Review
Preclinical trauma studies of recombinant factor VIIa

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Abstract

Preclinical studies in animals and ex vivo human blood have provided a solid rationale for conducting prospective randomized trials in trauma patients. Small animal models have been utilized to study the efficacy of recombinant activated factor VII (rFVIIa; NovoSeven®) in treating thrombocytopenic rabbits and for the reversal of anticoagulation. Safety models in the rabbit also exist to test for systemic activation of clotting and pathologic thrombosis. Animal models simulating traumatic injuries in humans have primarily been performed in pigs because of species similarities in terms of coagulation characteristics and the larger internal organs. The pig studies, utilizing human rFVIIa, have shown increased strength of clot formation, decreased bleeding, and improved survival. However, these findings are not uniform and are dependant on the model chosen. All of the animal models described have provided good safety data and suggest that the use of rFVIIa is not associated with systemic activation of coagulation or microthrombosis of end organs.

Introduction

Animal models utilizing human recombinant activated factor VII (rFVIIa; NovoSeven®; Novo Nordisk A/S, Bagsværd, Denmark) are limited by interspecies differences in terms of coagulation molecule interactions. The interaction between tissue factor and factor VIIa is species specific [1,2]. Human factor VII has been shown to have reduced activity when it is exposed to porcine thromboplastin. These considerations are compounded by the fact that the majority of standard in vitro coagulation assays are performed with rabbit brain thromboplastin. Both rabbit and human thromboplastin induce coagulation more efficiently in human plasma than in swine plasma [3]. Therefore, changes in coagulation parameters seen with the use of human rFVIIa in animals may not correlate with hemostatic end-points.

Because of these interspecies differences with regard to human rFVIIa activity, minimal conclusions concerning optimal dosing in humans can be drawn from animal experiments. In pharmacologic models the dose of rFVIIa needed for effect varies substantially between animal species, including the dog (60 µg/kg) [4], rabbit (2 mg/kg) [5], and mouse (10 mg/kg) [6].

Small animal models

Rabbits and rats are the basis of the small animal models. Tranholm and coworkers [5] produced thrombocytopenia in rabbits with γ radiation and platelet antibodies. Animals given 2 mg/kg rFVIIa had decreased time of bleeding and blood loss compared with control animals. There was no evidence of microthrombosis in the kidneys of these animals. The efficacy of reversing a bolus of low-molecular-weight heparin (LMWH) was studied in a rabbit ear puncture model [7]. Animals were given 1800 anti-factor X units/kg of LMWH, which raised the primary bleeding time approximately fourfold. rFVIIa did not reduce bleeding time or blood loss in this model. The absence of efficacy was hypothesized to be secondary to very high anti-factor Xa levels produced by bolus dosing of LMWH.

The efficacy of rFVIIa in reversing the effects of coumadin and the direct thrombin inhibitor melagatran has also been studied in a rat tail bleeding model. The use of rFVIIa resulted in decreased blood loss in animals given warfarin once but it did not reduce blood loss significantly in animals given warfarin twice [8]. In rats given melagatran, 1 mg rFVIIa resulted in a decreased bleeding time but no significant reduction in blood loss [9].

Studies assessing safety have also been performed in small animal models. The effect of rFVIIa administration on dissemination of clotting and thrombogenicity was investigated in the rabbit. In a model of stasis-induced thrombosis,

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FVII:C = factor VII clotting activity; LMWH = low-molecular-weight heparin; rFVIIa = recombinant activated factor VII.

administration of rFVIIa at a dose that enhanced clot formation at the site of injury resulted in no change in platelet count or the plasma concentration of antithrombin, indicating that rFVIIa was not associated with systemic activation of coagulation [10]. In an experimental model of arterial thrombosis, rFVIIa given in concentrations that decreased ear bleeding time and blood loss from incision sites in the liver and spleen did not affect arterial thrombosis [11].

Pig studies

Currently, three swine trauma models exist in the literature for the study of the efficacy and safety of rFVIIa. These models include a grade V liver injury model, a liver avulsion model, and an infrarenal aortotomy model. In addition to differences in the mechanism and site of injury, these models also differ with respect to animal temperature and coagulation status, timing and rate of resuscitation, timing and dose of therapy, and use of conventional therapies.

