Repeat Administration Of pDNA/Polyethylenimine (PEI) Aerosols To The Murine Lung Is Associated With A Loss Of Gene Transfer Efficiency

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Overview:

- We are investigating gene transfer to the terminally differentiated epithelial cells of the airways, the target for the treatment of cystic fibrosis lung disease
- As with many chronic diseases, gene therapy for cystic fibrosis will require long-term gene expression and/or repeated administration of gene therapy vectors
- Unlike many viral vectors where repeat administration may be severely compromised by neutralising antibodies, non-viral vectors such as plasmid DNA/PEI polyplexes are not associated with significant immune responses in the lung and may provide an appropriate alternative for long term gene therapy
- Gene expression produced by current non-viral gene transfer agents is transient in nature and successful gene therapy will require an efficient vector that can demonstrate robust gene expression upon readministration to the lung
- We have utilised aerosol delivery of pDNA/PEI complexes to the lungs of mice as a model to investigate repeat administration of this non-viral gene transfer agent

Results:

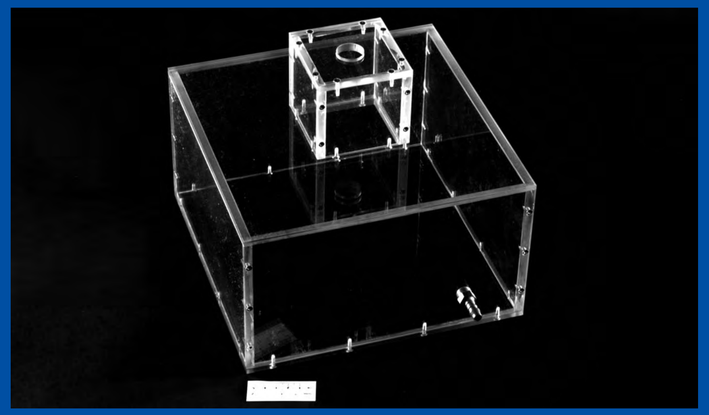

Figure 1: Aerosol exposure chamber

Female BALB/c mice (8-16 wks) housed in a whole body exposure chamber (Figure 1) were exposed to aerosols containing 25kDa branched PEI (Sigma, Poole, UK) complexed to the plasmid pCIKLux expressing the firefly luciferase gene under control of the CMV immediate/early promoter. pDNA/PEI complexes were prepared in sterile water at a DNA concentration of 0.2mg/ml and with a PEI nitrogen (N) to pDNA phosphate (P) ratio of 10:1. For each exposure 20 ml of the pDNA/PEI formulation was aerosolised using an Aerotech II nebuliser (CIS-US Inc, Bedford, MA USA) operating at 40psi with 5% CO_2 as the driving gas. Luciferase quantification was performed on whole lung homogenates using the Luciferase Assay System (Promega, Southampton, UK) and normalised for protein content prior to graphing.

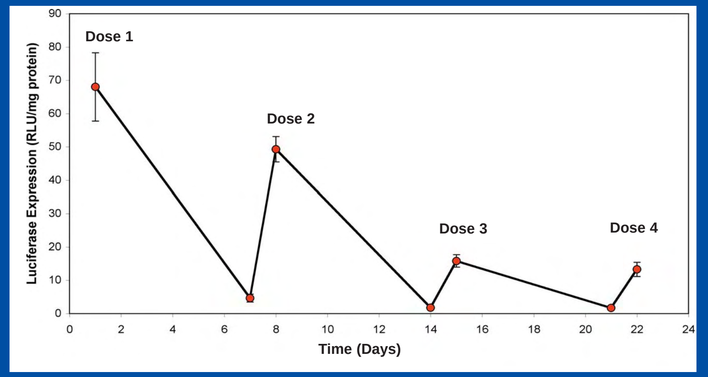

Figure 2: Repeat administration of DNA/PEI aerosols

Mice were exposed to repeated aerosols of pCIKLux/PEI delivered at 7 day intervals. Lung luciferase expression was measured in groups of mice (n=7) sacrificed 1 day and 7 days after each aerosol. Luciferase expression of 68.0 ± 10.3 RLU/mg (mean ± SEM) was detected in the lungs of treated mice 1 day after the initial aerosol dose. As expected, expression was transient and fell to 4.7 ± 1.2 RLU/mg by day 7, probably due to the attenuation of the CMV promoter (Gill *et al*, Gene Therapy 8: 1539-46, 2001). Following a second aerosol exposure, lung luciferase expression 1 day later was significantly lower than initial levels at 49.3 ± 3.8 RLU/mg (p=0.027 ANOVA/PLSD analysis). Further reductions in lung luciferase levels were observed 1 day after the third (15.8 ± 1.9 RLU/mg) (p<0.0001) and fourth (13.3 ± 2.1 RLU/mg) (p<0.0001) aerosol exposures. These results demonstrate that repeat administration of pDNA/PEI aerosols is associated with a reduction in subsequent gene expression.

