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A randomized, double-blind trial demonstrating bioequivalence of the current recombinant activated factor VII formulation and a new robust 25°C stable formulation

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Summary. Recombinant activated factor VIIa (rFVIIa) is a well-established treatment for bleeding episodes in patients with congenital or acquired haemophilia A or B with inhibitors to factors VIII and IX and patients with FVII deficiency. The aim of this trial was to demonstrate bioequivalence between the currently marketed (rFVIIa/NovoSeven®) and a new rFVIIa formulation (VII25) stable at up to 25°C. Furthermore, short-term safety and tolerability of VII25 and pharmacokinetics of both formulations were investigated. In this single-centre, randomized, double-blind, two-way cross-over trial, healthy male subjects received one intravenous bolus injection of rFVIIa and one of VII25, both at 90 μg kg^{-1}, in a randomized order 2–3 weeks apart. Mean VII25/rFVIIa ratio for area under the plasma activity-time curve from time 0 to last quantifiable activity (primary bioequivalence endpoint), was 0.93, 90% confidence interval (CI) (0.89–0.96), within the predefined bioequivalence range (0.80–1.25). Secondary pharmacokinetic parameters were comparable between formulations. No serious adverse events were observed. Six mild or moderate treatment-emergent adverse events were reported in five subjects. Coagulation-related parameter profiles were similar between rFVIIa and VII25. No clinically abnormal changes were observed for laboratory parameters and no subjects developed FVIIa antibodies. This trial demonstrated bioequivalence between the currently available rFVIIa and VII25 stable at up to 25°C. VII25's 'user-friendly' formulation removes the inconvenience of storing/transporting at 2–8°C, and as the drug substance is the same, the activity and safety established for rFVIIa is maintained.

Keywords: 25°C stable formulation, bioequivalence, pharmacokinetics, recombinant activated factor VII, safety and tolerability

Introduction

Recombinant activated factor VII (rFVIIa, NovoSeven®; Novo Nordisk, Maalöv, Denmark) is licensed in 72 countries worldwide. In Europe the indications are: treatment of bleeding episodes and for the prevention of bleeding during surgery or invasive procedures in patients with congenital haemophilia A and B with inhibitors to coagulation factors VIII (FVIII) or IX (FIX), or in those expected to have a high anamnestic response to FVIII or FIX, acquired haemophilia, congenital FVII deficiency or Glanzmann's thrombasthenia with antibodies to GP IIb/IIIa and/or human leucocyte antigen and refractory to platelet transfusions.

Recombinant activated FVII controls bleeding at the site of vascular injury. Its local action in areas where tissue factor is exposed and activated platelets are present [1] generates thrombin through activation of the prothrombinase complex [2], limiting activity to areas of injury and decreasing the risk of disseminated intravascular coagulation (DIC) [3]. At pharmacologic doses, rFVIIa can directly activate factor X on the surface of activated platelets forming a stable haemostatic plug that controls bleeding [4,5].

Based on a review of 13 clinical trials by Levy *et al.* [6] and European postmarketing surveillance of nearly 750 000 doses of rFVIIa (1996–2003) in

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patients with congenital or acquired haemophilia A or B with inhibitors [7], relatively few spontaneously reported thrombotic events, or cases of DIC, have been associated with rFVIIa use [6,7].

The current rFVIIa product is available as a sterile, white lyophilized powder in single-use glass vials as either 1.2, 2.4 or 4.8 mg $vial^{-1}$ to be reconstituted in sterile water for injection [8]. Storage and transport of the product are dependent upon refrigeration at 2–8°C. To make the product more convenient for users, and less dependent on cooling boxes and refrigerators, a new formulation intended for storage at up to 25°C has been developed (VII25).

The objectives of this trial were to demonstrate bioequivalence between the currently available rFVIIa product and the new formulation (VII25), to compare pharmacokinetic parameters of the two formulations, and to demonstrate short-term safety and tolerability of the new formulation.

