Biologics: Targets and Therapy

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REVIEW

Treatment of hemophilia B: focus on recombinant factor IX

This article was published in the following Dove Press journal:
Biologics: Targets and Therapy
11 February 2013
Number of times this article has been viewed

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Abstract: Hemophilia B is a recessive X-linked bleeding disorder characterized by deficiency of the coagulation factor IX (FIX). In hemophilia B patients the severity of the bleeding phenotype is related to the degree of the FIX defect. Hemophilia B treatment has improved greatly in the last 20 years with the introduction first of plasma-derived and then of recombinant FIX concentrates. Replacement therapy may be administered through on-demand or prophylaxis regimens, but the latter treatment modality has been shown to be superior in prevention of hemophilic arthropathy and in improvement of patients' quality of life. The purpose of this narrative review is to summarize the current knowledge on treatment strategies for hemophilia B, focusing on recombinant FIX products either clinically used or in development. There is only one rFIX product that is licensed to treat hemophilia B patients; from the analysis of the literature data presented in this review, the authors conclude that this rFIX product has demonstrated an excellent safety profile and excellent clinical efficacy for halting and preventing bleeds in hemophilia B patients. While prophylaxis has emerged as the best therapeutic strategy for such patients because of its ability to prevent hemophilic arthropathy and to improve patients' quality of life, the pharmacokinetically tailored dosing of rFIX is another key point when planning hemophilia B treatment, as it allows optimization of the factor concentrate usage. Further clinical studies are needed to better assess the safety and efficacy (ie, the incidence of adverse reactions and inhibitor development) of newer rFIX products.

Keywords: recombinant FIX products, plasma-derived FIX concentrate, bleeding, blood clotting disorder, on-demand treatment, prophylaxis treatment

Introduction

Hemophilia B is a recessive X-linked blood coagulation disorder leading to a deficiency of functional factor IX (FIX), one of the serine proteases of the intrinsic pathway of the coagulation cascade of secondary hemostasis.[1,2] FIX is synthesized as a single polypeptide chain that undergoes extensive posttranslational modifications including signal peptide cleavage, disulfide bond formation, glycosylation, vitamin K–dependent gamma-carboxylation of glutamic acid residues in the NH_2 terminal region, beta-hydroxylation, and propeptide cleavage.[3–5] The liver is the primary site of FIX synthesis and hepatocytes directly secrete FIX into the plasma.[4] FIX is proteolytically activated by factor XIa or factor VIIa to form FIXa, which in turn and together with other cofactors (activated factor VIII, phospholipid, and calcium ions) forms the "tenase complex" and activates factor X to form factor Xa, the first member of the final common coagulation pathway ultimately resulting in cross-linked fibrin.[5]

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Hemophilia B is the second most common form of hemophilia (after hemophilia A) and it is estimated to occur in one in 30,000 live male births across all ethnic groups.[1] Multiple mutations have been described in the FIX gene, located on the long arm of the X chromosome, with the most common being single base-pair changes that result in missense, frameshift, or nonsense mutations.[6] In hemophilia B, the deficiency of FIX results in the reduction of a functioning intrinsic tenase complex, leading to diminished thrombin generation and an inability to form and maintain a stable clot.[5,6] Accordingly, the bleeding tendency in hemophilia B patients depends on the levels of FIX coagulant activity, classified as mild (5%–40%), moderate (1%–5%), or severe (<1%).[7] Thus, patients with mild hemophilia tend to experience abnormal bleeding only in response to surgery, tooth extraction, or injuries. Conversely, patients with moderate hemophilia experience prolonged bleeding responses to relatively minor trauma, and patients with severe hemophilia experience frequent spontaneous bleeds, especially recurrent hemarthroses and soft-tissue hematomas, leading over time to severe arthropathy, joint contractures, and pseudotumors and, consequently, to chronic pain, disability, and a reduced quality of life.[1] Traditionally, hemophilias A and B have been considered clinically indistinguishable from each other.[8] Recent evidence, however, suggests that patients with hemophilia B have a less severe bleeding phenotype, a lower bleeding frequency, and better long-term outcomes (lower likelihood of joint arthroplasty).[9,10] The mainstay of treatment for hemophilia B involves replacing the missing blood coagulation FIX when bleeding episodes occur (on-demand treatment) or by scheduled infusions several times per week (prophylaxis treatment). Both plasma-derived (pd) and recombinant (r)FIX clotting factor concentrates are suitable for these different strategies of hemophilia B management.[11]

This narrative review summarizes both current and developing treatment strategies for hemophilia B, focusing in particular on rFIX products.

