Hemophilia clinical gene therapy: brief review

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Genetic correction of hemophilia A and B was long considered amenable to the available gene transfer technologies. This assumption has come to fruition with the recent results of a phase I/II trial for hemophilia B. Here we review the clinical application of gene therapy for the hemophilia's as a paradigm of the evolution of gene transfer science and technology. This review is not intended as comprehensive but rather to highlight current clinical developments of gene therapy for the hemophilias. (Translational Research 2013;161:307–312)

Abbreviations: AAV = adeno-associated virus; BP = branch point; FIX = factor IX; hFIX = human factor IX; PP = polypyrimidine tract; PTM = pre-trans-splicing molecule

Successful gene therapy will likely revolutionize the treatment for several congenital bleeding disorders. Hemophilia A (factor VIII [FVIII] deficiency) and hemophilia B (factor IX [FIX] deficiency) are the most commonly inherited bleeding disorders.[1] The United States alone has ~20,000 patients with hemophilia of which 80% are FVIII deficient. Alteration of the genetic mechanisms responsible for inadequate FVIII/IX protein production is the key to successful, long-term treatment of these bleeding disorders. To achieve this, the requirements for successful gene transfer include persistent expression of therapeutic levels of FVIII or FIX, lack of significant toxicity to the gene transfer vehicle (vector), and lack of a host immune response to the normal factor VIII/IX (ie, the development of inhibitors and/or the vector). Gene therapy clinical trials for both FIX and FVIII are the subject of this review[2]; here we review recently completed and newly opened clinical trials.

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Conflict of interest: None.

Submitted for publication October 25, 2012; revision submitted December 13, 2012; accepted for publication December 28, 2012.

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1931-5244/$ - see front matter

http://dx.doi.org/10.1016/j.trsl.2012.12.016

HEMOPHILIA: BASIC BIOLOGY

Factor VIII/IX deficiencies are sex-linked recessive disorders manifesting as frequent, spontaneous intra-articular joint and soft tissue bleeding episodes.[1] The severity of the disease is directly related to the amount of circulating functional plasma levels of FVIII or FIX. Patients with less than 1% of normal FVIII/FIX activity levels are phenotypically severe with frequent bleeding episodes (weekly) requiring treatment with either plasma-derived or recombinant factor products. Patients with mild disease maintain >5% up to 30% of normal factor levels and typically have few spontaneous bleeding episodes; they are, however, still at risk for trauma-induced bleeding. The majority of patients with hemophilia A or B have <1% of factor and will have frequent spontaneous bleeds unless they are receiving treatment. Chronic intra-articular bleeding leads to arthropathy and eventual loss of joint function. Treatment with plasma-purified or recombinant protein at the time of bleeding is the standard of care. However, clinical studies clearly demonstrate prophylactic factor infusion regimens have a dramatic effect on reducing the rate and severity of joint bleeding[3,4]; yet, prophylactic treatment is extremely expensive ($100–300,000/y) and inconvenient, requiring multiweek intravenous infusions. Thus, the aim with FVIII/FIX gene transfer is sustained long-term factor production at levels of >5% that would effectively convert severely affected patients to a milder phenotype. If a treatment strategy could produce

consistent factor levels approaching 50%, this would be considered curative (normal factor VIII/IX levels are 50%–150%).

The half-life of FVIII and FIX are ~12 hours and ~25 hours, respectively. Based on these pharmacologic parameters, frequent multiweek infusions of factor are required to maintain a trough level greater than 1% of normal factor activity.[5] If these prophylactic regimens are followed, a marked reduction in bleeding and arthropathy has been measured in patients. Currently, many biopharma companies have developed long-acting (LA)-factors that can extend the half-life of FIX 2.5 times and FVIII 1.6 times. These LA-factors would be used once every 1–2 weeks (FIX-LA), and every 4-5 days (FVIII-LA).[6] The LA-factors can be viewed as the next generation of treatment until gene transfer methods are improved. Thus, gene therapy offers the hope that patients could be free of intravenous infusions and have continuous production of adequate, functional factor that would prevent future bleeding episodes.

