A Survey On The Effect Of Antioxidant And Metabolism Modifiers On Lung Metabolism Using Hyperpolarized 13C NMR

Hoora Shaghaghi[1], Stephen Kadlecek[1], Mehrdad Pourfathi[1], Sarmad Siddiqui[1], Profka Harrilla[1], and Rahim R. Rizi[1]
[1]*Radiology, University of Pennsylvania, Philadelphia, Pennsylvania, United States*

Introduction: Lactate and pyruvate comprise 40-50% of glucose utilization in the lung under aerobic conditions, and only about 5% of glucose carbons are oxidized to CO_2 through to pyruvate dehydrogenase flux [1]. In the presence of pulmonary disorder such as anoxia, hyperoxia, and hypertension these proportions can be altered [2]. In this work, we investigate the underlying mechanism of these pulmonary metabolic changes in order to develop a more comprehensive understanding of lung disease. Hyperpolarized (HP) ^{13}C NMR is used to examine the effects of several notable compounds on the biochemical activity of isolated, perfused lungs. Such compounds include: dichloroacetate (DCA), a well-known pyruvate dehydrogenase kinase (PDK) inhibitor; glutathione (GSH), an antioxidant and lactate dehydrogenase (LDH) inhibitor; ascorbic acid (ASA), a vital antioxidant; dehydroascorbic acid (DHA), the oxidized form of ASA; and alpha-lipoic acid (ALA), a metabolism modifier present in lung tissue.

Method: A total of 25 Sprague-Dawley rats (300-400g) were used for this study. All 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, and the pH and temperature held relatively constant at a physiological value of 7.4 ± 0.1 and 36.5 ± 1°C, respectively. The health of the tissue was monitored using ^{31}P NMR spectroscopy. A HyperSense DNP system (Oxford Instruments) was then utilized to polarize 28.5mg of [1-^{13}C] pyruvate . The dissolution process was facilitated by heating 4 mL Tris-buffered saline with 100 mg/L EDTA to 190°C at 10 bar. After which, the sample was further diluted with oxygenated Krebs-Henseleit buffer (without BSA), yielding a neutral, isotonic 4mM solution. Excised rat lungs were bathed in the perfusate doped with either 2mM ascorbate (ASA), 2mM glutathione (GSH), 2mM alpha-lipoic acid (ALP), 2mM dehydroascorbate (DHA), 5mM or 10mM dichloroacetate (DCA), 5mM DCA+2mM ascorbate, or 1mM dehydroascorbate (DHA)+1mM ASA . HP pyruvate injections (4mM) were delivered to each rat about 50 minutes post-excision. The health of the tissue was measured intermittently with ^{31}P NMR. HP pyruvate was injected at 10mL/min, and low flip-angle (α=15°) carbon spectra were acquired for the duration of the hyperpolarized signal. The spectra were fitting and analyzed using custom MATLAB routines.

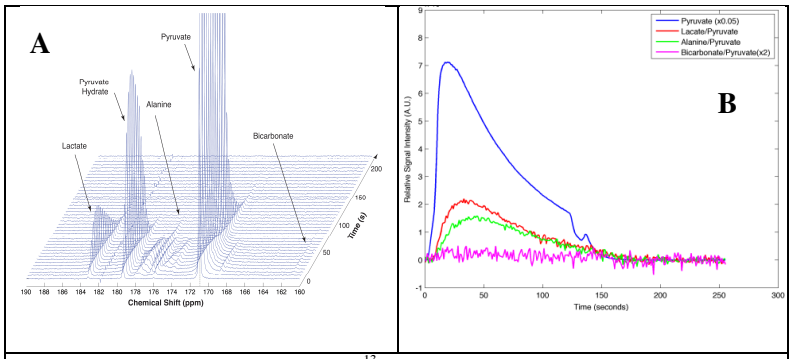

Figure 1 Typical time-series of stacked ^{13}C spectra (A) and fit peaks area (B) for perfused lung normalized by hyperpolarized pyruvate signal intensity.

