Journal of Thrombosis and Haemostasis, **12**: 220–228 DOI: 10.1111/jth.12477

ORIGINAL ARTICLE

Pharmacological characteristics of a novel, recombinant fusion protein linking coagulation factor VIIa with albumin (rVIIa-FP)

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To cite this article: Zollner S, Schuermann D, Raquet E, Mueller-Cohrs J, Weimer T, Pragst I, Dickneite G, Schulte S. Pharmacological characteristics of a novel, recombinant fusion protein linking coagulation factor VIIa with albumin (rVIIa-FP). *J Thromb Haemost* 2014; **12**: 220–8.

Summary. *Background:* Recombinant factor VIIa (rFVIIa) is approved for use in controlling bleeding episodes in people with hemophilia who have developed inhibitors to replacement therapy. Due to its short half-life ($t_{½}$), frequent injections are required, limiting its use as a prophylactic treatment. A novel, recombinant fusion protein linking coagulation factor VIIa with albumin (rVIIa-FP) has been developed to extend the $t_{½}$ of rFVIIa. *Objectives:* The aim of our studies was to investigate the pharmacokinetic/pharmacodynamic characteristics of rVIIa-FP in preclinical animal species. *Methods:* Pharmacokinetic (PK) parameters were derived after single intravenous dosing in hemophilia A mice, rats, rabbits and monkeys. PK analysis was based on human FVII plasma levels determined by measuring FVII antigen levels by ELISA in mice and rats, and FVIIa activity using STACLOT® VIIa-rTF in rabbits and monkeys. Induction of thrombin generation was investigated in mice, while hemostatic activity was assessed by thrombus formation in rabbits. *Results:* Compared with rFVIIa, rVIIa-FP displayed a prolonged $t_{½}$, enhanced *in vivo* recovery and reduced clearance in all species investigated. In mice, 16 h after treatment with rVIIa-FP, thrombin levels were quantifiable, indicating prolonged efficacy, whereas values had approached baseline at this time after treatment with rFVIIa. After 12 h, hemostatic efficacy was negligible in rFVIIa-treated rabbits, but sustained in animals receiving rVIIa-FP. *Conclusions:* These studies indicate that the longer $t_{½}$ of rVIIa-FP compared with rFVIIa translates into extended activity. These findings suggest that rVIIa-FP has the potential to be administered less frequently than rFVIIa-containing concentrates in clinical use.

Keywords: half-life; hemophilia; hemostasis; inhibitors; pharmacokinetics.

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Received 3 September 2013
Manuscript handled by: P. de Moerloose
Final decision: F. R. Rosendaal, 16 November 2013

Introduction

Hemophilia is a hereditary (X-linked) bleeding disorder characterized by a lack of clotting factor VIII (hemophilia A) or factor IX (hemophilia B). This congenital disease leads to inadequate clotting associated with serious consequences, including joint bleeding and swelling resulting in arthropathy, intracerebral bleeding and brain hemorrhage, damage to organs and pain, with some bleeding episodes being fatal [1].

The development of neutralizing antibodies to coagulation factor VIII (FVIII) or factor IX (FIX) remains the most serious complication of replacement therapy with factor concentrates in hemophilia patients. Up to 32% of hemophilia A patients develop inhibitors (anti-drug antibodies) to exogenous FVIII [2]. While incidence rates of inhibitors to exogenous FIX in hemophilia B are lower, ranging from 1% to 6% [3], the development of these inhibitors, when they do occur, is frequently associated with severe allergic reactions, including life-threatening anaphylactoid responses [4,5].

Although attempts have been made to eradicate inhibitors with plasmapheresis, immunosuppressive therapy or immune-tolerance induction (ITI), these therapies are not always effective. For example, ITI is successful in 70–85% of FVIII inhibitor cases and in 30% of anti-FIX antibody-positive patients [3]. Therefore, many patients with inhibitors rely on bypassing products to control or prevent hemorrhages [3]. The efficacy of available bypassing agents such as activated prothrombin complex concentrate (aPCC; FEIBA®, Baxter AG, Vienna, Austria) and recombinant activated factor VII (rFVIIa; NovoSeven®, Novo Nordisk A/S, Bagsvaerd, Denmark) is inferior at control-

ling bleeds when compared with FVIII/FIX substitution therapy in patients without inhibitors [6]. Furthermore, these bypassing products have short half-lives ($t_{½}$) that may limit their application in prophylactic treatment. For example, due to the rapid systemic clearance of rFVIIa (terminal $t_{½}$ of ~2.4 h in humans), use of this therapy often requires an inconvenient regimen of two to three doses given at 2- to 3-h intervals to achieve hemostasis following an acute bleed [7]. It should be noted, however, that the hemostatic activity of rFVIIa may endure for longer than its plasma kinetics, as suggested by uptake and binding to tissue factor (TF) in perivascular tissues [8].

