Hyperpolarized Helium Measurements of $P_{O_2}$ Correlate with Neutrophil Inflammation in the Rat Bleomycin Model


1Radiology, University of Pennsylvania, Philadelphia, Pennsylvania, United States, 2Pulmonary Division, University of Pennsylvania, Philadelphia, Pennsylvania, United States, 3Otolaryngology–Head & Neck Surgery, Johns Hopkins University, Baltimore, Maryland, United States, 4Radiology, University of Pennsylvania, Philadelphia, Pennsylvania

INTRODUCTION: Hyperpolarized (HP) $^3$He MRI is a technique sensitive to both lung structure (airway size through Apparent Diffusion Coefficient, ADC) and function (alveolar $P_{O_2}$ and fractional ventilation, $r$) through direct imaging of respiratory gas molecules. In this work, we investigated correlated changes of these metrics with bronchoalveolar lavage (BAL) measurements and biochemistry in a rat model of interstitial fibrosis secondary to bleomycin.

METHODS: Male Sprague Dawley rats ($n=9$, 300–350g body weight) were given intratracheal bleomycin and 7 (7BR) and 21 (21BR) days after intratracheal bleomycin, they underwent HP $^3$He MRI to measure $r$, ADC and $P_{O_2}$. The animals were intubated and connected to a custom-designed small animal ventilator. This ventilator is capable of delivering the breathing gas with an accuracy of ±100µL/breath and real-time monitoring of peak inspiration pressure (PIP). For ventilation imaging, a series of 10 HP gas breaths ($^3$He-$O_2$, FIO$_2$:20%) was delivered to the rat at the designated tidal volume $V_T=1.0$ml/100g, and one image was acquired after each breath during a 350-ms breath-hold. The HP $^3$He signal build up in the rat lung was then recursively solved for $r$ by yielding the fractional ventilation map, as described earlier [1]. For ADC imaging, rats were ventilated with five identical breaths of HP $^3$He-$O_2$ (4:1) at the designated inflation level followed by a 3-sec breath-hold during which five diffusion-weighted images were acquired corresponding to $b$-values = 0.00, 3.73, 2.18, 1.00 and 0.00 s/cm$^2$. This procedure was repeated immediately with identical but reversed polarity diffusion gradient $b$-values. These 10 diffusion-weighted images were then combined to yield the ADC map of the imaged slice according to a double-acquisition diffusion imaging scheme described earlier [2]. For $P_{O_2}$ imaging, rats inhaled a series of 5 breaths of $^3$He-$O_2$ followed by a short 6-sec breath-hold, during which images were acquired at a set of predefined delay times, and the resulting images corresponding to the same slice/delay combination were then averaged and fit to a model of $O_2$-induced decay and respiratory gas redistribution as described earlier [3]. Images were acquired using a diffusion-weighted gradient echo imaging pulse sequence with centric phase-encoding in a 50-cm bore 4.7-T MRI scanner (Varian Inc) equipped with a 12-cm 25-G/cm gradients and a 2-3/4-“ID quadrature 8-leg birdcage body coil (Stark Contrast). Images were acquired in the middle coronal slice of the rat lung with the following imaging parameters: FOV=5x5x5cm$^3$, ST=6mm, MS=6x6x6, $\delta$=4–5°, TR=6.6ms, and TE=4ms. Diffusion sensitizing gradient was applied along the phase-encoding (L–R) direction with the following timing parameters: $\Delta$=1ms, $\delta$=200µs, and $\tau$=180µs according to the naming convention of [4]. Upon conclusion of imaging, BAL was performed for measurement of white blood cell (WBC) numbers and differential, and BAL protein content as a measure of lung damage. The right lung was fixed for histology and the left lung for measurement of hydroxyproline, a marker of fibrosis. Healthy rats (HR) were similarly tested. Values were expressed as mean ± SD and statistical significance was determined by pairwise t-tests.

RESULTS AND DISCUSSION: Figure 1 shows a representative map of $P_{O_2}$, ADC, and $r$ in HR, 7BR, and 21BR rats. Figure 2 shows that the overall mean of ADC were not significantly reduced in 7BR (0.25±0.10 cm$^2$/s) and 21BR (0.24±0.07 cm$^2$/s) compared to HR (0.31±0.11 cm$^2$/s). The means of $r$ were also not significantly reduced in 7BR (0.23±0.15) compared to the HR (0.33±0.17) and returned towards normal in 21BR (0.31±0.16). In contrast, Figure 3 shows the $P_{O_2}$ was significantly ($p<0.05$) increased in 7BR (175.0±24.8 mbar) compared to HR (108.2±4.4 mbar) and returned toward HR in 21BR (99.07±21.55 mbar). Both the number ($r=0.868$) and percent ($r=0.833$) of neutrophils in the BAL fluid was significantly ($p<0.01$) correlated with the $P_{O_2}$ but there was no significant correlation between BAL cells or protein with ADC or $r$. Hydroxyproline was unchanged in 7BR but was significantly increased in 21BR (HR = 64.3, 7BR =53.1, 21BR = 95.7 ug/ug lung tissue, p < 0.05).

CONCLUSION: In the rat model of pulmonary fibrosis due to bleomycin, $P_{O_2}$ correlated with the extent of neutrophil inflammation in the lung. This suggests that $P_{O_2}$ measured by HP $^3$He MRI can be a sensitive indicator of pulmonary inflammation and may help with predicting prognosis, and helping with drug development as a non-invasive measure of lung function.