<u>Review Article</u>

Advanced Therapies for the Treatment of Hemophilia: An End of 'Royal Pathology' in the Third Millennium

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Abstract: Hemophilia is an ideal condition for gene therapy because of its monogenetic character and the fact that it requires only a small amount of the expressed protein to achieve palliation. It is not infrequently diagnosed in the absence of a family history and its initial presentation can be bleeding in the neonatal period. In a neonate with clinically significant ongoing haemorrhage, where Hemophilia is suspected based on a prolonged APTT, it may be appropriate to administer fresh frozen plasma (FFP) while the results of appropriate factor assays are awaited. There is an advanced therapies may involve the replacement of a deficient gene by a healthy gene so that it generates a certain functional, structural or transport protein (gene therapy); the incorporation of a full array of healthy genes and proteins through perfusion or transplantation of healthy cells (cell therapy); or tissue transplantation and formation of healthy organs (tissue engineering). For their part, induced pluripotent stem cells have recently been shown to also play a significant role in the fields of cell therapy and tissue engineering. In the third millennium, Hemophilia treatment should encompass more ambitious goals through gene replacement, to result in permanent and safe Hemophilia 'eradication', making Hemophilia a part of the history of medicine. Evidence-based guidelines are also presented for the management of Hemophilia in the foetus and neonate.

Keywords: Hemophilia, bleeding disorders, advanced therapies, Gene therapy, Cell therapy, Hemophilia A, Hemophilia B.

INTRODUCTION

"There is much reason for optimism, but caution is imperative in order not to raise false expectations in our patients."

Hemophilia is a recessive X-linked hereditary disorder caused by a deficiency of coagulation factor VIII (Hemophilia A) or IX (Hemophilia B). The disease is considered to be severe when factor levels are below 1% of normal values, moderate when they are between 1 and 5% and mild when levels range between 5% and 40% [1]. As the prevalence of the disease is 7.7/100,000, it is considered a rare hematologic disorder. Although Hemophilia is particularly amenable to treatment with these techniques given its monogenic nature and the lower levels of deficient coagulation factor required to achieve a moderate phenotype, research in the Hemophilia field is still at a teething stage and further work must be undertaken to determine whether advanced therapies can be safely applied to this patient population which present specific clinical characteristics [2].

Some gene therapy research trials have been performed in humans with mixed results. The future for gene therapy in Hemophilia is continuing albeit at a moderate pace. There are many projects continuing in animal models. Improved long-term expression of the new genes will require the development of better vectors (the means of delivering the new genes into the cells). Call NHF's toll free number (1-800-42-HANDI) for more information about gene therapy for Hemophilia. Several new technologies are also being implemented to advance Hemophilia treatment. These new technologies, once used to destroy viruses in blood, have been successful in virtually eliminating the risk of contracting HIV or hepatitis C from clotting factor today. Pharmaceutical companies are continuing to investigate genetically manufactured product alternatives derived from little to no human blood products. New products have consistently been developed which have an even higher purity than have ever been available before.

Etiopathogenesis of Hemophilia [3]

The etiopathogenesis of the disease is related to different kinds of mutations (large deletions and insertions, inversions and point mutations) that occur in the gene expressing the deficient coagulation factor. The clinical characteristics of both types of Hemophilia are very similar: spontaneous or traumatic hemorrhages, muscle hematomas, hemophilic arthropathy resulting from the articular damage caused by repetitive bleeding episodes in the target joints, or hemorrhages in the

central nervous system (Table 1). In the absence of appropriate replacement treatment with exogenous coagulation factors, these manifestations of the disease can have disabling or even fatal consequences thus negatively impacting patients' quality of life and reducing their life expectancy. Bleeding episodes may be spontaneous in the severe and (less so) in the moderate forms of the disease, with 70% of them being articular, 15% muscular and 15% visceral.

Goal

The chief goal of these new strategies will be to address some of the limitations associated with current treatment options such as the short in vivo half-life of administered factors, the risk of a pathogen-induced infection and the development of inhibitors. Another goal of the advanced therapies (cell therapies) will be palliative treatment of the articular consequences derived from haemophilic arthropathy [4].

Diagnosis of Hemophilia

Diagnosis of Hemophilia is aimed at identifying type of Hemophilia and degree of involvement as well as detecting symptomatic and asymptomatic carriers of the disease, either obligate (daughter of Hemophiliacs) or de novo (women by sporadic and spontaneous mutations).Diagnostic methods are based on determination of coagulation factor levels and the detection of the mutation in the DNA extracted from peripheral blood leucocytes by mean of direct or indirect genetic methods (detection of genetic polymorphism).