Methods

Trial design

The trial was designed as a single-centre, randomized, double-blind, two-way cross-over study. Each subject was randomized to receive two single-dose administrations, one 90 μg kg^{-1} body weight of rFVIIa and one 90 μg kg^{-1} body weight of VII25 respectively. Both doses were given as intravenous (i.v.) bolus injection with a 2–3 week washout period between each treatment. VII25 was supplied as a sterile, freeze-dried powder in single-use vials of 5 mg of rFVIIa to be reconstituted with a dedicated solvent containing 10 mM histidine.

The trial consisted of five visits: a screening visit (visit 1), two dosing visits (visits 2 and 3) and two follow-up visits (visits 4 and 5) after 2–3 weeks and 3 months respectively. The treatment at visits 2 and 3 was either sequence A (VII25 followed by rFVIIa), or sequence B (rFVIIa followed by VII25). Visits 2 and 3 were separated by a 2–3 week washout period. The trial was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice.

Subjects

Healthy subjects were recruited in the trial at a single centre in France. Eligible subjects comprised Caucasian males aged 18–45 years in good health. Written informed consent was obtained from all subjects. The key exclusion criteria included: known history of atherosclerosis, thromboembolic events or known high levels of troponin I, having increased thromboembolic risk, known or suspected allergy to rFVIIa or related products, overt bleeding, hepatitis B or C, or HIV infection, blood donation or other trial participation 3 months before screening visit, mental handicap, history of migraine, or alcohol or drug abuse within the last 12 months. Use of prescription or non-prescription medication was not permitted during the trial.

Pharmacokinetic evaluations

The primary objective was to demonstrate bioequivalence between rFVIIa and VII25 based on the primary pharmacokinetic endpoint, area under the plasma activity-time curve from time 0 to last quantifiable activity (AUC_{0-t}). Secondary objectives included comparison of the secondary endpoints: area under the plasma activity-time curve from time 0 to infinity (AUC), AUC from time 0 to 30 h postdosing (AUC_{0-30}), maximum plasma activity at 5 min post-trial product administration (C_{max}), terminal half life ($t_{½}$), total plasma clearance (CL), distribution volume at steady state (Vss), volume distribution in central compartment (Vc) and mean residence time (MRT) between the two formulations. Both primary and secondary endpoints were based on FVIIa activity measurements.

Fifteen serial blood samples for FVIIa activity were collected from each subject during each treatment visit (visits 2 and 3). Three samples were drawn at −10, −20 and −30 min predosing, followed by 12 samples taken at 5, 10, 20, 30 min, 1 h, 2, 3, 5, 8, 12, 24 and 30 h postdosing. Venous blood drawn from the opposite arm to the bolus injection was collected in 5 mL citrate tubes (3.2% citrate, 1:9 v/v, final concentration: 0.109 M) by trained personnel. Blood samples were centrifuged within 30 min of collection at 2500 *g* (room temperature) for 15 min. Plasma samples were thereafter frozen and stored at −80°C until assayed.

Factor VIIa activity in the plasma samples was determined by a clot assay (Capio Diagnostik A/S, Denmark) [9]. Imprecision of the assay, from analysis of quality control samples, was determined as <9.3% (mean inaccuracy −7.5% to −1.0%).

Safety

A physical examination was carried out predosing and 30 h postdosing; vital signs were assessed predosing, 10 and 30 min, 1 h, 5, 12, 24 and 30 h postdosing. A 12-lead electrocardiogram (ECG) was performed predosing, 1 and 30 h postdosing. Clinical laboratory tests (haematology, clinical

chemistry, urinalysis) and troponin I analysis were performed predosing and 24 h postdosing.

For the coagulation assessment screen (platelet count, prothrombin fragments 1 + 2 [F_{1+2}], activated partial thromboplastin time [aPTT], prothrombin time [PT], fibrinogen, antithrombin, D-dimer) blood was collected predosing, 30 min, 1 h, 5, 12 and 24 h postdosing. Samples were centrifuged at 2500 *g* (room temperature) for 15 min. Plasma was thereafter frozen and stored at −80°C until assayed.