The first of these studies, reported by Martinowitz and coworkers [12], utilized a specially designed clamp to create a grade V liver injury based on American Association for the Surgery of Trauma organ injury scaling criteria (Fig. 1) [13]. Prior to injury, 10 animals underwent a 60% isovolemic exchange transfusion with 6% hydroxyethyl starch and cooling to 33°C. Thirty seconds after injury, blinded therapy, consisting of either 180 µg/kg rFVIIa or saline, was given and liver injuries were packed with gauze. Resuscitation with 40°C lactated Ringer's was initiated at 250 ml/min 5.5 min after injuries. Animals were resuscitated and maintained at their baseline blood pressure for the 1-hour study period. Treatment with rFVIIa resulted in a statistically significant reduction in blood loss from 976 cc to 527 cc. Treatment also resulted in significant reduction in prothrombin time, and elevation in the FVII clotting activity (FVII:C) was observed. Postmortem analysis revealed no evidence of large clots in the hepatic veins or inferior vena cava and no evidence of microthrombosis.

In a follow-up study, Schreiber and coworkers [14] repeated the analysis with minor differences in the model used. The 60% isovolemic hemodilution was performed with 5% albumin instead of hydroxylethyl starch to avoid the potential coagulopathic effects of starch solutions. The creation of the dilutional coagulopathy resulted in a hypotensive state with a starting mean arterial pressure of 45 mmHg. Blinded therapy, packing, and initiation of resuscitation were performed simultaneously at 30 s and animals were resuscitated with lactated Ringer's at 100 ml/min. The temperature of the resuscitation fluid was varied to maintain animal temperature at 33°C. Three groups of 10 animals were given either 180 µg/kg rFVIIa, 720 µg/kg rFVIIa, or an equivalent amount of buffer solution. Similar to the study conducted by Martinowitz and coworkers, animals treated with rFVIIa had significantly less blood loss than did controls (control animals 2187 ml, 180 µg/kg group 1085 ml, and 720 µg/kg group

Figure 1
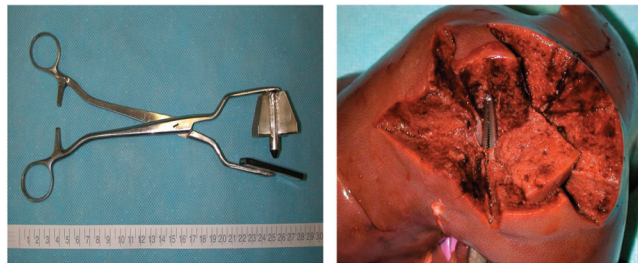
Clamp designed to create a grade V liver injury based on organ injury scaling criteria from the American Association for the Surgery of Trauma [12,13].

1086 ml). Nadir blood pressures following injury were significantly lower in control animals than in treated animals. Treated groups also had significantly lower prothrombin times and higher thrombin–antithrombin complexes, suggesting activation of thrombin. Death occurred in four out of 10 controls, three out of 10 in the 180 µg/kg group, and two out of 10 in the 720 µg/kg group. These differences did not achieve statistical significance. The only difference between the animals that received 720 µg/kg rFVIIa and 180 µg/kg rFVIIa was a dose-dependent increase in FVII:C levels following delivery of drug.

The same investigators also tested the efficacy of rFVIIa as sole therapy in noncoagulopathic, normothermic animals [15]. In this study, 30 animals were randomly assigned to receive either 150 µg/kg rFVIIa or an equivalent volume of buffer solution 30 s after injury. Animals were resuscitated with warmed lactated Ringer's solution at 100 ml/min initiated 15 min after injury to maintain their baseline blood pressure and normothermia (38°C) for the 2-hour study period. Liver injuries were not treated in this model. Despite documentation of a significant increase in FVII:C and decrease in prothrombin time, there was no difference in blood loss between groups. Blood pressure curves were similar and thrombin–antithrombin complexes became equally elevated between groups throughout the study. Potential explanations for the failure of rFVIIa to reduce blood loss in this model included the use of the drug as a sole agent in this large venous injury model and the normotensive state of the animals at the start of the study compared with the hypotensive state produced in the dilutional coagulopathy model.

Lynn and coworkers [16] described a reproducible grade IV liver injury whereby a clamp was utilized to crush and avulse the left median liver lobe and major vessels of the left lateral lobe. In the first study using this model, 13 animals were randomly assigned to receive 180 µg/kg rFVIIa or blinded placebo given when the mean arterial blood pressure dropped by 10% of baseline. The study was continued for 1 hour without resuscitation. Animals receiving rFVIIa had

significantly higher blood pressures after treatment than did control animals. Blood loss in the treatment group was 33 ml/kg versus 27 ml/kg in the control group ($P = 0.2$). Mortality was 43% in the control group and 0% in the treatment group ($P = 0.08$). All deaths occurred during the first 15 min of the study. Animals receiving rFVIIa had a significantly lower prothrombin time after treatment than did controls.

