Metabolism of Hyperpolarized [1-13C]Pyruvate in the Isolated Perfused Rat Lung – An Ischemia Study

Stephen Kadlecek[1], Benjamin Pullinger[1], Kiarash Emami[1], Harrilla Profka[1], Masaru Ishii[2], and Rahim Rizi[1]

[1]*Radiology, University of Pennsylvania, Philadelphia, Pennsylvania, United States,* [2]*Otolaryngology-Head and Neck Surgery, Johns Hopkins University, Baltimore, MD, United States*

INTRODUCTION: Very few lung NMR studies have been reported due to low signal density arising from low tissue density and line-broadening from air-tissue interfaces. These difficulties can be partially overcome using fast-dissolution ^{13}C-DNP NMR. This experiment studies the evolution of the [1-^{13}C] lactate signal in the isolated, perfused rat lung after injection of [1-^{13}C] pyruvate and fermentative glycolysis. The observed accumulation of lactate, which represents a combination of net metabolic flux through LDH and label exchange between the pyruvate and lactate pools, is then studied under normoxic and ischemic states. Changes in the oxidative state of the lung are integral to ischemia-reperfusion injury, a serious problem encountered in lung transplantation, and retention of aerobic metabolism during storage is highly beneficial [1]. In combination with ^{31}P-NMR spectroscopy, ^{13}C spectroscopy of hyperpolarized pyruvate is studied as a method to non-destructively probe the redox state in lung tissue, as well as for its potential to acquire regional information via imaging techniques.

METHODS: *Animal Handling.* Ten male Sprague-Dawley rats (300-450 g) were anesthetized with i.p pentobarbital, a tracheostomy was performed and 200 U herparin was administered via tail vein. The lungs were prepared for NMR study according to the previously reported method of degassing. In a subsequent thoracotomy, the heart was cut transversely, and the pulmonary artery was cannulated via the right ventricle. The lungs were perfused with a modified Krebs-Henseleit buffer (119 mMNaCl, 25 mM $NaHCO_3^-$, 1.3 mM $CaCl_2$, 1.2 mM Mg SO_4, 4.7 mMKCl, 10 mM glucose, 1% (w/v) bovine serum albumin). The perfusate was passed through an oxygenating column at a constant flow of 1 atm 95:5 O_2:CO_2 and was warmed to physiological temperature. After perfusion was started, lungs were excised and placed in a 20-mm NMR tube. *Hyperpolarized [1-^{13}C]pyruvate injection.* 37 mg [1-^{13}C] pyruvic avid were polarized to ~30% at 1.42 K and 94 GHz with a HyperSense DNP system (Oxford Instruments). 4.5 ml Tris-buffered saline was heated to 190ºC at 10 bar to dissolve the frozen sample. The solution was diluted in 12.5 mL oxygenated Krebs-Henseleit buffer, yielding 24 mM [1-^{13}C] pyruvate. The solution was injected at 14.6 mL/min in lieu of the steady-state perfusion buffer. *Ischemia study design.* Metabolism was observed under normoxic and ischemic conditions. Global ischemia was accomplished by stopping the flow of perfusate for 25 min. Both conditions were tested in each lung; five lungs were tested before ischemia and immediately after, and an additional five were tested after ischemia and after 30 minutes of post-ischemia reperfusion. Nominal 45º nonselective ^{13}C spectra were acquired at 2s intervals after injection of the agent. ^{31}P spectra were also collected before and during ischemia and during reperfusion to confirm the expected changes to PCr and ATP. *PCA Extracts*. Two lungs were freeze-clamped after 45 min of normoxic perfusion (corresponding to the time point of the initial pyruvate injection), two immediately after 25 min of stopped-flow ischemia, and two after ischemia followed by 30 min of reperfusion. The entire lung block was freeze-clamped and extracted into ice-cold perchloric acid. After neutralization to pH = 7.2±0.2, the extracts were kept at −85°C until lyophilized, redissolved in D_2O, and analyzed. Metabolites were quantified by peak integrals, which were normalized to total frozen weight. *NMR spectroscopy.* All NMR spectroscopy was performed with 20-mm ^{1}H-X probe on 9.4-T vertical magnet. ^{31}P spectra: α=75°, TR=1 s, NS=512 (total time=8.5 min), SW=39 kHz. ^{13}C spectra: individual spectra, TR=2s, α=45°, SW=24.5kHz. Peak integrals were determined by fitting the spectral peaks to Lorentzians in a custom MatLab routine.