Results: **Figure 1** illustrates a typical time-series of stacked HP ^{13}C spectra (A) and fit peaks area (B) for perfused lung. The inset bar graph shows (**Figure 2**) the mean and average integrated lactate, alanine, and bicarbonate signals in the control as well as the perfused with 5mM DCA, 10mM DCA, 2mM GSH, 2mM ALA, 2mM ASA 1mM DHA+1mM ASA, and 5mM DCA+2mM ASA cohorts. While no effect on lung pyruvate metabolism is observed at low dichloroacetate concentrations (~5mM), doubling its concentration to 10mM yields a noticeable ,increase in all metabolites . Perfusion of lung with glutathione increased total pyruvate metabolism but just alanine production was significantly changed by a factor of 1.7 relative to control group (P<0.06). Bicarbonate was also enhanced about 50% (not significant). Lactate signal was significantly increased (1.6 times) in lung perfused with alpha-lipoic acid compared to the control cohort. Alanine signal was enhanced 1.4 times (not significant), but Bicarbonate signal decreased 68% in presence of ALA. Ascorbate increased the signal of all ^{13}C metabolites, but the significant change appears in the bicarbonate signal (2.7 fold increase for 2mM ASA). Combination of 1mM ASA with 1mM DHA did not change the bicarbonate signal compared with 1mM ASA alone, but it increased the alanine signal. Perfusion of lung with 2mM ascorbic acid + 5mM DCA resulted in a significantly increased HP bicarbonate signal (two-fold, P<0.05) compared to both the control and 5mM DCA.

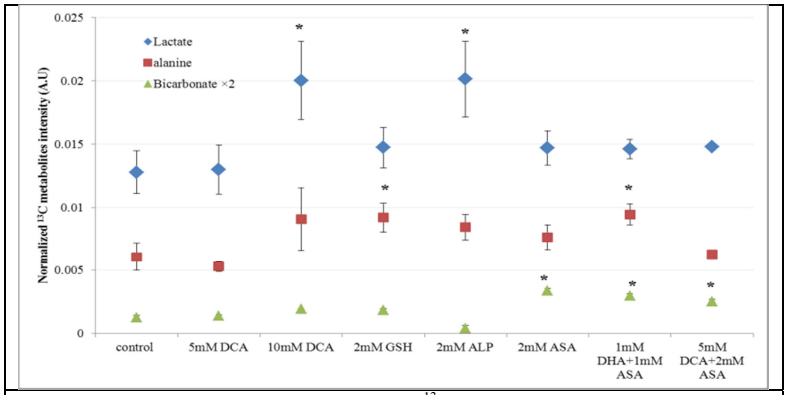

Figure 2 Comparison of the total averaged ^{13}C bicarbonate signal in presences of antioxidant or metabolism modifiers.* Significantly different (p<0.05) when compared with control.

Discussion and Conclusion: A significant increase in bicarbonate is only observed in the lung when ASA is present in the perfusate. Our results suggest that this increase is driven purely by a mechanism inherent to ASA activity. This pathway is distinct from DCA, which is widely recognized to inhibit PDK and increase PDH flux. We expected DCA to produce an increased bicarbonate signal from the HP [1-13C] pyruvate via the PDH flux and/or a decreased lactate signal from LDH. However, 5mM DCA did not have any effect on pyruvate metabolism in the perfused lungs. This is most likely due to the very low level of PDK2 isoform in lung that is the most sensitive to inhibition by DCA relative to other PDK isoenzymes [3]. Since reactive oxygen species (ROS) inhibit PDH activity, and GSH and ASA are the major antioxidants in the lung, we would expect the lung perfused with 2mM glutathione to exhibits a significant increase in bicarbonate production if antioxidant activity was the main cause of enhanced PDH flux. But the HP bicarbonate signal in the presence of GSH alone increased by only 50%, which was not significant relative to the controls. We can also conclude that ASA's enhancement of bicarbonate production is not predominantly due to its role as an antioxidant and radical scavenger, though it is clearly responsible for some of this effect. Additionally, ASA's effect on the conversion of pyruvate to bicarbonate does not seem to be related to the presence of ALP in lung tissue. Since it is a cofactor of the PDH complex, we anticipated that an increase in ALA concentration could accelerate the conversion of pyruvate into acetyl-CoA. However, no enhancement of the bicarbonate signal was observed in lung perfused with 2mM ALA. The significant increase in lactate signal in the presence of alpha-lipoic acid could be related to an increase in lactate pool size due to stimulation of glucose uptake and inhibition of PDH flux. Finally, the result of perfusion with 1mM ASA+1mM DHA illustrated that the effect of ascorbate on PDH flux is not related to its oxidized form.

References: 1. A. B. Fisher, Intermediary metabolism of lung. In D. Massaro. (Ed.), Lung Cell Biology (pp. 735-763). New York: Marcel Decker Inc. (1989) 2. Scholz, R. W. Biochem. J. 126: 1219-1224 (1972). 3. Bowker-Kinley MM, Biochem J. 1998 1;329 (Pt 1):191-6.