These investigators used the same model to study 24 animals divided into three groups [17]: control (group 1); 180 μg/kg rFVIIa (group 2); and 720 μg/kg rFVIIa (group 3). The study drug was again given following a 10% drop in mean arterial pressure following grade IV liver injury. This follow-up study was continued for 2 hours after treatment. Death occurred in four out of eight in group 1, two out of eight in group 2, and none of eight in group 3 during the first hour ($P = 0.02$ for control versus 720 μg/kg). All deaths in the first two groups occurred within 22 min and the only death in the third group occurred at 116 min. Blood loss was significantly less in group 3 than in group 1. Prothrombin times were significantly less in the treated groups than in the control group. Blood loss and prothrombin times were not statistically different between rFVIIa treated groups. There was no evidence of microthrombosis or macrothrombosis within viewed sections of kidney, mesentery of small intestine, small intestine wall, lung, heart, or brain in the treated groups.

The final animal study described in this review was designed by Sondeen and coworkers [18] to test the efficacy of rFVIIa in producing stronger clots and elevating the blood pressure at which rebleeding occurs in a pig aortotomy model. By elevating the blood pressure at which rebleeding occurs, rFVIIa would theoretically permit more effective resuscitation without increasing blood loss. Thirty pigs were randomly assigned to a control group, a 180 μg/kg group, or a 720 μg/kg group. Blinded treatment was administered 5 min before injury. A skin biopsy punch was used to make a 2 mm hole in the infrarenal aorta. Resuscitation was initiated 10 min after injury with lactated Ringer's at 100 ml/min. If rebleeding did not occur before the mean arterial pressure achieved a plateau after administration of 4 l resuscitation fluid, animals were given epinephrine (adrenaline) to raise the blood pressure to a maximum of 200 mmHg. Animals that failed to rebleed with this treatment were classified as non-rebleeders. Resuscitation was given to maintain the baseline blood pressure for the 2-hour study period.

The rebleeding blood pressure in the control group was significantly lower (45 mmHg) than that in the 180 μg/kg group (69 mmHg) and the 720 μg/kg group (66 mmHg) [18]. There was a trend toward greater rebleed volume in the control group (39 ml/kg in controls versus 22 ml/kg and 26 ml/kg for the 180 μg/kg and 720 μg/kg groups, respectively). Four of 10 animals required epinephrine in the high-dose group versus one out of 10 in each of the other

two groups. Significantly more animals in the control group (nine out of 10 animals) rebled at mean arterial pressures below baseline than in the rFVIIa groups (two out of 10 animals). rFVIIa-treated animals received significantly more fluid resuscitation before rebleeding than did control animals. Similar to prior studies, FVII:C exhibited a dose-related increase in the two treatment groups. Prothrombin times were equally lowered in the two treatment groups.

Ex vivo human blood studies

Cohort studies in humans have suggested that there is a population of patients who do not respond to treatment with rFVIIa [19,20]. This group of patients includes those extremely ill individuals with the lethal triad of hypothermia, coagulopathy, and acidosis who are on pressor therapy. It also includes those with irreversible hemorrhagic shock, who are unlikely to respond to any therapy. Based on these findings, Meng and coworkers [21] attempted to determine the effects of temperature and acidosis on factor VIIa activity utilizing blood from healthy adults. Factor VIIa activity on phospholipid vesicles and on platelets was measured by determining factor Xa generation in the presence and absence of tissue factor. Tissue factor dependent factor Xa generation increased with an increase in temperature from 24°C to 37°C, whereas tissue factor-independent factor Xa generation decreased to an equal degree. This resulted in no overall change in factor VIIa activity with changes in temperature. Alternatively, reduction in pH resulted in marked diminution in factor VIIa activity from both tissue factor dependent and independent mechanisms. The authors concluded that acidosis effects overall factor VIIa activity but that hypothermia does not.

Conclusion

The efficacy and safety of rFVIIa has been demonstrated in multiple small and large animal models. Because of species specific differences in the interaction between human rFVIIa and animal tissue factor, comments regarding dosing remain guarded. However, there does appear to be a maximum dose above which no additional benefit is achieved. Published *ex vivo* data suggest that rFVIIa may not be effective in severely acidotic environments. Preclinical data strongly support progression to prospective randomized trials in humans.

Competing interests

References
1. Janson TL, Stormorken H, Prydz H: **Species specificity of tissue thromboplastin.** *Haemostasis* 1984, **14:**440-444.
2. Kase F: **The effect of homo- and heterologous thromboplastins on plasmas of man, seven mammalian and two avian species: a comparative study.** *Comp Biochem Physiol* 1978, **61A:**65-68.
3. Pusateri AE, Ryan KL, Delgado AV, Martinez RS, Uscilowicz JM, Cortez DS, Martinowitz U: **Effects of increasing doses of activated recombinant factor VII on haemostatic parameters in swine.** *Thromb Haemost* 2005, **93:**275-283.
4. Brinkhous KM, Hedner U, Garris JB, Diness V, Read MS: **Effect of recombinant factor VIIa on the hemostatic defect in dogs**

with hemophilia A, hemophilia B, and von Willebrand disease. *Proc Natl Acad Sci USA* 1989, **86:**1382-1386.

