DELIVERY DYNAMICS AND DESTINATION OF GENE VECTOR INSTILLATIONS IN LIVE MOUSE AIRWAYS

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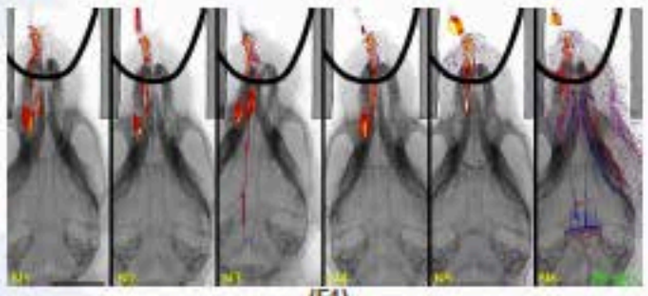
BACKGROUND: Most groups use the mouse nose as an *in vivo* model site for developing gene treatments for CF airway disease. Reliable dosing of an airway pre-treatment followed by a lentiviral vector is essential for the success of our gene transfer protocols. Despite using standardised delivery techniques we see variability in reporter-gene histological assessments and electrophysiological measurements. The aim of this experiment was to use synchrotron X-ray imaging at SPring-8 to accurately determine the fate of fluids delivered into live mouse nasal airways and the trachea.

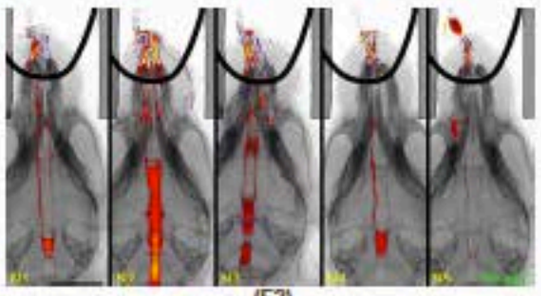
METHODS: Nembutal anaesthetised C57Bl/6 mice (n = 6 nasal & n = 4 trachea) were imaged on the BL20B2 beamline at the SPring-8 synchrotron (Japan). For the **nasal study** images were captured at 1Hz. After 1 minute of baseline a 4 µl sample of iodine-based contrast fluid (airway pre-treatment surrogate) was delivered over 10 sec. After 10 min of data collection an additional 20 µl bolus (gene-vector surrogate) was delivered over 30 sec. Imaging continued for a further 10 min. Fluid motion was revealed using a background subtraction method. For the **tracheal study** mice were intubated and ventilated at 80 br/min with 1 image captured per breath. After 1 min of baseline a 15 µl bolus (airway pre-treatment or gene-vector surrogate) was delivered over 30 sec. Following 20 min of data collection an additional 15 µl bolus was delivered over 3.6 sec. Image capture continued for a further 10 min. Frame differencing was used to reveal fluid motion.

NASAL AIRWAY RESULTS:

- The **4 µl dose (F1)** was retained on the treated side, supporting the strength of gene expression seen in nasal ciliated epithelium and validating the use of a 4 µl dose to retain an untreated nasal airway as a within-animal control.
- Confirms why gene expression is limited to the treated side when it extends into the tubular nasopharynx.
- Although the dose also distributes into the lateral transitional/olfactory region, expression from the gene vector was restricted to respiratory epithelium in our gene transfer studies.
- The **20 µl dose (F2)** overwhelms the 'holding capacity' of the dosed nostril, with some fluid continuing into the trachea and likely into deeper lung airways.
- Supports our finding of long term lung expression using a LV-Luc vector delivered to nose and suggests smaller vector doses may produce the same levels of nasal expression.
- Supports the proposed LPC mechanism of action since we only observe gene expression in areas reached by the 4 µl dose, despite demonstrating the wide reach of the 20 µl surrogate.



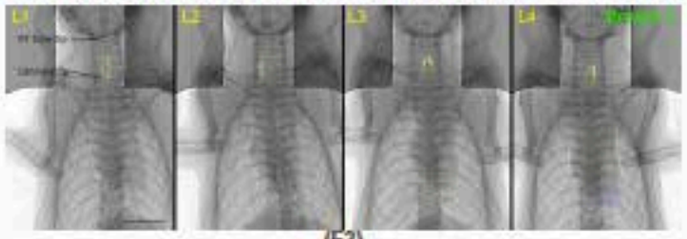
(F1)



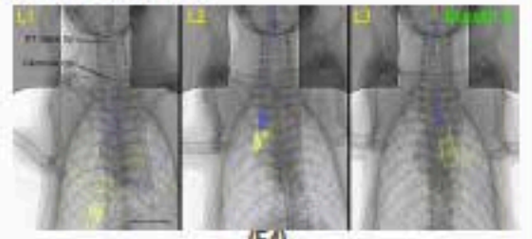
(F2)

TRACHEA RESULTS:

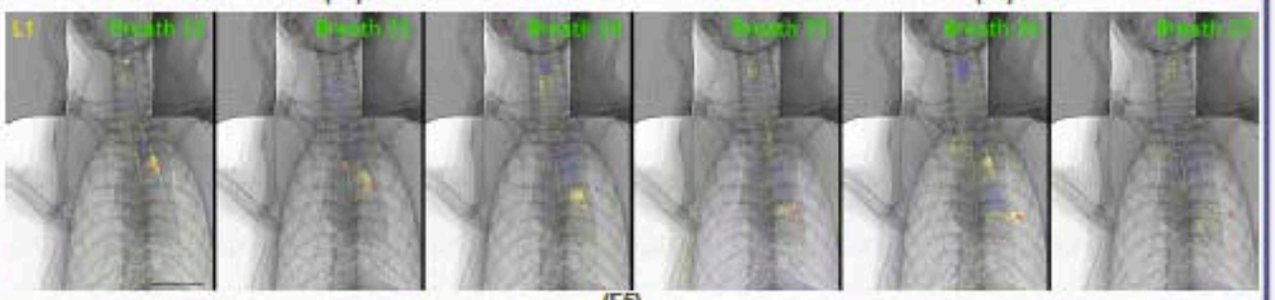
- Substantial dose losses may occur upon delivery via immediate retrograde fluid motion (F3).
- Speed of bolus delivery (**30 sec in F3 versus 3.6 sec in F4**) into the lung may influence the relative targeting of conducting airways and deep lung.
- The fast fluid bolus delivery created larger localized increases in the contrast fluid volume that were more easily visualized (F4) using this technique.
- A bolus of fluid, marked with a red X, can still clearly be seen moving down the left main bronchus (F5) of one mouse that received 15 µl of fluid over 30 seconds.



(F3)



(F4)



(F5)

CONCLUSION: Synchrotron imaging can help explain the mechanisms underlying published outcomes from our gene-transduction protocols in mouse nasal airways. Our findings suggest the need for, and permit, much greater attention to dosing specifics – animal orientation, volume, speed – and enable improvements in dosing technique design.

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