



Regular Article

Visions in haemophilia care

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ABSTRACT

Significant advances have been made in the treatment of patients with haemophilia, resulting in reduced mortality, improved joint outcomes, enhanced quality of life, and extended life span. Comprehensive haemophilia centres, the adoption of home therapy, and worldwide advocacy efforts have contributed to these improvements. Areas of more recent progress and ongoing research include the development of improved pathogen screening/inactivation, viral inactivation techniques, reduction in the development of inhibitory antibodies, and generation of recombinant clotting factors. These advances have helped facilitate aggressive therapeutic interventions such as prophylaxis. However, several barriers limit the adoption and adherence of effective prophylactic therapy. The establishment and utilization of biotechnology in the field of haemophilia potentially offers novel agents, as well as therapeutics with longer half-lives, increased potency and resistance to inactivation, increased secretion, and reduced resistance to inhibitors. The current pipeline of new technologies and products is promising, with some agents already advancing from the laboratory to clinical trials.

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Introduction

Haemophilia A is a severe bleeding disorder and the most common disorder of its kind. It is primarily an inherited disorder in which the functionally active coagulation factor VIII (FVIII) is partially or totally deficient. Haemophilia results in abnormal bleeding, depending on the degree of factor deficiency. Individuals with severe haemophilia are at high risk of repeated joint bleeds that can lead to progressive joint damage and long-term health issues [1]. Without treatment, these affected individuals die prematurely, secondary to bleeding, especially intracranial bleeding and its complications. Treatment with appropriate therapy can stop or prevent bleeding episodes and reduce the associated morbidity, as well as improve quality of life and normalize life expectancy. Unfortunately, current management of haemophilia is not optimal. The development of alloantibodies that inhibit the activity of infused replacement products remains a significant complication. Haemophilia is also associated with a significant financial burden with an estimated annual cost of over \$100,000 per patient in the USA [2]. Standard prophylaxis regimens (using 25–40 IU/kg of FVIII 3 times/week) is a barrier for some individuals where suboptimal adherence remains a problem [3]. There is also the frequent requirement for central venous access devices

to facilitate prophylactic infusion of FVIII. The use of these devices has been associated with infection and thrombosis [4]. Infusion of factor concentrates is also usually required frequently, owing to their short half-life in the circulation. Fortunately, there have been tremendous advances in worldwide advocacy efforts, improvements in clinical care – particularly prophylaxis and tailored management – and advanced developments in biotechnology, including improved pathogen screening/inactivation methods, development of recombinant clotting factors and advancements in bioengineering strategies – all of which are paving the way for a promising outlook for individuals with haemophilia. This report summarizes the current and future trends in haemophilia treatment, with a particular focus on aspects of targeted bioengineering strategies for improving FVIII.

Improving FVIII

Elucidation of the life cycle of FVIII as well as its structure and function have led to opportunities to develop modified forms of FVIII with improved clinical utility (Fig. 1). Omission of the B-domain of the recombinant FVIII (rFVIII) molecule enhances mRNA expression by up to about 20-fold without sacrificing procoagulant activity [5–7]. This finding facilitated a more efficient gene transfer into host cells for rFVIII production and, consequently, early gene therapy trials. However, this approach resulted in only a 30–50% increase in the amount of secreted protein. Building on these findings, we showed that it was possible to improve secretion efficiency by 15- to 25-fold using a B-domain-deleted (BDD) FVIII

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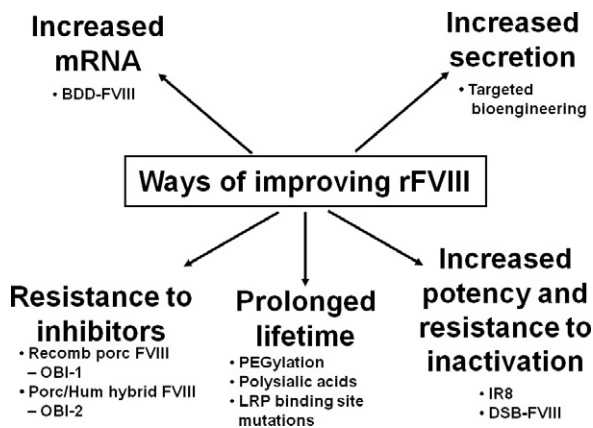


