Co-polarization of (1-13C) Pyruvate and 13C Sodium Bicarbonate by Dynamic Nuclear Polarization allows Simultaneous Assessment of in vivo pH and Tumor Metabolism

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INTRODUCTION: The molecular probes used in hyperpolarized ^{13}C spectroscopy have important advantages over contrast agents currently in clinical use, expected to have little or no toxicity in humans, even at relatively high concentrations. This feature is particularly appealing given recent concerns about contrast nephropathy associated with iodinated CT contrast, as well as nephrogenic systemic fibrosis (NSF) seen in patients receiving gadolinium chelates for MR[1,2]. Many new ^{13}C agents may be appropriate for metabolic imaging in humans. In addition to ^{13}C pyruvate, ^{13}C lactate itself is a promising primary agent for cancer imaging[3]. Other recent work has demonstrated in vivo pH mapping using ^{13}C bicarbonate, by hyperpolarization of ^{13}C cesium bicarbonate followed by an ion exchange method to exchange most of the Cs^+ for sodium[4]. In this abstract, a method for direct polarization of ^{13}C sodium bicarbonate is reported, that is suitable for use in humans. This method has been combined with a copolarization approach that allows simultaneous polarization of ^{13}C bicarbonate and ^{13}C pyruvate, to perform both pH and metabolic mapping in vivo using a single contrast bolus.

METHODS: ^{13}C sodium bicarbonate and 1-^{13}C pyruvic acid were hyperpolarized using the Hypersense (Oxford Instruments) DNP polarizer[5], and subsequently dissolved in EDTA/H_2O to a final concentration of 55mM for bicarbonate and 9mM for pyruvate. Percentage polarizations were quantified in solution by measuring the signal enhancement obtained by DNP polarization compared to the signal at thermal equilibrium on a 11.7T Varian INOVA spectrometer (125MHz ^{13}C, Varian instruments). T_1 studies were performed using a 3T GE Signa™ scanner (GE Healthcare, Waukesha, WI) equipped with the MNS (multinuclear spectroscopy) hardware package. The RF coil used in these experiments was a dual-tuned ^1H-^{13}C coil with a quadrature ^{13}C channel and linear ^1H channel construction based on an earlier design and also used in ^{13}C pyruvate mouse imaging studies. For the T_1 measurement experiments, a double spin-echo pulse sequence with a 5 degree flip non-selective RF excitation pulse and a pair of non-localized 180 degree hyperbolic secant refocusing pulses was used. A TE of 35 ms (half-echo collected), a repetition time of 3 s (for 3 minutes) and a readout filter of 5000 Hz / 2048 pts were used for these studies. Magnitude decay curves were obtained for pyruvate, pyruvate-H_2O, bicarbonate and CO_2, from which the T_1 fits were derived. The experimental points making up each T_1 decay curve were fit to a mono-exponential decay function. Imaging studies were performed on a B6SJL male wild-type mouse, and a Transgenic Adenocarcinoma of Mouse Prostate (TRAMP) mouse model. T_2-weighted ^1H images were in three planes using a fast spin-echo (FSE) sequence. ^{13}C spectroscopic imaging was acquired immediately upon completion of a 15 s injection of the dissolved copolarized solution. These used a half-echo FID acquisition, 8x8 matrix size, TR = 130 ms (wild-type) or 230 ms (TRAMP), TE = 5 ms, 10 mm slice, 10x10mm in-plane resolution for 1.0cc voxels, and a readout filter of 5000 Hz and 512 pts (wild-type) or 1024 pts (TRAMP). K-space data was acquired concentrically, and a progressive flip angle scheme to utilize all available magnetization. This resulted in a total acquisition time of 8 s (wild-type) or 15 s (TRAMP). A syringe with 8 M ^{13}C urea inserted alongside the mouse was used for RF pulse calibration.

