High Resolution Imaging of pH in an Isolated Perfused Rat with Hyperpolarized Bicarbonate Produced via Chemical Reaction

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Purpose: Dynamic nuclear polarization has enabled enhancements to the nuclear spin polarization by factors of over 10^4. This has facilitated the direct measurement of the kinetics of several metabolic pathways which are altered in disease states. Several parameters such as pH as altered in pathological conditions such as acute lung injury, cancer, ischemia, and inflammation. Measurement of pH would allow for the detection of disease, and enable to monitoring of response to treatment. Although techniques such as MRI, MRS, PET have had success in preclinical models, limitations have prevented translation to a clinical setting. Hyperpolarized (hp) bicarbonate has shown promise in measuring *in-vivo* pH. Unfortunately the low polarization of the non-toxic sodium bicarbonate salt limits the resolution of images. In this work we generate hp bicarbonate by rapid decarboxylation of hp pyruvate with H_2O_2. We subsequently image the pH in an isolated perfused rat lung. The ratio of pyruvate to bicarbonate can be tuned to allow for simultaneous monitoring of metabolism.

Methods: In general the alpha keto carboxylic acids undergo rapid 2nd order decomposition when catalyzed with H_2O_2 as shown below[1]:
R-CO-COOH + $H_2O_2 \rightarrow CO_2 + H_2O +$ R-COOH. (eq. 1). The CO_2 undergoes a pH dependent chemical equilibrium with bicarbonate, which is fast under typical aqueous conditions[2]: $CO_2 + H_2O \rightleftharpoons H_2CO_3$, $H_2CO_3 \rightleftharpoons HCO_3^- + H^+$, $HCO_3^- \rightleftharpoons CO_3^{2-} + H^+$ (eq. 1). Under conditions of physiological pH the HCO_3^- is the dominant form. The ratio between the products can be used to determine the pH. Excess H_2O_2 is eliminated prior to injection by rapidly reacting the solution with sulfite.

Samples were hyperpolarized using a commercial DNP polarizer (Hypersense Oxford) by dissolving OXO63 trityl radical in 1-^{13}C Pyruvic acid in a 1.7% by weight solution. The sample was irradiated with microwaves at ~94.062 GHz at a temperature of 1.42K. The perfused organ experiments used injections of hyperpolarized substrate over 2 minutes at concentrations 16-80mM. NMR experiments were performed in a 9.4T vertical bore Varian system and detected with a 5mm hetero-nuclear probe. The isolated rat lung was perfused at 10ml/min with a modified Krebs-Henseleit buffer. The buffer contained 119mM NaCl, 1.3 mM $CaCl_2$, 1.2 mM $MgSO_4$, 4.7 mM KCl, 25mM $NaHCO_3^-$, 10mM glucose and 1%(w/v) bovine serum albumin. The perfusate was passed through and oxygenating column operating with 95:5 O_2/CO_2 under a constant flow of 1atm. The perfusate was kept at 37°C by encapsulating the flow tube in a constant temperature water jacket. The pH image was obtained by utilizing a standard chemical shift imaging sequence (1).The k space points were traversed using a modified 35x35 point centric spiral encoding, where every 15th point returned to the k=0 point (2). After the k=0 point is samples the sequence returns to the next k space point it would have acquired in the spiral trajectory. The sampling of the k=0 point on multiple occasions allows us to correct for the effect of the decaying magnetization during the acquisition on the image resolution (3). The nominal tip angle was 30°, and the transmitter power was set to 55db. The repetition time t_r was 70ms. The transmitter offset frequency was set to ~165ppm. Experiments were conducted in a Varian 9.4T magnet in a 20mm heteronuclear probe. The 128x128 pixel projection image had an in plane resolution of 1.2mm x 1.2mm.