At present, patients with Hemophilia benefit from optimized treatment schedules based on the intravenous systemic delivery of exogenous coagulation factors, either prophylactically or on demand protocols. The current policy in developed countries is in general to administer a prophylactic treatment (2 or 3 times a week) from early childhood into adulthood18. Such prophylactic protocols result in a marked improvement in patients' quality of life on account of the prevention of haemophilic arthropathy and other fatal manifestations of the disease as well as a reduction in the long-term costs of treatment because of a decrease in the need of surgical procedures such as arthrodesis, arthroplasty or synovectomy [4].

Gene therapy strategies

Gene therapy in turn, consists of transplantation of genetically modified cells so that they may produce a deficient protein. Cells are useful in these therapies because of their ability to differentiate in to specific cell lines required for restoring a given type of tissue. The most significant breakthroughs in the field of advanced therapies and Hemophilia are chiefly related to both preclinical and clinical trials in the fields of gene therapy (through the use of viral and non-viral vectors) and cell therapy (using several types of target cell) The studies published so far have, in the most part, not reported any adverse event resulting from the application of such strategies in the clinical trials performed in terms of an immune-mediated trans gene rejection (factor VIII or IX expression) although factors such as innate cellular T cell toxicity to adeno associated capsid protein and the low efficacy obtained by non-viral vectors are impeding and limiting their success [5].

Cell therapy strategies

The use of cell therapy in the treatment of Hemophilia has to date consisted mainly in the transplantation of healthy cells in an attempt to repair or replace a coagulation factor deficiency. These procedures have been conducted mainly with adult stem cells and, more recently, with progenitor cells partially differentiated from iPSCs, albeit in most cases the mechanisms by which transplanted cells (to a greater or lesser extent) engraft and go on to proliferate and function remain unknown. Aronovich *et al.* [6] have shown that transplantation of embryonic day 42 spleen tissue in immunocompetent mice with Hemophilia A attenuates the severity of the disease in the 2–3 months after the procedure. These results would seem to indicate that transplantation of a fetal spleen —obtained from a developmental stage prior to the appearance of T-cells— may potentially be used to treat some genetic disorders. For their part, Follenzi *et al.*[7] reported that once liver sinusoidal endothelial cells were transplanted and successfully engrafted into mice with Hemophilia A, they were seen to proliferate and partially replace some areas of the hepatic endothelium. This resulted in a restoration of factor VIII plasma levels and in the correction of the bleeding phenotype.

Non viral gene transfer and chimerical oligonucleotide-based mutation repair have also been used. Cell therapy for Hemophilia is based, mainly, on the transplantation of healthy cells to repair or replace deficient functions, for example the transplantation of liver sinusoidal endothelial cells or endothelial progenitor cells derived from induced pluripotent stem cells. Of particular interest in the field of advanced therapies are the results obtained by High *et al.* who used zinc finger nucleases and adeno-associated vectors to correct Hemophilia B mutations through the 'editing' of DNA-mutated sequences [8]. Although in this case factor IX (FIX) expression is only 5% of normal levels, the advantage of this strategy is that it allows strict control of the integration of normal sequences into DNA, thus preventing the development of insertional mutagenesis-induced tumors.

Nathwani *et al.* have recently completed a clinical trial in patients with Hemophilia B. The trial included patients with severe Hemophilia B (<1% FIX) who were injected with aserotype- 8-pseudotyped, self-complementary AAV, that expresses FIX and can efficiently transduce hepatocytes. This is a more efficient vector as it obviates the need for second-strand

synthesis or re-annealing of positive and negative AAV strands to generate transcription-competent dsDNA templates. The results showed that patients expressed between 3 and 11% of normal FIX levels. Another encouraging finding was that no inhibitors (anti- FIX antibodies) were detected. These results must be considered taking into account, first of all, that the expression of FIX corresponds to a mild-to-moderate phenotype of the disease and, second, that concomitant glucocorticoid treatment is required in order to prevent immune rejection and an elevation of liver transaminases [9].

Advanced therapies in the hemophilic arthropathy

Lastly, it is important to consider the potential application of advanced therapies in the palliative treatment of the articular consequences of haemophilic arthropathy. Although adequate treatment is currently available for Hemophilia, which is specifically efficient regarding the negative consequences of haemophilic arthropathy, it cannot be forgotten that only 25% of Hemophiliacs, most of them living in developed countries, can benefit from such treatment. In the rest of the world, haemophilic arthropathy and its disabling sequelae are the norm. But even in the developed world many patients still present moderate or severe haemophilic arthropathy on account of the fact that they either developed inhibitors or started being treated a few decades ago when present day therapies were still unavailable. Against this background, advanced therapies may constitute a solution of these patients [4]. Chondrocyte implantation and cell therapy using bioreactors, growth factors, mesenchymal stem cells and genetically modified cells may be used as an adjunct or even as an alternative to the current approaches (bone marrow stimulation, osteochondral autograft or allograft transplantation) for the repair of chondral damage in advanced arthropathic disease. Mesenchymal stem cells appear hold great promise for chondral repair given their high differentiation ability and their proven therapeutic effects.[10] Implantation of autologous chondrocytes or mesenchymal stem cells was up to now able to address only highly localized chondral lesions, and the use of bioreactors and growth factors, which stimulate cartilage formation, may optimize such strategies.