RESULTS: On dissolution using EDTA/H_2O, the solution state polarization was 10.9% for bicarbonate and 16% for pyruvate, with a measured pH of 7.8-7.9. Calculated T_1's at 3T were 50 s and 67 s respectively. Representative data are shown in Figure 1, that demonstrated monoexponential decay for all species, with no significant impurities noted in the hyperpolarized dynamic spectrum. For animal studies in a wild-type mouse and TRAMP model, data were obtained for multiple 2D CSI studies with a 1cm^3 voxel size, with resolution limited by the SNR of the upfield ^{13}C CO_2 peak. As validated previously, integration of ^{13}C bicarbonate and ^{13}C CO_2 peaks, and application of the Henderson-Hasselbalch equation allowed calculation of pH on a voxel by voxel basis. Examples are presented in Figures 2 and 3. An initial study using a normal mouse demonstrated pH values in the normal physiologic range, whereas pH values for the prostate and hepatic tumor models were significantly lower, especially in those anatomic regions consumed by tumor.

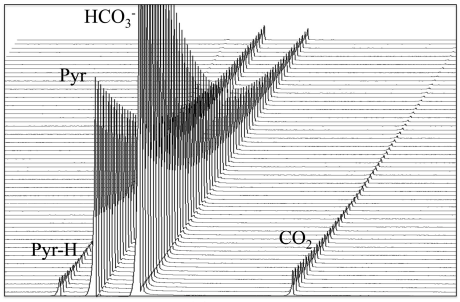
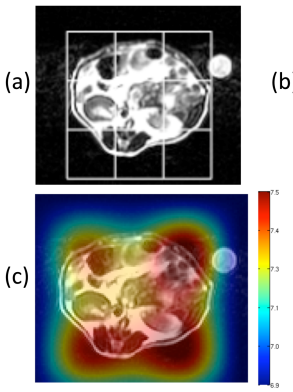


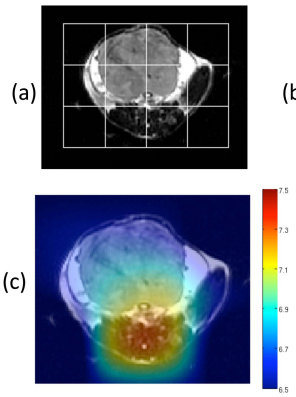
Fig 1. Copolarization of ^{13}C bicarbonate and 1-^{13}C pyruvate at 3T. Calculated T1's were 50 and 67s.

DISCUSSION: A significant advantage of hyperpolarized ^{13}C endogenous probes is their lack of toxicity. In particular, ^{13}C pyruvate, ^{13}C lactate, and ^{13}C bicarbonate are contrast agents expected to have little, if any untoward effects in humans. Since changes in pH are associated with a variety of pathologic processes, including inflammation, ischemia, and neoplasm, hyperpolarized ^{13}C bicarbonate holds special promise as a general MR contrast agent. This potential was realized in recent efforts to probe pH in vivo, with the methods described herein further limiting toxicity and/or complications associated with the technique. Initial work on ^{13}C sodium bicarbonate indicates that it can be polarized readily to high levels, and in conjunction with other benign agents, including ^{13}C pyruvate. The copolarization method described in this abstract allows simultaneous evaluation of in vivo pH as well as LDH activity, reflected in the relative concentrations of ^{13}C lactate and ^{13}C pyruvate observed in the hyperpolarized spectra. These processes are likely related in the setting of neoplasm, although much work needs to be done to define this relationship, in cancer and other diseased states.



7.35	7.27	7.54
7.28	7.32	7.40
7.24	7.39	7.48

Fig. 2



6.62	6.68	6.63	6.36
6.55	6.97	7.09	6.62
6.06	7.12	7.19	6.37

Fig. 3

Fig 1. Wild-type mouse. (a) T_2-weighted image through the abdomen (b) Hyperpolarized ^{13}C 2D-CSI with spectra obtained for voxels through abdomen. (c) pH map obtained using Henderson-Hasselbalch equation and pK_a=6.17 @37°C (4) Actual pH calculation for individual voxels.

Fig 3. TRAMP (prostate CA) mouse. (a) T_2-weighted proton study (b) Hyperpolarized ^{13}C CSI (c) pH map (d) Actual pH calculations

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