

Blood Coagulation, Fibrinolysis and Cellular Haemostasis

Effects of increasing doses of activated recombinant factor VII on haemostatic parameters in swine

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Summary

This study examined dose-response relationships between activated recombinant factor VII (rFVIIa) and (1) *in vivo* haemostasis and (2) *in vitro* measures of coagulation and platelet function. Anesthetized swine were used. Ear bleeding time (BT) was measured and blood was sampled following increasing doses of rFVIIa (0, 90, 180, 360 and 720 µg/kg; n = 6) or saline (n = 6). BT was not altered by rFVIIa. Prothrombin time (PT) using standard or pig-specific methods was decreased by rFVIIa. Activated clotting time (ACT) was decreased by rFVIIa. Thromboelastography using collagen (COLL) or pig thromboplastin (p-ThP) as agonist demonstrated shorter reaction times, shortened time to reach maximum velocity of clot formation, and increased α -angle in the presence of rFVIIa. rFVIIa dosing increased maximum veloc-

ity of clot formation when p-ThP was used to initiate the reaction but not when COLL was used. rFVIIa at the highest concentration increased maximum amplitude when COLL was used to initiate the reaction. Platelet aggregation was not altered by rFVIIa. Following completion of the dose escalation phase, a severe liver injury was produced. rFVIIa altered neither blood loss nor survival time following injury but improved mean arterial pressure. A small increase in systemic thrombin-antithrombin III complex occurred after administration of rFVIIa at doses of 180 µg/kg and above. However, there was no histological evidence of intravascular coagulation after rFVIIa administration. In summary, rFVIIa activity was detectable *in vitro* but did not change haemostasis in normal swine.

Keywords

Pharmacological haemostasis, liver injury, trauma, thromboelastography

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Introduction

Activated recombinant factor VII (rFVIIa) has been widely used to treat bleeding complications in haemophilia patients. Recent reports suggest broader applications (1). Currently, there is great interest in the potential for use of rFVIIa in the treatment of haemorrhage after trauma (2, 3). However, use for trauma is by no means proven and advances in this area will continue to require experimental study.

The few experimental studies published that examine the use of rFVIIa in trauma have exclusively used swine. In one study, severe liver injuries were induced in swine made coagulopathic by haemodilution and hypothermia; animals received either 180

µg/kg rFVIIa or saline in addition to gauze packing. Blood loss was reduced when rFVIIa was used (4). A similar study demonstrated that either 180 µg or 720 µg/kg rFVIIa reduced blood loss in coagulopathic pigs (5). In pigs with normal coagulation, rFVIIa decreased blood loss following liver injury in one study (6) but not in two others (7, 8).

There appears to be two mechanisms by which rFVIIa acts at pharmacologically relevant doses. One mechanism has been described that involves direct binding of rFVIIa to activated platelets (9). This leads to the generation of small amounts of activated factor X and subsequently thrombin, resulting in platelet activation, granule release, and further recruitment of platelets (9). The other mechanism of action requires binding of rFVIIa to

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exposed tissue factor on an appropriate surface followed by enzymatic activation of factor X (10). The tissue factor-FVIIa interaction is species-specific (11, 12). For example, human factor VII (FVII) appears to have reduced activity when exposed to porcine thromboplastin (11, 12). Furthermore, standard *in vitro* coagulation tests commonly use as a tissue factor source rabbit brain thromboplastin, which reacts differently with human versus pig plasma (11). When rabbit-derived reagents are used to assay pig blood that contains human rFVIIa, yet a third species is introduced into the assay system. This may produce a situation in which a given dose of rFVIIa has more apparent activity in pig blood when measured by *in vitro* tests than the same dose has *in vivo* when exposed solely to pig tissue factor. This could complicate interpretation of results.

This study was conducted to determine the effects of increasing doses of rFVIIa on *in vivo* haemostasis and *in vitro* measures of coagulation and platelet function using species-specific assays in swine with normal coagulation function.

Methods

Experimental procedures were conducted in two phases. The objective of the first phase (Dose Escalation Phase) was to determine the effects of increasing doses of rFVIIa on capillary bleeding time and *in vitro* haemostatic parameters using species-specific procedures. The second phase (Injury Phase) was a pilot study of the effects of rFVIIa pretreatment on the response to severe liver laceration.

Animals

Crossbred commercial swine (Duroc x Musclor x Large White x Yorkshire) weighing 37.2 ± 0.8 kg (mean \pm SEM) were used in this study. Animals were maintained in a facility accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care, International. This study was approved by the Institutional Animal Care and Use Committee of the US Army Institute of Surgical Research, Fort Sam Houston, Texas. Animals received humane care in accordance with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health publication 86-23, revised 1996).

Animals were assigned randomly to treatment groups. Treatment consisted of either 1) increasing doses of rFVIIa (rFVIIa group; $n = 6$), or 2) repeated infusions of physiological saline (saline group; $n = 6$). Investigators were blinded to treatment. Swine were fasted for 24 to 36 hours before the procedure, with water continuously available. Animals were sedated using glycopyrrolate, tiletamine HCl, and zolazepam. They were then intubated and anesthetized (isoflurane in oxygen) using a closed circuit system with mechanical ventilation. Digital temperature probes were placed on each ear, and heat lamps were positioned to maintain ear temperature between 40 and 41°C. An infusion catheter was placed occlusively in a femoral vein and advanced to the caudal vena cava. An 8.5 Fr catheter introducer was shortened to 3 cm and placed occlusively in a femoral artery for blood sampling. The opposite femoral artery was catheterized occlusively using a Paratrend7+ Multiparameter Sensor-catheter (Diametrics Medical Inc., Roseville, Minnesota) attached to a Trendcare TCM 7000 blood gas monitoring system (Diametrics Medical

Inc., Roseville, Minnesota) for continuous monitoring of body temperature and blood pH. A port in the Paratrend7+ catheter was coupled to a continuous data collection system (Micro-Med[®], Louisville, KY) for monitoring blood pressure and heart rate. Laparotomy, splenectomy, and cystotomy were performed on each pig. To compensate for removal of the spleen, each animal was infused with lactated Ringer's solution at a volume equivalent to three times the spleen weight. Animals were stabilized for 20 minutes at a body temperature of 38.5 to 39.5°C, a blood pH of 7.37 to 7.43, and a mean arterial pressure (MAP) between 50 and 80 mm Hg.