In recent years, different technologies have been developed to prolong the $t_{½}$ of recombinant coagulation factors. Amongst these, PEGylation and fusion technologies applied to wild-type or genetically modified proteins have been most widely explored. CSL Behring has developed the albumin fusion technology platform in which a fusion protein linking a human coagulation factor and human albumin is expressed as a single recombinant construct in Chinese hamster ovary (CHO) cells (i.e. recombinant human albumin is fused to the C-terminus of rVIIa via a flexible glycine-serine linker: rVIIa-FP) [9,10]. This technology was used to successfully prolong the plasma $t_{½}$ of recombinant coagulation FIX, and the respective fusion protein, rIX-FP, has a $t_{½}$ that is less than 5-fold longer in hemophilia B patients compared with plasma-derived and recombinant FIX products [11]. The aim of the present studies was to characterize the pharmacokinetic/pharmacodynamic (PK/PD) profile of rVIIa-FP in a range of animal models in comparison with rFVIIa.

Methods

Pharmacokinetic (PK) studies in hemophilia A mice, rats, rabbits and cynomolgus monkeys

Different doses were selected for each species investigated to reflect between-species variations in clotting response.

Female FVIII knockout mice [12] were used to compare the PK properties of rVIIa-FP and rFVIIa (NovoSeven®). After correction for the molecular weight of the fusion protein, an equimolar dose (based on the molecular weight of the rVIIa-moiety for rVIIa-FP only) of 100 μg kg^{-1} for both test items was administered as a single intravenous (i.v.) dose into the lateral tail vein. In total, 30 animals per treatment group were given either rVIIa-FP or rFVIIa. Blood samples were drawn at 2, 5 and 30 min, and 1, 2, 4, 6, 16, 24 and 48 h post-administration and pooled (n = 3 per time-point), then processed to 10% citrate (3.13% w/v) plasma. Three animals received vehicle to determine baseline levels.

The PK properties of rVIIa-FP and rFVIIa, both administered as a single 900 μg kg^{-1} i.v. dose, based on total respective molecular weights, were also assessed in eight rats per treatment group (with blood samples drawn at 5, 15 and 30 min, and 1, 2, 4, 8 and 24 h after injection), and in three rabbits per treatment group following single i.v. doses (based on the respective total molecular weight of each test item) of 2000 and 275 μg kg^{-1}, respectively (with blood samples drawn at pre-dose, 1, 5, 10 and 30 min, and 1, 2, 4, 8, 24, 48, 72, 96 and 168 h post-dose). Finally, the PK properties of rVIIa-FP and rFVIIa were evaluated in cynomolgus monkeys after single i.v. doses (based on total molecular weight) of 2700 and 270 μg kg^{-1}, respectively, with two animals in each treatment group. Blood samples were drawn at 5 and 15 min, and 1, 2, 4, 8, 24, 48, 72, 96 and 120 h post-dose. In the rabbit and monkey PK studies, the higher doses of rVIIa-FP vs. rFVIIa reflect the relative potency ratio of 8–10 found as a consequence of the higher molecular weight, and its reduced specific FVIIa activity due to the albumin moiety of the fusion protein. This potency ratio matches the difference in selective FVIIa activity between rVIIa-FP and rFVIIa as observed using the STACLOT® VIIa-rTF assay system (Diagnostica Stago, Asnières, France), selective for activated FVII.

A commercially available enzyme-linked immunosorbent assay (ELISA)-based system (Cedarlane Laboratories Limited, Burlington, ON, Canada) was used to evaluate human FVII antigen (FVII:Ag) plasma levels obtained from the rodent species. In the analysis of FVII:Ag data, the first value below the limit of quantification (250 ng mL^{-1}) was imputed to one-half of this limit (125 ng mL^{-1}) in the rVIIa-FP and rFVIIa groups. Subsequent values below the limit of quantification were ignored in the calculation of PK parameters. As enzymatic FVII activity is the more widely used PK parameter when monitoring FVIIa plasma levels in patients [13–15], the STACLOT® VIIa-rTF assay was used (in addition to FVII:Ag) in plasma samples derived from rabbits and monkeys to determine selective FVIIa activity.

From each preclinical study described above, PK parameter estimates were derived using WinNonlin® software version 6.2 (Pharsight, Cary, NC, USA), including maximum concentration (C_{max}), area under the curve (AUC) from t = 0 to last observation, $AUC_{0-\infty}$, $t_{½}$, mean residence time, clearance (CL), incremental recovery and *in vivo* recovery (IVR), which was calculated assuming a plasma volume of 40 mL kg^{-1}. IVR was the maximum observed plasma level multiplied by plasma volume and divided by dose; it is a dimensionless ratio and was expressed as a percentage. PK data are presented descriptively.

