Hyperpolarized 83Kr MR Relaxation Measurements in Excised Rat Lungs.

K. F. Stupic[1], N. D. Elkins[2], G. E. Pavlovskaya[3], J. E. Repine[2], and T. Meersmann[1,3]
[1]Department of Chemistry, Colorado State University, Fort Collins, CO, United States, [2]Health Science Center, Webb-Waring Institute, University of Colorado, Denver, CO, United States, [3]School of Medicine, University of Nottingham, Nottingham, United Kingdom

Introduction: Because of its spin I = 9/2, the noble gas isotope ^{83}Kr possesses a nuclear electric quadrupole moment that is a source for rapid relaxation. However, its surprisingly long gas-phase T_1 times of up to several hundred seconds at ambient pressure allow for ^{83}Kr hyperpolarization that is 3-4 orders of magnitude above the equilibrium polarization at 9.4 T field strength [1,2]. The quadrupolar dominated longitudinal ^{83}Kr relaxation can be utilized for MR studies of surrounding surfaces since it is susceptible to the surface-to-volume ratio, surface hydration, and surface temperature [3]. Hyperpolarized (hp) ^{83}Kr has been shown to provide T_1 relaxation weighted MRI contrast that is highly sensitive to the surface chemistry in low surface-to-volume model surface systems [1]. In a recent study, a special ventilation chamber was devised that allowed for *in situ* hp ^{83}Kr MRI of excised rat lungs [4]. In the present work an improved hp ^{83}Kr delivery system is used to obtain hp ^{83}Kr T_1 relaxation (spatially not resolved) in excised rat lungs as a function of lung inflation.

Methods: Following the University of Colorado Health Sciences Center approved protocol, 30 healthy, 30 male Sprague-Dawley rats (Charles River Laboratories, Inc., Wilmington, MA) rats (175 – 400 g) at the time of lung excision were anesthetized with ketamine (80 mg/kg) (University of Colorado Hospital pharmacy) and xylazine (16 mg/kg) (MWT Veterinary Supply, Meridian, ID). 100 USP units heparin (American Pharmaceutical Partners, Inc., Schaumburg, IL) were allowed to circulate for 10-15 s before the lungs were placed on a ventilator. The lungs were perfused with a 50 mL of Belzer-MPS solution (UW Kidney Preservation Solution -Trans-Med Corporation, Elk. River, MN) and the trachea was clamped at time of inhalation to avoid collapsing the airways while removing the heart and lungs from chest cavity. The excised lungs were then cannulated with an adapter tube positioned 5 mm above the bifurcation of the lungs. Following excision, the lungs, with the heart still attached, were immediately transferred in a Pyrex ventilation chamber shown in Fig. 1 (ID = 24 mm and height = 100 mm) and immersed in ~60 mL of Belzer-MPS solution. Two 40 W line narrowed diode array lasers (Spectra Physics 794.7 nm, line width 0.3 nm) were used to generate hp ^{83}Kr in cylindrical glass cells (Pyrex, no surface treatment) in stopped flow mode as previously described [1]. A 10-15 min optical pumping period was applied between consecutive hp gas deliveries. The krypton mixture was produced from research grade gases (Airgas, Radnor, PA) and contained 25% krypton (natural abundance of 12.4% ^{83}Kr), 5% N_2, and 70% helium. NMR Spectroscopy and MR Imaging experiments were performed on a Chemagnetics CMX II 400 MHz NMR spectrometer in a 9.4 T, 89 mm bore superconducting magnet equipped with a triple axis micro imaging system (Resonance Research, Billerica, MA). Spectra and images were obtained using a custom-built probe with a single 28 mm (i.d.) saddle coil for excitation and detection tuned to 15.4 MHz ^{83}Kr frequency. Images were acquired in 16 gradient steps using gradient-echo sequence for a single-phase increment. A variable excitation pulse FLASH sequence was used to fully utilize the initial ^{83}Kr polarization [5,6]. The raw data matrix size was 32 x 16 with a FOV of 2.656 cm and a BW of 14 KHz (NEX = 4, no slices election).