Dose escalation phase

Treatment administration

Treatments were administered at 0, 20, 40, 60, and 80 minutes. Treatments were infused via the femoral vein catheter over a four-minute period using a PHD 2000 infusion pump (Harvard Apparatus, Natick, MA). A four-minute circulation period was allowed prior to further procedures. Doses of rFVIIa were calculated to provide the following cumulative effective doses: 0 μ g/kg at time 0, 90 μ g/kg at 20 minutes, 180 μ g/kg at 40 minutes, 360 μ g/kg at 60 minutes, and 720 μ g/kg at 80 minutes. Dose calculations accounted for an estimated half-life for rFVIIa of 147 minutes in the circulation (13), with the assumption that half-life was similar between humans and swine. The actual doses administered at each respective time point were: 0 μ g/kg, 90 μ g/kg, 96 μ g/kg, 192 μ g/kg, and 384 μ g/kg at 0, 20, 40, 60, and 80 minutes.

Ear bleeding times

Ear bleeding time (BT) was determined at baseline and after each treatment administration and circulation period. BT was performed simultaneously in both ears by completely penetrating each using a #11 scalpel blade. The mean of the two ears was recorded as BT. Filter paper was used to wick away blood during the procedure.

In a pilot experiment, the sensitivity of the BT procedure was assessed in three additional anesthetized pigs in which activated clotting time (ACT) was extended by infusing heparin and reversed by infusing protamine sulfate. At baseline, BT and ACT were 158 ± 88 (mean \pm SEM) and 99 ± 95 seconds, respectively. Following heparin, BT and ACT were 317 ± 88 and 636 ± 95 seconds, respectively. Protamine decreased BT to 156 ± 88 seconds and ACT to 145 ± 95 seconds.

Injury phase

The peritoneal cavity was suctioned immediately before induction of the liver injury (at 100 minutes). Using a specially designed instrument (14), a penetrating laceration was made in the right central portion of the liver such that the right medial lobar vein was lacerated approximately 3 cm distal to the vena cava. The abdomen was temporarily closed and the animal was monitored without resuscitation. At 60 minutes post-injury, surviving animals were euthanized. Following euthanasia or earlier death, intraperitoneal blood was quantitated.

Blood sampling

Blood samples were collected by inserting a 20 cm single-use catheter made from Tygon[®] tubing (I.D. 0.9 mm; Saint Govaine

Performance, Akron OH) into the self-sealing port of the catheter introducer and gently withdrawing the blood to minimize shear-induced platelet activation. The first 3 ml of blood was discarded. In the Dose Escalation Phase, blood samples were collected at baseline and after each respective treatment infusion and circulation period. In the Injury Phase, a blood sample was collected at 30 minutes post-injury. Except for thromboelastography (TEG) samples, blood was anticoagulated with 3.2% sodium citrate at 1 part citrate solution to 9 parts blood.

At each time, hematocrit, platelet count, fibrinogen concentration, factor VII activity, standard prothrombin time (PT), activated partial thromboplastin time (aPTT), pig-specific PT (pPT), thrombin-antithrombin-III complex (TAT) concentration, and activated clotting time (ACT) were determined. At baseline, after the 180 ug/kg and 720 ug/kg doses, and at 30 minutes post-injury, thromboelastography (TEG) and platelet aggregometry were also performed.

Standard laboratory procedures

Hematocrit (Hct) and platelet (PLT) counts were performed as direct measurements using the ABX Pentra 120 hematology analyzer (ABX Diagnostics, Inc., Irvine, CA). ACT was performed using the Hemochron Response (International Technidyne Corp., Edison, NJ), according to manufacturer's instructions. FVII activity was measured with a one-stage clotting assay (FVII:C) using an ACL Futura Coagulation System (Instrumentation Laboratory, Lexington, MA). For the assay, normal and FVII deficient human plasma standards and rabbit brain thromboplastin reagent provided by the manufacturer were used. The ACL Futura methodology reports results in percent activity. Percentages were divided by 100 to convert to U/ml. Samples were diluted as necessary according to manufacturer specifications. Mean FVII activity was 1.06 ± 0.07 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), activated partial thromboplastin time (aPTT), and fibrinogen concentrations were determined at 37°C using the ACL Futura Coagulation System according to manufacturer's instructions. Mean clotting time for the standard PT assay using pooled normal pig plasma was 12.87 ± 0.15 sec, with intra-assay and interassay CV of 1.2% and 4.7%, respectively. Mean aPTT using pooled normal pig plasma was 14.40 ± 0.38 sec, with intra-assay and interassay CV of 1.2% and 2.6%, respectively. Fibrinogen concentration for pooled normal pig plasma was 215.0 ± 13.8 mg/dL, and intra-assay and interassay CV were 6.4% and 11.4%, respectively. Thrombin-antithrombin III complex (TAT) concentrations were quantitated using the Enzygnost TAT micro enzyme immunoassay (Dade Behring, Marburg, Germany), which has previously been demonstrated to cross-react with porcine TAT (15). Intra-assay CV was 5.6% and interassay CV was 10.3% for a pig plasma pool containing 38.4 ± 1.6 ng/ml TAT.

