Gaceta Médica de México

Volumen *Volume* 138 Suplemento *Supplement* 1 Marzo-Abril *March-April* 2002

Artículo:

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Catherine Scott-Manno*

A gene therapy or gene transfer approach to treatment of severe hemophilia has several attractive features. First, modest transgene expression resulting in small increases in the level of circulating F. VIII or F. IX levels would likely improve the clinical course of severe disease. The decreased bleeding observed in patients with moderate hemophilia (1–5% F. VIII or F. IX) or in those on prophylaxis regimens demonstrates this principle. Second, over-expression of the transgene product would be unlikely to result in hard to the patient. Finally, several animal models of hemophilia exist making the possibility of accurate pre-clinical experimentation a feasible enterprise.

Viral and Non-Viral Approaches to Gene Transfer

Both viral and non-viral gene therapy approaches are now under clinical investigation in patients with hemophilia. The viral approaches involve injection of a vector encoding either F. VIII or F. IX that has, in pre-clinical experiments, been demonstrated safe and effective in a specific target tissue. Non-viral approaches including chimeroplasty and naked DNA transfer are under pre-clinical investigation. Non-viral therapies avoid some of the toxicities associated with viral vectors such as pathologic inflammatory response and virus replication. Three Phase 1 (safety) clinical trials have now been completed, two using viral vectors and one that used an *ex vivo,* non-viral approach.

Viral Vectors

Retroviral Vectors

Retroviral vectors were the first used in gene therapy trials. Retroviruses are single s-tranded RNA viruses that integrate into the host genome upon introduction into dividing cells. Although integration may provide long-term transgene expression, it may also cause insertional mutagenesis. Limitations to the use of retroviral-mediated liver-directed gene transfer for hemophilia include the low number of cells transduced, the low level of expression seen per integrated copy of provirus, and the need for invasive procedures such as partial hepatectomy to induce cell division in the target tissue.

Adeno-Associated Viral Vectors

Vectors derived from wild-type adeno-associated virus (AAV) are among the more promising approaches to gene therapy in hemophilia. AAV is a replication defective parvovirus that is not associated with human disease. A disadvantage of AAV is its relatively small genome of 4.7 kb, which limits the size of coding sequence that can be inserted into AAV-derived constructs. Skeletal muscle, liver, and neuronal tissue have all been efficiently transduced with rAAV. Liver and skeletal muscle can sustain long-term transgene expression in animal models.

Adenoviral Vectors

Adenovirus is a double-stranded DNA virus that has been broadly used as a gene delivery vehicle. Adenoviral vectors are relatively easy to produce in large quantity, transduce non-dividing cells such as the liver when given intravenously and demonstrate high transduction efficiency. Disadvantages of adenoviral vectors include hepatotoxicity and gradual loss of transgene expression over time, likely caused by an immune response against the transduced cells. In efforts to reduce toxicity and immunogenicity, various viral genes have been eliminated from newer generations of adenoviral vectors. The vectors called gutless or gutted express no viral genes. Such vectors are thought to be essentially safer and may also allow more sustained transgene expression.

Lentiviral Vectors

Lentiviral vectors are relatively new to gene therapy but have not been yet used in human trials. Wild-type lentiviruses are retroviruses that are responsible for various neurologic and immunologic diseases in nature. One advantage of lenitiviral vectors over other retroviral vectors is their ability to infect non-dividing cells. Partial hepatic resection increases lentiviral transduction by 30% in mouse hepatocytes; other experiments raise the possibility that DNA synthesis may be required for transduction in mice.

* Children Hospital, Philadelphia, USA

Clinical Trials

All completed and open gene therapy clinical trials for hemophilia are intended to demonstrate safety in human subjects. Although clinical efficacy is always of interest in safety trials, phase II/III efficacy trials may commence only after the safety of a specific approach has been demonstrated. Prior to recruitment of research subjects, clinical gene therapy trials in the U.S. are carefully evaluated by the Food and Drug Administration (FDA) as well as local institutional review boards and institutional biosafety committees. The FDA has oversight responsibilities for clinical gene therapy trials through the Investigational New Drug (IND) mechanism. Trials supported by funding from the National Institutes of Health are reviewed by the Office of Biotechnology Activities and if novel technologies are involved, are reviewed by the Recombinant DNA Advisory Committee. Written informed consent, demonstrating that participants are cognizant of the risks, benefits (if any), and altematives to participation in the trial must be signed prior to enrollment of any human research subject. Recently, the tenets of informed consent have received renewed emphasis in the U.S. following the highly publicized, tragic death of a research subject enrolled in a gene therapy trial in 1999.

