Journal of Thrombosis and Haemostasis, **11**: 1977–1985 DOI: 10.1111/jth.12409

ORIGINAL ARTICLE

Safety and pharmacokinetics of a recombinant fusion protein linking coagulation factor VIIa with albumin in healthy volunteers

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To cite this article: Golor G, Bensen-Kennedy D, Haffner S, Easton R, Jung K, Moises T, Lawo J-P, Joch C, Veldman A. Safety and pharmacokinetics of a recombinant fusion protein linking coagulation factor VIIa with albumin in healthy volunteers. *J Thromb Haemost* 2013; **11**: 1977–85.

Summary. *Background:* Development of neutralizing antibodies remains the most problematic complication in treating congenital hemophilia. Control and prevention of bleeding events in such patients with recombinant factor VIIa (rFVIIa) is limited by the short half-life of the available product. Here, we report on the pharmacokinetics and safety of a novel, recombinant fusion protein linking coagulation FVIIa with albumin (rVIIa-FP) in a first-in-human study in healthy male subjects. *Methods:* Forty healthy male subjects between 18 and 35 years of age were included and dosed in five consecutive cohorts. In each cohort, six subjects were randomized to a single dose of rVIIa-FP (140, 300, 500, 750, or 1000 μg kg^{-1}) and two to placebo. All subjects received anticoagulation with an oral vitamin K antagonist to reach an international normalized ratio between 2 and 3 prior to dosing with rVIIa-FP/placebo. Dosing with oral vitamin K antagonist was continued at a fixed dose for 6 days after injection of rFVIIa. *Results and Conclusions:* Tolerance of rVIIa-FP was good at all dose levels. No serious adverse events were observed. None of the subjects developed anti-drug antibodies. The maximum baseline-corrected mean (SD) FVIIa plasma activity increased in a dose-proportional manner. Across the dose range, the median half-life was consistent, ranging from 6.1 to 9.7 h. At the highest dose of 1000 μg kg^{-1}, the median FVIIa activity-based half-life was 8.5 h. Clearance ranged from 7.62 to 12.74 mL h^{-1} kg^{-1}. Compared with the commercially available rFVIIa product, rVIIa-FP had a reduced clearance resulting in an approximately 3- to 4-fold increase in half-life.

Keywords: factor eight inhibitor bypassing activity; factor VIIa; half-life; hemophilia A; hemophilia B.

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Received 21 May 2013
Manuscript handled by: M. Levi
Final decision: F. R. Rosendaal, 4 September 2013

Introduction

The development of neutralizing antibodies to coagulation factor VIII (FVIII) or FIX remains the most serious complication of replacement therapy with factor concentrates in hemophilia A and B. Apart from attempts to eradicate these inhibitors with immune tolerance induction, immune suppressive therapy, or plasmapheresis, patients with inhibitors rely on bypassing products to control or prevent acute hemorrhages. The efficacy of available products—such as FVIII bypassing agent (FEIBA®; Baxter AG, Vienna, Austria), recombinant activated FVII (rFVIIa, NovoSeven®; Novo Nordisk A/S, Søborg, Denmark), and others such as porcine FVIII—is inferior to FVIII or FIX substitution therapy in controlling bleeds in hemophilia patients without inhibitors [1]. Furthermore, rFVIIa has a relatively short half-life ($t_{½}$) that limits the practicality of prophylaxis with this therapeutic protein.

In the past years, different technologies have been developed to prolong the $t_{½}$ of recombinant coagulation factors. Among those, glycopegylation and fusion technologies seem to be most widely used. CSL Behring developed the albumin fusion technology platform in which a fusion protein linking a recombinant human coagulation factor and recombinant human albumin is expressed as a single recombinant construct in Chinese hamster ovary (CHO) cells (i.e., recombinant FVIIa linked with albumin: rVIIa-FP). This technology was used

to successfully prolong the plasma $t_{½}$ of recombinant coagulation FIX [2].

In comparison to rIX-FP, the coagulation factor and albumin in rVIIa-FP are linked by a non-cleavable linker. The compound showed promising pharmacokinetic, pharmacodynamic activity and safety attributes in several non-clinical animal models [3]. Here, we report the results of a single dose first-in-human, placebo-controlled, dose-escalation safety and pharmacokinetic study in 40 healthy male subjects, pretreated with an oral vitamin K antagonist (OVKA).

Subjects and Methods

Aims

The goal was to investigate safety (with a focus on the occurrence of thromboembolic events and the development of anti-drug antibodies) and pharmacokinetics of rVIIa-FP in healthy male subjects, pretreated with OVKA.

Subjects

The study protocol was reviewed and approved by the respective national authority (Paul-Ehrlich-Institut, Frankfurt, Germany) and the ethics committee (Ethik-Kommission des Landes, Berlin, Germany). The study aimed to enroll a total of 40 healthy male subjects aged between 18 and 35 years. Main exclusion criteria consisted of personal or family history of thrombosis, cardiovascular events, or other prothrombotic risk factors such as smoking or obesity. All study participants underwent screening for congenital thrombophilia (resistance to activated protein C, protein C or S deficiency, lupus anticoagulants, elevated plasma homocysteine, and antithrombin deficiency). The study was double-blinded; thus, the subjects, the investigator, and the study physician were blinded to the treatment. Subjects were randomized to rVIIa-FP or placebo in a 3:1 ratio by means of a computer-generated randomization list.