Fig. 1. Strategies for improving recombinant factor VIII (rFVIII). BDD, B-domain deleted; DSB, disulfide-bridged; Hum, human; FVIII, factor VIII; IR8, inactivation-resistant FVIII; LRP, lipoprotein receptor-related protein; OBI, recombinant porcine BDD; PEGylation, polyethylene glycolation; porc, porcine.

variant with an A1 domain point mutation [7]. Similar approaches have also seen success in enhancing factor IX (FIX) expression.

Other areas for exploration include strategies to improve the biosynthesis, secretion, functional activity, half-life, and antigenicity/immunogenicity of rFVIII [8–10]. We genetically engineered a FVIII molecule that is resistant to spontaneous inactivation (IR8) and has a higher specific activity than that of wild-type FVIII [11]. This FVIII variant also demonstrated greater haemostatic efficacy at a significantly lower protein dose compared with wild-type FVIII in a haemophilia A mouse model [12]. Furthermore, a disulphide-bond engineered variant of FVIII exhibited improved potency and procoagulant activity compared with wild-type FVIII [13].

Some of the methods that are being explored to prolong the half-life of FVIII include biochemical modifications of the protein such as conjugation with polyethylene glycol (PEG) polymers or polysialic acids (PSA) – which are discussed in more detail below – stabilization with PEG-modified liposomes (PEGLip), and bioengineering with fusion proteins. Interference with FVIII receptor-mediated clearance through the low-density lipoprotein receptor-related protein and heparan sulphate proteoglycans is another potential target for prolonging FVIII half-life [9].

Another line of investigation involves developing FVIII with reduced antigenicity/immunogenicity. The most sophisticated constructions are human/porcine hybrid FVIII molecules. A human/porcine recombinant BDD-FVIII mutant has demonstrated reduced immunogenicity in the mouse model of haemophilia A without any apparent loss of function [14]. A significant theoretical advantage of this type of FVIII variant would be reduced immunogenicity in previously untreated patients; however, this is currently a topic for further research. A recombinant form of porcine FVIII is also being examined in clinical trials of acquired haemophilia A patients with auto-antibodies to FVIII [15].

Prolonging the half-life of FVIII

Prolonging the half-life of FVIII could greatly reduce the frequency and dose of infusions, thereby improving the efficacy of prophylaxis through better compliance, as well as improve convenience and patient health-related quality of life [16].

PEGylation

PEG incorporates many water molecules within its hydrophilic structure and PEGylation increases the effective size of its conjugated protein above the filtration size of the kidney. PEGylation has been successfully applied to extend the half-life

of several therapeutics including the commercially available PEG-asparaginase for the treatment of acute lymphoblastic leukaemia, PEG-adenosine deaminase for severe combined immunodeficiency disease, PEG-interferon for hepatitis C, PEG-granulocyte colony stimulating factor for chemotherapy-induced neutropenia, and PEG-growth hormone for the treatment of acromegaly (see reference [9]).

PEGylation as a strategy for extending the half-life of coagulation factors is generating great interest. Two broad approaches can be taken: direct and indirect modification. The former approach involves modification of the FVIII molecule biochemically such as with PEGylation or polysialylation. The indirect approach involves similar modification of von Willebrand factor (VWF), which serves as the FVIII carrier protein, or providing an alternative reconstitution of FVIII with PEGLip.