Adverse events (AEs) were monitored throughout the trial. An AE was defined as any undesirable medical event occurring to a subject during the trial, whether or not related to the trial product(s). AEs were graded as serious or non-serious and by severity (mild, moderate, severe) and relationship to trial drug(s) (none, unlikely, possible, probable). Injection site was visually inspected immediately after each bolus injection and at 1, 5, 24 and 30 h after drug administration for any reactions.

For the anti-FVIIa antibodies, blood samples were collected into heparin, centrifuged for 10 min (2000 *g*, room temperature) and plasma stored at −18°C until analysis using a validated radioimmunoassay (Novo Nordisk A/S, Maalöv, Denmark). In brief, I^{125} labelled hamster rFVIIa was added to the samples, resulting in the formation of antigen–antibody complexes. These immune complexes, together with unspecific immunoglobulins, were then precipitated using protein G sepharose mixed with anti-immunoglobulin antibodies. The radioactivity present in the precipitate is proportional to the amount of rFVIIa antibodies in the sample. Inter- and intra-assay variation were <15% (5.9–8.0 and 4.0–6.3 for inter- and intra-assay percentage coefficient of variation [%CV] for rFVIIa).

Statistical analysis

To demonstrate bioequivalence between the two formulations (VII25 and rFVIIa), a mixed model was applied to the logarithmically transformed AUC_{0-t}. The model included treatment, period, sequence and body mass index (BMI) as fixed effects, and subject as a random effect. The ratio VII25/rFVIIa with two-sided 90% confidence interval (CI) was estimated using least square means. If the CI was completely contained within the bioequivalence interval (0.80–1.25), the two formulations were considered bioequivalent [10]. Descriptive statistics of all pharmacokinetic parameters were presented as mean, standard deviation (SD), median, minimum and maximum values, geometric mean and %CV.

Results

Demographic characteristics

Forty-eight subjects were screened. Of these, 25 subjects were randomized and 23 subjects completed the trial. All 25 subjects were Caucasian males aged 22–44 years (median 29), with a BMI of 20.5–27.0 kg m^{-2} (median 23.7).

Of the 25 subjects who were randomized, 24 subjects received a single i.v. dose of VII25 and 24 subjects received a single i.v. dose of rFVIIa. The discontinuation of two subjects who failed to complete the trial was due to AEs during the washout period: one subject (VII25) reported skin urticaria and one subject (rFVIIa) reported abdominal pain and diarrhoea. All AEs were unlikely to have been related to trial drugs.

Pharmacokinetic evaluations

Overall, 22 subjects who received both trial products at dose 90 μg kg^{-1} body weight were included in the pharmacokinetic analysis. An additional subject received 81 μg kg^{-1} body weight rFVIIa (90% compliance) and was therefore excluded from the pharmacokinetic analysis.

The primary endpoint, AUC_{0-t}, was based on FVIIa activity. Predosing, FVIIa activity ranged from 0.03 to 0.12 IU mL^{-1} (rFVIIa) and from <0.02 to 0.10 IU mL^{-1} (VII25). During the first 5–10 min after dosing maximal FVIIa activity was observed in all subjects. Decrease in FVIIa activity was similar

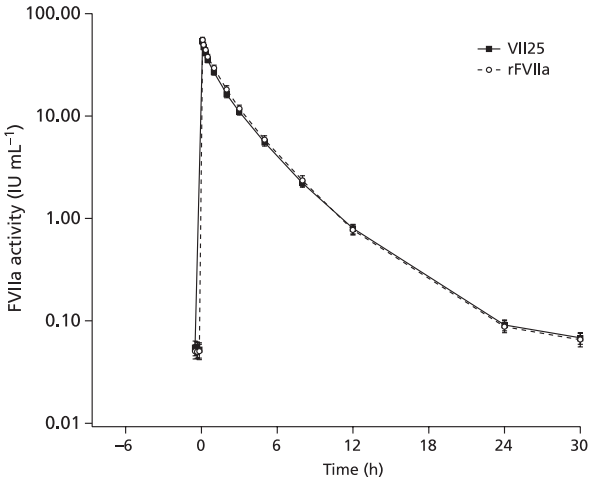

Fig. 1. Mean (SD) plasma activated factor VII activity vs. time after a single i.v. injection of 90 μg kg^{-1} of current product recombinant activated factor VII and VII25.