Anticoagulation

Before the first dose of study medication (rVIIa-FP or placebo), subjects underwent pretreatment with OVKA (warfarin) to achieve an international normalized ratio (INR) between 2 and 3 as an additional precaution against potential thromboembolic events. The OVKA dose needed to reach stable anticoagulation within the aforementioned INR range was continued as a fixed daily dose for 6 days after administration of study medication. The study team, except for an unblinded subinvestigator, was blinded for all INR results once the subject was dosed with the study medication. After administration of the study drug, the OVKA dose was only adjusted in the event of an increase of the INR above 3 (prompted after review by the unblinded subinvestigator). On day 7 after dosing with the study medication, residual anticoagulation was reversed with oral vitamin K (5 mg).

Administration of rVIIa-FP or placebo

Once a stable INR between 2 and 3 was reached, the subjects received a single intravenous injection of rVIIa-FP or placebo (normal saline in corresponding volumes), administered over 15 min. The starting dose was derived from the no observed adverse event levels in the single-dose toxicology studies in rats and cynomolgus monkeys according to CPMP/ICH/286/95, the dose escalation followed a modified Fibonacci series. Subjects were dosed in five sequential cohorts of eight subjects each, with six subjects randomized to rVIIa-FP and two subjects to placebo (140 μg kg^{-1} for the first, 300 μg kg^{-1} for the second, 500 μg kg^{-1} for the third, 750 μg kg^{-1} for the fourth, 1000 μg kg^{-1} for the fifth cohort). In each cohort, dosing was performed in a staggered fashion with two subjects dosed on day 1, three on day 2, and three on day 3. After each cohort, adverse event (AE) data were reviewed by a safety review committee consisting of the principal investigator, an independent expert, the sponsor safety physician, and program director before dosing of the subsequent cohort commenced.

Testing for rVIIa-FP plasma levels

Blood samples for pharmacokinetic analysis were obtained before injection (0 h), immediately after end of injection (nominally at 0.25 h), and at 0.50, 1, 4, 8, 12, 24, 36, 48, 72, 96, and 120 h after the start of infusion. FVIIa activity was determined using StaClot FVIIa-rTF clotting assay (Diagnostica Stago, Asnières, France) in combination with a coagulation analyzer (BCS XP; Siemens Healthcare Diagnostics, Marburg, Germany). Briefly, citrated plasma samples were diluted with StaClot dilution buffer into measuring range according to actual dosing regimen if necessary. The reference curve was prepared from World Health Organization 2nd International Standard Factor VIIa concentrate NIBSC Code 07/228. The calibration range was 7.8–250 mIU mL^{-1}.

Pharmacokinetic analysis and statistics

Pharmacokinetic analysis included determination of incremental recovery defined as the peak level recorded after infusion related to dose [(mU mL^{-1}) (μg kg^{-1})]; terminal elimination $t_{½}$; maximum observed plasma activity (C_{max}) (mU mL^{-1}); area under the activity-versus-time curve from time zero to the last sample with quantifiable drug activity (AUC) (h × mU mL^{-1}); area under the activity-versus-time curve from time zero extrapolated to infinity ($AUC_{0-\infty}$) (h × mU mL^{-1}); clearance (CL) (mL h^{-1} kg^{-1}); mean

residence time (h); and volume of distribution at steady state (mL kg^{-1}).

Pharmacokinetic parameters were derived for baseline-corrected FVIIa activity using Phoenix® WinNonlin® 6.2 (Pharsight, St. Louis, MO, USA). Baseline-corrected FVIIa activity was calculated by subtracting the preinfusion value from each measurement after infusion. An assessment of dose proportionality was performed for uncorrected and baseline corrected C_{max} and AUC_{0-t} using the power-law model.

Testing for anti-drug antibodies

A tiered approach to immunogenicity testing for rVIIa-FP was used during the study. First, an ELISA-based screening assay, allowing for 5% false-positive results, was performed. Citrated plasma samples were diluted 1:20 using Tris-buffered saline containing 3% non-fat milk. Diluted samples were applied to an ELISA plate coated with rVIIa-FP. All samples above cut-off were subjected to a confirmatory assay. For this assay, samples were diluted 1:50 and all samples above cut-off would be reported as positive for anti-rVIIa-FP antibodies. Cut-offs were defined according to guidelines for industry: Assay development for immunogenicity testing of therapeutic proteins (FDA, effective 2009 [4]).

To further discriminate between different epitopes, positive samples would have been assayed for antibodies against NovoSeven® or human albumin.

Inhibitors were titrated by a newly established and validated assay based on the Bethesda method, using the Nijmegen modification that was designed for detection of inhibitory FVIII or FIX antibodies. In detail, equal volumes of plasma samples—with and without dilution in FVII-deficient plasma—and a standardized FVII containing sample as well as FVII-deficient plasma (negative control) were mixed and incubated for 2 h at 37°C followed by measurement of FVII activity using a one-stage clotting assay based on Thromborel S® (Siemens Healthcare Diagnostics). During the incubation period, antibody–antigen complexes would be formed. Subsequently, FVII activity values would decrease, resulting in residual activity of plasma sample. This residual activity was converted to BU mL^{-1} using the published method. The cut-off of this method was determined to be 0.6 BU mL^{-1}; results above 0.6 BU mL^{-1} were reported as positive.

