A Next Generation Recombinant Factor VIII for Subcutaneous Hemophilia A Prophylaxis

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PB1144

INTRODUCTION

Despite significant progress in hemophilia A therapy within the last decade including the approval of non-factor treatment options, classical factor VIII (FVIII) replacement therapy is still administered intravenously while alternative routes remain elusive. In several patients, especially small children, vein access can be critical. As highly frequent intravenous applications are also a great burden for patients, subcutaneous prophylaxis with FVIII replacement therapies is highly desirable. Thus, we aimed for the development of a new recombinant FVIII with outstanding subcutaneous bioavailability, maintaining full prophylactic properties compared with intravenous treatment.

MATERIALS & METHODS

Biotest's novel recombinant FVIII protein variants containing four albumin-binding domains (Hemophilia A Therapeutic ≙ HAT) were injected intravenously and subcutaneously into hemophilia A mice (n=10/group) and Göttingen minipigs (n=3/group) with Moroctocog alfa used as comparison. Blood samples taken at defined time points (0 – 240 h) after administration, were analyzed for chromogenic FVIII activity or FVIII antigen levels. Non-compartmental analysis was performed to determine half-life, and area under the curve (AUC) comparison was done to assess subcutaneous bioavailability.

CONCLUSION

The next generation recombinant FVIII HAT containing four albumin-binding domains resulted in 50% bioavailability after subcutaneous injection in the Göttingen minipig model. Thus, HAT provides a feasible treatment option combining subcutaneous hemophilia A prophylaxis with the benefit of a substantial half-life extension (for further details see poster PB1160).

HEMOPHILIA A THERAPEUTIC (HAT)

In the course of a FVIII development project, four albumin-binding domains were incorporated into a single chain FVIII sequence resulting in a molecule designated as Hemophilia A Therapeutic (HAT), optionally comprising further deimmunizing amino acid substitutions (HAT RI, poster PB0223). An *in silico* structure model of HAT and HAT RI is demonstrated in Fig. 1. Both molecules offer similar *in vitro* and *in vivo* characteristics.

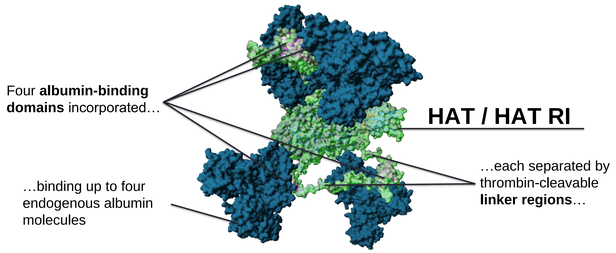


Fig. 1: *In silico* homology model of Hemophilia A Therapeutic (HAT, green) binding four endogenous albumin molecules (blue).

The subcutaneous bioavailability of HAT was first determined in hemophilia A mice by comparing area under the curve (AUC) values after identical dosing via either intravenous or subcutaneous application. For HAT a bioavailability of 15.3 % (mean evaluation) and 18.6 % (median evaluation) was determined in these mice, while Moroctocog alpha was not detectable after subcutaneous administration (Fig. 2).

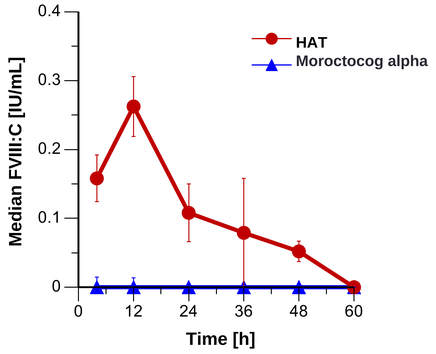
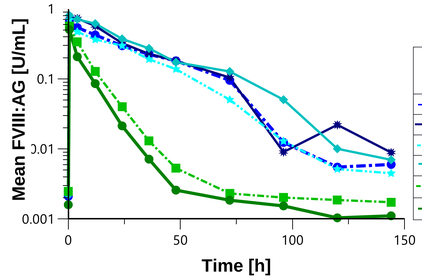


Fig. 2: Pharmacokinetic study in hemophilia A mice (B6;129S-F8tm1Kaz/J). 200 IU/kg bodyweight were injected subcutaneously and chromogenic FVIII activity was measured 4, 12, 24, 36, 48, and 60 h post injection. A non-compartmental analysis was performed and area under the curve (AUC) values of identical i.v. and s.c. dosings were used for bioavailability calculation. n= 10 mice/construct.

SUBCUTANEOUS BIOAVAILABILITY OF HAT

Evaluation of the subcutaneous bioavailability of HAT was performed by comparing the intravenous AUC as observed in a first study in Göttingen minipigs dosed with 30 U/kg bodyweight (Fig. 3) compared with AUC values obtained from a second study where animals were dosed with either 300 or 150 U/kg bodyweight (Fig. 4). Depending on the formulation, a subcutaneous bioavailability of up to 50% was observed in this close-to-human model.



Component	Half-Life extension	AUC_{inf} (U/mL*h)
HAT RI // 1% HSA	2.83	21.10
HAT RI // 10% HSA	3.1	24.92
HAT // 1% HSA	2.76	17.90
HAT // 10% HSA	3.06	27.48
Moroctocog alfa // 1% HSA	1	5.29
Moroctocog alfa // 10% HSA	1	3.58

Fig. 3: Pharmacokinetic study in Göttingen minipigs. 30 U/kg bodyweight FVIII antigen (FVIII:AG) were injected intravenously and FVIII:AG levels were measured up to 144 h post injection. A non-compartmental analysis was performed using Phoenix 8.1 to determine AUC and half-life. n= 3 animals/group.

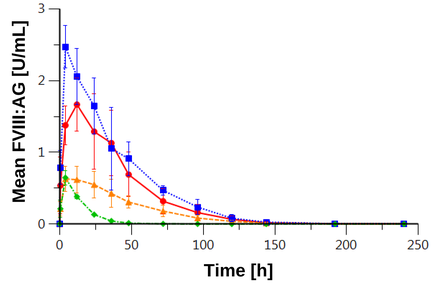


Fig. 4: Pharmacokinetic study in Göttingen minipigs. 300 or 150 U/kg bodyweight FVIII:AG were injected subcutaneously and FVIII antigen levels were measured up to 240 h post injection. A non-compartmental analysis was performed using Phoenix 8.1 to determine relevant parameters. Bioavailability was calculated by comparing dose-corrected AUC values of intravenous and subcutaneous treatment of each group. n= 3 animals/group.

Line	Component	Half-life (h)	Cmax (U/ml)	AUC_{inf} (U/mL*h)	Bioavailability (%)
Dose comparison	300 U/kg Moroctocog alfa // Formulation 1	8.55	0.65	10.70	20
	300 U/kg HAT RI // Formulation 1	20.33	2.47	**105.39**	**50**
	150 U/kg HAT RI // Formulation 1	23.47	0.63	35.61	34
	300 U/kg HAT RI // Formulation 2	19.54	1.67	**81.17**	**38**