HIGHLIGHTED TOPIC | Physiological Imaging of the Lung

In vivo lung morphometry with hyperpolarized $^3$He diffusion MRI in canines with induced emphysema: disease progression and comparison with computed tomography

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Tanoli TSK, Woods JC, Conradi MS, Bae KT, Gierada DS, Hogg JC, Cooper JD, Yablonskiy DA. In vivo lung morphometry with hyperpolarized $^3$He diffusion MRI in canines with induced emphysema: disease progression and comparison with computed tomography. J Appl Physiol 102: 477–484, 2007. First published July 27, 2006; doi:10.1152/japplphysiol.00397.2006.—Despite a long history of development, diagnostic tools for in vivo regional assessment of lungs in patients with pulmonary emphysema are not yet readily available. Recently, a new imaging technique, in vivo lung morphometry, was introduced by our group. This technique is based on MRI measurements of diffusion of hyperpolarized $^3$He gas in lung air spaces and provides quantitative in vivo tomographic information on lung microstructure at the level of the acinar airways. Compared with standard diffusivity measurements that strongly depend on pulse sequence parameters (mainly diffusion time), our approach evaluates a “hard number,” the average acinar airway radius. For healthy dogs, we find here a mean acinar airway radius of $\sim 0.3$ mm compared with 0.36 mm in healthy humans. The purpose of the present study is the application of this technique for quantification of emphysema progression in dogs with experimentally induced disease. The diffusivity measurements and resulting acinar airway geometrical characteristics were correlated with the local lung density and local lung-specific air volume calculated from quantitative computed tomography data obtained on the same dogs. The results establish an important association between the two modalities. The observed sensitivity of our method to emphysema progression suggests that this technique has potential for the diagnosis of emphysema and tracking of disease progression or improvement via a pharmaceutical intervention.

PULMONARY EMPHYSEMA, which is defined as “a condition of the lung characterized by abnormal, permanent enlargement of airspaces distal to the terminal bronchioles, accompanied by destruction of their walls, without fibrosis” (23), is a major medical problem worldwide. There are several conventional methods of diagnosing and evaluating emphysema: pulmonary function tests, chest radiography, and computed tomography (CT). These methods in general provide a gross assessment of the degree of emphysema and are used for qualitative, clinical applications. Pulmonary function tests are reportedly insensitive to early, mild changes of emphysema (24); the same is true for conventional chest radiography (25). CT is more sensitive in detecting early changes and local variations of emphysema (9, 10, 15, 17, 20), and it has shown good correlation with pathology specimens for assessing the degree of emphysema (7, 12, 18). However, CT cannot provide information on lung tissue microstructure and cannot readily distinguish between ventilated and nonventilated lung regions.

Magnetic resonance (MR) imaging of lung air spaces with hyperpolarized gases can provide new insights into lung physiology. In particular, diffusion lung imaging with hyperpolarized $^3$He gas has demonstrated substantial differences between the $^3$He gas apparent diffusion coefficient (ADC) in healthy and emphysematous lungs, both in humans (4, 21, 22, 26) and animals (rats with elastase-induced emphysema) (2, 19). This points to a large potential for identifying emphysema by means of hyperpolarized gas ADC measurements. However, before the technique can become a useful tool for characterizing emphysema, it is important to understand the relationship between the measured ADC and the underlying lung microstructure. Recently, an MR imaging technique, i.e., in vivo lung morphometry (27), was introduced by our group. In this approach, lung geometry at the acinar level was described in terms of cylindrical airways covered with alveolar sleeves, a model previously introduced by Haefeli-Bleuer and Weibel (11), as depicted in Fig. 1. The in vivo lung morphometry technique (27) is based on MRI measurements of anisotropic-restricted diffusion (along and perpendicular to the acinar airway axis) of hyperpolarized $^3$He atoms in lung air spaces and allows evaluation of acinar airway geometry and the integrity of alveolar walls. The method provides in vivo tomographic information on lung microstructure and may be considered as a virtual morphometry of the acinar airways without physically violating the lung parenchyma for tissue samples. The enlargement and destruction of the acini associated with emphysema increases the apparent diffusivity of the $^3$He gas. This alteration in diffusivity, along with ventilation images obtained with $^3$He spin density MRI, may be used to assess the structure-function relationship of the lung; the quantitative

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values can be used to follow the natural history of emphysema progression and treatment outcome.

