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Quantitative Assessment of Lung Ventilation and Microstructure in an Animal Model of Idiopathic Pulmonary Fibrosis Using Hyperpolarized Gas MRI

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Abstract

RATIONALE AND OBJECTIVES—The use of hyperpolarized ³He MRI as a quantitative lung imaging tool has progressed rapidly in the past decade, mostly in assessment of the airways diseases COPD and asthma. This technique has shown potential to assess both structural and functional information in healthy and diseased lungs. In this study, we apply the regional measurements of structure and function to a bleomycin rat model of interstitial lung disease.

MATERIALS AND METHODS—Male Sprague Dawley rats (300–350 g) were administered intra-tracheal bleomycin. After 3 weeks, apparent diffusion coefficient and fractional ventilation were measured by ³He MRI and pulmonary function testing using a rodent-specific plethysmography chamber. Sensitized and healthy animals were then compared using threshold analysis to assess the potential sensitivity of these techniques to pulmonary abnormalities.

RESULTS—No significant changes were observed in total lung volume and compliance between the two groups. Airway resistance elevated and forced expiratory volume significantly declined in the 3-wk bleomycin rats, and fractional ventilation was significantly decreased compared to control animals ($p < 4 \times 10^{-4}$). Apparent diffusion coefficient of ³He showed a smaller change, but still a significant decrease in 3-wk bleomycin animals ($p < 0.05$).

CONCLUSION—Preliminary results suggest that quantitative ³He MRI can be a sensitive and non-invasive tool to assess changes in an animal interstitial lung disease model. This technique may be useful for longitudinal animal studies and also in the investigation of human interstitial lung diseases.

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Keywords

bleomycin; interstitial lung disease; hyperpolarized ^3He MRI; idiopathic pulmonary fibrosis; interstitial lung disease

INTRODUCTION

Of all the pulmonary diseases, Idiopathic Pulmonary Fibrosis (IPF) is one of the most debilitating due to the fact that it is a progressive disorder for which the etiology and pathogenesis are unknown and there is no effective therapy [1]. IPF is a relatively rare disease with a prevalence in men of approximately 20/100,000 and in woman of 13/100,000. In addition, the incidence of this disorder appears to be rising over the last decade. The obstacles and shortcomings to learning about effectively treating this disease must be overcome in order to care for the 5 million people worldwide with IPF. Because of the current lack of knowledge and understanding of this disorder, patients diagnosed with IPF face a median survival time after diagnosis of 3.2 years [2]. To date, despite there being much interest in determining the causes of IPF and in finding effective treatments, no methods have yet come forward to solve either problem [2–4].

A major shortcoming which makes the discovery of new therapies a challenge to investigators, is the inability to find a reliable early marker of progressive disease [2–4]. The pathological changes noted in IPF are not specific and are regionally heterogeneous [5]. In addition, the changes can also be seen in other disorders such as asbestosis. Fibrosis appears to be an active process and it is characterized by the presence of fibrogenic foci. This is thought to cause remodeling of the lung with the destruction of alveoli and thickening of the interstitium [6]. These pathological changes are also thought to result in the physiological changes that are characterized by decrease in lung volume, an increase in lung stiffness or decreased compliance and finally a decreased ability of the lung to transfer oxygen from the airspaces to the red blood cells. Unfortunately, the techniques used to measure changes in lung volume, compliance and oxygen transfer are difficult and some that are not routinely performed (compliance) have high variability (diffusion capacity) and can be affected by other processes (forced vital capacity and total lung capacity). Several techniques to monitor progression [7] have been used to record changes in forced vital capacity (FVC), diffusion capacity (DLCO), six minute walk test [8] and/or changes on the chest CT scan. Changes in the chest CT have not proven useful and the FVC, DLCO and six-minute-walk test cannot measure regional changes but only global changes in function. To this end, we chose to explore IPF using a new technique, namely hyperpolarized gas imaging technology.

Over the past decade, hyperpolarized helium-3 ($\text{HP } ^3\text{He}$) MRI technology has been advancing and its applications directed towards many diseases in order to show regional changes in both lung structure and function [9–11]. For instance, this technique has been used to evaluate ventilation to perfusion ratio [12] as well as to investigate changes in the large and small airways [13]. Several recent papers have been published that present reproducible results regarding the administration of $\text{HP } ^3\text{He}$ MRI techniques in both human [14] and animal models [15]. Two metrics that have been commonly used to evaluate lung function and structure are the Apparent Diffusion Coefficient (ADC) and fractional ventilation. The ADC is understood to tightly correlate to distal airway or alveolar size [16]. Due to decreased compliance in IPF, the alveoli and distal airways should exhibit a marked reduction in size. The second metric, fractional ventilation, is a quantitative measure of the replacement of gas into the alveoli and small airways during each breath [17]. Because of this decreased compliance, there should also be a subsequent decrease in the fractional ventilation. By assessing these two metrics on a regional

level, it may be possible to detect regional changes in IPF that are not observed by conventional techniques, as demonstrated on other pulmonary diseases [18].

