Thrombosis Research 141S3 (2016) S9–S12

Contents lists available at ScienceDirect

Thrombosis Research

journal homepage: www.elsevier.com/locate/thromres

Review

Entering new areas in known fields: recombinant fusion protein linking recombinant factor VIIa with recombinant albumin (rVIIa-FP) – advancing the journey

Claude Négrier*

Hemophilia Comprehensive Care Center and Hematology Department, Louis Pradel University Hospital, Lyon, France

KEYWORDS

Hemophilia A
Hemophilia B
Inhibitors
NovoSeven®
rVIIa-FP
PROLONG-7FP

ABSTRACT

The novel fusion protein linking recombinant factor VIIa with recombinant albumin (rVIIa-FP) is designed to extend the half-life of recombinant factor VIIa (rFVIIa) and improve the care of hemophilia A or B patients with inhibitors. Preclinical studies in various animal models have demonstrated markedly improved pharmacokinetic and pharmacodynamic properties, as well as prolonged retention in the joint tissues, of rVIIa-FP compared with a commercially available rFVIIa (NovoSeven®). A phase I study in healthy volunteers – the first study in the PROLONG-7FP program – confirmed that rVIIa-FP has a good tolerability profile in doses of up to 1,000 μg/kg and has demonstrated enhanced pharmacodynamic activity relative to rFVIIa. The half-life of rVIIa-FP at the highest dose investigated in the study was 8.5 hours, which represents a 3- to 4-fold half-life extension compared with rFVIIa. Encouraging results from preclinical and phase I studies have led to the initiation of clinical studies of rVIIa-FP in patients with congenital hemophilia A or B and inhibitors, and in patients with confirmed factor VII deficiency. The results from these studies are awaited with interest by clinicians and patients alike.

Introduction

Inhibitory antibodies to factor VIII (FVIII) or factor IX (FIX) develop in approximately 30% of previously untreated patients with severe hemophilia A and in 3–5% of previously untreated patients with severe hemophilia B who initiate treatment with coagulation factor concentrates [1–4]. The management of patients who develop high-responding inhibitors (≥5 Bethesda units/mL) frequently includes the use of bypassing agents, such as recombinant factor VIIa (rFVIIa; NovoSeven®) or Factor Eight Bypassing Activity (FEIBA®) [5]. rFVIIa is administered with the aim of providing supraphysiological levels of FVIIa to enhance the rate of thrombin generation on thrombin-activated platelet surfaces in the absence of FVIII or FIX [6]. Although highly effective in treating bleeds in hemophilia patients with inhibitors, the short half-life of rFVIIa (estimated to be 2.3 hours in this population [7]) necessitates intravenous (IV) administration every 2–3 hours to achieve hemostasis [7].

Recombinant fusion protein linking recombinant factor VIIa with recombinant albumin (rVIIa-FP) is a novel fusion protein that has been designed to extend the half-life of rFVIIa with the aim of achieving more effective and convenient care for patients with hemophilia A or B and inhibitors. The molecule is produced by the fusion of "wild-type" rFVIIa to recombinant albumin using a non-cleavable 31-amino-acid glycine–serine linker and expressed as a single moiety by Chinese hamster ovary cells (Figure 1) [8,9]. No modification of the FVIIa amino acid sequence is required to produce rVIIa-FP and, upon activation, the FVII activity provided by rVIIa-FP mirrors that of wild-type rFVIIa [8,9].

