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REVIEW

Haemophilia gene therapy: Progress and challenges

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ABSTRACT

Current treatment for haemophilia entails life-long intravenous infusion of clotting factor concentrates. This is highly effective at controlling and preventing haemorrhage and its associated complications. Clotting factor replacement therapy is, however, demanding, exceedingly expensive and not curative. In contrast, gene therapy for haemophilia offers the potential of a cure as a result of continuous endogenous expression of biologically active factor VIII (FVIII) or factor IX (FIX) proteins following stable transfer of a normal copy of the respective gene. Our group has recently established the first clear proof-of-concept for a gene therapy approach to the treatment of severe haemophilia B. This entails a single intravenous administration of an adeno-associated virus vector encoding an optimised FIX gene, resulting in a long-term (>4 years) dose dependent increase in plasma FIX levels at therapeutic levels without persistent or late toxicity. Gene therapy therefore has the potential to change the treatment paradigm for haemophilia but several hurdles need to be overcome before this can happen. This review provides a summary of the progress made to date and discusses the remaining changes.

1. Historical perspective

Haemophilia A and B are X-linked recessive disorders resulting from mutations in the gene for blood clotting factor VIII (FVIII) or IX (FIX) respectively. The incidence of haemophilia A is approximately 1 in 10,000, and that of haemophilia B is 1 in 50,000 live male births. Collectively they are amongst the most common inherited bleeding disorders in the World. Despite the genetic and biochemical differences, these disorders are indistinguishable clinically with the severity of bleeding symptoms varying according to the residual factor activity in a patient's plasma. Patients with a mild bleeding phenotype have baseline plasma factor levels in excess of 5% of normal and typically have few spontaneous bleeding episodes, but they may have prolonged and even life-threatening bleeding after trauma or surgery. Over half of the patients with haemophilia A or B have factor levels of <1% of normal [1]. These individuals have a severe bleeding phenotype consisting of frequent spontaneous musculoskeletal and soft tissue haemorrhages in the absence of treatment. The ankles are most commonly affected in children, and the knees, elbows, and ankles in adolescents and adults. Chronic intra-articular bleeding leads to painful haemophilic arthropathy and eventual loss of joint function associated with chronic pain and disability.

Until the mid-1960s fresh frozen plasma (FFP) was the mainstay of treatment for the haemophilias. As plasma contains very small quantities of factor VIII and factor IX, large volume FFP transfusions were required to stop bleeding episodes, thus patients were frequently hospitalised for treatment and ran the risk of congestive cardiac failure from fluid overload. The ability to prepare cryoprecipitated plasma from FFP, which is rich in FVIII, in the mid-1960s was an important step change in the management of haemophilia A. Relatively smaller volume of cryoprecipitate were required to manage bleeding episodes, enabling outpatient based treatment for most patients with haemophilia A. Cryoprecipitate is still the main treatment in many parts of the developing world.

The next transformative treatment change occurred in the 1970s and 1980s as a consequence of the ability to fractionate both FVIII and FIX from pooled plasma. The resulting clotting factor concentrates allowed more consistent dosing of patients, thus facilitating challenging surgical procedures and home treatment of bleeding episodes. However, a great catastrophe beaconed as these clotting factor concentrates were made by combining thousands of plasma donations. This allowed rapid transmission of human immunodeficiency virus and hepatitis B and C to a large proportion of haemophilia patients in the 1980s. Since then the safety of plasma derived factor concentrates has been greatly improved by viral screening and inactivation measures. These changes to plasma processing have effectively eradicated the risk of hepatitis B, C and human immunodeficiency virus but not all blood borne pathogens as exemplified by the possible transmission of variant Creutzfeldt–Jakob [2–4]. The risk of blood borne pathogens has been

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mitigated by successful cloning of the FVIII gene in 1984, which galvanised the production of recombinant human FVIII and the subsequent licensure in 1992. The FIX gene was cloned in 1982, and a licenced recombinant FIX product became available in 1997.

2. Current standard of care for haemophilia

Recombinant clotting factor concentrates have revolutionised haemophilia care in the Western World where their prophylactic administration to maintain circulating factor levels above 1% of normal has resulted in a dramatic reduction in bleeding frequency and its associated complications such as arthropathy. The life expectation of patients with severe haemophilia has been normalised to that of age matched healthy males in their community. Moreover, there have been substantial improvements in the quality of life. However, considerable planning of daily activities that would be taken for granted by most people living without haemophilia is still required, particularly in children and adolescents. Additionally, the relatively short half-life of recombinant factor necessitates frequent intravenous administration of factor concentrates (at least 2–3 times a week) associated with peaks and troughs of circulating factor levels and occasionally breakthrough bleeding when levels drop below 1%. Prophylaxis, in addition to being demanding and invasive, is highly expensive with annualised costs being in access of £100,000/patient. The cost is a magnitude higher for those who develop inhibitory antibodies, which occurs in almost 40% of severe haemophilia A patients but only 3% of haemophilia B patients.