In this study, HP ^3He MRI was utilized to perform simultaneous and regional quantitative measurements of lung function and structure in an animal model of IPF. Our results suggest that changes in ADC and fractional ventilation may be useful parameters to monitor changes in pulmonary fibrotic disorders.

MATERIALS AND METHODS

Study animals

All animal experiments were conducted in accordance with protocols approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Pennsylvania. Male Sprague-Dawley rats weighing between 300 and 350 grams were used for all experiments. All rats were carefully age-matched and maintained under very similar environments and dietary conditions. Five healthy control rats and six, 3-week bleomycin rats were included in this study.

Bleomycin model induction

In order to induce the rats with bleomycin, the animals were first anesthetized and then intubated. Specifically, 8-week-old rats were put under an inhaled general anesthesia of 2.5% Isoflurane and were placed supine so that they could be intubated with a 2"-long 14-gauge angiocatheter. A 2.5 U/kg concentration of bleomycin (Bedford Laboratories, Bedford Ohio), was then instilled through the catheter, followed by a 3 ml injection of air to clear the catheter. Once the bleomycin was successfully introduced into the endotracheal tube, the animals were then gently rocked from side to side to evenly distribute the bleomycin throughout the lungs. The animals were then recovered from anesthesia and briefly ventilated with a rodent ventilator (CWE Inc., Ardmore, PA) using supplemental oxygen if they failed to recover from anesthesia in a timely manner. In total, eight animals were induced with the bleomycin model and six successfully underwent the MRI procedures.

Preparation of animals for imaging

Animals were sedated with 0.1 g/kg intraperitoneal ketamine and 10 mg/kg xylazine. The dose was repeated every 90 minutes or upon observation of movement, whichever came first. The rats were intubated with a 2-inch long, 14-gauge angiocatheter (BD, Franklin Lakes, NJ), modified with a sealant (UHU Tac adhesive putty; Saunders Mfg. Co., Readfield, ME) to create a tight seal around the entrance to the trachea. With this tight seal, it was possible to have a breath-hold of up to 25 cm H₂O for 5 seconds with negligible leakage. Pulmonary function tests (PFT's) were then performed on a rodent specific-plethysmography chamber (Buxco Electronics, Wilmington, NC), and the following parameters were measured on intubated rats prior to the MRI session: functional residual capacity (FRC), total lung capacity (TLC), forced expiratory volume in 100 ms (FEV₁₀₀), airway resistance (R_I), and dynamic compliance (C_{dyn}). Upon completion of PFT's, animals were connected to a custom-built MRI-compatible HP gas ventilator. In order to minimize the respiratory motion during imaging, the animals were temporarily paralyzed with a 1 mg/kg intravenous injection of pancuronium bromide (Abbott Labs, North Chicago, IL) immediately before commencing the HP ^3He MRI.

During the imaging session, mechanical ventilation was maintained at a respiratory rate of 60 BPM with a tidal volume equal to 15% TLC (when available from PFT measurements, otherwise set to 10ml/kg body weight) in order to normalize the forced ventilation effect among different animals. Temperature was monitored using a rectal probe, and was maintained at 37°C by a flow of warm air through the bore of the magnet. Heart rate and blood oxygen saturation

level was monitored using a veterinary pulse-oximeter (Nonin Medical, Inc. Plymouth, MN) with the optical probe attached to the rat's hind foot.

Hyperpolarization of helium

As was described in the introduction, hyperpolarized gas possesses several unique properties and its use has been shown to be among the most novel techniques for assessing lung function and structure. For this study, helium gas (Spectra Gases, Branchburg, NJ) with a nominal concentration of 99.19% ^3He and 0.81% N_2 was used. This mixture was hyperpolarized through spin exchange collisions with optically pumped rubidium (Rb) atoms, as previously described in published work [19], using a commercial polarizer (IGI.9600.He, GE Healthcare, Durham, NC). The ^3He gas was polarized to a level of approximately 30% over 14 hours.

