Subcutaneous Marzeptacog Alfa (Activated) Effectively Treats Bleeding in FVII Deficient Rats Both When Administered Prior To or After Bleeding Has Started

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Conclusions

+ SQ marzeptacog alfa (activated) (MarzAA) corrects the bleeding phenotype in FVII deficient rats when administered prior to or after bleeding has started in a tail vein transection (TVT) bleeding model
+ The data provides robust nonclinical evidence that SQ MarzAA has the potential for prophylaxis or on-demand treatment of bleeding in subjects with FVII deficiency (FVIID)

Key observations

+ Specific FVIID was induced by pre-treatment with warfarin as evidenced by a time-dependent prolongation of the prothrombin time (PT) but not activated partial thromboplastin time (aPTT)
+ SQ MarzAA corrected blood loss in a dose-dependent manner when administered both before and after TVT
+ SQ MarzAA reversed the prolongation of PT which correlated with increasing blood loss when administered both before and after TVT

Objectives

Primary

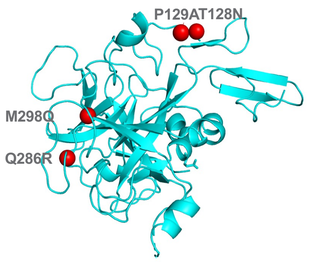
- Establish model of warfarin induced functional FVII deficiency in rats
- Investigate the effect of SQ MarzAA on TVT bleeding in FVII deficient rats

Secondary

- Determine the effects of SQ MarzAA on PT and aPTT

Background

+ MarzAA is a novel rFVIIa variant with enhanced potency and bioavailability enabling SQ administration
+ Two amino acid substitutions (Q286R and M298Q) in the protease domain and increase FX activation in the absence as well as presence of tissue factor
+ Two additional substitutions in the EGF2 domain of the light chain (T128N and P129A) create an additional N-linked glycosylation site
+ MarzAA has previously been shown to reduce blood loss in FVIII deficient mice and rats



Results

PT and aPTT in rats at different time points after IV warfarin treatment

The optimal timepoint for starting the TVT was at a time where the perturbations of the coagulation system induced by warfarin would be primarily exerted on the extrinsic but not the intrinsic pathway since this would represent an isolated FVII deficiency. The most suitable time for starting the TVT was deemed to be at 3 hours following the IV administration of warfarin where PT was markedly and aPTT was only mildly prolonged.

Treatment	Time (h)	PT (sec)	P vs. vehicle	aPTT (sec)	P vs. vehicle
Vehicle	4	26.4 (24.0-28.8)	-	15.9 (15.4-16.4)	-
Warfarin (0.3 mg/kg)	1	29.4 (27.7-31.1)	ns	16.1 (15.4-16.9)	ns
	2	41.8 (38.2-45.5)	< 0.0001	17.1 (14.8-19.3)	ns
	3	71.2 (61.7-80.7)	< 0.0001	19.7 (18.0-21.4)	< 0.001
	4	126.0 (121.6-130.5)	< 0.0001	28.7 (25.7-31.7)	< 0.0001

Table shows mean (95% C.I). Difference between groups were compared with vehicle by one-way analysis of variance (ANOVA) adjusted for multiple comparisons according to Dunnett's post-test.

Effect of SQ MarzAA on blood loss in rats pre-treated with warfarin or vehicle

Rats treated with warfarin showed a statistically significantly increase in blood loss compared to control rats treated with vehicle. The subsequent treatment of MarzAA significantly reduced blood loss in a dose-dependent manner. At the highest dose level (10 mg/kg), blood loss was normalized to the level of rats not receiving warfarin. In addition, when rats were administered MarzAA 2 hours before TVT or at +1 min after induction of bleeding, total blood loss was significantly decreased compared with control animals treated with warfarin or vehicle.

