Noninvasive in-vivo quantification of compensatory lung growth following pneumonectomy, via ^{1}H and ^{3}He MRI

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Introduction

Pneumonectomy (PNX), the surgical removal of one or more lobes of the lung, is a robust, established model of adult compensatory lung growth [1]. Understanding the time course and the mechanism of compensatory lung growth will shed light on understanding of post-natal lung growth and regeneration. While some research has been performed on dogs, rabbits and rats, characterization of post-PNX lung growth by histology was not reported in mice until recently [2, 3]. ^{3}He lung morphometry has been successfully implemented in humans for many years [4], and was recently developed and validated in dogs [5] and mice [6]. It can provide many of the same physiological parameters as in quantitative histology but non-invasively and with tomographic information. Here we employed this ^{3}He MRI technique to image *in-vivo* morphometry at baseline and to serially assess compensatory growth after left PNX in mice.

Methods

Ten 12-week-old C57BL/6 mice were used, with Animal Studies Committee approval: one group (n=6) underwent left lung PNX; the other group (n=4) was subjected to a sham operation (thoracotomy without tissue resection). All animals were attached supine to a custom MRI-compatible ventilator, ventilated at 120 breaths per minute with 0.25-mL tidal volume and anesthetized with inhaled isoflurane during imaging. Each breathing cycle consisted of an inhalation of pure O_2, a brief breath hold (pressure precisely monitored at 4 cm H_2O), an inhalation of a ^{3}He/N_2 gas mixture, a second breath hold (at 15 cm H_2O) and then passive exhalation. MRI was performed at baseline (immediately prior to PNX surgery), 3 days and 30 days after surgery, using a doubly resonant solenoid coil (tuned to both ^{3}He and ^{1}H frequencies, 151.1 MHz and 198.3 MHz respectively) on a Varian 4.7 T, horizontal, 12-cm clear-bore magnet. A trigger signal from the ventilator was used to control the image data acquisition beginning at the exact time point during one respiratory cycle. ^{1}H MR images were acquired twice (during two breath hold time respectively) for volume measurements at different pressure levels using a 2D multislice gradient-echo sequence with echo time (TE) = 0.99 ms, field of view = 4 cm × 3 cm at a resolution of 313 µm × 313 µm. 9 axial slices enabled the coverage of very top and bottom of the lung. The ^{3}He diffusion MR images were acquired at the peak of each inspiration at the center-five slice positions (covering nearly the entire lung) of ^{1}H MRI using a 2D multi-slice gradient-echo sequence with embedded bipolar diffusion-sensitizing gradients (matrix = 64 × 48, FOV = 4 cm× 3cm, thickness = 2.0 mm, TE = 2.44 ms; b = 0, 1, 2, 4, 6, 9 s/cm^2, diffusion time is 440 µs). Lung volume was calculated from ^{1}H images by counting all the voxels in the lung region excluding the large blood vessels, trachea, and large conducting airways. Compliance was determined by calculating the change in volume at two levels of pressure ($\Delta V/\Delta P$). ^{3}He MR images of 6-b values were fitted to the Weibel model of acinar airways on a voxel-by-voxel basis using Bayesian analysis software. This generates parametric maps of the airway radii R, the alveolar depth h, the mean linear intercept (Lm), the alveolar density N_a and the surface-to-volume ratio S/V. Total alveolar number was calculated by multiplying N_a by the lung volume measured from ^{1}H MRI.

Results and Discussion

Representative ^{1}H MR images for a PNX mouse are shown in Figure 1. For the PNX group, a striking increase of right lung volume (~30%) was observed at day 3 with further expansion (~15%) up to day 30; for the sham group, the right lung volume remained unchanged during the time period (Figure 3a). Representative *Lm* maps are shown in Figure 2. The compliance increased about 2-fold up to day 30 post-PNX (Figure 3b), suggesting that the overall static mechanical properties were restored to pre-PNX level by day 30. *Lm* significantly increased by day 3 (~+10% compared to baseline, P = 0.0004) and then decreased by day 30 (~-3% compared to day 3, P = 0.03) for the PNX group, indicating an initial size increase of existing alveoli, then an increase in number of total alveoli. Left-sided PNX removed 35% of the total alveoli; however, compensatory lung growth resulted in a significant gain in the total alveolar number in remaining right lung with a 21% increase at day 3 and a 39% at day 30 compared with sham right lungs (Figure 3c).

