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Absence of exaggerated pharmacology by recombinant ADAMTS13 in the rat and monkey

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Insufficiency of ADAMTS13 (a disintegrin and metalloprotease with thrombospondin motif repeats-13) is the cause of thrombotic thrombocytopenic purpura (TTP) and contributes in microangiopathy in sickle cell disease (SCD). Recombinant ADAMTS13 effectively cleaves prothrombotic ultra-large von Willebrand factor (VWF) multimers. It is being tested as replacement therapy for TTP, and at supra-physiologic concentrations, for moderating vaso-occlusive crisis in SCD. Deficiencies of VWF, or concomitant treatment with antithrombotic drugs, could pose risks for increased bleeds in these patient populations. The purpose of the experiments was to evaluate the potential of exaggerated pharmacology and temporary bleeding risks associated with rADAMTS13 administration. We utilized safety studies in monkey and tested the effects of administering maximum-feasible doses of rADAMTS13 on nonclinical safety and spontaneous or aggressive bleeds in the rat model. Evaluation of pharmacokinetics, toxicity profiles, and challenge in a tail-tip bleeding model show that treatment with rADAMTS13 did not increase bleeding tendency, either alone, or in combination with enoxaparin or acetylsalicylic-acid. These novel findings demonstrate absence of rADAMTS13 exaggerated pharmacology without spontaneous or aggravated bleeds even at supra-physiologic (>100-fold) plasma concentrations. *Blood Coagul Fibrinolysis* **33:56–60 Copyright © 2021 The Author(s). Published by Wolters Kluwer Health, Inc.**

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Introduction

ADAMTS13 (a disintegrin and metalloprotease with thrombospondin motif repeats 13), is the major protease regulating size and function of ultra-large von Willebrand factor (ULVWF) multimers. Vascular endothelial cells synthesize, store and secrete procoagulative VWF constitutively or upon demand. However, unprocessed ULVWF is also potentially harmful leading to spontaneous tethering of platelets and blockade of the microcirculation [1,2]. Thus, congenital or acquired ADAMTS13 deficiencies cause the clotting disorder thrombotic thrombocytopenic purpura (TTP) [3]. Multiple protein and cellular interactions orchestrate the physiological role of VWF (Fig. 1). Unfolding of VWF under shear stress allows proteolytic cleavage by ADAMTS13 within the VWF A2 domain, thereby reducing ULVWF procoagulant activity [4]. This process is competitively inhibited by hemoglobin (Hb) and thrombospondin-1 (TSP-1), or increased through allosteric effects by factor VIII and platelets [5–7].

Recombinant (r)ADAMTS13 (TAK-755) is a novel and effective experimental enzyme replacement therapy for the treatment of TTP [12,13]. However, preclinical studies in sickle cell disease (SCD) models [14–16] suggest that supra-physiological rADAMTS13 concentrations are required to overcome the inhibition by pathologic free Hb or TSP-1 content [5,6], and need to be maintained for achieving disease-modifying activities in SCD patients.

Thus, potential sequelae related to excessive VWF deficiency, and the concomitant use of antithrombotic and nonsteroidal anti-inflammatory drugs in the SCD patient population must be considered. Here we address the potential of exaggerated rADAMTS13 pharmacology and temporary bleeding risks within nonclinical safety studies.

Methods

Experiments complied with national legislation and were approved by Institutional Animal Care and Use Committees. Animals were housed under controlled conditions at 19–24 °C, and 40–85% relative humidity. Chow and municipal water were provided ad libitum. rADAMTS13 (TAK-755, SHP655) was provided by Baxalta Innovations GmbH, a Takeda company, Orth an der Donau, Austria.

