Accepted: 20 June 2017
DOI: 10.1111/hae.13312

ORIGINAL ARTICLE
Clinical haemophilia

WILEY Haemophilia

The pharmacokinetics and pharmacodynamics of single-dose and multiple-dose recombinant activated factor VII in patients with haemophilia A or B

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Summary

Monitoring recombinant activated factor VII (rFVIIa) treatment outcomes remains challenging. Thromboelastography (TEG) and the thrombin generation assay (TGA), measure coagulation dynamics over time and are being assessed as potential methods for evaluating and monitoring haemophilia treatment. Lack of standardized TEG/TGA methods makes it difficult to compare results and to establish a correlation with clinical outcomes.

Aims: To compare the pharmacokinetics (PK) of rFVIIa after 3×90 μg kg^{-1} doses vs a single dose (270 μg kg^{-1}) in haemophilia patients and to evaluate TEG/TGA results postdosing to determine how these assays relate to PK findings.

Methods: Patients in this open-label, single-centre, randomized, crossover trial received one injection of 270 μg kg^{-1} rFVIIa crossed over with three injections of 90 μg kg^{-1} rFVIIa in a non-bleeding state. For TEG, kaolin and tissue factor were used as activators; TGA was performed on frozen platelet-rich and platelet-poor plasma, with and without corn trypsin inhibitor. FVIIa activity was evaluated using in vivo samples.

Results: TGA showed a dose-dependent effect of rFVIIa on thrombin generation; TEG revealed lower dose-dependent effects. Both showed some differences between single-/multiple-dose rFVIIa; both supported the PK findings.

Conclusion: While TEG and TGA are not yet clinically predictive, both supported the PK results. Data suggest that, while a single dose of 270 μg kg^{-1} rFVIIa provides slightly higher haemostatic potential than the multiple-dose regimen of 3×90 μg kg^{-1}, the latter results in prolonged activity levels compared with a higher single dose.

KEYWORDS
pharmacodynamics, pharmacokinetics, rFVIIa, therapeutic drug monitoring, thrombin generation assay, thromboelastography

1 | INTRODUCTION

Recombinant activated factor VII (rFVIIa; NovoSeven®, Novo Nordisk A/S, Bagsværd, Denmark) has been available since 1996 for treatment of bleeds in haemophilia A or B patients with inhibitors. rFVIIa is licensed for use at standard (3×90 μg kg^{-1}) or high (1×270 μg kg^{-1}) doses world-wide[1] (the 270 μg kg^{-1} dose is however not approved in USA and Canada[2]), with a single dose of 270 μg kg^{-1} rFVIIa shown to be at least

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as effective as three consecutive doses of 90 μg kg^{-1}.[3,4] While rFVIIa provides high efficacy rates,[3-7] monitoring treatment outcomes remains challenging: the relationship between FVIIa clot activity and clinical efficacy is unclear and there are currently no validated, standardized assays to measure in vivo efficacy of rFVIIa or predict individual patient response.[8,9] Global haemostatic assays, such as thromboelastography (TEG) and the thrombin generation assay (TGA), measure coagulation dynamics over time[8-10] and are being assessed as possible methods for evaluating and monitoring haemophilia treatment.[10,11] However, lack of standardized TEG/TGA assay methods hinders result comparisons between laboratories/trials and establishing a correlation with outcomes in clinical trials.[10] For TEG, the choice of activators varies among studies and research centres, as well as diseases and treatments studied, with both intrinsic (using kaolin)[12,13] and extrinsic activation (using tissue factor [TF])[10,12,14,15] described in the literature. For TGA, both platelet-rich (PRP) and platelet-poor (PPP) plasma have been used[10,16]; the use of fresh or frozen plasma is another variable to consider,[16] along with the addition of contact activation inhibitors such as corn trypsin inhibitor (CTI).[10] The trial's aims were to compare pharmacokinetics (PK) of rFVIIa after multiple doses (3×90 μg kg^{-1}) vs a single dose (270 μg kg^{-1}) in haemophilia patients and evaluate TEG and TGA results postdosing to determine how these global assays relate to the observed PK findings. TEG/TGA methods were selected on the basis of an earlier evaluation, which determined the most suitable assay conditions for monitoring rFVIIa.

2 | MATERIALS AND METHODS

2.1 | Patients

Adult males (≥18 years) with severe congenital haemophilia A or B (<1% FVIII/FIX) with/without inhibitors were eligible. Key exclusion criteria were the presence of any other congenital or acquired coagulation disorder, clinical signs/known history of arterial thrombotic events and history of deep vein thrombosis or pulmonary embolism. Written informed consent was obtained from all patients.

2.2 | Trial design

This was an open-label, single-centre, randomized, crossover trial. Patients received a single intravenous (IV) injection of rFVIIa 270 μg kg^{-1} crossed over with three 90 μg kg^{-1} (every three hours [q3h]) IV injections in a non-bleeding state. The two dosing regimens were administered in a randomized order at least 48 hours, and no more than 4 weeks, apart (Figure 1). Prior to administration, all patients completed a 3-, 5- or 2-day washout period from the last FVIII administration, FIX/plasma-derived activated prothrombin complex concentrate (pd-aPCC) or rFVIIa respectively. Following rFVIIa administration, blood samples were collected for up to 24 hours for evaluation of PK and pharmacodynamics (PD), as outlined below. The study was conducted in accordance with the Declaration of Helsinki and the International Conference on Harmonisation Good Clinical Practice standards, and is registered on clinicaltrials.gov (NCT01949792).