Patients with congenital Hemophilia should be treated with recombinant products, particularly. They are as follows:

Recombinant concentrates (Tables 1)

In order to manufacture recombinant coagulation factors, the gene (or a modified gene) is inserted into a cell line. The cells are cultured and the secreted factor is purified from the culture medium. The factor must be stable throughout the production process and in the final formulation for lyophilisation. Concern has been expressed over the use of human and animal products in the culture media, and over the use of human albumin

as a stabilizer. If mouse monoclonal antibodies are used in the purification process, trace amounts may appear in the final product. There is the possibility of viral infection of the cell lines used to produce the coagulation factor and any monoclonal antibodies used so even if all animal and human proteins can be removed from the production process, a viral inactivation/removal step will enhance safety.

First generation recombinant coagulation factor concentrates have human albumin added to the final formulation as a stabilizer. The only such product now available, Recombinate (Baxter) is not distributed in the UK. Second generation recombinant coagulation factor concentrates stabilized without the addition of human albumin are Kogenate Bayer (Bayer) [also labelled as Helixate Nexgen (CSL Behring)] and ReFacto (Wyeth); both have human albumin in the cell culture medium. Recombinant VIIa (NovoSeven; NovoNordisk) does not contain any stabilizing protein, although bovine serum is used in the cell culture medium. Third generation recombinant coagulation factor concentrates in which animal products are not used in the culture media are Advate (Baxter) and Benefix (Baxter). Refacto (Wyeth) lacks the B-domain which leads to a discrepancy between values obtained with onestage and chromogenic FVIII assays [11]. The one stage assay is usually performed using a plasma standard. If the standard used is Refacto any difference are be abolished [11]. The manufacturers have supplied such a standard for use; however, in their latest Summary of Product Characteristics (SPC), the manufacturer strongly recommends that the chromogenic substrate assay be used to assay post infusion samples.

Fractionated plasma concentrates

All currently available plasma-derived clotting factor concentrates have an excellent safety record. Transfusion transmitted infection was a major problem with these products prior to the introduction of viral inactivation steps during their manufacture in the mid-1980s [10]. Even after this time infections still occurred rarely, because of ineffective viral inactivation processes but no such problem has been encountered with products that undergo dual viral inactivation as is currently used [9]. The current reduction in infective risk of fractionated plasma products has been achieved though a multi-step process that includes donor selection, screening and testing, testing of plasma pools to minimize contamination as well as fractionation and dual viral inactivation.

Non-fractionated blood products

Fresh frozen plasma (pooled virally inactivated, single donor virally inactivated and single donor non-virally inactivated) and cryoprecipitate (virally inactivated and non-virally inactivated) are available in the UK. The methylene blue treatment of cryoprecipitate results in a 40% loss of fibrinogen activity as measured with the Clauss assay (Lorna

Williamson, personal communication). Because the concentration of the relevant clotting factors in non fractionated plasma products is low and the viral inactivation (if performed) inferior, these products must be considered as second choice and only used if concentrates which have undergone two viral inactivation steps are not available.

Table 1: Summary of recombinant products

Products	Cell line	Gene	Protein in culture medium	Murine mAbs	Human albumin as stabilizer	Viral inactivation removal	Generation
Recombinate	CHO	VIII VWF	Bovine albumin, insulin, aprotinin	Yes	Yes	No	1
Helixate NexGen Kogenate-Bayer	BHK	VIII	Human albumin	Yes	No	SD and high salt concentration hold step	2
Refacto	CHO	B domain deleted VIII	Human albumin, insulin, aprotinin	Yes	No	SD	2
Advate	CHO	VIII VWF	No	Yes	No	SD	3
NovoSeven	BHK	VII	Foetal calf serum (Bovine)	Yes	No	SD	2
Benefix	CHO	IX	No	No	No	NF	3

BHK, baby hamster kidney; CHO, Chinese hamster ovary; SD, solvent detergent; NF, nanofiltration; mAbs, monoclonal antibodies.

Other therapeutic agents

Desmopressin, a synthetic analogue of the non-apeptide arginine vasopressin, remains the drug of choice in the prophylaxis and treatment of haemorrhagic episodes in patients with mild Hemophilia A or VWD (predominantly type 1) and symptomatic carriers of Hemophilia A. Typically, FVIII and VWF levels will increase 3–5 times above the basal levels. This drug has no clinically useful effects on levels of Factors V, X or XI [11] but can improve primary haemostasis and has proven useful in some patients with mild platelet function defects [10]. Desmopressin may be administered as a slow intravenous infusion (using a 4 lg mL) 1 preparation diluted in saline), or as a sub-cutaneous injection (using a 15 lg mL) 1 preparation). An intranasal spray preparation is also available, although some patients have difficulty mastering the administration technique. This is particularly suitable for home treatment and has been advocated for bleeding disorder patients with menorrhagia.