Species-specific PT

Preparation of pig thromboplastin (p-ThP)

Brains were collected from slaughterhouse pigs and stored at 4°C in 0.9% saline until washing with 0.9% saline and removal of large blood vessels. Each brain was homogenized in 500 ml of

phosphate-buffered saline (PBS) for five minutes at full speed in a Waring (Torrington, CT) commercial blender and the homogenate placed into 50 ml conical vials and centrifuged for 15 minutes at 2100 x g. The resulting supernatants were retained as p-ThP, pooled, and frozen at -20°C in 1 ml aliquots.

Modified PT assay

p-ThP was thawed and centrifuged at 2100 x g for 5 minutes. Supernatant was collected and diluted 1:20 with HEPES buffer (Sigma, St. Louis, MO) containing 100 µM 25% phosphatidylcholine: 75% phosphatidylserine (PCPS) lipid vesicles (Haematologic Technologies, Essex Junction, VT), and equilibrated for 10 minutes at 39°C. Assays were performed at 39°C using a Beckman-Coulter (Fullerton, CA) DU640 spectrophotometer equipped with a temperature-controlled cuvette holder. To perform the procedure, 200 µl of pig plasma and 400 µl of diluted p-ThP were added to each prewarmed cuvette and allowed to equilibrate for two minutes. The reaction was initiated by the addition of 200 µl of CaCl₂. Absorbance measurements were made at 880 nm every 0.4 seconds for a total of 120 seconds. Mean clot formation time for pooled normal pig plasma was 35.2 ± 0.7 seconds. Intra-assay and inter-assay coefficients of variation were 5.1% and 6.5%, respectively.

Thromboelastography

TEG monitors changes in the viscoelastic properties of a forming clot. Blood is delivered into a sample cup and clotting initiated spontaneously or using various agonists. A pin is suspended in the blood by a torsion wire and monitored for motion as the pin is repeatedly rotated through the blood in a 10-second cycle. Increased blood viscosity increases resistance to pin rotation and deflects the pin. The deflection is translated into output measured in mm of amplitude, which increases as blood viscosity increases. Various standard parameters are calculated, including reaction time (R), coagulation time (K), α-angle, maximum amplitude (MA), and time to reach MA (tMA). R reflects the period of latency from the start time to initial clot formation. K reflects the time from R until a standardized level of clot firmness is reached (amplitude = 20 mm). The α-angle is a measure of the kinetics of clot development. MA is the maximum amplitude attained and reflects the maximum firmness of the clot. For further description see Chandler (16).

Two additional TEG parameters were calculated, maximum velocity (MaxVel), and time to MaxVel (tMaxVel). Calculations were made using a variation of the methods reported by Sorensen et al. (17). Amplitude data were recorded at 5 second intervals. Velocity of clot formation was calculated every five seconds based on the change in amplitude within a 30 second window spanning 15 seconds on either side of each time point. The highest velocity within a given TEG run was termed MaxVel and expressed in mm/min. The time at the occurrence of MaxVel was designated tMaxVel and expressed in minutes.

TEG was performed at each blood sampling time, except immediately following the 90 and 360 µg/kg doses. The assay was performed at 39°C using the model 5000 TEG (Haemoscope, Skokie, IL). Fifty µl of p-ThP diluted 1:250 with 0.9% saline or 100 µg/ml equine tendon collagen (COLL; Helena Laboratories, Beaumont, TX) were preloaded into each reaction cup. The final

dilution of p-ThP in the reaction mixture was 1:1750. The final collagen concentration in the reaction mixture was 14 µg/ml. TEG was performed using 50 µl of saline as a control to allow confirmation that the clotting observed in response to the two agonists was predominantly due to agonist activity, as opposed to contact with the cup wall. Unaltered whole blood (300 µl) was delivered to each cup within one minute of collection to initiate clotting. The procedure was terminated shortly after MA was attained.

TEG was performed in triplicate. The single value for each calculated TEG parameter was derived by averaging, with elimination of a single value when the CV exceeded 10%. Using p-ThP as agonist, the mean CV within replicates (intra-assay) were 4.9%, 5.5%, 5.2%, 4.4%, 1.9%, 3.1%, and 5.7% for MaxVel, tMaxVel, R, K, α -angle, MA, and tMA, respectively. Mean CV were similar for COLL.

The dilution of p-ThP used was selected to be dilute enough to optimize sensitivity to rFVIIa activity (17), while ensuring that the tissue factor pathway would be dominant over reactions initiated by contact with the cup wall (intrinsic pathway). A titration was performed to characterize the response to a series of p-ThP dilutions. Blood was collected via catheter from four additional anesthetized swine. Whole blood was delivered to TEG cups containing diluted p-ThP to yield final dilutions of 1:7, 1:175, 1:350, 1:700, 1:1750, and 1:3500. The dose-response relationship between p-ThP dilutions and clot formation velocity profiles was similar to that reported for tissue factor dilutions in human blood (17). The dilution used in the present study gave satisfactory sensitivity to rFVIIa.