2.3 | Pharmacokinetic analysis

The PK of single-dose (270 μg kg^{-1}) and multiple-dose (3×90 μg kg^{-1}) rFVIIa were evaluated by assessing plasma FVIIa activity and FVII antigen concentration. Samples were drawn in regular 3.2% sodium citrate plasma tubes and immediately underwent two consecutive centrifugations (1600 g, 15 minutes each). The resulting samples were stored at −80°C until analysis. FVIIa activity was determined using a commercially available assay (Staclot® VIIa-rTF, Diagnostica Stago UK Ltd, Theale, UK) validated for human citrated plasma. FVII antigen concentration was measured using an enzyme-linked immunosorbent assay (ELISA) validated for human citrated plasma samples.

2.4 | Pharmacodynamic assessment

2.4.1 | Evaluation of optimal TEG/TGA assay conditions

Earlier ex vivo and in vivo studies were conducted in patients to assess optimal TEG/TGA assay conditions for monitoring rFVIIa treatment (see Supplementary Material). When evaluating rFVIIa administration response by TEG, kaolin activation is preferable to activation by TF+tissue plasminogen activator (tPA), as it is simpler and more reproducible.[10] TF needs to be diluted several-fold and this can cause variability in the results, which make comparison difficult. When TGA is used to evaluate rFVIIa treatment response, frozen PRP without CTI should be the preferred method as it provides better correlation between TGA parameters and FVIIa activity (vs frozen PPP). Therefore, for the current study, kaolin was chosen for TEG analysis of rFVIIa treatment, and frozen PRP without CTI was chosen for TGA evaluation of treatment response.

2.4.2 | Thromboelastography

The TEG assay was performed at the trial site in Spain (Hospital Universitario La Paz). The analyses were performed on TEG

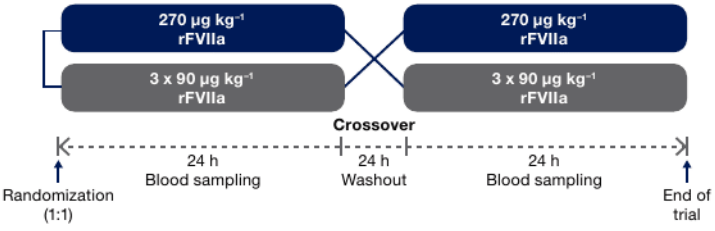

FIGURE 1 Study design. rFVIIa, recombinant activated blood coagulation factor VII [Colour figure can be viewed at wileyonlinelibrary.com]

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thromboelastographs (TEG® 5000 Hemostasis Analyzer System, Haemonetics Corporation, Braintree, MA, USA). Based on earlier TEG evaluation, coagulation activation was initiated using kaolin (Haemonetics Corporation, USA), thawed for 30 minutes at room temperature prior to use. Blood samples were collected in a 2.7 mL 3.2% sodium citrate tube and allowed to rest for 30 minutes before 1 mL of citrated whole blood was added to the kaolin tube. For re-calcification, 20 μL of $CaCl_2$ was added to each TEG cup, after which 340 μL of blood was added. Each sample was analyzed in duplicate using the two channels of each machine according to a predefined scheme, so for all subjects, all samples for a particular time point were analyzed on the same machine. Four TEG parameters were evaluated:

1. Reaction time (r-time [minute])—time to clot formation[17]
2. α-angle (degrees)—velocity of clot formation[11]
3. Maximum amplitude (MA [mm])—maximum clot strength
4. Maximum thrombus generation (MTG)—maximum velocity of clot formation.

2.4.3 | Thrombin generation

This was measured using the Calibrated Automated Thrombogram® (CAT; Fluoroskan Ascent™ Microplate Fluorometer, Thermo Fisher Scientific Inc., Waltham, MA, USA). Based on earlier evaluation of TGA, PRP without CTI was used. Blood samples were collected in standard 3.2% sodium citrate tubes and centrifuged to generate raw PRP (152 g for 10 minutes). Following platelet count adjustment to 150 000/μL with PPP, all samples were frozen at −80°C, then thawed for 10 minutes in a preheated 37°C water bath; 20 μL of buffer (20 mmol L^{-1} HEPES, 150 mM NaCl, 2% bovine serum albumin; pH 7.4) was added to the wells before adding 80 μL of the samples. TGA was triggered by the addition of 20 μL of starting solution (FluCa kit, Thrombinoscope™ BV, Maastricht, The Netherlands). Analyses were performed in triplicate using 96-well round-bottom microplates (Thermo Scientific Immulon® 2HB Plate). Four TGA parameters were assessed:

1. Lag time—when thrombin generation rises systematically above an arbitrary level (minute)[18,19]
2. Endogenous thrombin potential (ETP)—total enzymatic activity of the thrombin in the sample (nmol L^{-1}×minute)
3. Peak thrombin value (nmol L^{-1})
4. Time to peak thrombin (minutes).

2.5 | Other coagulation-related parameters

Activated partial thromboplastin time (aPTT), prothrombin time (PT), prothrombin fragments 1 and 2 (F1+2) and D-dimers were measured throughout the trial to allow for a more comprehensive picture of coagulation and to enable comparison of these "standard" coagulation parameters vs those for TEG/TGT. The aPTT analysis was performed using STA-R (Diagnostica Stago [SAS], Asnières sur Seine, France), with celphalin as reagent. PT analysis was performed using STA Stago, with STA-Neoplastin (both Diagnostica Stago) and calcium as reagents. F1+2 was measured by ELISA: Enzygnost F1+2 (monoclonal) (Dade Behring Inc., Deerfield, IL, USA). D-dimer levels were measured by ELISA VIDAS® D-Dimer Exclusion™ (BioMérieux, Marcy l'Etoile, France).

2.6 | Blood sampling

Blood samples for PK analysis of single-dose rFVIIa were collected predose and 10 minute, 1, 3, 6, 9, 12 and 24 hours postdose. For PK analysis of the 3×90 μg kg^{-1} regimen, samples were collected predose and 10 minutes, 1 and 3 hours, 3 hours 10 minutes (10 minutes after second administration), 6 hours, 6 hours 10 minutes (10 minutes after third administration) and 7, 9, 12 and 24 hours after the first dose. Sample collection for the TEG/TGA assays was performed at the same time points, except for the 1- and 12-hour time points.

