HEPATIC gene therapy could revolutionize the treatment of many inherited diseases. Although repeated infusion of the particular proteins and liver transplantation have been used for the treatment of serum protein deficiencies, the risk of viral infection, cost, donor organ availability, and rejection severely limit the usefulness of these therapeutic approaches. The transfer of a functional gene into the liver cells of a genetically deficient individual could correct the clinical manifestations by providing long-term production of the affected gene product. In contrast to other gene transfer methods, retroviral vectors consistently result in long-term expression due to their ability to integrate into chromosomal DNA.

We have reported a rapid, reliable method for the in vivo delivery of retroviral vectors using a 70% hepatectomy in rats followed by intraportal retroviral injection during a 3-minute period of hepatic inflow occlusion. Using a Moloney murine leukemia virus-derived retroviral vector containing the promoter for RNA polymerase II (Pol-II), and the human a1-antitrypsin (hAAT) reporter gene, we have achieved transduction efficiencies of 10% and sustained expression for over 70 weeks. However, further optimization of the delivery system should result in higher expression in vivo. We have previously shown that the concentration of retrovirus immediately prior to intraportal injection increased in vivo expression by as much as 10-fold. Herein, we describe our further attempts to optimize our retroviral delivery system.

**MATERIALS AND METHODS**

Creation of Retroviral Vectors and Packaging Cell Lines

The Pol-II-hAAT retroviral vector (Fig 1) was used in these studies. Amphotropic methotrexate-resistant (Mtx6) retroviral titers of 1 x 106 colony forming units (cfu)/mL were determined on NIH 3T3 cells, and packaging cell clones were proven free of replication competent helper virus as previously described.

![Fig 1](image)

**Fig 1.** The Pol-II-hAAT retroviral vector contains the hAAT cDNA, the encephalomyocarditis virus internal ribosome entry site (ires), the mutant, methotrexate-resistant dihydropyrimidine reductase (*DHFR*), and the promoter for murine RNA polymerase II. Transcription may initiate from either the retroviral LTR promoter or the internal Pol-II promoter as depicted by the black and gray arrows, respectively.

In Vivo Hepatocyte Transduction Protocol

Adult male Sprague-Dawley rats (Sasco, Omaha, Neb) weighing 200 to 275 g were cared for according to standard NIH approved institutional guidelines. Twenty-four hours after 70% hepatectomy, in vivo hepatocyte transduction was accomplished by temporarily occluding the hepatic artery and portal vein with microvascular clips and injecting 5 mL of the packaging cell conditioned medium which was concentrated (24-fold by volume and 10-fold by Mtx titers) by ultracentrifugation at 15,000 rpm in a Beckman SW27 rotor for 90 minutes, spiked with a final concentration of 8 µg/mL polybrene (unless otherwise specified), into the proximal portal vein. Hepatic inflow occlusion time was 3 minutes unless otherwise specified. The amount of hAAT protein in the transduced rat serum was quantitated by human-specific enzyme-linked immunosorbent (ELISA).

**RESULTS**

Effect of Polybrene on In Vivo Hepatocyte Transduction

Polybrene is a cation that enhances retroviral infectivity by coating virion envelopes and improving interactions with the negatively charged target cells. We reasoned that an increased polybrene concentration in the intraportal injectate and/or an increased preincubation time of the vectors with polybrene might increase transduction efficiency, resulting in increased levels of hAAT expression. Polybrene was added at a final concentration of either 8 or 200 µg/mL to conditioned medium from Pol-II-hAAT packaging cells and in vivo transduction was performed approximately every 60 minutes between 1 and 7 hours after the addition of polybrene. Comparison of serum hAAT protein levels measured 8 days after transduction (Fig 2) revealed that increasing either the polybrene concentration or the polybrene retrovirus preincubation time was unable to significantly increase in vivo gene expression as determined by one-way analysis of variance (ANOVA).

Effect of Hepatic Inflow Occlusion Time on In Vivo Transduced Gene Expression

We reasoned that prolonging the contact time between retrovirus and hepatocytes by increasing the duration of hepatic inflow occlusion might increase retroviral transduction efficiency and in vivo gene expression. Therefore,
conditioned medium containing the Pol-II-haAT retroviral vector was injected intraperitoneally into rats which had hepatic vascular inflow occluded for 3 minutes, 10 minutes, or had no hepatic inflow occlusion. The mean serum haAT protein levels measured 14 days after transduction in five rats which had the extended 10 minute HIFO period (59.5 ± 27.5 ng/mL) were not statistically different from the rats which had no HIFO (48.0 ± 12.8 ng/mL) (n = 5) or the standard 3 minute HIFO period (21.3 ± 8.3 ng/mL) (n = 5) (Fig 3) (two-tailed, unpaired Student’s t test).

DISCUSSION

Liver-directed gene therapy is increasingly attractive, given the recent success of ex vivo⁸ and in vivo²,⁵ transduction protocols. However, its widespread application would be facilitated by a delivery system that avoids prolonged, large-scale hepatocyte culture or complicated retroviral delivery methods. Although our rapid retroviral transduction method modifies up to 10% of hepatocytes, improved transduction efficiency should further increase the overall expression of retroviral vectors. While polybrene has been widely used in retroviral transduction experiments, a systematic appraisal of the effects of polybrene, upon in vivo transduction efficiency has not been reported. We found no benefit upon increasing the polybrene concentration or the preincubation time with the retrovirus. We also found that prolonging hepatic inflow occlusion time during the retroviral injection was not able to improve haAT gene expression in rats as compared with those that received injection without hepatic inflow occlusion. This indicates that a simple portal vein injection can achieve efficient retroviral transduction, and further simplifies the procedure for in vivo retroviral delivery, as retroviral transduction could be performed via a catheter placed at the time of partial hepatectomy. Although the need to perform a 70% hepatectomy for efficient in vivo transduction makes this technique somewhat less attractive for clinical use than the ex vivo method that is currently being used in humans,⁸ identification of alternative ways to induce hepatocyte replication might make an in vivo delivery system more applicable to the treatment of human genetic deficiencies.

REFERENCES