Repeated Application of Baxter's Recombinant Factor IX in Rats and Macaques

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Introduction

Baxter is developing a recombinant factor IX (rFIX) product which is produced by a genetically engineered Chinese hamster ovary (CHO) cell line in a cell culture medium free from any animal or human proteins. The objective of the studies in rats and macaques was to assess the safety and to determine the no observed adverse effect level (NOAEL) after systemic administration of Baxter's rFIX.

Methods

Assessment of toxicity was performed in rats and macaques after repeated administration of rFIX and a recovery phase. Animals were treated with Baxter's rFIX at 200 IU/kg/dose or 750 IU/kg/dose or with licensed rFIX at 200 IU/kg/dose for 4 weeks. Safety endpoints were body weight, clinical condition, clinical pathology, food consumption, urinalysis, ophthalmic status, organ weights, and macroscopic and microscopic pathology investigations. Additionally, toxicokinetics and anti-product antibody formation (AB Formation) were investigated. All animal experiments accorded with either Austrian or UK laws governing animal experimentation and were additionally approved by the Institutional Animal Care and Use Committee (IACUC).

Repeated Dose Toxicity Including TK in Rats and Macaques

Dose : Baxter's rFIX IU/kg BW*	Species	Animals / Group	Study Duration
200, 750	Rat	15 male / 15 female (Toxicity) 9 male / 9 female (TK) 10 male (AB Formation)	28 days (dosing every other day), 2 weeks' recovery
200, 750	Macaque	5 male / 5 female (Toxicity, TK, AB Formation)	28 days (dosing every other day), 2 weeks' recovery

* commercially available rFIX was used as the comparator product

Endpoints	
Macaques	**Rat**
clinical signs, body weights, ophthalmoscopy, cardiovascular investigations, clinical pathology, toxicokinetics, analysis of FIX activity in plasma, analysis of neutralizing and binding antibodies in plasma, organ weights, necropsy observations, and histopathological examinations.	clinical signs, body weights, ophthalmoscopy, clinical pathology, sperm analysis, toxicokinetics, analysis of FIX activity in plasma, analysis of neutralizing and binding antibodies in plasma, organ weights, necropsy observations, and histopathological examinations.

Conclusions

- Baxter's new rFIX was well tolerated in rats and macaques.
- macaques and rats did not show any signs of toxicity after 14 injections every other day at doses of 200 or 750 IU/kg.
- The no observed adverse effect level (NOAEL) in rats and macaques was 750 IU/kg.
- The good safety profile of Baxter's rFIX was the basis for proceeding with trials in humans

Results

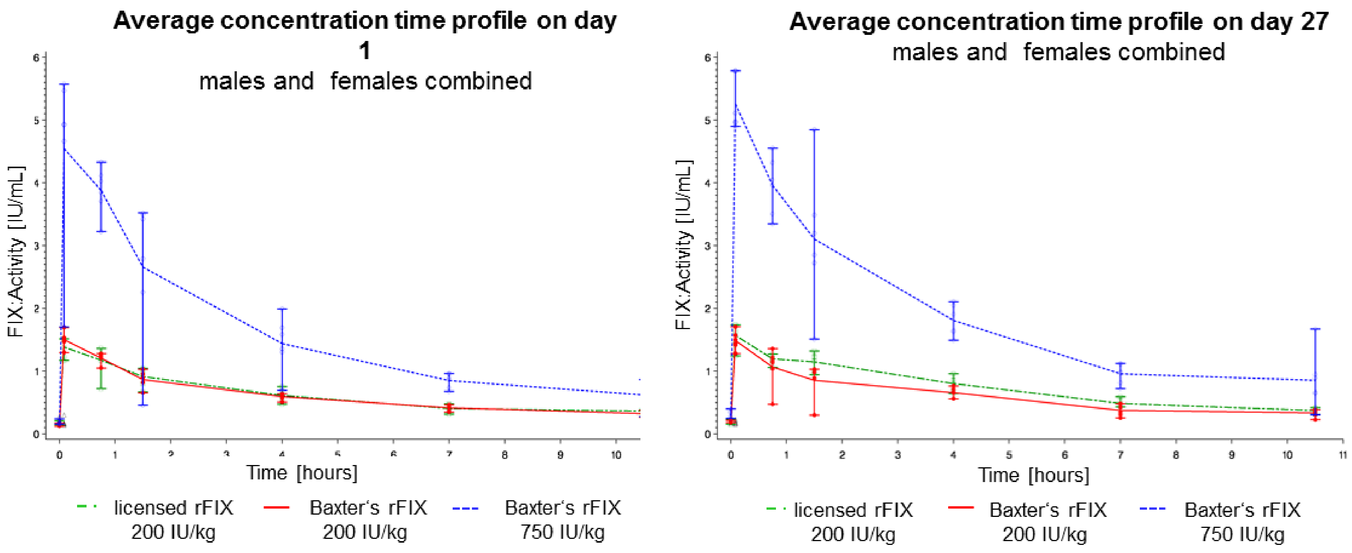
Repeated Dose Toxicity Study in rats

Treatment of rats with Baxter's rFIX had no adverse effects on the clinical condition of the animals, their body weight development, food consumption, ophthalmologic status, and clinical pathology variables or the macroscopic and microscopic examination.

- **NOAEL for rats following a 28-day period of treatment: 750 IU/kg**

Toxicokinetic analysis confirmed exposure throughout the entire study period.
There were no binding anti-human FIX antibodies detectable in any of the treatment groups after the end of the treatment period on day 28. Low titers of binding anti-human FIX antibodies were detected in single animals after the recovery phase following treatment with rFIX. The titers for neutralizing antibodies were below the detection limit in these animals.