Risks of Gene Therapy in Hemophilia

The major safety concerns identified in viral gene transfer approaches for hemophilia include germline transmission, insertional mutagenesis, and risk of inhibitor (antibody) formation to the transgene product. Parenteral administration of a vector might result in dissemination to the recipient's gonads and subsequent introduction into germ cells. If foreign gene sequences were part of a fertilization event, the seqnences could potentially disrupt the normal program of fetal development. Insertional mutagenesis would result if integration of a transferred gene disrupts a gene critical to tumor suppression, resulting in tumor formation. Formation of inhibitory antibodies to F. VIII and F. IX occurs in approximately 20% of hemophilia A and 3% of hemophilia B patients treated with factor concentrates. The likelihood of inhibitor development in patients whose endogenous factor production is a result of gene transfer is unknown, although surveillance for inhibitory and non-inhibitory antibody formation is critical to phase I safety trials. Safety assessments in current clinical trials focus on these issues as well as other general assessors of patient health.

Completed Clinical Trials

AAV-Muscle

A phase 1 dose-escalation trial was recently completed using a muscle-directed AAV vector encoding F. IX. This vector contains a CMV enhancer/promotor, a portion of a F. IX minigene, and the SV40 late polyadenylation sequence between two AAV inverted terminal repeats. Eight adult males with severe F. IX deficiency have received multiple intramuscular injections under ultrasound guidance (for the purpose of minimizing injection into intravascular structures). Subjects in the highest dose cohort received 2.0×10^{12} vg/kg. Muscle biopsies were performed 2 and 6 months after vector administration to look for the presence of vector sequences. Subjects tolerated the injections without significant systemic or local side toxicities. Vector shedding analyses showed an absence of vector sequences in the semen at all time points tested, consistent with the hypothesis that risk of vertical transmission in this scenario is low. No inhibitory or non-inhibitory antibodies against F. IX were demonstrated on Bethesda assay or Western blot. Analysis of muscle biopsy specimens showed evidence of gene transfer by PCR analysis or by Southern blot in seven of eight subjects. Immunohistochemical staining of injected skeletal muscle showed F. IX expression in seven of eight patients. Efficacy data from the first three subjects enrolled showed one with F. IX levels up to 1.6 %. The first two subjects reduced their use of factor concentrates.[12]

Retroviral Vector for F. VIII Deficiency

Chiron Inc. is currently sponsoring a multi-center trial of BDD-F. VIII infused intravenously into adult males with severe F. VIII deficiency at doses of $1\text{-}8 \times 10^{12}$ cfu/kg. As of June, 2000, 13 subjects were enrolled in this trial with an anticipated enrollment of 20 subjects. To date, no serious adverse events have been encountered; efficacy data are not yet available.

Non-Viral Approach

Results from a phase I trial investigating the safety of a non-viral gene transfer approach to subjects with severe hemophilia A were recently reported. In this study, a cooperative effort between Transkaryotic Therapies, Inc. and investigators at the Beth Israel Deaconess Medical Center in Boston, Massachusett, USA, skin fibroblasts were placed in cell culture and transfected with BDD F. VIII using electroporation. The plasmid construct contained a fibronectin promotor and the neomycin resistance gene. A clonal population of autologous cells expressing the transgene was laparoscopically injected into the subject's omentum. Six adult males with severe hemophilia A have been enrolled in this trial. All patients tolerated the procedures and the presence of transduced cells without serious adverse events and without inhibitor formation. Of note, three of six men had repeated F. VIII levels above baseline

(1–2% of normal) with a maximum of 4% measured in one subject. The increase in factor levels corresponded with reduced bleeding episodes. The effects of this therapy were transient, in that 12 months after omental implementation, F. VIII levels were back below 0.5% in all who had had a sustained rise.

Current Clinical Trials

Two clinical trials are currently underway. Because preclinical experiments have shown that targeting the liver results in higher expression levels, both new protocols use the liver as the target tissue. The first trial sponsored by Avigen, Inc. (Alameda, CA, USA) is based on an AAV-F. IX construct, which is to be infused into the hepatic artery of adult males with severe hemophilia B. Vector is given according to a dose-escalation strategy with low, medium and high dose groups. Nine subjects will be enrolled with three in each dose cohort. Two subjects have been enrolled to date.

The Genstar Therapeutics (San Diego, CA. USA) protocol is testing a gutted adenoviral vector encoding full-length F. VIII, administered by intravenous injection. The goal of this trial is to determine the potential toxicity of two doses of the vector. Six subjects with hemophilia A will be enrolled.

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