Preclinical studies of rVIIa-FP in animal models

The pharmacokinetics (PK) and pharmacodynamics (PD) of rVIIa-FP have been evaluated extensively across various animal models. In one of the first preclinical studies conducted, wild-type rFVIIa, NovoSeven®, rVIIa-FP (all at a dose of 100 μg/kg body weight), or plasma-derived human serum albumin (at a dose of 500 mg/kg body weight) were administered to CD® rats, with FVII and albumin antigen levels measured up to 24 hours postinjection using an enzyme-linked immunosorbent assay [9]. In this study, the half-life of rVIIa-FP was found to be 6.7-fold longer than that of wild-type rFVIIa and 5.8-fold longer than that of NovoSeven® [9]. The recovery (i.e. the percentage recovered 5 minutes after injection) of rVIIa-FP was 47.1% compared with just 19.5% after NovoSeven® and 34.8% after wild-type rFVIIa injection. The combination of an improved recovery, reduced clearance, and prolonged half-life with rVIIa-FP resulted in an area under the curve that was 9.5-fold and 14.5-fold higher with rVIIa-FP than with wild-type rFVIIa and NovoSeven®, respectively [9].

* Corresponding author at: Centre de Référence de l'Hémophilie, Hôpital Louis Pradel – Hospices Civils de Lyon, Université Claude Bernard, Lyon, France.
Tel.: +33 4 7211 8821; fax: +33 4 7211 8817.
E-mail address: claude.negrier@chu-lyon.fr (C. Négrier).

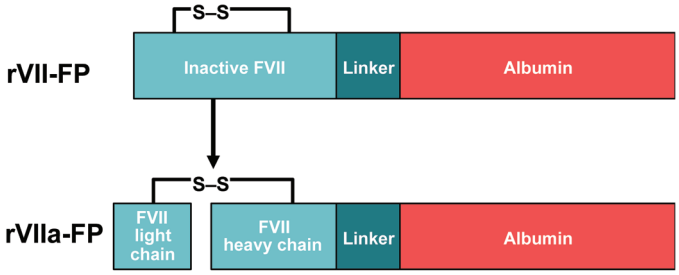

Fig. 1. Design of the recombinant fusion protein linking recombinant factor VIIa with recombinant albumin (rVIIa-FP): designed to extend the half-life of recombinant activated factor VII (rFVIIa) and improve patient care.

Subsequent PK studies comparing single IV doses of rVIIa-FP and NovoSeven® in hemophilia A mice, rats, rabbits, and cynomolgus monkeys confirmed these initial findings and demonstrated enhanced recovery, reduced clearance, and a substantial prolongation of the rFVIIa half-life after the administration of rVIIa-FP compared with NovoSeven® (Figure 2) [10].

The improved PK properties of rVIIa-FP relative to NovoSeven® have translated into enhanced PD activity in animal models [10]. Studies using thrombin generation assay in hemophilia A mice reported prolonged activity and enhanced thrombin generation after the administration of equimolar doses of rVIIa-FP compared with NovoSeven® [10]. The procoagulant activity of rVIIa-FP was also enhanced compared with NovoSeven® in a rabbit model of venous thrombosis [10].

The tissue distribution of rVIIa-FP and NovoSeven® has recently been studied in rats using quantitative whole-body and knee-joint autoradiography [11]. In this study, Sprague–Dawley rats received a single IV injection of tritium-labeled rVIIa-FP 10 mg/kg (n=8) or NovoSeven® 1.6 mg/kg (n=4) and underwent autoradiography for 240 hours or 24 hours, respectively, post-administration. The tissue distribution patterns observed for both products were similar, with both penetrating well into many tissues, including the kidney, bone endosteum, bone marrow, spleen, muscles, liver, and skin. NovoSeven® and rVIIa-FP penetrated rapidly into knee-joint structures, with the highest concentrations found in the calcified cartilage, endosteum, and periosteum. A detailed analysis of the knee joint showed high levels of radioactivity within 15 minutes of administration of both rVIIa-FP and NovoSeven® (Figure 3). However, by 24 hours after administration of NovoSeven®, levels were almost undetectable in the knee. In contrast, radioactivity was still detectable up to 120 hours after administration of rVIIa-FP, indicating markedly prolonged tissue retention [11].