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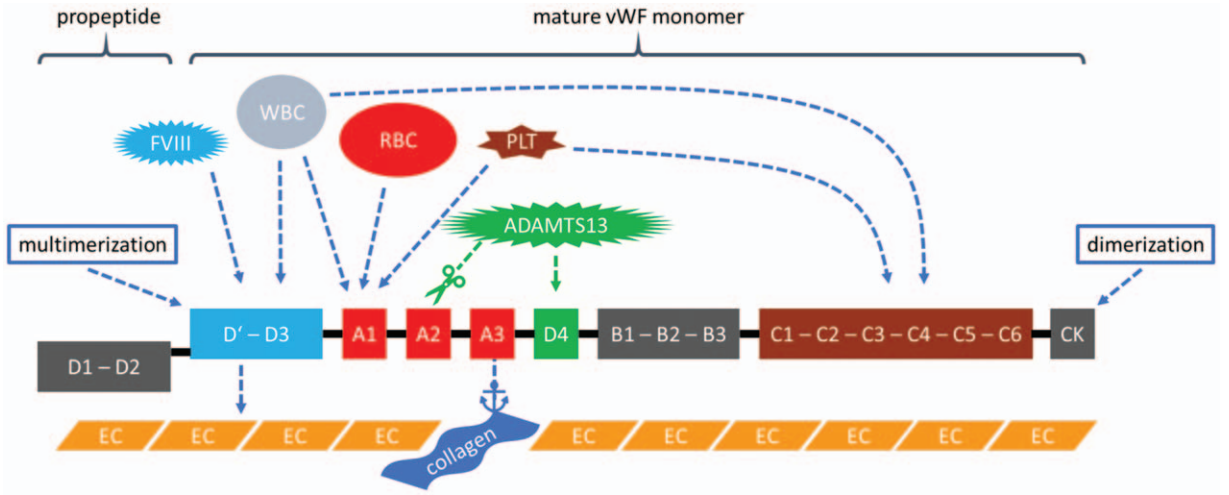
Fig. 1



The domain organization of von Willebrand factor. Propeptide domains D1 and D2 are removed by furin cleavage to generate mature von Willebrand factor monomer. The D′ and D3 domains are involved in multimer formation and the C-terminal cysteine knot domain is required for dimer formation. von Willebrand factor D′/D3 domains bind FVIII and von Willebrand factor D4 domain binds and activates ADAMTS13 by conformational changes [4]. In vascular injury, von Willebrand factor A3 domain anchors to exposed subendothelial collagen. Shear-stress unfolds von Willebrand factor A1 domain and allows binding of platelet GPIbα. Activated platelet integrin αIIbβ3 binds to von Willebrand factor C2/C3 domains and fibrinogen, leading to aggregation of the activated platelets and formation of the initial platelet plug. Shear-stress unfolds also the A2 domain, where ADAMTS13 now binds and exerts its proteolytic cleavage activity and thus reduces ultra-large von Willebrand factor procoagulant activity [4]. Further, von Willebrand factor binds to vascular endothelial cells [8], damaged erythrocytes (red blood cells) [9], leukocytes (white blood cells), like neutrophils [10] and T cells [11], and neutrophil extracellular traps [10]. Free hemoglobin, due to red blood cell hemolysis in sickle cell disease [5], or TSP-1 released from activated platelets [6], bind and compete directly with ADAMTS13 cleavage at domain A2.

Daily repeat and maximum feasible dose toxicity study in rats

CD-Sprague-Dawley rats (Charles River Limited, Margate, Kent, UK) were randomly assigned to study groups ($n = 15$/sex/group) by body weight and treated intravenously daily (30 days) with vehicle [L-histidine (20 mol/l), calcium chloride (2 mol/l), mannitol (3%), sucrose (1%), polysorbate-80 (0.05%), pH 6.9–7.1] or rADAMTS13 (800 or 1820 IU/kg). The high dose was the maximum feasible dose.

The potential toxicity or reversibility of any findings were evaluated by assessment of mortality, clinical signs, clinical pathology including hematology, coagulation, clinical chemistry, and urinalysis, as well as organ weights and histopathology. Toxicokinetic analyses on Day 1 and Day 30 used a sparse sampling design and evaluation of developing anti-ADAMTS13-antibodies predose, and after 2 weeks recovery period.

ADAMTS13 activity was determined by fluorescence resonance energy transfer (FRET) assay using synthetic quenched fluorogenic peptide of VWF A2 domain (FRETS-VWF73, PeptaNova GmbH, Sandhausen, Germany) [17]. Appropriately, diluted plasma was mixed with substrate (2 μmol/l) and fluorescence measured every 2 min for 60 min ($\lambda_{ex/em}$ 340/450 nm) at 30 °C, against reference pooled human plasma (1 U/ml, George King Bio-Medical Inc., Overland Park, Kansas, USA).