However, a randomized placebo-controlled crossover trial in this setting showed only a non-significant trend to reduced blood loss [11].

Tranexamic acid

Tranexamic acid is a lysine analogue which can reversibly bind both circulating and fibrinbound plasminogen and thus inhibit fibrinolysis. Such agents have proven efficacy in reducing blood loss, without increasing thrombosis risk, in cardiac and orthopaedic surgery in normal individuals. In patients with inherited bleeding disorders tranexamic acid is especially helpful in reducing mucosal bleeding where fibrinolytic activity is particularly active. It can be administered orally, intravenousl or topically (as a mouth wash). It is often given as an adjunct to desmopressin in treatment of oral bleeding, menorrhagia or prophylaxis for dental surgery [12-15].

General recommendations

Patient information and consent Good practice dictates that the necessity for treatment is appropriately explained to the patient and/or parent. This should include the advantages and risks of different therapies to allow an informed decision to be made. When consent has been obtained this should be recorded in the case notes.

Specific recommendations

Patients with congenital Hemophilia should be treated with recombinant products, particularly, if they have never been exposed to plasma products (Level IV, Grade C). Hemophilia A Recombinant FVIII (rFVIII) is the treatment of choice (Level IV, Grade C). Hemophilia B Recombinant FIX is the treatment of choice (Level IV, Grade C). If unavailable, the alternative is a high purity plasma-derived FIX (pFIX) concentrate as these cause less haemostatic activation than prothrombin complex concentrates [16, 17] (Level Ib, Grade A) which should be avoided because of the increased risk of thrombosis. Coagulation concentrates for treating acute bleeds in patients with inhibitors. The products, in addition to FVIII and FIX concentrates, recommended for the treatment of acute bleeds are FVIIa (NovoSeven) and FEIBA. Recommendations on their use have been made in a recent UKHCDO guideline [18].

Choosing a therapeutic product

The key issues in selecting a product are its efficacy and safety. Other features such as cost, volume and ease of reconstitution, storage conditions, shelf-life, the possibility of use by continuous infusion and the security of supply should also be considered.

Efficacy

The latest draft of guideline from the Europrean Medicines Agency (EMEA) suggests that clinical efficacy of factor VIII (FVIII) should be evaluated in at least 50 previously treated patients.

Safety

When selecting a plasma-derived or recombinant concentrate, the two most important safety issues are transmission of infectious agents and inhibitor formation. Clinical trials of new products are usually undertaken on relatively few patients and are therefore not large enough to assess the incidence of rare complications. The EMEA draft (see above) recommends at least 50 frequently treated patients should be followed and documented for a minimum of 50 exposure days, but a trial involving 3000 patients is required to rule out one in 1000 risk with 95% certainty. Safety from rare complications is usually assessed from vigilant follow-up of treated patients in formal post-marketing surveillance studies.

Vaccination against hepatitis A and B

Hepatitis A and B vaccination is highly effective in preventing infection (Level IIa, Grade B). All patients who currently receive, or may require, blood products should be vaccinated. Carers who are preparing and/or injecting blood products should also be offered vaccination. Hepatitis A vaccine is not licensed for those less than one year of age. In patients, the vaccines should be given subcutaneously, not intramuscularly, to reduce the risk of haematoma at the injection site.

Avoidance of exposure to concentrates, blood products and animal proteins

Mild Hemophilia A and von Willebrand disease (VWD) should be treated with desmopressin (and tranexamic acid) in preference to coagulation factor concentrates whenever possible (Level IIb, Grade B).

In the future, the different types of advanced therapies such as gene therapy, cell therapy and tissue engineering as well as the more recently developed induced puloripotent stem cells technology, may offer innumerable clinical application for the treatment of certain monogenic diseases including Hemophilia. There were different prophylactic regimens for the management of severe Hemophilia are described along with the use of adjuvant treatment options to achieve haemostasis. The development of biotechnology has resulted in the emergence of new therapies are bound to change medical practice. Advanced Gene, cell and tissue based therapies constitute new strategy for the treatment of some diseases.[4] Cell therapy consists of transplantation of living cells in to an organism in order to repair tissue and restore an absent or deficient protein.

Licensed products

Licensed products used within their product licence should be preferred to unlicensed products or products used outside their product licence unless there are clear advantages to an alternative treatment (Level IV, Grade C). Unlicensed products should be used, if possible, under formal clinical trial rather than on a named patient basis.

"There is much reason for optimism, but we need to act cautiously so as not to create premature expectations in our patients."

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