The purpose of the present study is the application of the in vivo lung morphometry technique (27) for evaluation of emphysema progression in dogs with experimentally induced emphysema and in healthy controls. The diffusivity measurements and the acinar airway geometrical characteristics so determined were correlated with quantitative CT measurements in the same dogs, providing an important association between two modalities.

**MATERIALS AND METHODS**

The experiments reported here are based on five mongrel dogs (weight between 18 and 20 kg). All of the procedures used were approved by the institutional animal studies committee.

**Animal preparation.** Emphysema was induced in only the right lungs of dogs, according to a previously developed protocol (3). In brief, porcine pancreatic elastase was instilled through one side of a specially designed, double-lumen tube that allows separate ventilation of each lung. The right lung was made to be atelectatic to facilitate a deep penetration of the elastase into the lung parenchyma. The right lung was first ventilated with 100% oxygen. Then the ventilation was sensitizing gradients, transverse to the axis, $D_T$, and a diffusion coefficient transverse to the axis, $D_T$. Given that a large number of acinar airways with different directions reside in each imaging voxel, the total MR signals can be expressed as a sum of the signals from airways with an isotropic distribution of directions, leading to the following analytical expression (27):

$$S = S_0 \exp(-bD_T) \left[ \frac{\pi}{4bD_{AN}} \right]^{1/2} \phi(bD_{AN})^{1/2}$$

(1)

Here, $S_0$ is the MR signal intensity in the absence of diffusion sensitizing gradients, $\phi(x)$ is the error function, and the anisotropy of the diffusion coefficient $D_{AN}$ is

$$D_{AN} = D_h - D_T$$

(2)

Hence, this model allows extraction of the microscopically $D_{AN}$ in lung airways, despite the macroscopically nearly isotropic nature of the lung and despite the airways being too small to be resolved by direct imaging. $Equation \ 1$ describes the non-monoeponential dependence of diffusion attenuated signal on $b$ value. Hence, the ADC, defined as $ADC = -\ln(S/S_0)/b$, is a function of $b$ value. It can be easily demonstrated that, for small $b$ values, $ADC$ coincides with mean diffusion coefficient ($D_m$):

$$ADC = D_m = \frac{1}{3}D_h + \frac{2}{3}D_T$$

(3)
The $D_M$ value reported by our method is related to ADC measurements reported previously (i.e., Refs. 2, 21, 22). However, the previous work employed only two $b$ values and effectively assumed exponential signal decay as a function of $b$ through the diffusion-sensitizing gradient strength. As is evident from the present results and analysis, the ADC in lungs determined from the two-$b$ method depends on $b$ value and approaches the true orientation-average value $D_M$ from below, only in the limit of small $b$. The $D_M$ values reported herein provide a more objective result because they do not depend on the diffusion-sensitizing gradient strength.

For $^3$He diffusion, MR image analysis, nine $b$-value images were utilized. The theoretical model, Eq. 1, was fit to the data on a pixel-by-pixel basis using Bayesian probability theory, and maps of $D_T$, $D_L$, and $D_M$ were generated. To reduce the influence of noise on our results, the minimum threshold value was set to three times the value of noise (typical SNR for the image with minimum $b$ value was 35–40). Thus only the pixels that had signal values in the largest $b$-value image greater than three times the value of noise were considered for all $b$-value images. The mean external radius ($R$) of acinar airways (see Fig. 1) in millimeters were calculated from $D_T$ using Equation 8 in Ref. 27 (see also Figs. 1 and 3 therein). This equation is rather complicated, and we do not reproduce it here. It was derived theoretically, and for a given gradient waveform establishes a unique relationship between transverse ADC $D_T$ (measured with our technique) and airway $R$.

