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Airway distension during lung inflation in healthy and allergic-sensitised mice *in vivo*

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ABSTRACT

We evaluated the airway distention during lung inflation of varying size in healthy and allergic-sensitised mice *in vivo*. Computed tomography (CT) images of healthy and ovalbumin-treated mice were acquired using a synchrotron *in vivo* CT system when lung pressures was 0 and 20 cmH_2O, and the morphometric distension (diameter, length, and volume) and the compliance of airway segments (to as small as ~150 μm internal diameter) were calculated. With respect to airway size, in healthy mice, the changes in airway diameter and compliance were larger in the small-airway group. In contrast, in allergic-sensitised mice, there were no significant differences in the changes in airway distension or compliance. Airway wall thickness in allergic-sensitised mice increased significantly in all airway groups, but the change was much larger in the small than in the large-airway group. Compared with healthy airways, the changes in diameter and airway compliance of the allergic-sensitised mice were significantly smaller in the small-airway group.

1. Introduction

During ventilation of the lung, both the bronchial tree and alveolar regions of the lung expand and contract in volume, but the manner in which this occurs is dependent on the local mechanoelastic properties of the structures. Due to the complexity of the arrangement of the airways and vascular structures within the parenchyma, the airway geometry—both its shape and volume—changes markedly and in a complex manner during respiration. From measurements of excised rat lungs using micro-computed tomography (micro-CT) (Sera et al., 2005), for smaller airways (diameter < 300 μm), the diameter and length increased dramatically at end tidal inspiration and total lung capacity compared with the values at functional residual capacity, and the airway volume for the smaller airways did not change linearly with those of lung volume. In addition, smaller airways were generally more compliant than larger airways with increasing airway generation.

The intrapulmonary airways *in situ* are surrounded by lung parenchyma and thorax, and the effective microscopic airway deformations during ventilation are influenced by the surrounding support. It is particularly important to evaluate airway compliance in the lung parenchyma and thorax because the alveolar membrane and the thorax act as a spring and a wall, respectively (Hyatt and Flath, 1966; Takishima et al., 1975; Sera et al., 2004, 2005). Previously, the airway compliance in humans (airway diameter > 2.5 mm) was evaluated *in vivo* from the relationship between the cross-sectional area and transmural pressure during forced expiration (Brackel et al., 2000). In addition, a new technique has been developed to measure the airway compliance of humans *in vivo* and to evaluate the bronchodilator response in those with asthma (Kelly et al., 2010, 2011). This method is based on the combination of airway diameter measurements by high-resolution computed tomography (HRCT) with pressure–volume recording. In particular, HRCT scans were obtained during voluntary breath-holding at various lung volumes (Kelly et al., 2010, 2011). The diameter–pressure relationship for airways of varying size could be obtained from the results of these studies.

Asthma is characterised by chronic inflammation of the airway walls. Structural changes reported in the airways of asthmatic patients include epithelial fragility, goblet cell hyperplasia, enlarged submucosal mucus glands, angiogenesis, increased matrix deposition in the airway wall, increased airway smooth muscle mass, wall thickening and elastin abnormalities (Bai and Knight, 2005). These structural changes within the airway wall alter the

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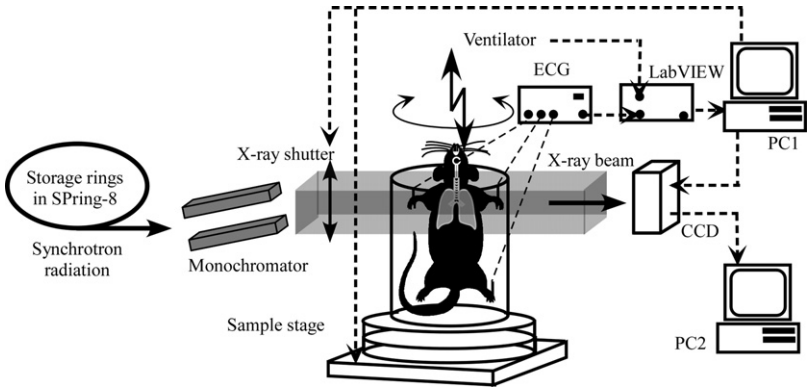

Fig. 1. Four-dimensional *in vivo* synchrotron CT system.

mechanical properties of the airway. Further, asthma is recognised as a disease of both the large and small airways (Roche, 1998; Tashkin, 2002; Bai and Knight, 2005; Tulic and Hamid, 2006; Usmani and Barnes, 2012). In particular, decreases in small airway diameter lead rapidly to increased airflow resistance (Wiggs et al., 1992; Hamid et al., 1997), because resistance is inversely related to the fourth power of the airway radius (Tashkin, 2002; Corren, 2008; van den Berge et al., 2011; Johnson and Hamid, 2012). The diameter may be reduced by an increase in the volume of tissue encroaching into the space and contraction of the smooth muscle.

