Single dose Pharmacokinetics of a PEGylated Variant of Recombinant FVIII in Factor VIII ko Mice, Rats and Macaques

Gerald Höbarth, Susan Kubik, Martin Wolfsegger, Alfred Weber, Herbert Gritsch, Werner Höllriegl, Alexandra Schiviz, Hartmut J. Ehrlich, Friedrich Scheiflinger, Peter L. Turecek, Hans-Peter Schwarz, Eva-Maria Muchitsch
Baxter Innovations GmbH, Vienna, Austria

Objective

Baxter and Nektar are developing a recombinant FVIII (rFVIII) called BAX 855 which is modified with polyethylene glycol (PEGylation) to prolong its circulation in blood.

Our studies evaluated the pharmacokinetic profile of Baxter's longer acting rFVIII in Factor VIII ko mice, Sprague Dawley rats and macaques. These studies were incorporated into a preclinical program including safety and efficacy studies[1, 2, 3, 4].

Methods

Baxter's longer acting rFVIII (BAX 855) and Advate, a licensed rFVIII product, were tested at a dose of 200 IU/kg in mice and rats. Both items were tested at a dose of 350 IU/kg in macaques. Mean residence time (MRT), terminal half-life ($t_{½}$), total clearance per kg body mass (CL), $AUC_{0\text{-tlast}}$ (the area under the concentration vs. time curve from 0 to the last measured time point) and in vivo recovery (IVR) were evaluated. Citrated plasma samples were analyzed for FVIII activity (chromogenic assay) in mice and monkeys and FVIII antigen (FVIII ELISA) in rats. Additionally, FVIII–bound PEG (PEG-FVIII ELISA) was analyzed in all species.

Conscious restrained FVIII ko mice (B6;129S4-*F8*^{tm1Kaz}) received Baxter´s PEGylated rFVIII or Advate as a single bolus injection via the lateral tail vein. 16 (BAX 855) and 8 (Advate) animals per time point were bled by cardiac puncture under anesthesia for blood sampling 5 min, 1, 3, 6, 9, 16, 24, 32, 40 and 48 hours after the administration of the test items following a serial sacrifice design.

Anesthetized rats (isoflurane) received an intravenous bolus injection of BAX 855 or Advate via the lateral tail vein. A single animal design was used with 16 (BAX 855) and 8 (Advate) animals. Blood samples for analysis of FVIII levels in citrated plasma were drawn from the ventral tail artery at base line, 5 minutes, 1, 4, 8, 12, 22, 32 and 48 hours after item administration.

Eight (BAX 855) and four (Advate) macaques received Baxter´s longer acting rFVIII or Advate by intravenous (bolus) injection into the saphenous vein. Blood samples were withdrawn from a suitable vein at the following timepoints: base line, 5, 30 minutes, 2, 6, 12, 24, 48, 60, 72, 96, 108 and 120 hours after dosing.

FVIII activity was measured with a chromogenic assay specific for FVIII (Immunochrom, Baxter in-house reagents). FVIII antigen was measured by ELISA using commercially available polyclonal anti-human factor VIII antibodies (Cedarlane, Birlington, Ontario, Canada). PEG-FVIII ELISA was done to check for the presence of PEGylated FVIII and measure its concentration. The principle of the assay included capturing PEGylated FVIII via its PEG moiety to the plate (generated and purified in-house) followed by the binding of a specific anti-FVIII antibody (Cedarlane, Birlington, Ontario, Canada). The integrity of PEGylated FVIII could be confirmed as both components of the conjugate, PEGylation and immuno-reactivity were required to yield an assay signal.
All statistical analyses were performed with SAS Version 8.2 for Linux.

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

References

1. Piskernik et al (2012) Preclinical safety pharmacology of a PEGylated variant of recombinant factor VIII
2. Höllriegl et al. (2012) Efficacy of a PEGylated Variant of Recombinant Factor VIII in Mouse Models of Hemophilia A
3. Spatzenegger et al. (2012) Safety of a PEGylated variant of recombinant factor VIII after repeated application in rats and macaques
4. Stidl et al. (2012) Absorption, Metabolism, Distribution, and Excretion of a PEGylated Variant of Recombinant Factor FVIII Following Intravenous Administration to Rats

Results

The results of pharmacokinetic studies of Baxter's rFVIII in FVIII ko mice, rats and macaques revealed that plasma concentrations of FVIII activity, FVIII antigen and FVIII-bound PEG declined in a bi-phasic manner. A prolongation in MRT of BAX 855 compared with Advate could be demonstrated in all species. FVIII activity analysis showed an increase in MRT from 4.9 to 7.9 hours in mice and from 7.5 to 11.5 hours in monkeys. This prolongation was also reflected in the terminal $t_{½}$ (4.3 to 5.9h in mice; 5.7 to 9.4h in monkeys). A lower clearance for BAX 855 than for Advate could be observed consistently. Similar pharmacokinetic results could be shown for FVIII-bound PEG in all three preclinical models and for FVIII antigen in rats.

In hemophilic mice a prolongation of MRT and the terminal $t_{½}$ of BAX 855 vs. Advate was observed (Table 1, Fig. 1).