Imaging equipment and parameters

For this study, imaging was performed on a 50-cm 4.7-T MRI scanner (Varian Inc., Palo Alto, CA) equipped with 12-cm 25 G/cm gradients and a quadrature 8-leg birdcage body coil with ID = 7 cm (Stark Contrast, Erlangen, Germany) tuned to the ^3He resonance frequency of 152.95 MHz. The animal was placed supine in the RF coil and pulse width calibration was performed on the loaded RF coil to estimate the applied flip angle. Ventilation imaging was performed using a fast gradient echo pulse sequence with the following parameters: field of view (FOV) = $6 \times 6 \text{ cm}^2$, slice thickness (ST) = 5 mm, $\alpha \approx 5^\circ$, matrix size (MS) = 64×64 pixels, $T_R = 6.6$ ms, and $T_E = 3.3$ ms. The middle coronal slice was selected by performing preliminary scout ^3He images to determine position in the three major planes while still assuring that the trachea would be included in the middle slice. ADC imaging was performed using a diffusion-weighted version of the same pulse sequence by adding a shaped bipolar diffusion sensitizing gradient along the phase encode (PE) direction with diffusion time $\Delta = 1.5$ ms, gradient duration $\delta = 200 \mu\text{s}$, and diffusion gradient b -values = 0, 5.27, 3.09, 1.41, and 0 sec/cm^2 all with a fixed ramp time of $\tau = 180 \mu\text{s}$.

Regional measurement of gas replacement: Fractional Ventilation

Regional ventilation is the metric used for regional assessment of lung function in both normal and bleomycin rats. Fractional ventilation, r , is defined as the ratio of the amount of fresh gas added to a region of interest (ROI) in the lung during inspiration, denoted as V_f , to the total gas space of that ROI at the end of inspiration, V_t (comprising V_f and the residual volume V_r); $r = V_f/V_t = (V_f/V_r)$. A voxel's gas content at end-inspiration under breath-hold pressure is assumed to be divided between r , consisting of the delivered fresh gas, and $q = 1 - r$, which represents the residual capacity of the ROI. A measurement of $r = 0$ indicates that no gas has been replaced, whereas $r = 1$ indicates a complete gas exchange with each breath. Fractional ventilation provides a quantitative measure of gas replacement efficiency in the lung. Fractional ventilation imaging was performed using the technique previously described [20]. Briefly a series of 1 to 10 HP gas breaths ($^3\text{He}:\text{O}_2=4:1$ mixture) were delivered to the rat at the designated tidal volume and breathing rate, and one ventilation image was acquired after each breath during a 350-ms breath-hold. The signal buildup in the rat lung was then fit to a recursive model of the form:

$$S(j) = r \cdot S_0 + (1 - r) \cdot S(j - 1) \cdot \exp[-D_{\text{RF}} + D_{\text{O}_2}], \quad S(0) = 0. \quad [1]$$

This model was used to yield fractional ventilation maps by solving for the r value on a pixel-by-pixel basis. S_0 is the signal proportional to the source magnetization of the HP ^3He in the ventilator reservoir and $S(j)$ represents the signal intensity for the j -th breath. The oxygen-induced depolarization of ^3He during each breath is governed by $D_{\text{O}_2} = -\tau/T_{1,\text{O}_2}$, where $T = \xi/\text{PO}_2$ is the oxygen-induced depolarization time constant of HP ^3He as a function of the partial pressure of oxygen (PO_2) present in the airways, with $\xi \approx 2.6 \text{ bar} \cdot \text{s}$ at normal body temperature

[21]. The RF depolarization effect $D_{RF} = N_{PE} \cdot \ln(\cos \alpha)$ represents the accumulative effect of repeated RF excitations on HP ^3He , where α represents the RF pulse flip angle, and N_{PE} is the number of pulses triggered per image (i.e. the number of phase encode lines). From a practical standpoint the oxygen decay effect has a negligible effect on signal buildup [20] and therefore a nominal value of $\text{PO}_2 = 140$ mbar was assumed to hold throughout the lung. On the other hand, the RF pulse effect on the other hand has a substantial effect on signal dynamics and is separately calculated on regional basis by acquiring a series of five back-to-back ventilation images during a 2-sec breath-hold at the end of the fractional ventilation maneuver.

