Hyperpolarized 1-13C pyruvate metabolism of inflamed lung via pulmonary delivery: A preliminary study

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Introduction: The pulmonary route is increasingly important both for targeted drug delivery to the lung and also as a rapid or patient-preferred (non-injection) method of systemic treatment. For pulmonary disease (e.g., asthma, Chronic Obstructive Pulmonary Disease, cystic fibrosis and acute lung injury), advantages of inhaled over systemic delivery are: 1) delivery of high drug concentrations directly to the disease site, 2) rapid clinical response, 3) minimized risk of systemic side-effects, and 4) lower barriers to therapeutic efficacy, such as poor gastrointestinal absorption and first-pass metabolism in the liver. These combine to allow similar or superior therapeutic effect at a fraction of the systemic dose. The main goal of this study is to assess the applicability of 13C-hypepolarized MRI for assessing pulmonary drug delivery. Efficient and reproducible drug delivery requires non-invasive monitoring and imaging of regional lung deposition, pharmacokinetics, and pharmacodynamics.

Method: A total of 12 Sprague-Dawley rats (300-400g) in two groups (healthy and 7-day-post Bleomycin-exposed inflammatory models) were used in this study. All the lungs were excised and placed in a 20-mm NMR tube (9.4T vertical bore magnet) while perfused with a modified Krebs-Henseleit buffer containing 3% (w/v) fatty acid free BSA. The perfusate was oxygenated, the pH maintained at a physiological value of 7.4 ± 0.1 and the temperature maintained at 36.5 ± 1°C. The health of the tissue was monitored using ^{31}P spectroscopy. 28.5mg [1-^{13}C] pyruvate was polarized with a HyperSense DNP system (Oxford Instruments). 4 mL Tris-buffered saline with 100 mg/L EDTA was heated to 190°C at 10 bar, and was used to rapidly dissolve the frozen sample. This sample was further diluted with oxygenated Krebs-Henseleit buffer (without BSA) yielding a neutral, isotonic 80, 16, and 4mM solution. The first HP pyruvate sample was injected at 10mL/min via the pulmonary artery (perfusion) into the lungs and the second injection via the trachea into the alveolar spaces (figure 1). Low flip-angle (α=15°) carbon spectra were acquired for the duration of the hyperpolarized signal (TR=1s). The spectra were fitted and analyzed using custom MATLAB routines.

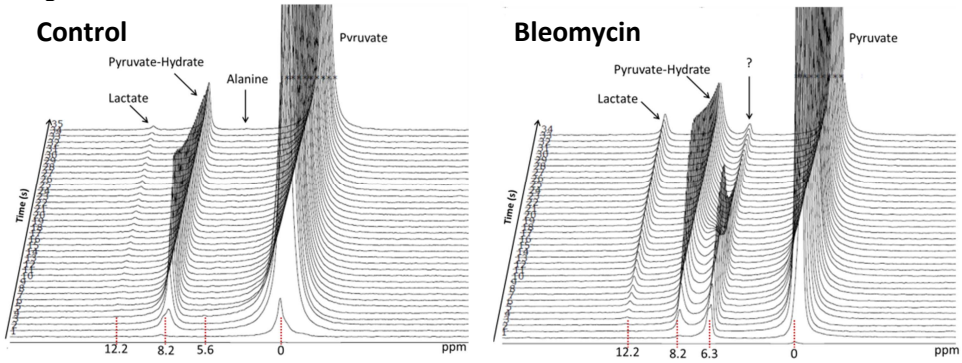

Figure 1 Time-series of stacked 13C spectra of isolated perfused lung for injection via the trachea into the alveolar spaces of Bleomycin-injured (right) and control (left). ? is the new peak that is appears right after pyruvate and before pyruvate hydrate. It is specific to the inflammatory model and is rapidly relaxed or converted to another form.

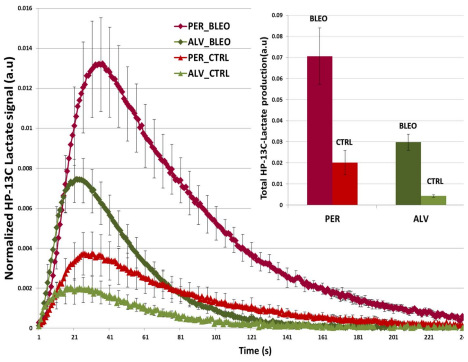

Figure 2 The full time-series of fit peak areas of HP-13C Lactate for 7-day Bleomycin-injured and control cohorts for injection via the pulmonary artery (PER, red) and trachea into the alveolar spaces (green, ALV)

Results and Discussion: Metabolism of hyperpolarized [1-13C]-pyruvate to lactate, alanine and bicarbonate was observed in both control and inflamed lungs (figure 1) after both methods of administration. The average 1-13C lactate signal of the inflammatory cohort exceeded that of the control cohort by approximately a factor of 3.5 and 7.1 for injection via pulmonary artery and trachea, respectively. The higher ratio for injection via trachea shows direct metabolism of infiltrated inflammatory cells into the airways and alveolar spaces. Although the increase may also be partially due to greater permeability of the alveolar epithelium in acute lung injury, we believe this to be of lesser importance because increased transamination to alanine, characteristic of the native lung cells, is not observed. Although, the lactate production of both control and inflamed lung cohorts were higher in injection of pyruvate from perfusion compared to alveolar (figure 3), PER/ALV ratio of normalized lactate for the injured cohorts is 50% lower than control cohorts. Furthermore, the first peaks to appear in the inflammatory model were pyruvate and a new peak 6.3 ppm downfield of pyruvate followed within one second by 1-13C pyruvate hydrate (right). The new peak is specific to the inflammatory model and is rapidly relaxed or converted to another form. Although its identity is unknown, this peak may result from an interaction between pyruvate hydrate and the increased mucus production or another feature of the lung's response to insult.

Conclusion: In this study we demonstrated preliminary data of hyperpolarized 13C-MRI for pulmonary drug delivery. The metabolite signal from hyperpolarized pyruvate shows alveolar uptake even in healthy cohorts. Enhancement of lactate signal in the injury model also shows the possibility of using hyperpolarized 13C-MRI for monitoring the arrival of the drug at the site of lung inflammation.

References: 1) Mata, M. et al. Eur. Respir. J. 2003, 22:6900-905 2) Shaghaghi, H. et al. NMR in Biomed. 2014, 27: 939–947