Platelet aggregometry (PA)

PA was performed in triplicate using a PACKS-4 optical platelet aggregometer (Helena Laboratories, Beaumont, TX). Standard adenosine diphosphate (ADP; Helena Laboratories, Beaumont, TX) and COLL reagent solutions containing 50 mM CaCl₂ were used. Stock preparations of these agonists were used undiluted and diluted 1:5, 1:10, and 1:20 with PBS. This yielded concen-

trations of 20, 4, 2, and 1 µM for ADP and 10, 2, 1, and 0.5 µg/ml for COLL, respectively, in the final platelet rich plasma reaction mixture. Citrated blood was centrifuged at 200 x g to yield platelet rich plasma and at 1000 x g to produce platelet poor plasma. All reactions were performed at 39°C using prewarmed reagents. Platelet poor plasma was used to represent 100% aggregation for each sample. To initiate aggregation, 50 µl agonist solution was added to 450 µl stirring platelet rich plasma.

Pathology

Samples from kidney, lung, mesentery, heart, and skeletal muscle were collected within 10 minutes *post-mortem* and fixed in formalin. All samples were embedded in paraffin, sectioned, and stained using hematoxylin and eosin, Masson's Trichrome, and phosphotungsten acid hematoxylin. Tissues were examined under light microscopy by a board-certified veterinary pathologist who was blinded to treatment group.

Statistical analysis

Continuous data were analyzed by analysis of variance using the General Linear Models (GLM) procedure of SAS (18). Where parameters were monitored over time, such as MAP, TAT, and others, effects of rFVIIa and injury were examined accounting for the repeated nature of the measurements. When examining within group changes over time, a new variable was generated by subtracting the time 0 value from each subsequent value within each animal. These variables were used in statistical analysis but not listed in results. All appropriate data are expressed as mean \pm SEM. Discrete data, such as survival, were analyzed by Fisher's Exact Test using the FREQ procedure of SAS.

Results

MAP was not affected by treatment during the dose escalation phase (Fig. 1). Ear capillary BT was not affected by treatment at any dose and averaged 139 \pm 6 seconds across times and treatment groups.

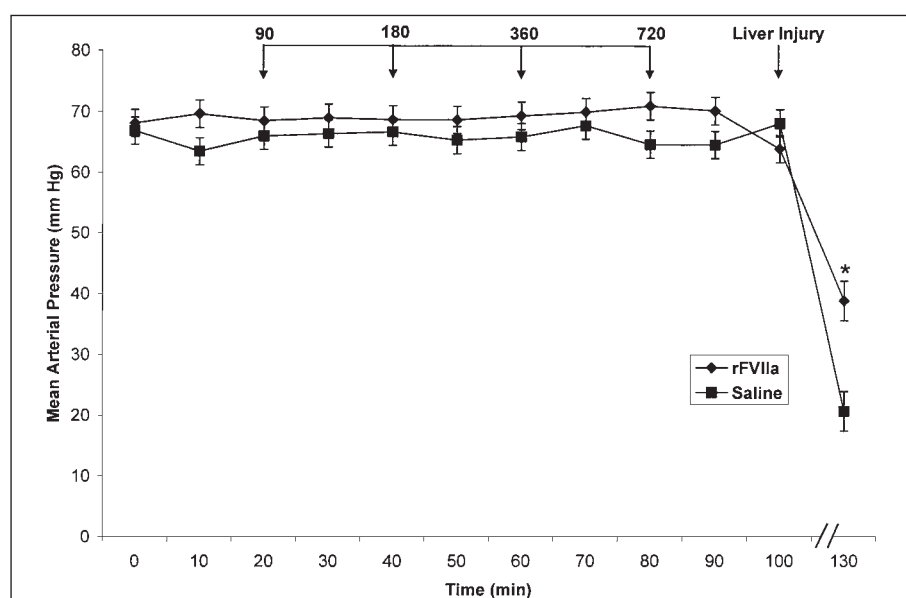


Figure 1: Mean arterial pressure in response to increasing doses of rFVIIa (0, 90, 180, 360 and 720 µg/kg) followed by severe liver injury. *Significant ($p < .05$) difference between treatment groups.

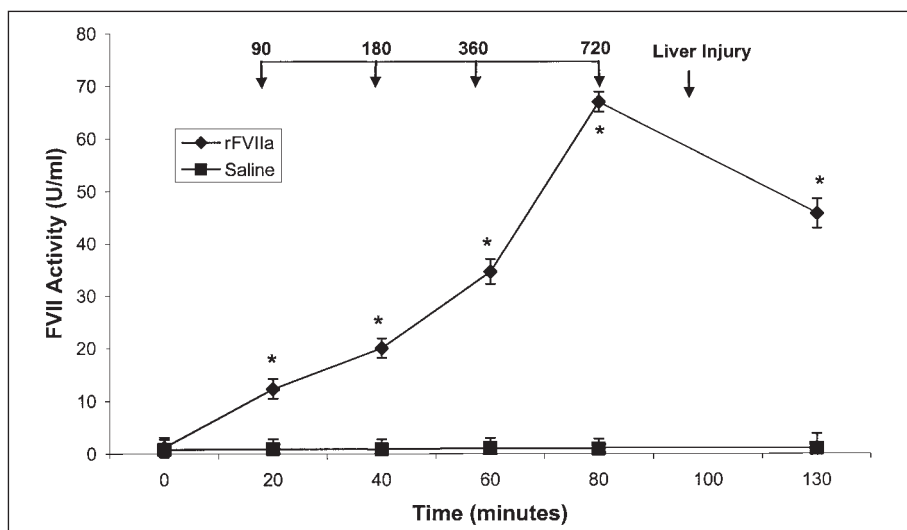


Figure 2: Plasma FVII levels following rFVIIa treatment (0, 90, 180, 360 and 720 µg/kg) and 30 minutes post-liver injury. *Significant (p<.01) difference between treatment groups.

