

The future of hemophilia treatment

The future of treatment in hemophilia is one of promises and expectations. It can be approached by tackling the present situations and challenges, as well as addressing current debatable issues. Hopes lie with the prospects of gene transfer and replacement therapy, the latter providing a possibility to improve currently available products and so dramatically change the lives of hemophilia patients.

The present as a prologue

Recombinant factors are efficacious and safe as both prophylactic and therapeutic agents, with little complications or problems associated with their use. However, in countries which have converted completely to recombinant products, measures must be taken to avoid shortages. Improvements have also been made with plasma-derived factors, becoming more efficacious and safer. Conversely, the costs of plasma-derived factors have increased, taking into account the additional tests and processes to increase the safety of these products. This poses difficulties for developing countries, which are already under-resourced. Despite the era of modern technology, only 10% of factor VIII (FVIII) is obtained from 1L of plasma. Attempts to increase this low yield have been made but efforts have not been greatly focused.

Continuous surveillance of treatment safety is mandatory and has proved beneficial. In collaboration with 140 hemophilia treatment centers, the Centers for Disease Control and Prevention carried out a monitoring process of 1,149 seroconversions for hepatitis viruses, identified among patients with bleeding disorders, from May 1998 until June 2002.¹ None of these cases were attributable to blood-borne products, indicating the safety of the processes used in preparing the factors.

Immune tolerance has dramatically changed the treatment paradigm of hemophilic patients who have developed inhibitors. Immune tolerance may effectively eliminate inhibitors in up to 70% of patients.² However, the issues of which dosages are better and which concentrates are more effective still remain unresolved, with variations existing between countries making it difficult to compare practices and to establish conventional procedures. The ongoing Immune Tolerance Induction Study may answer some of these questions.³ This study compares the efficacy, morbidity and cost effectiveness of low versus high-dose immune tolerance in hemophilia A patients.

Possibilities still exist to improve the treatment of patients with inhibitors, through regular prophylaxis,

FVIII inhibitor bypassing activity (Feiba VH®; Baxter AG, Austria) and activated recombinant coagulation factor VII (rFVIIa; NovoSeven®, Novo Nordisk A/S, Bagsvaerd, Denmark). Current ongoing trials show encouraging preliminary data and along with further trials which need to be conducted, good prospects exist to improve the treatment of patients with inhibitors or even to prevent them.

The remaining issues to be tackled include finding explanations for the anaphylactic reactions that develop in some hemophilia B patients. Current theories include the activation of the complement system but no evidence exists to support this. These patients are rare and hence definitive data difficult to obtain. Despite the risk of new blood-borne infections from viruses such as West Nile Virus and SARS, the successful viral inactivation process through which blood products are refined, indicates that this is not an issue.

The publication of the first transfusion-transmitted prion disease raises concern.⁴ However, the fractionation procedures to obtain plasma-derived products have shown high levels of safety and should therefore be able to clear prions that enter the plasma pool.

Gene transfer

More than a decade ago, it was predicted that gene transfer therapy would play a large role in hemophilia therapy, even making hemophilia the first genetic disease to be cured in the millennium. On the basis of animal studies, promising data were obtained, however results proved better and more efficient compared with data from human studies, with respect to efficacy of transfer and duration of gene expression.

Gene transfer trials in hemophilia A and B patients (Table 1) have focused on various methods of gene transfer with a range of vectors. Despite the different approaches, most of these trials have either been terminated or are on hold due to preliminary unsuccessful results. The initial results were encouraging with respect to measurable levels of FVIII and factor IX (FIX) in the circulation. However, expression levels of the transgene were only transient and not sustained, and not sufficient to correct the phenotype to provide considerable benefit. The aim of gene transfer would be to transform severe hemophiliacs to moderate-mild disease status, requiring the occasional administration of recombinant products when needed. This is far from being achieved.

Reasons for current problems include poor and transient expression of the transgene and the need for multiple dose transfers. Repeated dosing is required to sustain levels of the transgene and may not be acceptable to the patient – single dose transfer is still a remote target. However, repeated gene transfer may

Table 1. Gene transfer trials in hemophilia initiated from 1998 onwards.

<i>Trial</i>	<i>Vector</i>	<i>Method of gene transfer</i>	<i>Status to date</i>
Transkaryotic	Plasmid DNA	<i>Ex vivo</i> , autologous fibroblasts	On hold
Avigen 1	Adeno-associated virus	<i>In vivo</i> , intramuscular	Terminated
Chiron	Replication-deficient retrovirus	<i>In vivo</i> , intravenous	Terminated
Avigen 2	Adeno-associated, liver driven virus	<i>In vivo</i> , hepatic artery infusion	Terminated
Genstar	Gutted, liver-driven adenovirus	<i>In vivo</i> , intravenous	Terminated

not be an option due to safety issues. Two cases of T-cell leukemia were observed after insertional mutagenesis in two children with severe combined immunodeficiencies.⁵ This occurred during a trial of gene therapy based upon a Maloney retroviral vector. This has not been seen in hemophilia patients but raises concern over retroviral vectors. According to molecular biologists, this problem can be circumvented.

