Nasal application of the cationic liposome DC-Chol:DOPE does not alter ion transport, lung function or bacterial growth


ABSTRACT: Liposome-mediated gene transfer is commonly used for in vitro transfection of deoxyribonucleic acid (DNA) into mammalian cells. We and others have recently demonstrated that this can be an effective method for in vivo delivery of plasmid DNA containing the human cystic fibrosis transmembrane conductance regulator (CFTR) gene to mouse models of cystic fibrosis (CF). This suggests that cationic liposomes may be useful for transferring CFTR complementary DNA (cDNA) into the airways of CF subjects. In this study, measurement of nasal potential difference (PD) was used to monitor the efficacy of correction of the CF bioelectric defect and to provide a sensitive assay of epithelial integrity.

We therefore assessed whether the cationic liposome DC-Chol:DOPE altered nasal ion transport parameters, in six normal and three CF subjects. Lung function was also measured as a further marker of safety. Finally, as CF airways are chronically infected, we studied whether DC-Chol:DOPE or DC-Chol:DOPE-DNA complexes altered the bacterial growth and sensitivities of CF sputum.

No significant effect was seen on any of these parameters, suggesting that DC-Chol:DOPE may be appropriate for use in human trials of liposome-mediated gene therapy for CF.


Despite considerable advances in therapy over the past 20 yrs, most patients with cystic fibrosis (CF) still die from respiratory failure. Following the identification of the bioelectric defect characteristic of CF [1] and isolation of the CF gene [2], strategies for gene therapy have been suggested that may arrest the progressive lung damage typical of the disease.

Cationic liposomes are commonly used for gene transfer in vitro, forming stable complexes with the negatively charged deoxyribonucleic acid (DNA) more efficiently than neutral liposomes, and having a potential advantage over viral methods in vivo, since the latter may induce an immune response. Recently, we [3], and others [4], have demonstrated that cationic liposome-mediated cystic fibrosis transmembrane conductance regulator (CFTR) complementary DNA (cDNA) transfection of the airways of CF mouse models in vivo can correct the bioelectric defect characteristic of CF, suggesting that human gene therapy using these methods is feasible. Although a recent report has demonstrated some cytotoxic effects of cationic liposomes on isolated cells in vitro [5], no adverse reactions were found in our CF mice, either histologically or physiologically.

The principal means of determining the efficacy of gene therapy in human subjects will be the measurement of airway ion transport. The major abnormalities in CF include a raised (more negative) baseline potential difference (PD), increased sodium absorption and decreased chloride secretion. The baseline PD depends on a combination of active ion transport processes and the total epithelial resistance, which relates mainly to the integrity of the tight junctions. Thus, a decrease in the baseline PD of the CF airway epithelium following gene therapy could occur either as a result of damage, or through correction of the bioelectric defect. The increased sodium absorption in CF is commonly studied using the sodium channel blocker amiloride, and the decreased chloride secretion by the application of isoprenaline, which induces chloride secretion through cyclic AMP mediated pathways. Changes in the charge of a lipid membrane can alter ion channel open probability [6], so it is possible that the positively charged liposome could alter the characteristics of sodium or chloride channels. Thus, administration of cationic liposomes alone could theoretically alter both the baseline nasal PD and the response to various interventions. Another theoretical effect of cationic liposomes in CF airways in vivo would be their incorporation into the cell wall of the bacterial flora, altering their susceptibility to antibiotics that induce bacterial cell wall lysis.
We have, therefore, studied whether the cationic liposome DC-Chol:DOPE altered the bioelectric properties of a human respiratory epithelium in vivo with two aims: firstly, to confirm safety; and, secondly, to ensure that they do not affect the bioelectric properties which will be used in vivo to monitor gene expression. As an additional measure of safety, lung function was also performed prior to and following nasal inhalation of the liposome. Finally, in view of the chronic infection typically found in the airways of the CF subjects, we also studied the effect of liposomes and liposome-DNA complexes on the common pathogens found in the sputum of CF subjects.

Materials and methods

Liposome

Liposomes were prepared from DC-Chol and DOPE (3:2 molar ratio) by sonication as described previously [7], and were stored for less than 28 days at 4°C. DC-Chol:DOPE was diluted in sterile water and delivered to each nostril using a pump spray (mass median aerosol diameter of ~60 µm) in 10 equal doses (50 µg·200 µl⁻¹), administered every 15 min for a total of 135 min. This period was chosen to match that for our proposed gene therapy trial, with the aim of continuously bathing the nasal epithelium in liposome-DNA complexes for at least 2 h. As the time to transport exogenous substances along the floor of the nose of CF subjects is of the order of 15–30 min [8], we chose repeated doses every 15 min to maximize the contact time.