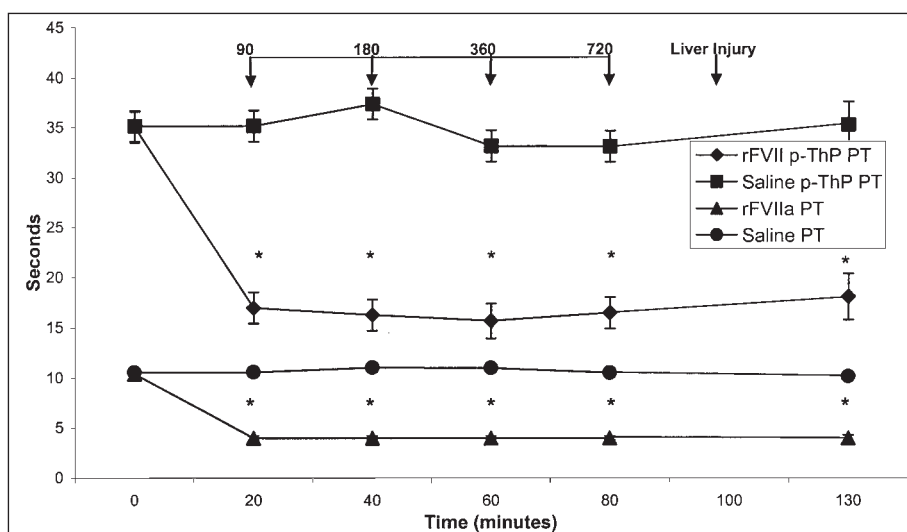


Figure 3: Standard and species-specific PT following rFVIIa treatment (0, 90, 180, 360 and 720 µg/kg) and 30 minutes post-liver injury. *Significant (p<.01) difference between treatment groups.

A progressive increase in FVII activity was observed with each increasing dose of rFVIIa, followed by a decline at 30 minutes post-injury (p<.01; Fig. 2). There was no effect of either rFVIIa or injury on Hct, PLT or fibrinogen concentration. Overall mean Hct, fibrinogen, and PLT were 25.6 ± 0.9%, 172 ± 2 mg/dL, and 384 ± 11 × 10³/µl, respectively. The coagulation times obtained from both the standard and pig-specific PT assays decreased significantly in the rFVIIa group beginning at the 90 µg/kg dose and remained shorter at each time point thereafter (p<.01), with no additional change associated with the injury phase (Fig. 3). Throughout the study period, aPTT was lower in the rFVIIa than in the saline group, with baseline means of 12.2 ± 0.2 and 14.6 ± 0.3 seconds, respectively (p<.01), and no subsequent changes in these baseline values in either group throughout the study. There were no differences in TAT concentration between groups at any time studied (Fig. 4). When examined on the basis of within group change from baseline, TAT was increased by both rFVIIa administration and by injury (Fig. 4).

TEG results for p-ThP and COLL are depicted in tables 1 and 2, respectively. Initial clot formation time (R) was consistently

shortened by rFVIIa, regardless of agonist. When p-ThP was used, clot development progressed more rapidly after rFVIIa treatment, as reflected by increased MaxVel and α-angle and shortened tMaxVel and tMA (Table 1). Using COLL, tMaxVel was decreased and both α-angle and MA were increased by rFVIIa treatment (Table 2). At 30 minutes post-injury, MaxVel increased (p<.05) in the rFVIIa group when COLL was the agonist. Using p-ThP, MaxVel for both groups decreased following injury, such that the rFVIIa group mean was no longer greater than baseline and the saline group mean declined below its respective within group baseline (p<.05). Also following injury, tMaxVel and R were increased (p<.01) compared to baseline within the saline group. In the rFVIIa group, tMaxVel remained statistically shorter than baseline, while R increased such that it was no longer statistically shorter than baseline (Table 1).

There was no effect of rFVIIa or injury on platelet aggregation in response to any concentration of agonist. Percent platelet aggregation decreased with each decreasing agonist concentration. Across treatments and times, aggregation in response to ADP was 43 ± 2, 19 ± 2, 9 ± 2, and 4 ± 1% for concentrations

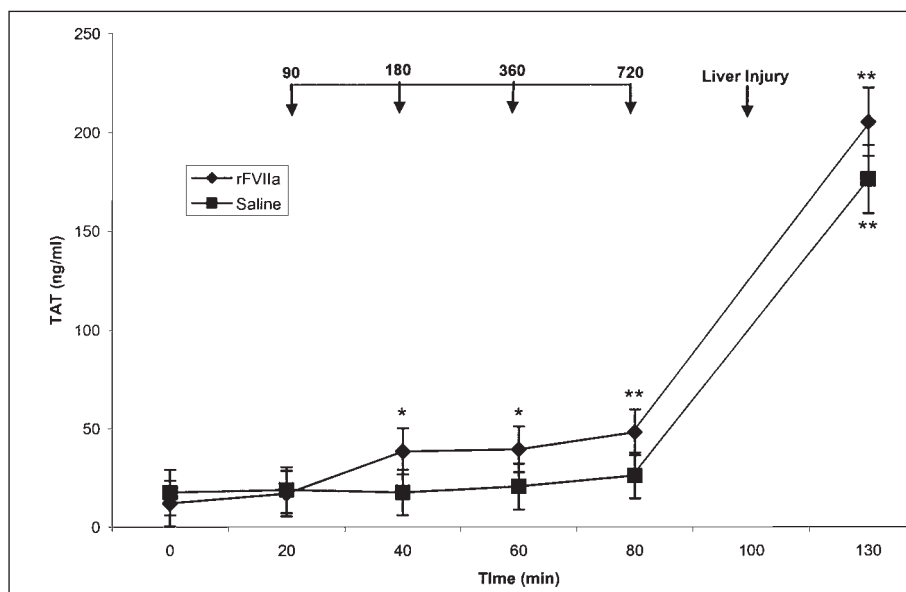


Figure 4: Thrombin-antithrombin complex (TAT) following rFVIIa treatment (0, 90, 180, 360 and 720 µg/kg) and 30 minutes post-liver injury. *Significantly different from baseline within group (p<.05).**Significantly different from baseline within group (p<.01).

