Medical Bio EX Proposal Report

Proposal number:

2009B1922

Title of experiment:

Improved synchrotron X-ray detection of pollutant particle behaviour after deposition onto lung airways

Name and affiliation of the project leader:

Dr David Parsons, Women's and Children's Hospital, North Adelaide, South Australia

Beamline used:

BL20XU Downstream – Biomedical Imaging Centre

Research purpose and background

Air pollution is a significant environmental and health concern. Man-made airborne pollutants produced by a wide variety of sources exist in liquid, gaseous or solid forms. Solid particulates include coal and mineral dusts from mines and quarries, fibreglass and asbestos fibres, lead fumes from smelters and vehicles to dusts from construction and demolition. We have previously reported a new technique to image these types of solid particulates in mouse nasal [1] and tracheal [2] airways. These particulates have the potential to produce deleterious acute or delayed health effects that can impact on asthma, cystic fibrosis (CF), bronchitis, emphysema, lung and heart disease, and respiratory allergies.

This experiment is part of our wider effort towards developing a cure for the CF gene defect in the airways of sufferers. In CF mucociliary transport (MCT) can be affected resulting in stagnation of airway secretions, obstruction establishment, unstoppable infection and eventual lung failure. In attempting to determine the effectiveness of genetic [3, 4] and other potential therapeutics for CF airway disease we have developed MCT monitoring methods that can be used *in-vivo* in mice. Existing methods for quantifying bulk particle clearance in airways [5] are unable to non-invasively detect and track the motion of individual pollutant particles in real time and with high resolution. Direct visualisation of the behaviour of deposited particles in animal model airways is expected to improve our understanding of airway surface function and suggest novel methods for prevention or treatment of respiratory disease.

In 2008B we demonstrated the ability to locate, track and quantify the motion of common inhaled particles in the nasal airways of live mice using phase contrast X-ray imaging (PCXI), and in 2009A we extended this technique to the trachea of live mice. The aim of this 2009B experiment was to improve our PCXI techniques for the non-invasive quantification of pollutant particle MCT by optimising our model and delivery systems. The specific aims were to:

- Test and validate an ex-vivo imaging system, including a custom designed tissue bath.
- Modify our animal restraint and mounting method, and improve our particulate delivery system.
- Accurately measure and minimise the radiation dose.

We hoped that technique developments would improve image quality and to allow us to perform significant new types of experiments, including: studies longer than 30 minutes; and studies in which animals are repeat-imaged over multiple days or months (ie in our CF gene therapy studies to determine therapy effectiveness and persistence, or to examine long-term effects of pollutant inhalation).

Experimental/analytical method

The PCXI experiment was performed in the downstream BL20XU hutch. The imaging and experimental setups are described in three publications [1, 2, 6]. The experiment consisted of an *ex-vivo* study to examine the visibility of the particulates in an excised mouse trachea, and an *in-vivo* study to examine the behaviour of the particles after deposition onto live mouse airways.

The same potentially respirable pollutant particles visible in the 2008B and 2009A studies (white chrysotile asbestos, fibreglass from a commercial pipe insulation, galena lead ore, dolomite quarry dust and reference 14 µm silver coated hollow glass beads) were prepared in physiological saline at a concentration 1% w/v. In addition, lead carbonate and iron oxide were also prepared in the same manner, and their visibility under PCXI established by imaging 15µl samples inside small steel washers placed between the adhesive surfaces of Kapton tape.

Ex-vivo experiments were performed using 6 C57Bl6 mice humanely killed via Nembutal overdose. In each mouse the trachea was surgically exposed and 20Ga i.v. catheters were inserted into each end secured in place with surgical suture prior to the complete excision of the trachea. The catheters were then placed into a modified Living Systems CH/1/AU tissue bath, containing PBS maintained at a constant temperature of 37ºC to minimise specimen dehydration during the experiment. The chamber was mounted on the x-y stage in the imaging hutch such that the X-ray beam passed through the excised trachea. Horizontal and vertical orientations were both tested. Images were captured at baseline and after flushing with 100 µl samples of the particulates suspended in saline. The stage was also translated in the appropriate direction to allow the full length of the excised trachea to be imaged using a number of exposures. In one excised trachea we also tested the delivery of particulates suspended in perfluorocarbon liquid.