Toxicokinetic parameters were calculated with baseline-adjusted mean concentrations per time point using noncompartmental analysis. Drug accumulation was assessed by comparison of C_{max} and AUC_{0-24} on Day 30 with those derived from Day 1 through a two-compartment model with first-order elimination, assuming linear pharmacokinetics.

Rat tail-tip bleeding study testing rADAMTS13 and cotreatments with enoxaparin or acetylsalicylic acid

Male CD-Sprague-Dawley rats (Charles River Laboratories, Sulzfeld, Germany) were randomly assigned to study groups ($n = 6$/group) by body weight and administered saline; rADAMTS13 (10 ml/kg, 3111 FRETS-U/kg); enoxaparin (30 mg/kg); rADAMTS13 + enoxaparin; acetylsalicylic acid (ASA) (30 mg/kg); or rADAMTS13 + ASA.

The dose of rADAMTS13 was the maximum feasible dose, and doses for enoxaparin and ASA were selected based on a previous report [18]. Combination treatments were administered by separate subsequent intravenous tail-vein injections 5 min before animals were anesthetized. Blood loss after cutting the tail tip (3 mm) was determined for 45 min as described previously [19].

Statistical analysis was done using unpaired one-tailed *t* test with Welch's correction (GraphPad Prism 8.0, San Diego, California, USA).

Table 1 Exposures to rADAMTS13 in animal studies and safety margins versus human exposures in a clinical trial

Species, study (dose interval and duration)	NOAEL U/kg BW	rADAMTS13 plasma exposure C_{max} (U/ml)/AUC_{0-t} (U/ml h) First dose	rADAMTS13 plasma exposure C_{max} (U/ml)/AUC_{0-t} (U/ml h) Last dose	Fold safety margin[a] C_{max}/AUC[b]
Rat toxicity study (every 3rd day repeat dose, 4 weeks) [20]	800	13.7/240	14.3/244	15.2/4.7
Rat toxicity study (every 3rd day repeat dose, 26 weeks) [20]	400	6.7/124	9.6/223	10.2/4.3
Cynomolgus toxicity study (once weekly repeat dose, 4 weeks) [20]	400	8.7/180	5.3/20	5.6/0.4
Rat toxicity study (daily repeat dose, 30 days)	1820	40.6/482.6	108.5/1153	115.3/22.0
Rat tail tip bleeding study (single dose)	3111	ND	NA	NA
Mouse SCD efficacy study (single dose) [15]	3270	44.6/864.4	NA	NA

Comparison of rat and cynomolgus monkey exposures at the no-observed-adverse-effect level (NOAEL) in pivotal toxicity and safety pharmacology studies with those in congenital thrombotic thrombocytopenic purpura (cTTP) patients treated with 40 U/kg (pooled data from both sexes) [13] and exposure in a mouse SCD efficacy study [15]. AUC_{0-t}, area under the concentration versus time curve from 0 to the last sampling time point; C_{max}, peak drug concentration; NA, not applicable; ND, not determined; SCD, sickle cell disease. [a] Safety margins are based on exposure at the respective end of the toxicity study. [b] Median C_{max} and AUC_{0-inf} in cTTP patients at 40 U/kg were 0.941 U/ml and 52.3 U/ml h [13].

Results

Absence of spontaneous bleeding events in safety pharmacology and toxicity studies

A completed rADAMTS13 toxicology package included single and repeat-dose toxicity studies in rats treated every third day up to 26 weeks; as well as in cynomolgus monkey treated weekly up to 4 weeks [20]. There were no adverse clinical signs, target organ pathologies, or other adverse findings directly related to rADAMTS13. Cardiovascular and respiratory parameters in cynomolgus monkeys showed no deleterious effects. Importantly, there were no spontaneous bleeding events in these studies at plasma concentrations exceeding endogenous ADAMTS13 levels 10-fold (Table 1).

