Aerosol Delivery of Concentrated pDNA/PEI Formulations to the Murine Lung

Lee A. Davies[1,2], Stephen C. Hyde[1,2] & Deborah R. Gill[1,2]

1. Gene Medicine Research Group, NDCLS, John Radcliffe Hospital, University of Oxford, Oxford, UK
2. The United Kingdom Cystic Fibrosis Gene Therapy Consortium

lee.davies@ndcls.ox.ac.uk http://users.ox.ac.uk/~genemed

Overview:

- The UK Cystic Fibrosis Gene Therapy Consortium is committed to the testing and development of gene therapy vectors for CF clinical trials
- Successful lung gene therapy vectors will require topical administration to the airway epithelium via aerosol delivery
- The cationic polymer 25kDa polyethylenimine (PEI) has demonstrated successful gene expression following aerosol administration to the mouse lung
- Viability of PEI formulations for clinical applications is currently limited by low maximal pDNA concentrations of <0.5mg/ml
- We have utilised ultrafiltration to generate concentrated PEI (cPEI) formulations containing >8mg/ml pDNA and we have examined gene expression and toxicity following administration of these formulations to the mouse lung

Results:

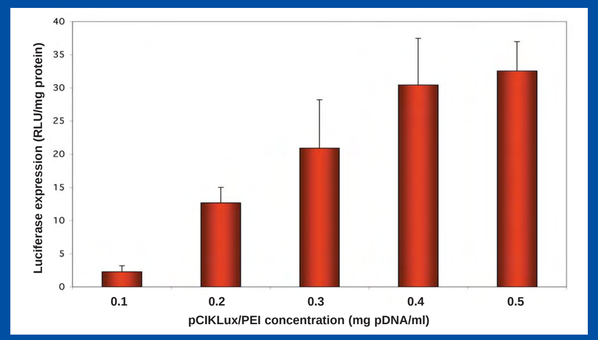

Figure 1: Aerosol administration of standard pDNA/PEI formulations

Gene expression following aerosol delivery of standard pDNA/PEI formulations was examined in a mouse lung model (see methods)

Mice were exposed to aerosols containing plasmid pCIKLux (expressing firefly luciferase) complexed with 25kDa PEI (N:P ratio 10:1) over a range of pDNA concentrations

Formulation of pDNA complexes >0.5mg/ml resulted in precipitation

No significant increase in lung luciferase expression was observed using formulation concentrations greater than 0.3mg/ml ($p>0.05$ ANOVA)

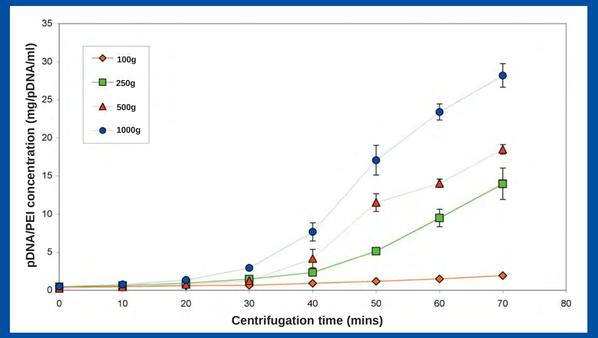

Figure 2: Concentration of pCIKLux/PEI formulations by ultrafiltration

Formulations of pCIKLux/PEI prepared at 0.2mg/ml were concentrated by centrifugation using a Centriplus YM-100 ultrafiltration unit (Millipore Ltd, Watford, UK) equipped with a 100kDa cutoff cellulose filter

Time dependent concentration of pCIKLux/PEI formulations to over 20mg/ml was observed

Rate of concentration by ultrafiltration was shown to be dependent upon centrifugation speed

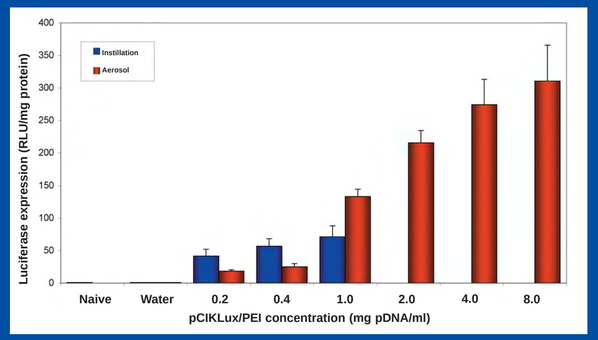

Figure 3: Gene expression following delivery of cPEI formulations

Gene expression in the mouse lung was investigated following instillation or aerosolisation of cPEI formulations

No significant increase in luciferase expression was observed following instillation of cPEI formulations

Instillation of 1mg/ml pCIKLux/PEI was associated with high mortality and higher concentrations were omitted from the study

