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Effect of Recombinant FVIIA in Hypothermic, Coagulopathic Pigs with Liver Injuries

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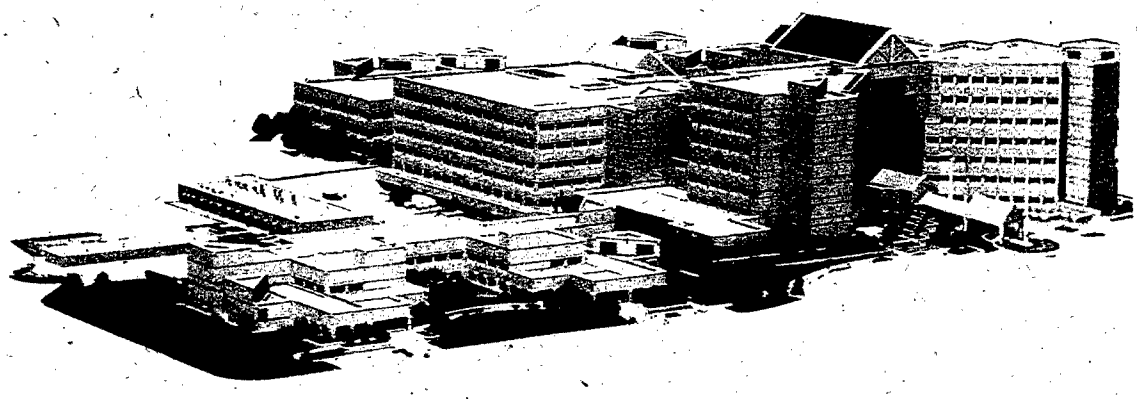

UNITED STATES ARMY INSTITUTE OF SURGICAL RESEARCH FORT SAM HOUSTON TEXAS

Effect of recombinant FVIIa in Hypothermic, Coagulopathic Pigs with Liver Injuries

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14. ABSTRACT

A study was conducted to evaluate effects of the drug, rcombinant activated Factor VII (rFVIIa) on survival, survival time, blood loss, and disseminated intravascular coagulation (DIC) in hypothermic, coagulopathic swine subsequent to severe liver injury and associated hemorrhage. Swine were anesthetized, and catheters were placed in the carotid artery, in the jugular, and in the femoral artery. After midline laparotomy, splenectomy and stabilization of blood pressure, pH, and body temperature, approximately 60% of the blood was removed and replaced with 6% Hetastarch (Hespan) maintained at 33 degrees C. Hemodilution was followed by adjustment of body temperature to 32.5 +/- .5 degrees C. Subsequently, a severe liver injury was produced using a specially designed clamp with associated cross-blades. After 30 sec, either vehicle control or treatments were administered intravenously during a 30 second period. Resuscitation with 33 degrees C Lactated Ringers was initiated at 4.5 min post injury.In this pig model, these data indicate an inability of these doses of rFVIIa to reduce blood loss, increase percent survival, or increase survival time.

15. SUBJECT TERMS
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Technical Report

Title of the Study

Effect of recombinant FVIIa in hypothermic, coagulopathic, anesthetized pigs with liver injuries.

Investigators

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Short Summary

A study was conducted to evaluate effects of the drug, recombinant activated Factor VII (rFVIIa) on survival, survival time, blood loss, and disseminated intravascular coagulation (DIC) in hypothermic, coagulopathic swine subsequent to a severe liver injury and associated hemorrhage. Swine (36.6 ± 0.3 kg body weight) were anesthetized, and catheters were placed in the carotid artery (for monitoring of blood pressure, pH, gases, and body temperature), in the jugular vein (for resuscitation and treatment administration), and in the femoral artery (for blood withdrawal). After midline laparotomy, splenectomy and stabilization of blood pressure, pH, and body temperature, ~ 60% of the blood was removed (calculations that include ongoing blood dilution indicate 49% of original blood volume) and replaced with 6% Hetastarch (Hespan) maintained at 33 C^{o}. Hemodilution was followed by adjustment of body temperature to 32.5 ± 0.5 C^{o}. Subsequently, a severe liver injury was produced using a specially designed clamp with associated cross-blades. After 30 seconds, either vehicle control (60 ml 0.9% sterile saline) or treatments (180 µg/kg BW or 720 µg/kg BW rFVIIa in 60 ml sterile saline) were administered intravenously during a 30 second period. Concomitantly, laparotomy pads were used to pack around the liver to reduce blood loss. Resuscitation with 33 C^{o} Lactated Ringers (260 ml/min) was initiated at 4.5 min postinjury. Animals were continuously monitored for 4 hr postinjury or until death. Liver injuries were comparable among all treatment and control groups ($P = 0.89$). Plasma Factor VIIa % activity increased 57 to 110-fold ($P < 0.0001$) within 10 min of treatment initiation, and declined thereafter. Some factors associated with *in vitro* coagulation (prothrombin time, activated partial thromboplastin time, thromboelastographic split-point time, and thromboelastographic R-time) were enhanced (i.e.; time periods were reduced) by rFVIIa administration ($P< 0.05$). However, neither percent survival ($P = 0.82$), survival time ($P = 0.56$), nor blood loss ($P = 0.73$) were affected by treatment. Disseminated intravascular coagulation (DIC) was not evident in lung or kidney tissue. In this pig model, these data indicate an inability of these doses of rFVIIa to reduce blood loss, increase percent survival, or increase survival time. There was no evidence of DIC, thereby providing evidence for safety of these doses of rFVIIA under conditions of this study.

Introduction

Hemorrhage is the primary cause of death in civilian trauma patients (Sauaia et al., 1995) and it is the principle cause of death on the battlefield (Bellamy,1984). Factor VIIa has been used successfully clinically for treatment of hemophilia and other bleeding disorders (Hedner and Ingerslev,1998). More recently, evidence is accruing for its ability to reduce/stop bleeding in trauma victims (Kenet et.al.,1999; Martinowitz et al., 2001; Kamphuisen et al.,2002; O'Neill et al., 2002; Dutton et al., 2003), and during a variety of surgical procedures (Danilos,2003; Friederich PW et al.,2003; Karadimov et al., 2003; Naik et al., 2003). In efforts to evaluate more critically the potential use of rFVIIA to reduce trauma-associated hemorrhage, laboratory studies using pigs have been conducted. Such studies generally can be divided into two categories dependent on the pig model used: 1) normothermic pigs with normal coagulation abilities, and 2) and hypothermic pigs with abnormal coagulation due to the hypothermia and concomitant blood dilution. Of the four studies involving normothermic pigs (Lynn et al.,2002; Jeroukhimov et al.,2002; Schreiber et al.,2003; Pusateri et al., submitted, 2003), only Jeroukhimov and colleagues demonstrated an increased survival time commensurate with a reduced blood loss at 1 hr after crushing and avulsion of part of a liver lobe. Two studies conducted using hypothermic, coagulopathic pigs (Martinowitz et al., 2001; Schreiber et al.,2002) used different preinjury blood dilution solutions (6% Hetastarch *vs* 5% human albumin solution respectively), different resuscitation rates (250 ml /min *vs* 100 ml/min respectively), and were conducted for different time periods (1 hr *vs* 2 hr respectively). Both studies demonstrated significant FVIIa-associated reductions in blood loss, and one also demonstrated numerical (80% *vs.* 60%) but not significant increases in survival at two hours post liver injury (Schreiber et al., 2002). For these latter two studies, there was some concern about small sample sizes, and the brevity of observation periods postinjury. Hence, as the coagulopathic, hypothermic pig model with a severe liver injury appeared to provide the best model for evaluation of rFVIIa, it was decided to conduct the current study with larger sample sizes, and to extend the observation period to 4 hours postinjury. The primary objective of this study was to determine in hypothermic, coagulopathic, anesthetized pigs subsequent to severe liver injury effects of rFVIIa on percent survival and survival time. Secondary objectives were to determine effects of rFVIIa on: 1) posttreatment blood loss; 2) coagulation function; and 3) degree of disseminated intravascular coagulation (DIC). Our hypothesis was that there would be a dose-dependent effect of rFVIIa to improve survival time and percent survival, and to decrease blood loss. Such effects would be mediated through an enhancement of coagulation function without causing DIC.

Materials and Methods

Recombinant Coagulation Factor VIIa (rFVIIa, lot number 25813) was supplied to the Institute of Surgical Research (ISR) by Novo Nordisk Pharmaceuticals, Inc (Denmark) in vials containing 1.2 mg (60 KIU) per vial. This drug was shipped in a frozen state (on dry ice) via Federal Express, and arrived frozen with dry ice still present in shipping containers. All vials were examined upon arrival, and stored in a refrigerator maintained

at 2-4 C°. Approximately 2 hours prior to use (average time 102 min), the contents of these vials were solubilized in 2.2 ml of sterile water. Contents of multiple vials were then combined to provide the two doses used in the study: 180 μg/kg of body weight, and 720 μg/kg of body weight. All volumes then were brought to a total volume of 60ml using sterile 0.9% saline.

Recombinant Factor VIIa prepared as detailed above, or sterile saline that served as vehicle control, were placed into sterile 60 ml syringes, which then were placed on an infusion pump (Harvard Apparatus, PHD 2000, Holliston, MA). Thirty seconds after the liver injury, 60 ml of rFVIIa or saline were administered intravenously in the jugular vein during a 30 second time period using this infusion pump. As this was a "blinded study", a randomization sheet was generated by one of the study directors, with treatments designated as A, B, and C. Study directors were not aware of the identity of treatments A, B, and C. The randomization sheet was given to the person in the laboratory who prepared the study drug (or vehicle) for administration to experimental animals. On each day of the study, a syringe labeled only with the animal number was delivered to the animal operating room. Study directors and other personnel performing animal procedures and data collection were not aware of the identity of the treatment groups until the code was broken at the end of the study.

Crossbred pigs (Musclor x Duroc) 32.2-40.3 kg were obtained from HDS SwineFarm (Boerne, Texas). Both female (gilts) and castrated males (barrows) were used and evenly distributed among treatments. Animals were housed in caging of appropriate size in a room with appropriate temperature and humidity. Standard Operating Procedure (SOP) A-5 entitled *Procedures for Receiving of* Laboratory Animals, SOP P-1 entitled Sanitation of the Animal House, SOP P-9 entitled Quarantine and Conditioning of Animals,and SOP P-10, entitled *Animal Bedding Changes* were fulfilled by animal caretaker personnel, under the supervision of the facility manager and attending veterinarian. SOP A-7 entitled *Maintenance of Animal Records* and SOP P -3 entitled *Daily Walk-Thrus* address attending veterinary care and were conducted by animal technicians under the supervision of the facility manager and the attending veterinarian. Pigs were fed ~1.3 kg daily of Agri Pride Pork grower (Country Acres Feed Company, Inc., Brentwood, MO) and had water available constantly. Pigs were assigned randomly to treatment groups. An acclimation period of seven days in the ISR animal facility was conducted prior to use of animals in the study. Animals underwent routine veterinary health screening in accordance with USAISR Vivarium Standard Operating Procedures. Additional screening included a blood sample for a standard prothrombin time (PT) test and a complete blood count (CBC) used for determination of platelet concentration during the acclimation period. For inclusion in the study, animals had to be in good general health (as determined by the veterinary staff) must not have received any experimental treatments for another study, and must have met the following hematological criteria: platelet count $\geq$ 200,000/μl and a standard PT $\leq$ 14.0 seconds. One pig was excluded due to a low platelet count.

Pigs were fasted for ~36 hours prior to surgery, but water was available continuously. Pigs were sedated using a combination of glycopyrrolate, tiletamine HCl, and zolazepam, and intubated. Anesthesia was maintained with isoflurane in air, utilizing a closed circuit system. A Paratrend7+ Multiparameter Sensor-catheter (Diametrics Medical Inc., Roseville, Minnesota) was implanted surgically into the left carotid artery occlusively, and attached to a Trendcare TCM 7000 Blood Gas monitoring system (Diametrics Medical Inc., Roseville, Minnesota) to allow continuous monitoring of body temperature, blood pH, paO_2, and $PaCO_2$. A port in the Paratrend7+ catheter was coupled to a transducer and attached to a Transpac IV Trifurcated Monitoring Kit (Abbott Laboratories, North Chicago, Illinois). An Ohmeda 5250 Respiratory Gas Monitor (Ohmeda Inc., Madison, WI) was used to measure respiratory gases, airway pressure, and respiratory rate. All instruments were connected to a Model 88 Viridia Continuous Monitoring System (Hewlett Packard, Palo Alto, CA), to integrate data. Blood pressure and blood gas data was recorded in real time at 5 sec intervals from the Viridia Central Monitoring System by a Dynamic Research Evaluation Workstation (D.R.E.W.) (US Army Institute of Surgical Research, Fort Sam Houston, TX). An infusion catheter was placed surgically into the left external jugular vein, and a 7.0 Fr. shortened cordice was implanted occlusively into one femoral artery. Next, a laparotomy was performed, the liver was inspected for obvious anomalies, and a splenectomy and cystotomy were performed. Lactated Ringer's solution (39C°) was administered at three times the weight of the spleen to offset blood loss associated with spleen removal.

A stable plane of anesthesia was maintained and ventilatory and anesthetic parameters were adjusted to attain the following physiological targets: core temperature 38.5 C° to 39.5 C°; arterial blood pH 7.35 to 7.45; and mean arterial pressure (MAP) of at least 40 mm Hg. After a 15-minute stable baseline, "Normal Baseline" blood samples were collected. Following the "Normal Baseline" sample, pigs were wrapped completely with cooling blankets set at 4 C°. Towels soaked in ice water were placed around the throat and in the groin regions to assist in cooling. Blankets were removed, repositioned or adjusted as necessary to achieve and maintain body temperature of 32-33° C.

Preinjury blood volume was estimated using the equation: blood volume (mL/kg) = $161.4751(\text{body weight}^{-0.2197})$, as previously reported (Martinowitz et al., 2001). Hemodilution was performed in three steps of ~ 10 min each. The infusion and controlled hemorrhage volumes for each of three hemodilution steps were 20% of calculated original blood volume. Hetastarch (Hespan) (6%; average molecular weight of 670,000, range 550,000-800,000; Abbott Laboratories, North Chicago, IL; 33°C) was infused (Easy Load II, Master Flex LS Pump, Cole-Parmer Instrument Co, Vernon Hills, IL) into the jugular catheter at the precalculated rate and time. Simultaneously, the predetermined blood volume was removed via the femoral artery by draining blood into a preweighed canister on a balance. If at any time during the hemodilution step MAP dropped more than 10%, blood removal was stopped temporarily while infusion continued. Two to three minutes of circulation time was allowed after the end of the Hetastarch infusion. Twenty minutes after the baseline blood sample, and then again at

30 minutes, the second and third hemodilution steps were performed using the same procedures.