**CT studies.** CT scans were performed using a four-channel multidetector row CT scanner (Plus 4 volume zoom; Siemens Medical Systems, Iselin, NJ). The scan parameters include spiral scan mode, 120 kVp, 120 mA, and $4 \times 2.5$-mm collimation. The CT images were reconstructed with 3-mm section thickness, except for one set reconstructed with 5-mm section thickness. The dogs were anesthetized, intubated, and positioned prone during the CT scan (similar to the position for the MR scan). The scans were obtained at an end-inspiratory volume of 600 ml above functional residual capacity, using air delivered from a calibrated cylinder.

**CT scan analysis.** Analysis of CT images was carried out using Analyze 3.1 image analysis software program (Biomedical Imaging Resource, Mayo Foundation, Rochester, MN) and a home software for image registration. CT images were first registered against proton MR images that were acquired during the same MR imaging session and image registration. CT images were then analyzed using the Analyze software. Analysis of CT images was carried out using Analyze 3.1 image analysis software program (Biomedical Imaging Resource, Mayo Foundation, Rochester, MN) and a home software for image registration. CT images were first registered against proton MR images that were acquired during the same MR imaging session and image registration. CT images were then analyzed using the Analyze software.

The $V$ of the lung (tissue and gas; $V_{tissue \& gas}$) was thus measured as:

$$V = V_{tissue \& gas} - V_{tissue} \quad (4)$$

where $V$ values are the inverse of densities and are measured in ml/g. The $V$ of the lung (tissue and gas; $V_{tissue \& gas}$) was thus measured from CT as:

$$V_{tissue \& gas} (ml/g) = 1,000/(HU + 1,000) \quad (5)$$

The $V$ of tissue was calculated as $V_{tissue} = 1/\rho_{tissue}$, where density of tissue was assumed to be $\rho_{tissue} = 1.065$ g/ml (13).

**RESULTS**

The canines were imaged at various stages of emphysema induction. Figure 2 demonstrates histograms of CT attenuation distribution for healthy untreated, emphysematous, and healthy control (the neighboring to emphysematous) lungs. The lower attenuation (more negative HU) in the emphysematous lungs is clear and as expected. We note a decrease in the volume of the healthy control lungs and a corresponding increase in their CT attenuation coefficient.

Typical diffusion-attenuated MR images for both healthy and emphysematous lungs along with their corresponding CT images are displayed in Fig. 3. In the top row, the small diffusivity of the lungs in untreated dogs is demonstrated by the weak attenuation of signal intensity with increasing $b$ value. In the bottom row with data from a dog with three lavage treatments, the larger diffusivity of the emphysematous lung and the smaller diffusivity of the untreated control lung are evident.

**Typical dependence of diffusion-weighted MR signal on $b$ value** is demonstrated for healthy untreated, healthy control lungs, and lungs with emphysema of varying severity in Fig. 4. This figure clearly shows that the attenuation of hyperpolarized helium gas MR signal is substantially dependent on the severity of emphysema. There is very rapid signal decay in the case of diffusion of $^3$He molecules in free air, as can be obtained in

**Fig. 2.** The frequency distribution curves of computed tomography (CT) Hounsfield units (HU), normalized to equal areas, demonstrate clear differences between the healthy and treated lungs. The untreated healthy lungs show a peak at −880 HU, whereas the healthy left lungs (LLs) of the treated dogs (healthy control) display a peak at −740 HU. The treated right lung (RL) group demonstrates a left shifted peak at −940 HU. For emphysematous and healthy control lungs, these HU values were measured in dogs with three or more lavage treatments.
the trachea. In contrast, the rate of signal decay in healthy control lungs is far slower. In between the two extremes are signal attenuation curves from normal healthy and emphysematous lungs from the same dog after each emphysema treatment, tentatively identified as being mild, moderate, or severely emphysematous.

