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D. J. Harris , J. Marshall , E. R. Lee , C. S. Siegel , D. Mcneilly , N. S. Yew , J. Nietupski , M. Nichols , M. Cherry , N. Wan , C. Jiang , M. Lane , E. Rowe , R. K. Scheule , A. E. Smith & S. H. Cheng

[a] Genzyme Corp. , One Kendall Square, Cambridge, MA 02139, USA

[b] Genzyme Corp. , One Kendall Square, Cambridge, MA 02139, USA

[c] Genzyme Corp. , One Kendall Square, Cambridge, MA 02139, USA

[d] Genzyme Corp. , One Kendall Square, Cambridge, MA 02139, USA

[e] Genzyme Corp. , One Kendall Square, Cambridge, MA 02139, USA

[f] Genzyme Corp. , One Kendall Square, Cambridge, MA 02139, USA

[g] Genzyme Corp. , One Kendall Square, Cambridge, MA 02139, USA

[h] Genzyme Corp. , One Kendall Square, Cambridge, MA 02139, USA

[i] Genzyme Corp. , One Kendall Square, Cambridge, MA 02139, USA

[j] Genzyme Corp. , One Kendall Square, Cambridge, MA 02139, USA

[k] Genzyme Corp. , One Kendall Square, Cambridge, MA 02139, USA

[l] Genzyme Corp. , One Kendall Square, Cambridge, MA 02139, USA

[m] Genzyme Corp. , One Kendall Square, Cambridge, MA 02139, USA

[n] Genzyme Corp. , One Kendall Square, Cambridge, MA 02139, USA
[o] Genzyme Corp. , One Kendall Square, Cambridge, MA 02139, USA
[p] Genzyme Corp. , One Kendall Square, Cambridge, MA 02139, USA
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THE DEVELOPMENT OF IMPROVED CATIONIC LIPIDS FOR GENE TRANSFER INTO CYSTIC FIBROSIS AIRWAY EPITHELIAL CELLS

D. J. HARRIS, J. MARSHALL, E. R. LEE, C. S. SIEGEL, D. MCNEILLY, N. S. YEW, J. NIETUPSKI, M. NICHOLS, M. CHERRY, N. WAN, C. JIANG, M. LANE, E. ROWE, R. K. SCHEULE, A. E. SMITH, AND S. H. CHENG.
Genzyme Corp., One Kendall Square, Cambridge, MA 02139, USA.

Abstract In an effort to improve the efficiency of cationic lipid mediated gene transfer, over 90 novel cationic lipids of diverse structural types were synthesized and evaluated *in vitro*. Four cationic lipids derived from phospholipids were examined. The most promising cationic lipid formulations were tested *in vivo* by intranasal or transtracheal instillation into the lungs of BALB/c mice. The most active formulations gave CAT reporter gene expression levels which are greater than 500 fold over that which could be attained using free DNA alone. Certain cationic lipid formulations have been shown to facilitate substantial expression of the CFTR (cystic fibrosis transmembrane conductance regulator) gene *in vitro* as determined by the SPQ and Ussing Chamber assays.

Key Words: cystic fibrosis, gene transfer, cationic lipids, phospholipids

INTRODUCTION

Cationic lipids are able to introduce genes into a variety of different cell types [1]. They offer a number of advantages over the use of viral vectors, the most important of which is their potential lack of immunogenicity. However, the efficiency of cationic lipid mediated gene transfer is less than that achieved by viral-based approaches. We at Genzyme have a multi-disciplinary program focused on the development of effective non-viral gene transfer technology for use in the treatment of cystic fibrosis.

RESULTS AND DISCUSSION

In an effort to improve the efficiency of cationic lipid mediated gene transfer, over 90 novel cationic lipids of diverse structural types were synthesized and evaluated using a high-throughput 96 well-based assay. In many cases, cationic lipid formulations that contained dioleoylphosphatidylethanolamine as a co-lipid were found to have the highest transfection efficiencies. A process for the synthesis of dioleoylphosphatidyl-ethanolamine was developed based on Genzyme phospholipid synthesis technology [2] and GLP grade material was prepared via this route (Figure 1). We have evaluated four cationic lipids based on phospholipids (Figure 2). DOGE and DMGE (Avanti Polar Lipids) were found to have transfection efficiencies which were approximately 1.5 to 2 fold greater than DMRIE or DC-chol *in vitro*. DMRIE [3] and DC-chol [3, 4] have been used in gene transfection clinical trials. Cationic Lipids #21 and #54 were only marginally active *in vitro* and not active *in vivo*, under the respective test conditions.