Testing of thrombin–antithrombin, F_{1+2} fragments, and antithrombin

Thrombin–antithrombin (TAT) levels and F_{1+2} were measured by commercially available ELISAs (Enzygnost TAT micro; Enzygnost F1 + 2 (monoclonal); both Siemens Healthcare Diagnostics). Antithrombin (AT) was measured by chromogenic determination of functional activity of AT in plasma samples using the Berichrom Antithrombin III kit (Siemens Healthcare Diagnostics) in combination with the Behring Coagulation System XP (BCS XP; Dade Behring, Deerfield, Il, USA). All kits were used according to manufacturer's instructions.

Testing for activated partial thromboplastin time and INR

The determination of the activated partial thromboplastin time (APTT) was performed by using a Dade Actin® FS Activated PTT Reagent (Siemens Healthcare Diagnostics). Factors of the intrinsic coagulation system are activated by incubation with phospholipids and surface activator. The coagulation process is triggered by the addition of calcium ions, and the clotting time is measured.

INR was tested using the Dade Innovin® Reagent (Siemens Healthcare Diagnostics). In this method, the coagulation cascade is activated by incubating plasma with the optimal amount of thromboplastin and calcium, and the clotting time is measured.

Testing for fibrinogen and D-dimers

The quantitative determination of fibrinogen in plasma was performed by a commercially available Dade Thrombin® Reagent (Siemens Healthcare Diagnostics).

D-dimer levels were measured by a commercially available INNOVANCE® D-Dimer Assay (Siemens Healthcare Diagnostics). This is a particle-enhanced, immunoturbidimetric assay for the quantitative determination of cross-linked fibrin degradation products (D-dimers) in human plasma for use on coagulation analyzers.

Results

A total of 103 healthy male volunteers aged 18 to 35 years were screened to enroll 40 subjects in this study. The most relevant reason for screening failure were deviations from the defined in/exclusion criteria within the lab safety parameters, particularly elevated homocysteine, decreased protein S, and elevated liver function test results or failure to reach a stable INR during the warfarin run-in phase. Flow of study subjects through the study is shown in Fig. 1. Subject characteristics are depicted in Table 1.

Evaluation of safety (primary end point)

The local tolerance of rVIIa-FP was good in all subjects. A total of 34 AEs and no severe AEs were observed. Three AEs (headache, infusion site hematoma, and flatulence) occurred in the placebo group. Thirty-one AEs occurred in the rVIIa-FP groups, with no specific accumulation at any dose level. All AEs except four were mild; four AEs were reported as moderate (nasopharyngitis [two subjects], ligament sprain, and occurrence of several angiolipomas at the forearms). One AE (pain at the infusion site after injection of 1000 μg kg^{-1}) was

