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Utility of the nasal model in gene transfer studies in cystic fibrosis*

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SUMMARY

Despite advances in the treatment for cystic fibrosis (CF), life expectancy for affected patients remains dramatically curtailed. Recent years have produced a spectacular increase in our understanding of the genetic, molecular and physiological bases of this disease. Gene transfer is a new and conceptually-attractive potential treatment for CF. A number of centres have undertaken preliminary human gene-therapy trials. Central to these trials has been the use of the nasal model in gene transfer studies. While the eventual target of gene therapy in CF will be the lungs, the nasal administration of vector offers a number of advantages over the tracheobronchial tree in early experimentation. Implicit in the use of the nasal model is the potential for rhinologic variables to influence the results. We review our own gene transfer studies as well as series from other institutions, considering the role of nasal factors in the experiments' outcomes. Rhinologic variables may, at least partially, potentially explain the sometimes disparate results reported in this emerging area of scientific interest.

Key words: gene transfer, cystic fibrosis

INTRODUCTION

Cystic fibrosis (CF) is the most common autosomal recessive disease in people of European Caucasian descent. In this population, it has an incidence of 1 in 2,500 live births (Welsh et al., 1995a). There are an estimated 30,000 people with CF in the United States and another 7 million people are carriers for the condition (Fitzsimmons, 1993).

The gene for CF, identified in 1989, is located on the long arm of chromosome 7 (Rommens et al., 1989). The CF gene encodes a protein, the *Cystic Fibrosis Transmembrane Conductance Regulator (CFTR)*, which resembles a family of proteins involved in transportation across cell membranes. Recently, CFTR has been shown to be a chloride channel, regulated by cAMP-dependent phosphorylation and intracellular nucleotides (Welsh et al., 1992). Mutations of the CFTR gene cause loss of function of the CFTR channels. The most common mutation, designated △F508, is caused by the deletion of a single phenylalanine residue at position 508 of CFTR and accounts for over 70% of CF patients. More than 450 mutations of the CF gene have been identified. Most non-△F508 mutations are rare, occurring in less than 1% of screened populations (Welsh, et al., 1995a). The loss of function of the CFTR chloride-channels produces the physiological hallmark of CF-defective chloride transport across affected epithelia (Quinton, 1990). While this produces diverse clinical manifestations, including paranasal sinus disease and nasal polyposis, it is the pulmonary sequelae which are the major cause of mortality and morbidity.

Advances in treatment for CF have produced increases in patient survival. Data from the CF Foundation's Annual Report put the mean survival time in 1992 at 29.4 years. Despite this improvement, the lifespan of CF patients remains markedly reduced. No clinically available treatment addresses the fundamental defect of CF, the CFTR abnormality. In this regard, gene transfer techniques hold some promise as a new and conceptually attractive technique designed to correct the fundamental, physiological defect of CF, the abnormal chloride transport.

VECTORS

For gene transfer techniques to be successful, the desired genetic material needs to be expressed in the target cell. The "vector" is the vehicle which transports the desired DNA past the target cell defenses. A variety of vectors have been used in gene transfer experimentation. In CF gene transfer studies, the adenovirus, adeno-associated virus and liposome vectors have been

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employed. Most information is available about the adenovirus vector.

Engineered adenoviruses offer the potential for successful gene transfer in CF (Welsh et al., 1994). The virus can be constructed to encode and express a desired gene product; in this case, the CF-associated protein, CFTR. The virus is attenuated, being unable to replicate in a normal, lytic viral life-cycle. Adenovirus has a natural tropism for the targeted cells of airway epithelia. The adenoviruses are able to infect the non-actively dividing airway cells. This is in contrast to retroviruses, also used in human gene therapy, which require actively replicating cells. Adenovirus expression is achieved without integration of the viral DNA into the host chromosome, alleviating potential concerns about insertional mutagenesis. The use of adenoviruses as live enteric vaccines over a number of years has been associated with an excellent safety profile. The importance of these safety issues is compounded by the likely need for repetitive administration because of the natural turnover of respiratory epithelium.