of 20, 4, 2, and 1 µM ADP, respectively. Aggregation was 77 ± 1, 54 ± 2, 20 ± 2 and 9 ± 3% in response to 10, 2, 1 and 0.5 µg/ml COLL. ACT remained stable in the saline group but declined significantly in the rFVIIa group (Table 3).

During the injury phase, blood loss was similar between groups, with mean blood losses of 812 ± 71 ml and 893 ± 71 ml for the rFVIIa and saline groups, respectively. At 30 minutes post-injury, MAP declined from 64 ± 2 mm Hg to 39 ± 3 mm Hg

Table 1: Thromboelastograph parameters using pig brain thromboplastin.

Parameter	Treatment	Baseline (0 µg/kg)	180 µg/kg	720 µg/kg	30 minutes post-injury
MaxVel (mm/min)	rFVIIa	29.7 ± 0.8	32.2 ± 0.8*	33.9 ± 0.8*	28.9 ± 1.4
	Saline	29.8 ± 0.8	30.3 ± 0.8	29.6 ± 0.8	26.5 ± 1.2*
	Between group p value	NS	NS	<.01	NS
tMaxVel (min)	rFVIIa	4.28 ± 0.13	3.86 ± 0.13*	3.51 ± 0.13*	3.74 ± 0.23*
	Saline	4.01 ± 0.12	4.21 ± 0.12	4.26 ± 0.12	4.92 ± 0.18*
	Between group p value	NS	NS	<.01	<.01
R (min)	rFVIIa	3.41 ± 0.09	2.68 ± 0.09**	2.68 ± 0.09**	2.99 ± 0.19
	Saline	3.14 ± 0.08	3.26 ± 0.08	3.24 ± 0.08	3.78 ± 0.16*
	Between group p value	<.05	<.01	<.01	<.01
K (min)	rFVIIa	0.92 ± 0.03	0.86 ± 0.03	0.87 ± 0.03	0.89 ± 0.06
	Saline	0.84 ± 0.03	0.87 ± 0.03	0.85 ± 0.03	1.03 ± 0.04*
	Between group p value	NS	NS	NS	<.05
α-angle (degrees)	rFVIIa	78.9 ± 0.5	80.6 ± 0.5	80.1 ± 0.5*	79.7 ± 0.9
	Saline	79.1 ± 0.5	79.4 ± 0.5	79.6 ± 0.5	77.3 ± 0.7
	Between group p value	NS	NS	NS	<.05
MA (mm)	rFVIIa	74.2 ± 0.8	74.5 ± 0.8	73.5 ± 0.8	71.7 ± 1.3
	Saline	73.4 ± 0.7	74.7 ± 0.7	74.3 ± 0.7	72.7 ± 1.1
	Between group p value	NS	NS	NS	NS
tMA (min)	rFVIIa	17.34 ± 0.41	15.18 ± 0.41*	14.97 ± 0.41*	15.95 ± 0.75
	Saline	16.21 ± 0.37	16.06 ± 0.37	15.47 ± 0.37	17.64 ± 0.60
	Between group p value	NS	NS	NS	NS

*Significantly different from baseline within treatment group (p<.05)
 **Significantly different from baseline within treatment group (p<.01)

Table 2: Standard thromboelastograph parameters using collagen.

Parameter	Treatment	Baseline (0 µg/kg)	180 µg/kg	720 µg/kg	30 minutes post-injury
MaxVel (mm/min)	rFVIIa	11.1 ± 0.6	11.6 ± 0.5	12.1 ± 0.5	14.2 ± 0.8*
	Saline	10.8 ± 0.5	10.2 ± 0.5	10.3 ± 0.5	11.0 ± 0.8
	Between group p value	NS	NS	<.05	<.01
tMaxVel (min)	rFVIIa	7.06 ± 0.22	5.56 ± 0.20**	5.09 ± 0.20**	5.24 ± 0.30**
	Saline	6.96 ± 0.20	7.11 ± 0.20	6.89 ± 0.20	7.47 ± 0.30
	Between group p value	NS	<.01	<.01	<.01
R (min)	rFVIIa	5.28 ± 0.19	3.86 ± 0.17**	3.48 ± 0.17**	3.97 ± 0.27**
	Saline	5.06 ± 0.17	5.24 ± 0.17	5.23 ± 0.17	5.24 ± 0.27
	Between group p value	NS	<.01	<.01	<.01
K (min)	rFVIIa	2.18 ± 0.10	2.08 ± 0.09	2.03 ± 0.09	1.78 ± 0.16
	Saline	2.41 ± 0.09	2.52 ± 0.09	2.35 ± 0.09	2.38 ± 0.16
	Between group p value	NS	NS	NS	NS
α-angle (degrees)	rFVIIa	61.7 ± 1.4	60.7 ± 1.2	63.2 ± 1.2	67.0 ± 1.9
	Saline	59.4 ± 1.2	56.0 ± 1.2	57.5 ± 1.2	57.4 ± 1.9
	Between group p value	NS	<.05	<.01	<.01
MA (mm)	rFVIIa	62.1 ± 0.8	64.5 ± 0.7	64.9 ± 0.7*	65.9 ± 1.1
	Saline	62.4 ± 0.7	62.2 ± 0.7	62.8 ± 0.7	62.4 ± 1.1
	Between group p value	NS	NS	NS	NS
tMA (min)	rFVIIa	25.74 ± 0.66	25.79 ± 0.58	24.14 ± 0.58	21.74 ± 1.17
	Saline	26.88 ± 0.58	27.99 ± 0.58	27.79 ± 0.58	26.45 ± 1.16
	Between group p value	NS	NS	NS	NS