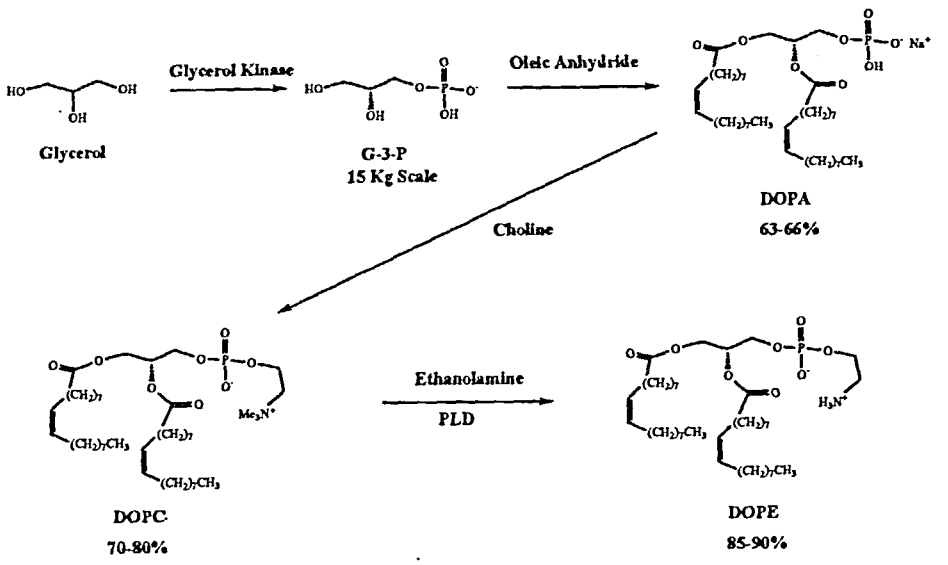

FIGURE 1 Synthesis of dioleoylphosphatidylethanolamine.

Several new cationic lipids with improved transfection efficiencies (greater than 3-fold DMRIE or DC-chol) were identified (Figure 3). Using the optimal conditions, transfection of greater than 80% of airway cells *in vitro* could be achieved. Transfection of CF airway epithelial cells using a CFTR-encoding plasmid restored cAMP-stimulated chloride channel activity as assessed using the SPQ assay [5] and corrected the cAMP-mediated chloride current using the Ussing chamber assay [6]. The most promising cationic lipid formulations were tested *in vivo* by intranasal or transtracheal instillation into the lungs of BALB/c mice. Using a formulation of cationic lipid #67 and the pCMVHI-CAT vector (a reporter gene vector), expression of up to 400 ng of chloramphenicol acyltransferase (CAT) enzyme per mouse lung was achieved. These levels are greater than 500 fold over that which could be attained using free DNA alone. Although expression decreased markedly after a few days, CAT activity could be detected up to 21 days post-instillation.

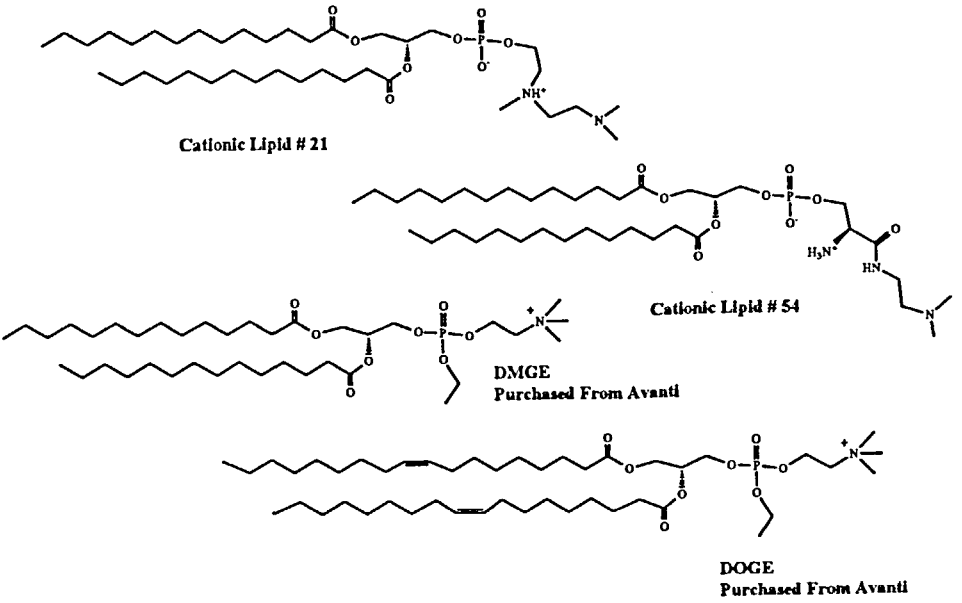

FIGURE 2 Phospholipid Based cationic lipids.

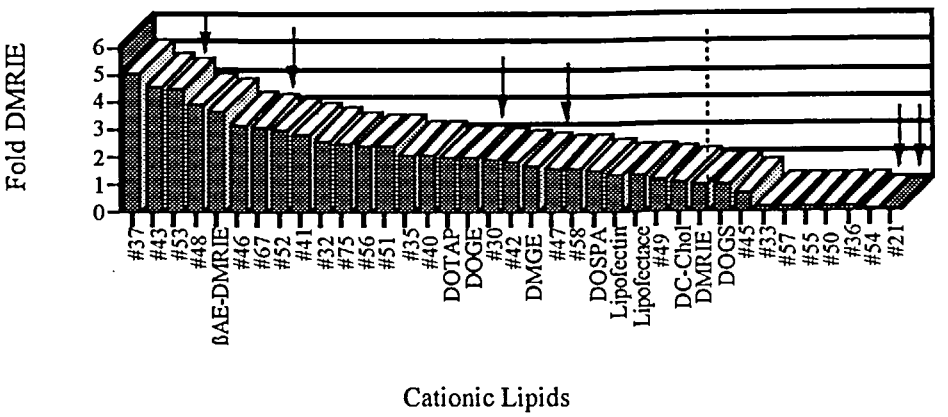

FIGURE 3 Rank order of cationic lipids on CFT1 cells.

CONCLUSIONS

Significant progress has been achieved in increasing the efficiency of cationic lipid mediated gene transfer. These results suggest that with further optimization, cationic lipid mediated gene transfer may be clinically efficacious for the treatment of cystic fibrosis.

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