THE NASAL MODEL

The feasibility of gene transfer to airway cells has been demonstrated, both *in vitro* and in animal experiments. Expression of cDNA for wild-type CFTR corrected the chloride-channel defect in cultured CF airway epithelia (Rich et al., 1990). Recombinant adenoviral vectors have been shown to deliver CFTR-cDNA safely in both primates and cotton rats (Zabner et al., 1994). Encouraging *in vitro* and *in vivo* results were the basis for progression to human experimentation to address questions that, ultimately, could only be answered in patient trials. Initial human trials had the objective of reducing potential patient toxicity to a minimum, at the same time as yielding measurable results. While the eventual goal of therapy in CF will require repeated administration of vector to the lower respiratory tract, the nasal cavity offers an excellent and relatively safe model for initial studies.

Since the turn of the century, it has been established that certain epithelial cells are capable of generating and maintaining potential differences across their surface. Melon (1968) showed that excised human nasal mucosa displayed this potential difference. Knowles et al. (1981) measured human nasal potentials *in vivo*, and measured voltages in patients with CF. They found that the nasal potential difference in 24 patients with CF exceeded, by more than three standard deviations, the mean voltage of healthy controls, patients with other respiratory disease or heterozygotes for CF (Knowles et al., 1981). The nasal voltage is present in areas of ciliated pseudostratified columnar epithelium. Although there is subject-to-subject variation, the voltage has a characteristic appearance in CF, both with respect to absolute negativity and its response to perfusion of terbutaline, a ß-agonist in a low-chloride environment, after pre-treatment with the sodium-channel blocker amiloride (Figure 1).

Gene transfer studies have sought to establish whether application of a recombinant adenovirus-encoding CFTR could correct the physiological hallmark of CF-altered epithelial chloride transport as manifested by the characteristic nasal potential. The nasal potential in CF provides a measurable endpoint for gene transfer experiments. Safety concerns aside, intrapulmonary administration of a vector does not offer a readily measurable endpoint. Measurement of epithelial potential in the pulmonary epithelium is cumbersome and difficult. Any acute potential improvement in lung function from experimental gene transfer may be difficult to quantify in patients with pre-existing pulmonary disease.

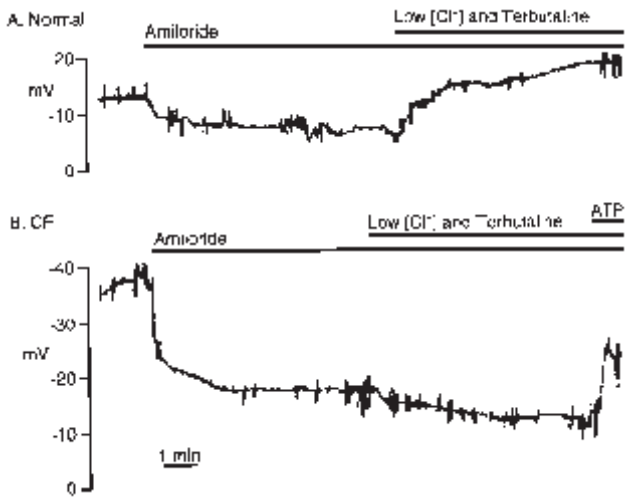

Figure 1. Nasal voltage traces in normal and CF patients demonstrating results at basal levels and after perfusion with terbutaline in a low-chloride environment, following pre-treatment with amiloride.

Perhaps the most compelling argument for the nasal model is its safety over intrapulmonary administration. A major concern in any human gene-transfer experiments is potential toxicity. Limited application of vector to a defined area of nasal epithelium reduces the likelihood of a serious reaction occurring. A serious reaction occurring in the lungs of a CF patient with pre-existing pulmonary compromise may produce a potentially life-threatening event. Indeed, adverse systemic events have been reported after pulmonary administration of recombinant adenovirus (Crystal et al., 1994).

Accessibility is another major advantage for the nasal model. Vectors can be applied in the nasal cavity in a less morbid and easier fashion than is needed for precise reproducible pulmonary application. Vector can be applied in the nose, pinpointing the location relative to anatomical landmarks. This requires little or no anaesthesia or vasoconstriction, depending on the method chosen. Ease of access for application is complemented by ease of access for observation, photographic documentation and measurement. The area of application is easily available for brushing and swabbing. Biopsy, if required, can be achieved almost as easily.