Two of the dogs were followed serially before and approximately 1 mo after each lavage. Figure 5 illustrates diffusion maps, proton density, and corresponding CT images from one of them. Baseline healthy diffusion MR maps (first column) display a relatively homogenous distribution of diffusion coefficients. The corresponding CT images also display lung parenchyma that appears healthy, with no evidence of emphysema. After the first lavage (second column in Fig. 5), the CT images show some evidence of tissue destruction in the right lung. As in Fig. 3, nine $b$-value MR images demonstrate good gas distribution in both lungs on the initial $b$-value images (very small $b$ value, so no diffusion weighting; images not shown). Increasing $b$-value images illustrate relatively faster signal decay in the regions, with greater tissue destruction in the right lung. This rapid signal decay is reflected in the
nonhomogenous distributions in the right lung on MR diffusivity maps (Fig. 5). After three lavage treatments, the changes in \(^3\)He diffusivities and CT attenuation are yet more pronounced. In the third column of Fig. 5, regions of very low ventilation appear in the treated lung (similar to the low-intensity regions in the treated lung shown in Fig. 3, second row). They manifest as voids in diffusion maps because the low SNR does not allow accurate calculation of diffusion coefficients in these voxels. Hogg et al. (14) measured the pressure-volume curves of centrilobular emphysematous spaces and showed that they were so compliant that they became fully inflated at very low transpulmonary pressures. We suspect that the same is true here in that, if the emphysematous spaces are fully inflated and on the flat part of their pressure-volume curve, it takes large transpulmonary pressure to further inflate them when \(^3\)He gas is delivered. Our experiments on excised lungs (not shown here) demonstrate that such regions can actually be filled with the \(^3\)He gas after several inhalation/exhalation cycles.

Figure 6 illustrates values of \(^3\)He diffusivities, airway \(R\), and lung-specific \(V\) from a serial follow-up in the same dog. This bar graph displays an increase in \(D_T, D_L, D_M, R\), and gas-specific \(V\) values from healthy to diseased lung. In contrast, if we look at the untreated left lung, its mean specific \(V\) decreases slightly after each treatment (the compliant emphysematous right lung reduces the transpulmonary pressure gradient, leading to effective “underinflation” of healthy control lung), while the \(^3\)He diffusivity values and, therefore, the airway \(R\) tend to remain relatively constant within experimental error.

**Comparison of quantitative analysis of CT and diffusion MR images.** Figure 7 demonstrates the relationship between mean values of \(D_T, D_L, D_M, R\), and gas-specific \(V\). The specific \(V\) calculated from Eq. 4 is a physiologically meaningful parameter that allows easy calculation of regional and total lung \(V\) using X-ray linear attenuation coefficient obtained from CT data. Data for \(D_T, D_L, D_M\), and \(D_M\) were approximated by the following mathematical equations:

\[
D_T = D_0 \frac{V}{V_T + V}; \quad D_L = D_0 \frac{V}{V + V_L}; \quad D_M = D_0 \frac{V}{V + V_M}
\]

where \(a\) is a dimensionless parameter. This provides a good fit to the experimental data for healthy and emphysematous lungs, as shown in Fig. 7. The lung tissue density, \(\rho_{\text{tissue}} = 1.065 \text{ g/ml}\), is introduced to Eq. 7 for convenience.

Figure 8 also shows our results in a more conventional way, plotted directly as functions of HU. Fitting curves represent the same Eqs. 6 and 7, where specific \(V\) is defined as a function of HU according to Eq. 4, and the values of \(V_T, V_L, V_M\), and \(a\) are the same as in Fig. 7.