2.7 | Data analyses

PK analysis was performed using non-compartmental methods (SAS version 9.3). TEG profiles and all parameters except MTG were analyzed using TEG® software v4.2.101 (database v1.0.17); MTG was analyzed using the TEG® analytical software, Lysis Tracker (research only), v4.1.73. For flat profiles, r-time was set to 180 minutes, ie the maximum duration of TEG analysis. TGA results were generated using the Thrombinoscope™ software program, v3.0.0.29 (Thrombinoscope™ BV, Maastricht, The Netherlands). For flat TGA profiles or where no distinct peak was identified by the program, lag time was set to 120 minutes (maximum duration of the assay); for TGA profiles with the error code "no tail found," only lag time was used.

2.8 | Safety

All patients were monitored for adverse events (AEs).

3 | RESULTS

3.1 | Patients

Seven patients were screened and six enrolled (haemophilia A, n=5; haemophilia B, n=1; mean age, 32 years [range, 24-46]). One patient was classed as a screening failure at Visit 1 and excluded from the study as he did not meet inclusion criteria (this patient had FIX activity level >1%). Of these six, two (one each with haemophilia A and B) had high-responding inhibitors; all completed the trial. Patient characteristics are listed in Table 1.

3.2 | Pharmacokinetics

PK profiles and parameters (Figure 2; Table 2) were in accordance with previously published PK data for single-dose rFVIIa in haemophilia patients[20,21] and healthy subjects.[22] PK data were consistent and comparable between single- and multiple-dose regimens, with

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TABLE 1 Patient characteristics

Patient number	Age (years)	Weight (kg)	Haemophilia type	Inhibitor status	Inhibitor titre (Bethesda units)	Inhibitor history
1	46	71.0	Haemophilia A (severe)	Negative	<0.4	No history of inhibitor
2	32	59.1	Haemophilia A (severe)	Negative	<0.4	History of transient inhibitor; no ITI
4	27	75.1	Haemophilia A (severe)	Negative	<0.4	History of inhibitor; successful eradication with ITI
5	26	72.7	Haemophilia A (severe)	Positive	62.7	High-responding inhibitor
6	38	72.5	Haemophilia B (severe)	Positive	<0.4	High-responding inhibitor
7	24	68.8	Haemophilia A (severe)	Negative	<0.4	No history of inhibitor

ITI, immune tolerance induction.

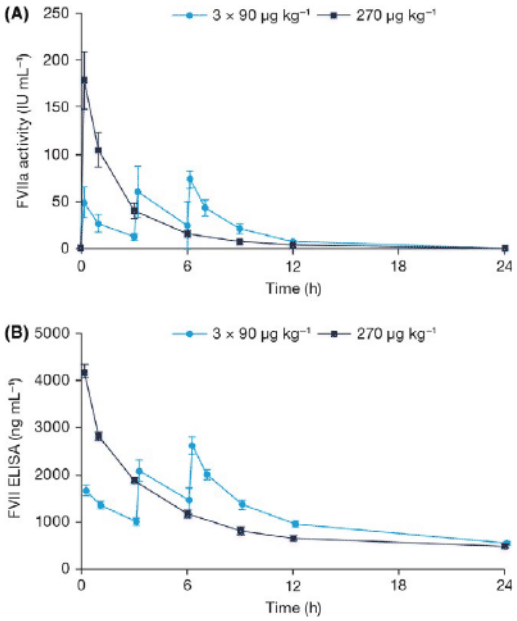

FIGURE 2 Mean FVIIa activity (A) and FVII concentration (B) following intravenous administration of 270 μg kg^{-1} rFVIIa and 3×90 μg kg^{-1}. FVII, blood coagulation factor VII; FVIIa, activated FVII [Colour figure can be viewed at wileyonlinelibrary.com]

low intersubject variability. In the FVIIa clot activity assay, mean (standard deviation) peak levels, observed 10 minutes after administration, were 178.8 (31.0) and 48.2 (16.6) IU mL^{-1} for rFVIIa 270 and 3×90 μg kg^{-1}, respectively, and the estimated half-life for each was 2.86 and 2.61 hours, respectively (Table 2). The accumulation ratio was 1.8, based on area under the plasma concentration–time curve for the multiple-dose regimen ($AUC_{0\text{-}3\ hours}$ third dose divided by $AUC_{0\text{-}3\ hours}$ first dose), with higher peak levels (73.2 IU mL^{-1}) following the third 90 μg kg^{-1} administration (Figure 2).PK parameters based on the plasma FVII antigen assay showed a reduced clearance compared with those based on FVIIa activity profiles (Table 2). This is as expected and caused by complex formation between rFVIIa and antithrombin (AT), the main clearance pathway for rFVIIa, as the FVII antigen assay does not distinguish between active and AT-bound FVII.[23]

3.3 | Thromboelastography

Despite intra- and interpatient differences, TEG profiles for individual patients showed good responses to both single-dose and multiple-dose rFVIIa, with a clear time effect and similar predose/24-hours postdose profiles.

