HIGH SPEED X-RAY IMAGING REVEALS DOSED-FLUID DYNAMICS AND FATE IN MOUSE NASAL AND LUNG AIRWAYS

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(F1)

BACKGROUND:

- Mice are commonly used for developing gene therapy treatments for CF airway disease, but little is known of how fluid doses distribute after delivery into the airways.
- To help understand the variability in reporter-gene expression and functional correction of CFTR gene function after vector delivery, we have developed synchrotron X-ray imaging techniques to determine the fate of fluid doses delivered into live mouse nasal airways.
- In this study we used high spatial and temporal resolution X-ray imaging to determine the destination and behaviour of (surrogate) fluid dose deliveries to the mouse nose and lung.

METHODS:

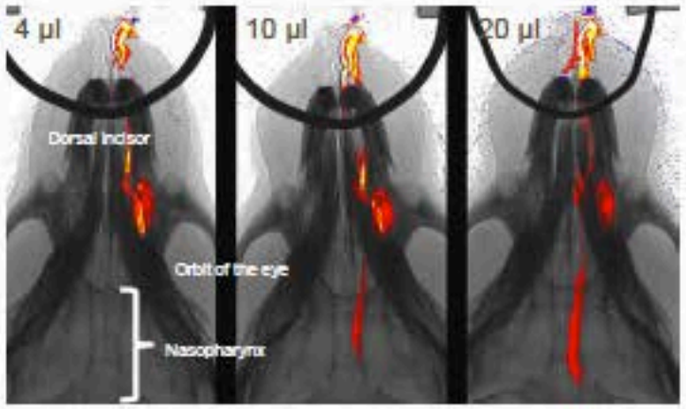
- Nembutal-anaesthetised C57Bl/6 mice were imaged at the SPring-8 synchrotron *(Fig. 1)*. Mice were freely breathing for nasal studies, and intubated and ventilated (80 br/min) for lung studies
- An iodine-based contrast fluid mixture was used to mimic a standard gene transfer dose delivery.
  - **Nose**: 4, 10 or 20 µl (n = 5 each)
  - **Lung**: 15 or 30 µl (n = 5 each)
- Images were acquired at 6.7 frames per second for 5 minutes following delivery initiation. Pseudo-colouring techniques revealed the progress of fluid along the airways over time.

RESULTS:

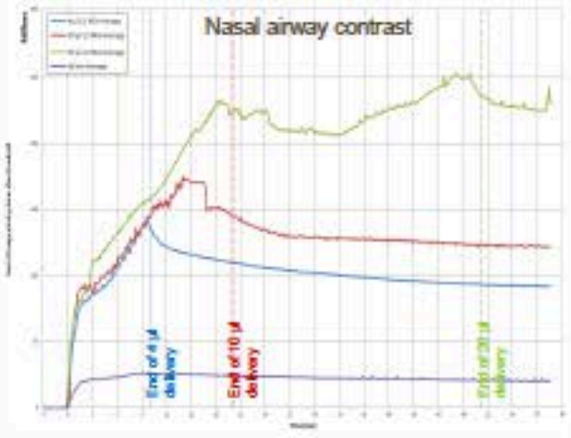
Nose:

- Fluid distributions were dose-dependent.
- The 4 µl dose typically remained in the anterior and olfactory regions. Higher doses 'overflowed' into the nasaopharyngeal airway towards the lung and into the contralateral nasal spaces. Residual dose in the distal nasopharynx often refluxed proximally.
- *Fig. 2* shows a comparison of the 4, 10 and 20 µl doses ~17 sec after delivery initiation, with the fluid artificially coloured using background subtraction.
- *Fig. 3* shows that the change in X-ray absorption contrast during nasal fluid delivery can act a surrogate for measuring the fluid volume in the nose.

(F2)



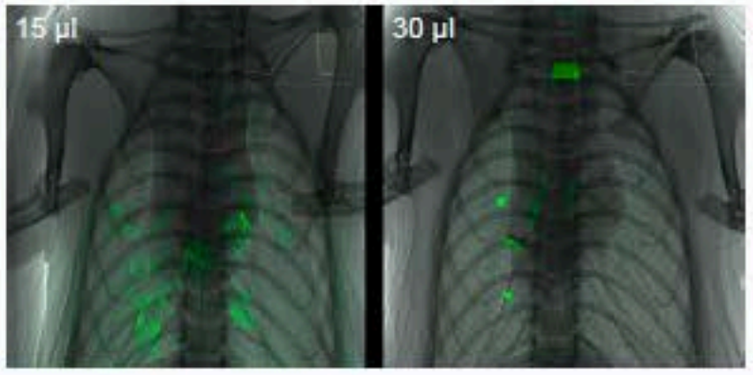
(F3)



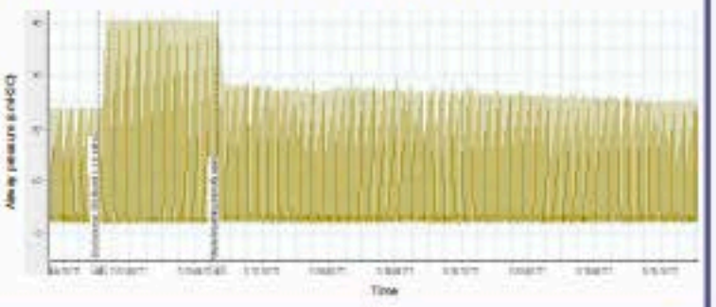
Lung:

- The advancing of dose fronts along conducting airways and into distal alveolar spaces was easily followed. Dose progress into the lung tree was patchy and not uniform across animals.
- Some deliveries induced a respiratory pause, and cases of subsequent fluid clearance (i,e. potential loss) also occurred.
- *Fig. 4* shows a 15 µl (left) and 30 µl dose, with fluid movement artificially coloured using frame differencing (same timepoint in successive breaths).
- *Fig. 5* shows the change in airway pressure during dosing associated with a 40 second respiratory pause.

(F4)



(F5) Respiratory pause during lung fluid dosing



CONCLUSION:

These novel non-invasive imaging techniques reveal the complex real-time dynamics of airway dosing in mice and could also be applied to understanding dose delivery in other animal models. The variability noted suggests therapeutic dosing outcomes may be influenced by heterogeneous dose distributions that naturally occur in the anatomically complex nasal and lung airways.

ACKNOWLEDGEMENTS:

Supported by NH&MRC Australia and the Cure4CF Foundation. SPring-8 experiments performed under proposal 2011A1306. Travel funding provided by the Australian Synchrotron ISA Program. We thank Naoto Yagi, Kentaro Uesugi and Andreas Fouras for their assistance.