin generation is necessary for activation of the thrombin activatable fibrinolytic inhibitor, which protects the stabilized hemostatic fibrin plug. In addition, the initial rate of thrombin generation is important for the formation of a tight fibrin gel with low permeability and high resistance to fibrinolysis. The addition of rFVIIa to hemophilia plasma in vitro also results in normalization of the fibrin clot permeability and tightening of the fibrin structure. This indicates a role for high concentrations of exogenous rFVIIa in enhancing thrombin generation initiating the formation of tight fibrin gels that are resistant to premature dissolution. Such fibrin plugs will maintain hemostasis more efficiently, so, it has been hypothesized that infusion of high-dose rFVIIa will result in acceleration of stable clot formation resulting in decreased bleeding after trauma.

The primary finding of this study was that rFVIIa did not reduce blood loss when used as a sole agent, in non-coagulopathic swine with Grade V liver injuries. This occurred despite documentation of elevated FVII:C levels and notable reductions in the prothrombin time. These results are very different from those obtained in prior studies using a similar model. In those studies, an identical injury was made, but rFVIIa was used as an adjunct to liver packing and the animals were cold and dilutionally coagulopathic. Of note, rFVIIa successfully corrected the coagulopathy of hypothermia and dilution based on prothrombin times and blood loss was reduced by approximately 50% in the treatment groups. Possible explanations for the differences between the findings presented in this article compared with the prior studies include the use of the drug as a sole agent and the normotensive state of the animals at the time of injury.

The described Grade V liver injury produces large venous lacerations as well as injuries to the hepatic parenchyma. The hepatic venous system is a low-pressure system. In the prior studies, abdominal packing was performed at the time of resuscitation, theoretically compressing the cut ends of open vessels. This allowed at least partial control of hemorrhage and facilitated the action of rFVIIa, which requires a biochemical process including complex formation with TF and a series of enzymatic reactions resulting in the generation of thrombin and the formation of tight hemostatic plugs. This process is unlikely to be optimized in the setting of uncontrolled bleeding from major vessels when rFVIIa is used as the sole hemostatic agent.

The animals in the prior studies underwent a 60% isotonic hemodilution and reduction of temperature to 33°C, resulting in preinjury hypotension. Alternatively, in the current study animals had a mean preinjury MAP of 97 ± 22 mmHg. The lower MAPs seen in the earlier studies and the presumed corresponding decrease in hepatic venous flow is likely to have facilitated the hemostatic efficacy of rFVIIa.

There is a theoretical possibility that high doses of rFVIIa can initiate a systemic activation of the coagulation system and cause the formation of thrombi in sites...
distant from the injury. This concern has not been substantiated in the literature. The formation of TAT complexes occurred in all animals undergoing surgery and hemorrhagic shock with no differences between the groups. This suggests that rFVIIa did not induce systemic activation of the coagulation system. In addition, there was no evidence of acute formation of intravascular thrombi on lung histology. These findings are in agreement with the prior studies.

The results of the current study, placed within the context of previous work, suggest that rFVIIa should be used as an adjunct to standard surgical therapy. Large-vessel bleeding should be controlled either by suture ligation, repair, or packing, facilitating the action of rFVIIa. Persistent bleeding produced by dilutional coagulopathy and hypothermia might then be concurrently controlled by rFVIIa.

**Author Contributions**

Study conception and design: Schreiber, Holcomb, Hedner, Hoots

Acquisition of data: Schreiber, Macaitis, Meng

Analysis and interpretation of data: Schreiber, Brundage, Macaitis, Aoki, Meng

Drafting of manuscript: Schreiber

Critical revision: Holcomb, Hedner, Twardy

Statistical expertise: Brundage, Aoki

Obtaining funding: Hedner, Twardy, Hoots

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**REFERENCES**