For a single dose of 270 μg kg^{-1}, the ability of the blood to form a clot in the assay was maximal 10 minutes after administration and then declined gradually to reach predose levels at 24-hours postdose (Figure 3). The effect on r-time, MA, MTG and alpha-angle was equally strong for the 3×90 μg kg^{-1} regimen (Figure 3). There were no statistically significant differences between the 3×90 μg kg^{-1} and the 270 μg kg^{-1} treatment regimens for any of the TEG parameters 10 minutes postdosing (95% CI: MTG, −2.33-1.63; r-time, 0.67-1.55; MA, 0.43-1.75; α-angle, 0.54-1.54). Interestingly, TEG results for the multiple-dose regimen showed a durable effect on the ability of blood to form a clot in vivo between the three consecutive doses not reflected in the more fluctuating activity data, with a clear difference in pre- and postdose activity (Figure 3). This was supported by the positive correlation observed between FVIIa activity and both MTG (correlation coefficient, 0.61; $P<.0001$) and α-angle (correlation coefficient, 0.53; $P=.0003$), where FVIIa activity increased as each of the two parameters increased (Figure 4). These results also demonstrate that FVIIa activity above 10-15 IU mL^{-1} is sufficient to generate a clot in the TEG assay, correlating well with clinical experience. Additionally,

1. **For a single dose of 270 μ g kg –1, the ability of the blood to form a clot in the assay was maxima...**
Anchor Name: For a single dose of 270 μ g kg –1, the ability of the blood to form a clot in the assay was maximal 10 minutes after administration *[Agency FCB Halesway Olga Kooi]*

TABLE 2 Pharmacokinetic profile parameters for single-dose (270 μg kg^{-1}) and multiple-dose (3×90 μg kg^{-1}) rFVIIa (mean [SD])

	FVIIa clot activity (IU mL^{-1})		FVII concentration (ng mL^{-1})	
PK parameter	270 μg kg^{-1}	3×90 μg kg^{-1}	270 μg kg^{-1}	3×90 μg kg^{-1}
$AUC_{(0\text{-inf})}$, IU h mL^{-1}/ ng h mL^{-1}	455.36 (75.41)	429.50 (116.88)	37 580 (9438)	37 040 (4980)
$C_{10\ minute}$, IU mL^{-1}/ ng mL^{-1}	178.81 (30.97)	48.82 (16.56)	4205 (350)	1675 (241)
CL, mL kg^{-1} h^{-1}*	33.54 (5.32)	36.65 (7.87)	7.52 (1.42)	7.46 (0.91)
$t_{½}$, hours	2.86 (0.29)	2.61 (0.14)	18.34 (9.82)	12.29 (1.24)
V_{ss}, mL kg^{-1}	91.09 (16.05)	105.85 (27.13)	154.38 (44.55)	108.55 (18.24)

AUC, area under the curve; $C_{10\ minute}$, activity/concentration measured 10 minutes after first injection; CL, clearance; rFVIIa, recombinant activated blood coagulation factor VII; SD, standard deviation; $t_{½}$, terminal half-life; V_{ss}, volume of distribution at steady state.
*Least-squares mean.

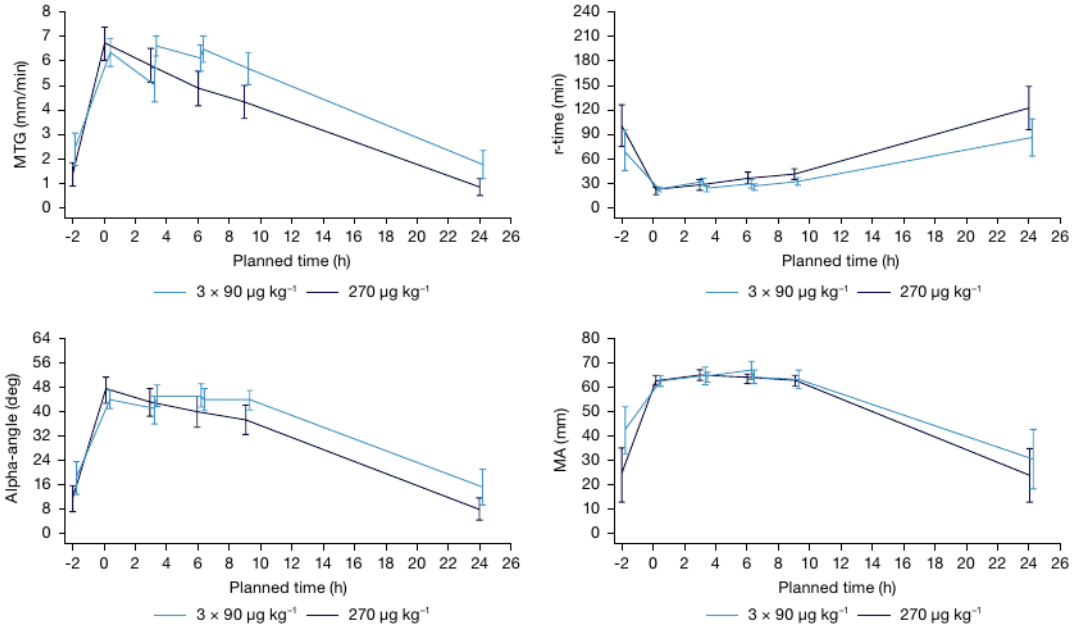

FIGURE 3 TEG mean profiles for MTG, r-time, α-angle and MA, using kaolin-activated samples. MA, maximum amplitude; MTG, maximum thrombus generation; r-time, reaction time; TEG, thromboelastography [Colour figure can be viewed at wileyonlinelibrary.com]

TEG profiles for both regimens show that blood is able to form a clot in the assay up to 9 hours after the first rFVIIa dose.

3.4 | Thrombin generation

Intra- and inter-patient differences were also evident with the TGA assay, with a clear time response after administration of both 270 and 3×90 μg kg^{-1} rFVIIa. The 24-hours postdose profiles were comparable to predose profiles. Peak thrombin generation and ETP showed a dose-dependent increase 10 minutes after 270 vs 3×90 μg kg^{-1} rFVIIa administration (dosing regimen ratio 3×90 μg kg^{-1}/270 μg kg^{-1} for peak thrombin, estimate 0.49 nmol L^{-1} [95% CI 0.35; 0.68] and ETP, estimate 0.61 nmol L^{-1}×minutes [95% CI 0.43; 0.85]) (Figure 5). Duration of the measurable effect of rFVIIa on thrombin peak and ETP was more pronounced with the 3×90 vs 1×270 μg kg^{-1} regimen (Figure 5), but fluctuation between pre- and postdose samples was higher than for the TEG parameter MTG, thus more in line with FVIIa activity levels. Some profiles, particularly those containing low activity levels, did not generate enough thrombin to provide reliable output.