Current FIX therapies

Since the introduction of plasma-derived FIX concentrates in 1992, the availability of pdFIX clotting factor concentrates has dramatically improved the quality of life and the life expectancy of hemophilia B patients, as it has permitted the safe execution of surgeries (particularly orthopedic operations) and the widespread adoption of home replacement therapy with the broad implementation of prophylactic treatment regimens.[12–14] Indeed, primary prophylaxis has become the optimal standard of care for severe hemophilia patients, shown to prevent joint damage, decrease the frequency of joint and other hemorrhages, and improve health-related quality of life (Table 1).[15]

The goal of replacement therapy for hemophilia B is to achieve a plasma FIX level of 60%–80% for major bleeds and 20%–40% for minor bleeds (Table 1). There are several high-purity pdFIX concentrates commercially available (Table 2). However, although the safety of such products has been greatly improved over the last 20 years, thanks to the adoption of multiple viral inactivation and purification steps for each product, there are still some concerns regarding prions and noncapsulated viruses.[16–19] Cloning of the FIX gene in 1982[20,21] paved the way for the production, using genetically engineered Chinese hamster ovary cells, of rFIX concentrate – this became commercially available in 1998.[22] Because it is manufactured without animal or human proteins (including albumin) in the culture medium or the final formulation and is therefore safe in terms of freedom from risk of infection, rFIX concentrate has occupied a significant place among the available products for hemophilia B therapy. Recent reports show that currently 28% of patients receive rFIX treatment in Spain and 64% of patients receive pdFIX.[23] Unlike for hemophilia A, where there are multiple recombinant products licensed for treatment, only one rFIX product is currently available for hemophilia B: nonacog alfa (BeneFIX®; Pfizer, Sandwich, UK).[24]

rFIX is a single-chain glycoprotein with a molecular mass of approximately 55,000 Da. Its primary 415-amino-acid sequence is identical to the Ala[148] allelic form of pdFIX, and it has structural and functional characteristics similar to

Table 1 Recommended dosages of factor IX (FIX) concentrates for the treatment or prevention of bleeding episodes in hemophilia B patients

Type of hemorrhage	FIX dose (U/kg)
Mild or moderate hemarthroses or hematomas	20–40
Severe hemarthroses or hematomas	40–60
External bleeding with anemia	
Moderate posttraumatic bleeding	
Cranial trauma	50–100*
Cerebral hemorrhage	
Surgery prophylaxis	
Primary prophylaxis	30–40 (two times weekly)

Note: *For surgical prophylaxis, FIX levels should be maintained above 50% for 7–15 days after surgery.

Table 2 Characteristics of licensed factor IX concentrates

Brand name	Company	Source	Fractionation	Viral inactivation	Specific activity (IU/mg)
Aimafix®	Kedrion	Plasma	Anion exchange, DEAE Sephadex®/Sepharose®, and heparin affinity chromatography	TNBP/polysorbate 80; dry heat, 100°C for 30 minutes; nanofiltration, 35 + 15 nm	>100
AlphaNine® SD	Grifols	Plasma	Dual polysaccharide ligand affinity chromatography	S/D: TNBP/polysorbate 80; nanofiltration	210
Berinin® P	CSL Behring	Plasma	DEAE Sephadex and heparin affinity chromatography	Pasteurization at 60°C for 10 hours	146
Betafact®	LFB	Plasma	Ion exchange and affinity chromatography	TNBP/polysorbate 80; nanofiltration, 15 nm	110
Factor IX Grifols®	Grifols	Plasma	Precipitation and multiple chromatography	S/D; nanofiltration, 15 nm	>150
Haemonine®	Biotest	Plasma	Anion exchange, immunoaffinity, and hydrophobic interaction chromatography	TNBP/polysorbate 80; nanofiltration, 15 nm	>70
Hemo-B-RAAS	Shanghai RAAS	Plasma	Ion exchange and affinity chromatography	S/D; dry heat; nanofiltration	>50
Immunine®	Baxter BioScience	Plasma	Ion exchange and hydrophobic interaction chromatography	Polysorbate 80; vapor heating, 60°C for 10 hours, 190 mbar, then 80°C for 1 hour, 375 mbar	~100
Mononine®	CSL Behring	Plasma	Immunoaffinity chromatography	Sodium thiocyanate; ultrafiltration	>190
Nanotiv®	Octapharma	Plasma	Ion exchange and affinity chromatography	TNBP/Triton® × 100; nanofiltration	>150
Nonafact®	Sanquin	Plasma	Immunoaffinity and hydrophobic interaction chromatography	TNBP/polysorbate 80; nanofiltration, 15 nm	≥200
Octanine F®	Octapharma	Plasma	Ion exchange and affinity chromatography	TNBP/polysorbate 80; nanofiltration	>120
Replenine®-VF	Bio products laboratory	Plasma	Metal chelate chromatography	S/D; nanofiltration, 15 nm	200
TBSF FIX	CSL biotherapies	Plasma	Ion exchange and heparin affinity chromatography	TNBP/polysorbate 80; nanofiltration	>50
BeneFIX®	Pfizer	Recombinant	Anionic chromatography	Nanofiltration	≥200

