Raising Factor VIII’s Half-Life to the Next Level

Steffen Kistner¹, Jens Daufenbach¹, Peter Herbener¹, Jörg Schüttrumpf¹
¹ Biolog AG, Dreieich, Germany

INTRODUCTION

The half-life of Factor VIII (FVIII) is a critical parameter for defining the frequency of injections along with the FVIII trough levels in patients. Thus, the half-life of FVIII directly correlates with the patient’s burden for a treatment. As currently approved FVIII products do not provide mean half-lives of more than 20 hours, it is one of the major issues of modern FVIII replacement therapy.¹

In this study, we aimed for the development of a novel recombinant FVIII with von-Willebrand-factor-independent half-life prolongation by endogenous albumin-binding, while this molecule was to maintain the characteristics of a FVIII wild type after its activation.

DEVELOPMENT OF HAT (HEMOPHILIA A THERAPEUTIC)

The screening of various FVIII-ABD fusion molecules demonstrated good expression in a human cell line and in vitro functionality of several candidates. An increasing number of candidates and their respective positions in FVIII-ABD candidates led to a reduced binding to Wf (Fig.1), known to be the limiting parameter of previous half-life extension approaches of FVIII.²

The inverse correlation between half-life extension and Wf-binding was in line with pharmacokinetic (PK) results in hemophilia A mice (Fig. 2). The most promising candidate was designated as Hemophilia A Therapeutic (HAT), incorporating four albumin binding domains, and demonstrating full in vitro functionality. An in silico model of HAT is shown in Fig. 3.

CHARACTERIZATION OF HAT

As murine albumin has a much shorter half-life compared to human albumin, hemophilia A mice are not an ideal model for PK investigations of HAT. Thus, an albumin-deficient, human FcRn Ω-chain-expressing mouse model was used demonstrating a 4-fold longer half-life of HAT compared with ReFacto AF, even with only 1% human albumin in the formulation (Fig. 4).

The in vivo efficacy of HAT was demonstrated in a dose-dependent manner using hemophilia A mice for the tail vein transaction assay. A dose of 20 U/kg bodyweight corresponding to approx. 10% of normal FVIII levels, resulted in bleeding times similar to wild type mice (Fig. 5).

MATERIALS & METHODS

Albumin-binding domains (ABD) were incorporated into a single chain FVIII molecule construct. Human cells were transfected with several FVIII-ABD variants and supernatants were screened for FVIII expression and activity. The most promising FVIII-ABD variants were produced, purified, and investigated for von-Willebrand-Factor binding potency as well as for thrombin-mediated activation. Half-life was assessed by pharmacokinetic studies in hemophilia A mice and in albumin-deficient mice which express the human instead of the murine neonatal Fc receptor. The in vivo functionality was investigated in hemophilia A mice using a tail transection assay determining bleeding time and blood loss after a standardized cut.

CONCLUSION

Here we present a next generation recombinant FVIII molecule with four albumin-binding domains incorporated (Hemophilia A Therapeutic ± HAT) resulting in a 4-fold half-life extension in a close-to-human model while preserving the wild type characteristics of activated FVIII with full in vivo functionality.

REFERENCES