Subjects

Six nonsmoking males, mean age 27 yrs, (range 21–36 yrs), with no history of any respiratory disorders, taking no regular medications, were studied as normal subjects. Three nonsmoking males with CF, aged 19–23 yrs, homozygous for the ΔF508 mutation were also studied - all had abnormal sweat tests, and were pancreas-insufficient. No subject had suffered an upper respiratory tract infection for at least one month prior to study. The study was approved by the Hospital Ethics Committee and all subjects gave informed consent.

Physiology

Nasal PD was measured along the floor of each nostril, using the method described previously [8], with a minor modification to allow perfusion of the surface epithelium. The maximal PD for each nostril was measured, and the catheter positioned at this point for subsequent perfusion. Following measurement of the baseline PD, the effect of sequential superfusion with the following solutions was measured: amiloride HCl (100 µM), amiloride in a low chloride (6 mM) solution (gluconate substitution), and finally amiloride HCl in low chloride solution with isoprenaline (10 µM as the hemisulphate salt). The results from both nostrils were averaged for each patient at each time-point.

These measurements were performed prior to, and at one and five days following nasal application of the DC-Chol:DOPE. Nasal PD was not measured immediately following the liposome inhalation, as perfusion may accelerate removal of the liposome from the nose, and also to maintain uniformity with our proposed gene therapy trial. Measurements of spirometry (Vitalograph Compact) were taken before, immediately after, and 1 and 5 days after liposome application.

Bacteriology

Samples of sputum from 10 CF patients were individually homogenized with an equal volume of Ringer’s saline solution, and three 1 ml aliquots were then taken. DC-Chol:DOPE (250 µg) was added to the first aliquot, DC-Chol:DOPE (250 µg) complexed with DNA (50 µg pCMVβ Clontech Inc.) to the second aliquot, and an equal volume of normal saline added to the control. Samples were then cultured in parallel on chocolate agar, McConkey’s agar, mannitol salt agar, Difco Pseudomonas sp. medium, MAST Pseudomonas cepacia medium, and Sabouraud medium, and incubated at 37°C. After 2 days, the cultures were quantitated, and sensitivities assessed [9]. The Wilcoxon signed rank test was used for statistical analysis, and the null hypothesis rejected at p<0.05.

Results

Physiology

Nasal inhalation of the liposome was well-tolerated by all subjects. Nasal PDs prior to and following liposome are shown in figure 1. There was no statistically significant difference between any of the measurements on the three different days for either group. Spirometry results are shown in table 1. Again, no significant changes were seen following liposome inhalation.

Bacteriology

Positive sputum cultures in the 10 CF patients included *Pseudomonas aeruginosa* (8), *Staphylococcus aureus* (4), and *Pseudomonas cepacia* (1). There was no significant change in the colony counts following the addition of either liposome or DNA-liposome complexes. One culture of mucoid *Pseudomonas Aeruginosa* showed a small change in antibiotic sensitivity following the addition of DNA-liposome, with resistance to 12 of 16 antibiotics, compared to 10 of 16 antibiotics in the liposome and control groups. All other samples showed identical antibiotic sensitivities.
We have recently demonstrated that in vivo CFTR cDNA transfection with DC-Chol:DOPE can correct the characteristic ion transport defect in the airways of the mouse model of CF created by insertional mutagenesis [3]. No deleterious effect of cationic liposomes on airway ion transport or histology was seen. Although DC-Chol:DOPE has been approved for use in human trials of gene therapy for advanced malignancy [10], prior to its use in the airways of human subjects with CF, we sought confirmatory evidence that DC-Chol:DOPE is safe, and does not affect the ion transport properties of the human respiratory epithelium.

Nasal PD is a sensitive indicator of epithelial integrity, and can be used to measure subtle changes of epithelial damage [11]. Thus, the constant baseline nasal PD throughout the study suggests that no significant damage occurred. This, together with the stable lung function and the lack of effect on sputum bacteriology, provides preliminary evidence for the safety of a single dose of this cationic lipid in the human respiratory tract, at least over the five day period studied. As we did not measure the nasal bioelectrics until 24 h following inhalation, we may have missed a transient effect resolving completely by this time. Further safety studies will be required before DC-Chol:DOPE is administered to the lower airways, or given repeatedly. To the best of our knowledge, no studies of the effect of cationic liposomes on the human respiratory epithelium in vivo have been reported. Our findings are in accord with a previous study showing that phosphatidylcholine, a neutral lipid, has no acute deleterious effect on lung function as measured by spirometry in 10 human subjects [12].

The nasal epithelium also demonstrates the characteristic chloride impermeability found in CF [11], and will be used to measure the degree of correction obtained in trials of gene therapy for CF. The stable responses to amiloride, low chloride and isoprenaline solutions throughout the study suggest that DC-Chol:DOPE does not directly change the nasal ion transport properties measured.

In conclusion, the lack of effect of the cationic lipid DC-Chol:DOPE on any of the parameters studied suggests that this lipid is appropriate for our proposed trials of gene therapy for CF.

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References