Abbreviations: DEAE, diethylaminoethanol; S/D, solvent–detergent; TNBP, tri-n-butyl phosphate.

those of endogenous FIX. rFIX concentrate is purified by a chromatographic process and a final membrane filtration step is included for additional viral safety. Although rFIX shares nearly identical hemostatic characteristics with pdFIX, there are differences in posttranslational modification of the FIX molecule that appear to affect the in vivo recovery time (ie, the ratio between the observed and the theoretical maximum FIX activities) of the recombinant product.[25] Specifically, rFIX exhibits an approximately 30% reduced recovery in plasma at equivalent dosing to pdFIX.[26,27] Björkman[28] made efforts to summarize these different rFIX properties in clinical practice and reviewed 17 studies on the difference in pharmacokinetics between rFIX and pdFIX, drawing a general conclusion that conversion factors between rFIX and pdFIX of 1.5 for single doses and 2 for prophylactic dosing could be tentatively applied. However, interindividual patient pharmacokinetics greatly influence the response to treatment, so tailored treatment should be the general practice.[29] These altered pharmacokinetics are particularly observed in young children, mostly because of their higher plasma volume of distribution.[30,31] However, when the dose is adjusted accordingly, rFIX has proven to be safe and effective in the treatment of bleeding episodes in previously untreated and previously treated patients with hemophilia B,[32–34] with a low incidence of serious adverse effects such as allergic events, thrombosis, or inhibitor development (the current most challenging complication of replacement therapy in hemophilia).[35–37] The same studies showed that no adverse effects such as thrombotic events or viral transmission could be related to rFIX administration. Table 3 summarizes the most important published clinical studies on rFIX products.[25–27,32,38–41] Only a few studies have specifically compared pdFIX and rFIX concentrates,[25,26,35,38,41] confirming the lower recovery rate of recombinant product but documenting their equivalence in terms of efficacy rate.

Table 3 Summary of the main clinical studies on recombinant factor IX (rFIX) concentrates

Reference	Study design (patient population)	Main results
White et al[34]	Double-blind, randomized, crossover (rFIX. n = 11; pdFIX, n = 11)	Significantly lower recovery for rFIX; safe and effective
Roth et al[32]	Prospective PK, safety, and efficacy (rFIX, n = 56)	Low recovery; safe and effective
Poon et al[25]	Retrospective observational (rFIX, n = 126; pdFIX, n = 75)	Significantly lower recovery for rFIX; in boys aged < 15 years, decreased recovery for both products; inhibitors, 2/244 (0.8%)
Ewenstein et al[26]	Double-blind, two-period crossover (rFIX, n = 43; pdFIX, n = 43)	Wide product-related (decreased for rFIX) and patient-related variability in recovery
Kisker et al[38]	Double-blind, two-period crossover (rFIX, n = 15; pdFIX, n = 15)	Decreased recovery for rFIX rFIX more expensive because of higher doses
Shapiro et al[27]	Open-label, single-cohort (rFIX, n = 63)	Recovery depending on age; safe and effective
Lambert et al[39]	Double-blind, randomized, PK crossover (rFIX, n = 34)	Recovery, safety, and efficacy of reformulated rFIX is comparable with original
Monahan et al[40]	Prospective PK, safety, and efficacy (rFIX, n = 25)	One or two rFIX infusions per week as prophylaxis is well tolerated
Recht et al[41]	Retrospective, safety (rFIX, n = 163; pdFIX, n = 88; rFIX and pdFIX, n = 71)	No difference in the frequency of allergic reactions or inhibitor development between pd- and rFIX concentrates
Berntorp et al[33]	Prospective, observational cohort (rFIX, n = 218)	A low incidence of SAEs was detected (inhibitors 0.9%, thrombosis 0.5%, allergic events 3.7%)

Abbreviations: pdFIX, plasma-derived factor IX; PK, pharmacokinetic; SAEs, serious adverse events.