In-vivo experiments were performed using 10 C57Bl/6 mice. Mice were anaesthetised with Nembutal and then intubated using a 20Ga i.v. catheter as an endotracheal (ET) tube. A significant advance during these experiments was the use of an optic fibre connected to a bright light source as an introducer for the ET tube. This provided increased illumination of the tracheal opening, increased the intubation success rate and reduced the intubation time dramatically. After intubation the mice were secured to a polyethylene imaging board, with dorsal incisors hooked over a stainless-steel wire loop and the limbs, shoulders and torso were taped to minimise respiratory movements. The imaging board was mounted on the x-y-rotation stage in the hutch such that the X-ray beam passed laterally through the mouse trachea, at approximately three cartilage rings below the tip of the ET tube. Importantly, and in contrast to previous experiments, the animal was mounted in a supine position rather than a head-high position. The ET tube was connected to a flexiVent small animal ventilator, and anaesthesia was maintained using a passively humidified isoflurane O_2 mixture. The ventilatory profile was configured with a 100ms end-inspiratory pause to allow unblurred image capture.

Images were captured using our standard pco.4000 CCD camera, but a thicker 26µm scintillator and 0.5NA objective lens were used in the SPring-8 beam monitor. Image capture was triggered by the ventilator once every 7 breaths (5.25 sec). After 2 minutes of baseline collection (~23 images), a 15 µl sample of the particulate suspended in saline was remotely delivered via a heat-pulled PE10 catheter contained within the ET tube using a syringe pump in a single bolus delivered over ~10 seconds. Image acquisition was resumed at the same rate for a further 20 minutes (~240 images). In all experiments the number of counts from an ion chamber mounted downstream from the imaging shutter were closely monitored and recorded to allow an estimation of the X-ray dose to be calculated.

Research results

Due to the short time between conducting the experiment and the deadline for reports we have not

performed any substantial analysis of the results, so only preliminary results and observations (no images) are presented here.

The *in-vitro* PCXI visibility of the lead carbonate and iron oxide particles in saline was similar to that of the other particulates tested [1, 2]. Based on our *in-vitro* observations we continued to use a concentration of 1% w/v for both *ex-vivo* and *in-vivo* studies, to ensure sufficient particulates were present to potentially be visible when deposited into the mouse trachea.

The *ex-vivo* visibility of the particulates was also verified in a segment of excised mouse trachea. All particulates could be easily detected. Although all particulates were detectable, the smaller and less visible quarry dust particles were harder to detect than fibreglass, galena and the hollow glass beads. In addition, only one bundle of asbestos fibres was visible in the trachea in contrast to the other particulates, of which many individual particles could be detected. The use of an enclosed chamber did prevent the excised trachea from visible dehydration, and thus we attempted to use this system to analyse MCT behaviour.

During this experiment mice did not exhibit the large unpredictable respiratory excursions that previously caused image blurring and limited the usable imaging time to less than 30 minutes. Using this setup it was possible to image for substantially longer, and in some cases animals responded well even after 120+ minutes of ventilation in a supine position. We suspect that changing from head-high to supine imaging reduced loading on the diaphragm that likely causes muscle fibre shortening, ventilatory loading and potential hypoxaemia and hyercapnia. It is also likely that venous return problems when vertical reduced cardiac output and potentially metabolism. In addition, we also suspect that supine imaging imposed more physiologically realistic gravitational effects on particle motion, assisting us to quantify true post-deposition MCT behaviour.

During this experiment we made a number of important technique improvements that will be useful for future PCXI studies at SPring-8 and the Australian Synchrotron:

- The intubation system was improved and now utilises an optic fibre ET introducer connected to a bright light source rather than an arterial guide wire. This system illuminates the vocal cords and trachea much more clearly, facilitating rapid and accurate intubations.
- We tested a range of ET tube sizes and determined that a larger ET tube and internal delivery cannula could allow better delivery of large particulates (ie asbestos) to the trachea.
- A passive humidifier was integrated into the isoflurane delivery circuit to the ventilator. The relative humidity of the mix was measured at 83%, and is likely to allow more physiologically realistic MCT measurements than a 0% RH dry oxygen / isoflurane mix.
- The supine mounting orientation of the mouse allowed for substantially longer imaging sequences.
- Particulate delivery was performed using a remotely controlled syringe driver that allowed us to acquire images while the particulates were delivered to the airways. This is a significant improvement over our previous setup in 2009A [2] where it was necessary to manually instill particulates.
- The exposure time was reduced through the use of a thicker scintillator and a higher NA objective lens, which also meant that the delivered dose was reduced.
- The use of a ex-vivo tissue bath for examining particulate deposition and post-deposition behaviour in a segment of trachea was demonstrated.
- Tracheal excision was improved for the ex-vivo studies.