Regional measurement of lung microstructure: Apparent Diffusion Coefficient

Apparent diffusion coefficient maps of ^3He were acquired using a double-acquisition ADC imaging technique previously described in [22]. This method is based on the geometric mean of two separate sets of ADC images which only differ in the order of positive and negative lobes of bipolar diffusion gradients. This is achieved by performing two independent measurements with a single pair of gradients having inverted amplitudes and taking the image product of the results. The double-acquisition technique was shown to be able to eliminate the cross terms between diffusion gradients and imaging gradients [23], which if ignored, could potentially yield to an over-estimated value of the diffusion coefficient. Briefly, a series of five HP gas breaths ($^3\text{He}:\text{O}_2=4:1$) were delivered to the rat at the designated tidal volume to acquire the first series of ADC images during a 3-sec breath-hold for the given series of b -values, given

by: $b(j) = (\gamma G_m)^2 \left[\delta^2 \left(\Delta - \frac{\delta}{3} \right) + \tau \left(\delta^2 - 2\Delta\delta + \Delta\tau - \frac{7}{6}\delta\tau + \frac{8}{15}\tau^2 \right) \right]$, where $\gamma = 32.43$ MHz/T is the gyromagnetic ratio of ^3He , δ is the diffusion gradient duration, Δ is the diffusion time, and $G(j)$ represents the diffusion gradient amplitude corresponding to j -th image in the series at time $t(j)$. This procedure was then repeated in an identical manner with the reverse order of bipolar diffusion gradient amplitudes (i.e. $+/-$ versus $-/+$) in a second breath-hold. The geometric mean of the signal values for corresponding b -values from each breath were then calculated and fit to the following governing equation to yield ADC values on a pixel-by-pixel basis:

$$S(j) = S_0 \cdot \exp[N_{PE} \cdot \ln(\cos(\alpha)) \cdot j - b(j) \cdot \text{ADC} - t(j)/T_{1,\text{O}_2}], \quad [2]$$

where all other parameters represent the same quantities as in the fractional ventilation model.

Histology

Upon completion of imaging, all animals were euthanized using intracardiac, high-concentration potassium chloride. After the animals were confirmed deceased, their lungs were filled with a 10% formalin solution administered through the previously placed angiocatheter to a pressure of 25 cm H_2O for 24 hours as described in published works [24,25]. The trachea and lungs were then removed *en bloc* and placed for one week in a bath of 10% formalin solution, after which they were sectioned and stained with hematoxylin and eosin for microscopic analysis.

Data analysis

Data analysis was performed using custom MATLAB (Mathworks, Natick, MA) programs that were developed by this group. Regional fractional ventilation and apparent diffusion coefficient analysis was performed on a pixel-by-pixel basis at a sub-millimeter planar resolution (approximately 0.94×0.94 mm²). In order to distinguish lung tissue from background, bins with an SNR below a threshold were excluded from analysis. The SNR threshold varied between 5 and 10 for each dataset and was visually inspected to ensure that the entire lung parenchyma was included in the analysis. After excluding background pixels

from the image, time evolution of signal intensity of valid pixels were fit to Equations 1 and 2, to yield maps of regional r and ADC values respectively.

Reducing a distributed measurement to its mean value can potentially mask important information regarding the heterogeneity of the distribution. To circumvent this problem, a percentile threshold metric was used in order to incorporate the distribution heterogeneity of regional r and ADC maps in our analysis. The marker is the percent population of pixels above/below a certain threshold values for each r and ADC quantities ($r_{\text{threshold}}$ and $\text{ADC}_{\text{threshold}}$ respectively). Threshold curves for r or ADC maps were generated and pixels with $r \approx 1.0$ and $\text{ADC} \geq 0.5 \text{ cm}^2/\text{s}$ (corresponding to conductive airways) were masked. For each lung, the $r_{\text{threshold}}$ and $\text{ADC}_{\text{threshold}}$ values were then varied from 0 through 1, and from 0 through 0.5 cm^2/s respectively, and the percent population of pixels with r and ADC value above and below the respective thresholds was calculated accordingly.

For statistical analysis a significance level of 0.05 was used. Separate one-way ANOVA tests were performed for analysis of variance of fractional ventilation and apparent diffusion coefficient values between the two cohorts of rats. The mean r and ADC values of rats in each group were treated as independent samples from each population. The mean and standard deviation of these individual mean values were then calculated as the representative distribution of the r and ADC quantities for the two cohorts were subsequently used to perform the ANOVA test for significance. A similar comparison was performed for the thresholded quantities in order to compare the relative sensitivity of each set of parameters in stratifying the diseased animals from healthy ones.

