Assessment of Pulmonary Inflammation in a Rat Bleomycin Model using Oxygen-weighted Hyperpolarized 3He MRI

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INTRODUCTION: Idiopathic pulmonary fibrosis (IPF) is a serious lung disease whose epidemiology and pathogenesis are still not completely known. Over a period of time, the lung tissue including alveolar membranes becomes thicker and less compliant. The scarring of lung in the air sac walls and the spaces around them limits the amount of oxygen passable through the membranes into the bloodstream. In the absence of a surgical lung biopsy, the diagnosis and stratification of IPF remains uncertain. In this study, hyperpolarized (HP) ^{3}He MRI is utilized to acquire functional maps of oxygen tension, oxygen uptake and fractional ventilation in a rat model of interstitial fibrosis secondary to bleomycin, and sensitivity of each parameter is assessed with respect to presence and stage of fibrosis formation and progression.

METHODS: Male Sprague Dawley rats (body weight 300~350g) were utilized in three groups: healthy controls (n=3), 7-day post-administration of intra-tracheal bleomycin (n=3), and 21-day post-administration of intra-tracheal bleomycin (n=3). The rats were sedated, intubated, and mechanically ventilated with an MRI-compatible mechanical ventilator for imaging experiments. Fractional ventilation (r) imaging was performed as described in [1], briefly using a series of 10 normoxic HP gas breaths (^{3}He:O_2=4:1). One ventilation image was acquired after each breath during a 350-ms breath-hold. The HP gas signal buildup in the rat lung was then recursively solved to yield the r map. P_AO_2 imaging was performed as described in [2], briefly using a series of 5 HP gas breaths followed by a short 6-s breath-hold. During the breath-hold, images were acquired after a set of predefined delay times and repeated for several (3–5) cycles. Signal dynamics was fit using an iterative algorithm to determine alveolar oxygen concentration (P_AO_2) and oxygen depletion rate (ODR) according to:

$$S(t) = S(0)\cdot(1+f\exp(-t/\tau))\cdot\exp\left(-t\cdot(P_AO_2/\xi - \mathrm{ODR}/2)\right) \qquad R = (F_iO_2\times 1\ \mathrm{atm} - P_AO_2)\cdot r\cdot \mathrm{BR}$$

with breathing rate BR, ξ=2.6 mbar·s, and two additional free parameters: respiratory gas redistribution time constant τ, and directional gas flow term f. Imaging was performed in a 50-cm bore 4.7-T MRI scanner (Varian, Inc.) equipped with a 12-cm, 25-G/cm gradient and a 2-3/4"-ID quadrature eight-leg birdcage body coil (Stark Contrast) tuned to the ^{3}He resonance frequency of 152.95 MHz. The animal was placed supine in the RF coil. All imaging was performed using a fast gradient-echo pulse sequence with: FOV=6×6cm^2, ST=6mm, MS=64×64, α=5°, and TR/TE=6.6/3.3ms. At the conclusion of MRI, animals underwent a bronchoalveolar lavage (BAL) to obtain white blood cell (WBC) numbers and differentials, as well as BAL protein content as a measure of lung damage to compare with MRI results. Right lung was fixed for histology and the left lung was used for measurement of hydroxyproline, a measure of lung fibrosis.

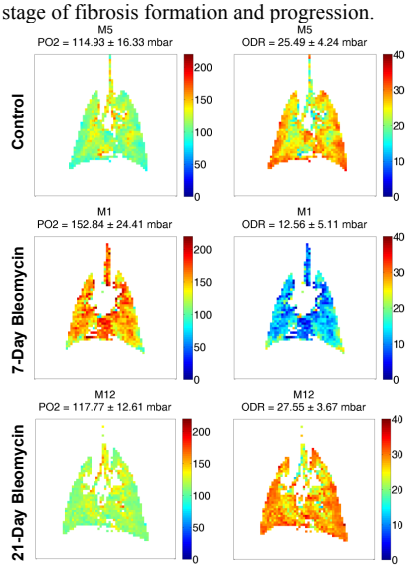

Figure 1. Representative maps of P_AO_2 and ODR in rat lungs from healthy, 7-day Bleomycin and 21-day bleomycin groups.