Hemostatic potency of rVIIa-FP and rFVIIa under acute bleeding conditions after tail clip in hemophilia A mice

In an acute bleeding study, hemophilia A mice were administered rVIIa-FP at dose levels of 0.5, 1, 2, 4 and 8 mg kg^{-1} and rFVIIa at dose levels of 0.5, 1 and 2 mg kg^{-1} on an equimolar basis for FVIIa, with 15 ani-

mals per treatment group. Both agents were administered 2 min before a tail clip. The tail was cut with a scalpel knife at the start of the observation period under deep anesthesia (sodium pentobarbital, 74.5 mg kg^{-1}), removing approximately 3–4 mm of the tail tip. Immediately upon lesion, the tail tip was submerged in isotonic saline solution (0.9%), which was kept at the physiological body temperature of the mice using a water bath, until hemostasis occurred. The volume of total blood loss was calculated over an observation period of 30 min, or until hemostasis occurred, by measuring the hemoglobin (Sysmex F-820, Sysmex Europe GmbH, Norderstedt, Germany) present in the isotonic saline. The procoagulant effects of rFVIIa and rVIIa-FP were dose-proportional with parallel dose–response curves and maximum responses obtained at approximately 4 and 11 mg kg^{-1}, respectively, with rFVIIa having a 2.7-fold higher potency (Figure S1). When equimolar doses for both activated FVIIa concentrates were adjusted according to their selective FVIIa activity, they showed similar hemostatic activity (Figure S2).

Pharmacodynamics (PD) in hemophilia A mice: thrombin generation assay (TGA)

The duration of the PD effect of rFVIIa and rVIIa-FP was assessed in hemophilia A mice by TGA. Citrate (10% v/v) and corn trypsin inhibitor-stabilized (50 μg mL^{-1}) blood was collected (3–10 animals per treatment group and time-point) at 5 min, and 4, 7 and 16 h after administration of equimolar doses of 400 μg kg^{-1} of either rVIIa-FP or rFVIIa, based on FVIIa molecular weight. TGA was performed by calibrated thrombinography (Calibrated Automated Thrombogram [CAT®], Thrombinoscope BV, Maastricht, the Netherlands) after extrinsic activation using PPP-Reagent 5 pM (Thrombinoscope BV). Thrombin generation and time to onset of observed thrombin generation (lagtime) were calculated for each treatment group.

Procoagulant activity and prothrombin time in rabbits

The procoagulant activity of rVIIa-FP and rFVIIa up to 24 h was assessed in a rabbit venous thrombosis model after inducing temporary venous stasis (modified Wessler test [16]). In a pilot study, New Zealand white or Chinchilla Bastard rabbits were treated with either rVIIa-FP (n = 6–9) at a dose of 450 or 900 μg kg^{-1}, or rFVIIa (n = 6–9) at dose levels of 125 or 450 μg kg^{-1} (based on the respective total molecular weight). Activated prothrombin complex concentrate (aPCC) was used as a positive control (n = 3; 50 U kg^{-1}). Incidence of thrombosis was the primary endpoint, and observed thrombi were graded according to a scoring system of 0–3 (0 = no clot; 1 = one or a few small clots, no measurable weight; 2 = not fully occluding clot, one or several clots of bigger size, weight can be measured; 3 = segment fully occluded by clot, weight can be measured). Based on this pilot study, the procoagulant activity of rVIIa-FP and rFVIIa was determined as being equivalent at a dose ratio of 7.2 : 1 based on total protein weight. New Zealand white rabbits (2.3–3.1 kg) were therefore allocated to rVIIa-FP (n = 22) or rFVIIa (n = 16) at dose levels of 2000 and 275 μg kg^{-1}, respectively, or placebo (n = 6). Doses were administered intravenously via the ear vein to anesthetized animals, with the negative control (placebo) group receiving isotonic saline. To assess the procoagulant potential of the test substances after 10 min, 12 h or 24 h, the animals were anesthetized before or at 12 or 24 h after administration of test substance with an i.v. injection of 5 mg kg^{-1} ketamine (10%) and 0.5 mg kg^{-1} xylazine (2%) solution, and maintained by i.v. infusion of 25 mg kg^{-1} h^{-1} ketamine (10%) and 2.5 mg $kg^{-1}h^{-1}$ xylazine (2%). At the 10-min, 12- and 24-h time-points, the left and right jugular veins were exposed (n = 6–10 per time-point) and a segment of approximately 2 cm was isolated. Stasis was produced in these segments by ligation with cotton threads. Side branches were occluded using titanium clips. Blood was allowed to fill the vein segments, then a second ligature was placed approximately 1.5 cm cranial to the first one, causing complete stasis in the isolated segment. Ten minutes after stasis induction, the right vein segment was excised and dissected in a petri dish filled with sodium citrate solution. The same procedure was followed for the left vein segment 20 min after stasis. Any observed thrombi were graded from 0 to 3 and the distribution of the thrombus score in the two groups was compared by an exact Wilcoxon rank sum test.