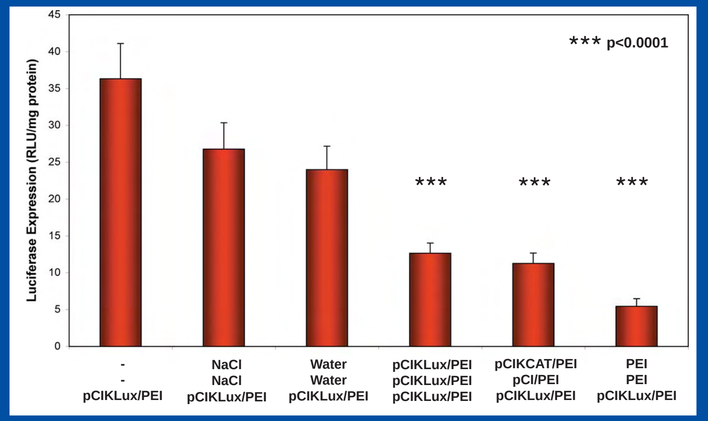

Figure 3: PEI mediates inhibition of gene expression

Groups of mice (n=6) were exposed to two aerosols (7 day interval) containing either 0.9% NaCl, water, pCIKLux/PEI, PEI complexed to control plasmids or PEI prepared alone. All groups received a final aerosol containing pCIKLux/PEI and lung luciferase expression 1 day later was compared with levels observed in control mice receiving only the final pDNA/PEI aerosol. Luciferase expression was significantly reduced (p<0.0001 ANOVA/PLSD) in all groups that received prior treatment with aerosols containing PEI or pDNA/PEI complexes. These results demonstrate that exposure to PEI alone was sufficient to mediate the observed inhibition of gene expression upon administration of pCIKLux/PEI aerosols.

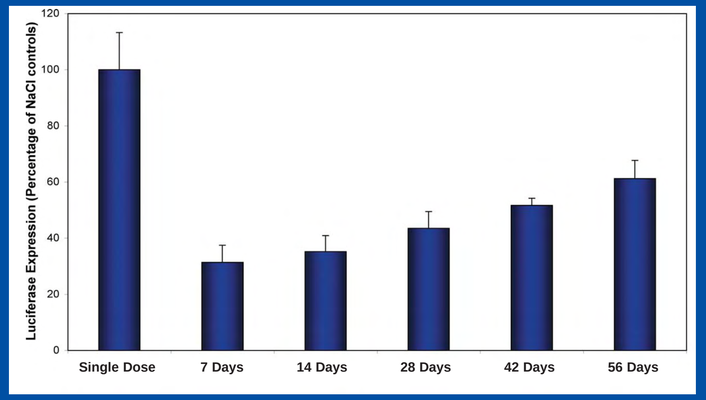

Figure 4: Refractory period for pDNA/PEI aerosols in the murine lung

Groups of mice (n=6) were exposed to 3 successive pCIKLux/PEI aerosols delivered at time intervals ranging from 7 days to 56 days. For each group, a control group of mice received 2 initial doses of NaCl aerosol followed by a final pCIKLux/PEI aerosol delivered at corresponding time intervals. 1 day after the final aerosol exposure, lung luciferase expression was determined for each treatment group and expressed as a percentage of the expression observed in the corresponding NaCl control group. All treatment groups demonstrated reduced luciferase expression compared to control animals (p<0.02 ANOVA/PLSD). Increasing the time between aerosol exposures resulted in higher levels of luciferase expression in treatment groups. However, even with a dosing interval of 56 days luciferase expression attained only 61% of the levels seen in control animals. Calculations determined that a 120 day dosing interval would be required to observe complete restoration of luciferase expression levels. These results demonstrate that there is a refractory period for readministration of pDNA/PEI aerosols in the mouse lung.

Conclusions:

- Repeat administration of pDNA/PEI aerosols to the murine lung is associated with a modest reduction in subsequent gene expression
- Prior exposure to PEI alone is sufficient to mediate the observed inhibition of gene expression upon administration of pDNA/PEI aerosols
- There is a measurable refractory period for the repeat administration of pDNA/PEI aerosols to the murine lung

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