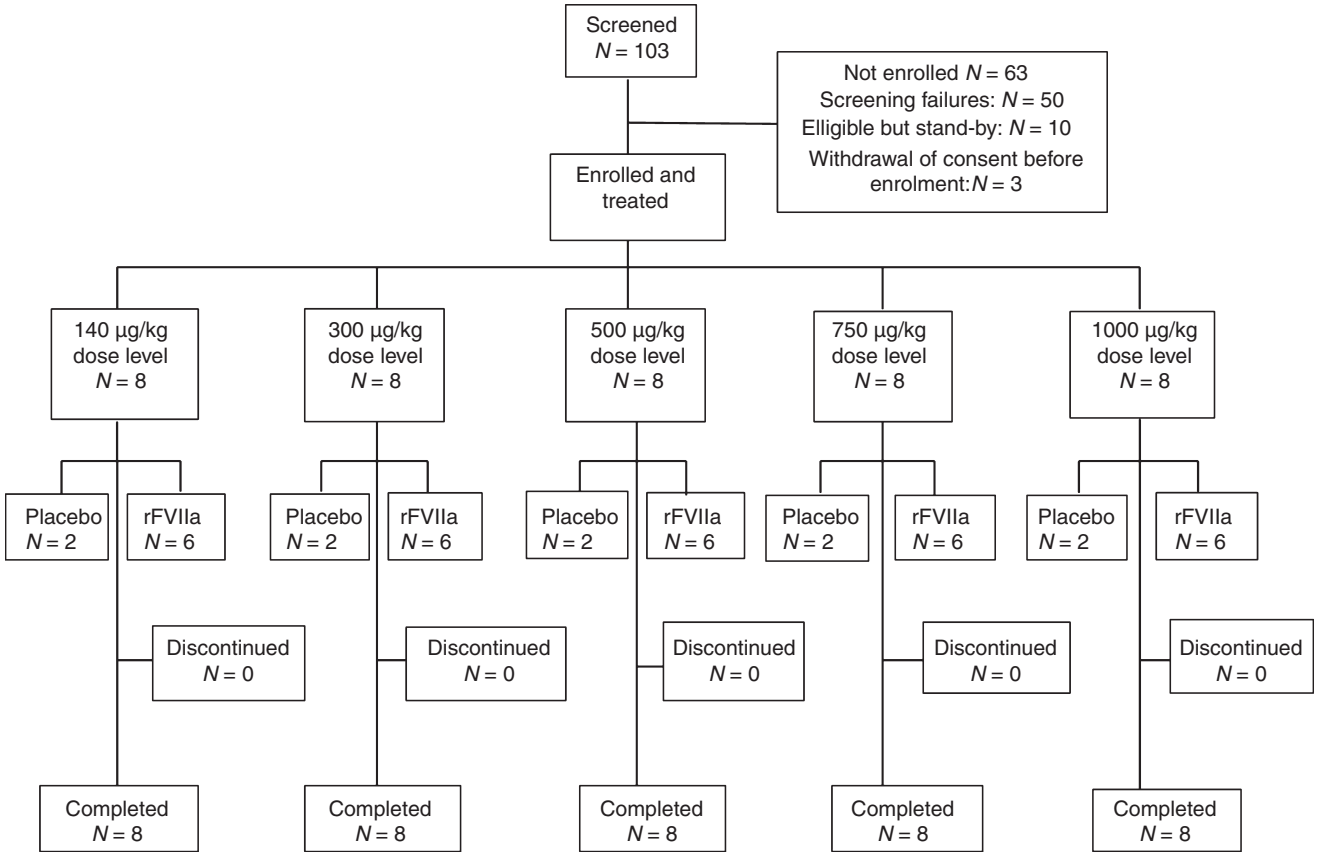

Fig. 1. Allocation of study participants. CONSORT diagram showing flow and allocation of study participants through the study.

judged to be related to rVIIa-FP. No subject tested positive for anti-drug antibodies or inhibitors before, at day 8, or on day 28 after study drug administration.

One subject receiving the 500 µg kg^{-1} dose of rVIIa-FP reported the sudden appearance of several subcutaneous nodules on the left forearm and the upper right arm. This subject had multiple preexisting lipomas on both forearms. The newly reported nodules were manually movable subcutaneous nodules and showed no signs of inflammation. A biopsy was performed, and histology showed nodular tumors of lipocytes crossed by capillary proliferations with individual microthrombi. These results are consistent with an angiolipoma (teleangiectatic lipoma), which is considered normal for a man of that age. In addition, immunohistochemical investigation showed no proliferative activity (Ki-67) in the lipocytes. There was nuclear expression in some endothelial cells of the capillary proliferations within the angiolipoma (< 5%). This result is not consistent with rapid growth resulting in a sudden appearance as reported by the subject. No relationship to administration of rVIIa-FP could be established.

All AEs resolved without sequelae with the exception of angiolipoma that was reduced in size but still detectable at the end of study.

The safety analysis had special focus on thromboembolic complications. No thromboembolic events were observed during the study. One subject (receiving rVIIa-FP at the 1000 µg kg^{-1} dose) experienced pain at the infusion site, but this was not related to a thrombophlebitic or thrombotic reaction.

Evaluation of the pharmacokinetic profile of rVIIa-FP (secondary end point)

Before the infusion of rVIIa-FP, FVIIa activity was below the level of quantitation of the assay (7.8 mU mL^{-1}) in all subjects except one who had a baseline FVIIa activity of 8.6 mU mL^{-1}. The pharmacokinetic parameters are summarized in Table 2. Following single intravenous infusions of 140–1000 µg kg^{-1}, FVIIa activity peaked near the end of infusion (Fig. 2). The median t_{max} was 0.267–0.500 h across dose levels, which corresponded to the end of infusion for approximately half of the subjects. FVIIa baseline-corrected mean C_{max} (SD) values increased in a dose-proportional manner, with an approximately 7-fold increase in C_{max} over the 7-fold increase in dose (9240 [515] mU mL^{-1} for the 140 µg kg^{-1} dose to 63 520 [13 515] mU mL^{-1} for the 1000 µg kg^{-1} dose). Therefore, the incremental recovery was fairly consistent across dose levels and ranged from 56.35 [(mU mL^{-1}) (µg kg^{-1})] at the 500 µg kg^{-1} dose level to 71.58 [(mU mL^{-1}) (µg kg^{-1})] at the 300 µg kg^{-1}

Table 1 Characteristics of subjects in the trial

Characteristic (Unit) Statistic	Placebo (N = 10)	rVIIa-FP					
		140 μg kg^{-1} (N = 6)	300 μg kg^{-1} (N = 6)	500 μg kg^{-1} (N = 6)	750 μg kg^{-1} (N = 6)	1000 μg kg^{-1} (N = 6)	Overall (N = 40)
Age (years)							
Median (min; max)	25.5 (21; 33)	31.0 (22; 35)	30.5 (24; 35)	27.5 (23; 35)	33.0 (27; 35)	29.0 (24; 35)	30.0 (21; 35)
Height (cm)							
Median (min; max)	182.0 (173; 190)	186.0 (173; 189)	184.0 (170; 192)	176.0 (170; 188)	186.5 (170; 194)	182.5 (173; 186)	183.0 (170; 194)
Weight (kg)							
Median (min; max)	78.75 (71.1; 88.4)	80.65 (65.2; 90.9)	89.65 (65.6; 100.2)	79.85 (62.9; 97.9)	96.65 (69.5; 99.9)	82.90 (65.2; 95.5)	82.55 (62.0; 100.2)
BMI (kg m^{-2})							
Median (min; max)	24.10 (21.3; 25.9)	24.25 (20.6; 26.3)	24.55 (22.6; 29.6)	24.70 (21.5; 27.7)	26.45 (24.0; 28.4)	25.30 (19.3; 28.8)	24.80 (19.3; 29.6)