Prothrombin time (PT) was determined *ex vivo* using Thromborel® S (Siemens Healthcare Diagnostics Products GmbH, Marburg, Germany) with a Schnitger and Gross coagulometer.

Results

Pharmacokinetic (PK) studies in hemophilia A mice, rats, rabbits and cynomolgus monkeys

PK parameters from mice and rats based on FVII:Ag, and rabbits and monkeys based on FVIIa activity, are shown in Table 1. In all species, pivotal PK characteristics (i.e. *in vivo* recovery, CL and $t_{½}$) were improved for rVIIa-FP compared with rFVIIa. In both mice and rats, CL of rVIIa-FP was lower and $t_{½}$ was prolonged compared with rFVIIa. Furthermore, rVIIa-FP showed a 2-fold enhanced *in vivo* recovery compared with rFVIIa (Table 1).

Similarly to the rodent studies, in which PK properties of FVIIa were calculated only measuring FVII:Ag levels, the PK profile of rVIIa-FP was also improved compared with rFVIIa in rabbits and monkeys based

Table 1 Pharmacokinetic parameters of rVIIa-FP and rFVIIa in hemophilia A mice, rats, rabbits and cynomolgus monkeys

	Variable							
	IVR observed (%)		MRT (h)		CL (mL h^{-1} kg^{-1})		$t_{½}$ (h)	
	rFVIIa	rVIIa-FP	rFVIIa	rVIIa-FP	rFVIIa	rVIIa-FP	rFVIIa	rVIIa-FP
Mice (n = 3/time-point) 100 μg kg^{-1}	21	51	1.0	5.3	212	19	0.9	3.7
Rats (n = 4/time-point) 900 μg kg^{-1}	32	72	1.1	6.7	127	10.8	0.8	5.1
Rabbits (n = 3/group) rVIIa-FP: 2000 μg kg^{-1} rFVIIa: 275 μg kg^{-1}	10	53	3.3	16.9	212	6.1	2.3	12.5
Monkeys (n = 2/group) rVIIa-FP: 2700 μg kg^{-1} rFVIIa: 270 μg kg^{-1}	44	75	2.9	11.9	45.8	4.9	2.2	8.6

Data for MRT, CL and $t_{½}$ are presented based on FVII antigen measurements for rodents, and based on selective FVIIa activity for rabbits and monkeys. For mice, samples from three animals per time-point were pooled, resulting in one assay result per time-point, a single PK curve and a single set of PK parameters. For rats, samples from four animals per time-point were tested individually and averaged to give a single PK curve and a single set of PK parameters. For rabbits and monkeys, each animal provided a full PK curve and geometric means are reported. CL, clearance; IVR, *in vivo* recovery; MRT, mean residence time; rFVIIa, recombinant factor VIIa; rVIIa-FP, fusion protein; $t_{½}$, terminal elimination half-life.

on selective FVIIa activity (Table 1). In both species, CL was again lower and $t_{½}$ extended when comparing rVIIa-FP with rFVIIa. There was a 5-fold enhanced *in vivo* recovery in rabbits when comparing rVIIa-FP with rFVIIa, while in monkeys there was a 1.7-fold enhanced *in vivo* recovery when comparing rVIIa-FP with rFVIIa (Table 1). PK profiles for rVIIa-FP and rFVIIa in mice, rats, rabbits and monkeys are shown in Fig 1.

Thrombin generation assay (TGA) in hemophilia A mice

The improved PK profile of rVIIa-FP, with an extended $t_{½}$, translated into a prolonged PD activity compared with rFVIIa (Fig 2A,B). The lagtime to thrombin generation for rFVIIa time-dependently increased, reaching baseline values (derived from the control group) at 16 h from start of treatment (Fig. 2A). This was confirmed by the thrombin generation curves at 16 h after start of treatment, which were superimposable for the vehicle- or rFVIIa-treated animals (Fig. 2B). In contrast, prolonged activity of rVIIa-FP was clearly evident, showing an average 2-fold reduction in the lagtime until 16 h after start of treatment with rVIIa-FP, despite the potency difference observed during tail-clip studies, and in the absence of selective FVIIa activity adjustments (Fig. 2A). The extended activity of rVIIa-FP was also observed as enhanced thrombin generation at 16 h after start of treatment (Fig. 2B).

Procoagulant activity and prothrombin time in rabbits

The initial procoagulant activity elicited by rVIIa-FP and rFVIIa was similar at 10 min after treatment, with thrombus scores for both ranging from 2 to 2.5 following 20 min of venous stasis (these scores increasing from about 1.0 after 10 min of stasis) (Figure S3). Following 20 min of stasis at the time-point of 12 h after treatment, however, thrombus formation in animals treated with rFVIIa was negligible, but sustained thrombus formation was observed in rVIIa-FP-treated animals, resulting in a significantly different thrombus score (P = 0.0325). There was an estimated probability of 75% that the thrombus score of a rVIIa-FP-treated animal was higher than that of a rFVIIa-treated animal at 12 h (Fig. 3). No thrombus formation was observed following placebo treatment, or at 24 h, and no difference between rVIIa-FP and rFVIIa was seen 12 h post-administration following 10 min of stasis (Figure S4).