Result and discussion: The experimental setup previously described in [3] has been improved using a line narrowed laser system to obtain 13,000 fold polarization enhancement at 9.4 T (6% spin polarization) with a 25 % krypton N_2/He mixture. Two problems prevent the full benefit from the obtained polarization: (1) Unlike xenon, hp ^{83}Kr cannot easily be separated from the other gases in the mixture after optical pumping through a freeze-thaw cycle because of very fast hp ^{83}Kr depolarization at liquid nitrogen temperatures. Therefore the 25% krypton mixture leads to a 4-fold loss in signal intensity because of dilution. Alternatively optical pumping with 95% krypton (5% N_2) in the current setup leads to about 1 – 1.5 % polarization only. (2) Hp ^{83}Kr depolarizes rapidly in rat lungs (T_1 around 1 – 1.5s). An improved delivery system using a combination of vacuum shuttling, pneumatic valves and a gas delivery syringe reduced the hyperpolarization loss to about a factor of 3. The lung inflation can be followed in Fig. 2 that shows a series of small flip angle (12°) experiments separated by TR = 200 ms. It can be seen that gas delivery to final inflation of the lung takes about 1s (i.e. marked 'inflation stopped' in Fig 2). After this point the decay of the krypton signal is analyzed, in the simplest case through the assignment of a single T_1 time constant (more careful data analysis is also provided). Note that hp ^{83}Kr is delivered to the lung trachea at ambient pressure and that the inflation of lung takes place solely due to the suction applied to the ventilation chamber that allows for any lung inflation level to be selected. Fig. 3 shows an example of T_1 relaxation times at various levels of inflation with hp ^{83}Kr. The T_1 times in Fig 3 are decreasing with increasing lung inflation volume most likely due to prolonged ^{83}Kr relaxation in the major airways that slows the decay of the overall signal. As the lung inflates further, the signal originating from krypton in the airways contributes less to the overall signal, thus leading to a faster apparent decay. This effect is largely removed when only an initial 6 ml of hp ^{83}Kr is inhaled followed by unpolarized gas to the final inflation volume. In this case (not shown in Fig. 3) the signal decay is largely independent from the lung inflation level. This is somewhat surprising since the surface to volume ratio is expected to decrease with increasing inflation level and therefore slower relaxation at high inflation should be observed. Fig. 4 shows FLASH hp ^{83}Kr MRI (no slice selection) to demonstrate the currently obtained signal intensity.

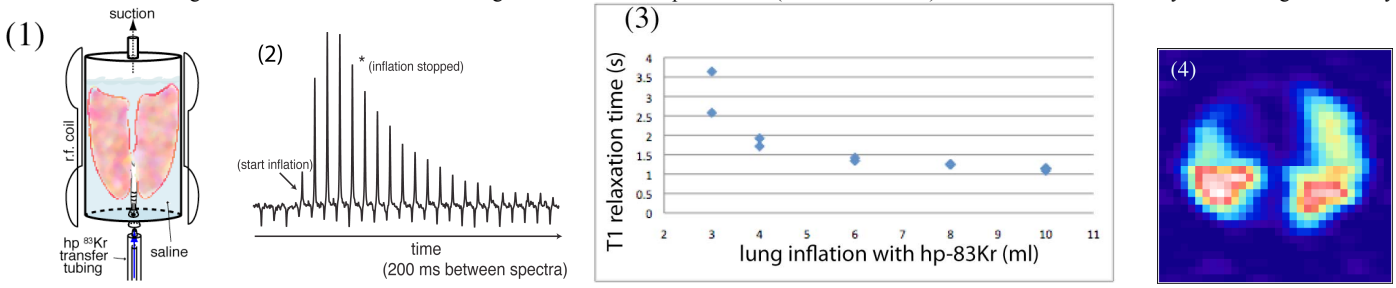

Figure 1. Sketch of excised rat lung in ventilation apparatus submerged in Belzer-MPS solution as used in [4]. **Figure 2**. Series spectra with 12° excitation pulses each separated by TR = 200 ms. Suction is applied at 'start inflation point' The lung is inflated at 'inflation stopped' point as estimated from displacement stoppage of the Belzer-MPS solution surrounding the lung. **Figure 3**. Hp ^{83}Kr T_1 relaxation time as a function of lung inflation. **Figure 4**. Hp ^{83}Kr FLASH transverse plane MRI of rat lung (no slice selection, NEX = 4).

Conclusions: Improvements in spin exchange optical pumping and a specialized hp-^{83}Kr delivery system allow for spatially non-resolved ^{83}Kr T_1 relaxation studies as a function of lung inflation and the first hp-^{83}Kr FLASH image of *in situ* rat lungs at 9.4T. The measured relaxation of $T1 \geq 1$s is slow enough to permit future *in vivo* studies. Surprisingly, the relaxation in ex vivo lungs does not change with increased lung inflation (when the effects of airways are eliminated) despite the presumably changing surface to volume ratios in the alveoli. This effect will need further investigation that should also be extended to pathological lungs. Further improvements in the signal intensity should be possible with improved optical pumping utilizing more powerful laser systems and advanced pump cell design. A further 8-fold improvement is possible using isotopically enriched ^{83}Kr.

References: [1] Pavlovskaya et al. PNAS 2005; 102:18275-18279. **[2]** Cleveland et al. JCP 2008; 129: 244304-1 – 6. **[3]** Cleveland et al. JACS 2007; 129:1784-1792. **[4]** Cleveland et al. JMR 2008; 195: 232-237. **[5]** Gao et al. MRM 1997; 20:448-458.**[6]** Cleveland et al. MRI 2008; 26:270-278.