All fitting curves in Figs. 7 and 8 demonstrate good correlation \((r^2 = 0.71, 0.56, 0.54, \text{ and } 0.56 \text{ for } D_T, D_M, D_L, \text{ and } R, \text{ respectively})\) with experimental data. Among all of the diffusivity values, \(D_T\) demonstrates the greatest change (4–5 times) from healthy to affected lung. Figure 8 also demonstrates a large interdog variability in HU values among healthy dogs. For the same data points, diffusivity values lie within a comparatively narrower range. In addition, a closer look at Figs. 7 and 8 in the emphysematous region demonstrates a few data points (from one dog) clearly deviating from the norm, showing change in diffusivities after one treatment but very little or no change in HU value.

**DISCUSSION**

Two modalities that work by entirely different physical processes were used to detect the presence and severity of emphysema and follow its progression. The X-ray attenuation determined by CT was employed to directly assess the density and specific \(V\) of the lungs, and the MRI technique, utilizing measurements of diffusion of spins of hyperpolarized \(^3\)He gas, was employed to estimate the airway microgeometry. A quantitative measure reflective of acinar airway size and morphology contained within the corresponding voxel can be obtained by hyperpolarized \(^3\)He multi-\(b\)-value diffusion imaging during

\[\text{Figure 7 demonstrates the relationship between airway } R \text{ and gas-specific volume } V \text{ in canine emphysema, showing a change in specific } V \text{ values.} \]
health and disease. It is expected that, after emphysema induction with porcine pancreatic elastase, there should be panacinar-like emphysema changes in the acinar airways and their alveolar sleeves. These alterations in shape and size should cause an increase in $\text{D}_\text{T}$ values in emphysematous regions of the lungs. Using our laboratory’s technique, in vivo lung morphometry (27), we quantified these changes in lung parenchyma of mongrel dogs and evaluated the $R$ of the acinar airways in health and disease.

Our study demonstrates that $D_T$, $D_L$, $D_M$, $R$, and specific $V$ in healthy canine lungs are smaller than those in emphysema lungs. This result reflects that the alveoli of intact lung parenchyma are smaller, resulting in more restricted gas diffusion, in agreement with previous MR studies in humans (21, 22, 27). With progression of emphysema, the alveolar air space is enlarged, leading to the reduction in CT attenuation along with progressive increase in $D_T$, $D_L$, $D_M$, $R$, and specific $V$. The overall correlation between CT attenuation and diffusivity values is good, but a closer look at the data shows that the healthy lungs show large intersubject variability in CT values, whereas the diffusivity values do not. For example, data in Fig. 8 demonstrate that $D_T$ for healthy subjects changes approximately between 0.02 and 0.04 cm$^2$/s, which corresponds to ~12% of the $D_T$ dynamic range in all of the dogs. The same figure demonstrates that $D_T$ for healthy dogs changes approximately between ~800 and ~700, which corresponds to 40% of the whole dynamic range for all dogs. In addition (as previously pointed out), a twofold increase in the value of $D_T$ was observed after the first lavage treatment in one of the dogs without any significant change in HU value. These observations point to the fact that X-ray attenuation may be affected by various factors that are not directly associated with changes in the disease progression.

Comparing previously obtained results in healthy humans ($D_T$ in the range of 0.09 to 0.13 cm$^2$/s and $D_M$ in the range of 0.16 to 0.23 cm$^2$/s) (27) and our current results for dogs ($D_T$ in the range of 0.03 to 0.06 cm$^2$/s and $D_M$ in the range of 0.13 to 0.2 cm$^2$/s), we suggest that $D_T$ is much more sensitive to lung microstructural differences than the commonly used $D_M$. Indeed, the data show the average value of $D_T$ to be 240% higher in healthy humans than in healthy dogs, whereas $D_M$ is only slightly increased (~15%) in healthy humans compared with healthy dogs. This result becomes even more convincing if we note that exactly the same parameters for diffusion times were used in the pulse sequences employed herein and in the human studies (27).

