Efficacy of a New Recombinant Factor VIIa in Animal Models of Hemophilia

Alexandra Schiviz, Marlene Resch, Peter Leidenmühler, Bettina Bischetsrieder, Hartmut Ehrlich, Friedrich Scheiflinger, Hans-Peter Schwarz, Werner Höllriegl and Eva-Maria Muchitsch
Baxter BioScience, Vienna, Austria

Objective

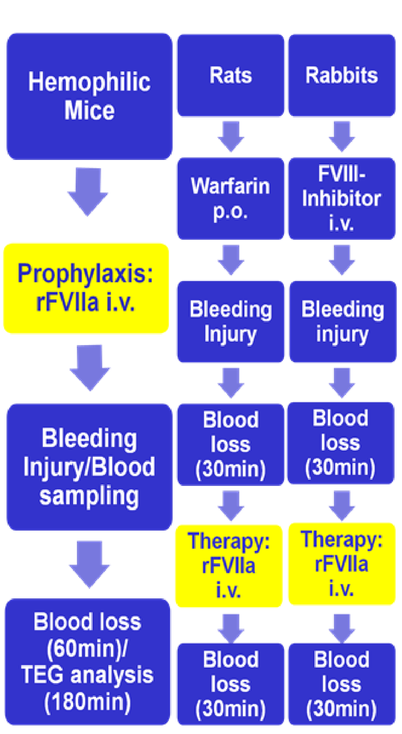
Baxter is developing a new recombinant factor VIIa (rFVIIa) product for treating patients with hemophilia A or B who have inhibitors. Our studies evaluated the efficacy of Baxter's new rFVIIa in hemophilia A (FVIII ko) and B (FIX ko) mice, warfarin-pretreated rats and factor VIII (FVIII)-inhibited rabbits. These studies were incorporated into a preclinical program including pharmacokinetic and safety studies[1,2].

Methods

Baxter's rFVIIa was tested at different doses to obtain dose-effect curves. A commercially available rFVIIa served as an active control and formulation buffer as a negative control item.
Mice received prophylactic treatment. Groups of 20 FVIII ko mice (B6;129S4-*F8*^{tm1Kaz}; 10m/10f) and 20 FIX ko mice (B6;129P2-*F9*^{tm1Dws}; 10m/10f) were used in a tail-tip bleeding model. Baxter's rFVIIa was tested at 3 doses (0.6-2.7mg/kg), the active control item at 2.7mg/kg. Blood loss [mg] was assessed over 60 min. The influence of rFVIIa on the thrombelastogram of hemophilic mice was assessed using 10 FVIII ko mice/group. Both items were tested at 3 doses (0.1-1.2mg/kg). Citrated whole blood was drawn by venipuncture of the V. cava caudalis. Blood was mixed with $CaCl_2$ and TEG analysis was started immediately. The main variable assessed was R-time [min], which is the latency from the time that the blood is placed in the TEG analyzer to initial fibrin formation. This represents the clot initiation phase and resembles the enzymatic portion of coagulation (function of coagulation factors).

Rats and rabbits were treated after infliction of the bleeding injury. 6 male Sprague Dawley rats/group were used in a tail-tip bleeding model. They were pretreated with 11mg/kg warfarin to deplete vitamin-K-dependent coagulation factors. Baxter's rFVIIa was tested at 3 doses (0.25-2mg/kg), the control item at 2.0mg/kg. 6 male New Zealand White rabbits/group were used in a nail-cut model. They were pretreated with a goat polyclonal FVIII-antibody to deplete endogenous FVIII. Baxter's rFVIIa was tested at 5 doses (0.1-3mg/kg), the control item at 2.0mg/kg. Relative blood loss [%] before (100%) and after (x%) treatment with the test or reference item was assessed in both models.

All animal experiments accorded with Austrian laws governing animal experimentation and were additionally approved by the Institutional Animal Care and Use Comittee (IACUC).