Phase I study of rVIIa-FP in healthy volunteers

A phase I safety and PK study of rVIIa-FP in healthy volunteers has also been completed as part of the PROLONG-7FP clinical development program [12]. The study was a prospective, double-blind, placebo-controlled study that enrolled 40 healthy, young (aged

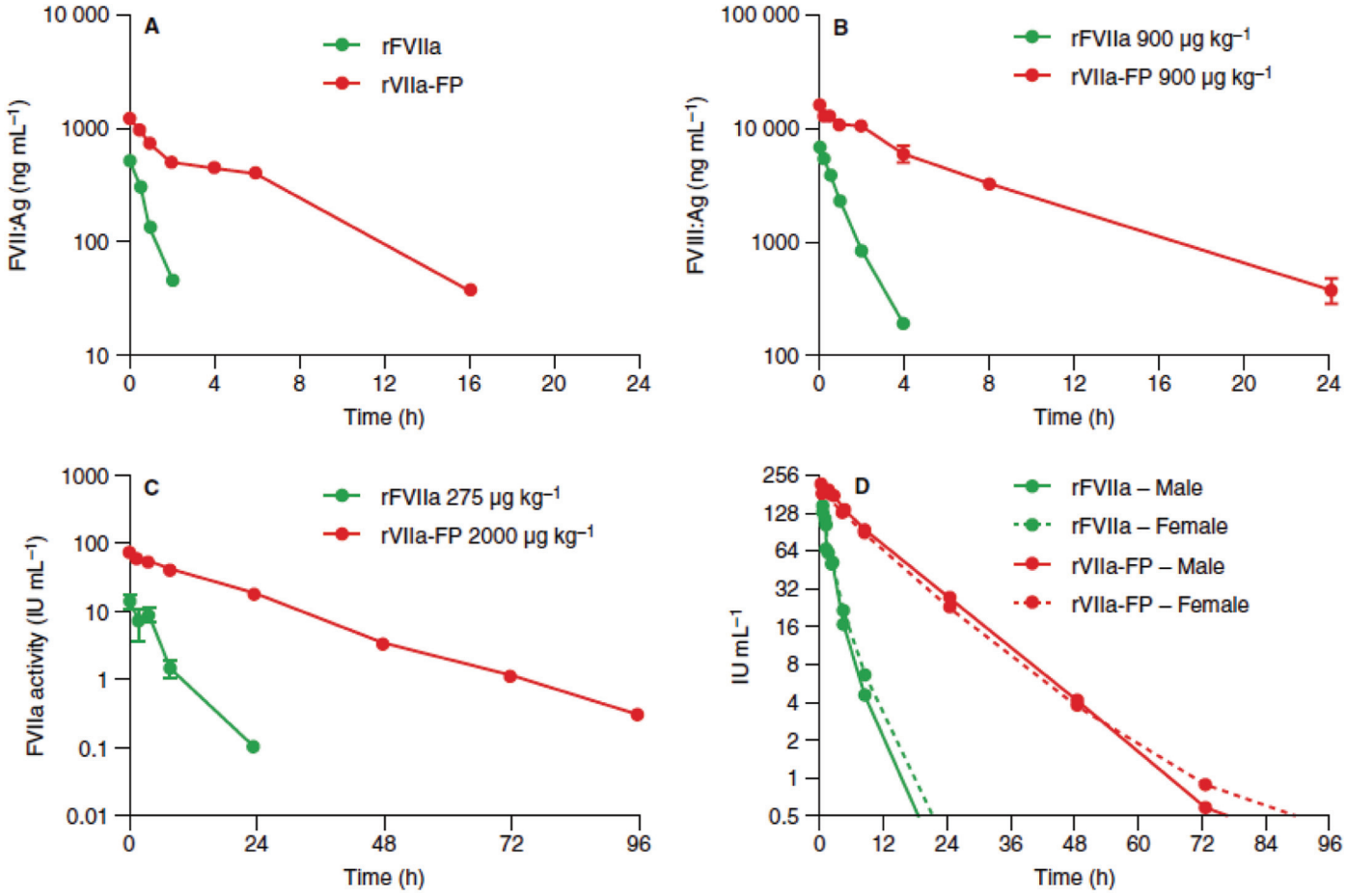

Fig. 2. Plasma concentration–time profiles of recombinant fusion protein linking recombinant factor VIIa with recombinant albumin (rVIIa-FP) and recombinant activated factor VII (rFVIIa; NovoSeven®) in (A) hemophilia A mice, (B) rats, (C) rabbits and (D) cynomolgus monkeys [10]. Data shown are means, except for mice (plasma pool) and monkeys, where the curve for each animal is shown. FVII:Ag, factor VII antigen. Reproduced from Zollner S et al. Pharmacological characteristics of a novel, recombinant fusion protein linking coagulation factor VIIa with albumin (rVIIa-FP). J Thromb Res 2014; 12: 220–228. CC-BY-NC-ND © 2013 International Society on Thrombosis and Haemostasis.

