Efficacy of a PEGylated Variant of Recombinant Factor VIII in Mouse Models of Hemophilia A

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Objective

FVIII concentrates are used in patients with hemophilia A to treat and prevent bleeding episodes. Multiple prophylactic administrations are necessary each week to maintain a FVIII level of at least 1% of normal. A longer acting FVIII concentrate would reduce the frequency of infusions. Baxter and Nektar are developing a recombinant FVIII (rFVIII) modified with polyethylene glycol (PEGylation) to achieve longer circulation (BAX 855).

Our studies evaluated the efficacy of BAX 855 in two different pharmacodynamic models in hemophilia A (FVIII ko) mice: the tail-tip bleeding model and the carotid occlusion model. These studies were incorporated into a preclinical program including pharmacokinetic and safety studies.

Methods

The aim of the presented studies was to evaluate the efficacy of BAX 855 over time. Advate, a commercially available rFVIII, served as an active control and formulation buffer as a negative control item.

The dose of 200 IU/kg rFVIII was chosen based on the results of a dose-response study with rFVIII in the tail cut model. In this study, 200 IU/kg led to the least inter-individual variation of all efficacious doses tested[1].

The test or reference items were administered prophylactically 12 to 40h and buffer was administered 5 to 15min before the tip of the tail was cut off or the endothelium was denuded.

Animals were anesthetized using ketamine and xylazine and humanely killed by cervical dislocation immediately after the end of the observation period. All animal experiments accorded with Austrian laws governing animal experimentation and were additionally approved by the Institutional Animal Care and Use Committee (IACUC).

16 FVIII ko mice (B6.129S4-*F8*^{tm1Kaz}; 8m/8f) per group were used in the tail-tip bleeding model. The tip of the tail was cut off and total blood loss [mg] was assessed over 60min.

10 FVIII ko mice (5m/5f) per group were used in the carotid occlusion model. The left carotid artery was exposed and the endothelium was denuded by topical application of $FeCl_3$. Time to occlusion [min] was assessed. The observation period was 30min.

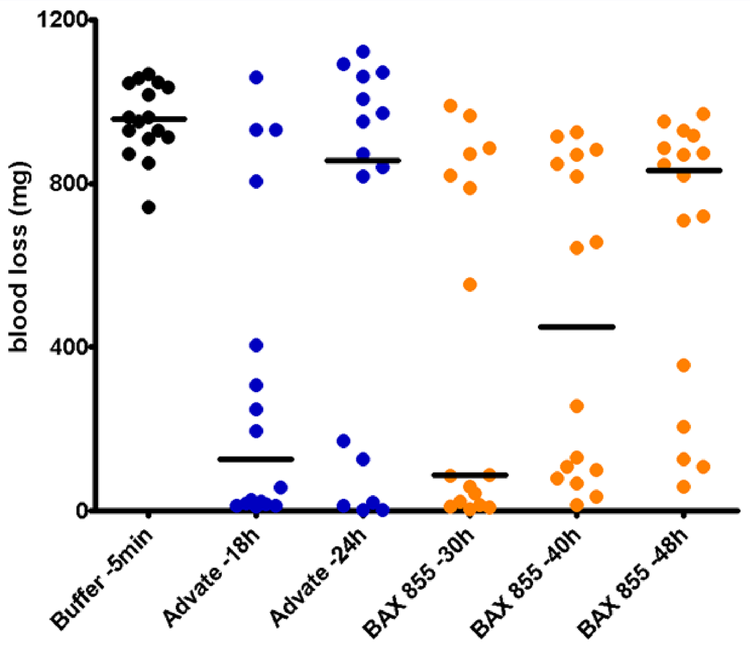

Fig. 1: Blood loss of BAX 855 or Advate-treated FVIII ko mice in the tail-tip bleeding model

Median total blood loss in buffer-treated animals was 951mg (black). Efficacy is shown by a distinct decrease in blood loss.