Results

Efficacy was defined as a bleeding phenotype statistically significantly different from buffer-treated animals. A linear, dose-dependent hemostatic effect ($p<0.0001$) of treatment with Baxter's rFVIIa was shown in all studies. Furthermore, reduction of blood loss or time to clotting after treatment with Baxter's rFVIIa did not differ from the licensed rFVIIa product administered at the same doses. The i.v. administration of Baxter's rFVIIa was well tolerated in all animals across all treatment groups without any signs of acute toxicity.

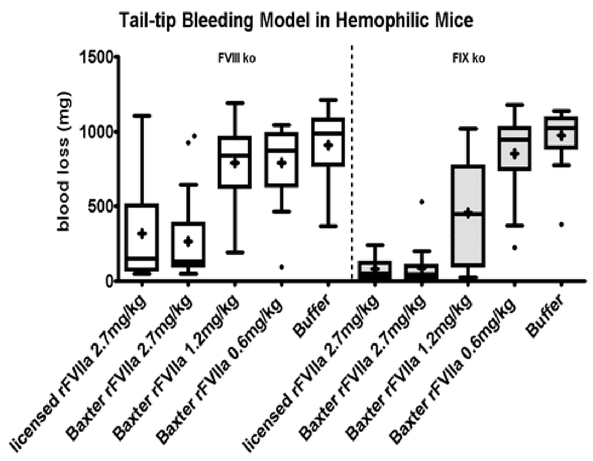


Fig. 1: Blood loss of rFVIIa-treated and buffer-treated FVIII ko and FIX ko mice

In both mouse strains, treatment with Baxter's rFVIIa led to a dose-dependent reduction of mean blood loss ($p<0.0001$) compared with buffer-treated animals (908mg/972mg). The minimum effective dose was 2.7mg/kg in FVIII ko mice (white; 266mg) and 1.2mg/kg in FIX ko mice (grey;458mg).

Efficacy of 2.0mg/kg of Baxter's rFVIIa and the licensed rFVIIa did not differ significantly ($p\geq0.7213$; 266mg vs. 318mg/84mg vs. 81mg).

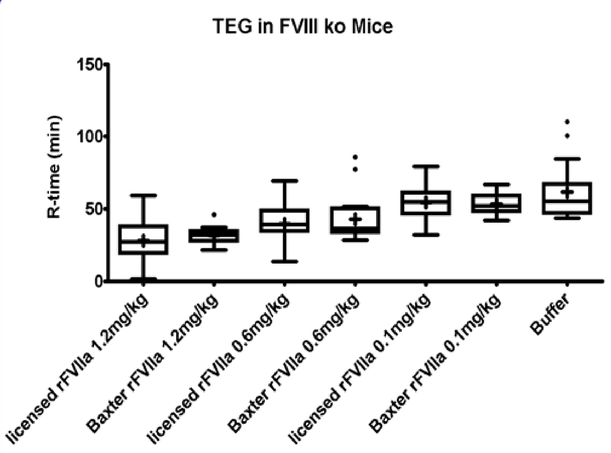


Fig. 2: Time to clotting (R-time) of rFVIIa-treated and buffer-treated FVIII ko mice

Mean R-time in buffer-treated animals was 61.5min. Treatment with Baxter's rFVIIa led to a dose-dependent reduction of R-time ($p<0.0001$). The minimum effective dose was 0.6mg/kg (42.7min).

R-times of groups treated with the same doses of either Baxter's rFVIIa or the licensed rFVIIa did not differ significantly ($p\geq0.5392$; 31.6min vs. 27.9min/42.7min vs. 39.6min/53.3min vs. 54.4min).