N, total number of subjects; min, minimum; max, maximum; BMI, body mass index.

dose level. This is consistent with the reported behavior of rFVIIa [5].

The baseline-corrected mean FVIIa AUC_{0-t} increased in a slightly more than dose-proportional manner across the dose range, from 49 546 (7109) h × mU mL^{-1} for the 140 μg kg^{-1} dose to 616 638 (125 522) h × mU mL^{-1} for the 1000 μg kg^{-1} dose.

The individual values of mean $t_{½}$ were documented in the range of 6.1–13.5 h. The coefficient of variation for $t_{½}$ was high for the 300 and 500 μg kg^{-1} dose groups (92% and 70%, respectively). This was due to one subject in each cohort with a long $t_{½}$ compared with the other subjects in those dose groups. For one subject in each of the lower three dose groups, the elimination rate constant and related pharmacokinetic parameters could not be estimated due to variation of the last several time points around baseline. Across the dose range, the median $t_{½}$ was quite consistent, ranging from 6.1 h to 9.7 h. At the highest dose (1000 μg kg^{-1}), the median $t_{½}$ was 8.5 h.

Assessment of dose proportionality using the power-law model showed that baseline-corrected and uncorrected C_{max} increased in a dose-proportional manner across the 140 μg kg^{-1} to 1000 μg kg^{-1} dose levels; the slope estimate was 0.9351 with a corresponding 95% confidence interval of 0.8606–1.0095. In contrast, AUC_{0-t} increased in a more than dose-proportional manner, since the baseline corrected slope estimate was 1.2205 with a corresponding confidence interval of 1.1250–1.3160. Therefore, the increase in mean AUC_{0-t} was 22% higher than expected compared with the corresponding increase in dose.

CL was consistent across dose levels, ranging from 7.62 to 12.74 mL h^{-1} kg^{-1}. Volume of distribution was also consistent, ranging from 81.70 to 97.97 mL kg^{-1}.

Changes in platelets, fibrinogen, AT, TAT, F_{1+2} fragments, and D-dimer after the administration of rVIIa-FP

Monitoring of platelets, fibrinogen, and AT did not show significant changes to baseline; no differences between the various dose groups and placebo could be detected. As expected, a moderate increase in D-dimer, F_{1+2} fragments, and TAT was detected after injection of rVIIa-FP (Fig. 3). D-dimers, F_{1+2} fragments, and TAT are markers of thrombin generation, and a transient increase of similar magnitude and duration has been published previously in studies investigating rFVIIa, N7-GP, and vatreptacog alpha in healthy volunteers [6–8]. A dose dependency could not be established.

Changes in APTT and INR after the administration of rVIIa-FP

Changes in APTT after the injection of rVIIa-FP did not differ between dose groups or from placebo at any time point.

Table 2 Descriptive Summary of Pharmacokinetic Parameters – Baseline Corrected (Pharmacokinetics Population)

	Statistic	140 μg kg^{-1} (n = 6)	300 μg kg^{-1} (n = 6)	500 μg kg^{-1} (n = 6)	750 μg kg^{-1} (n = 6)	1000 μg kg^{-1} (n = 6)
IR [(mU mL^{-1}) (μg kg^{-1})]	Mean	67.97	71.58	56.35	60.08	65.39
	SD	3.867	8.718	5.271	5.030	13.913
C_{max} (mU mL^{-1})	Mean	9240.0	20 873.3	27 451.9	43 760.0	63 520.0
	SD	515.36	2552.79	2544.67	3668.59	13 515.05
$t_{½}$ (h)	Mean	6.055*	13.471*	12.267*	7.549	8.512
	SD	0.9609	12.4203	8.5886	0.7392	0.9640
AUC_{0-t} (h × mU mL^{-1})	Mean	49 546.4	151 200.7	234 641.0	371 649.2	616 637.5
	SD	7108.88	16 601.70	35 680.36	71 741.77	125 521.98
$AUC_{0-\infty}$ (h × mU mL^{-1})	Mean	50 760.5*	155 913.6*	237 998.6*	371 774.4	616 805.8
	SD	7359.02	13 830.68	38 761.94	71 774.41	125 547.08
CL (mL h^{-1} kg^{-1})	Mean	12.74*	8.81*	9.78*	9.45	7.62
	SD	1.760	0.774	1.791	1.769	1.499
V_{SS} (mL kg^{-1})	Mean	85.20*	81.70*	97.09*	97.97	84.57
	SD	9.338	12.395	13.420	13.263	12.760
$MRT_{0-\infty}$ (h)	Mean	6.771*	9.264*	10.189*	10.556	11.187
	SD	1.0487	1.0242	2.4951	1.6909	0.7229

SD, standard deviation; IR, incremental recovery; $t_{½}$, terminal elimination half-life; C_{max}, maximum observed plasma activity; AUC_{0-t}, area under the activity-versus-time curve from time zero to the last sample with quantifiable drug activity; $AUC_{0-\infty}$, area under the activity-versus-time curve from time zero extrapolated to infinity; CL, clearance; MRT, mean residence time; V_{SS}, volume of distribution at steady state.
*n = 5.

