Correlation of Hyperpolarized ^{3}He Q-space Diffusion MRS Parameters with in vitro Lung Data Across Animal Species

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Introduction

Q space formalism provides information about the translational root mean square (rms) displacements in terms of a displacement probability profile (DPP). For ^{1}H q space diffusion analysis, the parameters extracted from DPP have been shown to be well correlated with structural information [1]. Recently, a hyperpolarized ^{3}He q space spectroscopy technique has been utilized for detecting pathological changes of lung micro-structure in emphysema and shown to be sensitive to age related changes in lung microstructure [2]. The parameters extracted from ^{3}He diffusion DPP *in vivo* were found to agree well with historical morphometry data. The purpose of this study is to directly assess the correspondence of those parameters with histology from different animal species.

Methods and Materials

Lung Harvesting and Fixation: Lungs from eight animals (4 rabbits, 3 rats and 1 pig) were harvested post mortem by making an incision along the anterior midline and removing en block the heart and lungs. The heart was removed, and the lungs fixed by inflating them with dry air [3]. The air pressure and inflation periods were as follows: Rat lungs: 20 - 25 cm of H_2O, 24 hours, Rabbit lungs: 25-30 cm of H_2O, 48 hours. Pig Lung: 30-35 cm of H_2O, approximately 7 days. **Q-space Spectroscopy:** All the studies were performed on 1.5 T Siemens Sonata MRI system. For each lung, 20 global lung spectra were collected, by using a nonselective 400 µs Gaussian RF pulse, bipolar trapezoidal gradients (δ=3.5 ms, Δ=7 ms, diffusion sensitization along A-P direction, q_{min} .= 0 mm^{-1}, q_{max} of 4.3 mm^{-1} and b_{max} of 428 s/cm^2, spectral width =10 kHz, and 256 complex data points) with TE = 9.4 ms, TR = 43.9 ms. To correct for T_1 and flip angle related attenuation, five q =0 mm^{-1} were interleaved with every fifth diffusion weighted signal acquisition [2]. The displacement resolution was 230 µm, which was digitally enhanced to 58 µm by zero-filling. **Spectroscopic Examination:** The fixed lungs were placed in adequately loaded, flexible ^{3}He coil. The transmitter frequency was adjusted by infusing a small volume of gas (~ 5 cc) in the dried lung and acquiring a low flip angle (<1°) signal to calibrate the transmitter frequency. Following the frequency adjustments, the lungs were flushed with ~ 20 cc of N_2 gas, after which ^{3}He gas was infused into the lungs. The amount of ^{3}He used varied by species with 30 cc of ^{3}He used in rabbit lungs, 4 cc in rats and 60 cc of ^{3}He mixed with 200 cc of N_2 in the pig lung. The q-space spectroscopy sequence was then executed twice with 14° and 20° flip angles. **Q-space Data Analysis**: The datasets were then processed as previously described. The displacement probability profiles (DPP) were fit with multi-Gaussian model: $DPP(x) = \sum_{n=1}^{m} Z_n e^{-0.5\left(x/X_{rms,n}\right)^2}$, $m \geq 1$. A F-test was used to determine whether next higher order Gaussian significantly improved the fit over the lower order model. The weighted average rms displacement was defined as: $X_{rms,WA} = \sum_{n=1}^{m} Z_n X_{rms,n} \Big/ \sum_{n=1}^{m} Z_n$. However, rms displacements > 800 µm were excluded since these large displacements were thought to be generated by gas that leaked out of lungs. **Histology:** For histology, approximately 10mm x 14 mm x 3mm sections of lung tissue were excised from upper, middle and lower portions of both the right and left lobes of the lung for a total of 6 sections per lung. The slides were prepared using a Miles/Sakura Tissue-Tek VIP Processor and then embedded in a paraffin block, before being sliced in approximately 5 µm slices and stained using Hematoxylin and Eosin (H&E) stain. **Histology Analysis:** The stained histology slides were digitized using a high–magnification clinical microscope system (Olympus BX-51, Melville, NY) equipped with a 12.5 MP digital camera. Four to five non-overlapping fields per histological slide were digitized. Mean chord length (MCL) was calculated, excluding large airways, as suggested by Lum et.al [4]. Chord lengths < 10 µm for rabbit and pig lungs and < 4 µm for rat lungs were excluded from analysis.

