

## Gene therapy and liver diseases

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### INTRODUCTION

We have shown previously that cell-surface receptors have been used as natural internalization sites for targeting genes to hepatocytes<sup>[1]</sup>. This targeting is based on the fact that parenchymal liver cells are the only cells that have large numbers of high affinity receptors that can recognize galactose-terminal(asialo-)glycoproteins. Binding of these glycoproteins by the receptor leads to invagination of the plasma membrane and internalization of the ligand- receptor complex in membrane-bound vesicles.

### METHODS AND RESULTS

A carrier system was developed consisting of two components: (1) an asialoglycoprotein ligand covalently bound to (2) a polymer containing multiple positive charges, a polycation. The asialoglycoprotein component provided the recognition signal for hepatocyte targeting, while the positively charged polycation served to bind DNA in a strong, non-damaging electrostatic manner to form a soluble protein-DNA complex<sup>[2]</sup>.

To determine whether normal genes could be targeted *in vivo* to correct metabolic dysfunction in genetically defective animals, the WHHL rabbit model for familial hypercholesterolemia was used. A plasmid carrying a full-length cDNA for the human low density lipoprotein (LDL) receptor was constructed using regulatory

elements from the mouse albumin gene inserted upstream to drive the LDL receptor gene. The plasmid was complexed with the carrier and injected intravenously into rabbits. Cellular DNA extracted and analyzed for the presence of human LDL receptor sequences demonstrated approximately 1000 copies of plasmid per cell, 10 min after injection. These levels declined progressively, and by 48 h plasmid DNA was less than 0.1 copies/cell. The transcriptional activity of the recombinant gene was analyzed by RNase protection assay for the presence of human LDL receptor transcripts. Exogenous LDL receptor mRNA was detected at 4 h, reached a peak at 24 h and decreased to undetectable levels by 72 h after transfection. Maximal levels of LDL receptor mRNA were found at 24 h and estimated to be 2%-4% of normal endogenous levels. In order to determine the metabolic effects of hepatocyte-directed gene transfer *in vivo*, WHHL rabbits injected with complexed LDL receptor gene or CAT gene were analyzed for changes in total serum cholesterol. Administration of the LDL receptor gene complex resulted in a rapid. But transient decline in serum cholesterol that lasted 6 d. The drop in cholesterol levels was maximal at 2 d post-injection and was 25% to 30% of pretreatment values<sup>[3]</sup>.

Based on the known requirement of migration of endosomal vesicles on microtubules, a microtubule-disrupting drug, colchicine, was administered to try to block lysosomal degradation of targeted DNA. The gene for normal glucuronyl transferase in a targetable complex was injected intravenously following a single colchicine injection into Gunn rats which possess defective glucuronyl transferase enzyme. This resulted in substantial decreases in serum bilirubin and production of bilirubin conjugates in bile that lasted at least 60 d<sup>[4]</sup>.

It was recognized that the carrier system could potentially target single-stranded DNA in the form of antisense oligomers. Because of the remarkable specificity of the action of antisense DNA in the inhibition of the synthesis of specific proteins based on hybridization of antisense to target mRNA, it was hypothesized that receptor-mediated delivery of antisense DNA specific for hepatitis B viral (HBV) mRNA sequences could inhibit viral gene expression in target cells. A 21-mer oligo DNA sequence complementary to the polyadenylation signal for human hepatitis B virus (HBV) was complexed to a soluble DNA-carrier system. A cell line, HepG2 (2.2.15) that possesses asialoglycoprotein receptors<sup>[5]</sup> and which is permanently transfected with hepatitis B virus (ayw subtype) was exposed to complexed antisense DNA or controls. In the presence of complexed antisense DNA, the concentration of hepatitis B surface antigen in medium was decreased by 80% by day 1, and by greater than 95% through the 6<sup>th</sup> day compared to untreated cells. There was no significant increase in surface antigen concentration in the presence of complexed antisense DNA after the first day of exposure. This inhibition was blocked by competition with an excess of free asialoglycoprotein. Protein secretion from cells was not affected and could not account for the decrease in HBV surface antigen concentration in the medium after exposure to antisense DNA. Also, total protein synthesis remained unchanged by exposure to complexed antisense sequences under identical conditions.

Finally, HBV DNA in the medium and cell layers after 24 h exposure to complexed antisense sequences was 80% lower than in controls. Exposure of cells to a random 21-mer oligo DNA sequence under identical conditions failed to alter HB V surface antigen concentration or HBV DNA in medium or cells<sup>[6]</sup>.

Bartholomew *et al.*<sup>[7]</sup> showed that targeted delivery of an antisense sequence against the poly A signal and 5' -upstream region of Woodchuck Hepatitis Virus (WHV) using a complex with an ASGP-based conjugate in a woodchuck hepatitis model, significantly decreased the virus particles in bloodstream by 5 to 10 fold, and this decrease was maintained over two weeks.

The DNA delivery system was tested to determine whether antisense oligonucleotides against the 5'-NTR of HCV genome could be targeted to inhibit HCV gene expression. The strategy was based on the fact that the 5'-Non-Translated Region (NTR) of Hepatitis C Virus (HCV) contains important elements that control HCV translation. Antisense oligonucleotides directed against a sequence in the internal ribosomal binding site of the NTR (Anti-III), and a portion of the NTR (Anti-IV) overlapping the core protein translational start site of HCV were prepared. In transient transfections of a plasmid containing a luciferase gene immediately downstream from an HCV NTR insert, oligonucleotides Anti-III and Anti-IV in the form of asialoglycoprotein-polylysine complexes were administered to Huh7 cells, and luciferase activity generated by CMV HCVluc measured. Anti-III inhibited luciferase activity by 75%, and 99% at 0.01  $\mu\text{mol/L}$ , and 0.1  $\mu\text{mol/L}$ , respectively. Similarly, Anti-IV inhibited luciferase activity 88%, and 99% at 0.01  $\mu\text{mol/L}$  and 0.1  $\mu\text{mol/L}$ , respectively. In cell lines stably transfected with CMV HCVluc plasmid, complexed Anti-III inhibited luciferase activity in Huh7 cells by 20% at 10  $\mu\text{mol/L}$  and 85% at 60  $\mu\text{mol/L}$ , and was compatible by an excess asialoglycoprotein<sup>[8]</sup>.

## CONCLUSION

A DNA carrier system can target polynucleotides to hepatocytes to provide new gene expression or to inhibit endogenous gene expression in a cell-specific manner.

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