The FIX complementary DNA (cDNA) is 1.4 Kb in size while the FVIII cDNA is ~9.0 Kb. Each protein is produced as an inactive molecule that, when cleaved by proteases, creates an active molecule. Subsequent proteolytic cleavage inactivates the molecule. Both proteins are post-translationally modified by the addition of sugar moieties.[7,8] FIX must also be carboxylated for full activity.[9] Although the liver appears to be the major source of both proteins, fully active FIX can be produced by nonhepatic tissues. FVIII also requires binding to the von Willebrand factor (vWF) protein for stability. vWF is produced normally in the liver endothelium and megakaryocytes, the latter of which is responsible for platelet production. The activity of both FVIII and FIX are readily assayed in plasma by clotting assays, biochemical assays (Coatest; Thrombin Generation) and biophysical assays (thromboelastography). Based on the cDNA size and tissue specificity, investigators have realized that the packaging of FVIII into available viral vectors would be more challenging than its smaller counterpart, FIX.

Another significant consideration in the effective treatment of FVIII/IX deficient diseases is inhibitor formation. Inhibitors are antibodies that bind and inactivate FIX or FVIII molecules. Inhibitor rates are ~20% in previously untreated FVIII patients and ~2-4% in untreated FIX patients.[1] Inhibitor development can lead to a more unpredictable and severe bleeding phenotype requiring specialized treatment either to eradicate the inhibitor antibodies or to use bypassing agents and treat the active bleeding. Although the discussion of inhibitor formation and treatment is beyond the scope of this review, gene therapy clinical trials must address inhibitor development as a potential devastating clinical consequence.

HEMOPHILIA GENE THERAPY TRIALS

Gene therapy: Approaches. *Ex vivo vs in vivo.* *Ex vivo* cell-based transfer is 1 major approach for factor gene transfer. Genetically modifying autologous cells *ex vivo*, reintroducing the cells and allowing for their engraftment and subsequent secretion of either FVIII or IX is one method that was attempted in an early trial by Chinese investigators. Fibroblasts were cultured from patients' skin and transduced with a FIX construct.[10] Cells carrying the human FIX DNA were then cloned, expanded, and introduced subcutaneously. Despite large numbers of cells, no significant FIX was measured. No assessment of the introduced cells was described; it is unknown whether the lack of effect was at the cellular or molecular level.

A similar study was performed using a FVIII construct introduced into skin fibroblasts. Here the cloned, expanded cells were introduced into the omental fat in the abdomen. In each cohort, 3 patients received either a high or low number of cells. There were no serious adverse events either from the use of factor VIII-producing fibroblasts or from the implantation procedure. No long-term complications developed and no FVIII inhibitors were detected. In 2 of the 6 patients, plasma levels of FVIII activity rose above 1% for periods of weeks to months before returning to baseline. These patients had received a high dose of cells (4×10^8 cells), whereas the low dose group (1×10^8 cells) did not demonstrate any significant FVIII plasma levels above baseline. Despite this, 2 low dose patients and 1 high dose patient self-reported a decrease in bleeding and a reduction in the use of exogenous FVIII.[11] Again, analysis of the inserted cells was not described.

More recently, investigators using retroviral gene transfer of both FVIII and FIX into hematopoietic stem cells in mice were able to measure detectable levels of both factors in the appropriate knockout mouse model.[12] Interestingly, although the plasma level of FVIII was quite low, it was complexed to vWF in mouse platelets. Experiments in large animal models (ie, sheep) are still ongoing. Whether the risks potentiated by transplantation and the possibility of insertional mutagenesis outweigh the benefits of continuous endogenous factor production remain to be determined.

The cell type used for *ex vivo* factor expression may be critical. Experiments using blood-derived endothelial progenitor cells suggest that in mice, a dose-relationship was achieved if these progenitors were gene-modified, expanded and reintroduced.