Measurement of PT supported the results obtained from thrombus score assessment, because reduced PT times were noted for rVIIa-FP until 24 h post-administration compared with rFVIIa-treated animals (Fig. 4). No general activation of the coagulation system was recorded when measuring fibrinogen, thrombin-antithrombin (TAT) and D-dimer as prothrombotic biomarkers (data not shown).

Discussion

The PK/PD characteristics of rVIIa-FP were assessed in a series of non-clinical studies, and rVIIa-FP was found to be effective as a hemostatic agent, with consistently longer half-lives and mean residence times, and greater *in vivo* recovery across the species studied. For the rodent studies, PK was assessed using an ELISA, and it should be noted that using polyclonal antibodies in an ELISA to measure FVII protein might additionally capture partially degraded FVII and/or FVII that has been inactivated by binding to circulating plasma proteins such as antithrombin [17], TF pathway inhibitor or alpha-2 macroglobulin [18]. In the rabbit and monkey PK studies, however, we also used the STACLOT® system, which specifically measures active FVII only and therefore represents the clini-

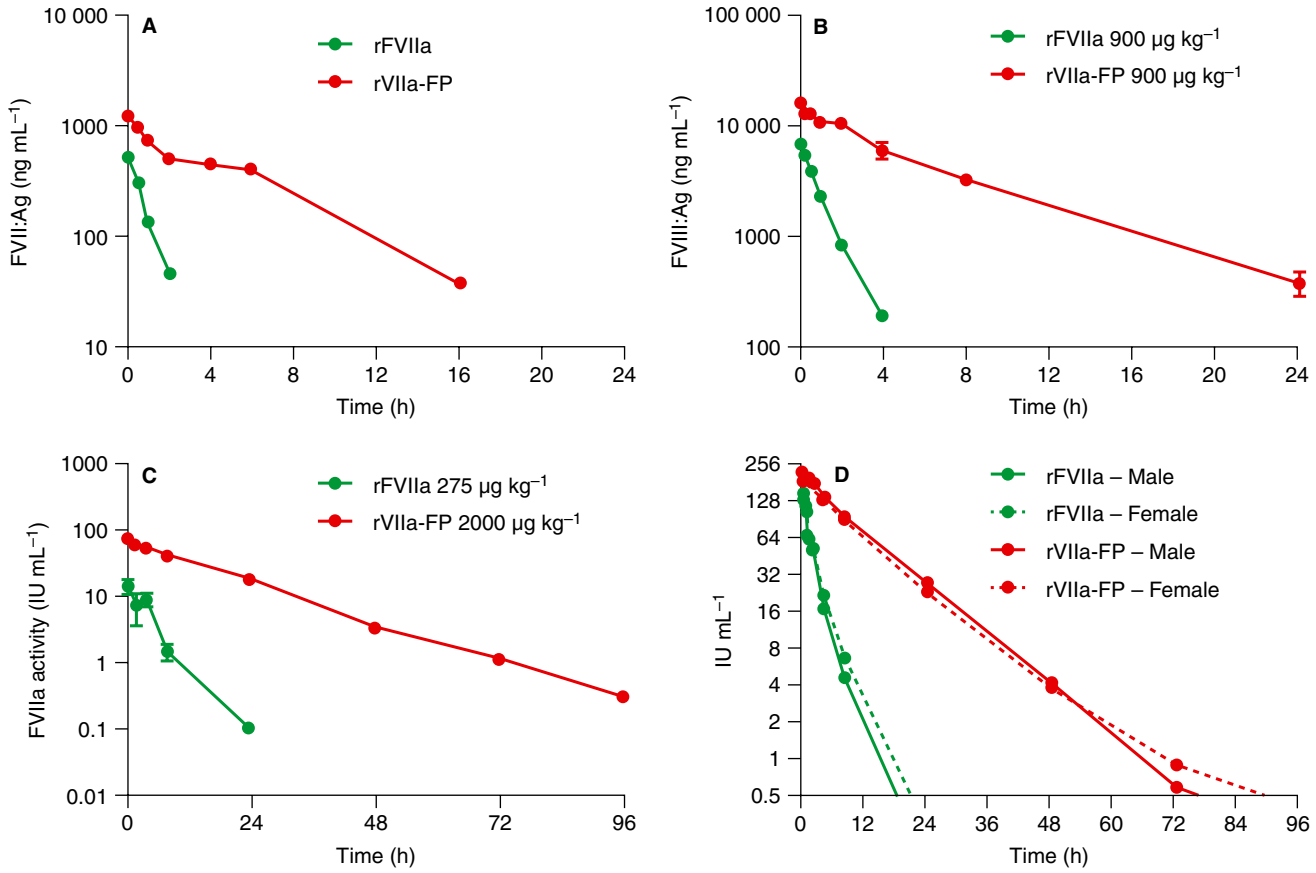