RESULTS: *^{31}P Spectroscopy.* In all spectra, peaks corresponding to the following metabolites were clearly observed, listed from downfield to upfield: phosphomonoesters (PMEs), inorganic phosphate (P_i), phosphodiesters (PDEs), phosphocreatine (PCr), γ-ATP, α-ATP, NAD^+/NADH, and β-ATP. Within 10 minutes of ischemia onset, reduction in peak amplitude was observed for PCr and ATP peaks. Within 30-40 minutes of reperfusion, the peaks recovered to a fraction of their original amplitudes. No significant differences were observed between the two experimental groups. Spectra could not be acquired sooner than 10 min after the injection, but no changes from the normoxic injection were exhibited in the ^{31}P spectra (**Fig. 1**). *^{13}C Spectroscopy.* Lactate "signal" was parameterized by normalizing the maximum lactate peak area to the maximum pyruvate peak area over the spectral time course. The increase in lactate signal for the post-ischemic injection was highly significant for both cohorts. When ischemic injection preceded normoxic injection, the lactate signal decreased from 48±25 to 6.9±2.9 (α=0.05,p= 0.025); when ischemic injection followed normoxic injection, the lactate signal increased from 7.5±2.7 to 31±14 (α=0.05,p= 0.01). Although the former group showed a higher average ratio of increase in lactate signal (8.6±5.4 vs 4.9±3.6), the means were not statistically different for a two-tailed T-test. Both groups contained a single outlier, in which lactate signal resembled that of a typical normoxic injection (**Fig. 2**). *PCA Extracts.* A prominent lactate signal was observed in the ^{1}H extract spectra. Comparison with a proton standard capillary allowed calculation of the tissue concentration of lactate, which was observed to increase significantly during ischemia and return to near pre-ischemia baseline after reperfusion of duration identical to that used in the hyperpolarized pyruvate experiments. The average lactate increase observed post-ischemia (a factor of ~4.5) approximates that of the hyperpolarized lactate signal increase under identical conditions.

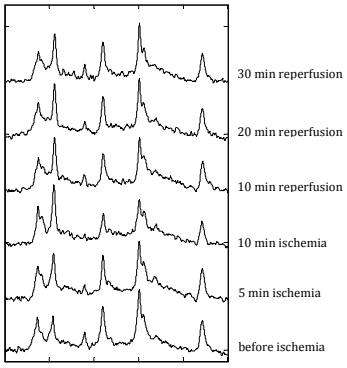

Fig. 1: Quantifiable but small changes in ^{31}P high-energy phosphate peaks associated with ischemia-reperfusion.

DISCUSSION AND CONCLUSION: Reduction and recovery of PCr and ATP are observed during ischemia-reperfusion of various tissues and our ^{31}P measurements confirmed the metabolic changes observed by other investigators. The increased hyperpolarized lactate signal post-ischemia correlates well with a comparable increase in endogenous lactate concentration measured in the PCA extracts, and was largely independent of the order of administration (before and after ischemia, or after ischemia and post-reperfusion). This lends support to the idea that hyperpolarized lactate signal is representative of lactate pool size in the cytoplasm. We found that despite ligating the trachea at end-exhalation, some lungs contained small regions of inflation which would collapse over the course of the experiment. It is possible that this represented residual oxygen which could protect regions of the ischemic lung from entering fermentative metabolism. Furthermore, hypoxic pulmonary vasoconstriction would tend to redirect perfusate flow to the least hypoxic pulmonary tissues, which may in part explain the two post-ischemic measurements which were in the normal range. Reperfusion in both outliers yielded a typical recovery of ATP and PCr, so a loss of tissue viability is not a likely explanation. The lung was observed to have an overall lower rate of lactate production than has been observed in solid organs, suggesting that direct extension to chemical shift imaging *in vivo* will be difficult. However, this low metabolic activity might provide additional contrast to distinguish more dense and active tumor tissue in the lung, and confusion between tumor and ischemic regions is unlikely because of this low baseline metabolic rate.

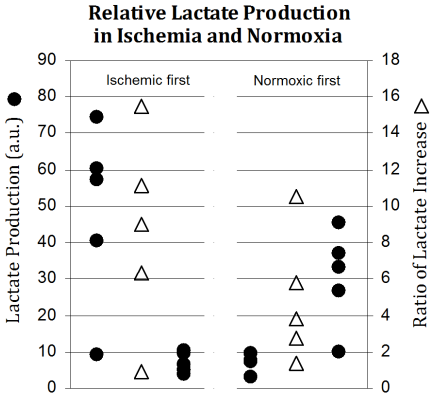

Fig. 2: Normalized lactate production increases by several times after ischemia and recovers to near baseline levels after reperfusion.

REFERENCES: [1] de Perrot M, et al. *Am J RespirCrit Care Med* 2003; 167(4):490-511