Thrombosis Research 131S2 (2013) S2–S6

Contents lists available at SciVerse ScienceDirect

Thrombosis Research

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

Original Article

Innovative coagulation factors: albumin fusion technology and recombinant single-chain factor VIII

Stefan Schulte *

Research & Development, CSL Behring GmbH, Marburg, Germany

ARTICLE INFO

Keywords:
Hemophilia A
Hemophilia B
Albumin fusion
Activated factor VII
Factor VIII
Factor IX
Half-life

ABSTRACT

Albumin fusion technology has been used to enhance the pharmacokinetic properties of recombinant coagulation factors. The goal of linking albumin to coagulation factors is to extend the half-life of the coagulation factor, thereby allowing for less frequent dosing for patients with bleeding disorders, such as hemophilia. The novel recombinant fusion proteins linking coagulation factors VIIa and IX with albumin (rVIIa-FP and rIX-FP, respectively) have a longer half-life and similar hemostatic efficacy compared with available recombinant coagulation factor products. Clinical evaluation of these fusion proteins is underway, and preliminary results with rIX-FP in patients with hemophilia B are encouraging. Other advances in coagulation factor therapy include a unique recombinant single-chain factor VIII (FVIII) protein, which has improved intrinsic stability and a higher affinity for von Willebrand factor (VWF), relative to other recombinant FVIIIs, and a recombinant VWF-albumin fusion protein (rVWF-FP), which has a significant longer half-life compared to available VWF products. Evaluation of these novel recombinant proteins continues and will help determine their potential to enhance the management of patients with bleeding disorders.

Coagulation factor products are important in the prevention and treatment of bleeding episodes in patients with bleeding disorders, such as hemophilia A, hemophilia B, and von Willebrand disease. Use of available coagulation factor products can help reduce bleeding complications and improve patient quality of life, but there is still room for improvement. Current research continues to help refine the efficacy, tolerability, and convenience of coagulation factor replacement therapy for patients with bleeding disorders. This field continues to evolve rapidly, and new coagulation factor products with enhanced properties have now entered the clinical evaluation phase of development. This article provides an update on some of the ongoing work at CSL Behring aimed at improving the properties of therapeutic coagulation factor products.

Avenues for improving coagulation factors

There are different avenues that researchers can take towards improving the properties of recombinant coagulation factors, and the choice of avenue depends on the intended goal. One could aim to enhance the product's functional activity, for example, by introducing genetic mutations that increase the potency of the recombinant protein. The same technique can also be applied in order to reduce the immunogenicity of the product, which is an important safety concern. The product may also be reformulated to allow for alternative routes of delivery (e.g. subcutaneous injection).

Another approach to improve coagulation factors is to extend their half-life; this has been an important research focus at CSL Behring. Coagulation factors are large, complex proteins, and their half-life is relatively short, which necessitates frequent dosing to maintain therapeutic levels. Modifications that extend the product's half-life could theoretically allow for extended dosing intervals, resulting in less frequent injections, improved compliance, and better prophylactic outcomes. There are several methods for prolonging the half-life of proteins, including chemical modifications and fusion of the target protein to a protein with a longer half-life. In the context of bleeding disorders, the optimal approach to half-life extension should ensure that the biological activity of the coagulation factor is not compromised and the risk of immunogenicity and safety is not increased, compared with currently available products. In addition, it is important to define what constitutes a clinically relevant extension of half-life: this depends on practical considerations, such as the dosing schedule, and the intended clinical application (e.g. on-demand vs. prophylaxis). Chemical modification, for example, may be less suitable for products intended for chronic use, because it is difficult to predict how this unnaturally, chemically modified protein will be degraded and what potentially toxic byproducts will accumulate and how this may affect

Abbreviations: aPTT, activated partial thromboplastin time; FcRn, neonatal Fc receptor; FIX, factor IX; FVIIa, activated factor VII; FVIII, factor VIII; IgG, immunoglobulin G; PK, pharmacokinetics; rFIX, recombinant factor IX; rFVIIa, recombinant activated factor VII; rFVIII, recombinant factor VIII; rIX-FP, recombinant fusion protein linking coagulation factor IX with albumin; rVIIa-FP, recombinant fusion protein linking coagulation factor VIIa with albumin; rVIII-SingleChain, recombinant single-chain factor VIII; rVWF, recombinant von Willebrand factor; rVWF-FP, recombinant fusion protein linking VWF with albumin; TGA, thrombin generation assay; VWF, von Willebrand factor.