Table 1. Summary statistics for pharmacokinetic parameters based on factor activated VII activity after a single i.v. injection of 90 μg kg^{-1} recombinant activated factor VII and VII25.

Treatment	AUC_{0-t} (h*IU mL^{-1})	C_{max} (IU mL^{-1})	AUC_{0-30} (h*IU mL^{-1})	AUC (h*IU mL^{-1})	$t_{1/2}$ (h)	MRT (h)	CL (mL h kg^{-1})	Vc (mL kg^{-1})	Vss (mL kg^{-1})
rFVIIa (n = 22)									
Mean	122.04	55.44	122.05	122.05	3.48	2.97	37.63	82.68	111.31
SD	18.03	7.89	18.01	18.01	0.27	0.26	5.99	12.50	17.52
Geom. mean	120.71	54.88	120.73	120.72	3.47	2.96	37.20	81.83	110.08
%CV	15	14	15	15	8	9	16	15	16
VII25 (n = 22)									
Mean	113.26	52.83	113.26	113.26	3.54	3.05	40.43	86.36	122.96
SD	17.36	7.31	17.36	17.36	0.28	0.27	6.23	12.31	20.42
Geom. mean	111.99	52.34	111.99	111.99	3.53	3.04	39.98	85.53	121.46
%CV	15	14	15	15	8	9	15	14	17

between both formulations. Figure 1 shows the logarithmic mean plasma FVIIa activity over time.

Table 1 shows that geometric mean (%CV) AUC_{0-t} for rFVIIa was 120.71 (14.77) h*IU mL^{-1} and 111.99 (15.33) h*IU mL^{-1} for VII25. The mean VII25/rFVIIa ratio of AUC_{0-t} was 0.93, 90% CI (0.89–0.96), within the predefined bioequivalence range (0.80–1.25). Although bioequivalent, AUC_{0-t} was slightly lower (8%) after VII25 than after rFVIIa administration (P = 0.1076). All secondary pharmacokinetic parameters were comparable between the two formulations (Table 1). The 90% CI for AUC_{0-30} was also within the 0.80–1.25 range.

Safety

Physical examination and clinical laboratory tests

Twenty-five subjects were included in the safety analysis. No clinically relevant changes were observed for ECG, vital signs, laboratory parameters (haematology, clinical chemistry, urinalysis) or troponin I levels from screening to post-treatment follow-up.

Coagulation-related parameter screen

Overall, coagulation-related parameter profiles were similar for rFVIIa and VII25. There were no clinically abnormal changes in platelet count, fibrinogen levels or antithrombin values during the 24-h sampling. aPTT mean values decreased after administration of both formulations, from 36.7 to 31.8 s (rFVIIa) and 36.8–31.2 s (VII25) at predose and 30 min respectively. Mean PT values were given in per cent, where 100% corresponded to the shortest detectable clotting time. The administration of rFVIIa and VII25 led to an increase of the PT as measured in per cent. For all subjects, the PT values (%) were above 100% until 5 h after administration of both formulations. (rFVIIa: 87.25%, 100.00%, 94.29%; VII25: 85.88%, 100.00%, 93.58% at predose, 5 and 24 h, where 100% = shortest detectable clotting time).

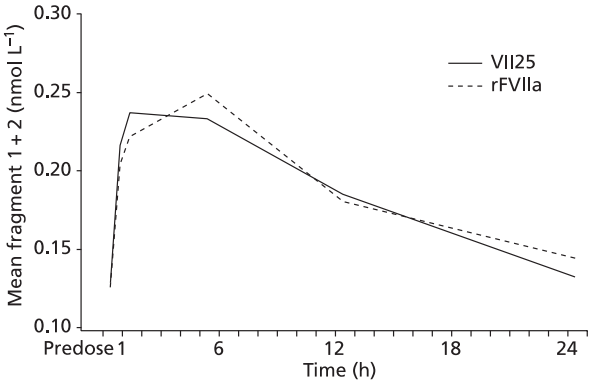

Fig. 2. Mean prothrombin fragment F_{1+2} levels (nmol L^{-1}) pre- and postdosing for current product recombinant factor VIIa and VII25.