CLINICAL TRIALS: PROGRESS, PROBLEMS AND RHINOLOGIC VARIABLES

The initial human study of gene transfer in CF was performed by Zabner et al. (1993). In this single-administration experiment, virus in escalating doses was applied to the nasal epithelium of three patients. The three patients were either homozygous for the △F508 mutation or △F508 compound heterozygotes. In the first two patients, vector was localized to a

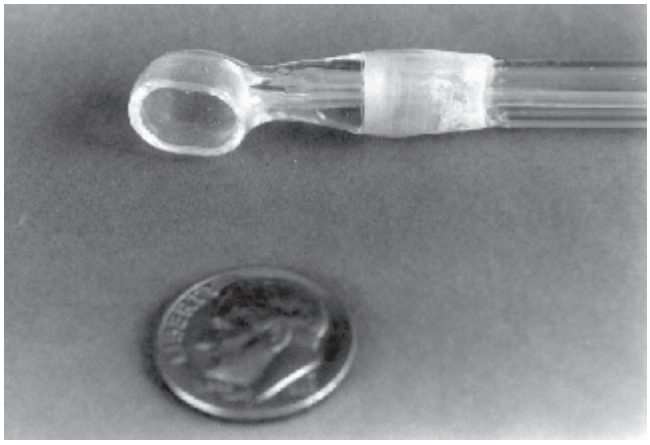

Figure 2. Nasal "spoon" for application of vector.

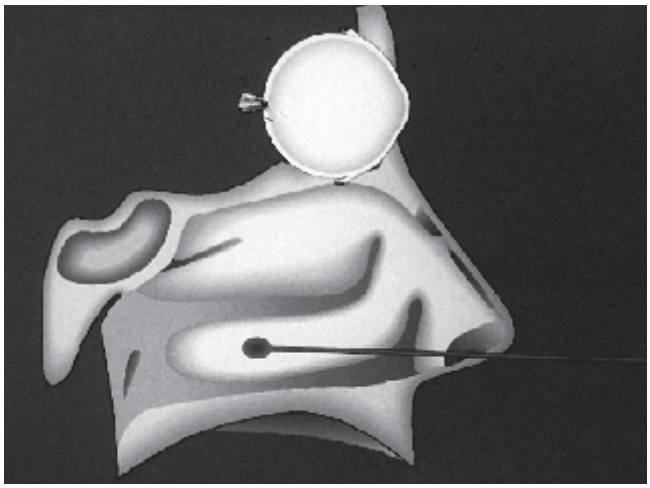

Figure 3. Schematic representation of area of application of vectors.

defined area on the medial aspect of both inferior turbinates. The nasal mucosa was pre-treated with 0.5% oxymetazoline and 2% tetracaine spray to effect vasoconstriction and anaesthesia. A purposely-built nasal spoon (Figure 2), allowing infusion of fluid after achieving an occlusive seal, was used to apply the solution of recombinant adenovirus to the defined area of nasal epithelium (Figure 3). Preliminary methylene blue studies suggested the area of application was approximately 0.5 cm^2. A 0° endoscope was used for guidance in positioning the applicator in the nose, relative to fixed landmarks such as the anterior end of the middle turbinate, septal morphology and, in individual cases, a small polyp and accessory maxillary ostium. The procedure was videotaped and multiple still-photographs were taken to aid in subsequent identification of the area of vector application. The videotape also allowed us to ensure that the area of application remained constant throughout the planned, timed 30 min. At the end of 30 min, the applicator was removed and excess solution suctioned away. Two other patients with CF served as controls. They had identical procedures with applications of normal saline, instead of vector solution. At Day 1 or 3 after application, the treated mucosa on one side was removed by endoscopic biopsy. The treated mucosa on the other side was used for nasal electrical potential difference measurements, swabs and brushings.

The first two patients appeared to sustain a mild mucosal injury which was attributed to the method of application of vector. After the anaesthesia and vasoconstriction had resolved, patients complained of nasal congestion and, in one case, mild rhinorrhoea. These were self-limiting symptoms which had disappeared by a day or so. Endoscopic examination of the nasal cavity revealed oedema, erythema, and exudate. The same symptoms and endoscopic appearances were present in the two control CF patients treated with saline. Some degree of swelling and congestion also occurred in patients who only had application of vasoconstrictor and anaesthetic. This suggested that the application of anaesthesia/vasoconstriction was traumatic in itself and this, coupled with the plastic applicator (Figure 2), produced mild epithelial trauma. Nasal swabs performed at the time of application produced a neutrophil increase, paralleling the injury. Independent histological evaluations of the biopsy specimens suggested changes consistent with mild trauma.

