

Gene Therapy: If At First You Don't Succeed . . .

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"Study Details Success of First Gene Therapy"

Headline, *Los Angeles Times*, October 20, 1995

"Scientists Report the First Success of Gene Therapy"

Headline, *New York Times*, April 28, 2000

"Teen Dies Undergoing Gene Therapy"

Headline, *Washington Post*, September 29, 1999

Gene therapy represents the culmination of medical research and its application to human health. Less than 60 years ago, Linus Pauling and others¹ described at the molecular and genetic level what was to be the first of many molecular diseases. In the most simple form, a mutation of just one base pair in a gene can alter the production or function of a protein that is vital to health and life. A "rough draft" of the human genome sequence has been completed and, with this, the sequence of every human gene is now theoretically available to scientists, physicians and the general public. Subsequently, the mapping of diseases to individual genes will be only a matter of time and effort. Over the past 50 years, investigators have teased out the mechanisms of many diseases at the physiologic and genetic levels and have made great progress in developing pharmacologic drugs to alleviate these maladies. The next step, already in progress, is to use genes themselves as the drugs—replacing or altering the expression of defective or misregulated genes—to treat patients at the molecular level.

Unfortunately, while gene therapy may be to the 21st century what antibiotics were to the last, we have a long way to go before success is at hand. As can be seen in the headlines above, gene therapy has had multiple "first" successes followed by the realization that

much of the enthusiasm for each success has been perhaps premature or overstated. Further, much of the early excitement about this approach has been dampened following the recent death of a young male involved in one clinical trial, as noted in the third headline above. However, as with all discoveries and new fields, problems do exist, and they need to be identified, studied and overcome. Indeed, these are exciting times to practice medicine, but much of the initial unbridled enthusiasm for gene therapy has worn off, and now the real work has begun.

Gene therapy began in the mid-1970s when it was discovered that DNA could be manipulated in the test tube. Forward-thinking investigators quickly realized that the possibility of expressing normal or foreign genes in human cells (and ultimately in humans themselves) could usher in a new era of medicine. Theoretically, mutant genes, in patients with such diseases as sickle cell disease and cystic fibrosis, could be replaced and the diseases corrected by transferring the wild type, or normal, genes into these patients.² Other diseases (e.g., cancer) might be treated with genetic therapy by adding genes to increase antitumor immune responses or by inhibiting angiogenesis and cutting off the tumor's blood supply.³ Furthermore, infectious diseases might be combated by altering the body's immune response.⁴

As more and more of the human genome was discovered, new associations between genes and diseases were identified, and the possibilities for genetic medicines became apparent. All of these treatments require moving desired genes into the appropriate cells in both animals and people—and this is where problems have been encountered over the past 10 years.^{5,6}

Two major approaches have been taken to ▶

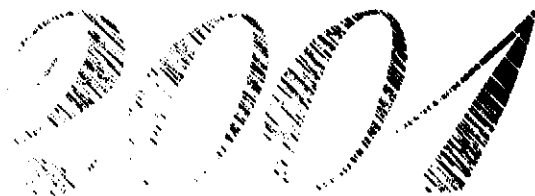
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developed, and as soon as a second dose of the viral vector is given, the virus and the cells it infects will be targeted for destruction when a second dose of the viral vector is administered, thereby greatly limiting the expression of the transferred therapeutic gene. Although these drawbacks may seem daunting, investigators are actively engaged in moderating these responses and finding ways to circumvent the problems. To this end, novel and modified viruses are being tested and show great promise in terms of significantly reduced inflammatory responses and the ability to administer repeated doses for multiple gene therapies.

The second approach for gene delivery and therapy is to use nonviral systems. The latter include plasmid DNA that is either delivered directly (termed "naked DNA") or complexed with carriers such as liposomes that encase the DNA, add stability and increase cellular delivery and entry.¹⁰ Based on design, plasmids have the ability to express in dividing and non-dividing cells, thus making them appropriate for administration to all cells in the body. Further, the lack of viral proteins and genes that the body sees as foreign and worthy of reaction means that little inflammation is induced; thus, no immune response is mounted against the DNA, which, in turn, makes multiple effective treatments possible. Unfortunately, the level of expression obtained with nonviral methods is usually much lower than that occurring with viral methods.

Other potential delivery vehicles that produce increased gene expression are in development. They include delivery to the skin by Star-Trek-like pneumatic guns and the use of electric fields and sound waves to drive DNA into cells.

Although the levels of nonviral DNA delivery and gene expression are much lower than those obtained using viruses, the lack of an immune response may lead the field to favor these systems over the potential hazards associated with viral methods. As time goes on, it will most likely be found that each system has



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BRIEF SUMMARY

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INDICATIONS AND USAGE

CIPRO[®] HC OTIC suspension is indicated for the treatment of acute otitis externa in adult and pediatric patients, one year and older, due to susceptible strains of *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Proteus mirabilis*.

CONTRAINDICATIONS

CIPRO[®] HC OTIC is contraindicated in persons with a history of hypersensitivity to hydrocortisone, ciprofloxacin or any member of the quinolone class of antimicrobial agents. This nonsterile product should not be used if the tympanic membrane is perforated. Use of this product is contraindicated in viral infections of the external canal including varicella and herpes simplex infections.

