Safety of a PEGylated variant of recombinant factor VIII after repeated application in rats and macaques

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Introduction

Factor VIII (FVIII) is a critical component of the intrinsic coagulation pathway. FVIII concentrates are used in patients with hemophilia A to provide a hemostatic FVIII level sufficient to treat and prevent bleeding episodes.
Prophylactic treatment regimens aim to maintain a FVIII level of at least 1% of normal to effectively prevent or reduce spontaneous bleeding episodes. The desired FVIII level can only be maintained by administering several infusions weekly depending on the individual pharmacokinetic profile, the bleeding phenotype, and the individual life style.

A longer acting FVIII concentrate would reduce the frequency of infusions, increase compliance and reduce the number of musculoskeletal bleeds with long-term sequelae and life-threatening bleeds.
Baxter and Nektar have developed BAX 855, a PEGylated form of Baxter's recombinant FVIII (rFVIII) product based on the Advate manufacturing process. The product is derived from a CHO cell line using a plasma-protein-free method and a virus inactivation step.
The objective of this preclinical program was to evaluate the safety of BAX 855 in different species. The systemic toxic potential of BAX 855 was tested in rats and macaques.

Methods

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

Macaques:

Two lots of BAX 855 were administered intravenously to groups of 5 male and 5 female animals at doses of 150, 350 and 700 IU/kg every fifth day for a period of 4 weeks. 2 males and 2 females of each group were monitored over a treatment-free period of 2 weeks.

Rats:

Two lots of BAX 855 were administered intravenously to groups of 10 male and 10 female animals at doses of 350 and 700 IU/kg every other day for a period of 28 days. 5 additional males and 5 additional females of each group were monitored over a treatment-free period of 2 weeks.

Endpoints

Macaques	Rats
Clinical signs, body weights, ophthalmic examinations, toxicokinetics, binding and neutralizing antibodies, hematology, clinical chemistry, urine analysis, histopathology	Clinical signs, body weights, ophthalmic examinations, toxicokinetics, binding and neutralizing antibodies, hematology, bone marrow smears, clinical chemistry, urine analysis, histopathology, male reproductive evaluation

Conclusions

- **Treatment with BAX 855 was well tolerated in macaques and rats.**
- **The NOAEL for both studies was 700 IU/kg, the highest dose tested.**

Results

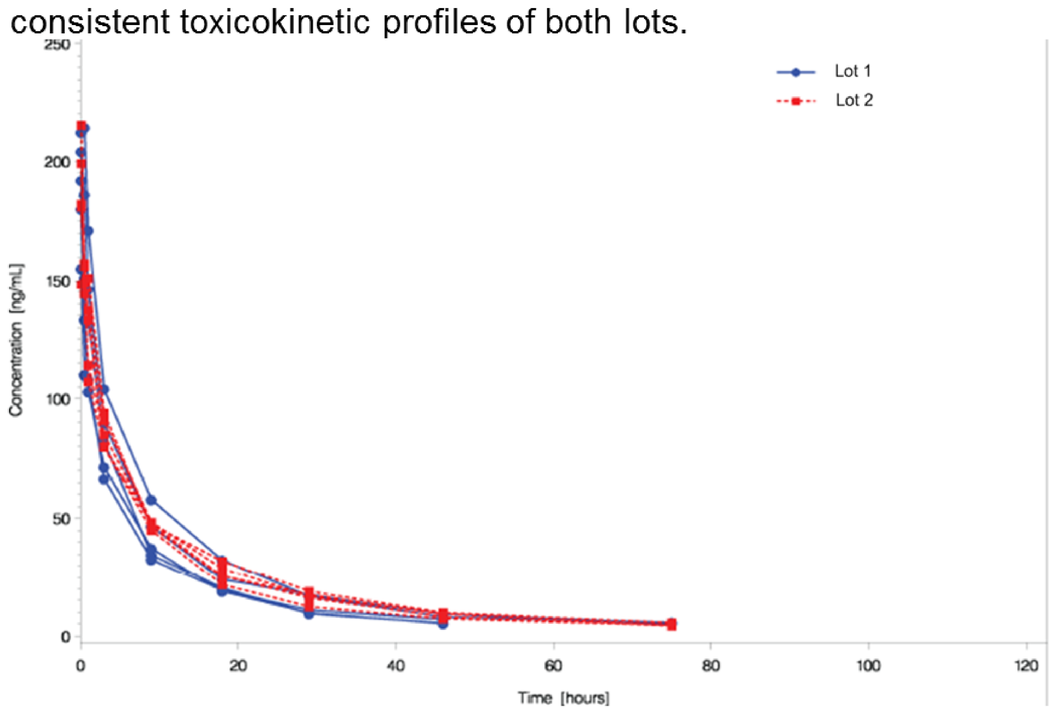
Macaques:

