Interaction of BAX 826 (PSAylated rFVIII) With VWF and LRP1 – An in vitro and in vivo Assessment


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INTRODUCTION

- BAX 826, Shire’s second investigational extended half-life candidate based on AWFVIII (antifibrinolytic factor recombinant), is a full-length recombinant FVIII molecule with an established safety and efficacy profile, and is modified using a hydrophilic polymer, polyaspartic acid (PSA), to extend dosing intervals.
- Extended FVIII circulation times would reduce the frequency of infusions, increase patient compliance, reduce the number of bleedings, and allow higher platelet levels of FVIII to be reached.
- Most FVIII molecules in the circulation are complexed with von Willebrand factor (VWF), which prevents binding of FVIII to LRPs (low density lipoprotein receptor-related protein), an important receptor involved in the clearance of FVIII. While the half-life of free FVIII is 2.3 h, that of FVIII bound to VWF is approximately 17 h, demonstrating that VWF is a major determinant of FVIII circulation time.

OBJECTIVE

- To investigate the interaction of BAX 826 with von WVF and LRP1 using appropriate in vitro methods.
- To confirm the VWF-independent clearance between BAX 826 and FVIII in mice deficient in FVIII and VWF.

METHODS

- The kinetics of FVIII binding to LRPs and to VWF were determined using surface plasmon resonance technology (Biacore, GE Healthcare, Uppsala, Sweden).
- LRP1 (Belz, Leipzig, Germany) was immobilized on the flow cells of a CM1 biosensor chip at a density of 10,000 response units (RU). A series of dilutions (1 to 327.5 mM) of FVIII or BAX 826 were then applied to the chip, allowing 10 min for association and 5 min for dissociation. The equilibrium dissociation constant (KD) was determined assuming a 1:1 interaction.
- Polysialic acid (PSA) was immobilized on the flow cells of a CM5 biosensor chip. A series of dilutions (0.1 to 100 nM) of rFVIII or BAX 826 were then applied to the chip, allowing 10 min for association and 5 min for dissociation. The equilibrium dissociation constant (KD) was determined assuming a 1:1 interaction.
- ELISA combined chromogenic assays were used as an orthogonal method to the Biacore assays to study FVIII-LRP1 and FVIII-VWF binding in brief.
- A dilution series (0 to 10 nM) of FVIII or rFVIII was then added to the wells of a microplate coated with LRP1. After incubation and removal of unbound FVIII by a washing step, bound FVIII was quantified by a FVIII chromogenic assay (Technologon, Technonics, Technonoc, Vienna, Austria).
- The pharmacokinetics of FVIII and BAX 826 were evaluated using a VWF-driven knock-out mouse model. After intravenous administration of 200 μg/kg FVIII, blood samples were collected by cardiac puncture at several time points from 5 min to 7 h post injection. FVIII levels in plasma were determined by a chromogenic assay. The endpoints for statistical evaluation were terminal half-life and mean residence time (MRT).

RESULTS – in vitro Binding Studies

- Binding kinetics of FVIII of BAX 826 were determined using a Biacore assay. The overall binding response of BAX 826 to LRPs was markedly lower than to FVIII. The reduced binding capacity is considered to be the result of FVIII modification with PSA, which yields a FVIII complex where specific binding epitopes for VWF are shielded from PSA.
- The binding affinities of BAX 826 and FVIII were similar, with Kd values ranging from 12.6 to 41.0 mM for BAX 826 (n = 6) and from 18.8 to 30.0 mM for rFVIII (n = 4). These results indicate functional intact binding epitopes on BAX 826 for LRPs when not shielded by PSA.
- Data from the ELISA–combining chromogenic assay for FVIII were in agreement with those from Biacore analysis, confirming reduced binding of BAX 826 to LRPs.

RESULTS – Pharmacokinetics in FVIII/VWF Double Knockout Mice

- Due to the absence of VWF, FVIII was rapidly cleared from the circulation and undetectable 3 h after injection. The estimated half-life was 0.3 h. In contrast, the terminal half-life of BAX 826 was prolonged to 5.5 h in this mouse model, suggesting a lower clearance of BAX 826 in the absence of the chaperone VWF.

CONCLUSION

- Two independent in vitro methods demonstrated that modifying FVIII with PSA markedly decreased its interaction with LRP1, an important clearance receptor for FVIII.
- Binding of BAX 826 to FVIII’s chaperone protein VWF was also reduced, which could render BAX 826 independent of clearance of the VWF-FVIII complex, likely escaping the half-life limitation determined by VWF.
- BAX 826 showed a substantially longer circulation time in a selective FVIII/VWF double knockout mouse model system.
- In conclusion, these results qualify BAX 826 as an attractive new extended half-life FVIII candidate with an alternative mechanism for half-life prolongation. Extensive clinical investigation of BAX 826 is required to further address these findings.

DISCUSSIONS

- All authors are employees of Basel (Basel Innovations GmbH), now part of Shire.