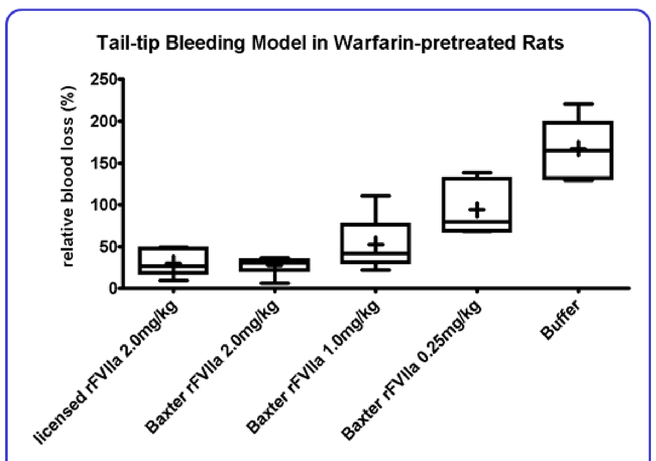


Fig. 3: Blood loss in anticoagulated rats after therapeutic treatment with rFVIIa or buffer

Blood loss after the first collection period (BL1) in animals treated with warfarin was assessed as 100%. The proportion of blood loss in the second blood sampling period (BL2) after treatment with test or control items was calculated by comparison with the first collection period (BL2/BL1x100).

Treatment with Baxter's rFVIIa led to a dose-dependent reduction of mean relative blood loss ($p<0.0001$) compared with buffer-treated animals (167%). The minimum effective dose was 0.25mg/kg (94%).

Efficacy of 2.0mg/kg of Baxter's rFVIIa and the licensed rFVIIa did not differ significantly ($p=0.7123$; 27% vs. 30%).

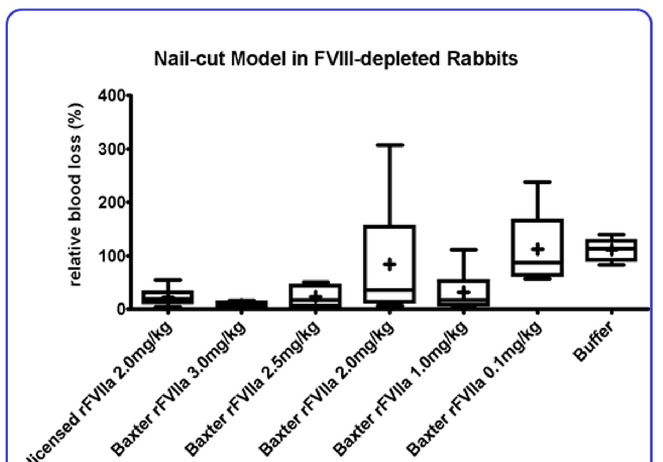


Fig. 4: Blood loss in FVIII-depleted rabbits after therapeutic treatment with rFVIIa or buffer

Relative blood loss was assessed as described in Fig. 3.

Treatment with Baxter's rFVIIa led to a dose-dependent reduction of mean relative blood loss ($p<0.0001$) compared with buffer-treated animals (111%). 2.5mg/kg (23%) was statistically evaluated as the minimum effective dose. However, inter-animal variation was high.

Efficacy of 2.0mg/kg of Baxter's rFVIIa and the licensed rFVIIa did not differ significantly ($p=0.2379$; 84% vs. 23%), nor was any statistically significant difference shown after treatment with 1.0mg/kg (32%) and 2.5mg/kg (23%).

Conclusions

- **A dose-dependent hemostatic effect of Baxter's rFVIIa could be shown in all primary pharmacodynamic studies.**
- **Furthermore, statistical evaluation of the efficacy of Baxter's rFVIIa did not reveal any statistically significant differences from the commercially available rFVIIa product after treatment at the same doses.**
- **The results of our studies show that Baxter's new rFVIIa is prophylactically and therapeutically effective in animal models closely reflecting the conditions in hemophilia A or B patients with inhibitors.**

References

1. Dietrich et al. (2011) Preclinical safety of Baxter's recombinant Factor VIIa, GTH 2011,P02-4
2. Höbarth et al. (2011) Single dose pharmacokinetics of a recombinant FVIIa in Factor VIII ko mice, rats and cynomolgus monkeys, GTH 2011, P02-6

Poster presented at the WFH – World Federation of Hemophilia Congress, July 8-12, 2012, France, Paris

Baxter BioScience

DOI: 10.3252/pso.eu.WFH2012.2012