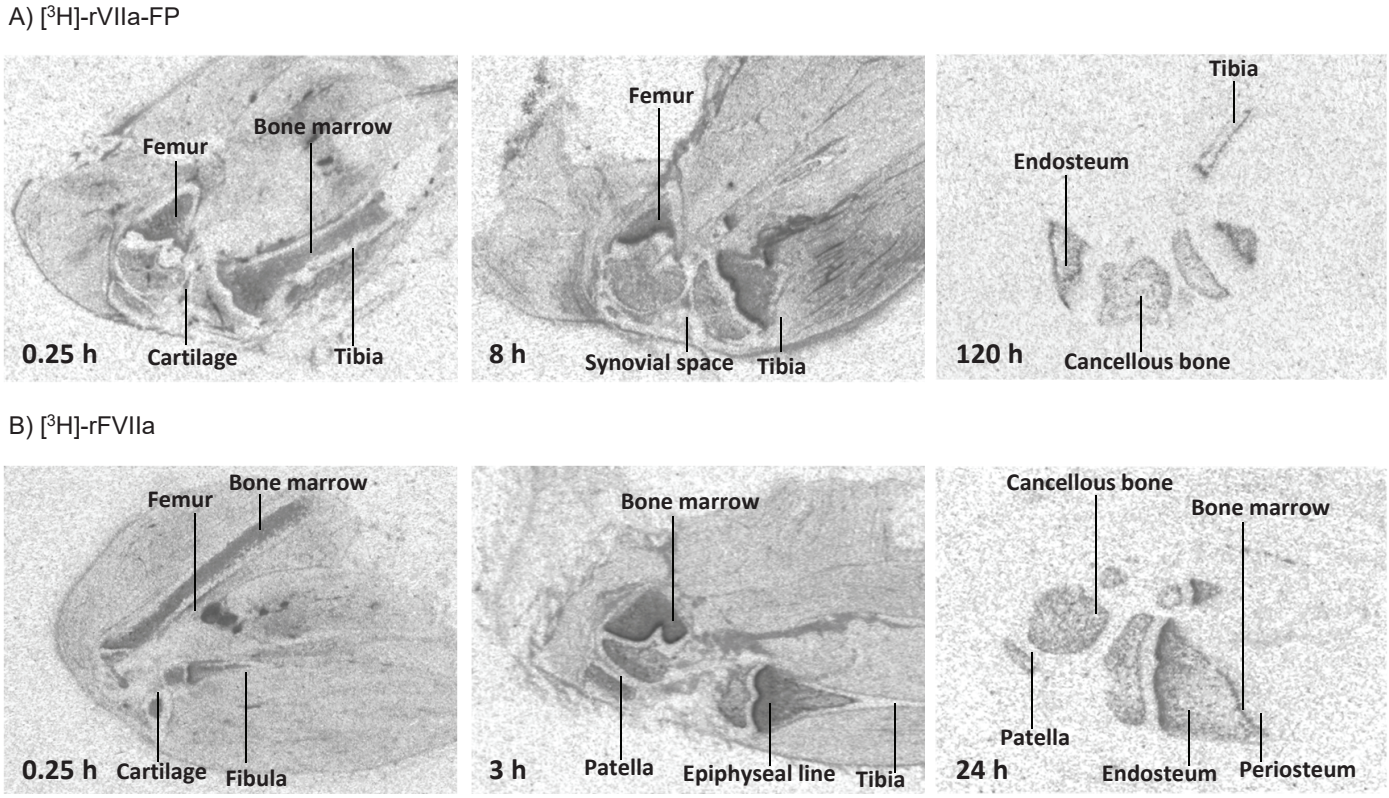

Fig. 3. Quantitative whole-body autoradiography images of knee-joint tissue obtained 0.25–120 hours (h) after dosing with tritium-labeled (A) recombinant fusion protein linking recombinant factor VIIa with recombinant albumin ([3H]-rVIIa-FP) or (B) recombinant activated factor VII (NovoSeven®) ([3H]-rFVIIa) in rats [11]. Reproduced from Herzog E, et al. Recombinant fusion protein linking factor VIIa with albumin (rVIIa-FP): tissue distribution in rats. Thromb Res 2014;134:495-502. CC-BY-NC-ND © 2014 Elsevier Ltd.

18–35 years), male volunteers who had been rigorously screened for thrombotic risk factors, including congenital thrombophilia. Before the first dose of study medication was administered, and throughout the study, all subjects were anticoagulated with a daily dose of an oral vitamin K antagonist (warfarin) to achieve a prothrombin international normalized ratio (INR) between 2 and 3. Once a stable INR had been reached, subjects received a single IV injection of rVIIa-FP or placebo (normal saline) administered over 15 minutes. Blood samples for PK analysis were obtained before each injection and up to 120 hours after the start of each injection. Subjects were dosed in a staggered manner in five cohorts, with six subjects per cohort receiving a single dose of rVIIa-FP (140, 300, 500, 750, or 1000 μg/kg) and two subjects per treatment arm receiving placebo. A safety monitoring board reviewed the data from each cohort before the next dose-escalation cohort could enter the study.

rVIIa-FP was well tolerated in this study. There were no serious adverse events and no thromboembolic events of any severity observed. Only one adverse event was judged to be related to rVIIa-FP, and that was described as pain and hardening of the vein at the injection site. No antidrug antibodies developed during up to 28 days of postinfusion monitoring, and there was no evidence of systemic activation of coagulation in any subject after the administration of rVIIa-FP.