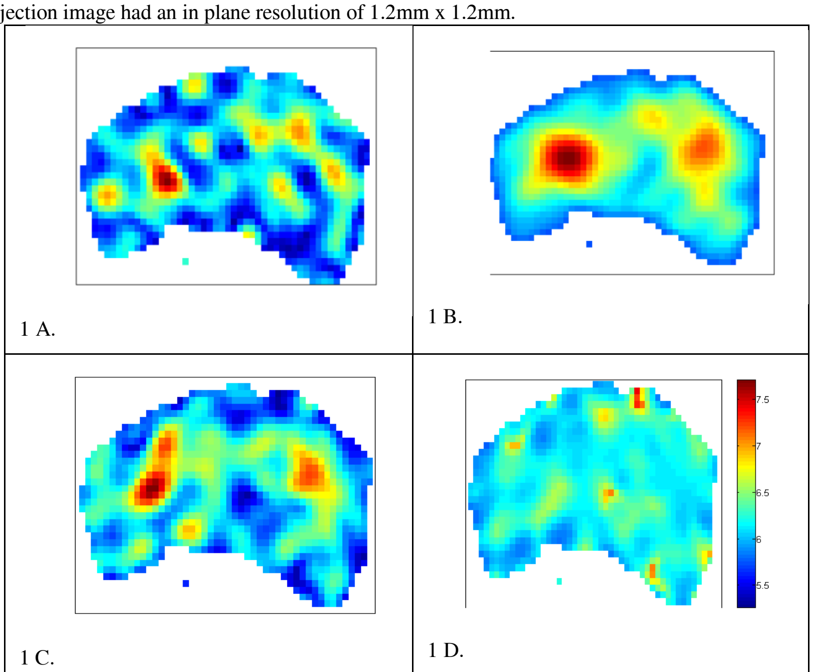

Fig. 1: Distrubution of A) hyperpolarized pyruvate B) bicarbonate, and C) CO_2 in a perfused rat lung. The distribution shown is an axial projection of the lung. A pH map of the isolated perfused lung is shown in D).

Results and Discussion: Images of the pyruvate, carbon dioxide, and bicarbonate species in an isolated perfused rat lung during injection are shown in (figure 1A-C). The variation of pH is shown in (figure 1D) as a proof of concept. We are able to generate images with 1.2 x 1.2mm in-plane resolution. However this can be increased to 0.5mm x 0.5mm by utilizing a different DNP polarizer which can polarize pyruvate to ~75%. Thus far the best images have had a resolution of 2mm x 2mm, while using toxic salts which make it inappropriate for translation to the clinic.

The health of a perfused lung was monitored by recording the relative amplitudes of ATP and inorganic phosphate concentrations with ^{31}P NMR both before and after the hyperpolarized injection. The similar ^{31}P spectra implied that the injection was not toxic to the lung tissue. The ^{31}P ratio in the ATP signal was the same before and after injection indicating that the lung tissue was healthy. This was expected as the injected volume of H_2O_2 was well below the LD50.

The bicarbonate polarization is approximately 70% of the pyruvate polarization after dissolution due to T_1 decay during the 15s reaction. At this point approximately 61% of the initial pyruvate species have been decarboxylated. The total ^{13}C polarization of all species at the 15s mark is approximately 70% of the initial polarization. However the decay of polarization during chemical reaction is not a limitation of the technique as the reaction rate can be increased either by increasing the H_2O_2 concentration, elevating the temperature, or by adding Ca^{2+} ions for catalysis.

Conclusion: Thus far the utilization of bicarbonate and carbon dioxide in hyperpolarized studies has been limited by four obstacles. Of primary concern is the low solubility of sodium bicarbonate,~1M in liquid DNP samples, leading to reduced signal in the dissolved fluid. Since the molarity of liquid pyruvate is 15M we can increase the molarity of the DNP target by 15 fold by producing bicarbonate via decarboxylation. Second, the best polarization reported was 16%, limiting the image resolutions possible. Since the reaction can be made exceedingly rapid by altering the concentration of H_2O_2 the bicarbonate can be polarized to the same polarization as the pyruvate molecule. The maximum polarization reported for pyruvate is 75%. This would lead to a polarization increase of 5 fold to approximately 75%.

Third, previous studies utilized cesium bicarbonate, of which even trace amounts are highly toxic. This work eliminates the use of toxic alkaline salts making it more appropriate for translation into a clinical setting. Finally, while the longitudinal relaxation is an intrinsic property of the molecule we note that we are able to utilize the longer relaxation time of pyruvate during the transport to the magnet prior to conversion in order to minimize polarization loss during the transport phase of the experiment. The best reported resolution of hp Bicarbonate is 2mmx2mm. We are able to achieve 1.2mmx1.2mm resolution. Extension of this technique will allow 0.5mmx0.5mm resolution on the pH maps without loss in the signal to noise.

References: **[1]** L. Brateman, Chemical shift imaging: a review, American journal of Roentgenology,May 1986 Vol. 146, No. 5 pp 971-980 **[2]** M. Salerno,TA. Altes,JR. Brookeman,EE. de Lange,and JP. Mugler III, Dynamic Spiral MRI of Pulmonary Gas Flow Using Hyperpolarized ^{3}He: Preliminary Studies in Healthy and Diseased Lungs, Magnetic Resonance in Medicine 46:667–677 (2001) **[3]** MH Deppe, JM Wild, Variable flip angle schedules in bSSFP imaging of hyperpolarized noble gases, Magnetic Resonance in Medicine June, vol 67, No. 6 pp 1656-64, 2012