Aerosol delivery of cPEI formulations resulted in a dose dependent increase in lung luciferase activity

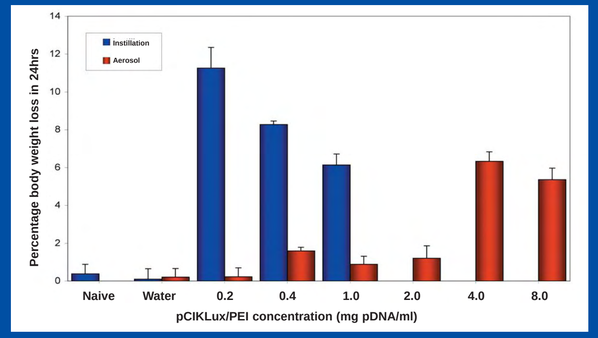

Figure 4: Weight loss following administration of cPEI formulations

To assess toxicity of cPEI formulations, weight loss in the 24 hr period after treatment was recorded for all mice following cPEI delivery

Instillation of all PEI formulations resulted in a significant loss of body weight in all treated mice

Aerosol delivery resulted in significant weight loss only in mice exposed to aerosols containing pCIKLux/PEI concentrations of 4mg/ml and above

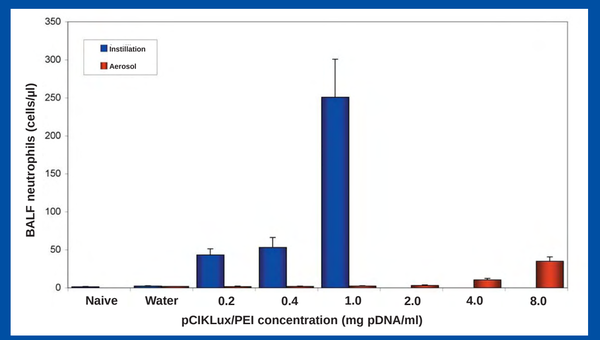

Figure 5: BALF neutrophils following administration of cPEI formulations

To quantify lung toxicity, the number of neutrophils detected in bronchalveolar lavage fluid (BALF) was determined in all mice following cPEI delivery

Instillation of all PEI formulations resulted in significant elevation of BALF neutrophils but highest levels were seen in mice dosed with 1mg/ml cPEI

Aerosol delivery resulted in significant elevation of BALF neutrophil levels only in mice exposed to aerosols containing pCIKLux/PEI concentrations of 8mg/ml

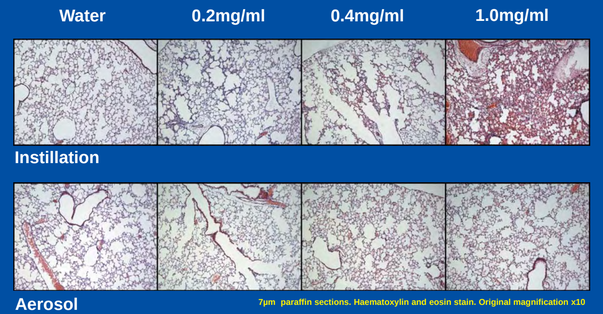

Figure 6: Histological analysis of lung sections following administration of cPEI formulations

Instillation of PEI formulations was associated with dose dependent histological changes ranging from patchy areas of interstitial inflammation and necrosis in mice receiving 0.2mg/ml pCIKLux/PEI to severe and widespread inflammation in mice receiving 1mg/ml pCILux/PEI

Aerosol administration of all cPEI concentrations up to 8mg/ml was associated with minor inflammatory changes similar to animals receiving water aerosols and consisting of mild oedema and small numbers of inflammatory foci

Conclusions:

- Concentrated pDNA/PEI formulations can be generated by ultrafiltration of standard low concentration formulations
- Aerosol administration of cPEI formulations is associated with high levels of gene expression and low levels of vector toxicity in the mouse lung model
- Delivery method is an important determinant for lung toxicity following cPEI administration
- Concentrated pDNA/PEI formulations demonstrate improved viability for lung gene therapy applications

Methods:

Aerosol delivery
Female BALB/c mice (n=6) were placed in an 8L perspex whole body aerosol exposure chamber and aerosol was administered using an Aerotech II jet nebuliser (CIS-US Inc, Bedford, MA, USA) operating at 40psi with 5%CO_2 as the driving gas. A total volume of 10ml aerosol was delivered in each study

Nasal instillation
Female BALB/c mice (n=6) were anaesthetised with isofluorane and a total volume of 100µl of complex was administered via nasal insufflation

Produced by Lee Davies, Oxford University GeneMedicine and the Medical Informatics Unit, Nuffield Department of Clinical Laboratory Sciences, University of Oxford. Telephone +44 (0)1865 220545