The maximum (C_{max}) baseline-corrected mean FVIIa plasma activity increased in a dose-proportional manner. Clearance ranged from 7.62 to 12.74 mL/hour and the median FVIIa activity-based terminal half-life ranged from 6.1 hours (140 μg/kg cohort) to 9.7 hours (300 μg/kg cohort) after a single dose of rVIIa-FP. At the highest dose level investigated (1000 μg/kg), the median terminal half-life of rVIIa-FP was 8.5 hours – a 3- to 4-fold half-life extension compared with NovoSeven® [12].

An immediate decrease in the prothrombin INR was observed after the administration of rVIIa-FP, with a normal INR achieved within 60 minutes. By 24 hours postadministration, more than 96% of subjects who received rVIIa-FP had maintained an INR <1.5 and by 48-hours postdose, more than 50% of these subjects still had an INR <1.5, indicating the sustained biological activity of the fusion protein. By contrast, in a similar study assessing NovoSeven® in doses ranging from 5 to 320 μg/kg [13], more than 50% of subjects had an INR >1.5 within 24 hours of dosing.

Conclusions

There are significant unmet needs in the management of hemophilia patients with inhibitors, not least of which is the need to extend the dosing interval of bypassing agents to simplify treatment, improve clinical outcomes, and, potentially, enable the use of prophylactic regimens. rVIIa-FP is a novel fusion protein that has been developed to meet these needs, offering a prolonged half-life compared with NovoSeven®, sustained clinical activity, and a good tolerability profile. Additional clinical studies in patients with hemophilia A or B and inhibitors, and in those with FVII deficiency, are now underway, and the results are awaited with interest.