Transient positive vector DNA was found in semen samples of hemophilia A and B patients in the Chiron and Avigen trials.^{6,7} Germinal cells were not transfected, but these findings raise concerns over whether mature sperm are affected and the possibility of a sustained integration of vector DNA.

Other concerns have been raised with side-effects related to the host-immune reaction to the vector, especially adenoviruses. Abnormalities of transaminases, thrombocytopenia and inflammatory symptoms were observed in a hemophilia A patient in the Genstar trial, although these were reversible.⁸ The high vector dose in the Avigen 2 trial may have resulted in the elevated transaminase levels in a patient with haemophilia B.⁹ At present, hemophilia is a disease with good available treatment options. Therefore, strong considerations should be made before the use of gene transfer in patients with hemophilia, especially with respect to the potential associated risks.

The future of gene transfer therapy may yet show improvement. Progresses in plasmidology and vectorology have shown promising results. However, for greater advances to be made, a more enhanced understanding of tissue and vector-specific adaptive and innate immune responses are required, with intervention to modulate such responses if needed.

The future of replacement therapy

This provides the most promising alternative to gene therapy and may be achieved by improving recombinant products through the engineering of coagulation factors and the use of transgenic bioreactors. Current restrictions especially for recombinant FVIII include limited expression of this protein in mammalian cells, and the high cost associated with their production,

purification and formulation. A high-efficiency production or expression of the recombinant factors would not only increase product availability, but also hopefully reduce the cost of the factor. Oral delivery of recombinant factors is a possibility and would be more acceptable to the patient, however, a large bulk of material would be required which may not be realistic.

The theoretical possibilities for the future of recombinant factors include increasing their expression, intracellular transport and secretion. Although increased expression has been achieved, this does not translate to an increase in secretion. Increase in activation of recombinant factors and reducing inactivation by physiological mechanisms, such as activated protein C and thrombin, provide promising targets.¹⁰ Prolongation of FVIII and FIX half-lives could result in longer intervals between prophylactic treatments, and once again questions the need for gene transfer therapy. A product with decreasing immunogenicity and antigenicity is highly desirable. Molecular engineering can attempt to achieve all these goals.

PEGylation of molecules may improve the half-life of factors. Prolonged half-life may also be achieved by disrupting the receptors involved in the clearance of FVIII and FIX by genetic alteration/mutations of the proteins. Improved mutants may be more useful in gene transfer, especially those which interact with the two main clearance receptors, lipoprotein receptor-related protein, and heparan sulfate proteoglycan.^{11,12} However, mutant molecules may be more immunogenic and surveillance is strongly recommended.

The use of bioreactors to produce recombinant factors may play a promising future role in the treatment of hemophilia. Transgenic pigs are able to produce milk containing FVIII and FIX, therefore providing an additional source of coagulation factors besides human donors.^{13,14} The milk produced, as for human plasma, requires fractionation. Few data have been published on transgenic pigs, however, early reports show great potential of these bioreactors. Factor IX protein can be produced in high concentrations (100–1,000 U/mL) with specific activity (250–350 U/mg) and with good yields (> 75%).¹⁵ More importantly, FIX is

produced with proper post-translational modifications that are required for suitable recovery and clearance of the protein.

The advantages of pig bioreactors include a similar biochemistry to humans, with the protein produced being comparable to that produced by humans. Support is provided by a history of FDA approved products from transgenic bioreactors, with safety substantiated by the pathophysiology of the pigs which are resistant to bovine encephalopathies. Each pig can produce up to 3 liters of milk per day for 100 days a year, hence providing excellent yields. The productivity of the pigs themselves is also fairly substantial, with one pig having the ability to produce 21 offspring per year. Subsequently, it is easy to scale-up with minimal capital investment.

Preliminary data of the recombinant milk FIX show efficacy even when administered orally. Animal studies demonstrated that a single feed of FIX-containing pig milk was able to normalize the clotting time in haemophilia B mice for up to two days.¹⁶ Additionally, administration of the protein induced immune tolerance; despite infusion with a heterologous factor, inhibitor development was not seen. These animal studies demonstrate the successful oral administration of recombinant factors and hence provide great potential for future treatment of hemophilia.

Conclusions

Despite the advances in haemophilia treatment and the future potentials on the horizon, 80% of hemophiliacs around the world do not receive any treatment at all. The World Federation of Haemophilia is attempting to tackle this problem, but face an enormous and complex challenge. Hope exists to improve the future of hemophilia treatment by improving the safety and efficacy of current recombinant factors, with focus on immune tolerance therapy to treat and prevent the development of inhibitors. Gene transfer therapy still provides the greatest potential for sustained effect and cure. However, safety and efficacy of the process still requires extensive research. Generation of further recombinant products through transgenic bioreactors have progressed farthest in the treatment of hemophilia, providing a new source of recombinant factors which may hopefully extend to benefit the lives of all haemophilia patients.

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