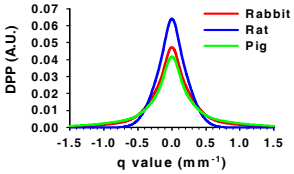

Figure 1. DPP in different lungs.

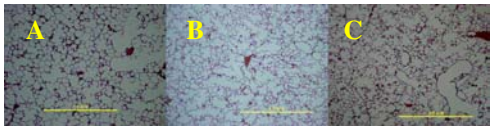

Figure 2. Histology sections in A. Rat, B. Rabbit and C. Pig lung. Note that scale on A is half of that on B and C.

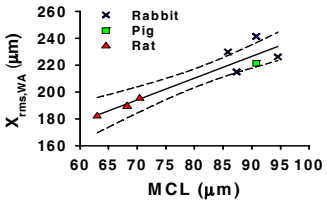

Figure 3. Correlation between MCL and $X_{rms,WA}$. The dashed line indicate the 95% CI interval.

Table. 1

	Bi-Gaussian	Tri-Gaussian	Quadra-Gaussian
Rat	1	1	1
Rabbit	--	2	2
Pig	--	--	1

Results and Discussion

Table. 2

Species	MCL (µm)	Z_1	$X_{rms,1}$ (µm)	Z_2	$X_{rms,2}$ (µm)	Z_3	$X_{rms,3}$ (µm)	Z_4	$X_{rms,4}$ (µm)	$X_{rms,WA}$ (µm)
Rat	67±4	0.014±0.003	92±6.5	0.026±0.014	246±9	0.006±0.001	794±24	0.001	1557	188±6.5
Rabbit	90 ± 4	0.014±0.002	84±3.8	0.025±0.004	230±18	0.007±0.003	533±109	0.002±0.001	874±95	231±20
Pig	91	0.016	94	0.018	234	0.005	585	0.007	1067	222

Q Space: The DPP obtained from the rat lung was narrower and sharper compared to those obtained from rabbit and pig lungs (Figure 1), reflecting a much more restrictive environment for diffusing ^{3}He atoms in rat lungs. Table.1 lists the order of the best multi-Gaussian model fit to the DPP for different animal lungs. For each species, the mean and standard deviations were calculated for the multi-Gaussian parameters pooled together (Table 1). The larger RMS displacements ($X_{rms,4}$) across the species, is similar to the rms displacements of freely diffusing ^{3}He gas (~1587 µm) or ^{3}He in air (~1100 µm) and suggests a probable gas leak. **Histology:** As expected, different species had differing alveolar sizes (Figure 2), which is reflected in the MCL measurements (Table 2). **Comparison**: Excellent correlation (r = 0.93, p< 0.001) was obtained between the MCL and $X_{rms,WA}$ for all the animals together (Figure 3). Within each species, the displacements Xrms,1 showed some correspondence to the MCL (figure 4), but for all animals together only weak correlation was present between MCL and X_{rms} (Xrms,1 : r = -0.53, p = 0.16, Xrms,2: -0.73, r = 0.03). The larger displacements observed in rats, compared to MCL; especially Xrms,1 might be due to the particular sequence parameter used or reflect differences in physical property of lung tissue such as surface relaxivity.

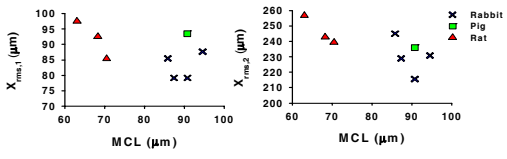

Figure 4. Comparison of MCL with $X_{rms,1}$ and $X_{rms,2}$ displacements

Conclusion

We observed a strong correlation between alveolar size as measured by mean chord length (MCL) on histology and the weighted average rms displacement ($X_{rms, WA}$) derived from the q-space displacement probability profile, suggesting that hyperpolarized ^{3}He q-space MRS is measuring the length scales of lung microstructure. The variation among species for the individual displacement components might reflect inter-species structural or physical variations which can be detected with q-space, but not the MCL analysis.

References: [1]. Assaf Y, et.al. *Magn. Reson. Med.* 2002; **48**: 71-81. [2]. Shanbhag DD, et. al. *J Magn Reson Imaging*. 2006; **24**: 84-94. [3]. Mata JF, *Ph.D. Dissertation*, University of Virginia, Charlottesville, USA, 2006. [4]. Lum H, et.al. *J. Appl Physiology*, 68: 2280-2286.