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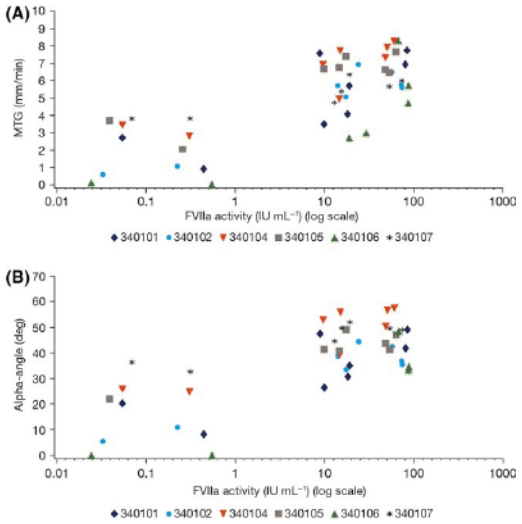

FIGURE 4 FVIIa activity vs TEG (kaolin-activated samples): (A) MTG and (B) α-angle for rFVIIa 3×90 μg kg^{-1}. MTG, maximum thrombus generation; rFVIIa, recombinant activated factor VII ; TEG, thromboelastography [Colour figure can be viewed at wileyonlinelibrary.com]

3.5 | Coagulation-related parameters

The results for the coagulation-related parameters PT, aPTT, F1+2 and D-dimers are shown in Figure 6. For F1+2, a delayed response to rFVIIa dosing was seen compared with FVIIa activity, with peak concentration measured 1 hour after administration. Additionally, there was a trend towards a response difference after 270 μg kg^{-1} and after the first 90 μg kg^{-1} dose, with a generally higher amount of F1+2 being formed after 270 μg kg^{-1}. However, after the third dose of 90 μg kg^{-1}, the difference seems to be in favour of the 3×90 μg kg^{-1} regimen, with a higher F1+2 concentration and the maximum level detected 1 hour after the third dose.

3.6 | Safety

Four non-serious AEs in four patients were reported: dizziness, lingual itching, superficial haematoma and trauma-related bleeding. All AEs were mild and not considered by the Investigator to be related to rFVIIa. There were no deaths, serious AEs or AEs leading to withdrawal. No other significant safety issues were identified.

4 | DISCUSSION

rFVIIa is licensed for the treatment of bleeding episodes in haemophilia A or B patients with inhibitors to Factor VIII or Factor IX at standard (3×90 μg kg^{-1}) or high (1×270 μg kg^{-1}) doses worldwide[1]; though the high dose is not approved in the USA and Canada.[2] Available data suggest that single-dose rFVIIa is at least as effective as the multiple-dose regimen while offering easier administration, improved treatment compliance, less injection-related pain and faster bleed control.[24] Our PK data, based on FVIIa activity and FVII antigen assessments, were

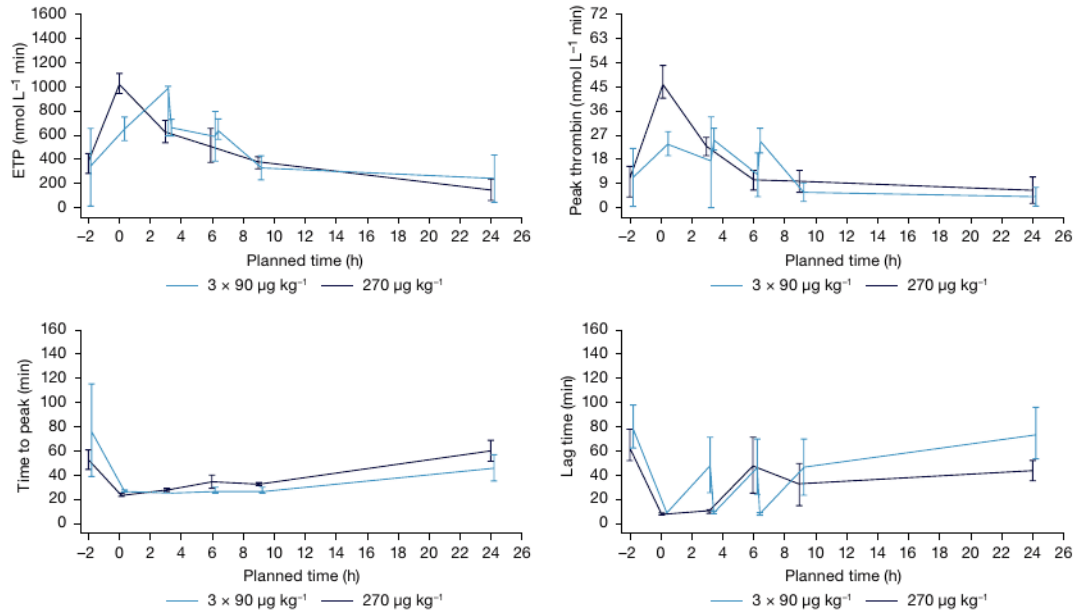

FIGURE 5 TGA mean profiles of ETP, peak thrombin value, time to peak and lag time using PRP. ETP, endogenous thrombin potential; PRP, platelet-rich plasma; TGA, thrombin generation assay [Colour figure can be viewed at wileyonlinelibrary.com]