RESULTS

Lung histology

Figure 1 shows example histological images from lungs of a healthy animal and a rat after 3 weeks of exposure to bleomycin. The healthy animals exhibited normal lung structure concurrent with their baseline measurements, whereas the 3-wk bleomycin animals demonstrated mild interstitial inflammation along with areas of confluent fibrosis, as demonstrated in the shown histology images.

Respiratory Physiology

The pulmonary function test results are summarized in Table 1. PFT results were not available for two of the healthy rats due to a technical problem with the plethysmography system. Even though the small sample size in the control group may preclude a proper statistical analysis on PFT results, the trends are worth noting. No significant difference was observed in TLC and C_{dyn} values between the healthy and 3-wk bleomycin rats. RI and FEV_{100} however were significantly different between the two groups ($p < 0.03$ and $p < 5 \times 10^{-4}$ respectively). These two quantities were modestly correlated with each other ($R^2 = 0.74$) and possibly indicate the presence of inflammation in the small airways as a result of exposure to bleomycin.

Regional fractional ventilation

The results from the analysis of the regional fractional ventilation metric can be seen in the representative maps and histograms of fractional ventilation shown in Figure 2. These are images of both representative healthy control rats and 3-week bleomycin rats. Even though no systematic difference in distribution of fractional ventilation between the two animals is visually observable, the overall r mean value for the bleomycin rat is lower than the healthy animal, with conductive airways (corresponding to $r \approx 1.0$) excluded from the distribution. With respect to the frequency distribution of fractional ventilation, the bleomycin rat represents an overall shift to the left in its r histogram without much difference in the shape, width or

peak population of the histogram and it reflects a relatively uniform decline of r throughout the lung. Mean and standard deviation of r values for all study animals are reported in Table 2, along with the combined average r value for all animals in each group, corresponding to $r_{\text{control}} = 0.58 \pm 0.03$ versus $r_{\text{bleomycin}} = 0.49 \pm 0.02$. Figure 3(a) shows the comparison between the population of healthy ($n = 5$) and bleomycin ($n = 6$) rats, depicting a statistically significant difference in overall r values ($p = 3.4 \times 10^{-4}$). The mean fractional ventilation in all rats modestly correlated with RI and FEV₁₀₀ ($R^2 = 0.50$ and $R^2 = 0.74$), whereas no significant correlation was observed with C_{dyn} .

Regional apparent diffusion coefficient

Representative maps and histograms of ADC are also shown in Figure 2 for the same sampling of healthy control and 3-week bleomycin rats. In this case, the bleomycin rat lung shows a more uniform distribution of ADC values as compared to the healthy animal. However, the overall ADC value for the bleomycin rat is lower than that of the healthy animal, with conductive airways (corresponding to $\text{ADC} \geq 0.5 \text{ cm}^2/\text{s}$) excluded from the distribution. The more uniform distribution of ADC value in the bleomycin rat is also evidenced by the narrower width of the ADC frequency distribution histogram in the bleomycin rat and a larger percentage of pixels at the peak value (20% for the bleomycin vs. 15% for the healthy rat). Mean and standard deviation of ADC values for all study animals are reported in Table 3, along with the combined average ADC value for all animals in each group, corresponding to $\text{ADC}_{\text{control}} = 0.19 \pm 0.02 \text{ (cm}^2/\text{s)}$ versus $\text{ADC}_{\text{bleomycin}} = 0.14 \pm 0.04 \text{ (cm}^2/\text{s)}$. Figure 2(b) shows the comparison between the two populations of healthy ($n = 4$) and bleomycin ($n = 6$) rats and depicts a significant difference in overall ADC values ($p = 0.049$). No significant correlation was observed between the ADC and any of the PFT parameters. The ADC results of control rat #1 had to be excluded from the analysis due to a technical acquisition problem related to incorrect RF pulse width calibration. This artifact was not discovered until the data analysis stage, and made it practically impossible to reliably calculate the ADC values.

Heterogeneity analysis

In order to utilize the inherent heterogeneity of the distribution of r and ADC in the rat lungs and to better quantify the difference between the two groups of animals, a threshold analysis was performed on the maps of each respective parameter. The calculations were performed for $r > r_{\text{threshold}}$ and $\text{ADC} < \text{ADC}_{\text{threshold}}$, using $r_{\text{threshold}} = 0.42$ and $\text{ADC}_{\text{threshold}} = 0.22 \text{ cm}^2/\text{s}$, respectively. The threshold values for r and ADC were selected as the median of the dynamic range of each parameter. End results are reasonably insensitive to the exact value of the threshold quantity as long as it is reasonably close to the center of the respective distribution.