The method of application for the third patient was changed because of the mild mucosal trauma in the first two. A Foley catheter, which would not require preliminary vasoconstriction or anaesthesia, was selected as the means of application for one side of the nose in the third patient. The vector solution was confined by surface tension to a relatively-defined similar area of medial inferior turbinate. This was achieved without anaesthetic or vasoconstriction. Endoscopic localization was employed in the same manner. Video-endoscopic documentation of the entire procedure ensured that the catheter tip did not move or slip during the timed 30 min. On the right side, recombinant adenovirus was administered with the custom applicator as before. Similar changes suggestive of inflammation ensued. On the left side, where the Foley catheter was used, the nose appeared normal and the patient had no symptoms of congestion. In this single-administration study, the nasal potential difference (V_t) became less negative in all three patients after application of an adenovirus-encoding CFTR. This decrease in basal V_t suggests that application of the vector corrected the CF-chloride transport defect in nasal epithelium in all three patients. This did not occur in the two control patients treated with saline. The authors concluded that *in vivo* application of a recombinant adenovirus-encoding CFTR can correct the defect in airway epithelial chloride transport that is characteristic of CF epithelia.

The next two publications on this subject by Crystal et al. (1994) and Knowles et al. (1995) did not support the correction of nasal potential reported by Zabner et al. (1993). Knowles et al. (1995) administered adenoviral vector to four cohorts of three patients each in logarithmically increasing doses. While finding molecular evidence of low-efficiency gene transfer, they found no correction of nasal potential difference. In their study, vector was infused under direct vision onto the inferior and medial surfaces of the inferior turbinate and nasal floor. The study of Crystal et al. (1994) involved administration of an adenovirus-containing human CFTR-cDNA to the upper and lower respiratory tracts of four patients with CF. The intrapulmonary vector administration produced pulmonary toxicity and further reinforced the special advantages of the nasal model in preliminary administration. CFTR was detected by immunohistochemistry. No statement, however, was made concerning nasal potential changes.

A second series of experiments by Zabner et al. (1996b) involved repeat administration of vector. These experiments sought to approximate the likely real-life need for repeat administration required by transient vector effects and cell turnover. The study involved six CF patients in two geographic centers. They received 4-5 administrations of a recombinant adenovirus-encoding CFTR at increasing doses, starting with 10^6 infectious units and escalating up to 10^{10} on the fifth dose. On average, the doses were given 44 days apart. One nasal cavity was randomly chosen as the treated side with the other being the "mock" control side where saline was similarly administered.

The method of vector administration was different from either technique in their single-administration study. The Foley catheter was retained as the vector delivery device in the hope of preventing mucosal injury. An endoscope, however, was used only for the initial placement - judged to be 6 cm from the caudal aspect of the columella. The site chosen was the inferior aspect of the inferior turbinate. Methylene blue studies suggested that, in fact, much of the nasal cavity ended up in contact with the applied solution. The V_t was measured using a Foley catheter placed endoscopically in the same position as the perfusing catheter under the inferior turbinate. This study did not demonstrate changes in basal V_t. There were, however, more subtle electrophysiological changes consistent with generation of CFTR-chloride channels, but the correction of the CF-associated electrophysiological changes was markedly less dramatic. More hopeful results have been conveyed by two further studies. Hay et al. (1995) produced improvements in the V_t toward those of the normal population. These corrections lasted approximately two weeks. Their study involved single escalating-dose administration to nine patients with CF. The vector was administered to the inferior aspect of the inferior turbinate, using a catheter. They concluded that the adenoviral vector delivered sufficient CFTR-cDNA to improve the V_t.

The study by Caplan et al. (1995) employed a cationic liposome vector, rather than a recombinant adenovirus. Their results followed a middle course, reporting a 20% correction in V_t in nine CF patients. In this study, the liposome vector was administered by a nasal pump spray. This spray, perhaps, most closely resembles a clinically attractive and practical delivery mechanism, should gene transfer eventually become therapeutically helpful.