Fig. 1. Plasma concentration–time profiles of rVIIa-FP and rFVIIa in (A) hemophilia A mice, (B) rats, (C) rabbits and (D) cynomolgus monkeys (mean ± SD). PK curves in mice and rats are based on FVII:Ag data, while the curves shown for rabbits and monkeys are based on selective FVIIa activity. Data are mean, except for mice (plasma pool) and monkeys, where the curve for each animal is shown. FVII:Ag, factor VII antigen; PK, pharmacokinetic; rFVIIa, recombinant factor VIIa; rVIIa-FP, fusion protein; SD, standard deviation.

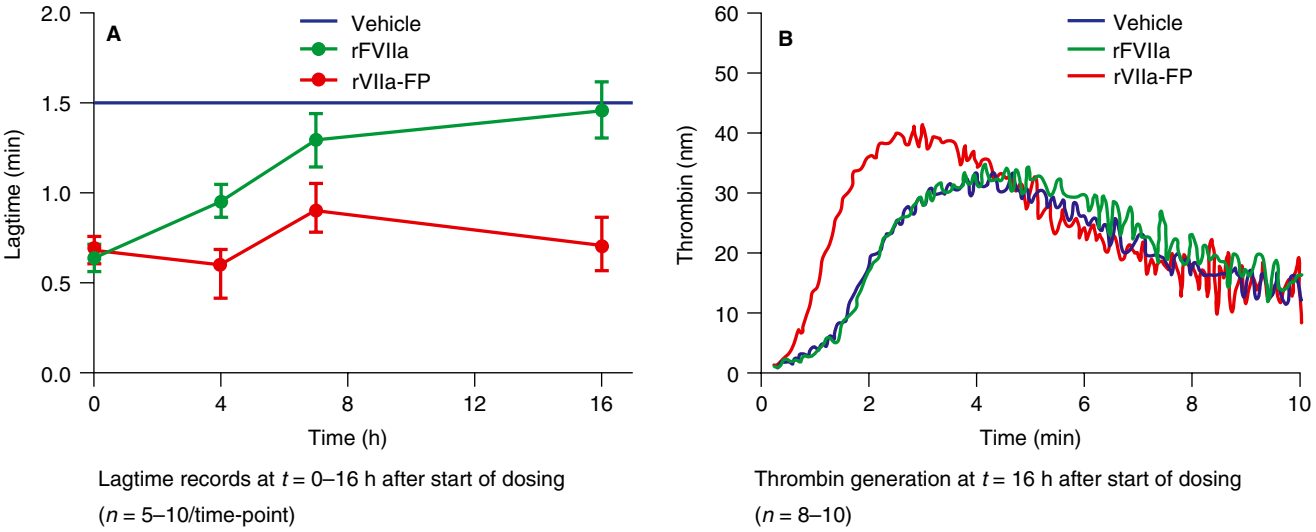

Fig. 2. PK/PD assessment measuring TGA hemostasis parameters *ex vivo* in hemophilia A mice. (A) Lagtime to thrombin generation; (B) thrombin generation–time profile at 16 h. PD, pharmacodynamic; PK, pharmacokinetic; TGA, thrombin generation assay.

cally more relevant and predictive parameter for expected clinical efficacy in humans. The STACLOT® data were qualitatively similar to the antigen data (not shown) in these species, confirming that rVIIa-FP has a more desirable PK profile than rFVIIa, which is currently in clinical use.

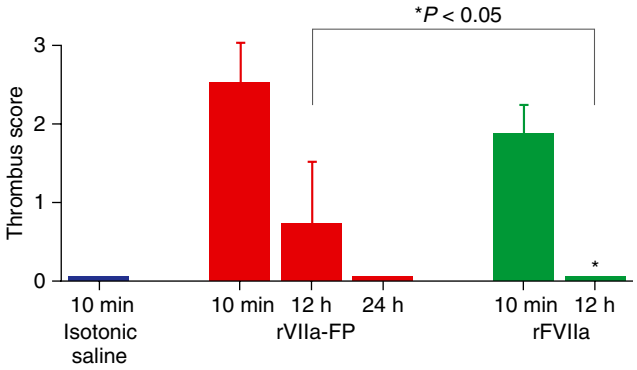

Fig. 3. Pharmacodynamic assessment measuring thrombus formation in rabbits. Thrombus scores are shown after 20 min of stasis from veins dissected at three time-points after administration of test substances. Definition of thrombus scores: 0 = no clot; 1 = one or a few small clots, no measurable weight; 2 = not fully occluding clot, one or several clots of bigger size, weight can be measured; 3 = segment fully occluded by clot, weight can be measured.