WARNINGS

NOT FOR OPHTHALMIC USE. NOT FOR INJECTION.

CIPRO[®] HC OTIC should be discontinued at the first appearance of a skin rash or any other sign of hypersensitivity. Serious and occasionally fatal hypersensitivity (anaphylactic) reactions, some following the first dose, have been reported in patients receiving systemic quinolones. Serious acute hypersensitivity reactions may require immediate emergency treatment.

PRECAUTIONS

GENERAL: As with other antibiotic preparations, use of this product may result in overgrowth of non-susceptible organisms, including fungi. If the infection is not improved after one week of therapy, cultures should be obtained to guide further treatment.

Information for Patients:

If rash or allergic reaction occurs, discontinue use immediately and contact your physician.

Do not use in the eyes.

Avoid contaminating the dropper with material from the ear, fingers, or other sources.

Protect from light.

Shake well immediately before using.

Discard unused portion after therapy is completed.

Carcinogenesis, Mutagenesis, Impairment of Fertility: Eight *in vitro* mutagenicity tests have been conducted with ciprofloxacin, and the test results are listed below:

Salmonella/Microsome Test (Negative)

E. coli DNA Repair Assay (Negative)

Mouse Lymphoma Cell Forward Mutation Assay (Positive)

Chinese Hamster V₇₉ Cell HGPRT Test (Negative)

Syrian Hamster Embryo Cell Transformation Assay (Negative)

Saccharomyces cerevisiae Point Mutation Assay (Negative)

Saccharomyces cerevisiae Mitotic Crossover and Gene Conversion Assay (Negative)

Rat Hepatocyte DNA Repair Assay (Positive)

Thus, 2 of the 8 tests were positive, but results of the following 3 *in vivo* test systems gave negative results:

Rat Hepatocyte DNA Repair Assay

Micronucleus Test (Mice)

Dominant Lethal Test (Mice)

Long-term carcinogenicity studies in mice and rats have been completed for ciprofloxacin. After daily oral doses of 750 mg/kg (mice) and 250 mg/kg (rats) were administered for up to 2 years, there was no evidence that ciprofloxacin had any carcinogenic or tumorigenic effects in these species. No long term studies of CIPRO[®] HC OTIC suspension have been performed to evaluate carcinogenic potential.

Fertility studies performed in rats at oral doses of ciprofloxacin up to 100 mg/kg/day revealed no evidence of impairment. This would be over 1000 times the maximum recommended clinical dose of otological ciprofloxacin based upon body surface area, assuming total absorption of ciprofloxacin from the ear of a patient treated with CIPRO[®] HC OTIC twice per day.

Long term studies have not been performed to evaluate the carcinogenic potential or the effect on fertility of topical hydrocortisone. Mutagenicity studies with hydrocortisone were negative.

Pregnancy: Teratogenic Effects. Pregnancy Category C:

Reproduction studies have been performed in rats and mice using oral doses of up to 100 mg/kg and IV doses up to 30 mg/kg and have revealed no evidence of harm to the fetus as a result of ciprofloxacin. In rabbits, ciprofloxacin (30 and 100 mg/kg orally) produced gastrointestinal disturbances resulting in maternal weight loss and an increased incidence of abortion, but no teratogenicity was observed at either dose. After intravenous administration of doses up to 20 mg/kg, no maternal toxicity was produced in the rabbit, and no embryotoxicity or teratogenicity was observed.

Corticosteroids are generally teratogenic in laboratory animals when administered systemically at relatively low dosage levels. The more potent corticosteroids have been shown to be teratogenic after dermal application in laboratory animals.

Animal reproduction studies have not been conducted with CIPRO[®] HC OTIC. No adequate and well controlled studies have been performed in pregnant women. Caution should be exercised when CIPRO[®] HC OTIC is used by a pregnant woman.

Nursing Mothers: Ciprofloxacin is excreted in human milk with systemic use. It is not known whether ciprofloxacin is excreted in human milk following topical otic administration. Because of the potential for serious adverse reactions in nursing infants, a decision should be made whether to discontinue nursing or to discontinue the drug, taking into account the importance of the drug to the mother.

Pediatric Use: The safety and efficacy of CIPRO[®] HC OTIC have been established in pediatric patients 2 years and older (131 patients) in adequate and well-controlled clinical trials. Although no data are available on patients less than age 2 years, there are no known safety concerns or differences in the disease process in this population which would preclude use of this product in patients one year and older. See DOSAGE AND ADMINISTRATION.

ADVERSE REACTIONS

In Phase 3 clinical trials, a total of 564 patients were treated with CIPRO[®] HC OTIC. Adverse events with at least remote relationship to treatment included headache (1.2%) and pruritus (0.4%). The following treatment-related adverse events were each reported in a single patient: migraine, hypesthesia, paresthesia, fungal dermatitis, cough, rash, urticaria, and alopecia.

NDC 0085-8531-10

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5,843,930; and Pat. Pending.

