Differentiating Injured and Normal Lungs by the Ratiometric Analysis of Hyperpolarized-13C-NMR Data

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Target audience: NMR or lung researchers, particularly in the field of metabolism, and clinical practitioners would benefit from this study.

Purpose: Early diagnosis of Acute Respiratory Distress Syndrome (ARDS) is critical because of its high death rate. There is currently no effective therapy for patients once their condition progresses into ARDS. This predicament demands a non-invasive method for assessing lung injury. Here, we induce lung injury in rats using the toxic anti-tumor drug bleomycin. Our goal is to identify an early metabolic predictor of lung inflammation using hyperpolarized ^{13}C-NMR (HP ^{13}C-NMR) techniques.

Methods: The rats (250-300 g) were randomly divided into the control and the treatment group. Bleomycin was injected into the lungs of the treatment group. In 7 or 21 days, the lungs were resected and immediately perfused with 400 ml PBS buffer suppl. with 3% albumin and 10mM glucose. All lungs were first observed by ^{31}P-NMR spectroscopy to confirm normal ATP metabolism (ATP/Pi>1) and lung viability. 10 ml of 32mM HP 1-^{13}C pyruvate was then infused through the pulmonary artery at 10 ml/min, and the signals corresponding to the agent and its metabolites were acquired using a non-selective, 15-degree pulse every 1s over a time period of 300s. All hyperpolarized NMR experiments were performed with a 20mm ^{1}H/^{13}C dual-tuned coil in a 9.4-T Varian vertical bore NMR spectrometer. The time-course data of the monitored species were first extracted from the FID signals using a customized MATLAB program and further analyzed using the ratiometric method [1] to obtain the apparent rate constants and other kinetic parameters of the LDH-catalyzed reaction.

Results and Discussion: Fig. 1 shows the typical time-courses and ratio fitting results of the control (a & b) and the bleomycin-treated group (7 days post-treatment, c & d). The treated lung has higher lactate signal than the control. The ratiometric analysis results appear in Fig. 2 (a-c). The quantitative H&E staining for neutrophils (N), macrophages (M), OP foci (O), and lymphocytes (L) are shown in Fig.2d, confirming the inflammatory condition and its change with time. The treated group yielded significantly larger apparent forward (k_p) and reverse (k_l) rate constants, indicating faster bidirectional pool labeling in inflammatory lungs. The rate constant ratio, k_p/k_l, also increased significantly indicating the alterations in cellular redox state NAD$^+$/NADH [2]. The bleomycin group measured at 21 days post-treatment (N=3) did not differ significantly from the control. The rate constants are volume-averaged values which do not distinguish intra- and extracellular contributions; thus, the accuracy of these data could be further improved by volume selective sequence or imaging sequence to exclude the contribution of ^{13}C-pyruvate in the perfusate. Nevertheless, our data indicate that the LDH activity was elevated in the inflammatory lungs. The elevation of LDH and its isoenzymes has been associated with inflammation in the lung. It has thus been proposed to be a marker for inflammation or a potential driving force of the initiation and progression of fibrotic disorders.

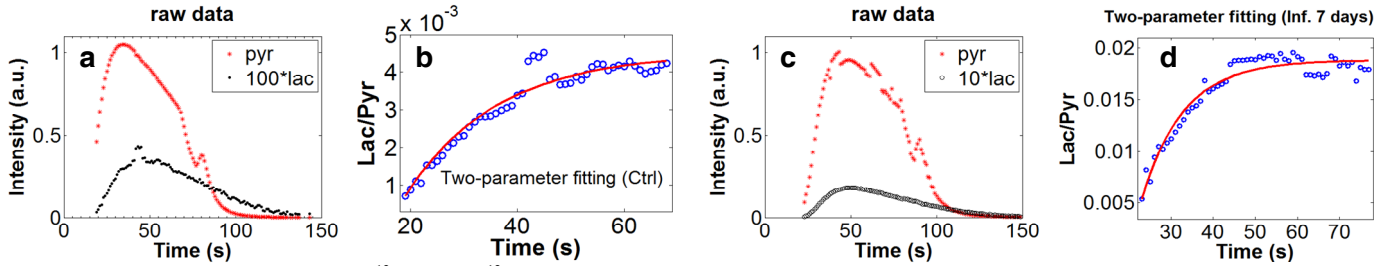

Fig.1. Time-courses of ^{13}C-pyr and ^{13}C-lac (scaled by a factor) and their ratio fitting. **a**&**b**-Ctrl; **c**&**d**-bleomycin-treated

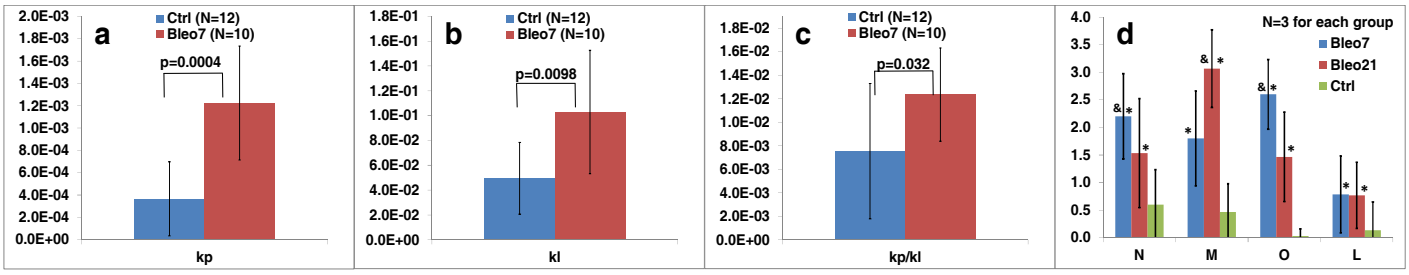

Fig.2. Rate constants and their ratio: a-c; bleomycin-treated: d, (*p<.05 vs Ctrl, &p<.05 between 7 and 21 days post-treatment)

Conclusions: The kinetic analysis of HP-^{13}C-NMR data shows substantial potential for probing lung injury. Our ratiometric analysis indicates that inflammation sped up both the forward and reverse rate of the LDH-catalyzed reaction and altered the cellular redox state in the lung tissue. This effect could be further developed to provide *in vivo* biomarkers for lung injury detection.

References:

1. Li LZ, Kadlececk S, Xu HN, et al. Ratiometric analysis in hyperpolarized NMR (I): test of the two-site exchange model and the quantification of reaction rate constants. NMR in Biomedicine. 2013. 2. Li LZ, Xu HN, Kadlececk S, et al. Proceeding of the Annual Meeting of the International Society of Magnetic Resonance in Medicine, 2012:4308