Ascorbic Acid Enhances Pyruvate Dehydrogenate Flux In Isolated Perfused Rat Lungs

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

Introduction: Ascorbic acid (ASA), a water-soluble vitamin, is a well-known antioxidant and key factor in pulmonary defense against endogenous and exogenous oxidant reactive species. In addition to acting as a strong radical scavenger in animal tissue, ASA is a coenzyme in several hydroxylase enzymes. Because of ASA's vital pulmonary roles, depletion of ascorbate in blood and lung intracellular fluids has been reported in several lung diseases [1]. In this work, we applied hyperpolarized (HP) NMR to detect ascorbate's effect on metabolic activity of the perfused lung. Here, we report for the first time, the ability of ascorbate to activate pyruvate dehydrogenase complex.

Method: A total of 40 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. The health of the tissue was monitored using ^{31}P NMR spectroscopy. Approximately 28.5mg of [1-^{13}C] pyruvate was polarized with a HyperSense DNP system (Oxford Instruments). After which, 4 mL of Tris-buffered saline with 100 mg/L EDTA was heated to 190°C at 10 bar, and used to rapidly dissolve the frozen sample. 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 with an ascorbate concentration that varied between 0 and 16 mM with or without 0-0.1mM TMPD (N,N,N′,N′-tetramethyl-p-phenylenediamine). HP pyruvate injections (4mM) were delivered to each rat at 50 (short time perfusion) and 120 (long time perfusion) minutes post-excision. The health of the tissue between injections was checked with ^{31}P spectroscopy. 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.

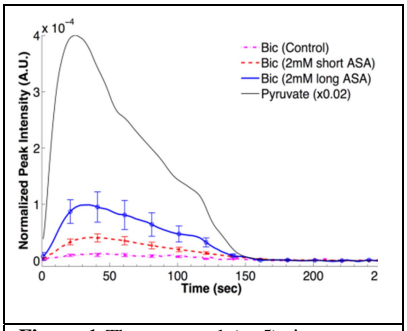

Figure 1 The averaged (n=5) time course of hyperpolarized bicarbonate signal for control (pink), short (red), and long (blue) time perfusion with 2mM ascorbic acid. Black line is averaged pyruvate signal for all 15 experiments

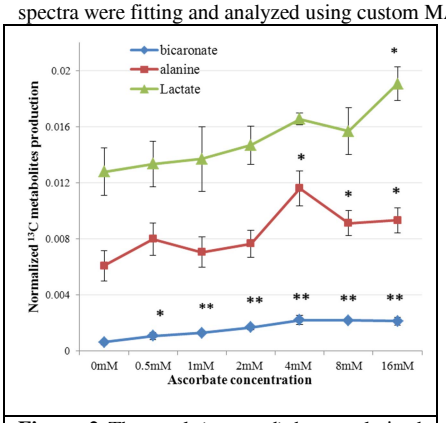

Figure 2 The total (summed) hyperpolarized lactate, alanine, and bicarbonate signals as a function of ascorbic acid concentration in perfusate.

Results: As is shown in **Figure 1**, the bicarbonate signal increases 2.7 and 6.2 times in lungs perfused with 2mM ascorbate for short (<60min), and long (>120min) duration, respectively. In both cases, the bicarbonate signal increase was significantly different compared to the control group ($p < 0.005$). In **Figure 2**, bicarbonate production increased as ascorbic acid concentration was raised to 4mM. Barring the 0.5mM ASA perfusion ($p<0.05$), bicarbonate production was significantly different when compared with controls ($p<0.005$). Lactate production increased with increasing ascorbate concentration but the changes were only significant in the 16mM case ($P<0.05$). Alanine production also increased as a function of ascorbate concentration. However, the changes were only found to be significant at ascorbate concentrations greater than 4mM. Of the quantified peaks in **Figure 3**, the three ATP peaks were significantly increased in the 2mM ascorbate ($P<0.05$) and 0.25mM ascorbate + 0.025mM TMPD ($P<0.005$) perfused lungs compared to the controls. The α-ATP, β-ATP, and γ-ATP peaks were 16.8%, 17.8%, and 23.1% greater in the presence of 2mM ascorbic acid and were 32%, 42%, and 30% greater in the perfusate with 0.25mM ASA + 0.025mM TMPD compared to controls, respectively. PCr, which is known to be active in the energy metabolism of other organs, showed no difference between the two cohorts. Addition of TMPD in concentration of 25 μM and 50 μM to the perfusate contains 0.25mM ascorbate resulted in a significant increase in the bicarbonate signal (2.4-fold, and 2.7-fold increase, respectively). As can be observed in **Figure 4**, the effect is comparable to that of perfusate with 1mM and 2mM ascorbic acid (2.1 and 2.7 fold enhancement, respectively). Furthermore, increasing the concentrations of TMPD (100, 250 μM) did not induce any further changes in the bicarbonate signal. This is most likely due to TMPD's toxicity at higher concentrations.