In the *in-vivo* experiment we measured parameters to calculate the radiation dose delivered to the animals, but little effort was made to limit it due to the need to maximise the number of useful images and image quality that could be acquired from these non-recovery animals. The dose was calculated to be approximately 0.44Gy/sec or 0.04Gy/exposure for the 100ms exposures. Due to the thicker scintillator and better objective lens we were able to reduce the exposure length from ~300ms (as used in 2009A and 2008B experiments) to 100ms, producing a large reduction in the delivered dose. However, at present the dose remains too intense to consider animal recovery and repeated imaging, although the current rapid development of synchrotron and imaging technology suggest there will continue to be improvements in imaging hardware and analytical techniques such as motion-detection and particle tracking. Combined, they should lead to improved image resolution and higher light sensitivity, allowing smaller — and therefore more physiologically relevant — particulates to be detected and tracked.

In summary, this project examined how different types of particles moved along the airways; and established the potential of this new non-invasive imaging method to assist in research into the initiation of childhood and other respiratory diseases that are related to particulate inhalations.

Current and future issues/challenges

The aerodynamic diameter of inhaled particulates must be less than 10 μm to be inhaled into the lung of humans. An improvement in the minimum size detectable under PCXI will be important for ensuring biological relevance in future instillation or inhalation studies.

The deposition of particles suspended in a carrier fluid is not physiologically normal. Particles are normally inhaled from the surrounding air where they are suspended in a dry form, and then deposit on the wet mucus covered airway surfaces. In future experiments we propose to deposit dry particles to examine their deposition characteristics and post-deposition behaviour, and to determine how these differ to the behaviour when the particulates are delivered suspended in a carrier fluid.

References

[1] M. Donnelley*, et al.*, "Real-time non-invasive detection of inhalable particulates delivered into live mouse airways," *Journal of Synchrotron Radiation,* vol. 16, pp. 553-561, 2009.
[2] M. Donnelley*, et al.*, "A new technique to examine individual particle and fibre deposition and transit behaviour on live mouse lung airways," *Respirology,* vol. Submitted Jan, 2009.
[3] M. Limberis*, et al.*, "Recovery of airway cystic fibrosis transmembrane conductance regulator function in mice with cystic fibrosis after single-dose lentivirus-mediated gene transfer (vol 13, pg 1961, 2002)," *Human Gene Therapy,* vol. 13, pp. 2112-2112, Nov 2002.
[4] A. Stocker*, et al.*, "Single-dose lentiviral gene transfer for lifetime airway gene expression," *The Journal of Gene Medicine,* 2009.
[5] B. R. Grubb*, et al.*, "Mucociliary transport determined by in vivo microdialysis in the airways of normal and CF mice," *Am J Physiol Lung Cell Mol Physiol,* vol. 286, pp. L588-595, March 1, 2004 2004.
[6] D. W. Parsons*, et al.*, "High-resolution visualization of airspace structures in intact mice via synchrotron phase-contrast X-ray imaging (PCXI)," *Journal of Anatomy,* vol. 213, pp. 217-227, Aug 2008.

Status of publication and patent

We are currently preparing a manuscript that includes results obtained during this experiment. Some of these results were also presented at the Medical Applications of Synchrotron Radiation 2010 conference (Melbourne) in February, will be presented at the Australian and New Zealand Respiratory

Scientists Meeting (Brisbane) in March, and are contained within the following submitted paper:

M. Donnelley, D. Parsons, K. Morgan, K. Siu, “Animals In Synchrotrons: Overcoming Challenges For High-Resolution, Live, Small-Animal Imaging”, AIP Proceedings, 2010. (Currently Under Review).

Keywords and annotations

particles; pollution; airway surface; lung; trachea; mucociliary transit; non-invasive; synchrotron radiation; X-ray imaging; X-ray phase-contrast; radiography; mouse;