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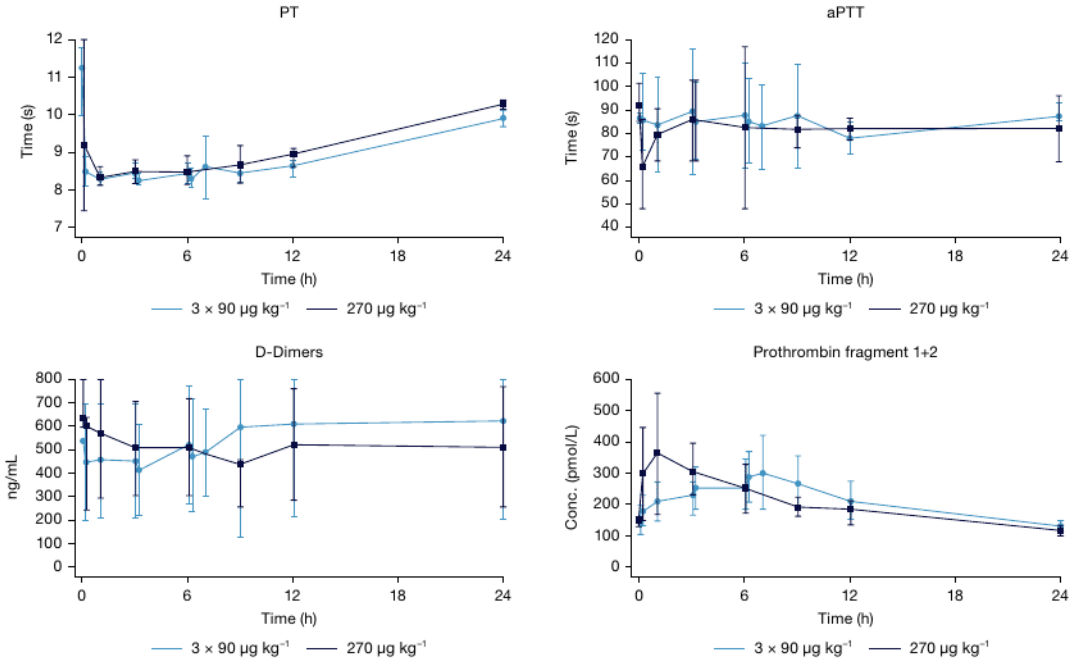

FIGURE 6 Mean coagulation-related parameter curves. aPTT, activated partial thromboplastin time; PT, prothrombin time [Colour figure can be viewed at wileyonlinelibrary.com]

similar between the two regimens and consistent with previous studies evaluating rFVIIa PK.[20-22] As expected, there was a build-up of FVIIa activity following repeated rFVIIa doses and the accumulation ratio of 1.8 supports the increased efficacy provided by additional doses following the first dose of 90 µg kg^{-1}. Both TEG and TGA showed some differences between single- and multiple-dose rFVIIa; both supported the PK results, as clot formation and thrombin generation were observed for FVIIa activity levels >15-20 IU mL^{-1}. We performed the TGA with frozen PRP, as the presence of platelets in the sample allows the haemostatic effect of rFVIIa to be captured more effectively. Both ETP and peak thrombin concentration were sensitive to FVIIa activity level and showed higher values for a single dose than for the first dose in the multiple-dose regimen, suggesting that 270 µg kg^{-1} has a higher haemostatic potential than obtained after each 90 µg kg^{-1} dose. The ETP and peak thrombin profiles over time also correlated with PK profiles, showing a reduction in thrombin generation correlating to PK trough levels; there were some outliers in TGA parameter profiles, but this was due to lost samples. These findings may support use of a single rFVIIa dose when a faster haemostatic response is needed, but additional clinical data are necessary. TEG data showed a FVIIa level-dependent effect but was incapable of depicting differences between single- and multiple-regimen doses. MTG response showed an activity-dependent decline with a 270 µg kg^{-1} single dose, whereas repeated doses of 90 µg kg^{-1} maintained almost unvariable MTG for up to 9 hours, suggesting a "carry over" effect. Therefore, when using a single dose of rFVIIa, additional doses may be needed after 6-9 hours to maintain activity levels at around 20 IU mL^{-1}, as achieved with the multiple-dose regimen. Indeed, registry data reported that a single dose was frequently administered 24 hours after high-dose rFVIIa treatment. Together, these findings suggest a rFVIIa dosing regimen of 3×90 µg kg^{-1} may produce lower, but more sustained, thrombin generation than a single 270 µg kg^{-1} dose. In contrast, the single 270 µg kg^{-1} dose may produce a higher initial peak than multiple doses. In clinical practice, it is often hard to determine bleed severity, which can make treatment decisions difficult. Our data suggest that a single 270 µg kg^{-1} dose may be an attractive option due to its higher initial thrombin generation and longer dose interval; these data are in line with several clinical studies supporting the use of a single high dose.[3,25-27] However, if a sustainable clinical response is not obtained after a single dose of 270 µg kg^{-1}, further doses may be necessary to maintain stable haemostatic levels.

The relationship between TEG/thrombin generation test (TGT) profiles and clinical response to rFVIIa has been previously evaluated in patients with haemophilia. Young et al. observed a good correlation between kaolin-activated TEG and clinical response to rFVIIa in three patients with inhibitors.[28] Interestingly, this work showed improvements in TEG profiles, similar to our results, more than 2 hours after rFVIIa dosing, suggesting the possible use of longer time intervals of rFVIIa dosing depending on individual clinical response. Although, recently, kaolin-activated-TEG has been reported as helpful in differentiating patients based on inhibitor status,[29] other studies have questioned the value of kaolin-activated TEG for evaluating response

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to treatment in patients without inhibitors.[30] In this latter study, only r-time correlated with levels of FVIII/FIX and no correlation between TEG profile and clinical outcome were found. These results are difficult to compare with our data, however, as clinical response to rFVIIa was not evaluated in this study; moreover, authors did not mention which final TEG condition was used (citrated or native whole blood) or the time to testing; both variables are of paramount importance for interpretation of the test.[31] Nonetheless, the same study reported good correlation between TEG peak thrombin in PPP and clinical outcome after FVIII/FIX treatment emphasising the value of TGT for evaluating response to treatment in patients with haemophilia without inhibitors. More studies are needed to clarify the relationship between TEG and clinical response in HA patients with or without inhibitors.