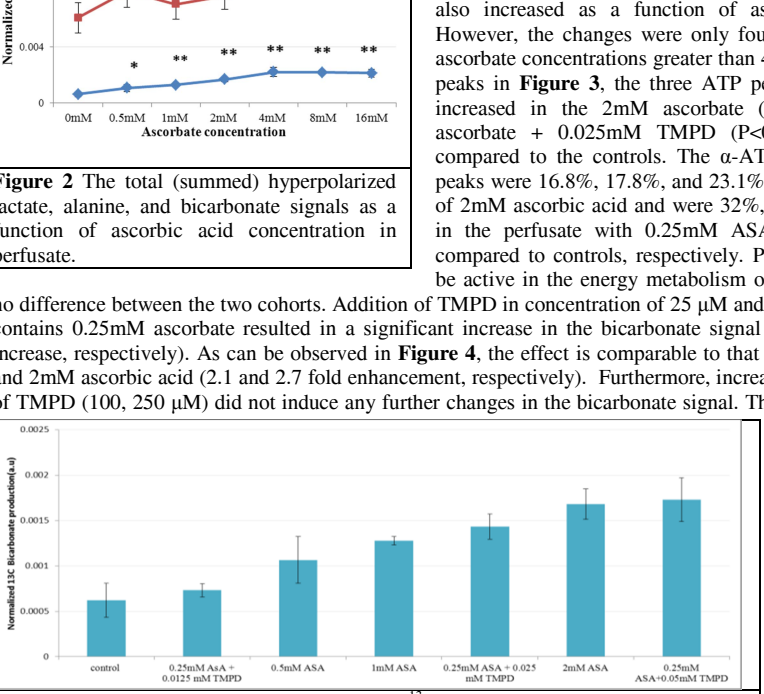

Figure 3 A typical ^{31}P NMR spectra and the comparison of four quantified peaks (α, β, γ ATP and PCr) in the following cohorts: control, perfusate with 2mM ascorbate, and perfusate with 0.25mM ascorbate + 0.025mM TMPD

Figure 4 the comparison of the hyperpolarized ^{13}C bicarbonate signal of perfused lung in the presence of ascorbate alone verse ascorbate plus TMPD

Discussion and conclusion: Based on these results, we hypothesize that ascorbate's effect on bicarbonate signal is related to increasing PDH flux due to an electron transfer chain-linked effect. This hypothesis is supported by two aspects of our results. First, the only possible pathway for the conversion of hyperpolarized pyruvate to bicarbonate in lung is PDH flux, a result of limited phosphoenolpyruvate carboxykinase (PEPCK) enzyme that produces carbon dioxide in gluconeogenesis. Secondly, TMPD is a well-known compound that makes the reaction rate of ascorbate 30 times faster [2]. As it shown in **Figure 4**, the effect of 25μM and 50 μM TMPD in the perfusate is comparable to that of only 1mM and 2mM ascorbate; this effect is in agreement with the the results of ^{31}P NMR spectra, which show a 20% and 35% increase in the ATP signals in the presence of ascorbic acid alone and TMPD + ascorbate, respectively (Fig. 3). One possible mechanism for the effect of ascorbate on PDH may be the polarization of the mitochondrial membrane by ascorbate and the resultant reuptake of Ca+2 by the mitochondria. The accumulation of Ca^{+2} in mitochondria activates PDP which dephosphorylates PDH.

References: [1] Koike, K. et al., Am J Physiol Lung Cell Mol Physiol. 2010, 298(6):L784-92 **[2]** Kimelberg et al., Arch. Biochem. Biophys. 1969, 133, 327–335.