

PROGRESS AND BARRIERS TOWARD LENTIVIRAL AIRWAY GENE TRANSFER DEVELOPMENT WITHIN THREE ANIMAL MODELS

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Introduction

CFTR gene transfer to treat or cure the airway disease in CF remains a challenge, although problems with efficiency, appropriate targeting, and sufficient longevity have limited progress. We have examined airway gene transfer in mouse nose and development, sheep lung, and marmoset lung to assess suitability in a primate lung. Using a lentiviral gene vector coupled with a brief airway instillation designed to access airway stem/progenitor cells, we examined successful gene transfer techniques with potential to produce permanent or transient CFTR gene expression to reverse CF airway disease.

Methods

Airways were dosed in two steps, starting with LPC (lysophosphatidylcholine, a component of lung surfactant that is essential for airway gene transfer and followed by a second step. The effect of the dosing was examined in mouse nasal airways. Acute effects were assessed after 7 days, while long-term effects were followed for up to two years.

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References

- a) Stocker et al J Gene Med 2009; b) Parsons et al J Gene Med 2009; c) Donnelley et al, J Synch Radiation 2009.

Results

Mice: In nasal airway, a single brief dose can produce reporter gene expression that lasts for a mouse lifetime. Single dose airway gene transfer into CF mouse nasal airway produced significant gene correction for at least 12 months (Fig 1b); an (incomplete) confirmatory study shows sustained ~40% gene correction towards normal levels for more than 6 months (not shown).
Sheep: In lung airway we observed successful gene transfer (Fig 3). In part, the low level reporter gene expression in mouse lung (not shown) were due to the small dose volumes we were able to generate for studies in this human-sized lung. Improved vector dose production is required and studies of improved techniques are planned.
Marmosets: In the two marmoset monkeys studied to date LacZ reporter gene expression was observed in the conducting airways (Fig 4), showing for the first time that airway gene transfer is well tolerated, but we noted some evidence of (recovering) epithelial cell disturbance, this is to be investigated in further studies. A transient (day 2) serum antibody response to the virus-vector surface protein (VSV-G) was lost by day 3.
Transduced cell types: Mouse, sheep and marmoset airway gene transfer was observed in the desired ciliated surface cells as well as basal cells (Fig 5).

New measurements of airway surface imaging technique using synchrotron X-rays that we recently described is providing the first insights into mucociliary transport behaviour of individual particles in live mice. This method may be able to track therapeutic improvements in airway mucociliary function in mice, with potential to monitor airway function in CF mice.

Conclusions

Single dose lifetime airway gene transfer is possible in mice, indicating involvement of airway progenitor cells in the persistence of gene expression. Lung gene transfer is possible in sheep and marmoset lung, but larger doses are needed to produce significant gene correction.



method for delivering therapeutic genes into lung airways for cystic fibrosis. Finally, our new X-ray method has the potential to provide rapid non-invasive measurement of airway physiology associated with a