DISCUSSION

The majority of the current CF-related rhinologic literature focuses on the relationship of CF and paranasal sinus disease and nasal polyposis. Rhinologic aspects of CF have, however, a wider importance in aiding understanding of the molecular and electrophysiological basis of the disease. While, clearly, the eventual clinical target for gene transfer in CF will be the bronchial tree, the nasal model can serve as a useful experimental substitute in answering a number of questions. Nasal voltage measurements in normal and CF populations differ, both in their absolute negativity and in their abnormal response to pharmacological challenge with terbutaline, a ß-agonist, after pre-treatment with the sodium-channel blocker amiloride. These measurements reflect the basic electrophysiological defect of altered chloride and sodium transport in CF. Easy measurement of V_t and its response to vector administration in CF patients offers an insight into the potential correction of this electrophysiological hallmark of the disease. The V_t has been referred to as the CF bioelectrical phenotype (Hay et al., 1995). How changes in this electrical potential might translate into clinical benefit, however, remains an issue of supposition (Welsh et al., 1995b).

How are we to explain the differences in results of these six studies? The potential reasons for this are numerous, indeed, dealing with the vector, host responses, and rhinologic variables. Zabner et al. (1996a) have listed experimental error, changes in vector, changes in doses, and immune response as potential culprits. Hay et al. (1995) have questioned which aspect of nasal voltage measurements best reflect CFTR function in gene transfer studies. Of particular interest to the rhinologist is the potential for "rhinologic factors" to influence the experiments' outcomes.

In the studies by Zabner et al. (1993, 1996b) the means of delivery of the vector changed twice. These changes reflect the need to get maximum information from experiments approved for only small numbers of patients. The area of application of vector became less constrained as the experiments proceeded. The custom-made applicator offered the greatest area of concentration of transfer, which could later be most easily located for electrophysiological studies. In the "repeat-administration" study, probably much of the nasal cavity was perfused with vector producing a more diffuse effect.

Grubb et al. (1994) have suggested that injured epithelium more rapidly takes up adenovirus vectors. The mild mucosal injury in the first two patients of the single-dose trial may have enhanced gene transfer. This does not, however, explain the correction of V_t seen in the third patient in this series. In the first study, the nose had been pre-treated with tetracaine, which probably reduced mucociliary clearance (Ingels et al., 1994) and increased vector exposure time. Zabner et al. (1996a), in an epithelial culture experiment, have shown that adenovirus-mediated gene transfer to airway epithelia increases with prolonged incubation time. Mucociliary clearance, although altered in CF, may, therefore, impede efficient gene transfer. Furthermore, the abnormal mucus encountered in CF (Smith et al., 1996) may, in itself, represent a physical barrier to transduction. These variables, however, were present in all six reports. If simple pre-treatment with a ciliostatic agent after nasal saline irrigations increases gene transfer, this is of potential utility in subsequent studies. Lastly, the anatomical site of administration was different. The medial aspect of the inferior turbinate was used in the singe-application study and the inferior aspect of the inferior turbinate was used for repeat applications. Potential histological differences in these sites must vary from patient to patient (Kaplan et al., 1995); however, adenoviral transfection is less efficient in columnar epithelium, and squamous metaplasia would adversely affect gene transfer.

The question of the usefulness of the contralateral nasal cavity as a control also arises. Rhinovirus infections can spread from one nostril to another; however, in a series of monkey experi-

ments, Zabner et al. (1994) only occasionally derived RT-PCR positivity in cells from the control nasal cavity. All six studies suffer from the difficulties of drawing conclusions from generally small patient numbers. These difficulties are implicit in the need for maximum safety in human gene-transfer experimentation. In the United States, human gene-transfer protocols undergo an extra layer of review by the Recombinant DNA Advisory Committee (RAC) and permission to proceed is granted for only small numbers of subjects in each early experiment (Blau and Springer, 1995). Notwithstanding the discussed variations, the nasal model for gene transfer in CF is an essential tool in this emerging area of scientific interest. The nasal cavity has morphological features and CF-associated electrophysiological defects very close to those of the lower respiratory tract. It offers considerable benefits over pulmonary sites on initial testing of new gene transfer techniques. These benefits include ease of access for application and observation as well as measurability of response. Most important, however, is the increased safety profile associated with localized intranasal vector administration.

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