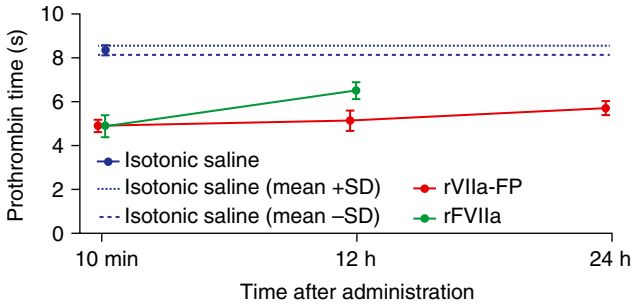

Fig. 4. Assessment of pharmacodynamic activity measuring prothrombin time in rabbits. n = 6–10; mean ± SD; SD, standard deviation.

The prolonged systemic availability of rVIIa-FP in plasma also translated into extended procoagulant activity in our PD studies, suggesting that rVIIa-FP has the potential to control and prevent bleeding episodes in hemophilia patients with inhibitors, with less frequent dosing than rFVIIa in clinical practice. Fewer injections should facilitate long-term prophylactic use of rVIIa-FP and be useful during surgical procedures [9].

Our studies in hemophilia A mice showed a 2-fold enhanced *in vivo* recovery of rVIIa-FP compared with rFVIIa. Furthermore, rVIIa-FP (0.5–8.0 mg kg^{-1}) and rFVIIa (0.5–2.0 mg kg^{-1}) demonstrated a dose-proportional decrease in total blood loss over the dose range investigated in hemophilia A mice under acute bleeding conditions. The prolonged $t_{½}$ of rVIIa-FP in hemophilia A mice was reflected in the extended PD activity, with thrombin generation observed 16 h after the start of rFVIIa-FP administration, whereas hemostatic efficacy had completely ceased 16 h post-dose with rFVIIa. Consistent with our findings, other studies in mice show a mean hemostasis $t_{½}$ of 1.2 h after treatment with rFVIIa at a dose of 10 mg kg^{-1} [19], and, similarly, no hemostatic effect was seen 24 h after administration of rFVIIa in mice at the same dose level [20].

Our studies in rats and rabbits also showed that rVIIa-FP had a longer $t_{½}$ compared with rFVIIa. Our findings are consistent with results of a previous study reporting a mean $t_{½}$ of 4.5 h for rFVIIa (based on a FVIIa clotting activity assay) administered to rabbits at a dose of 2 mg kg^{-1} [21]. While rVIIa-FP and rFVIIa showed similar procoagulant activity in our study in rabbits at 10 min after dosing, by 12 h thrombus formation had become negligible in the rFVIIa group, whereas it persisted in the rVIIa-FP group.

We also investigated activation of the coagulation system in rabbits, using the International Society on Thrombosis and Haemostasis (ISTH) scoring system of disseminated intravascular coagulation (DIC). This stipulates that initial signs of systemic activation of the coagulation system, non-overt DIC, are shown by changes in a score of platelet count, prothrombin time, fibrinogen and D-dimer [22,23]. Fibrinogen, TAT and D-dimer, as prothrombotic markers, indicated no increased risk of thrombosis in our study. These results confirm findings by Johansen *et al.* [21], in which rabbits treated with rFVIIa also showed no signs of systemic activation of the coagulation system.

Our study in monkeys again showed a longer mean $t_{½}$ in the rVIIa-FP group (8.6 h vs. 2.2 h with rFVIIa) and the CL rate was improved 10-fold (4.9 mL h^{-1} kg^{-1} vs. 45.8 mL h^{-1} kg^{-1} with rFVIIa). These PK variables found in monkeys seem to correlate with PK data obtained for rVIIa-FP during phase 1 in healthy human volunteers. Administration of five, single escalating doses (140 and 1000 μg kg^{-1}) revealed a median $t_{½}$ between 6.1

and 9.7 h, with 8.5 h at the highest dose. Furthermore, rVIIa-FP had a reduced mean CL (i.e. 7.62 mL h^{-1} kg^{-1} at 1000 μg kg^{-1}), resulting in an approximately 3- to 4-fold increase in $t_{½}$ [24] compared with rFVIIa [25–27]. In two clinical studies conducted in healthy human volunteers, rFVIIa (5–320 μg kg^{-1}) had a mean $t_{½}$ (determined measuring FVII clotting activity) between 2.43 and 2.45 h and CL rates ranging between 31 and 35 mL h^{-1} kg^{-1} [25,26]. In a third study conducted in healthy Japanese and Caucasian adults [27], a slightly longer $t_{½}$ was reported for FVIIa (dosed at 40–160 μg kg^{-1}), ranging from 3.9 h to 6.0 h, with CL rates of 34–37 mL h^{-1} kg^{-1} consistent with the other healthy volunteer studies [25,26]. Similarly to rVIIa-FP, the PK profile of rFVIIa in the monkey appears indicative of the profile in humans with regard to $t_{½}$ and CL, and may therefore aid allometric species scaling of preclinical animal data.