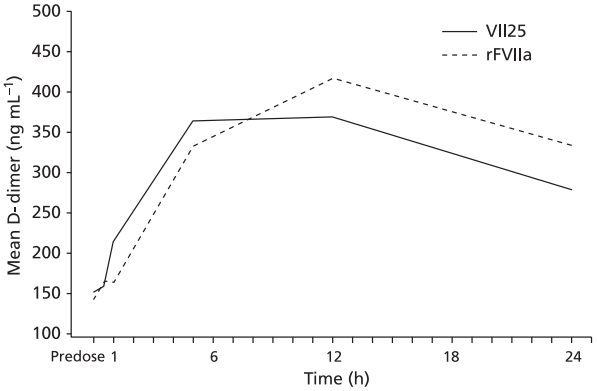

Fig. 3. Mean D-dimer levels (ng mL^{-1}) pre- and postdosing for current product recombinant factor VIIa and VII25.

Prothrombin fragment F_{1+2} levels increased up to 5 h after administration of rFVIIa and up to 1 h after administration of VII25. Mean peak values were similar for the two formulations (0.249 and 0.237 nmol L^{-1} for rFVIIa and VII25 respectively; Fig. 2). Profiles were similar for the two formulations with mean D-dimer levels increasing up to 12 h after administration of rFVIIa and VII25 respectively (mean peak values were 421.3 and 372.8 ng mL^{-1} for rFVIIa and VII25 respectively; Fig. 3).

Adverse events

There were no serious AEs or severe AEs. Six mild or moderate treatment-emergent AEs (TEAEs) were reported in five subjects treated with rFVIIa. These included two probably related TEAEs: injection-site pain; two possibly related TEAEs: arthralgia (23 h after dosing) and blurred vision (1 h after dosing); and two unlikely TEAEs: herpes virus infection and injection-site haematoma (25 h after dosing). All subjects recovered without treatment. No adverse events were reported after administration of VII25.

Within each treatment sequence, the values of FVIIa antibodies did not show any significant changes between the screening and the follow-up 2–3 weeks after the administration of the second trial drug. At the 3-month immunogenicity follow-up, none of the subjects had developed treatment-related antibodies to FVIIa during the course of the trial.

Discussion

Based on the data, this trial demonstrated bioequivalence between the currently marketed rFVIIa product and the new formulation (VII25) stable at up to 25°C. The 90% CI (0.89–0.96) for the ratio VII25/rFVIIa for AUC_{0-t} was within the bioequivalence range (0.80–1.25). Mean pharmacokinetic profiles were similar for the two formulations. The pharmacokinetic calculations are based on a bioassay (FVIIa activity) rather than on an antigen assay. Clotting activity assays are commonly used when assessing the pharmacokinetic parameters for coagulation factors [11].

The single 90 μg kg^{-1} dose of VII25 was well tolerated and no TEAEs were reported in subjects administered VII25. No clinically abnormal changes were observed for laboratory parameters (haematology, clinical chemistry, urinalysis).

Prothrombin fragment F_{1+2}, a marker of activation of the coagulation system, increased after administration of rFVIIa and VII25; this marker has previously been reported to be slightly elevated following rFVIIa administration, but without any adverse clinical consequences [12]. D-dimer levels showed similar profiles for both formulations. Measures of coagulation and fibrinolysis were consistent between the two formulations, indicating a comparable haemostasis profile. There was no evidence of significant systemic activation, consistent with a local haemostatic effect of the two formulations.

VII25 provides a convenient, robust formulation that is stable at temperatures up to 25°C. This new formulation therefore removes the need for storing and transporting factor at 2–8°C and maintains the established activity profile as known from rFVIIa. This formulation holds the promise of improved treatment of haemophilia patients with inhibitors, as they will be able to have faster access to treatment.

In conclusion, VII25 is well tolerated with similar activity and pharmacokinetic profiles to the current rFVIIa product, but with the added advantage of a formulation that is stable at temperatures up to 25°C.

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