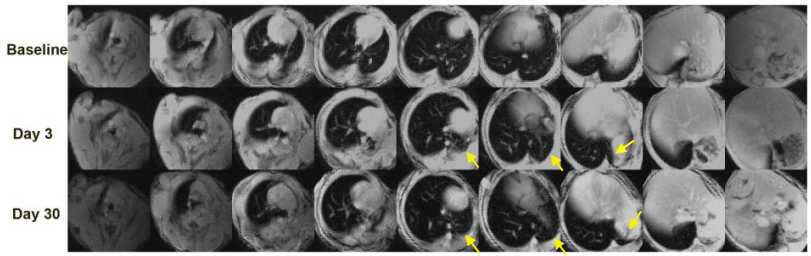

Figure 1 Representative 1H MR images at different time points for a PNX mouse (axial imaging plane). Before PNX, two lungs are shown. After PNX, only the right lung (appearing on the left in the images) remains in the thorax with great expansion of the volume. Part of the lung extending into the left thorax (yellow arrow) is the accessory lobe of the right lung.

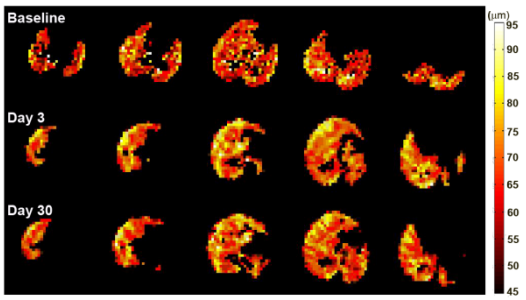

Figure 2 Representative *Lm* map of a PNX mouse at baseline, days 3 and 30 after PNX from ^{3}He morphometry.

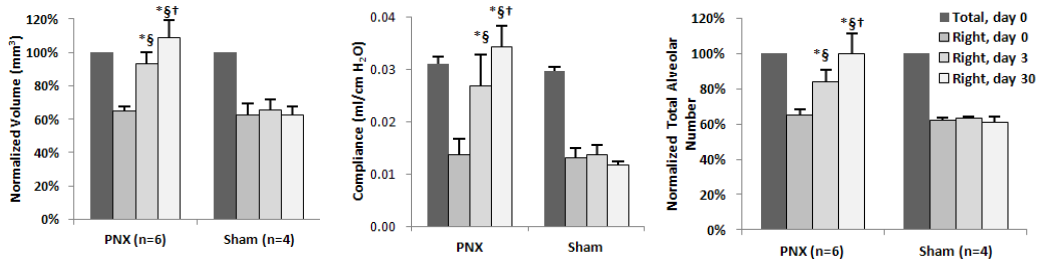

Figure 3 Lung volume (a), total alveolar number (b) and lung compliance(c) for both lungs and right lung at baseline (pre-PNX) and residual right lungs at days 3 and 30 post PNX. Each mouse's measurements were normalized by its own baseline measurement from both lungs. Data are presented as mean ± SD. * $p < 0.001$ *vs* sham right lung at baseline, § $p < 0.001$ *vs* PNX right lung at baseline, † $p < 0.05$ *vs* PNX at day 3

Conclusion

These studies demonstrate the first *in vivo* quantitative assessment of longitudinal, compensatory lung growth after unilateral PNX in mice via ^{1}H and hyperpolarized ^{3}He diffusion MRI. Our results show complete restoration in lung volume and total alveolar number with enlargement of alveolar size, which is consistent with prior histological studies conducted in different animals at various time points [1-3]. The unique strength of this imaging technique is the ability to longitudinally study lung growth in the same animal and also to reveal the regional variation of microstructural changes. It has great potential to become a valuable tool in understanding the time course and the mechanism of lung growth in individual animals and may provide insight into post-natal lung growth and lung regeneration.

References

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