The PEGylation approach to extending FVIII half-life is likely to have a more limited benefit compared to similar approaches with other biologics, as the FVIII molecule is already large enough that it is not filtered by the kidney. Rather, PEGylation may reduce interaction with clearance receptors in organs such as the liver. Another benefit is that this procedure could also potentially reduce access to inactivating proteases and immune-mediating cells. Although this is an established technique with proof-of-principle for complex proteins, several drawbacks still remain. PEGylation may reduce the specific activity of FVIII. There might also be reduced accessibility for key activating proteases or other protein–protein interactions that are essential to the therapeutic protein's biological activity; PEGylation may reduce the interaction of FVIII with VWF, thereby compromising its plasma stability. PEGylation that is sufficient to block cellular clearance may also result in decreased specific activity. PEG, itself, could potentially accumulate in tissues responsible for its clearance, and its long-term toxicity and impact on immunogenicity are not known.

Polysialic acids (PSA)

PSA modification of therapeutic proteins is an alternative strategy to PEGylation for increasing the size of a protein. PSA are linear, hydrophilic polymers of N-acetylneuraminic acid that occur abundantly on the surface of many cells and proteins. They can be conjugated to therapeutic proteins and potentially alter their pharmacokinetics (PK), including prolonging the half-life [17]. PSA produce a 'watery cloud' around the therapeutic molecule protecting it from immune-mediating cells, proteolytic enzymes, and clearance receptors.

The use of PSA has already been successfully applied to several therapeutic proteins such as PolyXen™-asparaginase, PolyXen™-interferon and PSA-insulin. Some of the advantages that have been demonstrated with PSA technology include reduced immunogenicity and antigenicity, and preservation of function with increased stability. Furthermore, PSA, unlike PEGylation, are naturally occurring and biodegradable, which offers the advantage of administration in large doses over a prolonged period of time.

PEGylated liposomes

PEGLip is an established process that has been used to extend the half-life of a broad range of therapeutic proteins including FVIII [18]. In this approach, the therapeutic protein is reconstituted with a PEG liposome carrier. Because liposomes are typically cleared quickly from the circulation, the addition of PEG can extend the circulatory half-life of the liposomes considerably. This approach can effectively modify the immunological, PK, and pharmacodynamic properties of proteins and has been utilized to develop a longer-acting FVIII. With this strategy, rFVIII molecules remain unmodified and so there

is no loss of normal protein–protein interactions and functional activities.

In preclinical trials with a haemophilic mouse model, prophylactic infusion of rFVIII reconstituted with PEGlip prolonged some PK parameters compared with standard rFVIII [19]. These findings correlated with an enhanced haemostatic efficacy with PEGlip-rFVIII [19]. In addition, PEGlip-rFVIII has been examined in patients with severe haemophilia A in a blinded, controlled, crossover, multicentre trial [16]. A single prophylactic infusion of PEGlip-rFVIII prolonged the bleeding-free period compared with standard rFVIII. The PEGlip formulation was well tolerated and no significant adverse events were reported during the trial. These findings generated great interest and a subsequent double-blind, randomized, crossover phase I trial was conducted to compare the PK of a single infusion of PEGlip-rFVIII with that of rFVIII in 26 men with severe haemophilia A [20]. However, PEGlip-rFVIII and standard rFVIII demonstrated similar PK parameters [20]. So the mechanism underlying the improved clinical efficacy that was previously observed in the study by Baru et al. remains unknown [19]. These observations clearly require further investigation.

Innovations with other coagulation factors

Factor IX

An innovative expression technology is being explored as a means of enhancing the expression of FIX in stable cell lines [21]. The technology involves flanking the recombinant protein with transcriptional control regions from a highly expressed gene from a Chinese hamster ovary cell utilizing the homologous Chinese hamster elongation factor 1 α (CHEF-1). These CHEF-1 vectors do not require gene amplification, thus accelerating cell-line development. With this technology, the expression levels of FIX are increased 10-fold. The technology is currently being exploited for production of recombinant FIX.

Approaches for extending factor half-life are also being applied to FIX. Some of these approaches include direct, targeted modification with PEGylation and fusion technology, which links the FIX protein to albumin to extend its half-life (see the article by Schulte in this supplement).