Figure 4 shows the threshold analysis results for fractional ventilation. Each line in Figure 4 (a) represents the distribution of pixels from a single animal with controls and bleomycin rats plotted with different markers. Varying the $r_{\text{threshold}}$ along the x-axis, the y-axis shows the percentage of pixels that are *above* the given threshold of fractional ventilation. Therefore, all curves vary from 100% to zero for the minimum and maximum threshold values. The evidence clearly demonstrates that the group of 3-week post-bleomycin rats has a larger number of pixels lower than that of the controls at a vast majority of the $r_{\text{threshold}}$ values, indicating the ventilation decline in this model. Specifically using $r_{\text{threshold}} = 0.42$ yields the percentage populations reported in Table 2 for both groups of rats with healthy controls at $(86.4\% \pm 4.0)$ versus $(63.3\% \pm 3.0)$ after 3 weeks of bleomycin exposure. The separation between the two groups is highly significant ($p = 2.1 \times 10^{-6}$), as shown in Figure 4(b), with better confidence compared to mean r values, Figure 3(a). Furthermore, Table 2 reports the commonly used 80% population threshold values for comparison which also suggests decreased fractional ventilation in the 3-week post-bleomycin rats.

The results of a similar threshold analysis on ADC maps are shown in Figure 5. Varying the $ADC_{\text{threshold}}$ along the x-axis, the y-axis shows the percentage of pixels that are below the given threshold of apparent diffusion coefficient. Therefore, all curves by construct vary from zero to 100% for the minimum and maximum threshold values respectively. On gross inspection it is evident that ADC in 3-week bleomycin rats shows a trend towards a smaller value than the healthy control animals. Specifically using $ADC_{\text{threshold}} = 0.22 \text{ cm}^2/\text{s}$ yields the percentage populations reported in Table 3 for both groups of rats with healthy controls at $(70.1\% \pm 10.8)$ versus $(89.1\% \pm 6.5)$ after 3 weeks of bleomycin exposure. Although the difference between ADC distributions between the two groups are smaller compared to fractional ventilation, the separation is still highly significant ($p = 0.008$), as shown in Figure 5(b), showing a much better separation of control and bleomycin rats when compared to mean ADC values, Figure 4(b). Similar to fractional ventilation, Table 3 also shows the 80% population ADC threshold values for comparison.

DISCUSSION

In this first study utilizing ^3He MRI to assess lung function in an animal model of mild pulmonary fibrosis, results imply that fractional ventilation declined and was associated with a decrease in ADC value in small airways and alveoli. The decline in total lung capacity and compliance measure by PFT was insignificant over the time frame of 3 weeks, even though a significant change in small airway resistance was observed as measured by forced exhale maneuvers. The forced maneuver parameters only modestly correlated with measurements of fractional ventilation. These findings suggest that the changes noted by ^3He MRI may be more sensitive measures to follow fibrosis lung disease in animal models and possibly in humans.

Bleomycin is the most widely studied rat model of IPF, and a review of the literature shows that it continues to be the model of choice for researchers [26]. The histology is somewhat different than usual interstitial pneumonia (UIP), but shares similarities. After intra-tracheal instillation, there is an intense inflammatory reaction where inflammatory markers IL-1, TNF-alpha, and IL-6 are elevated along with concomitant histological changes compatible with acute lung injury. At approximately day 9, there is a switch from inflammatory mediators to pro-fibrotic mediators like TGF-beta-1 and fibronectin, along with a bronchocentric fibrotic response on pathology. Associated with this fibrosis, there is also a decrease in TLC and compliance, as confirmed by PFT results in Table 1. However, this fibrotic response is not always progressive, and may begin to recede after day 21. It is the time period between days 9 and 21, that the model is most representative of human IPF.

Although not completely perfect, the bleomycin model has helped elucidate our most recent hypothesis of IPF being a disease of pro-fibrotic cytokines causing an increase in fibroblasts and myofibroblasts which subsequently produce collagen and other matrix components [27]. The initial insult may be to the epithelial cells which then lead to epithelial-mesenchymal cross-talk. The bleomycin model is also where several new anti-fibrotic agents like imatinib [28], bosentan [29], and anticoagulants [30] have had initial success, prompting their progression to human studies.