Besides rVIIa-FP, several other candidate agents have been developed as potential hemostatic therapies with modified PK properties. These have employed various principles other than albumin fusion, but not all met with success in early studies. Firstly, a glycoPEGylated rFVIIa (N7-GP) was produced in an attempt to prolong the $t_{½}$ [28,29]. In hemophilia A mice, N7-GP had a six-fold longer $t_{½}$ compared with rFVIIa [20], a trend also observed in humans [30]. The development of N7-GP was discontinued, however, due to a lack of dose–response linearity and one case of hypersensitivity during phase 2 clinical trials [31].

Secondly, vatreptacog alfa (NN1731), a genetic variant of rVIIa with a similar $t_{½}$ to rFVIIa but a more rapid onset of action, was effective as an acute treatment in 98% of joint bleeds in a phase 2 study. However, in phase 3 trials, one patient developed anti-drug antibodies with a potentially neutralizing effect, indicating potential tolerability issues and leading to its discontinuation [31].

BAY 86-6150 was another long-acting FVIIa agent in clinical development until recently. BAY 86-6150 is a genetic variant of rFVIIa, with a modified amino acid sequence, resulting in a slightly extended $t_{½}$ and increased binding to activated platelets. A phase 1, single-dose escalation study in hemophilia patients with or without inhibitors showed a dose-linear relationship over the dose range investigated (6.5–90 μg kg^{-1}), and a $t_{½}$ of 5–7 h for the two highest dose groups (50 and 90 μg kg^{-1}). One patient in this study developed anti-drug antibodies to BAY 86-6150, cross-reactive with endogenous rVIIa at baseline and indicative of possible undue immunogenicity [15]. Development of inhibitory antibodies to BAY 86-6150 during the phase 2/3 clinical trial led to discontinuation of development of this agent too [32].

Finally, the immunoglobulin G (IgG1) Fc domain is being investigated as another potential fusion partner to confer an extended $t_{½}$ to rFVIIa. FVIIa as a monomeric Fc fusion demonstrated a 5-fold longer $t_{½}$ than rFVIIa in hemophilia A mice [33]. In addition, rFVIIa-Fc variants are in development, with further studies needed to determine the efficacy and safety of IgG1. It is therefore possible that hemophilia patients with inhibitors might benefit from new treatments in forthcoming years.

Conclusions

Our preclinical studies confirm the proposed concept that a longer terminal elimination $t_{½}$ of rVIIa-FP compared with rFVIIa by albumin fusion translates into extended PD activity. Although inter-species scaling is complex, the observed improvement in the PK profile of rVIIa-FP compared with rFVIIa was consistent across all species studied. Thus, rVIIa-FP is a promising candidate for a long-acting bypassing agent. This is encouraging because there is a need for therapies with low injection-frequency regimens that can provide effective control of bleeding episodes during surgical procedures, and long-term prophylactic treatment of hemophilia patients with inhibitors. The potential clinical benefits of rVIIa-FP will, of course, require testing in clinical trials in patients with hemophilia and inhibitors.

Addendum

S. B. Zollner: study concept, study design and data analysis. D. Schuermann: study design, study execution and data analysis. E. Raquet: study design, study execution and data analysis. J. Müller-Cohrs: statistical analysis. T. Weimer: product concept and production. I. Pragst: study concept. G. Dickneite: product concept. S. Schulte: product concept.

Acknowledgements

This work was sponsored by CSL Behring GmbH and the authors were fully responsible for the content of this manuscript. The authors gratefully acknowledge the editorial assistance of Neel Misra, Murray Edmunds and Daria Renshaw, from Watermeadow Medical, Witney, UK, in the development of this manuscript. This assistance was funded by CSL Behring. This full article has not been previously published nor is it currently submitted for consideration for publication elsewhere. Abstracts including some of the study results were accepted for presentation in posters at the: 56th GTH Annual Meeting, 1–4 February, St Gallen, Switzerland; 5th EAHAD Annual Meeting, 22–24 February, Rome, Italy; 30th WFH Annual Meeting, 8–12 July, Paris, France; and 54th ASH Annual Meeting and Exposition, 8–11 December 2012, Atlanta, GA, USA.

Disclosure of Conflict of Interests

All authors are employees of CSL Behring GmbH (Marburg, Germany), whose product rVIIa-FP was studied in this work. D. Schuermann was an employee of CSL

Behring at the time of writing this manuscript but has since left the company.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig. S1. Dose–response curves for rVIIa-FP and rFVIIa from mouse tail-clip study.
Fig. S2. Mean total blood loss in hemophilia A mice following equimolar dosing, adjusted for selective FVIIa activity.
Fig. S3. Pharmacodynamic potency assessment measuring thrombus formation in rabbits.
Fig. S4. Pharmacodynamic assessment measuring thrombus formation in rabbits.

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