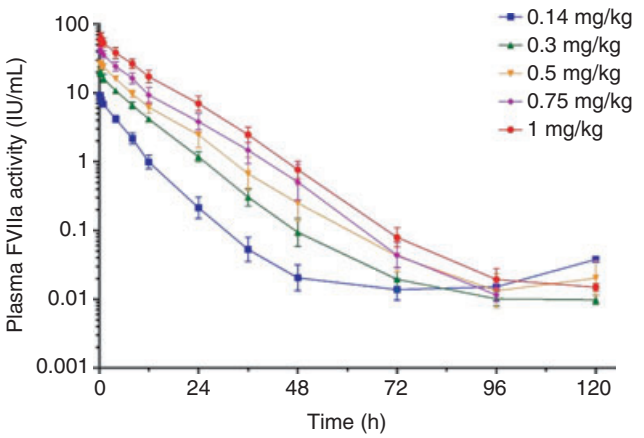

Fig. 2. Baseline corrected factor VIIa (FVIIa) plasma levels in subjects receiving recombinant fusion protein linking coagulation FVIIa with albumin (rVIIa-FP). Figure showing baseline corrected FVIIa plasma levels in subjects receiving rVIIa-FP over time per dose group.

INR normalized from the pretreatment level of 2–3 (all subjects received anticoagulation with OVKA) to an INR of 1 within 60 min after administration of rVIIa-FP, while subjects on placebo maintained an INR between 2 and 3 (Fig. 4). Since all subjects continued on a fixed daily dose of OVKA from day 1 until day 6 (after which the anticoagulation was reversed with vitamin K), all subjects receiving placebo maintained an INR above 2 until day 5, whereas in the rVIIa-FP dose groups, a decrease in the range of −1.392 and −1.530 was observed at 60 min after dosing. Due to long plasma $t_{½}$ of rVIIa-FP, almost all of the treated subjects (96.7%) had an INR below 1.5 for 24 h and more than half (53.3%) had it for 48 h. A *post hoc* analysis evaluating the difference of dose groups to placebo was performed using one-sided step-down superiority testing with an adjusted P-value of 0.005. It was found that subjects in the 300 μg group had statistically significantly lower INRs compared with the placebo group up to 24 h and with all other treatment groups up to 48 h after the administration of rVIIa-FP.

Discussion

In this study, we report on the first-in-human administration of a novel fusion protein, linking coagulation factor FVIIa with human albumin and expressed as a single recombinant construct from CHO cells. The aim of this study was to investigate the safety and pharmacokinetics of this fusion protein in healthy adults, using a standard design for first-in-human studies with a five-cohort dose escalation scheme (dosing at 140, 300, 500, 750, and 1000 μg kg^{-1}), random allocation to placebo and study product at each dose level, as well as data review by a safety board before each dose-escalation step and at the end of the study. The main safety concerns associated with all newly developed FVIIa compounds are potential thromboembolic events and the development of antibodies. Study participants were anticoagulated with warfarin to provide an additional protection against the occurrence of thromboembolic events.

No severe AEs were observed in this study. Furthermore, no thromboembolic events of any severity were observed throughout the study. The distribution of observed AEs did not show any dose relation and, with