A stable plane of anesthesia was maintained and ventilatory and anesthetic parameters were adjusted to attain a stable physiological status with the following requirements: body temperature 32.0 to 33.0 C° as measured in the arterial blood; arterial blood pH 7.35 to 7.45; and a stable MAP of 60 to 90 mm Hg. To satisfy preinjury stabilization requirements, these parameters had to be stable for 20 minutes prior to the "preinjury baseline" blood sample that then was collected. The average of the MAP readings for the final minute of the stable baseline period was designated the "resuscitation MAP target".

Liver injuries were induced as previously reported (Holcomb et al., 1999a, 1999b). Briefly, the liver was retracted by manually elevating the left and right medial lobes to allow adequate exposure. Next, a specially designed clamp with two 4.5 cm sharpened tines configured in the form of an 'X' were positioned with the center approximately 2-3 cm dorsal to the intersection of the left and right medial lobes, on the diaphragmatic surface of the liver. The base plate of the instrument was positioned beneath the quadrate lobe, on the visceral surface. The injury was induced by clamping the tines of the instrument through the parenchyma and underlying vessels of the two medial lobes so that the tines were seated in corresponding grooves in the base plate of the instrument. After the first penetration of the liver, the instrument was opened and the tines withdrawn and repositioned laterally, such that the second application overlapped the first by ~50 percent. Following this repositioning, the liver was penetrated a second time. The injuries appeared as large stellate wounds with a small island of tissue in the center, and measure approximately 10 x 8 x 4 cm. The injuries completely penetrated the liver, with one or more of the left medial lobar vein, right medial lobar vein, and portal hepatic veins lacerated.

During the first 30 seconds following injury, blood was collected by suction. This blood was designated as "pretreatment blood loss". Starting at 30 sec postinjury and conducted during a 30 sec time period, treatment infusion was initiated via a port in the jugular infusion catheter as described above under "In vivo administration". At 4 minutes 30 seconds postinjury, fluid resuscitation (lactated Ringer's solution at 33 C°) was initiated at 260 ml/minute using an infusion pump as described above for Hetastarch infusion. This resuscitation regimen was continued for 3 minutes--until 7.5 min postinjury--regardless of MAP.

The liver packing procedure also was initiated at 30 seconds postinjury. Gauze laparotomy pads were placed perihepatically as needed to attain effective packing. Effective packing was defined as that which resulted in the pig displaying a positive MAP response to resuscitation for 60 seconds. The packing procedure was completed within 7 minutes (i.e. by 7 minutes and 30 seconds postinjury). Whether packing was effective or not, packing attempts stopped at 7 minutes and 30 seconds postinjury, and further experimental procedures were continued. Packing was recorded as effective or not effective. Following packing, the abdominal wall was sutured closed. Beginning at 7

minutes and 30 seconds postinjury, resuscitation became dependent on MAP. The end point of resuscitation was the "resuscitation MAP target". If reached, resuscitation was stopped and was not resumed again until the MAP declined to a MAP that was 90% of the resuscitation MAP target; i.e., after a 10 % decline in MAP.

Animals were monitored until 240 minutes postinjury. At normal baseline, preinjury baseline, and at 10, 60, 120, 180, and 240 minutes postinjury, blood samples were collected (4.5 ml for thromboelastograph analysis; 10 ml for CBC and subsequent coagulation characteristics). Death prior to 240 minutes was defined as a heart rate of zero, confirmed by auscultation. At 240 minutes, surviving animals were euthanatized with an intravenous overdose of sodium pentobarbital (10 ml at 390 mg/ml). Immediately following death, liquid blood was collected from the peritoneal cavity by suction. Clotted and liquid blood, along with blood retained in the perihepatic packs (difference in weight of gauze packs before and after use) was combined to determine posttreatment blood loss.

The liver was excised and the injury characterized. Sections of right and left kidney, including full thickness cortex and medulla, as well as sections of right and left lower lung lobes were placed in 10% neutral buffered formalin within 10 minutes of death. Samples were sent via Federal Express to the Armed Forces Institute of Pathology where they were processed in paraffin within 48 hours of fixation by routine techniques. Processed tissue samples were sectioned on a standard microtome at 5 microns thickness and stained by routine histologic techniques (hematoxylin-eosin and Movat pentachrome) and immunohistochemical techniques (Streptavidin-biotin method). Monoclonal antibodies against fibrin II (Accurate Chemical Co., Westbury NY, 1:100) and factor VIII-related antigen (Strategic Biosolutions, Newark DE) were applied to detect intravascular deposition of fibrin and platelets, respectively. Cross-reactivity for these antibodies in porcine tissues has previously been confirmed (Ravanat et al., 1995). Stained sections were examined to determine the presence or absence of antemortem intravascular thrombi. The presence of any thrombi containing stainable fibrin admixed with platelets was considered evidence of intravascular coagulation, as these do not form postmortem or premortem in the absence of activation of the coagulation cascade. As the numbers of thrombi were small, the numbers of positive vessels were counted manually. The pathologist (Dr. Allen Burke) was "blinded" to treatment.

Hematocrit (Hct) and platelet (PLT) counts were performed as direct measurements using the ABX Pentra 120 hematology analyzer (ABX Diagnostics, Inc., Irvine, CA). 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 percent activity. 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. Average clotting time for the standard PT assay using pooled normal pig plasma was 9.56 ± 0.13 (mean $\pm$ SD) sec, with intra-assay (based on duplicate analyses) and inter-assay CVs of 0.32% and 1.36%,

respectively. Mean aPTT was 14.79 ± 0.62 seconds, with intra-assay and inter-assay CVs of 2.74% and 4.19%, respectively. Fibrinogen concentration for pooled normal pig plasma was 197.1 ± 4.97 mg/dL, and intra-assay and inter-assay CVs were 1.79% and 2.52%, respectively. Thrombin-antithrombin III complex (TAT) concentrations were quantitated using the Enzygnost TAT micro enzyme immunoassay (Dade Behring, Marburg, Germany), which previously has been demonstrated to cross-react with porcine TAT (Ravanat et al., 1995). Average concentrations in pig plasma were 21.89 ± 2.26 µg/L and intra-assay (based on triplicate analyses) inter-assay CVs were 9.55% and 10.33%, respectively.

For preparation of pig thromboplastin (p-ThP), brains were removed from slaughterhouse pigs and stored at 4°C in 0.9% saline. Brains were washed with 0.9% saline and all large blood vessels were removed. Brains were blended with 500 ml of phosphate-buffered saline (PBS) for five minutes at full speed in a commercial blender Waring (Torrington, CT). Homogenized tissue was placed into 50 ml conical vials and centrifuged for 15 minutes at 2100 x g. The resulting supernatant was retained as p-ThP, separated into 1 ml aliquots and frozen at –20C°.

Thromboelastography (TEG) was performed at each blood sample time. Split-point time (SP), reaction time (R), coagulation time (K), alpha-angle (ANG), and maximum amplitude (MA) (Kaufmann et al., 1997; Srinivasa et al., 2001) were determined using the model 5000 TEG (Haemoscope, Skokie, IL), using a modification of the fresh whole blood method. TEGs were set at 39C°. Fifty µl of either 0.9% saline, or p-ThP (diluted 1:250 with saline) was preloaded into each reaction cup. TEG was performed using 50 µl of saline as a control to allow confirmation that the clotting observed in response to the agonist 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. No other reagents were added. The procedure was terminated at 60 minutes.

Statistical Methods

Data were analyzed using the Statistical Analysis System (SAS, 1999) statistical package. For this study our null hypothesis is that there are no differences among the three groups (Control (µ1) *vs.* 180 µg/kg rFVIIa (µ2) *vs.* 720 µg/kg rFVIIa (µ3); H_0: µ1 = µ2 = µ3). The alternative hypotheses depend on the measure being evaluated. For survival (H_A: µ1 < µ2 < µ3) and blood loss (H_A: µ1 > µ2 > µ3) alternative hypotheses are opposite. Survival data were analyzed using PROC FREQ and associated Fisher's Exact test. Analysis of all survival time data--that contained pigs which were euthanized at the end of 4 hours and, therefore, their actual survival time is unknown (censored data)--was conducted using the PROC LIFETEST procedure of SAS with associated Log-Rank and Wilcoxon nonparametric tests. Blood loss, severity scores, and other single point measures were analyzed using 1-way ANOVA (PROC MIXED with pig's sex as a random variable). Analysis of covariance also was used to assess the appropriateness of various measures (e.g., pretreatment blood loss, blood vessel severity scores, preinjury MAP) to be used as covariates for adjusting other variables (e.g., survival time,

posttreatment blood loss). If the covariate was found not to be significant it was dropped from the SAS model. Measures taken in each pig at multiple time points were analyzed using 2-way repeated measures (PROC MIXED; with pig within treatment as a random variable). Means separation tests were conducted using robust, orthogonal contrasts and the conservative t-test with Bonferroni adjustment for multiple comparisons (Maxwell, 1980). All data were tested for homogeneity of variance (PROC ANOVA with associated Levene's test) and normality of distribution (PROC Univariate Normal with associated Kolmogorov-Smirnov test). Data were transformed where necessary to meet assumptions of ANOVA. In a few instances, transformations were incapable of providing data with a normal distribution for ANOVA. However, non-normality does not usually lead to misinterpretation of data (Sokal and Rohlf, 1969). To verify this with the pathology data, a nonparametric procedure was also used, PROC NPAR1WAY, Kruskal-Wallis Test. All data are presented as arithmetic means ± SEM (in some graphs error bars are very small and are not observed at the scale of the graph).

Results

In vivo and surgery-associated measures

Blood pH, body temperature, and mean arterial pressure (MAP) did not differ among treatments at either the normal, predilution base line, or at the preinjury baseline subsequent to hemodilution and induction of hypothermia (Table 1). Body weights, number of blood vessels cut, and pretreatment blood loss (30 sec blood loss after injury and before treatment) did not differ among treatment groups (Table 2). At necropsy, not only were livers inspected for the number of blood vessels cut, but also a severity score of 1 was assigned to a blood vessel with a cut < 5 mm and a severity score of 2 was assigned to a blood vessel with a cut of ≥ 5mm. These severity scores were then summed for the complete liver injury in each pig. Severity scores did not differ among treatments (Table 2). Subsequent to liver injury the number of gauze pads used for packing did not differ among treatment groups (Table 2). Postinjury blood loss averaged 4.4-fold greater than pretreatment blood loss, and did not differ among treatments (Table 2). Analysis of covariance did not reveal any relationship between this blood loss and the number of blood vessels cut ($P = 0.31$), the severity score ($P = 0.54$), or the posttreatment blood loss ($P = 0.97$). It was, however related to pretreatment MAP ($P = 0.02$), but when this measure was used in the overall statistical model, there was still no difference among treatment groups ($P = 0.61$). The volume of resuscitation fluid used was 14% less for pigs receiving 720 µg/kg rFVIIa than for pigs receiving 180 µg/kg rFVIIa ($P = 0.049$) (Table 2). Analysis of covariance did not reveal any relationship between resuscitation fluid volume and number of blood vessels cut ($P = 0.49$), severity score ($P = 0.28$), preinjury MAP ($P = 0.39$), or pretreatment blood loss ($P = 0.49$). After injury and treatment, there were no differences in survival time (Table 2; Fig 1), or in percent survival at 1, 2, 3, or 4 hours postinjury (Table 3).

There were no significant effects of treatment on mean arterial pressure (MAP) (Fig 2, $P = 0.30$; for number of pigs at each time interval see Table 4). During the 16 min just prior to liver injury, MAP was constant and across all treatment groups averaged 71.2 ± 1.24

mm Hg (Fig 2). At the time intervals tabulated, MAP decreased rapidly after injury, but by 10 min postinjury had returned to an average across all treatment groups of 63 ± 1.5 mm Hg as a result of cardiovascular compensation and resuscitation. After 10 min, MAP essentially remained constant ($P = 0.26$).

There were no treatment effects on blood pH (Fig 3, $P = 0.52$). At the time intervals tabulated, blood pH increased modestly but significantly during the 4 min post trauma (Fig. 3; $P < 0.001$). Across all treatment groups, pH declined rapidly between 4 and 10 min ($P < 0.01$) and remained constant thereafter ($P > 0.09$).

The overall ANOVA did not detect treatment effects on body temperature (Fig 4, $P = 0.37$). At the time intervals tabulated and across all treatment groups, body temperature measured in the arterial blood decreased modestly by 2 min post liver injury ($P <0.001$), then increased by 4 min ($P = 0.04$). Body temperature decreased modestly (0.9%; $P = 0.0018$) between one (32.5 ± 0.06; $n = 26$) and four hours (32.3 ± 0.09; $n = 20$) in pigs that survived. This response was most obvious in pigs treated with 720 μg/kg rFVIIa, which, at 4 hours postinjury, had body temperatures 1.5% lower than those in the other two groups ($P = 0.03$).

Plasma FVIIa and coagulation-associated measures

Factor VIIa activity was low (range 77-314 % activity) but measurable in all pigs at pre and post dilution sampling periods. There were no differences in FVIIa % activity among treatments within these time periods, nor between time periods (Figs 5a, 5b; $P = 0.59$). However, subsequent to liver injury and rFVIIa administration, marked treatment, time, and interaction effects were evident (Fig 5b; $P < 0.0001$). Within 10 min of treatment plasma concentrations of FVIIa had increased 57.5 and 110-fold in pigs injected with 180 μg/kg rFVIIa and 720 μg/kg rFVIIa, respectively. At each blood-sampling period, FVIIa % activity was greater in pigs given 720μg/kg rFVIIA compared with saline treated control pigs (Fig 5b). At 10 through 180 min, this latter dose produced plasma concentrations that also exceeded those present in pigs injected with 180 μg/kg rFVIIa. The 180 μg/kg rFVIIa dose produced plasma concentrations greater than those present in control pigs at 10 through 120 min (Fig 5b).

Thrombin-antithrombin (TAT) complexes increased (Fig 6; $P < 0.01$) 47% after hemodilution and induction of hypothermia. The overall ANOVA did not detect treatment ($P = 0.08$) or interaction ($P = 0.59$) effects, but did demonstrate significant time effects ($P < 0.0001$). Indeed, TAT complexes increased steadily ($P < 0.01$) in all treatment groups between 10 and 120 min post liver injury, and remained constant thereafter (Fig 6). Robust, *a priori* orthogonal contrasts demonstrated that TAT complexes at 60 ($P = 0.02$) and 180 min ($P = 0.04$) post injection in pigs receiving 720 μg/kg rFVIIa were greater than TAT complex concentrations in control pigs (Fig 6). Fibrinogen concentrations were unaffected by treatment (Fig 7; $P = 0.96$), but decreased 54% subsequent to hemodilution and hypothermia ($P < 0.01$), and 31% subsequent to liver injury ($P< 0.01$). Thereafter, fibrinogen concentrations did not change (Fig 7).

