Revealing the Hyperpolarized ^{129}Xe Red Blood Cell Resonance using Transgenic Mice

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TARGET AUDIENCE: Hyperpolarized Gas MRI, Preclinical MRI Lung Imaging

PURPOSE: Hyperpolarized ^{129}Xe provides a unique means of probing gas exchange, because this gaseous MR contrast agent is soluble in tissues and displays a large *in vivo* chemical shift range (>200 ppm). These properties allow alveolar ^{129}Xe to be detected separately from ^{129}Xe dissolved in red blood cells (RBCs) and interstitial tissues. This capability has been exploited to enable spectroscopic, 2D MRI[1], and 3D MRI[2] studies of impaired gas exchange in rat models of lung fibrosis. However, mice are typically favored in preclinical research, because of the relative ease with which transgenic and knockout models can be generated. Unfortunately, it was discovered early after the introduction of HP ^{129}Xe MRI that mice do not display a unique RBC resonance.[3] The absence of a unique RBC peak limits the utility of ^{129}Xe MR to study mouse models of interstitial lung disease. Here we overcome this experimental limitation using transgenic mice that exclusively express human hemoglobin[4] and demonstrate a unique RBC peak identical to that observed in humans.

METHODS: Wild-type control mice (C57BL/6, n=3) and 3 transgenic mice (129-Hba$^{tm1(HBA)Tow}$ Hbb$^{tm2(HBG1,HBB*)Tow}$/Hbb-/J, Jackson Laboratory, Bar Harbor, ME) were ventilated (75% Xe, 25% O_2 mixture) on a constant volume, HP-gas compatible ventilator[5]. ^{129}Xe spectra (α = 90°, points = 512, TR 20–360 ms) were acquired at 2 T using a GE Excite 12.0 Console (GE Healthcare) both during a 360-ms breathhold and at end expiration. Spectra were processed using matNMR[6] and additional routines written in MATLAB (Mathworks, Inc., Natick, MA). 3D, 1-point Dixon imaging of dissolved ^{129}Xe was also performed to spatially separate the signal from ^{129}Xe dissolved in the interstitial tissues and the RBCs. All studies were approved by our Institutional Animal Care and Use Committee.

RESULTS: Consistent with previous observations, wild-type mice exhibited a single NMR resonance at 198 ppm (Fig. 1A). In contrast, transgenic mice clearly displayed two dissolved-phase NMR peaks at 198 and 220 ppm. The interstitial tissue peak saturates after ~200 ms, whereas the RBC continues to increase out to 360 ms (Fig. 1B). Dissolved signal was higher for data acquired during breathhold compared to end expiration, but otherwise signal dynamics were similar. Dissolved ^{129}Xe SNR is sufficient to simultaneously image both dissolved ^{129}Xe in both the parenchymal tissues and RBCs of these transgenic mice (Fig. 1C).

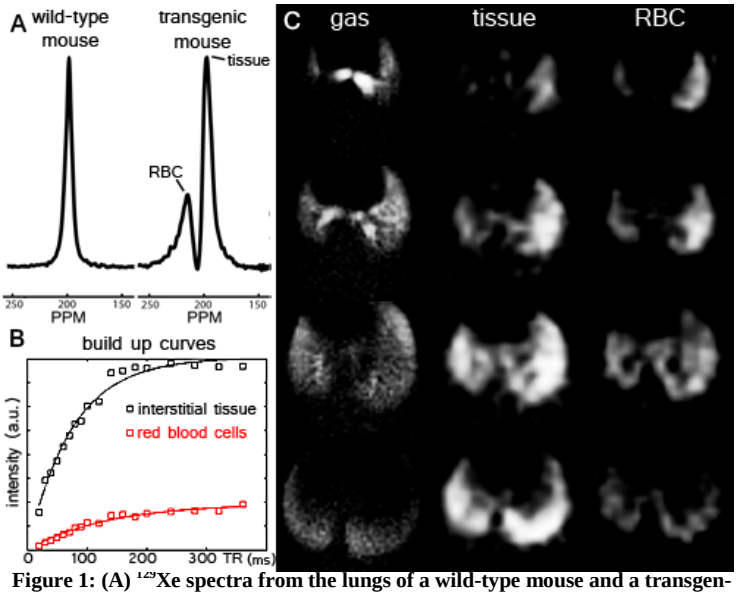

Figure 1: (A) ^{129}Xe spectra from the lungs of a wild-type mouse and a transgenic mouse expressing exclusively human hemoglobin referenced to the gas-phase at 0 ppm. (B) Signal dynamics in a transgenic mouse fit to the theory of ref 7. (C) Standard ventilation imaging (left), and 1-point Dixon image of ^{129}Xe in interstitial tissues (center) and RBCs (right).

DISCUSSION: When wild-type mouse hemoglobin (both α- and β- subunits) is knocked out and replaced by human hemoglobin, the ^{129}Xe NMR spectra observed from the lungs of these animals are almost identical to those seen in humans, which also display unique interstitial tissue (197 ppm) and RBC peaks (218 ppm)[7, 8]. During imaging, short TRs minimize RBC signal, while longer TRs increase the RBC signal relative to tissue signal.

CONCLUSION: It is possible to probe pulmonary gas-exchange on a timescale of milliseconds using dynamic ^{129}Xe spectroscopy and spatially using 3D MR imaging of ^{129}Xe uptake in transgenic mice expressing human hemoglobin. These capabilities will provide global and regional physiological information about gas transit to the RBCs in mouse models of disease and injury. Further, when combined with quantitative[9] ventilation imaging, these methods will enable translational "Mouse-to-Human" studies of impaired gas exchange in a variety of pulmonary diseases.

ACKNOWLEDGEMENTS: Duke Center for In Vivo Microscopy (NIBIB P41 RR005959), NHLBI 1R01-HL-105643, and NHLBI 1K99-HL-111217-01A1. **REFERENCES: 1.** Driehuys B et al. PNAS 2006; 103 (48) **2.** Cleveland ZI et al. ISMRM 2013 **3.** Wagshul ME et al. MRM 1996; 36 (2) **4.** Ryan TM et al. Science 1997 278 (5339) **5.** Nouls J et al. CMR 2011; 39B (2) **6.** van Beek JD et al. JMR 2007; 187 (1) **7.** Cleveland ZI et al. PlosOne 2010; 5 (8) **8.** Mugler JP et al. MRM 1997; 37 (6) **9.** Virgincar R et al. NMR Biomed. 2012; in press