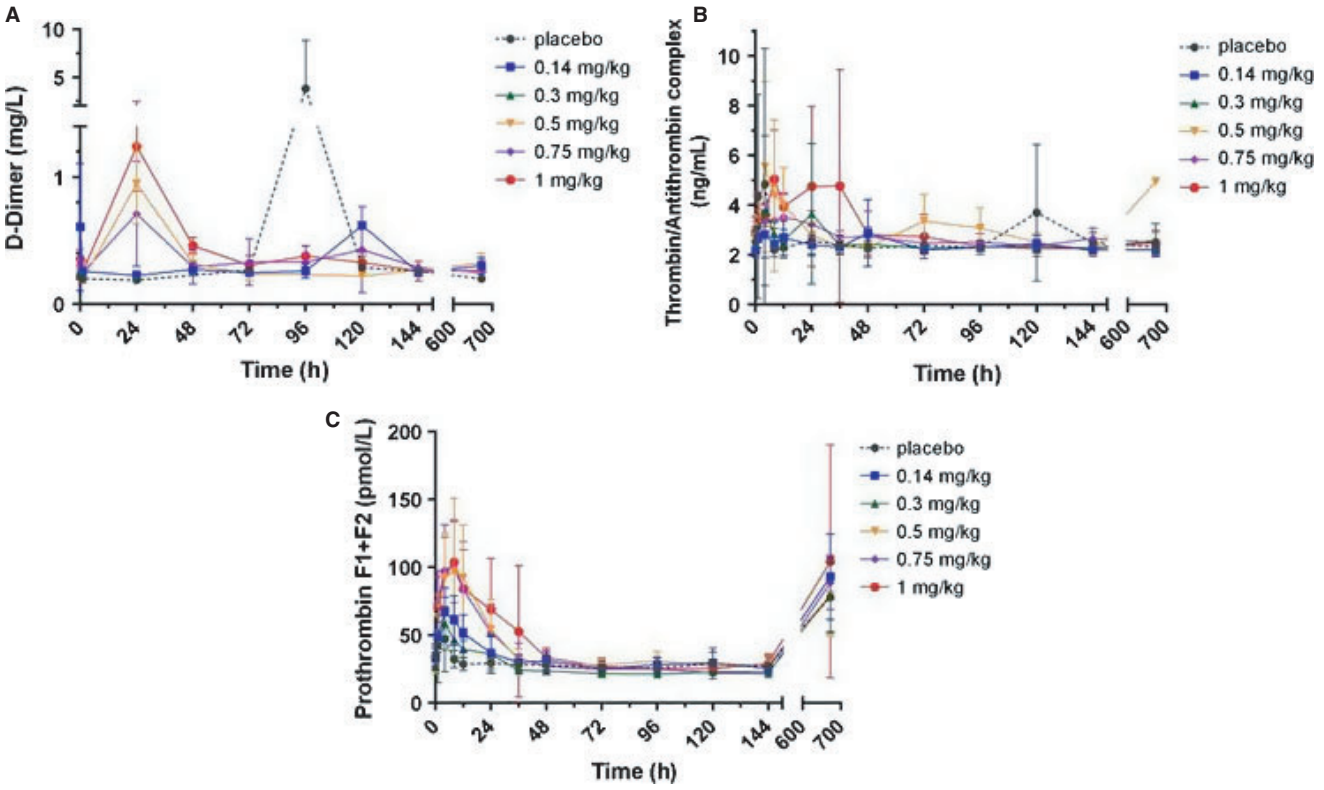

Fig. 3. D-dimers, F_{1+2} fragments, and TAT after injection of recombinant fusion protein linking coagulation FVIIa with albumin (rVIIa-FP). Figure showing baseline and course of D-dimers (A), TAT (B), and F_{1+2} fragments (C) as detected after injection of rVIIa-FP. TAT, thrombin–antithrombin complex.

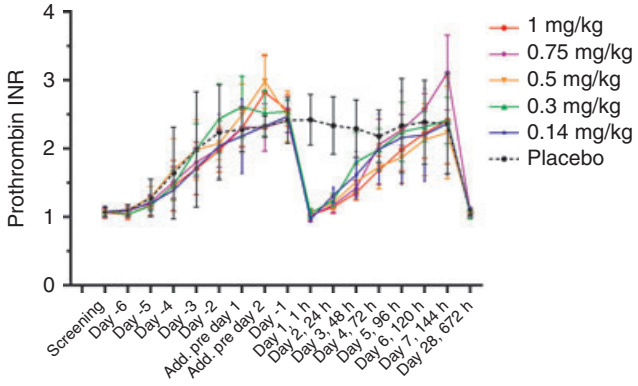

Fig. 4. INR measurements all subjects. Figure displaying INR measurements in subjects receiving recombinant fusion protein linking coagulation FVIIa with albumin (rVIIa-FP) and placebo over time per dose group. INR, international normalized ratio.

the exception of one episode of pain at the infusion site, the AEs have not been judged to be related to the administration of rVIIa-FP. No significant changes in the ECG have been observed in any of the study groups. Also, none of the participants tested positive for rFVIIa antibodies up to 28 days after study product administration, when the last test sample was drawn.

Neither platelet counts nor AT or fibrinogen levels indicated systemic activation of coagulation in any subject after the administration of rVIIa-FP. Expectedly, some increase in thrombin generation markers (such as TAT and F_{1+2} fragment) as well as a mild and transient elevation of D-dimers were observed after the administration of rVIIa-FP. This phenomenon is well described after the injection of rFVIIa and its newer variants and was found to be similar to the published literature in terms of magnitude and duration in this study [6–11].

In this study, subjects were anticoagulated with an OVKA to provide additional safety. After the injection of rVIIa-FP, but not after placebo, the INR normalized from > 2 to 1 within 60 min. This effect on warfarin reversal, that is interestingly not associated to a reduction in bleed risk if assessed by skin biopsy bleeding time, is well described in the literature [10–12]. Of note, the effect of rFVIIa on the INR in subjects anticoagulated with warfarin lasts for ≤ 24 h [10,11], while a post-hoc analysis provided statistically significantly differences in INR measurements between rVIIa-FP groups and placebo up to 48 h after injection despite continuation of daily warfarin administration at a fixed dose.