Prothrombin time (PT) increased 11% ($P < 0.01$) after hemodilution and hypothermia (Fig 8). After liver injury and treatment, PT in control pigs increased ($P < 0.001$), whereas PT in rFVIIa-treated pigs remained comparable to preinjury values (Fig 8). Accordingly, at 10 through 240 min PTs in pigs receiving either dose of rFVIIa were shorter than in control pigs ($P < 0.01$), and did not differ between doses. A species-specific PT assay produced identical results (data not shown). Activated partial thromboplastin times (aPTT) increased after hemodilution and hypothermia (Fig 9; $P < 0.01$). Ten min after injury, aPTT increased further in control pigs and in those treated with 180 μg/kg aFVIIa ($P < 0.01$; Fig 9). At 10 min postinjury and treatment, aPTT was shorter in pigs treated with 720 μg /kg rFVIIa than in control pigs. Subsequently, no further changes occurred in aPTT.

Thromboelastography and hematological measures

In order to measure better the coagulation process from initial clotting cascade to platelet interaction and clot strengthening (Srinivasa et al., 2001), thromboelastography (TEG) was conducted. The split-point time represents that latency time between placing blood into the TEG cup, and initiation of clot formation as measured by the initial detectable movement of the baseline from the horizontal plane. Subsequent to hemodilution and hypothermia there was a reduction in split-point time in pigs treated with rFVIIa compared with control pigs (Fig 10; $P < 0.01$). After liver injury and treatment, split-point time in rFVIIa-treated pigs again was shorter than in control pigs ($P < 0.01$). The decrease in split-point time after liver injury and treatment was greater ($P < 0.01$) compared with the decrease after hemodilution and hypothermia for pigs receiving either 180 or 720 μg/kg rFVIIa. The reduced split-point time due to treatment with rFVIIa continued through 60 min posttreatment (Fig 10; $P < 0.01$).

For thromboelastography, the R (reaction) time represents the latency time from placing blood into the cup to initiation of clot formation, as measured by the TEG tracing reaching a distance of 2 mm from horizontal. Hence, it is very similar to the split-point time. As with the split-point, R-values decreased in rFVIIa-treated animals compared with control animals subsequent to hemodilution and hypothermia (Fig 11; $P = 0.03$). The R-values decreased again for rFVIIa-treated pigs compared with controls ($P < 0.01$) when measured at 10 minutes posttreatment. The decrease in R-values for rFVIIa-treated pigs was greater after treatment than after hemodilution and hypothermia ($P < 0.001$). At 60 minutes posttreatment, only R-values in pigs treated with 180 μg/kg rFVIIa were shorter ($P < 0.01$) than those in control pigs. Unexpectedly, at 180 min after treatment, R-values in pigs receiving 720 μg/kg rFVIIa were longer ($P = 0.04$) than those in control pigs.

The K-time of the thromboelastograph represents the time for the TEG trace to pass from the 2mm to the 20 mm distance above baseline. It is considered to be a measure of speed of clotting to a certain level of clot strength. There were no treatment effects associated with K time (Fig 12; $P = 0.57$), but across all treatments, K-time increased as a result of

hemodilution and hypothermia, increased further subsequent to liver injury, then decreased between 10 and 60 minutes postinjury (Fig 12; $P < 0.01$).

The α–angle represents the slope of the line joining the TEG tracing at 2mm and at 20 mm. Like the K-time, the α-angle measures the kinetics of clot development. Again there were no treatment effects (Fig 13; $P = 0.37$). The α-angle decreased 22% ($P<0.01$) with hemodilution and hypothermia, decreased 5% ($P < 0.01$) 10 minutes after liver injury, then increased 9% ($P < 0.01$) by 60 min postinjury and remained constant thereafter.

The maximal amplitude (MA) is measured as the greatest vertical amplitude of the TEG trace and represents the greatest strength of the clot. There were no treatment effects on MA (Fig 14; $P = 0.87$). In a manner very similar to time-related effects on K-time, the MA decreased after hemodilution and hypothermia ($P < 0.01$), decreased slightly further after liver injury ($P < 0.01$), then increased modestly ($P < 0.01$) at 60 min postinjury and remained constant thereafter.

A number of additional hematological measures were unaffected by treatment ($P \geq 0.49$), but decreased in very similar manners post hemodilution and post liver injury ($P <0.01$): numbers of red blood cells (Fig 15), numbers of white blood cells (Fig 16); hematocrit (Fig 17); and hemoglobin concentrations (Fig 18). Red blood cell counts, hematocrit, and hemoglobin concentrations increased slightly ($P < 0.01$) between 10 and 60 min.

Histopathology

A major objective of this study was to determine potential effects of rFVIIa on disseminated intravascular coagulation (DIC), and in its absence provide evidence for the safe use of rFVIIa in patients. Using immunohistochemical techniques to detect fibrin II as a marker for DIC, there was no evidence of fibrin II in renal arterioles or capillaries in any of the pigs (Table 5, C & D; Appendix 9). There were very few thrombi in large vessels of the kidney, but these did not differ among treatments (Table 5; $P = 0.22$). When lung tissue was evaluated, and considering all pigs, small numbers of thrombi were present in large vessels, arterioles and in capillaries, but these did not differ among pigs (Table 5,A; $P > 0.35$). In evaluating individual pig data, it was evident that significant numbers of lung-vessel thrombi were evident primarily in pigs that survived the entire four hours and were euthanized. If these pigs were eliminated from the statistical evaluation, then the average numbers of thrombi present were lowered greatly (Table 5, B), but statistical evaluation now indicated a 3.7-fold greater number of thrombi in large vessels in pigs treated with 180μg/kg rFVIIa when compared with control pigs ($P = 0.04$).

Horowitz coefficient and inspiratory airway pressure

Although the number of gauze packs did not differ among treatment groups, there was concern that the number used per pig (~23, Table 2) was greater than in previous studies (13-17 gauze packs; Holcomb et al., 1999a; Holcomb et al.,1999b), and that the increased

gauzes might be associated with increased abdominal pressure and compartment syndrome. As an indirect measure of this potential complication (Ertel et al., 2000) the ratio of partial pressure of arterial oxygen to the fraction of inspired oxygen (PaO_2/FiO_2; Horowitz coefficient) was examined. This ratio was not affected by treatment ($P = 0.92$), nor did it change subsequent to liver injury and packing (Table 6; $P = 0.53$). Another indirect indicator of increased abdominal pressure, peak inspiratory airway pressure (Ertel et al., 2000; Toens et al., 2002; McNelis et al., 2003) was measured continuously in all pigs, but continuously recorded in only 25 of the 54 pigs. In these 25 pigs, peak inspiratory airway pressure (iPaw, cm H_2O) was not different among treatments (Fig 19; $P = 0.32$), but did change with time ($P < 0.001$). The time effect was due to an 18 % increase ($P < 0.01$) in iPaw within 2 min after liver injury and packing, a further 15.6% increase in the next 2 minutes ($P = 0.02$), a gradual decrease between 4 and 10 minutes postinjury ($P = 0.049$), and a 7.7 % increase in iPaw between 10 and 15 min postinjury ($P = .03$). Subsequently, the iPaw remained constant ($P = 0.20$). In any pig the highest individual iPaw was 25 cm H_20. The maximum average iPaw at any time period was 18.3 ± 1.1 cm H_2O at 210 min postinjury. This would have occurred in pigs surviving the longest.

Peak inspiratory airway pressure was recorded manually in all pigs at four time intervals: 1) after all initial surgeries and at a body temperature of 39 C°; 2) after hemodilution and hypothermia induction; 3) immediately prior to liver injury; and 4) 7.5 minutes after liver injury (at completion of the packing procedure). There was no measurable treatment effect on these iPaw measures ($P = 0.85$), but a time effect was evident ($P < 0.001$) due to a 27% increase in iPaw after liver injury and gauze packing (Fig 20; $P < 0.001$). There were no significant correlations of iPaw after packing with survival time ($r = 0.12$; $P = 0.37$), nor between survival time and the percent change in iPaw occurring subsequent to packing ($r = 0.12$; $P = 0.41$).

Discussion

Pigs assigned randomly among the three treatment groups did not differ in any of the pretreatment criteria used to assess their physiological and hematological condition. Similarly, the nature of the liver injury applied to each experimental group did not differ as determined by the number of blood vessels cut, the severity score, and the pretreatment blood loss. Packing around the liver as measured by the number of gauze packs, and any potentially adverse effects of packing (compartment syndrome) as measured by iPaw and the Horowitz coefficient (PaO_2/FiO_2) did not differ among experimental groups. Treatments among groups differed as demonstrated by the rapid, graded, and prolonged rises in plasma FVIIa activity (Table 7). Administration of rFVIIa enhanced some *in vitro* measures of coagulation (TAT-complexes, prothrombin time, activated partial thromboplastin time, TEG-associated split-point and reaction times). There were, however, no effects of rFVIIa administration on blood loss, survival time, or in mortality (i.e.; percent survival) measured during a four-hour period. Volume of resuscitation fluid used was modestly lower in pigs treated with 720μg/kg rFVIIa compared with pigs treated with 180μg/kg rFVIIa. Thus, with respect to the primary objective of the study, one must conclude that in this hemodiluted, hypothermic pig model with a Grade V liver

injury, administration of rFVIIa at the doses used did not reduce blood loss, prolong survival time, or increase survival. It was concluded by the pathologist at the Armed Forces Institute of Pathology that no DIC was present in either lung or kidney tissue. Absence of lung or kidney DIC provides evidence for the safety of administration of the two doses of rFVIIa used under the conditions of this study.

Because of previous positive results with rFVIIa administration to hypothermic, coagulopathic pigs (Martinowitz et al., 2001; Schreiber et al., 2002) this pig model was chosen to further evaluate the hemostatic abilities of rFVIIa subsequent to trauma-induced hemorrhage. Indeed, appropriate hypothermia ($32.4 \pm 0.06C°$) was achieved prior to liver injury, and this temperature range was maintained with few exceptions post injury. Hemodilution and hypothermia produced coagulopathy as evidenced by significant *in vitro* increases in prothrombin time, activated partial thromboplastin time, and increased thromboelastographic K-time concomitant with decreased α-angle and maximal amplitude. As there is *in vitro* evidence that FVIIa activity decreases significantly at pH 7.0 and lower, the maintenance of a nearly physiological pH is critical to optimal function of FVIIa (Meng et al., 2003). In the current study, pH levels were maintained above pH 7.2 in 49 pigs throughout their survival period. Of the remaining 5 pigs, four survived 2-4 hours with pH 7.05 to 7.2. Only one pig (a control) died in less than 30 min with modestly lower pH (7.1 to 7.2). Therefore, although pH was lowered subsequent to liver injury, in most pigs it did not reach levels that are highly detrimental to FVIIa activity (Meng et al., 2003).

Previous hemorrhage-related studies with pigs using rFVIIa administration have had somewhat contradictory outcomes (Tables
8 a, b, c). All such studies, but one, have made use of liver injuries and associated hemorrhage. Four studies have involved normothermic pigs, an absence of hemodilution prior to injury, and did not supplement rFVIIa therapy with liver packing when liver injury was involved (Tables 8a,c; Lynn et al., 2002; Jeroukhimov et al., 2002; Schreiber et al., 2003; Pusateri et al., 2003). In one of these studies, resuscitation was used beginning 15 min postinjury (Schreiber et al., 2003), and at a rate lower than that used in the current study (100 ml/min). Of these four studies, the most positive results occurred in the study by Jeroukhimov and colleagues (2002) in which 720 µg/kg of rFVIIa decreased blood loss by 39%, and increased the percent of surviving pigs at 1 hour by 50%. However, there were no differences in percent survival after two hours. The authors claimed an increase in survival time using statistical procedures that did not take into account the fact that the actual survival time of many of the pigs was unknown; i.e., that the animals were euthanized at 2 hours. Hence, this latter statement needs to be reexamined using statistics such as the LifeTest procedure that appropriately examines such "censored" data. In that study (Jeroukhimov et al., 2002) there were no significant effects of 180 µg /kg rFVIIa on blood loss and survival, substantiating previous results (Lynn et al., 2002). In this latter study, a higher mean arterial blood pressure was maintained by rFVIIa treatment.

In more recent work involving normothermic, non-coagulopathic pigs (Schreiber et al., 2003), a lower dose of rFVIIa (150 µg/kg) in moderately resuscitated pigs (100 ml/min)

did not reduce blood loss. As all pigs survived the 2-hour observation period, there were no measurable differences in survival time or percent survival. Hence, these three studies in similar--but not identical--pig models indicate a lack of efficacy of the lower rFVIIa dose. Similarly, normothermic, non-coagulopathic pigs were administered rFVIIa (0, 90, 180, 360, and 720 μg/kg) during an 80 min period, and at 20 minutes after the last dose (100 minutes after the initial dose), pigs were subjected to a Grade V liver injury. Survival time was monitored for up to 1 hour with no resuscitation (Pusateri et al, 2003). There was no effect of rFVIIa on blood loss, survival time or percent survival even though in vitro procedures to monitor coagulation (PT, activated clotting time, and TEG-associated R-time) were enhanced (Pusateri et al., 2003).

A final study involving normothermic, non-coagulopathic pigs made use of an aortotomy injury (2 mm diameter hole punch) and concomitant resuscitation at 100 ml/min to evaluate effects of rFVIIa (180 and 720 μg/kg, 5 minutes preinjury) on blood loss, survival time, and rebleed MAP (Table 8c;Sondeen et al., 2002). No effect was observed on blood loss, percent survival, or survival time, but both doses of rFVIIa equally increased the MAP at which rebleeding occurred. Such data suggest enhanced clot strength due to the presence of rFVIIa.

Two studies previous to the current one used the hypothermic, coagulopathic pig model with a severe liver injury (Table 8b; Martinowitz et al., 2001; Schreiber et al., 2002). The three studies are quiet similar with but few exceptions: surgical anesthesia, solutions used for hemodilution, resuscitation rates, number of replicates, doses of rFVIIa used, and observation time post trauma. In both previous studies there were significant reductions in blood loss, but no significant effects on percent survival or survival time. Schreiber and colleagues (2002) also reported significant improvements in blood pressure measures throughout much of the 2-hour post trauma period.