Recombinant activated factor VII

Many modifications have been made to recombinant activated factor VII (rFVIIa) in an attempt to improve its functionality. Some of these include glycoPEGylation, targeted PEGylation to specific residues of FVII, formulation with PEGlip, fusion of rFVIIa with albumin, and even the development of a high-potency form of FVIIa that is more efficient in generating factor Xa thereby enhancing thrombin generation. Several of these approaches have already moved to the clinical testing phase.

Alternative haemostatic agents

There are other novel areas of investigation examining alternative therapeutic agents. One interesting area involves the generation of peptides to neutralize inhibitors to FVIII [22]. These peptides are synthetically designed sequences that are screened and tested for their ability to bind to inhibitory antibodies. The promising peptides typically have sequences that are similar to FVIII. However, owing to their short half-life, strategies such as PEGylation may be required to extend their circulation time. Preliminary proof-of-principle data indicate that this approach is possible.

Another interesting class of therapeutics that are being investigated is the non-anticoagulant sulphated polysaccharides (NASP), which are heparin-like molecules that are devoid of

anticoagulant activity [17]. They are believed to act through blockade of a natural inhibitor of clotting, tissue factor pathway inhibitor. NASP can accelerate the clotting times of plasma from haemophilia patients, improve haemostasis when administered subcutaneously to haemophilic mice [17] or to dogs with low FVIII [23], and improve haemostasis when administered orally to severe haemophilia A dogs [23].

A new technology that employs aptamers, which are single-stranded nucleic acids that bind to the protein of interest, may offer the promise of enhancing haemostasis. Aptamers directly inhibit function by folding into a specific three-dimensional structure with high affinity for the target [24]. They can theoretically be generated to bind to any protein target. This technology is currently being explored in clinical trials of anti-cancer and anti-viral therapeutics. Moreover, opportunities also exist for therapeutic aptamers as anti-thrombotic and haemostatic agents.

Conclusions

We have witnessed a growing interest in attempts to develop novel agents and tools as well as advance current factor replacement therapies to optimize the treatment of patients with haemophilia. Although a cure for haemophilia is not yet actualized, there is a tremendous pipeline of future products with promising utility as haemostatic/anti-thrombotic agents, some of which have already progressed from basic research to the clinic (Fig. 2). The future of haemophilia looks bright and it will be exciting to overcome some of the remaining challenges that we have in the treatment of haemophilia.

Haemophilia research 2009

Novel Clotting Factors: The New Frontier		
Basic Studies	Preclinical Development	Clinical Trials
Structure/function of FVIII/IX/VIIa <ul style="list-style-type: none"> • mRNA stability • secretion efficiency • LRP interaction • phospholipid/VWF binding • collagen binding • active site mod • FVIIIa stabilization • inhibitor epitope mapping • peptidomimetics • targeted chemical modification • small molecule inhibitors 	Transgenic livestock Bioengineered FVIII <ul style="list-style-type: none"> • inactivation resistant • improved secretion • porcine/human hybrids • half-life extension <ul style="list-style-type: none"> – direct (FVIII) or indirect (VWF) <ul style="list-style-type: none"> • PEGylation • polysialylation Bioengineered FIX <ul style="list-style-type: none"> • half-life extension <ul style="list-style-type: none"> – albumin fusion – PEGylation Bioengineered rVIIa <ul style="list-style-type: none"> • PEGylation • albumin fusion 	Recomb porc FVIII PEGylated liposomes Novel rVIIa <ul style="list-style-type: none"> • high potency • PEGylated Recomb VWF High-expression FIX FIX:Fc fusion

Fig. 2. Overview of innovative research in haemophilia treatment in 2009. Fc, crystallizable antigen-binding fragment; FIX, factor IX; FVIII, factor VIII; FVIIIa, activated FVIII; FVIIa, activated factor VII; LRP, lipoprotein receptor-related protein; mod, modification; PEGylation, polyethylene glycolation; porc, porcine; Recomb, recombinant; rFVIIa, recombinant FVIIa; VWF, von Willebrand factor.

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