Limitations of our trial included the small patient numbers and rFVIIa assessment in a non-bleeding state, which prohibit evaluation of clinical efficacy (the main limitation of the trial). However, conducting an extensive PK/PD assessment based on multiple blood samples from patients in an ongoing bleeding state would be very difficult and result in even fewer patient numbers. We also observed inter- and intra-individual variability in TEG/TGA results, so it is possible that residual FVIII activity and FVIII/FIX inhibitory antibodies (in 2/6 patients) influenced the findings; no correlation was seen in baseline samples. TEG may be limited by its sensitivity to low FVIIa activity levels; results observed may be due to a saturation effect with maximum response achieved at low activity levels, resulting in little dose dependency. This would be consistent with reports that fibrin clot detection by TEG is achievable with very low levels of thrombin.[32,33] Finally, limitations of the TGA assay include restricted readouts, despite obtaining reasonable profiles.

5 | CONCLUSION

The TGA, performed using frozen PRP without CTI, showed a dose-dependent effect of rFVIIa on thrombin generation; TEG using kaolin activation revealed lower dose-dependent effects. While TEG and TGA are not yet clinically predictive, both supported PK results. The data suggest that a single dose of 270 μg kg^{-1} rFVIIa provides a slightly higher initial haemostatic potential than the multiple-dose regimen of 3×90 μg kg^{-1}, whereas the multiple-dose regimen results in prolonged activity levels compared with the higher single dose. Further studies are needed to define clinically relevant parameters of TEG and TGA and describe their relationship with clinical response. In addition, standardization of these assays is needed, as global procedures vary significantly. However, current data provide further insight into both the TEG/TGA utility in haemostasis evaluation following rFVIIa therapy and optimal assay conditions to implement in future clinical trials.

ACKNOWLEDGEMENTS

This program was sponsored by Novo Nordisk A/S (Måløv, Denmark). The investigators, trial staff and patients are thanked for their participation. Editorial assistance to the authors during the preparation of this manuscript was provided by Katherine Ayling-Rouse (medical writer, PAREXEL) and was financially supported by Novo Nordisk in compliance with international guidelines for good publication practice.

AUTHOR CONTRIBUTIONS

I.F.B contributed to the design of the laboratory conditions for TEG and CAT, performance of the CAT and TEG experiments, patient recruitment, analysis of the results, and the writing and reviewing of the paper. C.S. contributed to the design and planning of the trial, the design of the laboratory conditions for TEG and CAT, analysis of the results, and the writing and reviewing of the paper. N.B. contributed to the design of the laboratory conditions for TEG and CAT, performance of the CAT experiments, analysis of the results, and the writing and reviewing of the paper. V.L. contributed to the planning and design of the laboratory conditions for TEG and CAT, analysis of the results and reviewing of the paper. M.E. contributed to the planning and design of the laboratory conditions for TEG and CAT, analysis of the results, and reviewing of the paper. V.J.Y. contributed to patient recruitment, analysis of the results, and the writing and reviewing of the paper.

DISCLOSURES

I.F.B. and N.B. have received research funding from Novo Nordisk. N.B. has also received grants from Fondo de Investigación Sanitaria-Fondos FEDER CP14/00024 and PI15/01457. V.J.Y. has acted as a consultant for Novo Nordisk. C.S., M.E. and V.L. are employees of Novo Nordisk.

REFERENCES

1. Novo Nordisk. NovoSeven® summary of product characteristics. http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/000074/WC500030873.pdf. Accessed July 18, 2017.
2. Novo Nordisk. NovoSeven® summary of product characteristics. Food and Drug Administration. http://www.fda.gov/downloads/.../ucm056915.pdf. Accessed January 10, 2017.
3. Kavakli K, Makris M, Zulfikar B, et al. Home treatment of haemarthroses using a single dose regimen of recombinant activated factor VII in patients with haemophilia and inhibitors. A multi-centre, randomised, double-blind, cross-over trial. *Thromb Haemost*. 2006;95:600-605.
4. Young G, Shafer FE, Rojas P, et al. Single 270 μ kg^{-1}-dose rFVIIa vs. standard 90 μg kg^{-1}-dose rFVIIa and APCC for home treatment of joint bleeds in haemophilia patients with inhibitors: a randomized comparison. *Haemophilia*. 2008;14:287-294.
5. Key NS, Aledort LM, Beardsley D, et al. Home treatment of mild to moderate bleeding episodes using recombinant factor VIIa (NovoSeven) in haemophiliacs with inhibitors. *Thromb Haemost*. 1998;80:912-918.
6. Parameswaran R, Shapiro AD, Gill JC, et al. Dose effect and efficacy of rFVIIa in the treatment of haemophilia patients with inhibitors: analysis from the Hemophilia and Thrombosis Research Society Registry. *Haemophilia*. 2005;11:100-106.
7. Young G, Cooper DL, Gut RZ. Dosing and effectiveness of recombinant activated factor VII (rFVIIA) in congenital haemophilia with inhibitors by bleed type and location: the experience of the Haemophilia