*Significantly different from baseline within treatment group (p<.05)
**Significantly different from baseline within treatment group (p<.01)

in the surviving rFVIIa animals and from 68 ± 2 mm Hg to 21 ± 3 mm Hg in the surviving saline animals, and was higher (p<.05) in the rFVIIa group (Fig. 1). Post-injury survival time was similar for the two groups; animals in the rFVIIa and saline groups survived 33.5 ± 8.8 and 28.5 ± 8.8 minutes after injury. Survival to 30 minutes was 50% in each group; by 60 minutes post-injury, 33% of animals survived in the rFVIIa group, compared with 0% in the saline group. No evidence of intravascular coagulation was noted during histopathological examination of collected tissues.

Discussion

FVII activity was elevated in a dose-dependent manner in the treated animals, consistent with previous reports in humans

(19–21) and swine (5, 8). The decline in concentration after the final dose was approximately twice that expected based on the half-life of rFVIIa in humans (147 min; 13). This may reflect a difference in rFVIIa metabolism between humans and swine, or may be related to the period of haemorrhagic shock that followed the final rFVIIa dose.

Administration of rFVIIa consistently shortens PT in normal (21), coagulopathic (2, 19, 22, 23), and anticoagulated (21) humans, as well as in normal (6–8) and coagulopathic pigs (4, 5). The standard reagent used to initiate coagulation in the PT is rabbit brain thromboplastin (24). A confounding factor in animal research with rFVIIa is that the tissue factor-FVII interaction is species-specific (11, 12). Because rabbit thromboplastin efficiently induces coagulation in human plasma (11), species-spe-

Table 3: Activated clotting times.

Treatment	Activated Clotting Time (seconds)					
	Baseline (0 µg/kg)	90 µg/kg	180 µg/kg	360 µg/kg	720 µg/kg	30 minutes post-injury
rFVIIa	90.2 ± 3.1	80.2 ± 3.1*	76.9 ± 3.1*	76.0 ± 3.1*	82.2 ± 3.1*	84.2 ± 3.1*
Saline	94.2 ± 3.1	98.3 ± 3.1	100.5 ± 3.1	97.8 ± 3.1	96.9 ± 3.1	98.7 ± 4.6

*Significantly (p<.05) different from baseline value within treatment

cificity is not an issue in human clinical practice and research with rFVIIa. In studies with swine, however, species-specificity must be considered. Both rabbit and human thromboplastins induce coagulation more efficiently in human plasma than in porcine plasma. Additionally, porcine thromboplastin induces coagulation more efficiently in porcine plasma than in human plasma (11, 12). When rFVIIa is administered to swine, a mixture of native porcine FVIIa and human rFVIIa exists; both of these must react with pig tissue factor *in vivo*. In contrast, both FVIIa's must interact with rabbit tissue factor in a standard PT *in vitro*. It is therefore conceivable that a dose of rFVIIa that is not active in the pig *in vivo* could yield a "false positive" *in vitro* when using a standard PT with rabbit-derived reagents. To minimize this potential problem, we used a modified PT that incorporated p-ThP. Beginning with the 90 µg/kg dose, rFVIIa maximally shortened pig-specific PT, suggesting that rFVIIa at 90 µg/kg in pigs maximally enhances the tissue factor pathway to the extent detectable in a PT. In humans, 5 µg/kg rFVIIa submaximally shortened PT while a 10 µg/kg dose maximally shortened PT (20). The dose required to submaximally shorten species-specific PT in swine cannot be determined from the present data but that dose appears to be less than 90 µg/kg.

In both the aPTT and ACT, clotting is initiated by a negatively charged reagent through the intrinsic (factor XII) pathway. Shortened aPTT after administration of rFVIIa has been reported in humans (2, 19, 23) but not swine (4). Although aPTT was not altered by rFVIIa administration in this study, ACT was shortened after rFVIIa treatment, with the effect first observed with the 90 µg/kg dose. This effect may have been due to direct activation of factor X by rFVIIa (25) but this is not probable since aPTT was not altered. Alternatively, ACT may have been shortened by an effect of rFVIIa on platelets (9). Tissue factor-independent binding of rFVIIa is believed to enhance the capacity of platelets to generate thrombin, leading to enhanced platelet recruitment and clot formation. We therefore examined the platelet aggregation response to maximal and submaximal doses of ADP and COLL, hypothesizing that rFVIIa may enhance platelet aggregation in response to submaximal agonist doses. We observed no effect of rFVIIa on platelet aggregation, however, suggesting either that platelet function was not enhanced or that the methods used were not able to detect alterations in function.