Toxicokinetics in Rats



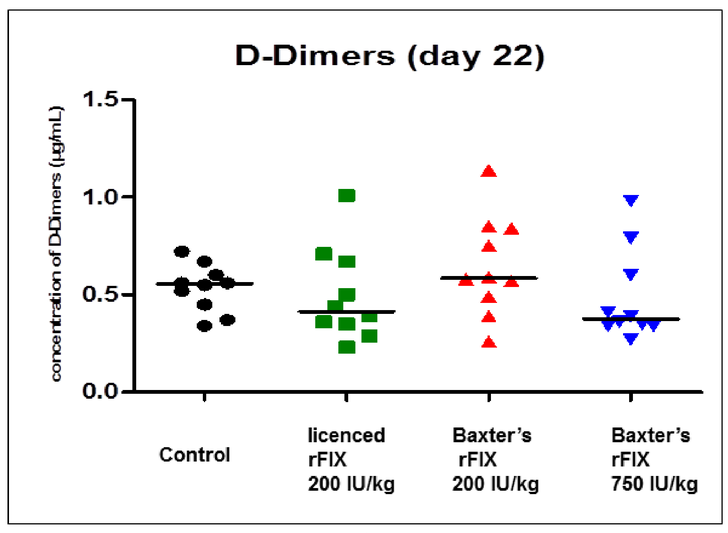
Repeated Dose Toxicity Study in Macaques

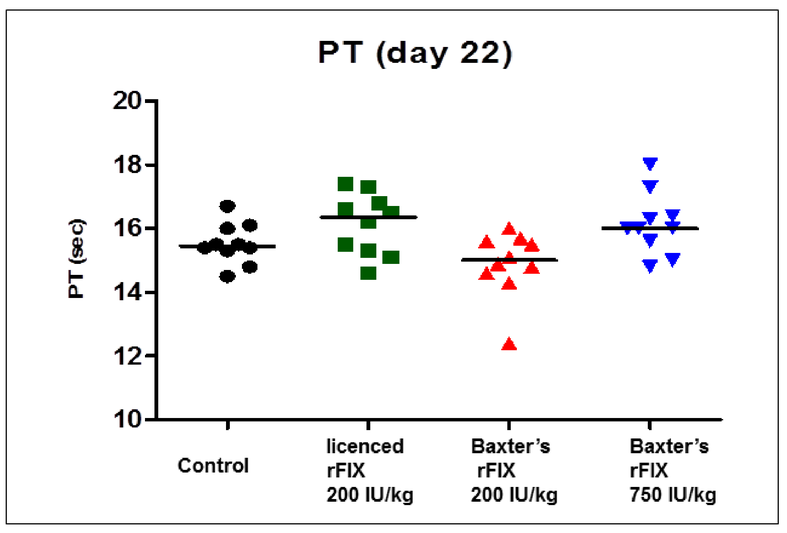
Repeated intravenous administration of Baxter's rFIX at 200 or 750 IU/kg/dose every other day for 4 weeks did not result in any evidence of adverse effects. There were no apparent changes in standard coagulation variables (prothrombin time (PT), activated partial thromboplastin time (APTT), fibrinogen) and D-dimers in the treated animals when compared with the controls or corresponding predose values.

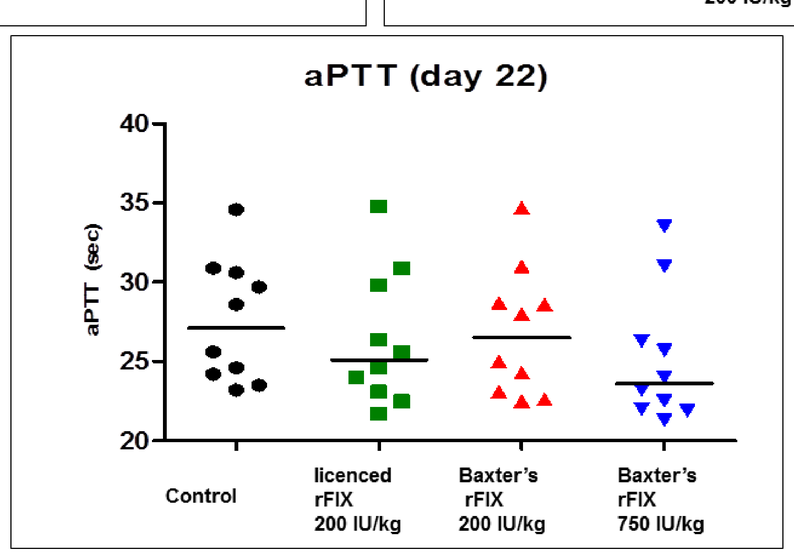
- **NOAEL for macaques following a 28-day period of treatment: 750 IU/kg**

Toxicokinetic analysis confirmed exposure within the whole study period. Systemic exposure of rFIX activity increased in an approximately dose-proportional manner across the dose range. No apparent sex-related differences were observed. Toxicokinetics revealed similar results for Baxter's rFIX and licensed rFIX.
As expected, binding antibodies were detectable in single animals treated with Baxter's rFIX or licensed rFIX. None of the antibodies had neutralizing activity except in one animal treated with licensed rFIX.

Coagulation variables in Macaques







Disclosure The authors of this presentation make the following disclosure of financial or personal relationships with commercial entities that may have a direct or indirect interest in the subject matter of this presentation: Barbara Dietrich, Wilfried Auer, Martin Wolfsegger, Hartmut J. Ehrlich, Friedrich Scheiflinger, Hans Peter Schwarz and Eva-Maria Muchitsch are full-time employees of Baxter Innovations GmbH, Vienna, Austria.

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