Pegylation of clotting factor proteins or fusion of FVIII or FIX proteins with albumin or Fc region of immunoglobulin A has recently generated a family of clotting factor with an extended half-life. Amongst these the new FIX preparations show the greatest promise as they raise the possibility of once every two weeks prophylaxis as opposed to the twice/week regimen in use currently with standard FIX preparations. However, these extended half-life products do not remove the problems of lifelong intravenous administration, breakthrough bleeding, mounting cost and induction of inhibitory antibodies against protein concentrates. In addition, the consequences of lifelong administration of pegylated proteins are unknown as is the immunogenic potential of using fusion proteins [5].

3. Rationale for gene therapy for haemophilia

Gene therapy offers the potential of a cure through persistent, endogenous production of either FVIII or FIX following the transfer of a normal copy of the cognate gene. The haemophilias are excellent candidates for gene therapy because their clinical manifestations are attributable to the lack of a single protein that circulates in minute amounts in the plasma. Additionally, years of clinical experience indicates that an increase of 1–2% in circulating levels of the deficient clotting factor can significantly modify the bleeding diathesis, therefore, the therapeutic goal for gene therapy is modest. Tight regulation of transgene expression is not necessary since a wide range of FIX or FVIII is expected to be beneficial and nontoxic. The availability of animal models including FVIII and FIX-knockout mice [6,7] and haemophilia A and B dogs [8,9], has facilitated extensive preclinical evaluation of gene therapy strategies. The therapeutic end point can be readily assessed in most coagulation laboratories by measuring plasma levels of FVIII or FIX. The gene encoding FIX is relatively small and lends itself well for most gene transfer strategies. In addition, its expression pathway is significantly less complex than that of the FVIII gene. Consequently, more gene transfer studies have focused on haemophilia B instead of the more common haemophilia A.

4. Vehicles for gene transfer

The success of gene therapy critically depends on effective vehicles for gene transfer, known as vectors, which introduce the therapeutic genes into target somatic cells, a process referred to as transduction. A number of gene transfer vehicles have been developed that can broadly be divided into two categories: non-viral and viral vectors. The properties of the commonly used vectors are described in Table 1. Non-viral vectors may consist of naked DNA or plasmids as well as RNA. These vectors have several advantages but are very inefficient at mediating gene transfer and maintaining stable expression of the transgenic protein. In contrast, viruses have evolved mechanism for efficient delivery of their payload and are therefore more efficient at the transfer of gene when compared to non-viral vectors. For the purposes of gene therapy virus-based vectors harness the viral infection pathway but are devoid of viral genes that are required for replication within and death of target cells. Viral vectors can be further divided into integrating and non-integrating vectors. Integrating vectors such as onco-retroviral and lentiviral vectors enhance integration of the transgene into the host chromosome, thus ensuring stable propagation of the transgene to the daughter cells. In contrast, non-integrating viral vectors mediate transgene expression from episomally retained transgene that remains outside the host chromosomal DNA. This reduces the risk of insertional oncogenesis but makes these vectors unsuitable for gene transfer into rapidly dividing cells such as haematopoietic stem cells (HSC). This is because transgene expression is the episomally maintained proviral DNA that is lost with each cell division resulting in only transient expression of the transgenic protein following gene transfer. Non-integrating viral vectors, however, are very useful for post-mitotic tissues such as the liver, neurons or the muscle as they can be stably maintained for much longer periods of time [10]. The properties of the commonly used vectors are described in Table 1.

5. Previous haemophilia gene therapy trials

Ten phase I clinical trials have been conducted in subjects with haemophilia using a variety of different approaches (Table 2). The first study used DNA encoding a B-domain-deleted factor VIII gene (BDD-hFVIII) which was introduced into autologous fibroblasts ex-vivo by electroporation (application of an electric shock to permeabilise the cellular plasma membrane, see Fig. 1) prior to their implantation into the omentum of subjects with severe haemophilia A. This procedure was well tolerated with no major side effects [11,12]. However, expression of FVIII was transient because of silencing of the transgene through DNA methylation. In another study, onco-retroviral vectors were used to introduce a normal copy of the FIX gene into ex-vivo expanded autologous fibroblasts prior to their implantation into skin of patients with severe haemophilia B. No toxicities were reported but FIX transgene expression was detectable for only a few days [13]. Subsequently, a phase I dose-escalation study, conducted by Chiron Corp, involving in-vivo, systemic, administration of onco-retroviral vectors encoding the BDD-hFVIII gene showed that vector doses as high as 9×10^8 transduction units/kg were well tolerated with no adverse events in subjects with haemophilia A [14]. Vector sequences were detectable in peripheral blood mononuclear cells for as long as 1-year post-treatment, providing evidence of in-vivo gene transfer. An increase in plasma FVIII activity of ~1% of normal was transiently detected in 6 of 13 subjects. Enthusiasm for these onco-retroviral vectors was tempered by the occurrence of insertional oncogenesis in children with SCID-XI following transplantation of gene modified CD34+ HSC [15–18]. Systemic administration of new type of adenovirus vectors, called helper-dependent adenoviral vectors or "gutless" adenoviral vectors (almost completely stripped of wild type adenoviral genes), encoding full length FVIII gene resulted in a transient increase in FVIII activity to ~3%. However, elevation of liver enzymes associated with thrombocytopenia was observed resulting in the study being closed early [19].

Recombinant adeno-associated viral vectors (AAV) have the best safety profile among gene transfer vectors of viral origin, as wild-type AAV has never been associated with human disease. Safety is further enhanced by the dependence of AAV on co-infection of the target cell with