Differences in hemodilution solutions among the three studies may potentially be of importance. Martinowitz and coworkers (2001) used a solution of 6% hetastarch that had an average molecular weight (MW) of 200,000. The study conducted by Schreiber and colleagues (2002) made use of 5% human albumin solution. Finally, in the current study a solution of 6% hetastarch (Hespan) with an average MW of 670,000 was used. Both studies using hetastarch (Martinowitz et al., 2001; current study) infused a solution maintained at 33 C^{o}, whereas the albumin solution was at room temperature (Schreiber et al., 2002). Hetastarch (hydroxyethyl starch) and albumin are the two colloids administered most frequently for intravascular volume expansion in human surgery (Warren and Durieux, 1997). Hydroxyethyl starch (HES) products have been associated with some adverse reactions in humans including: anaphylactoid reactions, pruritus (itching), and conflicting reports on coagulation (Warren and Durieux, 1997). For pigs there have been no studies formally comparing the above-noted three intravascular volume expanders. For humans one study compared albumin, HES with an average MW of 200,000, and HES with an average MW of 450,000 (Boldt et al., 1993). In this study with 75 cardiac surgery patients, use of the higher MW HES was associated with significantly higher blood losses during the first postoperative day when compared with controls and other treatments. With *in vitro* studies, HES with MW of 200,000 and

400,000 equally impaired coagulation subsequent to 50% dilutions as measured by TEG (increased R-time + K-time, decreased alpha-angle and maximum amplitude) when compared with undiluted controls (Niemi and Kuitunen, 1998). When compared with blood comparably diluted with Ringer's acetate solution, or with 4% albumin (50% dilution) HES (MW 450,000) increased R+K time whereas HES (MW 200,000) did not. Hence, differential effects both *in vivo* (blood loss) and *in vitro* (coagulation function) have been documented with studies relating to humans. For the current study Hespan (MW 450,000) was chosen because it caused "more" of a coagulopathy (e.g., Gan et al., 1999), and it had been successfully used previously for testing dry fibrin sealant dressings (Holcomb et al., 1999b). The possibility exists, therefore, that differences in blood loss outcome between the current study and previous work (Martinowitz et al., 2001; Schreiber et al., 2002) could in part reflect different hemodilution solutions.

In addition to the above-noted differences in experimental design, one also must consider the possibility that response differences might be associated with the use of different pig breeds. Pigs used in the current study are a Musclor x Duroc composite. In previous studies, the breeds were not documented. In the swine industry there are many production-related characteristics associated with certain breeds that have been selected via many generations of breeding to increase prolificacy, growth rates, carcass composition, and resistance to diseases (e.g., Haley and Lee, 1993; Tummaruk et al., 2000; Johnson et al., 2002; Berg et al., 2003; Chen et al., 2003). As response to hemorrhage is of no concern to swine producers, it has not been characterized in different breeds and little-to-no-information is available. However, potential unknown breed differences related to this characteristic must also be considered as one potential causative factor associated with study differences.

Hence, using very similar experimental models and procedures, results differed from the current study in terms of effects on MAP and blood loss. The relative influences of the modest experimental differences in the reported outcomes must remain speculative. In addition to study differences noted above, gauze packing differences were also considered. Earlier studies used fewer packing gauzes (13-17 per pig; Holcomb et al., 1999a; Holcomb et al., 1999b) compared with the current study (19-25 per pig). Increased packing suggests a potential problem of creating increased abdominal pressure that might cause compartment syndrome, thereby adversely affecting survival time. To address this concern, data were presented on the ratio of arterial oxygen partial pressure (PaO_2) to the fraction of inspired oxygen (FiO_2). In human trauma patients, respiratory compromise associated with abdominal compartment syndrome is associated with a significant decrease in this ratio (Meldrum et al., 1997; Ertel et al., 2000). In our animals, however, this ratio did not change after packing.

The peak or inspiratory airway pressure (iPaw) has also been found to be highly predictive of abdominal compartment syndrome in human trauma patients (Raeburn et al., 2001; McNelis et al., 2003). For such patients, a peak airway pressure of >40 cm H_20 associated with an intra-abdominal pressure of > 20 mm Hg was indicative of compartment syndrome (Meldrum et al., 1997). For pigs with a CO_2-induced intra-abdominal pressure of 30 mm Hg, the peak airway pressure rose to approximately 40

mbar (40.8 cm H_20; 30 mm Hg; Toens et al., 2002). Taken together, these data suggest strongly that in pigs of the current study, even though significant increases in peak or inspiratory airway pressure increased after packing, levels reached were far from those associated with excessive abdominal pressure indicative of compartment syndrome (Toens et al., 2002).

A major objective of this study was to demonstrate safety of use of rFVIIa as indicated by the lack of disseminated intravascular coagulation (DIC). Histological slides from lung and kidney sections were evaluated using immunohistological procedures that detected fibrin II and factor VIII as indicators of fibrin and platelet deposition respectively, and hence as indicators of the presence of thrombi. Although thrombi in large vessels of lung tissue were present in greater numbers in pigs receiving 180 µg/kg of rFVIIa than in control pigs-- in pigs that died during the 4-hour study course, and were not euthanized-- it was the opinion of the pathologist that thrombi in these vessels was not consistent with DIC. The conclusion of the pathologist--who was "blinded" to treatments assigned to each pig--was that there was no treatment-related effect on "activation of the coagulation system throughout the animals' circulation". Hence, this study provides evidence for the safety of rFVIIa when administered at the doses used and in the hypothermic, coagulopathic pig model.

In summary, to obviate potential shortcomings of previous studies, the current study used larger sample sizes (n= 18 per treatment group), and a more prolonged observational period of 4 hours. With these design changes, the current study with two doses of rFVIIa (180 and 720 µg/kg body weight) administered into hypothermic, coagulopathic pigs after a Grade V liver injury was unable to demonstrate either decreases in blood loss or increases in survival. Data detailed above suggest that blood loss associated with the liver injury was the primary cause of death. Based on lack of demonstrable DIC in lungs and kidneys, this study, did, however, provide additional evidence for the safe administration of rFVII--in the doses used--to pigs under the conditions of this study.

This study was conducted and in compliance with Good Laboratory Practices.

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Table 1. Average values for blood pH, mean arterial pressure (MAP), and body temperature in pigs prior to hemodilution and hypothermia (normal baseline), and after hemodilution and hypothermia (preinjury baseline).

Data Represent Mean ± SEM of the number of pigs indicated in parentheses

Measure Time Period	Normal Baseline				Preinjury Baseline			
Treatment	Control	180 ug rFVIIa/kg	720 ug rFVIIa/kg	Statistical Evaluation	Control	180 ug rFVIIa/kg	720 ug rFVIIa/kg	Statistical Evaluation
Blood pH	7.40 ±.003 (18)	7.40 ± .004 (18)	7.40 ± .003 (18)	P= 0.79	7.42 ± .004(18)	7.41 ± .005 (18)	7.41 ± .003(18)	P = 0.53
MAP (mm Hg)	87.2 ± 3.3 (18)	81.5 ± 3.1 (18)	86.1 ± 4.2 (18)	P= 0.49	71.1 ± 2.2 (18)	69.1 ± 1.7 (18)	72.7 ± 1.98 (18)	P = 0.43
Temperature (C)	38.9 ± .04 (18)	39.0 ± .04 (18)	39.0 ± .03 (18)	P= 0.29	32.4 ± .05 (18)	32.4 ± .07 (18)	32.5 ± .06 (18)	P = 0.24

Table 2. Measures in pigs subjected to severe liver injury and treated with saline or recombinant factor VIIa. Data represent means ± SEM of the numbers of pigs in parentheses.

	Treatment			
	Control	180 ug/kg rFVIIa	720 ug/kg rFVIIa	Statistical Evaluation
Sponges	23.2 ± .41 (18)	23.0 ± .38(18)	23.4 ± .37(18)	Treatment P =0.72
Body Weight (kg)	36.4 ± 0.4 (18)	36.2 ± 0.5(18)	37.2 ± 0.4 (18)	Treatment P =0.30
Injury Score (# blood vessels cut)	1.89 ± .20 (18)	1.78 ± .15 (18)	1.83 ± .15 (18)	Treatment P =0.89
Severity Score (# blood vessels cut plus size of injury)	3.56 ± .35 (18)	3.33 ± 30 (18)	3.44 ± .31 (18)	Treatment P =0.89
Pretreatment Blood Loss (ml)	262.7 ± 27.4 (18)	327.4 ± 26.6 (18)	314.7 ± 25.1 (18)	Treatment P =0.20
Post Treatment Blood Loss (ml)	1656.5 ± 207.4 (18)	1769.2 ± 170.9 (18)	1518.4 ± 282.5 (18)	Treatment P =0.73
Resuscitation Fluid (ml)	6862.3 ± 994.7 (18)	7683.0 ± 895.9 (18)	4973.8 ± 742.0 (18)	Treatment P =0.049
Survival Time (min)	118.3 ± 24.4 (18)	140.4 ± 24.8 (18)	107.3 ± 26.0 (18)	Treatment P =0.56; (PROC LifeTest)

Table 3. Effects of recombinant Factor VIIa on survival in pigs after a severe liver injury and associated hemorrhage.

Survival Ratios				
Treatments	**Control**	**180 ug rFVIIa/kg**	**720 ug rFVIIa/kg**	**Probability**
Time				
1 hr	**9/18**	**10/18**	**7/18**	**0.70**
2hr	**8/18**	**10/18**	**7/18**	**0.70**
3hr	**7/18**	**9/18**	**7/18**	**0.83**
4hr	**6/18**	**8/18**	**6/18**	**0.82**

Table 4. Sample sizes for each treatment group at designated time intervals associated with Figs. 2-4.

Treatment	Time Interval (minutes)																						
	-16	-11	-6	-4	-2	Cut	2	4	6	8	10	15	20	30	40	50	60	90	120	150	180	210	240
Control	18	18	18	18	18	18	18	18	18	18	18	16	16	13	10	9	9	8	8	7	7	6	6
180 µg/kg rFVIIa	18	18	18	18	18	18	18	18	18	18	18	18	16	13	13	10	10	10	10	9	9	8	8
720 µg/kg rFVIIa	18	18	18	18	18	18	18	18	18	17	17	16	14	10	7	7	7	7	7	7	7	7	6

Table 5. Histopathological evaluation of lung and kidney tissue. Numbers represent the Mean ± SEM of the number of thrombi detected per histological section based on fibrin II staining. A and C represent all animals. B and D represent animals remaining after deleting animals that survived the complete 4 hours and were euthanized with an overdose of sodium pentobarbital.

	TREATMENT	TISSUE	(n)	LOCATION		
				Large Vessels	Arterioles	Capillaries
A	Control	Lung	18	6.9 ± 4.8	4.8 ± 3.0	8.8 ± 6.2
	180 ug/kg rFVIIa		18	2.1 ± 0.6	1.4 ± 0.8	4.1 ± 1.9
	720 ug/kg rFVIIa		18	6.4 ± 3.5	7.3 ± 4.3	7.0 ± 3.2
	Statistical Evaluation			P=0.52 *; P = 0.10#	P=0.36 *; P = 0.83#	P=0.66 *; P = 0.98#
B	Control	Lung	12	0.3 ± 0.2[b]	0.4 ± 0.3	1.0 ± 0.6
	180 ug/kg rFVIIa		10	1.4 ± 0.4[a]	0.4 ± 0.3	4.0 ± 2.9
	720 ug/kg rFVIIa		12	1.2 ± 0.4	0.5 ± 0.5	5.1 ± 4.0
	Statistical Evaluation			P=0.05 *; P = 0.04#	P=0.99*; P = 0.80#	P=0.56 *; P = 0.73#
C	Control	Kidney	18	0.4 ± 0.2	0	0
	180 ug/kg rFVIIa		18	1.7 ± 1.2	0	0
	720 ug/kg rFVIIa		18	0.2 ± 0.1	0	0
	Statistical Evaluation			P=0.22 *; P = 0.65#		
D	Control	Kidney	12	0.4 ± 0.2	0	0
	180 ug/kg rFVIIa		10	1.8 ± 1.7	0	0
	720 ug/kg rFVIIa		12	0.1 ± 0.1	0	0
	Statistical Evaluation			P=0.37 *; P = 0.52#		

*Based on parametric procedure,PROC MIXED, ANOVA, data non-normal but homogeneous; # Based On nonparametric procedure, PROC NPAR1WAY,Kruskal-Wallis Test

a vs b P = 0.02

Table 6. Horowitz Coefficient (P_aO_2/FiO_2) for pigs subjected to severe liver injury. Data represent mean ± SEM at specified time intervals (minutes) before and after liver injury.

Time (min)	-45	-30	-15	15	30	45	60	Statistical Evaluation	
Control	532 ± 12	527 ± 13	517 ± 15	519 ± 24	514 ± 28	488 ± 27	485 ± 32	Treatment	P=0.92
180µg/kg rFVIIa	493 ± 16	513 ± 23	518 ± 29	552 ± 63	566 ± 78	477 ± 22	470 ± 23	Time	P=0.53
720µg/kg rFVIIa	515 ± 12	509 ± 13	512 ± 12	516 ± 13	519 ± 15	503 ± 18	500 ± 19	Interaction	P=0.60

Table 7. Summary of responses (compared with controls) to administration of recombinant factor VII activated (rFVIIa) to coagulopathic pigs (hemodiluted and hypothermic).

Measure	Response
Mean Arterial pressure	No effect
Plasma FVIIa	**Increased**
Plasma Fibrinogen	No effect
Thrombin-Antithrombin	**Time-dependent increase**
Prothrombin time	**Decreased**
Activated partial thromboplastin time	**Time-dependent decrease**
Thromboelastography	
Split point time	**Decreased**
R-time	**Decreased**
K-time	No effect
α-angle	No effect
Maximal amplitude	No effect
Blood Loss	No effect
Survival Time	No effect
Percent survival	No effect
Disseminated Intravascular Coagulation	No effect

Table 8a. Comparison of experimental conditions associated with the currently available studies involving use of recombinant factor VII activated (rFVIIa) in normothermic pigs.

Observations	(ref #14) Lynn M et al., J Trauma 52: 703-707, 2002	(ref # 15) Jeroukhimov I et al., J trauma 53:1053-1057, 2002	(ref #16) Schreiber et al. J Am Coll Surgeons 196: 691-97, 2003
Pig Breed	Yorkshire	*******	Yorkshire crossbred
Pig Weight	19+/- 2 kg	33.5 +/- 3 kg	~ 40 kg
Sample Size/Group	(6-7)	8	15
Anesthetic	ketamine sedation; sodium pentobarbital maintenance	IV infusion ketamine, xylazine, fentanyl	Telezol sedation, then isoflurane
Body Temperature during study	None given; assume normothermic	Normothermic	Normothermic (38C)
Pre-injury blood dilution	None	None	None
Solution used	N/A	N/A	N/A
Injury	Crushing and avulsion of left Median lobe & left lateral lobe; Grade IV	Crushing and avulsion of left Median lobe & left lateral lobe; Grade IV	Stellate cut of medial lobes; Grade V
Veins cut - control group	*******	*******	1.5 +/- 0.5
Veins cut - treatment 1	*******	*******	1.7 +/- 0.8
Veins cut - treatment 2	N/A	*******	N/A
Packing	No	No	No
Length of study	1 hr	2 hrs	2 hrs
Treatments			
Control	Placebo of unstated composition	Saline	Buffer
Treatment 1	180 ug/kg rVIIa	180 ug/kg rFVIIa	150 ug/kg rFVIIa
Treatment 2	N/A	720 ug/kg rFVIIa	N/A
When given	After injury when MAP decreased by 10%	After injury when MAP decreased by 10%	30 sec post injury
Resuscitation	No	No	Yes
Rate	N/A	N/A	100 ml/min
When Started	N/A	N/A	15 min post injury
Solution	N/A	N/A	Lactated Ringers(?C)
Treatment Effects On:			
Blood Loss	NE	Decreased (High dose)	NE
Survival Time	******	Increased ?	NE
% Survival	NE	Increased at 1 hr (high dose) ; NE at 2 hr	NE
Prothrombin Time	Decreased	Decreased (Both doses)	Decreased
Mean Arterial Pressure	Increased	********	NE

NE = No effect **N/A = Not Applicable** ******** = Not stated in paper**

Table 8b. Comparison of experimental conditions associated with the currently available studies involving use of recombinant factor VII activated (rFVIIa) in coagulopathic pigs (hemodiluted and hypothermic).