In this study in healthy adults pretreated with an OVKA, rVIIa-FP had a median terminal $t_{½}$ of 8.5 h and a median CL of 7.69 mL kg^{-1} h^{-1} at the 1000 μg kg^{-1} dose level. Friedberg *et al.* [6] reported the terminal $t_{½}$ of rFVIIa with 3.9–5.99 h with a plasma CL of 33–37 mL kg^{-1} h^{-1} in

healthy white and Japanese subjects; however, these data were obtained using FVII.C assays and not the FVIIa Sta-Clot assay used in the study reported here. In a review by Klitgaard *et al.,* [13] the terminal $t_{½}$ of rFVIIa measured by FVIIa clotting assay is reported to be 2.3–3.1 h in adult patients with hemophilia (CL 53–60 mL kg^{-1} h^{-1}) and 2.49–2.62 h in patients with FVII deficiency (CL 65–68 mL kg^{-1} h^{-1}). While it is difficult to directly compare the pharmacokinetics of rVIIa-FP to reports in the literature due to differences in assays and study populations, this study demonstrated that the fusion of recombinant albumin to recombinant FVIIa decreased the CL and thereby increased the $t_{½}$ of FVIIa in plasma. This is consistent with the observation of an increased duration of effect on warfarin-induced INR increase noted here earlier.

Other rFVIIa compounds with extended plasma $t_{½}$ or increased activity have recently been developed using different techniques. Moss *et al*. [7] reported on a glycopegylated rFVIIa variant (N7-GP) that was tested in healthy adults and demonstrated to have a longer $t_{½}$ than rFVIIa, however, this development program was later abandoned by the company. Mahlangu *et al*. [9] published a phase I study on a genetically modified rFVIIa variant (BAY 86–6150, program since stopped due to anti-drug antibody development) with both increased activity and prolonged plasma $t_{½}$ in 16 patients with hemophilia. In this study, the $t_{½}$ of BAY 86–6150 based on FVII antigen was reported as 5.8 h in the highest dose tier, but activity-based data are not reported. Of note, nonclinical tests of rVIIa-FP (and other coagulation factors) in several animal species showed that the antigen-based plasma $t_{½}$ of rVIIa-FP exceeds the activity-based measurements by 1.5–2 fold (CSL Behring, data on file).

An alternative development is the generation of rFVIIa variants with increased peak plasma activity and improved thrombin generation potential with the aim to provide a more rapid, reliable, and sustained resolution of acute bleeding events in patients with hemophilia and inhibitors. DePaula *et al*. [14] reported on a large, multicenter clinical study investigating the rFVIIa variant vatreptacog alpha in the treatment of joint bleeds in patients with hemophilia and inhibitors. In a previous phase I study in healthy volunteers, vatreptacog alpha demonstrated biphasic pharmacokinetics with a rapid initial distribution of 73% FVIIa activity ($t_{½\alpha}$ = 20 min) followed by a slower elimination of the remaining 27% of the compound ($t_{½\beta}$ = 3.1 h). The median CL of the highest dose group was 108 mL kg^{-1} h^{-1}, which is higher than that of rFVIIa [8]. Like the glycopegylated rFVIIa N7-GP and the BAY 86–6150 programs, the vatreptacog alpha development program was terminated by the sponsor after analysis of a phase III study, of which the results are not yet published.

Treatment strategies using rFVIIa in patients with hemophilia and inhibitors vary greatly. In treating acute bleeding events, Sorensen *et al*. [15] reported that the majority of physicians start using an initial dose of 270 μg kg^{-1} in both adult and pediatric patients, since most bleeding events were found to require more than one dose of rFVIIa if treatment was initiated with 90 μg kg^{-1}. Published data have indicated that prophylaxis regimens with rFVIIa to prevent bleeding events in patients with hemophilia and inhibitors are probably not mostly driven by the pharmacokinetics of rFVIIa. The sparse data in the literature report prophylaxis dosing frequencies ranging from twice daily to once weekly [16–18]. The prolonged $t_{½}$ of rVIIa-FP might enable prophylaxis in this group of patients with either a reduced frequency of factor administration or increased efficacy.

Limitations of this phase I study include, by design, the single dose exposure with a consequently limited ability to detect clinically relevant safety signals (i.e., thrombogenicity and immunogenicity) and the lack of an active comparator.

In summary, rVIIa-FP has a markedly improved pharmacokinetic profile in terms of prolonged $t_{½}$ and reduced CL compared with the reported pharmacokinetics for marketed and published rFVIIa compounds. The safety and tolerability in this first-in-human study were good. Further studies in patients with hemophilia and inhibitors will show if the prolonged plasma $t_{½}$ and reduced CL of rVIIa-FP translate into a relevant medical benefit in the treatment of these patients, both for control of acute bleeding events and as prophylaxis.

Addendum

G. Golor and S. Haffner executed the study. R. Easton analyzed pharmacokinetic data, K. Jung supervised data collection and management, T. Moises designed, performed, and supervised laboratory analysis, J.P. Lawo performed statistical analysis, C Joch supervised safety analysis and AE reporting, and A. Veldman and D. Bensen-Kennedy designed the study, supervised data analysis and interpretation, and wrote the manuscript.

Disclosure of Conflict of Interest

G. Golor and S. Haffner are employed by Parexel. D. Bensen-Kennedy, R. Easton; T. Moises, K. Jung, J. P. Lawo, C. Joch, and A. Veldman are employed by CSL Behring.

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