Results show that fractional ventilation was significantly decreased in a 21-day bleomycin model of interstitial lung disease. In addition to analyzing the change in mean fractional ventilation, we also utilized the metric of threshold analysis to indicate the relative area (i.e. number of pixels) above or below a specific fractional ventilation value. While a difference in the mean values was observed (Figure 4), a greater spread and increased significance was noted when the threshold analysis was utilized. This approach is most likely a better-suited technique to assess the distribution of a heterogeneous disease in the lungs. The cause of decreased fractional ventilation could be due to three processes. First, decreased tidal volumes could

result in decreased ventilation to the lungs. Since ventilation in these animals was maintained at 15% of TLC, it is not expected that this was the cause of the differences in observation. Second, in small airways, narrowing causing increased resistance may decrease the flow into each alveolus and thus decrease fractional ventilation. This is most likely the cause of decreased fractional ventilation in emphysema and probably does not occur in this model, although presence of residual inflammation in the airways after three weeks may have an adverse effect on gas replacement efficiency, as it was also evident by declined FEV₁₀₀ and elevated R_I values. Finally, a decrease in compliance would decrease the ability of the alveoli to expand during ventilation and could also result in decreased alveolar ventilation. It is suggested that this mechanism is the most likely explanation for the decreased fractional ventilation observed in the rats treated with bleomycin since changes in compliance have been reported earlier in the 21 day rat bleomycin model [31].

In addition to showing a decrease in fractional ventilation, the ADC measurements were shown to be significantly reduced (Figures 3 and 5). As with the measurements of fractional ventilation, the metric of threshold analysis was also used to demonstrate the change in ADC in bleomycin-treated rats. Threshold analysis of ADC indicated a greater difference between the bleomycin and the control animals than did the difference between the mean values of the ADC. ADC has been measured in humans [32, 33] and animal models of emphysema and asthma [34, 35]. This study however presents the first utilization of this radiologic measure of lung microstructure to interstitial lung disease. The decreased ADC is consistent with the finding of decreased fractional ventilation and supports the theory that a decrease in compliance was the cause of the decreased fractional ventilation.

Results suggest that quantitative imaging of lung ventilation and microstructure by ³He MRI may be sensitive measures for monitoring the progression of fibrotic disease in animal models and potentially human models in a noninvasive manner while eliminating the need for ionizing irradiation. Idiopathic pulmonary fibrosis is the most common fibrotic lung disease in adults and unfortunately there is no known therapy for this disease. Clinical trials of this condition have used either death or global assessments of lung function (changes in forced vital capacity or diffusion capacity) to gauge progression [3,4,36]. We expect that the ability to perform regional radiographic assessment of heterogeneous lung changes will lead to a more sensitive measurement of progression in this disorder. A possible challenge in implementing the proposed imaging technique in affected human populations is the necessity for presence of hyperpolarized ³He in the alveolar space to make the imaging possible. If no hyperpolarized ³He enters the airway, then no assessment of airway function could be made. In addition, many patients with fibrotic lung disease require oxygen supplementation, which can adversely affect the MRI signal through faster depolarization of hyperpolarized gas.

CONCLUSION

This study shows for the first time that quantitative ³He MRI is a potential tool for assessing pathology in an interstitial lung disease animal model. The primary benefit of this technique is that it combines both structural and functional information on a regional and inherently co-registered basis. Additionally, images can be acquired noninvasively and without exposure to ionizing radiation. Fractional ventilation may be the more useful of the two metrics that were used, and its change from baseline is likely a reflection of reduced compliance. Apparent diffusion coefficient of gas in the airways may also be of utility for further characterization of disease models and their progression. The most immediate implication of this work is in the realm of functional assessment of animal models in drug studies. It would also be worthwhile to conduct future studies in human populations to see if these novel metrics, which assess disease on a regional basis, have any utility in either diagnosis or in predictions of natural history.

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(a) Control

(a) 3-wk Bleomycin

Figure 1.
Representative lung histology slides of (a) healthy controls and (b) 3-week bleomycin rats.

Figure 2.

^3He spin density MR images of a representative healthy control and 3-week bleomycin rat along with their respective maps and histograms of fractional ventilation (r) and apparent diffusion coefficient (ADC).

Figure 3. Box plots comparing mean (a) r and (b) ADC values between healthy control and 3-week bleomycin rats showing the measure of statistical significance between the two groups.

Figure 4.

(a) Threshold heterogeneity analysis of fractional ventilation (r) between healthy control rats in blue and 3-week bleomycin rats in red. The x-axis is the range of r from 0 to 1.0 and the y-axis is the percent of pixels with an r greater than the specific r indicated on the x-axis. (b) Box plots illustrating the statistical significance between the two groups using a threshold of $r = 0.42$. The decreased population of pixels with a threshold $r = 0.42$ indicated a decreased fractional ventilation in the bleomycin treated rats.