Observations	(ref # 18) Martinowitz et al., J Trauma 50: 721-729, 2001	(ref # 19) Schreiber et al., J Trauma 53:252-259, 2002.	(current) Klemcke et al.,2004
Pig Breed	*******	Yorkshire crossbred	Large White x Yorkshire x Duroc x Musclor Crossbred
Pig Weight	35 +/- 2 kg	30 kg	36.6 +/- 0.4
Sample Size/Group	5	10	18
Anesthetic	isoflurane and nitrous oxide:oxygen (50:50)	Telezol sedation, then isoflurane	Telezol sedation, then isoflurane
Body Temperature during study	33C	33 C	32.4 +/- .06
Pre-injury blood dilution	~60% isovolemic, hypothermic exchange	~60% isovolemic, hypothermic exchange	~60% isovolemic, hypothermic exchange
Solution used	6% Hetastarch (Hespan); MW 200,000	5% human albumin, room temperature	6% Hetastarch; MW 670,000
Injury	Stellate cut of medial lobes; Grade V	Stellate cut of medial lobes; Grade V	Stellate cut of medial lobes; Grade V
Veins cut - control group	1.2	2.1 +/- 0.7	1.89 +/- 0.2
Veins cut - treatment 1	2	2.0 +/- 0.8	1.78 +/- 0.15
Veins cut - treatment 2	N/A	2.1 +/- 0.6	1.83 +/- 0.15
Packing	Yes	Yes	Yes
Length of study	1 hr	2 hrs	4 hrs
Treatments			
Control	Saline	Buffer	Saline
Treatment 1	180 ug/kg rFVIIa	180 ug / kg rFVIIa	180 ug / kg rFVIIa
Treatment 2	N/A	720 ug/kg rFVIIa	720 ug/kg rFVIIa
When given	30 sec post injury	30 sec post injury	30 sec post injury
Resuscitation	Yes	Yes	Yes
Rate	250 ml/min	100 ml/min	260 ml/min
When Started	5.5 min post injury	Post Packing. Time ?	4.5 min post injury
Solution	Lactated Ringers(40C)	Lactated Ringers (temp variable)	Lactated Ringers (33C)
Treatment Effects On:			
Blood Loss	Decreased	Decreased (both doses combined)	NE
Survival Time	NE	NE	NE
% Survival	NE	NE	NE
Prothrombin Time	Decreased	Decreased (both doses)	Decreased
Mean Arterial Pressure	*******	Increased	NE

NE = No effect **N/A = Not Applicable** ******** = Not stated in paper**

Table 8c. Comparison of experimental conditions associated with the currently available studies involving use of recombinant factor VII activated (rFVIIa) in normothermic pigs.

Observations	(ref #13) Sondeen et al., Shock 2004 (in press)	(ref #17) Pusateri et al., FASEB J 16: 131-132, 2002
Pig Breed	Large White x Yorkshire x Duroc x Musclor Crossbred	Large White x Yorkshire x Duroc x Musclor Crossbred
Pig Weight	39 +/- 1 kg	37.2 +/- 0.8 kg
Sample Size/Group	10	6
Anesthetic	Telezol sedation, then isoflurane	Telezol sedation, then isoflurane
Body Temperature during study	Normothermic (37-39C)	Normothermic (38.5 - 39.5C)
Pre-injury blood dilution	None	None
Solution used	N/A	N/A
Injury	Aortotomy	Stellate cut of medial lobes; Grade V
Veins cut - control group	N/A	1
Veins cut - treatment 1	N/A	1
Veins cut - treatment 2	N/A	N/A
Packing	N/A	No
Length of study	2 hrs	1 hr
Treatments		
Control	Vehicle	Saline
Treatment 1	180 ug/kg rFVIIa	720 ug/kg rFVIIa
Treatment 2	720 ug/kg rFVIIa	N/A
When given	5 min pre-injury	20 min pre-injury
Resuscitation	Yes - to promote rebleeding	No
Rate	100 ml/min	N/A
When Started	10 min post-injury	N/A
Solution	Lactated Ringers(37C)	N/A
Treatment Effects On:		
Blood Loss	Decreased rebleed blood loss (p=0.055)	NE
Survival Time	NE	NE
% Survival	Increased at 2 hr	NE
Prothrombin Time	Decreased	Decreased
Mean Arterial Pressure	rFVIIa increased MAP at which rebleeding occurred	Increased

FIG 1. Kaplan-Meier Survival Graph of survival times (minutes) .

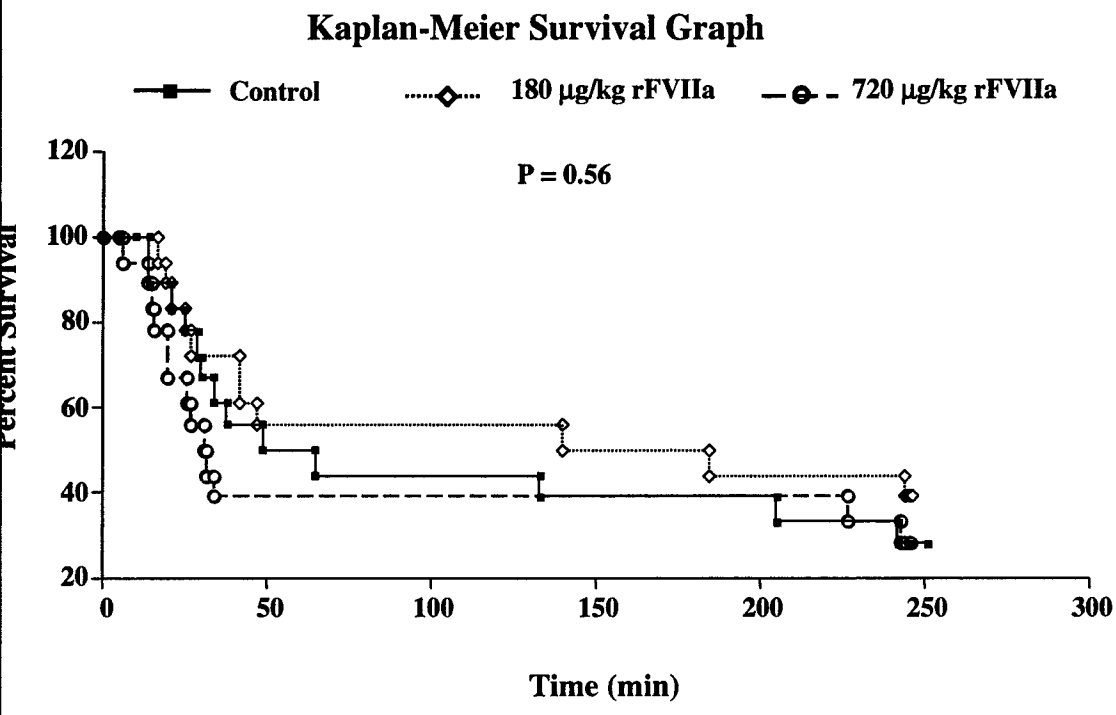

FIG 2. Mean arterial pressure measured at various time intervals before and after liver injury. Data represent Mean ± SEM of the number of animals at each time period indicated in Table 4.

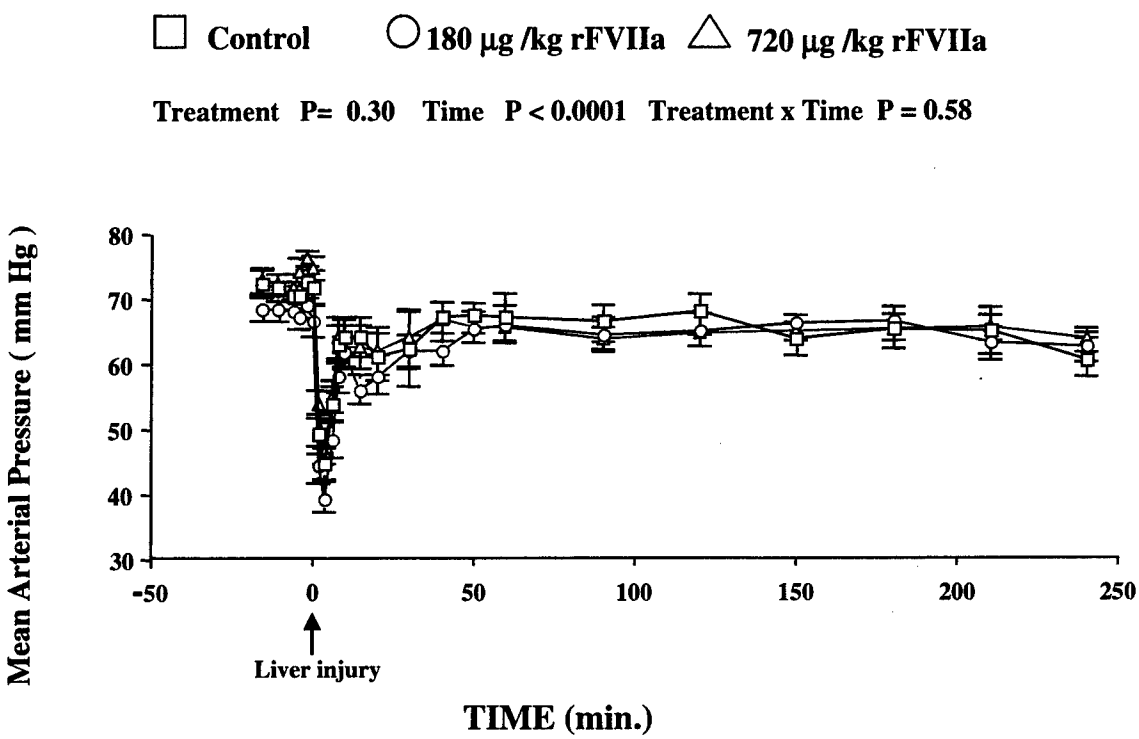

FIG 3. Blood pH measured at various time intervals before and after liver injury. Data represent Mean ± SEM of the number of animals at each time period indicated in Table 4.

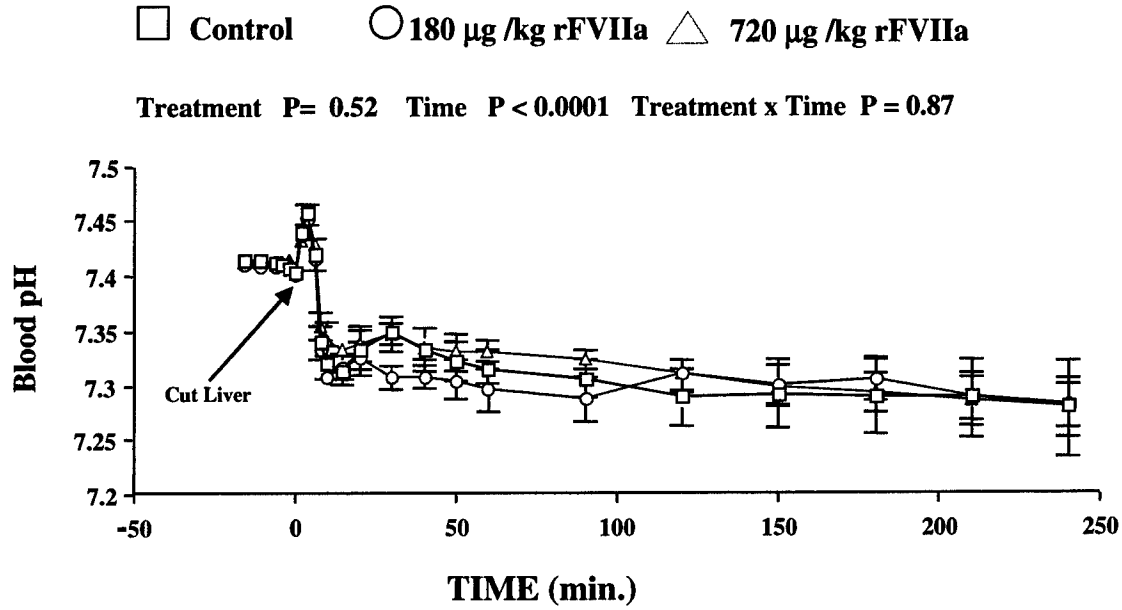

FIG 4. Body temperature measured at various time intervals before and after liver injury. Data represent Mean ± SEM of the number of animals at each time period indicated in Table 4.

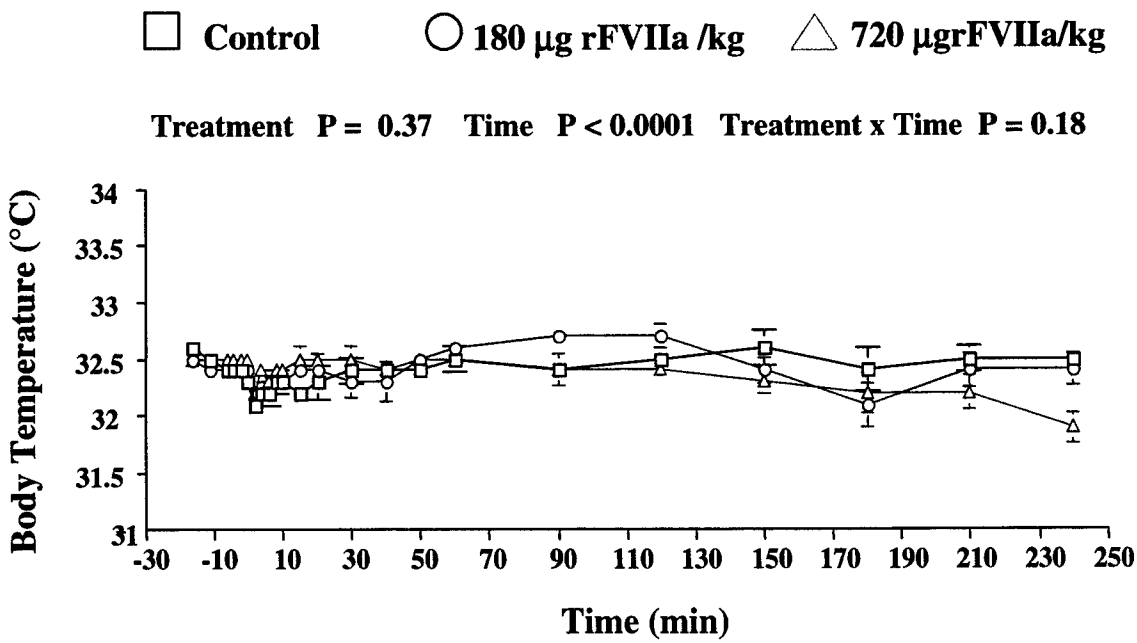

FIG 5A. Activated Factor FVII (FVIIa) in pig plasma--as measured by a one-stage clotting assay-- before and after hemodilution and induction of hypothermia. There were no differences for any treatment groups between pre and post-dilutional concentrations, nor among treatments at either time period.