* Correspondence: Stefan Schulte, CSL Behring GmbH, Emil-von-Behring Strasse 76, 35041 Marburg, Germany. Tel.: +49 6421 395 635; fax: +49 6421 394 663.
E-mail address: Stefan.schulte@cslbehring.com (S. Schulte).

0049-3848/$ – see front matter

long-term safety. The optimal approach to half-life extension of therapeutic proteins must therefore be assessed specifically on a case-by-case basis. The following discussion illustrates the different approaches our research program has taken to address the challenges in extending the half-life of three key factors in the coagulation cascade.

Half-life extension of factor VIIa and factor IX

We selected albumin fusion technology as the method of half-life extension for factor VIIa (FVIIa) and factor IX (FIX). Fusion of a therapeutic protein to a protein with a long half-life can be accomplished using single-step expression techniques and minimal processing, which yields a homogenous product [1–3]. Fusion to naturally occurring proteins, such as albumin, can also improve the solubility and stability of the therapeutic protein. Albumin has several characteristics that make it particularly well suited for this application [4]. It is the most abundant protein found in the plasma of the human body and has a naturally long half-life: in humans, the half-life of albumin is approximately 20 days [3,5,6]. The prolonged half-life of albumin is due to neonatal Fc receptor (FcRn)-mediated recycling, whereby the intracellular salvage receptor FcRn rescues albumin from lysosomal degradation by binding to albumin (or immunoglobulin G [IgG], another naturally abundant protein with a long half-life) under acidic conditions in the endosome and transporting it back to the cell surface, where it is released at neutral pH [7]. Albumin is a carrier protein and, unlike IgG, has no involvement in immune defense, suggesting that the impact of albumin fusion on coagulation factor immunogenicity would be minimal [5]. Albumin has been studied extensively, and much is known about its molecular structure and clearance [8–10]. It has been successfully fused to several therapeutic proteins, including insulin [11] and hirudin [12], as well as some more complex proteins, such as interferon [13,14], interleukin-2 [15,16], and butyrylcholinesterase [17].

To generate an albumin fusion protein, a DNA construct is created that encodes for both the target protein and albumin [1]. This DNA construct is transfected into a eukaryotic cell line (mammalian cells are required for coagulation factors for proper post-translational modification like gamma-carboxylation and glycosylation), and high expressing clones are selected. The clones secrete the recombinant fusion protein during fermentation, and standard protein purification techniques are applied to isolate the fusion protein. Our hypothesis was that fusing a recombinant coagulation factor with a short half-life to recombinant albumin would produce a novel recombinant albumin fusion protein that basically maintained the biological activity of the original coagulation factor, while extending its half-life [1,8]. We spent some time optimizing the linker sequence that connects the therapeutic protein to albumin to provide maximal hemostatic efficacy of the coagulation factor, and we found that different approaches worked best for different proteins, as explained below in more detail for recombinant fusion protein linking coagulation factor VIIa with albumin (rVIIa-FP) and recombinant fusion protein linking coagulation factor IX with albumin (rIX-FP).