New developments in rFIX products

Recombinant DNA technology and bioengineering have been applied to FIX to extend its half-life, thereby decreasing the frequency of infusions. Investigators are focused on the development of new strategies, which mainly include polymer modification with polyethylene glycol (PEG) and protein fusion technology.[24]

The covalent conjugation of PEG to a therapeutic protein, named PEGylation,[42] seems to be the ideal target for the research in this field. Indeed, the PEG polymers create a diffusion cloud around the protein, shielding it from exposure to proteolytic enzymes, clearance receptors, and immune effector cells (involved in the recognition of antigenic peptide epitopes).[11] The glycoPEGylated rFIX nonacog beta pegol (Novo Nordisk, Bagsvaerd, Denmark) is a 40 kDa molecule that has demonstrated a half-life five times longer than that of commercially available rFIX in FIX-knockout mice.[43] A phase I clinical trial of glycoPEGylated rFIX has now been completed and the results, documenting the enhanced pharmacokinetic properties and the safety of this new molecule, have recently been published.[44] A population pharmacokinetic model extrapolated from the results of this trial predicts that nonacog beta pegol may allow prophylaxis, surgical dosing regimens, and on-demand treatment of bleeding episodes with less-frequent injections and lower factor concentrate consumption than with standard pd- and rFIX concentrates.[45]

The fusion of the Fc-portion of immunoglobulin G to a single molecule of rFIX (rFIX-Fc) (Biogen Idec, Inc, Weston, MA, USA) has also been explored to increase its circulation time.[46] In animal models of hemophilia B, rFIX-Fc fusion proteins exhibited an extended half-life of up to 48 hours, compared with the standard rFIX half-life of approximately 18 hours, and they showed normal procoagulant activity.[47] The prolonged half-life of rFIX-Fc, in addition to its safety, emerged in a phase I/II dose escalation study conducted in 14 previously treated severe or moderately severe hemophilia B patients.[48] The molecule is currently being evaluated in two phase II/III clinical trials in hemophilia B patients.[49,50]

Another technology that uses rFIX fusion protein with albumin (CSL Behring LLC, King of Prussia, PA, USA) has achieved in preclinical studies a fivefold lengthening in half-life compared with licensed rFIX.[51] The improved pharmacokinetics of rFIX albumin fusion protein was also confirmed in a recently published phase I/II trial,[52] and a phase III clinical trial in patients with severe hemophilia B receiving an albumin-fused rFIX molecule is also under way.[53]

Finally, preclinical safety evaluations, including markers of thrombogenicity (performed in dog, rabbit, and rat models) and pharmacokinetics (performed in rats and hemophilic dogs), of the biosimilar rFIX IB1001 trenacog alfa

(Inspiration Biopharmaceuticals, Inc, Cambridge, MA, USA) demonstrated findings similar to those observed with identical nonacog alfa doses.[54] The equivalent pharmacokinetics properties were confirmed in a recent randomized, double-blind, noninferiority, crossover study comparing these two rFIX products in severe or moderately severe hemophilia B patients, supporting the use of IB1001 as an alternative recombinant FIX product.[55]

Conclusion

From the analysis of the literature data presented in this review, the only licensed rFIX product has demonstrated an excellent safety profile and clinical efficacy for halting and preventing bleeds in hemophilia B patients. While prophylaxis has emerged as the best therapeutic strategy for such patients because of its ability to prevent hemophilic arthropathy and to improve patients' quality of life, the pharmacokinetically tailored dosing of rFIX is another key point when planning hemophilia B treatment, as it allows optimization of the factor concentrate usage. The actual research of the pharmacotherapy of hemophilia B is directed to overcome one of the most important disadvantages, the need for frequent infusions, by the development of rFIX molecules (ie, PEGylated and fusion proteins) with a longer half-life. Further clinical studies are needed to better assess the safety and efficacy (ie, the incidence of adverse reactions and inhibitor development) of newer rFIX products.

Disclosure

The authors report no conflicts of interest in this work.

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