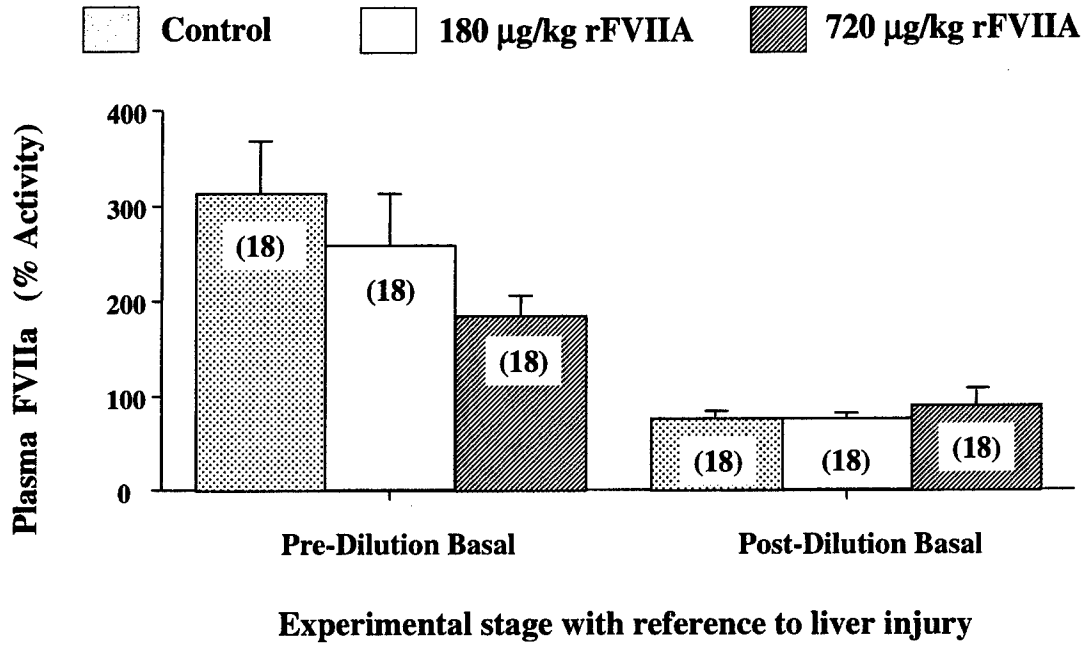

FIG 5B. Activated Factor FVII (rFVIIa) concentrations in pig plasma--as measured by a one-stage clotting assay-- before and after severe liver injury. Bars represent means + SEM . Bars with different letter superscripts within a time period are different ($p < 0.05$). At the scale shown, some bars and SEM are too small to be observed. Statistical comparisons indicated by brackets are time effects across all treatments.

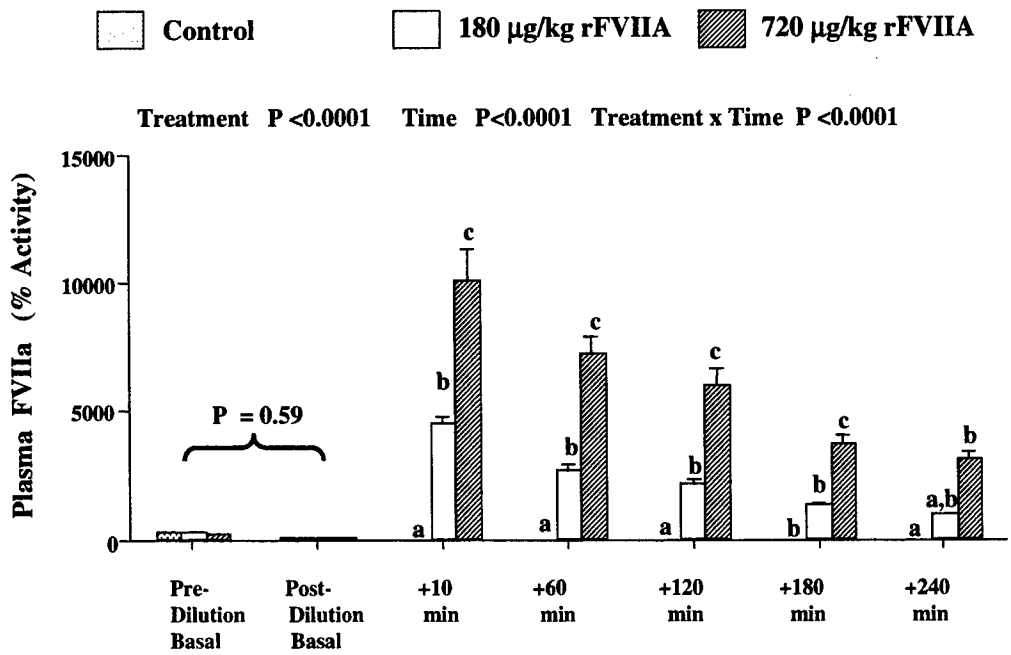

FIG 6. Thrombin-antithrombin (TAT) complexes in pig plasma before and after severe liver injury. Bars represent means + SEM. Bars with different letter superscripts within a time period are different ($p < 0.05$). At the scale shown, some SEM are too small to be observed. Numbers of replicates contributing to a mean are presented within bars. Statistical comparisons indicated by brackets are time effects across all treatments.

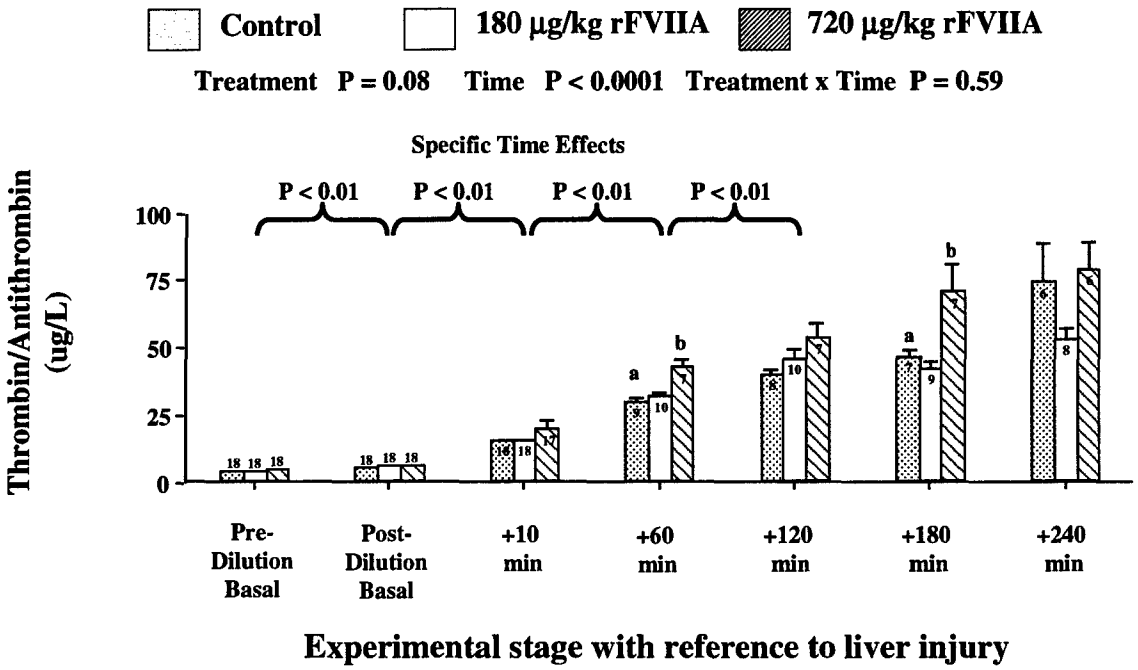

FIG 7. Fibrinogen concentrations in pig plasma before and after severe liver injury. Bars represent means + SEM. At the scale shown, some SEM are too small to be observed. Numbers of replicates contributing to a mean are presented within bars. Statistical comparisons indicated by brackets are time effects across all treatments.

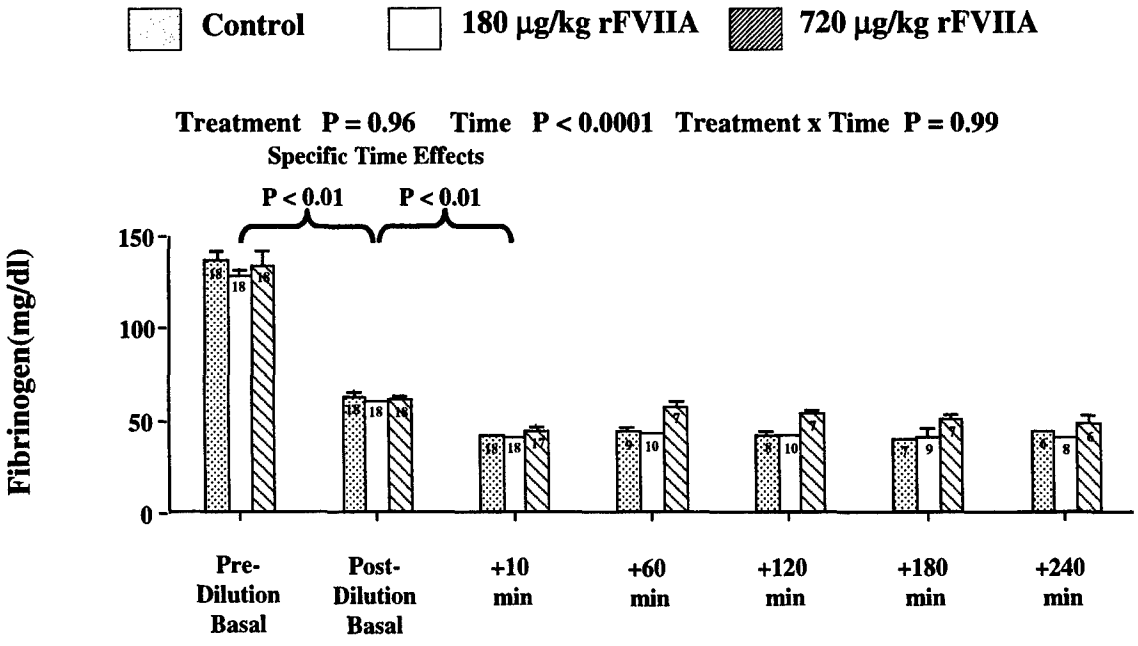

FIG 8. Prothrombin time (PT) for pig plasma before and after severe liver injury. Bars represent means + SEM. At the scale shown, some SEM are too small to be observed. The numbers of replicates contributing to a mean are presented within bars. The large bracket compares pre and post dilution effects across all treatments. Smaller brackets compare control *vs.* rFVIIa-treated values.

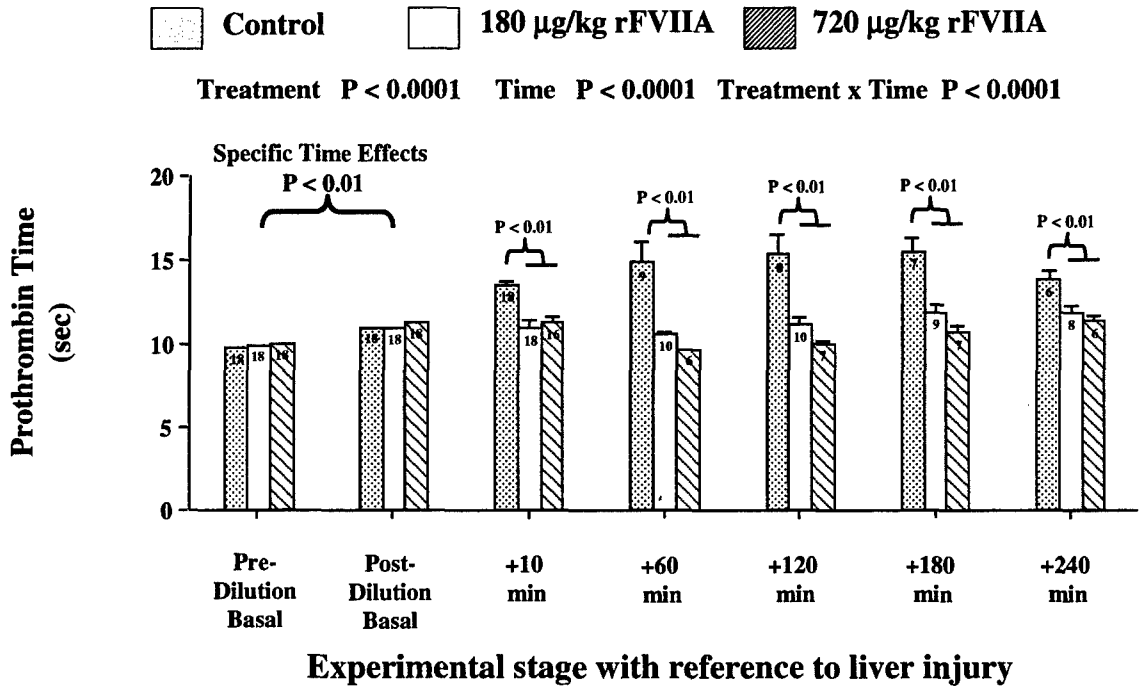

FIG 9. Activated partial thromboplastin time (aPTT) for pig plasma before and after severe liver injury. Bars represent means + SEM. At the scale shown, some SEM are too small to be observed. The numbers of replicates contributing to a mean are presented within bars. Bars with different letter superscripts within a time period are different ($p < 0.05$). The large bracket compares pre and post dilution effects across all treatments.