Figure 5.

(a) Threshold heterogeneity analysis of apparent diffusion coefficient, ADC, between healthy control rats in blue and 3-week bleomycin rats in red. The x-axis is the range of ADC from 0 to 0.5 and the y-axis is the percent of pixels with an ADC less than the specific ADC indicated on the x-axis. (b) Box plots illustrating the statistical significance between the two groups using an ADC threshold of 0.22 cm²/s. The increased population of pixel with a threshold less than 0.22 cm²/s indicates a decrease in alveolar size.

Table 1

Summary of pulmonary function testing measurements in the two groups of rats.

	No.	TV [mL]	FRC [mL]	TLC [mL]	FEV100 [mL/100ms]	RI [cmH2Oms/mL]	Cdyn [mL/cmH2O]	FEV100/FRC [100ms-1]	TV/(TV+FRC)	FRC/TLC
Healthy Controls	2	2.7	4.7	17.4	9.2	0.11	0.43	1.97	0.36	0.27
	3	2.7	5.3	17.6	10.2	0.05	0.34	1.91	0.34	0.30
	4	3.4	6.8	22.9	8.5	0.08	0.59	1.24	0.33	0.30
	MEAN	2.9	5.6	19.3	9.3	0.08	0.45	1.71	0.34	0.29
	SD	0.4	1.1	3.1	0.9	0.03	0.13	0.40	0.02	0.02
3-wk Bleomycin	1	1.5	5.2	10.1	2.3	0.30	0.33	0.44	0.23	0.51
	2	3.1	5.9	20.7	4.5	0.19	0.42	0.77	0.35	0.28
	3	2.5	5.7	16.6	5.3	0.21	0.56	0.93	0.30	0.34
	4	2.8	8.3	18.6	3.8	0.41	0.47	0.45	0.25	0.45
	5	1.9	10.2	12.7	1.4	0.47	0.13	0.13	0.16	0.81
	6	3.3	12.7	21.8	2.0	0.65	0.50	0.15	0.20	0.58
	MEAN	2.5	8.0	16.7	3.2	0.37	0.40	0.48	0.25	0.50
	SD	0.7	3.0	4.6	1.6	0.17	0.15	0.32	0.07	0.19

Table 2

Summary of measurements of fractional ventilation in the two groups of rats along with the 80% threshold values.

	No.	Mean <i>r</i>	Population Cutoff % @ <i>r</i> = 0.42	80% Threshold
Healthy Control Rats	#1	0.58 ± 0.13	90.2	0.47
	#2	0.59 ± 0.16	88.2	0.45
	#3	0.62 ± 0.16	89.2	0.49
	#4	0.56 ± 0.16	80.8	0.42
	#5	0.53 ± 0.12	83.6	0.44
		0.58 ± 0.03	86.4 ± 4.0	0.46 ± 0.03
3-week Bleomycin Rats	#1	0.50 ± 0.19	66.2	0.34
	#2	0.47 ± 0.16	64.2	0.35
	#3	0.50 ± 0.17	63.5	0.36
	#4	0.47 ± 0.17	58.6	0.33
	#5	0.51 ± 0.18	67.3	0.34
	#6	0.47 ± 0.16	63.0	0.35
		0.49 ± 0.02	63.3 ± 3.0	0.35 ± 0.01

Table 3

Summary of measurements of apparent diffusion coefficient in the two groups of rats along with the 80% threshold values.

	No.	Mean ADC [cm ² /s]	Population Cutoff % @ ADC = 0.22 cm ² /s	80% Threshold [cm ² /s]
Healthy Control Rats	#1	—	—	—
	#2	0.18 ± 0.08	75.3	0.23
	#3	0.17 ± 0.07	81.1	0.22
	#4	0.20 ± 0.09	68.0	0.25
	#5	0.22 ± 0.09	56.1	0.29
		0.19 ± 0.02	70.1 ± 10.8	0.25 ± 0.03
3-week Bleomycin Rats	#1	0.17 ± 0.07	84.1	0.21
	#2	0.18 ± 0.06	84.2	0.21
	#3	0.11 ± 0.05	97.4	0.14
	#4	0.09 ± 0.06	97.5	0.11
	#5	0.16 ± 0.06	84.7	0.21
	#6	0.16 ± 0.07	86.6	0.20
		0.14 ± 0.04	89.1 ± 6.5	0.18 ± 0.04