

Should Gene Therapy be Used for Newborns with Hemophilia?

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Hemophilia results from a deficiency of blood coagulation factors and leads to spontaneous bleeding. The disease is an attractive model for gene therapy because small amounts of the deficient protein can reduce the risk of bleeding. Therapeutic levels of coagulation Factor VIII (deficient in hemophilia A) and Factor IX (hemophilia B) have been achieved in mice and dogs, and five gene therapy trials have been initiated in adult patients [1–4]. These human trials include intramuscular or hepatic artery injection of an adeno-associated virus (AAV) vector for hemophilia B, intravenous injection of retroviral or adenoviral vectors for hemophilia A, and implantation of genetically-modified fibroblasts for hemophilia A.

The Food and Drug Administration has ruled out gene therapy trials on children unless their disease is lethal at an early age. An important question is whether gene therapy trials for newborns with hemophilia would be appropriate. I argue that there is strong rationale for early treatment. However, such trials should only proceed if studies in animals and initial evaluation in humans with other genetic deficiencies demonstrate safety and efficacy.

The use of gene therapy to treat hemophilia at birth provides two potential advantages. First, early treatment would obviously correct the bleeding diathesis sooner. For patients in countries that support frequent administration of the deficient coagulation protein (referred to here as factor), this could eliminate years of inconvenient and expensive treatment, complications such as catheter or viral infections, and the development of irreversible joint disease or other adverse effects of bleeding. Gene therapy might have an even greater impact in developing countries, where factor use is often limited to severe bleeds and is sometimes not available at all [5–8]. Worldwide, 23% of patients with hemophilia still die of bleeding [8], demonstrating that existing treatments are imperfect or unaffordable.

A second advantage is that early gene therapy might prevent the development of antibodies that inhibit the function of the infused coagulation factors because the immune system of humans is relatively immature at birth [9,10]. These antibodies are often referred to as inhibitors and occur in 20% of patients with hemophilia A [11] and 1% to 3% of those with hemophilia B [12]. For hemo-

philia A, inhibitors develop in approximately 35% of patients with large deletions and nonsense mutations [11], which is presumably due to the fact that many or all epitopes of the transfused Factor VIII are foreign. In contrast, inhibitors develop in only 8% of patients with single amino acid changes in Factor VIII [11], probably because the patients are tolerized to most epitopes during development. Inhibitors usually develop after about 8 to 12 days of treatment [12]. Patients with inhibitors can be treated with immune tolerance induction, which involves frequent injections of high doses of the deficient factor for a year or longer and costs approximately \$1 million U.S. dollars for a 5 year old child [13]. Furthermore, 20% of patients do not respond to this treatment. Alternative options for achieving hemostasis exist but are either more expensive or less reliable than simple administration of the deficient factor. Thus, development of inhibitors is a common and serious consequence of episodic factor infusion for hemophilia A.

A first important question is whether early gene therapy is sufficiently effective and safe to justify testing in newborns. Clearly, studies should proceed only if the vector achieves at least 1% of normal activity in animals for a significant period of time without any immediate toxicity. It will be more difficult to assess the long-term risk of cancer development, as this process might take years. A patient with severe combined immunodeficiency who received a retroviral vector that expressed the common γ chain for cytokine receptors has recently developed leukemia [14]. In addition, cancers developed in neonatal Mucopolysaccharidosis VII mice that received an AAV vector [15] and normal fetal mice that received an adenoviral vector [16], although there is no evidence that the gene transfer played a role. It would therefore be prudent to test neonatal gene therapy only in patients with otherwise-lethal genetic diseases and assess the risk of cancer for up to a decade prior to applying this approach to hemophilia.

A second question is whether or not neonatal gene transfer will indeed prevent immune responses to the coagulation factor. Neonatal gene therapy with AAV [17] and adenoviral [18] vectors failed to induce antibody formation to Factor IX in mice, although antibody formation to this protein usually occurs after gene transfer of these vectors into adults. Similarly, retroviral vectors led

to less frequent antibody formation to foreign proteins when transferred to newborn mice rather than adult mice ([19,20] and our unpublished data), yet some mice did produce inhibitory antibodies to Factor VIII [19]. Although these results are encouraging, the early immune systems of large animals are believed to be more mature than those in mice. On the other hand, the production of cytokines that are very important for antibody responses, such as interleukin 4 (IL-4) and IL-5, are still markedly reduced in lymphocytes from neonatal humans, and robust responses in newborns appear to require inflammatory stimuli [10]. Indeed, we have not observed antibody responses after neonatal delivery of a retroviral vector expressing canine β -glucuronidase to MPS VII dogs [21] or a vector expressing human Factor IX to normal dogs (J. Zhang, M. Haskins, KPP, unpublished data). Thus, existing data support the hypothesis that neonatal gene transfer may induce tolerance to a therapeutic protein, although additional studies in large animals including primates will be necessary to confirm this.

The question of whether or not gene therapy should be used to treat newborns with hemophilia cannot currently be fully answered, as there are insufficient data available regarding the relative risks and benefits. In the United States, hemophilia is treated reasonably well by factor replacement, and patients without HIV infection have an almost normal life span. According to the mandate of physicians, "Primum non nocere" (First, do no harm), we must answer these questions about efficacy and safety prior to using gene therapy to treat newborns with hemophilia. However, a blanket statement that gene therapy should not be considered for these patients does not make sense. Should neonatal gene therapy prove safe and tolerogenic, it would be an ideal solution for patients with hemophilia, particularly those with hemophilia A, who are at high risk of developing inhibitors.

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