Revised January, 1998

CHCOB-0198

References:

1. Pistorius B, Westberry K, Drahobi M, et al. Prospective, randomized, comparative trial of ciprofloxacin otic drops, with or without hydrocortisone, vs. polymixin B-neomycin-hydrocortisone otic suspension in the treatment of acute otitis externa. *Infect Dis in Clin Pract* 1998;6:387-395.

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deliver genes to cells: viral and nonviral. In the case of viral delivery, the vectors (delivery agents) are modified viruses that have had their genomes largely replaced with therapeutic genes.⁵ This allows the desired genes to be packaged into virus particles that are effective at entering cells within the body. By removing most of the viral genes, the chance for viral replication and aberrant infection in the host is, hopefully, removed. Thus far, adenoviruses and retroviruses are the two families of viruses that have been used in the majority of clinical trials.

Adenoviruses are good at infecting the non-dividing or very slowly dividing cells that make up most of our tissues. Conversely, the application of retroviruses is limited because they can infect only dividing cells; however, this application results in life-long expression of the carried gene because it actually incorporates itself into the host's chromosome.⁷ Unfortunately, such effective gene delivery comes at a price: viral vectors, especially adenovirus, can induce significant localized and systemic inflammation, and a sustained immune response. While each person will vary in inflammatory response to viral administration, the response can range from unnoticeable to severe.

Indeed, in the recent case at the University of Pennsylvania, administration of recombinant adenovirus carrying a gene to alleviate ornithine transcarbamylase (OTC) deficiency in a 17-year-old male resulted in activation of his innate immune system and fever, and ultimately led to his death.^{8,9} Although the preceding 17 patients in the same study had received the same viral vector, they did not exhibit anywhere near these levels of inflammation or response, thus revealing no indication predictive of the patient's death. This case only exemplifies the variations that are possible.⁹

Another result of an immune response to viral vectors is that the response limits the number of times the virus can be administered. Once antibody and T-cell responses are

its unique applications and both may ultimately find their way to the physician's office.

So, has gene therapy succeeded? In the realm of theory and laboratory experimentation, the answer is a resounding yes. Results from experiments have demonstrated that genes can be transferred to animals and humans and can exert therapeutic effects. In animal models, diseases can be corrected transiently and, in some cases, even on a long-term basis. In the earliest "first success" of gene therapy, Blaese and colleagues¹¹ transferred the gene for adenosine deaminase (ADA) using a retrovirus to T cells isolated from two young females with severe combined immunodeficiency as a result of ADA deficiency. When it was transfused back into the patients, small increases in their ADA levels were detected, as were minor increases in T cells and immune responses. However, the benefit was incomplete and did not fully "cure" the patients.¹¹

In a more recent "first success," researchers in France took a similar approach to this earlier work to treat a different form of severe combined immunodeficiency but, rather than target T cells, they targeted stem cells isolated from the bone marrow of three different infants, also using retrovirus.¹² When transfused back into the infants, the successfully transduced stem cells propagated and

expanded, outgrowing and replacing the endogenous defective cells. Indeed, success may be at hand, but only time will tell. Thus far, more than 390 other clinical trials involving gene therapy have been conducted over the past 10 years.

In conclusion, how close are we to using gene therapy in the family practice setting? When will an injection of recombinant virus or a plasmid infusion be used to control hypertension, sickle cell disease or cancer instead of using the traditional approaches? When will the first success of clinical importance occur? Hopefully soon, but to be realistic, definitely not before you have to take the Boards again.

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REFERENCES

1. Pauling L, Itano HA, Singer SJ, Wells IC. Sickle cell anemia: a molecular disease. *Science* 1949;110:543-8.
2. Mulligan RC. The basic science of gene therapy. *Science* 1993;260:926-32.
3. Blaese RM. Gene therapy for cancer. *Sci Am* 1997;276:111-5.
4. Weiner DB, Kennedy RC. Genetic vaccines. *Sci Am* 1999;281:50-7.
5. Verma IM, Sornia N. Gene therapy—promises, problems and prospects. *Nature* 1997;389:239-42.
6. Friedman T. Overcoming the obstacles to gene therapy. *Sci Am* 1997;276:96-101.
7. Wilson JM. Adenoviruses as gene-delivery vehicles. *N Engl J Med* 1996;334:1185-7.
8. Fox JL. Gene therapy safety issues come to fore. *Nat Biotechnol* 1999;17:1153.
9. Preliminary findings reported on the death of Jesse Gelsinger. IHGT, University of Pennsylvania. 1999. Retrieved February 2001 from: <http://www.med.upenn.edu/ihgt/finding/html>.
10. Felgner PL. Nonviral strategies for gene therapy. *Sci Am* 1997;276:102-6.
11. Blaese RM, Culver KW, Miller AD, Carter CS, Fleisher T, Clerici M, et al. T lymphocyte-directed gene therapy for ADA-SCID: initial trial results after 4 years. *Science* 1995;270:475-80.
12. Cavazzana-Calvo M, Hacein-Bey S, de Saint Basile G, Gross F, Yvon E, Nussbaum P, et al. Gene therapy of human severe combined immunodeficiency (SCID) - XI disease. *Science* 2000;288:669-72. ■

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