Detection of lung mitochondrial dysfunction using hyperpolarized [1-13C] pyruvate metabolism

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Introduction: Mitochondrial dysfunction is associated with various forms of lung injury and disease. Although, mitochondrial dysfunction is an all-purpose term for panoply of possible adverse effects on mitochondrial respiration and other mitochondrial functions, a key readout that may also underlie certain manifestations of lung injury is impairment of ATP-generating capacity. Pyruvate as intermediate of energy metabolism is a key compound to study mitochondrial dysfunction due to its ability to enter mitochondria or aerobic glycolysis. To address the effect of mitochondrial dysfunction on lung metabolism, hyperpolarized [1-13C] pyruvate metabolism was studied in presence of electron transport chain (ETC) inhibitor and activators.

Method: A total of 18 Sprague-Dawley rats (300-400g) were used for 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.

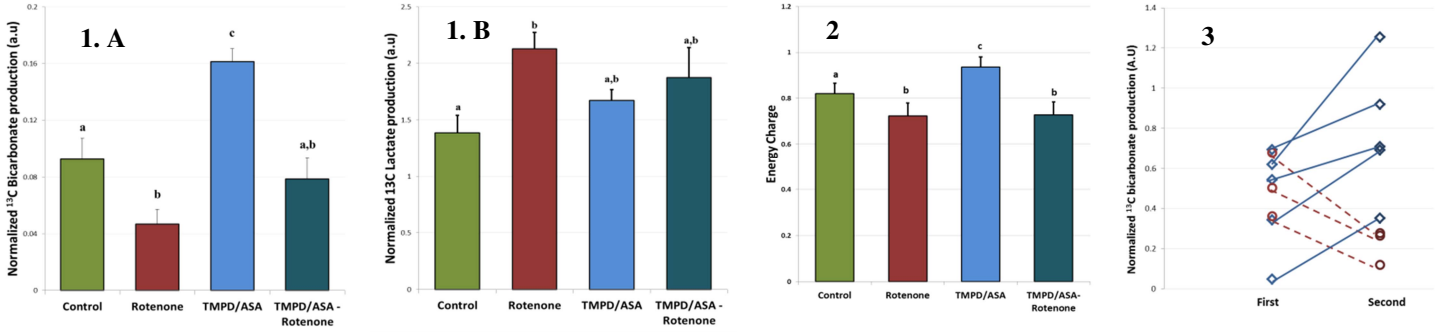

Figure 1 Hyperpolarized 13C bicarbonate (A) and lactate (B) signal in perfused lung treated with 20 µM rotenone for 10 min, with 25µM TMPD+0.25mM ASA for 40min, and with 20 µM rotenone for 1 min followed by 25µM TMPD+0.25mM ASA for 40 min.

Figure 2 31P spectroscopic ally-derived energy charge for four cohorts described in figure 1, EC= (ATP+½ADP)/(ATP+ADP+AMP).
Statistical analysis (figure 1and 2): Means with different letter designations are significantly different from one another ($p<0.05$) and means with similar letter designations are not significantly different ($p>0.05$).

Figure 3 Comparison of the 13C bicarbonate production for two hyperpolarized pyruvate administrations in each individual lung. First and second indicate first and second injection, respectively. All of the first injection was done after rotenone perfusion (blue and red). Connected points are the second HP pyruvate administration for each individual lung. Blue squares: perfusion with perfusate contained 25µM TMPD+0.25mM ascorbate after rotenone administration. Red circles: perfusion with normal perfusate after rotenone administration

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). To assess the effect of ETC complex I inhibition (1), lungs were perfused with buffer containing 20 µM rotenone for 10 minute, after which steady-state perfusion was restored for 10 minutes. To assess the effect of ETC complex IV activation, lungs were perfused with or 25µM (N,N,N′,N′-tetramethyl-p-phenylenediamine + 250µM ascorbic acid (TMPD+ASA) for 40 min. Immediately afterward, 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 [1-^{13}C] pyruvate sample. This sample was further diluted with oxygenated Krebs-Henseleit buffer (without BSA) yielding a neutral, isotonic 4mM solution, which was injected at 10 ml/min in lieu of the steady-state buffer. To assess the effect of simultaneous ETC complex inhibition and activation, the rotenone-treated lungs were further separated into two cohorts; five were treated with TMPD+ASA after the first hyperpolarized injection and three were not. After an additional period of one hour of perfusion, a second hyperpolarized sample was injected as above. Spectra were fitting and analyzed using custom MATLAB routines. The adenylate energy charge was calculated as: EC = (ATP+½ADP)/(ATP+ADP+AMP). Adenine nucleotides ratio were calculated by least-squares fitting of eight distinguishable peaks in 31P NMR spectra to Lorentzian peaks.

Results and Discussion: Rotenone decreased bicarbonate production significantly (on average by 49% with respect to control, fig. 1) and increased lactate label exchange (53%, fig, 2). TMPD increased bicarbonate production significantly (74%, fig. 1) with respect to control (fig. 1). After the first hyperpolarized measurement, rotenone-treated lungs continued to decline (-53% bicarbonate production, fig. 3) but this decline was reversed by TMPD-ASA (+1.7-folds) such that the latter cohort was indistinguishable from controls (-15%). Neither treatment significantly altered alanine production. Compared to control rotenone depressed the energy charge about 11% and TMPD increased it about 14%, respectively (figure 2). Although treatment of rotenone perfused lungs with TMPD did not significantly improved oxidative phosphorylation (energy charge) of lung, it protect lungs from more mitochondria dysfunction and 13C-bicarbonate production improved in all of the lungs treated with TMPD (figure 3).

Conclusion: In this study, we demonstrated the effect of mitochondrial dysfunction on lung energy metabolism, and that this effect is detectable using non-ionizing, HP-13C spectroscopy. In a previous study (2), injured lungs were shown to display increased lactate labeling, which could indicate direct metabolism by inflammatory cells or mitochondrial dysfunction. However, the present study indicates that mitochondrial dysfunction is accompanied by decreased bicarbonate production, which is not observed in inflammation.

References: 1) Bongard. RD. et al. 2013, 65:1455–1463 2) Shaghaghi, H. et al. NMR in Biomed. 2014, 27: 939–947.