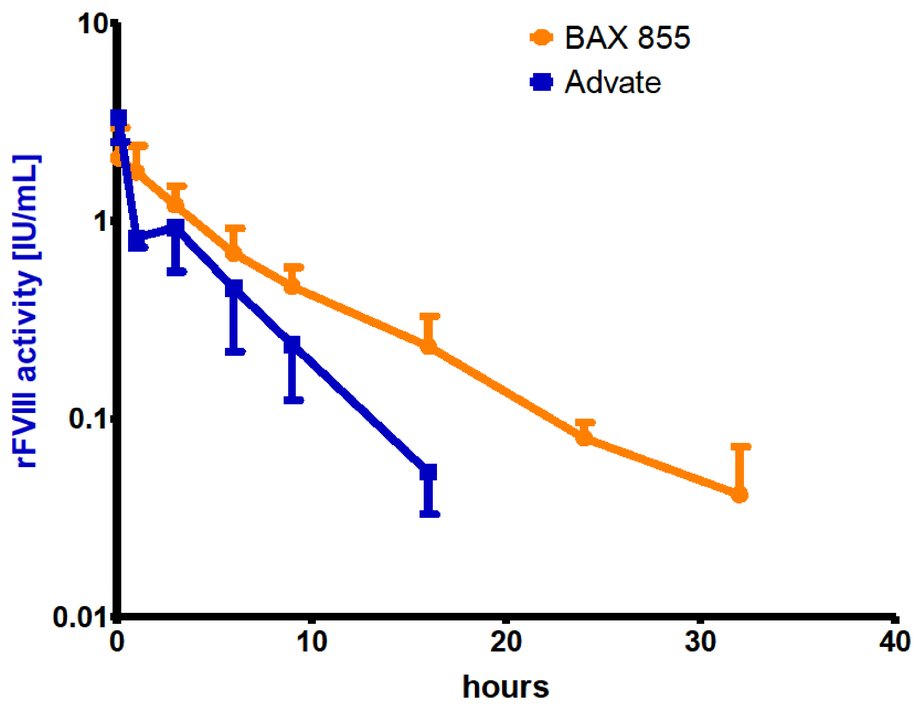

Fig. 1: FVIII activity (mice) time profiles (mean) were measured for BAX 855 (orange) and Advate (blue)

Table 1: FVIII activity / FVIII antigen and FVIII-bound PEG estimates of pharmacokinetic parameters of Baxter´s PEGylated rFVIII in rodents

			$AUC_{0\text{-tlast}}$	MRT [h]	terminal half-life [h]	IVR [%]	CL [mL/h/kg]
Mice	Advate	FVIII activity	0.042	4.9	4.3	69.5	22.1
	BAX 855	FVIII activity	0.080	7.9	5.9	56.3	12.2
		FVIII-bound PEG	0.050	8.0	6.1	62.5	16.9

$AUC_{0\text{-tlast}}$ [IU/mL*h / IU/kg] or [ng/mL*h / ng/kg]

In animals with a physiological endogenous level of FVIII, Sprague Dawley rats and macaques, a prolongation of MRT and the terminal $t_{½}$ of BAX 855 vs. Advate was observed (Tab. 2).

Table 2: FVIII antigen (rats) and activity (monkeys) and FVIII-bound PEG PK profile (geometric means) for Baxter´s longer acting rFVIII after adjustment for endogenous FVIII concentration

			$AUC_{0\text{-tlast}}$	MRT [h]	terminal half-life [h]	IVR [%]	CL [mL/h/kg]
Rats	Advate	FVIII antigen	0.050	6.2	5.5	47.1	19.1
	BAX 855	FVIII antigen	0.071	7.5	6.1	41.7	13.3
		FVIII-bound PEG	0.051	10.7	8.3	35.1	15.1
Monkeys	Advate	FVIII activity	47.2	7.5	5.7	86.0	8.1
	BAX 855	FVIII activity	68.7	11.5	9.4	88.6	4.9
		FVIII bound PEG	1942	18.9	19.0	107.0	4.7

$AUC_{0\text{-tlast}}$ [IU/mL*h] or [ng/mL*h]

Conclusions

- A prolongation in MRT and terminal half-life as well as an increase of $AUC_{0\text{-tlast}}$ of BAX 855 compared with Advate after IV administration was observed in FVIII ko mice, Sprague Dawley rats and macaques
- The integrity of the PEGylated recombinant FVIII-protein was confirmed by the FVIII-bound PEG analysis
- Pharmacokinetic data indicated that PEGylation of human rFVIII increases the circulation time of FVIII activity and FVIII antigen

WORLD FEDERATION OF HEMOPHILIA
FÉDÉRATION MONDIALE DE L'HÉMOPHILIE
FEDERACIÓN MUNDIAL DE HEMOFILIA
Treatment for All

Poster presented at the WFH 2012 World Congress
July 8-12, 2012 Paris, France

Disclosure

All authors are employees of Baxter Innovations GmbH

Baxter BioScience

WFH2012
175--Tu
Pharmacovigilance
Gerald Hbarth
DOI: 10.3252/pso.eu.WFH2012.2012
Poster presented at: WFH 2012 WORLD CONGRESS PARIS, FRANCE
PosterSessionOnline.com