Efficacy for Advate (blue) was shown in animals treated 18h before the tail cut (121mg). Efficacy for BAX 855 (orange) was shown in animals treated 30h before the tail cut (73mg), but efficacy in animals treated 40h before the tail cut was questionable, as the median total blood loss was 436mg. Efficacy could not be shown for longer treatment intervals (Advate -24h and BAX 855 -48h).

The prolongation of efficacy of BAX 855 observed was additionally tested by pair-wise comparison of mean total blood loss for BAX 855 -30h versus Advate -18h. This comparison did not show a statistically significant difference ($p = 0.6383$) and thus, similar efficacy at these time points can be concluded.

Results

Efficacy was defined as a bleeding phenotype markedly different from buffer-treated animals.

Buffer-treated animals had a median blood loss of 951mg in the tail-tip bleeding model and did not show vessel occlusion within 30min in the carotid occlusion model.

In the tail-tip bleeding model, a prophylactic effect of Advate could be observed for up to 18h. Prophylactic efficacy after treatment with BAX 855 could be observed for up to 40h.

In the carotid occlusion model, treatment with Advate 12h before the experiment shortened the time to occlusion to 3.8min.

With BAX 855, a similar efficacy could be observed for up to 24h after administration.

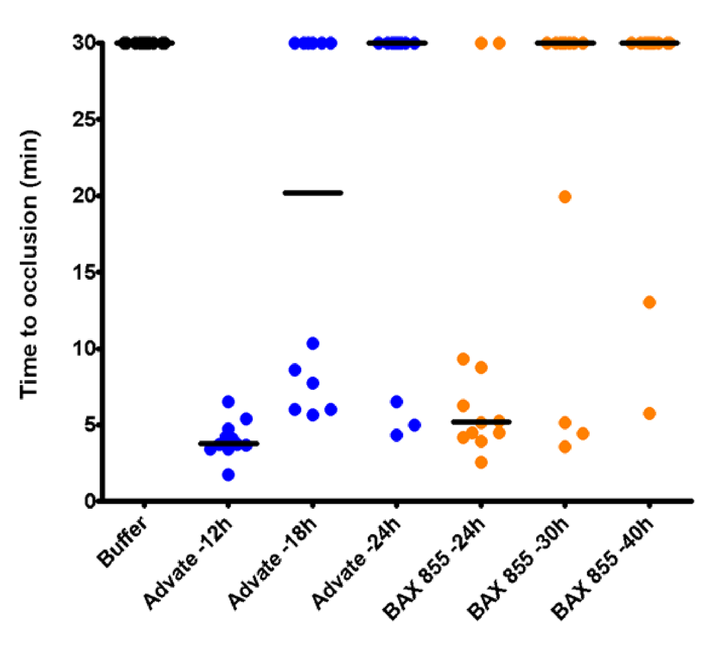

Fig. 2: Time to occlusion in BAX 855 or Advate-treated FVIII ko mice in the carotid occlusion model

No vessel occlusion was observed in buffer-treated animals (black) within the observation period of 30min. Efficacy is shown by a distinct decrease in time to occlusion.

Advate (blue) was considered efficacious when administered 12h prior to the experiment (median time to occlusion of 3.8min). Time to occlusion was 20.2min in animals treated 18h before the endothelium was denuded, already showing decreasing efficacy.

BAX 855 (orange) was considered efficacious when administered 24h prior to the vessel injury (5.2min). After 30 hours, efficacy was minor and not considered to be clinically relevant, although statistically significantly different from buffer. At later time points (Advate -24h and BAX 855 -40h), no efficacy could be observed anymore.

Conclusions

- **BAX 855 was efficacious in mouse models of hemophilia A.**
- **The efficacy of BAX 855 was prolonged compared with that of an unmodified rFVIII.**

References

1. Baumgartner et al (2010) Optimization, refinement and reduction of murine in vivo experiments to assess therapeutic approaches for haemophilia A. Laboratory Animals 44: 211–217

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