Treatment with BAX 855 was well tolerated at both doses.

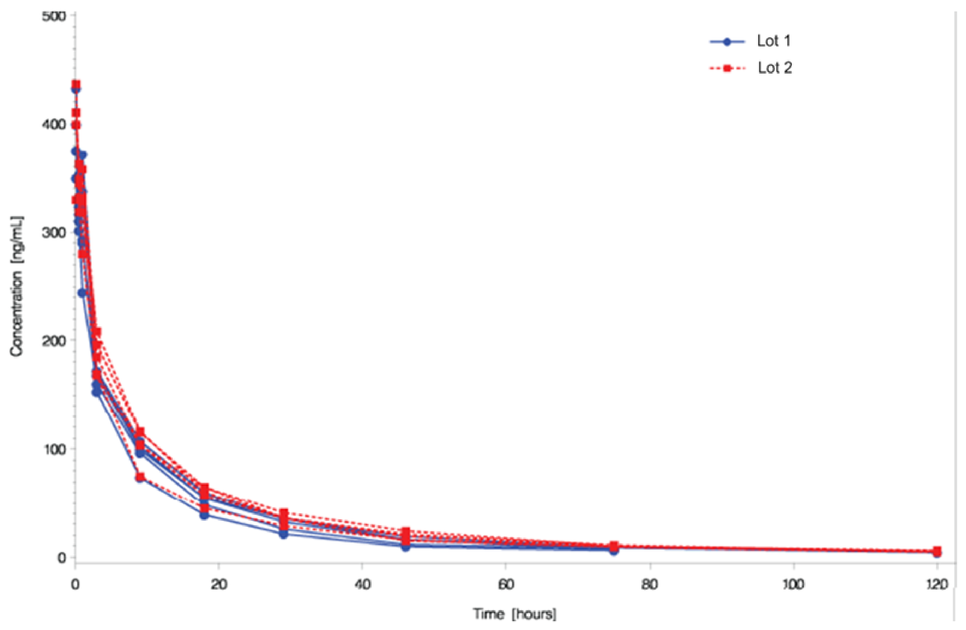
At the study end, lower levels of FVIII activity and FVIII-bound PEG in plasma correlated with the presence of neutralizing antibodies. Minor findings, including a prolonged APTT in all animals treated with BAX 855, were noted during the last week of dosing. These observations were likely caused by the development of cross-reactive neutralizing antibodies. Formation of antibodies against BAX 855 is an expected immune reaction after repeated application of heterologous human proteins to animals, which is also well known for non-PEGylated FVIII products.

There was no macroscopic or histopathological evidence of target organ toxicity due to the test item.

The results of the toxicokinetics of the two lots of BAX 855 demonstrated consistent toxicokinetic profiles of both lots.



Individual time concentration profiles on day 1 in macaques for FVIII- bound PEG at a nominal dose of 350 IU/kg of BAX 855



Individual time concentration profiles on day 1 in macaques for FVIII- bound PEG at a nominal dose of 700 IU/kg of BAX 855

Rats:

There were no drug-related adverse changes for any of the endpoints investigated.

At the study end, lower levels of FVIII activity and FVIII-bound PEG in plasma correlated with the presence of neutralizing antibodies.

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