5. Tranholm M, Rojkjaer R, Pyke C, Kristensen AT, Klitgaard B, Lollike K, Blajchman MA: **Recombinant factor VIIa reduces bleeding in severely thrombocytopenic rabbits.** *Thromb Res* 2003, **109:**217-223.

6. Tranholm M, Kristensen K, Kristensen AT, Pyke C, Rojkjaer R, Persson E: **Improved hemostasis with superactive analogs of factor VIIa in a mouse model of hemophilia A.** *Blood* 2003, **102:**3615-3620.

7. Chan S, Kong M, Minning DM, Hedner U, Marder VJ: **Assessment of recombinant factor VIIa as an antidote for bleeding induced in the rabbit by low molecular weight heparin.** *J Thromb Haemost* 2003, **1:**760-765.

8. Diness V, Lund-Hansen T, Hedner U: **Effect of recombinant human FVIIa on warfarin-induced bleeding in rats.** *Thromb Res* 1990, **59:**921-929.

9. Elg M, Carlsson S, Gustafsson D: **Effect of activated prothrombin complex concentrate or recombinant factor VIIa on the bleeding time and thrombus formation during anticoagulation with a direct thrombin inhibitor.** *Thromb Res* 2001, **101:**145-157.

10. Diness V, Bregengaard C, Erhardtsen E, Hedner U: **Recombinant human factor VIIa (rFVIIa) in a rabbit stasis model.** *Thromb Res* 1992, **67:**233-241.

11. Fattorutto M, Tourreau-Pham S, Mazoyer E, Bonnin P, Raphael M, Morin F, Cupa M, Samama CM: **Recombinant activated factor VII decreases bleeding without increasing arterial thrombosis in rabbits.** *Can J Anaesth* 2004, **51:**672-679.

12. Martinowitz U, Holcomb JB, Pusateri AE, Stein M, Onaca N, Freidman M, Macaitis JM, Castel D, Hedner U, Hess JR: **Intravenous rFVIIa administered for hemorrhage control in hypothermic coagulopathic swine with grade V liver injuries.** *J Trauma* 2001, **50:**721-729.

13. Moore EE, Cogbill TH, Jurkovich GJ, Shackford SR, Malangoni MA, Champion HR: **Organ injury scaling: spleen and liver (1994 revision).** *J Trauma* 1995, **38:**323-324.

14. Schreiber MA, Holcomb JB, Hedner U, Brundage SI, Macaitis JM, Hoots K: **The effect of recombinant factor VIIa on coagulopathic pigs with grade V liver injuries.** *J Trauma* 2002, **53:**252-257.

15. Schreiber MA, Holcomb JB, Hedner U, Brundage SI, Macaitis JM, Aoki N, Meng ZH, Tweardy DJ, Hoots K: **The effect of recombinant factor VIIa on noncoagulopathic pigs with grade V liver injuries.** *J Am Coll Surg* 2003, **196:**691-697.

16. Lynn M, Jerokhimov I, Jewelewicz D, Popkin C, Johnson EW, Rashid QN, Brown M, Martinowitz U, Cohn SM: **Early use of recombinant factor VIIa improves mean arterial pressure and may potentially decrease mortality in experimental hemorrhagic shock: a pilot study.** *J Trauma* 2002, **52:**703-707.

17. Jeroukhimov I, Jewelewicz D, Zaias J, Hensley G, MacLeod J, Cohn SM, Scalea TM: **Early injection of high-dose recombinant factor VIIa decreases blood loss and prolongs time from injury to death in experimental liver injury.** *J Trauma* 2002, **53:**1053-1057.

18. Sondeen JL, Pusateri AE, Hedner U, Yantis LD, Holcomb JB: **Recombinant factor VIIa increases the pressure at which rebleeding occurs in porcine uncontrolled aortic hemorrhage model.** *Shock* 2004, **22:**163-168.

19. Dutton RP, Hess JR, Scalea TM: **Recombinant factor VIIa for control of hemorrhage: early experience in critically ill trauma patients.** *J Clin Anesth* 2003, **15:**184-188.

20. Dutton RP, McCunn M, Hyder M, D'Angelo M, O'Connor J, Hess JR *et al.*: **Factor VIIa for correction of traumatic coagulopathy.** *J Trauma* 2004, **57:**709-718.

21. Meng ZH, Wolberg AS, Monroe DM, III, Hoffman M: **The effect of temperature and pH on the activity of factor VIIa: implications for the efficacy of high-dose factor VIIa in hypothermic and acidotic patients.** *J Trauma* 2003, **55:**886-891.