Recombinant fusion protein linking coagulation factor VIIa with albumin (rVIIa-FP)

Factor VII plays an important role in the coagulation cascade, and recombinant activated factor VII (rFVIIa) is indicated for the treatment of people with hemophilia A or B who develop inhibitors to their respective therapies, factor VIII (FVIII) or FIX. The half-life of rFVIIa, however, is very short (approximately 2.5 hours in humans), which often necessitates multiple infusions for a single bleeding episode [18]. Joint bleeding typically requires two or more infusions, while patients undergoing surgery require infusion every 2–3 hours for 2 or more days [10,19–21]. The short half-life of rFVIIa also limits its use as long-term prophylaxis [22]. Our goal was to extend the half-life of rFVIIa so that one infusion per bleeding episode would be sufficient, and long-term prophylaxis could be feasible [10,23].

A recombinant fusion protein linking coagulation factor VIIa with albumin (rVIIa-FP) was generated using the albumin fusion technology described above (Fig. 1A) [10,22–24]. A short glycine/serine linker sequence was inserted between the 406-amino acid rFVIIa sequence and the 585-amino acid albumin sequence; this flexible linker was found to be essential for achieving maximal potency of the rFVIIa portion of the molecule.

In rats, the half-life of rVIIa-FP was extended significantly, and was nearly 6-fold greater than that of commercially available recombinant FVIIa (NovoSeven®) [10,24]. Results of the thrombin generation assay (TGA) in a FVIII knock-out mouse model indicated that both rVIIa-FP and rFVIIa strongly reduced the lag time initially; however, lag time returned to baseline levels within 16 hours in mice exposed to rFVIIa, but remained reduced in those exposed to rVIIa-FP (CSL Behring, data on file).

These preclinical data demonstrate that rVIIa-FP has a significantly longer half-life than commercially available rFVIIa, as well as a 2-fold increase in recovery. The efficacy of rVIIa-FP *in vivo* is comparable to that of commercially available and wild-type rFVIIa. No adverse effects of treatment have been observed after intravenous infusion of rVIIa-FP in all species tested. A Phase I clinical evaluation of rVIIa-FP is underway (*PROLONG-7FP* clinical trial program).

Recombinant fusion protein linking coagulation factor IX with albumin (rIX-FP)

Recombinant FIX (rFIX) is indicated for the treatment of hemophilia B, but its relatively short half-life (approximately 18 hours) necessitates two or three infusions per week to maintain adequate levels for bleeding prophylaxis [25–27]. Our goal was to extend the half-life of rFIX to allow for a more convenient schedule of at least once-weekly dosing.

Initially, a recombinant fusion protein linking coagulation FIX with albumin was created (rIX-FP); in a design similar to that used for rVIIa-FP (Fig. 1B) [19,24]. These attempts to fuse FIX to albumin were unsuccessful because the albumin portion of the fusion protein interfered with the biological activity of the FIX portion [10,28–30]. The design was then modified to include a cleavable linker sequence between the FIX and albumin sequences. This linker sequence contains a cleavage site that is identical to the natural site found within the activation peptide from the FIX zymogen; cleavage at this site converts FIX to its activated form FIXa. Consequently, when rIX-FP is activated, the albumin portion is simultaneously removed, thereby minimizing potential interference with the biological activity of activated FIX [10,29,30].

Characterization of rIX-FP in rodent and rabbit pharmacokinetic (PK) models indicated improved half-life and recovery compared with commercially available rFIX (BeneFIX®) [10,29,31]. In rats, for example, the terminal half-life of rIX-FP was nearly 5-fold higher than that of rFIX. Hemostatic efficacy was observed in a tail-tip bleeding model of FIX knock-out mice, and the dose–response curve was comparable to that seen with rFIX (CSL Behring, data on file). Other studies using surface plasmon resonance analysis indicate that binding patterns of rIX-FP to FcRn are similar to that of albumin (i.e. both bind to FcRn at pH 6.0, but not at pH 7.4), whereas rFIX had negligible interaction with FcRn at any pH level [7].

The PK of rIX-FP were evaluated at doses of 50–500 IU/kg in cynomolgus monkeys and hemophilia B dogs [32]. Terminal half-life was extended in both species compared with rFIX. When FIX-FP and