High dosing frequency and high-dose challenge with rADAMTS13 does not result in safety signals or increased bleeds

We tested daily administration of rADAMTS13 for 30 days up to the maximum feasible dose level in the rat. Treatments were not associated with any unscheduled deaths, clinical signs, ophthalmology changes or clinical pathology. There were no treatment-related gross findings, organ weight differences or histological findings. Importantly, there were no spontaneous bleeding events at plasma concentrations exceeding endogenous ADAMTS13 levels by 100-fold (Table 1). One animal in the low-dose group and three animals in the high-dose group developed antirADAMTS13 binding or neutralizing anti-drug antibodies at the end of the 2-week recovery period, however, this did not affect ADAMTS13 exposures in the respective animals. Although anti-drug antibodies could possibly have cross-reacted with rat ADAMTS13 and resulted in acquired-TTP-like clinical signs, we did not observe signs reminiscent of TTP. This is in contrast to a previous study in cynomolgus monkeys [20], where anti-drug antibodies reduced the exposure to rADAMTS13 (Table 1), and induced signs of TTP. Toxicokinetic comparisons of C_{max} and AUC_{0-24} on Day 1 and Day 30 showed a two-fold increase of plasma rADAMTS13 activity and expanded the previous AUC-based safety margins five-fold (Table 1).

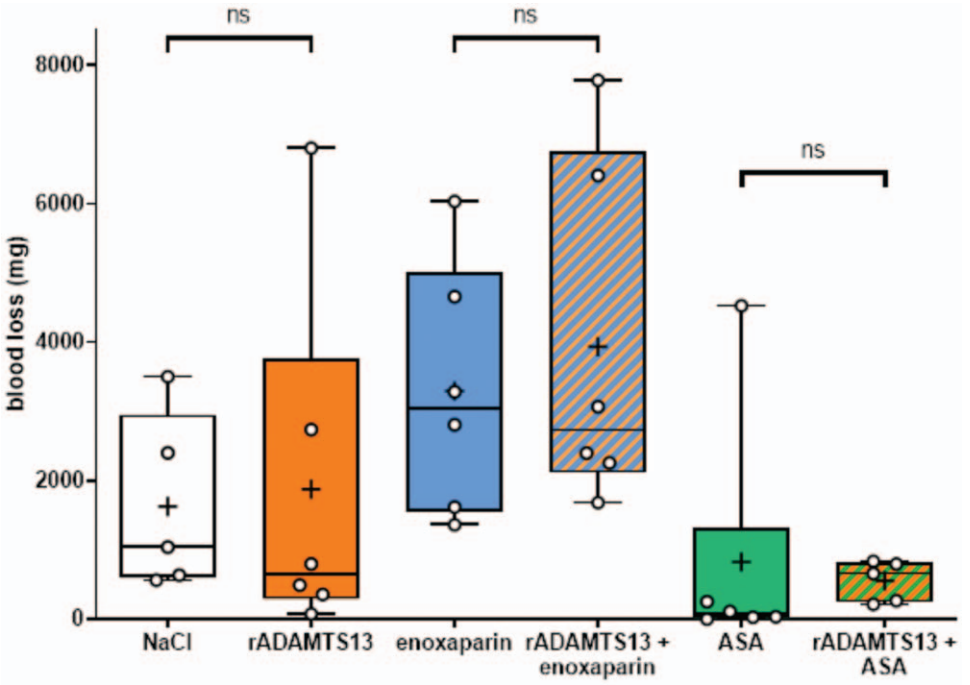
High-dose rADAMTS13 alone and in combination with enoxaparin or acetylsalicylic acid does not exacerbate acute bleeds

We examined the safety of rADAMTS13, alone and in combination with the antithrombotic drugs enoxaparin or ASA, on acute bleeding tendency in a rat tail-tip bleeding model. Saline-treated animals had a mean blood loss of 1630 ± 1282 mg (mean $\pm$ SD; Fig. 2). Mean blood loss in the rADAMTS13 treatment group (1880 ± 2595 mg) was increased but the difference versus the saline treatment group was not statistically significant ($P = 0.420$). As expected [18], treatment with enoxaparin led to a substantial increase in mean blood loss (3298 ± 1797 mg, $P = 0.054$) when compared with the saline treatment group. Treatment with ASA alone, decreased the mean blood loss (828 ± 1817 mg) as reported previously in rats [21], but the difference versus control group was not statistically significant ($P = 0.207$). Combined treatment with enoxaparin and rADAMTS13 significantly increased the mean blood loss (3935 ± 2528 mg) compared with saline treated group ($P = 0.044$), however there was no statistically significant difference when compared with enoxaparin treatment group ($P = 0.313$), indicating that supra-physiologic rADAMTS13 did not exacerbate the antithrombotic activity of enoxaparin. Similarly, combination treatment of rADAMTS13 and ASA decreased the mean blood loss (558 ± 295 mg), but group differences were not statistically significant when compared with the saline-treatment ($P = 0.068$) or with the ASA-treated group ($P = 0.367$). Thus, treatment with rADAMTS13 did not affect the activity of ASA.