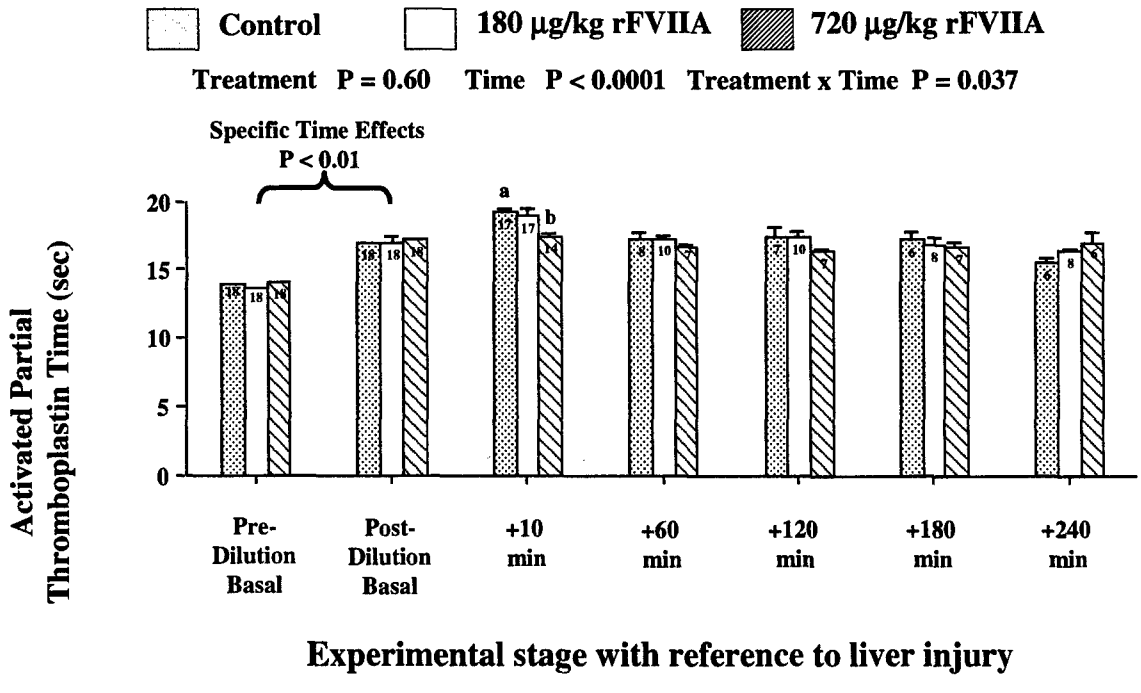

FIG 10. Thromboelastograph (TEG) split-point time for pig plasma before and after severe liver injury. Bars represent means + SEM. At the scale shown, some SEM are too small to be observed. The numbers of replicates contributing to a mean are presented within bars. Brackets compare control concentrations *vs* rFVIIa-treated at both doses.

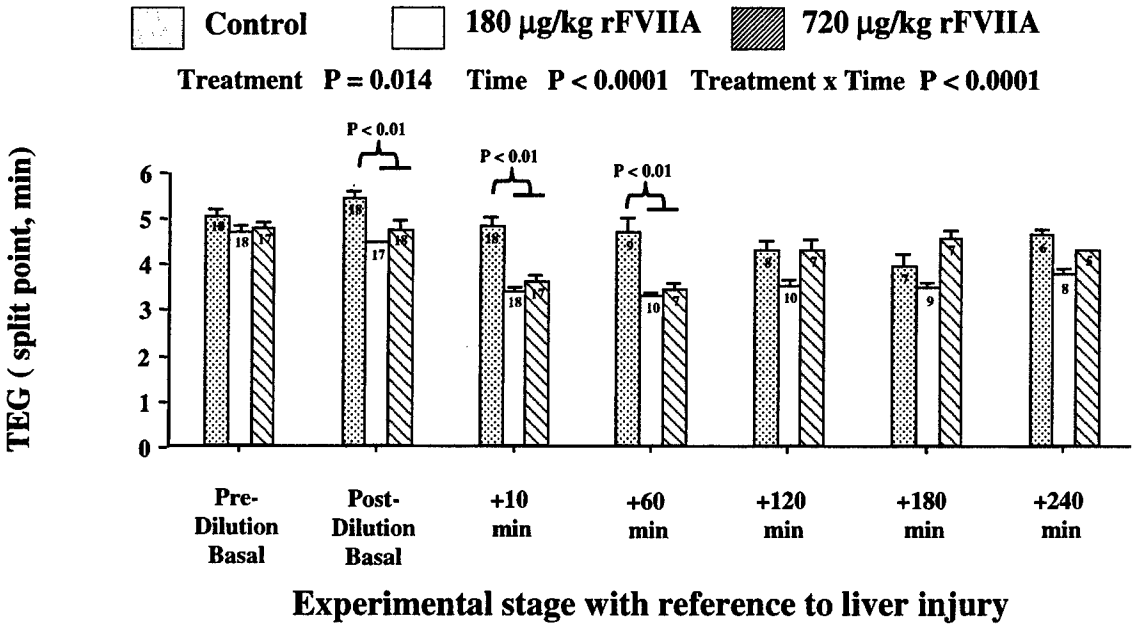

FIG 11. Thromboelastograph (TEG) reaction (R)-time for pig plasma before and after severe liver injury. Bars represent means + SEM. At the scale shown, some SEM are too small to be observed. The numbers of replicates contributing to a mean are presented within bars. Brackets (post-dilution and +10 min) compare control concentrations *vs* rFVIIa-treated at both doses. Brackets (+60 and + 180 min) compare treatments illustrated.

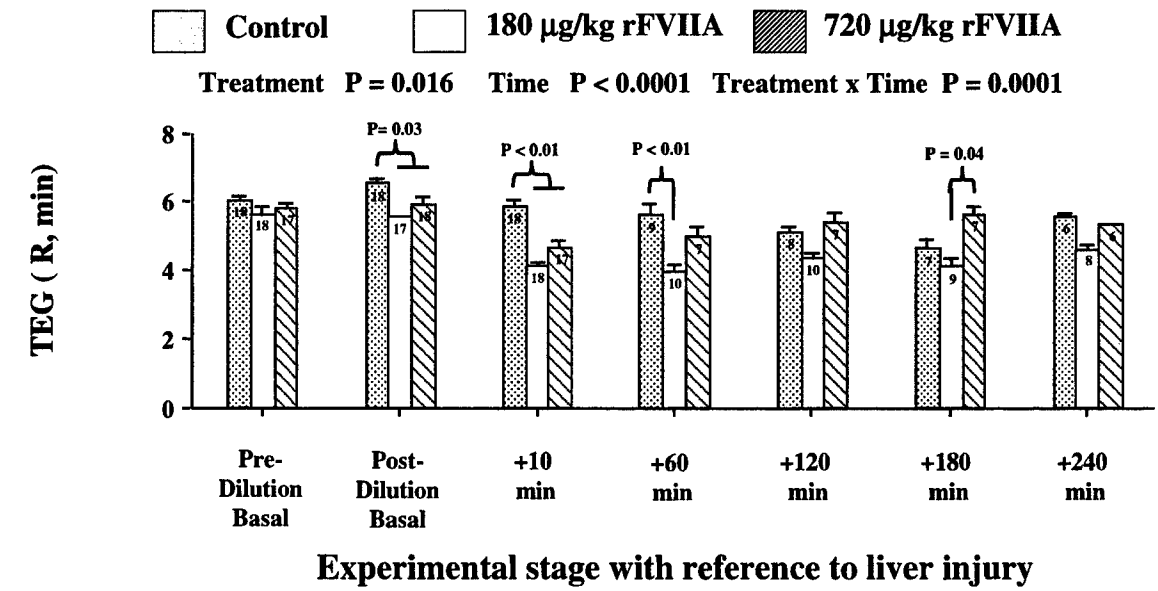

FIG 12. Thromboelastograph (TEG) K-time for pig plasma before and after severe liver injury. Bars represent means + SEM. At the scale shown, some SEM are too small to be observed. The numbers of replicates contributing to a mean are presented within bars. Statistical comparisons indicated by brackets are time effects across all treatments.

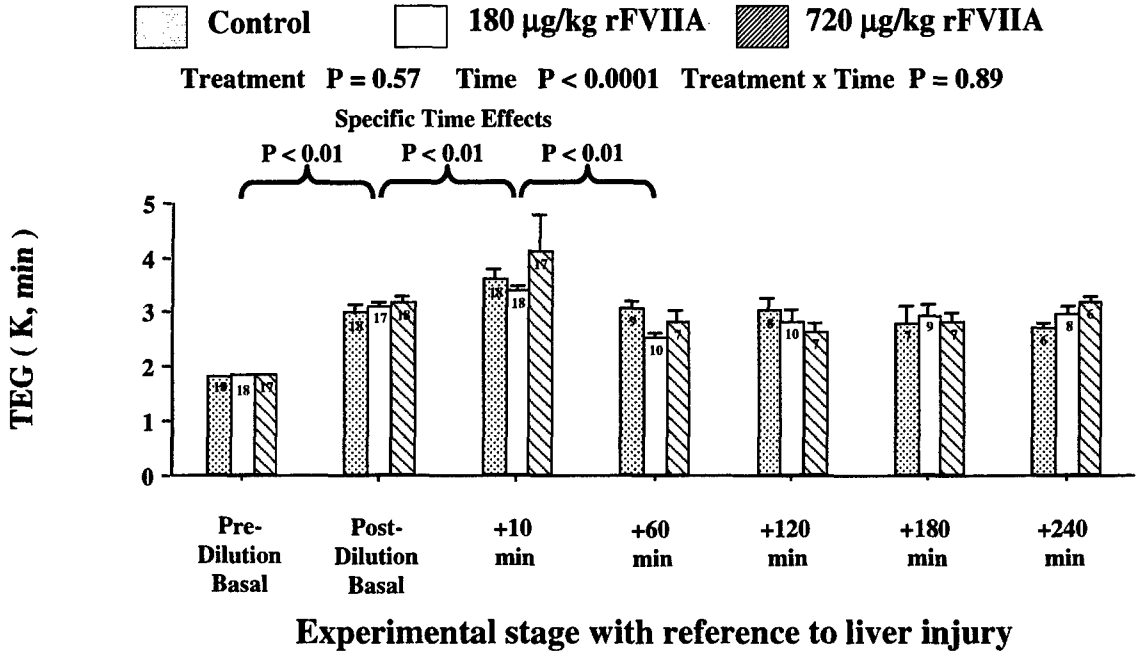

FIG 13. Thromboelastograph (TEG) α-angle for pig plasma before and after severe liver injury. Bars represent means + SEM. At the scale shown, some SEM are too small to be observed. The numbers of replicates contributing to a mean are presented within bars. Statistical comparisons indicated by brackets are time effects across all treatments.

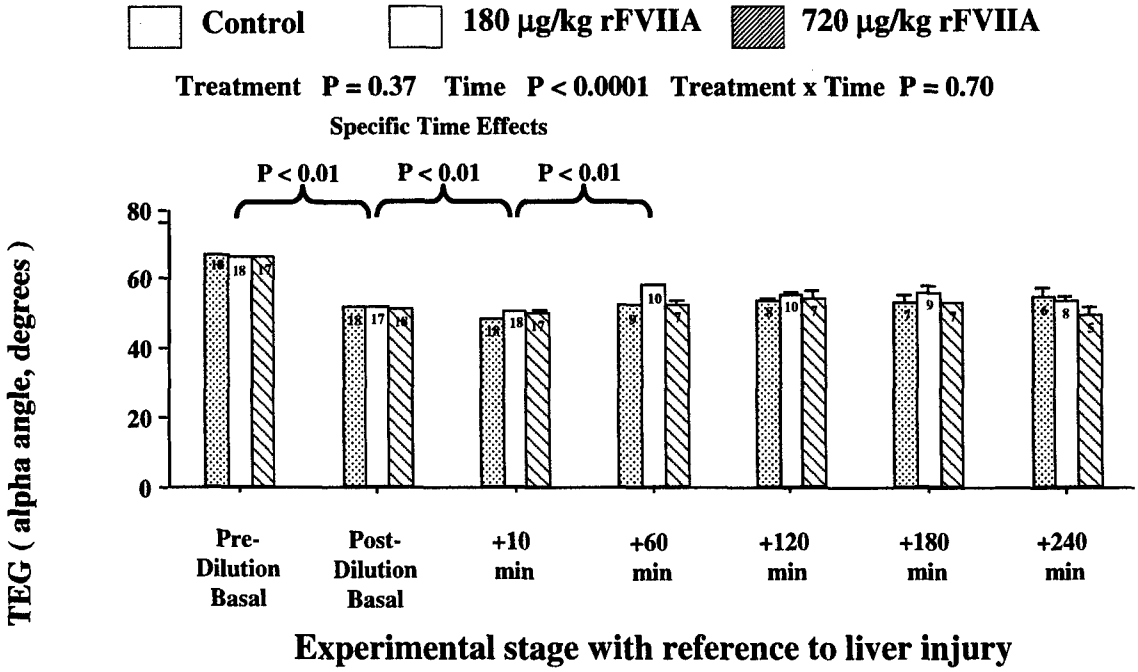

FIG 14. Thromboelastograph (TEG) maximal amplitude for pig plasma before and after severe liver injury. Bars represent means + SEM. At the scale shown, some SEM are too small to be observed. The numbers of replicates contributing to a mean are presented within bars. Statistical comparisons indicated by brackets are time effects across all treatments.