We specifically emphasize an important feature of our approach: the ability to evaluate a geometrical parameter of lung microstructure, the mean $R$ of acinar airways (Fig. 1). Compared with ADC measurements that strongly depend on pulse sequence parameters (mainly diffusion time), the array $R$ is a “hard number” that directly reflects the size of acinar airways.
For healthy dogs, our analysis yields a mean acinar airway $R$ of \( \sim 0.3 \) mm compared with 0.36 mm in healthy humans. While human data are in good agreement with previous direct measurements (11), to the best of our knowledge, there are no data for canine acinar airway geometry available for comparison.

We note that the theoretical model (27) that we have used for analysis is based on the description of lungs in terms of airways covered with alveolar sleeves (see Fig. 1) as proposed by Haefeli-Bleuer and Weibel (11) for healthy lungs. We can expect that this model can also be applied for initial stages of emphysema when only minor deformation and destruction of acinar airways and alveoli take place. With the further progression of emphysema, acinar airways become enlarged and alveolar walls undergo destruction; hence, the description of acinar airways in terms of cylinders covered with alveolar sleeves becomes less accurate (8, 27). Hence, the description of acinar airways in terms of cylinders covered with alveolar sleeves becomes less accurate (8, 27). Hence, in these cases, the parameters $D_T$, $D_L$, and $R$, as determined by our method, can only be considered as apparent. As we demonstrated herein, the values of these parameters increase with emphysema progression; consequently, they still can characterize the level of emphysema and serve as biomarkers of emphysema evolution.

The phenomenological Eqs. 6 and 7 arising from the comparison of diffusion measurements of lung microstructure and CT measurements of lung density and inflation $V$ (ml/g) (Figs. 7 and 8) can be used to estimate the microstructure of the acinar airways and the regional and overall specific $V$ of the lung at which the scan was obtained. With progression of emphysema, there is an observed increase in $D_M$ of $^3$He gas, which is associated with an increase in specific lung $V$ and acinar airway $R$. On a slice-by-slice comparison, the healthy lungs show reduced scatter, i.e., less variability in diffusivity and regional lung $V$ values. As emphysema progresses, this variability increases, amplifying the scatter. This finding is expected, as the general appearance of emphysema under magnification is extremely variable. It includes holes in the alveolar walls, coalescence of alveoli that form larger cavities. There are also groups of “intact” alveoli larger in size. This distention may be due to damage to elastin in the alveolar walls, making the walls weak before they break, or it is simply air trapping in those alveoli causing their distention (1).

The strong correlation between quantitative CT values and numbers obtained from measurement of multi-$b$-value diffusion MRI is important. CT is currently considered to be the best imaging option for diagnosis, quantification, and follow-up of emphysema. However, the presence of ionizing radiation limits its utility in following the progression of disease. Our in vivo lung morphometry technique, based on diffusion imaging, using hyperpolarized $^3$He gas (27), is, by contrast, free of ionizing radiation, and our results show that its value is well correlated with CT. In fact, it promises even improved results for diagnosis of emphysema; in addition to providing data concerning destruction of lung parenchyma, another advantage includes the identification of regions of poor

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**Fig. 8.** Scatter plots demonstrating relationships between $D_T$, $D_L$, $D_M$ (in cm$^2$/s), $R$ (in mm), and HU for all dogs. Each data point represents a mean value for a healthy or treated lung in a given slice at a specific time point in the treatment. *, Healthy lung in untreated dogs; □, untreated lung in treated dogs; ▲, treated lung. Treated lungs generally have larger diffusion coefficients and lower X-ray attenuations than the lungs of untreated dogs, while the control lungs have smaller diffusion coefficients and greater X-ray attenuations.
lungs ventilatory function. Therefore, not only can this technique be used for early diagnosis of emphysema, it also has potential for following disease progression or improvement via pharmaceutical treatments.

In conclusion, we suggest that the in vivo lung morphometry technique based on diffusion MRI with hyperpolarized $^3$He gas provides important regional information on lung microstructure and could potentially add to the evaluation of emphysema progression. It is safer and could be more sensitive for the diagnosis of emphysema than CT, especially at early stages of the disease.

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