In standard TEG, the clotting reaction is initiated via the intrinsic system (16). In recent years, however, it has become apparent that the tissue factor pathway (extrinsic system) plays the major role in haemostasis (24). Thus, we used the physiological agonists p-ThP and COLL to initiate clotting through the swine tissue factor pathway and through a cellular mechanism, respectively. Clear effects of rFVIIa were observed with TEG. In blood from rFVIIa-treated pigs, initial clot formation was hastened, regardless of agonist. In addition, clot development proceeded more rapidly in clots initiated by p-ThP and maximum clot firmness was increased in clots initiated by COLL. In patients undergoing liver transplantation, rFVIIa shortened R and K, and increased α -angle, indicating more rapid clot initiation and development (23). In a previous study in swine, TEG parameters were not affected by rFVIIa (4). In that study, however, animals were haemodiluted and hypothermic, which may have influenced TEG results. The present data confirm in swine the findings of

Sorensen et al. (17) that identified MaxVel and tMaxVel as sensitive parameters for detecting rFVIIa-induced changes in human blood. Results also indicate that sensitivity of MaxVel to rFVIIa may differ with different agonists.

Despite evidence from laboratory tests suggesting enhancement of the coagulation system, rFVIIa did not alter BT. BT is commonly used to monitor changes in haemostasis associated with platelet disorders (26). The reason for the lack of effect of rFVIIa on BT may have been that rFVIIa did not affect platelet adhesion under the noncoagulopathic conditions of the pig model used. This possibility is supported by the absence of an observable effect of rFVIIa on platelet aggregation.

In two studies using swine with induced coagulopathies, treatment with rFVIIa reduced blood loss when used as an adjunct to packing after severe liver injury (4, 5). In normal swine, rFVIIa did not reduce blood loss after severe liver injury in two studies (7, 8), but did reduce blood loss in another (6). In a study involving aortic injury, rFVIIa did not reduce blood loss but increased rebleeding pressure after resuscitation (27). In the present study, blood loss was not reduced. Differing results among studies may be explained by the differing uses of rFVIIa (adjunct to packing versus alone), to different types of haemorrhage (arterial versus venous), or to differences in coagulation status. In two of the three studies in which blood loss was reduced by rFVIIa, the pigs were coagulopathic (4, 5). In studies in which blood loss was unchanged, coagulation status was normal (7, 8, 27). Reports of clinical efficacy of rFVIIa in patients with normal coagulation are rare (21), while reports of efficacy in patients with abnormal coagulation have been numerous (1). It is possible that the potential for haemostatic improvement with rFVIIa is greater in the presence of a coagulopathy.

MAP was not affected by rFVIIa prior to the injury phase of the experiment. At 30 minutes post-injury, however, MAP in the surviving animals in the rFVIIa group was nearly double that of the saline group. Similar findings have been reported by some (7) but not others (8). It is possible that this increased ability to maintain MAP following rFVIIa reflects an increased ability of formed clots to maintain in the face of increasing blood pressure following auto- or fluid resuscitation, as recently reported in an aortic injury model (27). In spite of the apparently beneficial effect on MAP, survival was not affected by rFVIIa in the present study.

The primary safety concern pertaining to the use of rFVIIa is the possibility for intravascular coagulation. Although this drug has been safe to date, it remains important to specifically consider this question as the potential uses for the drug expand (2). In the present study, TAT, an indicator of thrombin generation, was elevated after rFVIIa treatment in the rFVIIa group and was elevated in both treatment groups after 30 minutes of hypotension following severe hepatic injury. Schreiber et al. (8) also reported elevated TAT after rFVIIa administration in swine but concluded that intravascular coagulation was not present because PLT and fibrinogen concentrations were stable and there was no evidence of *premortem* thrombus formation. In the present study, neither PLT nor fibrinogen concentration were affected either by rFVIIa or liver injury. TEG results were equivocal. Following haemorrhage, MaxVel in response to p-ThP declined in both groups but remained numerically higher in the

rFVIIa group. Both R and tMaxVel were prolonged in the saline group but not the rFVIIa group. These changes may reflect the early stages of a potentially coagulopathic state resulting from haemorrhage. The p-ThP TEG data suggest that rFVIIa did not exacerbate the situation and may have been somewhat protective in this regard. Histologic examination revealed no evidence of intravascular coagulation in either group. Taken together, these data indicate that rFVIIa treatment was associated with a slight systemic activation of thrombin. Systemic activation occurred to a greater degree in both groups as a result of severe haemorrhage and shock but was still without apparent consumption of fibrinogen and platelets in either treatment group.

We have demonstrated that *in vivo* administration of as little as 90 µg/kg rFVIIa in swine results in changes in coagulation that are detectable *in vitro*, using physiologically relevant agonists in a variety of assays. More rapid clot formation after rFVIIa administration was detected in both whole blood and plasma. Despite clear evidence of rFVIIa activity in the porcine system, *in vivo* haemostasis was not measurably altered by rFVIIa. These results suggest that rFVIIa may not improve upon the normal

haemostatic mechanism to produce more rapid haemostasis in the absence of coagulopathy. Although there was a mild systemic activation of thrombin, there was no evidence of systemic intravascular coagulation associated with rFVIIa. Human rFVIIa possesses sufficient activity in the porcine system to allow meaningful interpretation of results. The use of species-specific *in vitro* assays to monitor rFVIIa therapy adds to that interpretation.

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Blood Coagulation, Fibrinolysis and Cellular Haemostasis

Gender differences in the administration of prophylaxis to prevent deep venous thrombosis

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Summary

We investigated gender differences in the prescription of prophylaxis against deep vein thrombosis (DVT) in 2,619 patients who developed acute DVT while being hospitalized for reasons other than DVT or were diagnosed with acute DVT as outpatients but who had been hospitalized within 30 days prior to

DVT diagnosis. Men were 21% more likely than women to receive prophylaxis (OR 1.21, 95% CI 1.03–1.43; $p = 0.021$) after adjusting for DVT risk factors, including surgery, trauma, prior DVT, age, and cancer.

Keywords

Prophylaxis, deep vein thrombosis, gender

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