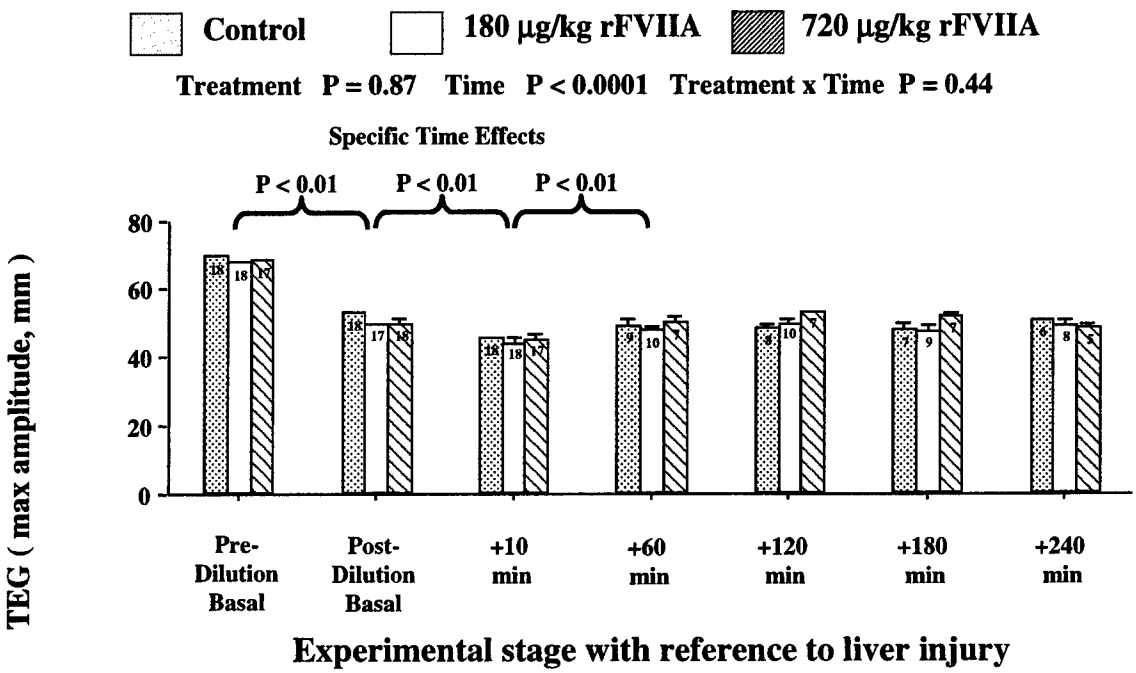

FIG 15. Intravascular red blood cells before and after severe liver injury. Bars represent means + SEM. At the scale shown, some SEM are too small to be observed. The numbers of replicates contributing to a mean are presented within bars. Statistical comparisons indicated by brackets are time effects across all treatments.

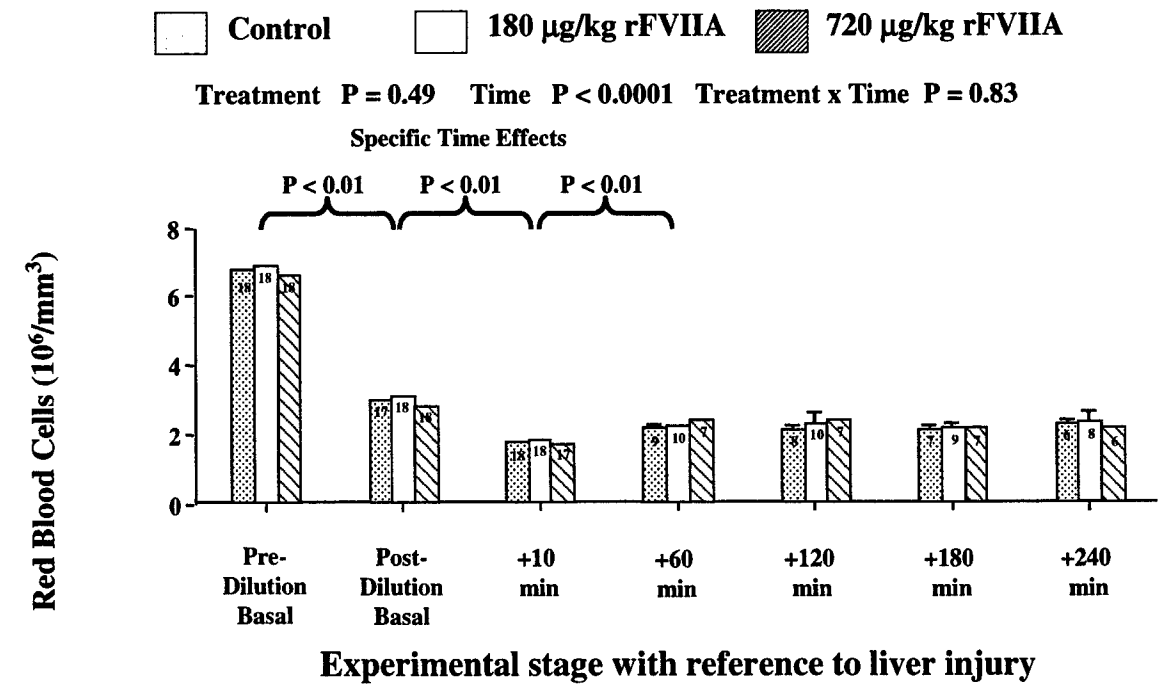

FIG 16. Intravascular white blood cells before and after severe liver injury. Bars represent means + SEM. At the scale shown, some SEM are too small to be observed. The numbers of replicates contributing to a mean are presented within bars. Statistical comparisons indicated by brackets are time effects across all treatments.

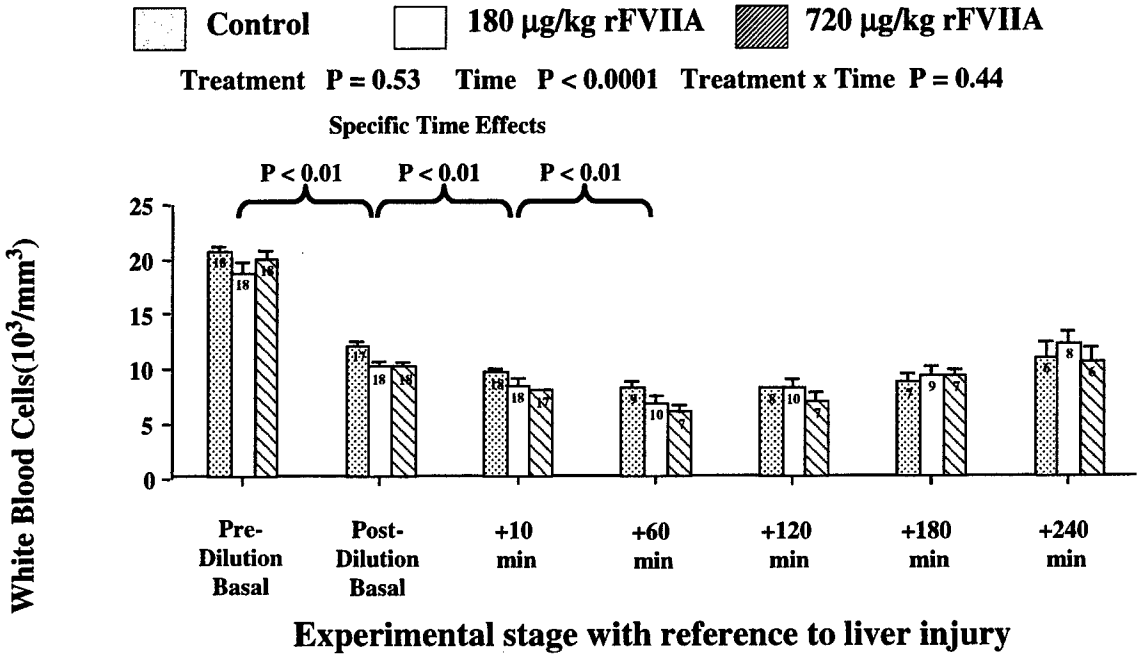

FIG 17. Hematocrit before and after severe liver injury. Bars represent means + SEM. At the scale shown, some SEM are too small to be observed. The numbers of replicates contributing to a mean are presented within bars. Statistical comparisons indicated by brackets are time effects across all treatments.

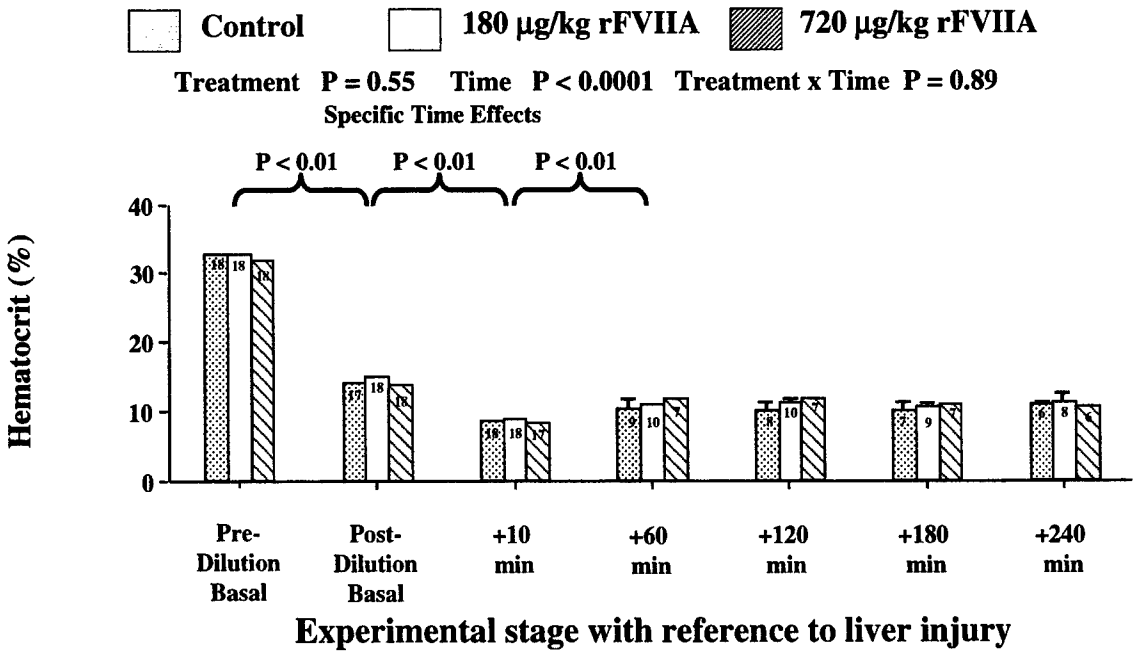

FIG 18. Hemoglobin concentrations before and after severe liver injury. Bars represent means + SEM. At the scale shown, some SEM are too small to be observed. The numbers of replicates contributing to a mean are presented within bars. Statistical comparisons indicated by brackets are time effects across all treatments.

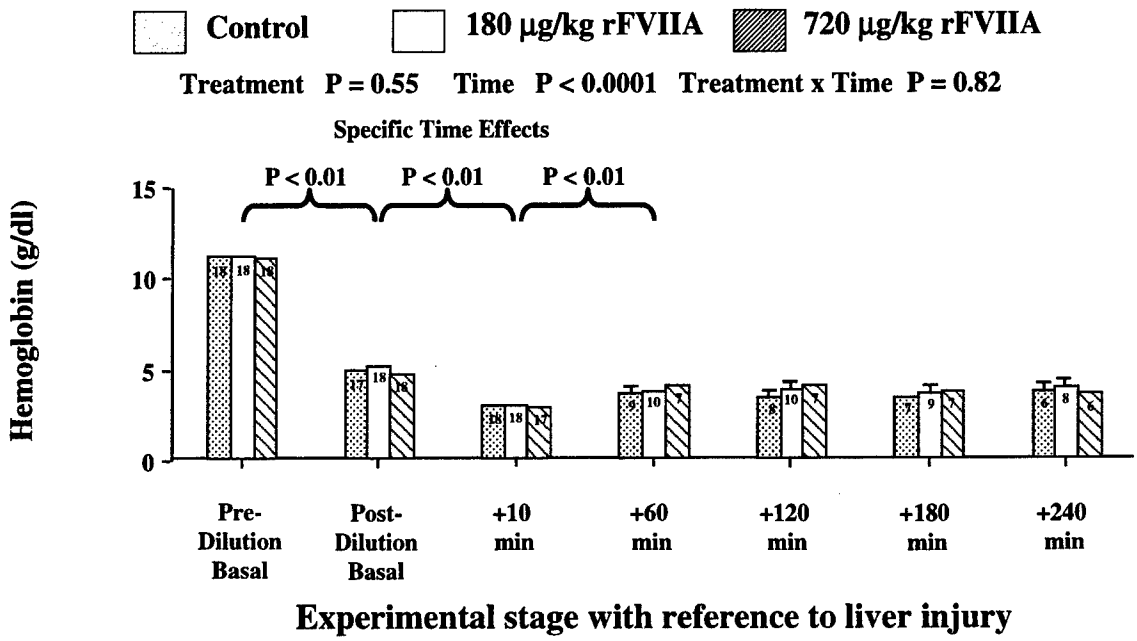

FIG 19. Peak inspiratory airway pressure measured in 25 of 54 pigs. Data points represent means ± SEM combined across all treatments. At the scale shown some SEM are too small to be observed. The number of replicates contributing to a mean is presented next to data points.

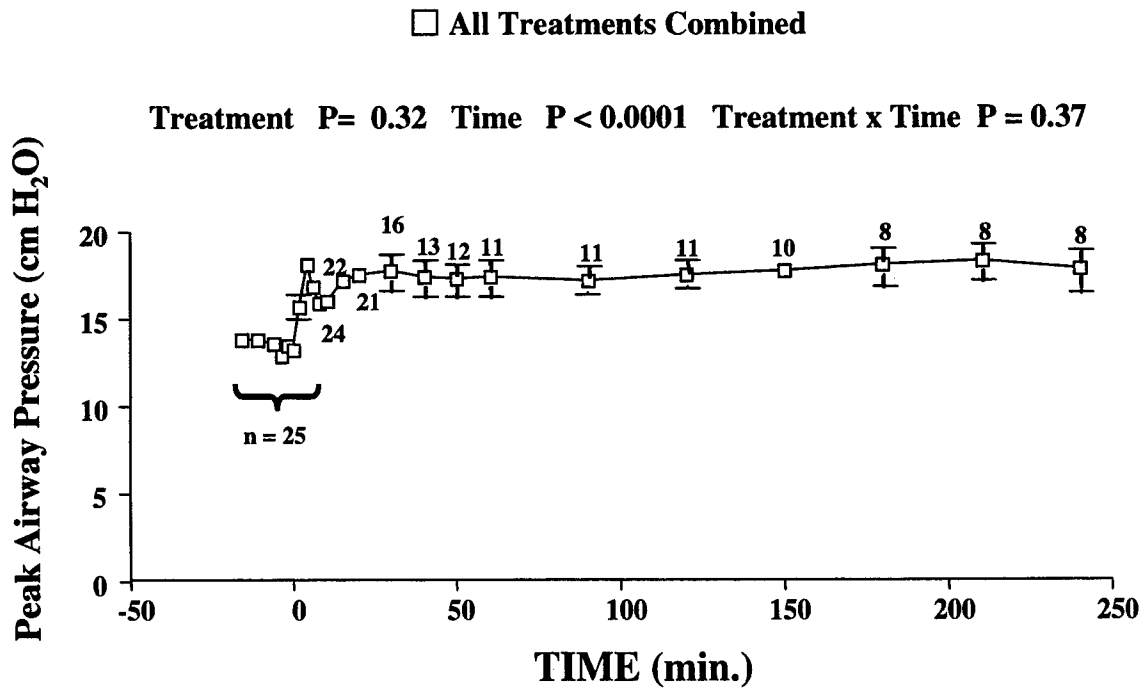

FIG 20. Peak inspiratory airway pressure measured in 54 pigs at each stage. Data points represent means combined across all treatments. At the scale shown SEM are too small to be observed.