Discussion

Enzyme replacement therapy with rADAMTS13 for the treatment of TTP is well tolerated and effective in clinical

Fig. 2



Administration of rADAMTS13 alone or in combination with antithrombotic drugs does not increase bleeding in the rat tail-tip bleeding model. rADAMTS13 (TAK-755, 3111 U/kg), enoxaparin (30 mg/kg), acetylsalicylic acid (30 mg/kg) or saline were administered prophylactically 5 min before the tail cut. Blood loss was assessed by weight for 45 min ($n = 6$/group). In boxplots the box extends from the 25th to 75th percentiles; the line in the middle is the median and the '+' is the mean; the whiskers include the smallest and largest values.

trials [13]. Supra-physiologic rADAMTS13 levels are required in SCD mice to overcome the inhibitory activity of free Hb, to reduce ULVWF and to alleviate the sequelae of vaso-occlusion (Table 1), suggesting higher clinical exposures for achieving disease-modifying activity in human SCD. Deficiency of VWF quantity or activity results in the bleeding disorder von Willebrand disease (VWD) and can be congenital or acquired through various underlying diseases and also through the exaggerated pharmacology of drugs; however, dysregulated high-ADAMTS13 activity has not been associated with VWD [22,23]. Our data are in line with this observation as treatment with rADAMTS13 neither induced spontaneous bleeds in rats or monkeys nor exacerbated acute bleeding in the rat. The absence of exaggerated pharmacology in rat was recorded at daily peak exposures (up to 108.5 U/ml) exceeding physiologic ADAMTS13 levels 100-fold, and exceeding efficacious C_{max} in SCD mice at least 2.5 fold (Table 1). For extrapolation to human, the testing of mouse, rat, and cynomolgus monkey VWF, demonstrated appropriate rADAMTS13 activities in vitro [20], although with lesser efficiency on murine VWF [24]. When considering the enzymology of ADAMTS13 and the pathologic high-VWF concentrations in SCD patients [25–27], treatment with rADAMTS13 is expected to increase the cleavage rate of VWF initially, and the rate reduces as the concentration of VWF multimers is reduced. Reductions of high-molecular weight (HMW) VWF-multimers were seen in plasma of treated rats and monkeys and were related to dose (400 U/kg), with a loss of the highest molecular weight VWF multimers from the band patterns (data not shown). Although this effect was indicative of the pharmacodynamic activity of rADAMTS13 we did not observe a depletion of the HMW VWF spectrum or bleeds reminiscent of VWD even after dosing over 29 days. The development of antibodies was monitored as an expected immune response after repeated application of a heterologous protein in both rat and monkey species and is not predictive of the human situation. The data presented here also suggest that the risk of bleeds by antithrombotic treatments is not exacerbated by pharmacologic exposures to rADAMTS13. Nevertheless, patients with SCD may be predisposed to bleeding [28], and clinical cases of drug-induced bleeding events reminiscent of VWD have been reported with anti-VWF antibody [29], valproic acid, hydroxyethyl-starch, ciprofloxacin, and thrombolytic agents, previously [22].

In conclusion, the data reported here suggest that treatment with rADAMTS13 at supra-physiologic plasma concentrations entails a low risk for increased bleeding events due to exaggerated pharmacology or drug interactions with enoxaparin and ASA.

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Conflicts of interest

P.L. is a full-time employee and stockholder of Takeda. All authors are current employees or were employees of Baxalta Innovations GmbH at the time experiments were conducted.

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