In asthma, structural changes of airway components occur in both the large and small airways but predominantly in the small airways (Carroll et al., 1993). We hypothesised that airway distension during lung inflation and airway compliance in hyperresponsiveness were small, and that the decrements would be greater in peripheral airways. Airway lumen is covered with lung surfactant, and airway distension and compliance are determined by airway pressure, tissue elasticity, and surface tension. Previously, we showed that airway distension and compliance in hyperresponsiveness were small and that the decrements would be greater in peripheral airways (Sera et al., 2007). However, the previous evaluation was based on the measurement of euthanised mice during quasi-static inflation. Moreover, the total scan time was over 3 h.

In the present study, we calculated the airway distension during inflation (morphometric changes in diameter, length and volume) and airway compliance of varying size in healthy and ovalbumin (OVA)-treated mice as a model of hyperresponsiveness *in vivo*. Recently, we developed a four-dimensional *in vivo* computed tomography (CT) system at SPring-8 (Super Photon ring-8 GeV) to analyse small airway mechanics in mice (Sera et al., 2008). SPring-8 is a third-generation synchrotron radiation source in Japan, and provides a much higher X-ray flux than a typically laboratory X-ray source (Goto et al., 2001). The synchrotron CT images of lung fixed sample depict the peripheral structures including peripheral airways, airspaces, and alveolar walls individually, and these findings in normal and diseased lung are correlated well with the microscopic images of the corresponding histological section (Ikura et al., 2004). In our system, anaesthetised mice are connected to a custom mechanical ventilator that can induce iso-lung pressure holding intermittently and arbitrarily during ventilator cycles. Projections are also acquired prospectively in synchrony with airway pressure, ECG, X-ray shutter and a Charge Coupled Device (CCD) shutter during iso-pressure hold to reduce motion artefacts and radiation dose. Consequently, 3D changes in airway geometry during lung inflation can be evaluated in four-dimensions *in vivo*.

2. Methods

2.1. Animals

All experimental protocols were approved by the SPring-8 Experimental Animal Care and Use Committee. Five-week-old specific pathogen-free male A/J mice ($n = 10$; SLC Japan Inc., Shizuoka, Japan) were divided into two groups: healthy ($n = 5$) and allergic-sensitised mice ($n = 5$). Anaesthetised mice were instilled with OVA (1 mg/mL) intranasally using a micropipette (50 μL) (Shinagawa and Kojima, 2003). OVA was administered three days per week for 10 weeks. In the healthy group, the mice received no treatment for 10 weeks. Twenty-four hours after the last administration of OVA, mice were anaesthetised with 50 mg/kg of sodium pentobarbitone by intraperitoneal (ip) injection and the lungs were imaged using 4D *in vivo* CT (Sera et al., 2008). Anaesthesia was maintained during the CT scan with 1% isoflurane. After imaging, mice were exsanguinated to allow measurement of total IgE.

2.2. X-ray imaging

The 4D *in vivo* CT system was constructed in medical imaging beamline (BL20B2) at SPring-8 (Sera et al., 2008). Briefly, the X-ray beam was monochromated using a double-crystal monochromator, and then transported into the experimental hutches. We placed a precision-rotation stage (Kouzu, Kawasaki, Japan), a high-resolution CCD camera (ORCAII-HR; Hamamatsu Photonics K.K., Japan), an X-ray shutter, a custom mechanical ventilator and an ECG (BMA-200; CWE, Inc., Ardmore, PA) in the experimental hutch (Fig. 1). In our CT system, the lung pressure was held at the same level when each projection was acquired. The programme that controlled synchronisation between the ventilator, X-ray exposure, X-ray shutter, and physiological motion was developed using LabVIEW (National Instruments, Austin, TX). So that exposures were made at a specific phase of the ventilator and ECG cycle, the programme generated triggers for the X-ray shutter and CCD detector from the airway pressure.

In this study, to analyse the morphometric distension in the same branching airway networks, images were acquired at two points in the pressure cycle: 0 and 20 cmH_2O, between T and next P wave of the ECG cycle (T-P segment, diastolic phase; Fig. 2). The triggers for the X-ray shutter were generated after 20 ms of the first R peak of the ECG signal during iso-pressure hold (300 ms). The X-ray energy was 20 keV, and the exposure time was 40 ms. In this protocol, iso-pressure was maintained prospectively during a respiratory cycle, and a projection image was acquired at each constant airway pressure. First, the lungs at 0 cmH_2O were imaged while the ventilator parameter was kept at the normal settings