Abbreviations

FEIBA®, Factor Eight Bypassing Activity; FIX, factor IX; FVII, factor VII; FVII:Ag, factor VII antigen; FVIIa, activated factor VII; FVIII, factor VIII; INR, international normalized ratio; IV, intravenous; PD, pharmacodynamics; PK, pharmacokinetics; rFVIIa, recombinant factor VIIa; rVIIa-FP, recombinant fusion protein linking recombinant factor VIIa with recombinant albumin.

Acknowledgments

The author would like to thank Swiss Medical Press GmbH for editorial assistance.

Conflict of interest

The author has received research support from Alnylam, Baxter, Bayer, Biogen, Idec/SOBI, CSL Behring, Inspiration, Novo Nordisk, Octapharma, Pfizer, has participated as a scientific advisory board member for Baxter, Bayer, Biogen, Idec/SOBI, CSL Behring, LFB, Novo Nordisk and Pfizer, has received honoraria from Biogen, Idec, Baxter, Bayer, CSL Behring, LFB, Novo Nordisk and Pfizer, and has received travel support from Baxter, CSL Behring and Novo Nordisk.

References

[1] Scharrer I, Neutzling O. Incidence of inhibitors in haemophiliacs. A review of the literature. Blood Coagul Fibrinolysis 1993; 4: 753–758.
[2] Scharrer I, Bray GL, Neutzling O. Incidence of inhibitors in haemophilia A patients–a review of recent studies of recombinant and plasma-derived factor VIII concentrates. Haemophilia 1999; 5: 145–154.
[3] Wight J, Paisley S. The epidemiology of inhibitors in haemophilia A: a systematic review. Haemophilia 2003; 9: 418–435.
[4] UK Haemophilia Centre Doctors' Organisation. The incidence of factor VIII and factor IX inhibitors in the hemophilia population of the UK and their effect on subsequent mortality, 1977–99. J Thromb Haemost 2004; 2: 1047–1054.
[5] Srivastava A, Brewer AK, Mauser-Bunschoten EP, Key NS, Kitchen S, Llinas A, et al. Guidelines for the management of hemophilia. Haemophilia 2013; 19: e1–e47.
[6] Franchini M. The use of recombinant activated factor VII in platelet disorders: a critical review of the literature. Blood Transfus 2009; 7: 24–28.
[7] NovoSeven® Summary of Product Characteristics. Novo Nordisk A/S, Denmark. December 2013.
[8] Schulte S. Use of albumin fusion technology to prolong the half-life of recombinant factor VIIa. Thromb Res 2008; 122 (Suppl 4): S14–S19.
[9] Weimer T, Wormsbächer W, Kronthaler U, Lang W, Liebing U, Schulte S. Prolonged in-vivo half-life of factor VIIa by fusion to albumin. Thromb Haemost 2008; 99: 659–667.
[10] Zollner S, Schuermann D, Raquet E, Mueller-Cohrs J, Weimer T, Pragst I, et al. Pharmacological characteristics of a novel, recombinant fusion protein linking coagulation factor VIIa with albumin (rVIIa-FP). J Thromb Haemost 2014; 12: 220–228.
[11] Herzog E, Harris S, McEwen A, Henson C, Pragst I, Dickneite G, et al. Recombinant fusion protein linking factor VIIa with albumin (rVIIa-FP): biodistribution in rats. Thromb Res 2014; 134: 495–502.
[12] Golor G, Bensen-Kennedy D, Haffner S, Easton R, Jung K, Moises T, et al. Safety and pharmacokinetics of a recombinant fusion protein linking coagulation factor VIIa with albumin in healthy volunteers. J Thromb Haemost 2013; 1: 1977–1985.
[13] Girard P, Nony P, Erhardtsen E, Delair S, Ffrench P, Dechavanne M, et al. Population pharmacokinetics of recombinant factor VIIa in volunteers anticoagulated with acenocoumarol. Thromb Haemost 1998; 80: 109–113.