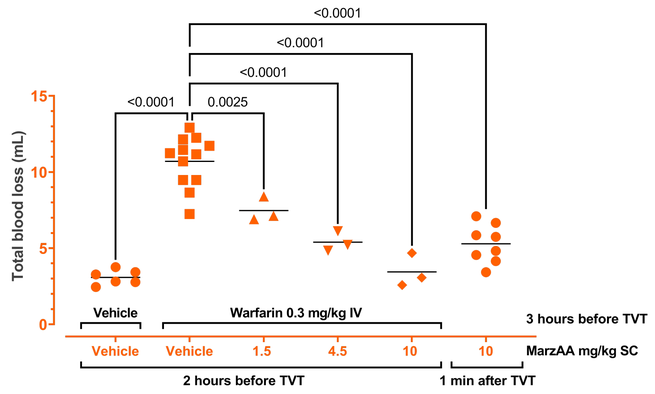


Figure shows means with each point indicating an individual value. Differences between groups were compared with vehicle + warfarin by one-way analysis of variance (ANOVA) adjusted for multiple comparisons according to Dunnett's post-test.

Results

Effect of SQ MarzAA on PT in rats pre-treated with warfarin or vehicle

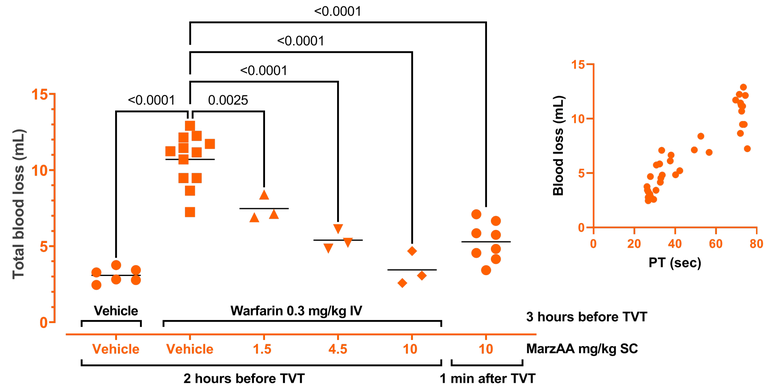


Figure shows mean values with each point indicating an individual value. Difference between groups were compared with vehicle + warfarin by one-way analysis of variance (ANOVA) adjusted for multiple comparisons according to Dunnett's post-test. Insert shows the relationship between PT and blood loss. Persson r squared 0.8437, P< 0.0001.

Methodology

+ Sprague Dawley rats were pretreated IV with vehicle or 0.3 mg/kg warfarin and a blood sample was obtained at 1, 2, 3, and 4 hours after administration for measuring PT and aPTT in order to select a therapeutic window for TVT.
+ Three hours before TVT, FVIID rats were pre-treated with vehicle or 0.3 mg/kg warfarin. For prophylaxis regimen, 2 hours before TVT (prophylactic), rats were administered with different doses of SQ MarzAA (1.5, 4.5 and 10 mg/kg). For the on-demand regimen, a single dose of SQ MarzAA (10 mg/kg) was administered 1 minute after TVT (on-demand).
+ TVT was performed during isoflurane anesthesia. The tail was immersed into pre-warmed saline. After 10 minutes, the tail was lifted from the saline and transecting 3 times the left lateral tail vein at exactly 3.3 mm in diameter using a specially designed blade guard. The tail was returned to the saline and the bleeding was monitored for a total of 60 minutes.
+ Total blood loss was calculated by a colorimetric assay for hemoglobin analysis and presented as volume of blood loss.
+ For measuring PT and aPTT, blood samples from the eye orbital plexus were obtained as close as possible to time of TVT in the prophylactic setting and about 5 min after TVT in the on-demand setting.

Discussion

Warfarin affects the hepatic production of the Vitamin K dependent coagulation factors. The factor concentrations were not measured in the present study. However, it is well-accepted that the initial effect on PT (but not aPTT) is caused by a decrease in functional FVII/FVIIa levels in circulation as this coagulation factor has the shortest half-life (Vainieri & Wingaard, J Pharmacol Exp Ther 1977, 201, 507-517).