[no notes on this page]

and Thrombosis Research Society (HTRS) Registry (2004-2008). *Haemophilia*. 2012;18:990-996.
8. Dargaud Y, Lienhart A, Negrier C. Prospective assessment of thrombin generation test for dose monitoring of bypassing therapy in hemophilia patients with inhibitors undergoing elective surgery. *Blood*. 2010;116:5734-5737.
9. Qi X, Zhao Y, Li K, et al. Evaluating and monitoring the efficacy of recombinant activated factor VIIa in patients with haemophilia and inhibitors. *Blood Coagul Fibrinolysis*. 2014;25:754-760.
10. Young G, Sorensen B, Dargaud Y, et al. Thrombin generation and whole blood viscoelastic assays in the management of hemophilia: current state of art and future perspectives. *Blood*. 2013;121:1944-1950.
11. Chitlur M, Rivard GE, Lillicrap D, et al. Recommendations for performing thromboelastography/thromboelastometry in hemophilia: communication from the SSC of the ISTH. *J Thromb Haemost*. 2014;12:103-106.
12. Brophy DF, Martin EJ, Christian BJ, et al. Monitoring rFVIIa 90 μg kg^{-1} dosing in haemophiliacs: comparing laboratory response using various whole blood assays over 6 h. *Haemophilia*. 2011;17:e949-e957.
13. Viuff D, Andersen S, Sorensen BB, et al. Optimizing thrombelastography (TEG) assay conditions to monitor rFVIIa (NovoSeven) therapy in haemophilia A patients. *Thromb Res*. 2010;126:144-149.
14. Kenet G, Stenmo CB, Blemings A, et al. Intra-patient variability of thromboelastographic parameters following in vivo and ex vivo administration of recombinant activated factor VII in haemophilia patients. A multi-centre, randomised trial. *Thromb Haemost*. 2010;103:351-359.
15. Sorensen B, Ingerslev J. Thromboelastography and recombinant factor VIIa-hemophilia and beyond. *Semin Hematol*. 2004;41:140-144.
16. Vila V, Aznar JA, Moret A, et al. Assessment of the thrombin generation assay in haemophilia: comparative study between fresh and frozen platelet-rich plasma. *Haemophilia*. 2013;19:318-321.
17. Salooja N, Perry DJ. Thrombelastography. *Blood Coagul Fibrinolysis*. 2001;12:327-337.
18. Hemker HC, Giesen P, Al Dieri R, et al. Calibrated automated thrombin generation measurement in clotting plasma. *Pathophysiol Haemost Thromb*. 2003;33:4-15.
19. Hemker HC, Kremers R. Data management in thrombin generation. *Thromb Res*. 2013;131:3-11.
20. Morfini M, Jimenez-Yuste V, Eichler H, et al. Pharmacokinetic properties of two different recombinant activated factor VII formulations. *Haemophilia*. 2012;18:431-436.
21. Tiede A, Friedrich U, Stenmo C, et al. Safety and pharmacokinetics of subcutaneously administered recombinant activated factor VII (rFVIIa). *J Thromb Haemost*. 2011;9:1191-1199.
22. Bysted BV, Scharling B, Moller T, et al. A randomized, double-blind trial demonstrating bioequivalence of the current recombinant activated factor VII formulation and a new robust 25 degrees C stable formulation. *Haemophilia*. 2007;13:527-532.
23. Agerso H, Brophy DF, Pelzer H, et al. Recombinant human factor VIIa (rFVIIa) cleared principally by antithrombin following intravenous administration in hemophilia patients. *J Thromb Haemost*. 2011;9:333-338.
24. Pan-Petesch B, Laguna P, Mital A, et al. Single-dose (270 μg kg^{-1}) recombinant activated factor VII for the treatment and prevention of bleeds in haemophilia A patients with inhibitors: experience from seven European haemophilia centres. *Haemophilia*. 2009;15:760-765.
25. Salaj P, Brabec P, Penka M, et al. Effect of rFVIIa dose and time to treatment on patients with haemophilia and inhibitors: analysis of HemoRec registry data from the Czech Republic. *Haemophilia*. 2009;15:752-759.
26. Salaj P, Kubes R, Cetkovsky P, et al. Economic evaluation of rFVIIa high initial dose compared to rFVIIa standard initial dose in patients with haemophilia with inhibitors using the Czech HemoRec registry. *Thromb Res*. 2014;133:162-167.
27. Santagostino E, Mancuso ME, Rocino A, et al. A prospective randomized trial of high and standard dosages of recombinant factor VIIa for treatment of hemarthroses in hemophiliacs with inhibitors. *J Thromb Haemost*. 2006;4:367-371.
28. Young G, Blain R, Nakagawa P, et al. Individualization of bypassing agent treatment for haemophilic patients with inhibitors utilizing thromboelastography. *Haemophilia*. 2006;12:598-604.
29. Salinas V, Carmona R, Mohammed BM, et al. Is some better than none: are TEG and TGA profiles different in severe FVIII-deficient patients with inhibitors? *Haemophilia*. 2015;21:398-404.
30. Ay Y, Balkan C, Karapinar DY, et al. Feasibility of using thrombin generation assay (TGA) for monitoring of haemostasis during supplementation therapy in haemophilic patients without inhibitors. *Haemophilia*. 2012;18:911-916.
31. Ingerslev J, Poulsen LH, Sorensen B. Potential role of the dynamic properties of whole blood coagulation in assessment of dosage requirements in haemophilia. *Haemophilia*. 2003;9:348-352.
32. Brummel KE, Paradis SG, Butenas S, et al. Thrombin functions during tissue factor-induced blood coagulation. *Blood*. 2002;100:148-152.
33. Rivard GE, Brummel-Ziedins KE, Mann KG, et al. Evaluation of the profile of thrombin generation during the process of whole blood clotting as assessed by thrombelastography. *J Thromb Haemost*. 2005;3:2039-2043.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

How to cite this article: Fernández-Bello I, Stenmo C, Butta N, Lind V, Ezban M, Jiménez-Yuste V. The pharmacokinetics and pharmacodynamics of single-dose and multiple-dose recombinant activated factor VII in patients with haemophilia A or B. *Haemophilia*. 2017;23:868–876. https://doi.org/10.1111/hae.13312

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