RESULTS AND DISCUSSION: Representative maps of P_AO_2 and ODR are shown in **Figure 1** for healthy, 7-day bleomycin and 21-day bleomycin. **Figure 2** summarizes the mean and SD of P_AO_2, ODR and biological assays for all three groups. Group P_AO_2 values were 122.2±6.3, 132.6±22.8, and 101.9 ± 16.8 [mbar] for control, 7-day and 21-day bleomycin groups, respectively. There was a tendency for P_AO_2 to increase at 7-day bleomycin group compared to the healthy group. Group ODR values were 28.5±3.0, 15.1±5.8, and 23.6±4.8 [mbar/s] for the three groups, in the same order. Alveolar oxygen uptake rate was significantly reduced for 7-day bleomycin group ($p<0.03$) compared to healthy group and returned toward normal levels for 21-day bleomycin group. The biological assays after 7 days of instillation of bleomycin showed a significant influx of inflammatory cells confirmed by the excessive recruitment of neutrophil, BAL Protein, polymorphonuclear leukocyte, and white blood cell counts. After 21 days of bleomycin induction, bronchoalveolar lavage shows a reduction of inflammatory cells similar to that from the healthy group. While there was some correlation of the alveolar P_AO_2 with the numbers of neutrophils in the BAL fluid (R = 0.60), there was a significant negative correlation of ODR with the total numbers of WBC (R = –0.84), % neutrophils (R = –0.86) and absolute number of neutrophils (R = –0.92) in the BAL fluid, as shown in **Figure 3**. No significant correlation was observed between the alveolar oxygen uptake and the size of the alveoli on histology, lung weight, or the hydroxycholine content of the lung.

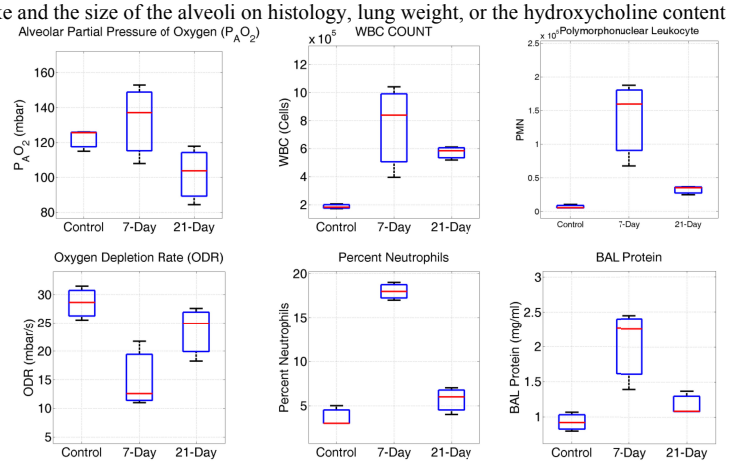

Figure 2. Box plot representation of imaging markers (P_AO_2, ODR) and biological markers of inflammation, fibrosis and lung damage for all groups.

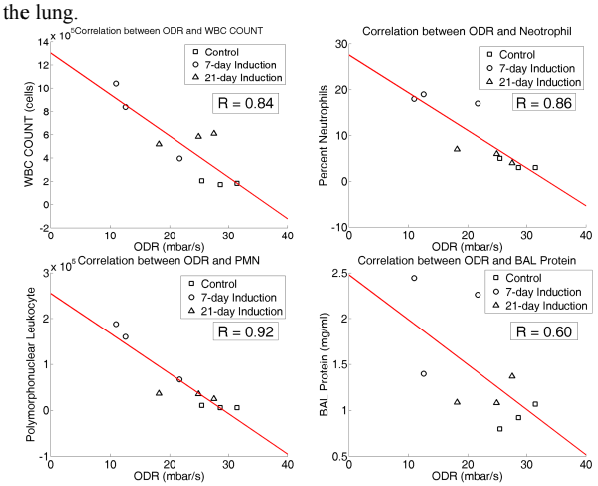

Figure 3. Correlation between oxygen depletion rate and biological markers of inflammation and pulmonary fibrosis.

CONCLUSION: Preliminary results demonstrate that P_AO_2 imaging using HP ^{3}He MRI can have utility in elucidating alterations in gas exchange as a function of inflammatory and fibrotic changes in lung tissue. This study highlights the potential of imaging oxygen uptake rate as a noninvasive and sensitive marker with potential to diagnose, monitor and stage idiopathic pulmonary fibrosis. While it is very encouraging to observe a strong correlation between the overall ODR with biological markers of inflammation, fibrosis and lung damage, the real value of this imaging biomarker is certainly in its regional nature and the fact that it can be potentially used to discriminate confined areas of fibrosis or inflammation in heterogeneous IPF. This idea is possibly extendable to other interstitial lung diseases (ILD) and is the subject of ongoing research both in humans and animal models. Establishing the relationships in a larger study can test the real value of oxygen-weighted HP gas MRI as a tool to investigate fibrosis development, predicting prognosis and drug development in humans.

Reference: [1] Emami K., *et al.* Magn Reson Med 2010; [2] Kadlecek S., *et al.* NMR Biomed 2011