Method for robust pH measurement using hyperpolarized bicarbonate and carbon dioxide

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Introduction: In the presence of carbonic anhydrase (CA) enzyme activity, the pH of the local environment can be estimated using the Henderson-Hasselbalch equation if the HCO_3^-/CO_2 ratio (~10 at normal physiological pH) is known. It has been demonstrated that in some tumors the extracellular HCO_3^-/CO_2 ratio can be measured *in vivo* by following the infused hyperpolarized ^{13}C-bicarbonate, and in the heart the intracellular HCO_3^-/CO_2 ratio can be measured *in vivo* by following metabolism of hyperpolarized [1-^{13}C] pyruvate (1-4). In these studies, $H^{13}CO_3^-$ and $^{13}CO_2$ signals have been measured by applying MRS with a small tip angle RF pulse that tip the spins of both species equally. Thus, a much smaller, possibly inadequate SNR of the $^{13}CO_2$ resonance may prevent accurate measurement of pH. Theoretically it is possible to excite the $^{13}CO_2$ resonance independently with a chemically selective RF pulse of larger tip-angle (to achieve higher SNR), while the other resonances of interest (including $H^{13}CO_3^-$) are acquired with a smaller tip angle. Even when repeated measurements are required to obtain temporally or spatially resolved data, this method would still result in accurate pH measurement since the ratio between the two pools is rapidly restored by CA-mediated exchange (CA has an extraordinarily high turnover rate, near 10^6 s^{-1} in human red blood cells) (5), and the robustness of the measurement would be improved due to the higher SNR for $^{13}CO_2$. In this study, a pulse-acquire MRS pulse sequence which interleaved a conventional small tip angle pulse and a spectrally selective pulse was designed and tested in a phantom and in *in vivo* pig hearts, with the aim of measuring intracellular pH using hyperpolarized $H^{13}CO_3^-$ and $^{13}CO_2$ signals.

Methods: Hardware and Agents: All studies were performed using a 3T GE MR750 scanner (GE Healthcare, Waukesha, WI). A micro-strip dual-tuned ^{1}H-^{13}C volume coil (8cm ID.) was used for the phantom experiments (Magvale, San Francisco, CA). A custom build ^{13}C transmit/receive surface coil (5") was used in the animal studies. A HyperSense DNP polarizer (Oxford Instruments, Abingdon, UK) was used to polarize the substrates (5). $NaH^{13}CO_3$ (Isotec, Miamisburg, OH) was prepared in glycerol with OX63 radical (Oxford Instruments) as previously described (4). Neat [1,2-$^{13}C_2$] pyruvic acid (Isotec) was doped with 15mM of OX63 radical and 1mM Gd chelate (Prohance®, Bracco International). Phantom Experiments: A pulse-acquire pulse sequence was modified to allow toggling of the excitation RF pulses between a 10ms spectrally selective pulse (150 Hz pass-band/10^{-4} stop-band) and a 200μs hard pulse on consecutive transients. Pre-polarized $H^{13}CO_3^-$ (~4ml/15mM in H_2O) was mixed with buffered CA enzyme (Sigma Aldridge, St. Louis, MO) solution (4ml/500mM phosphate buffer, pH =7.25, 6μg CA), and dynamic MRS experiment with the interleaved sequence was performed on ~5ml of the mixture (TR=2s, 40° selective pulse, 10° hard pulse). *In vivo* Experiments: Cardiac gated, dynamic MRS data was acquired from pig hearts *in vivo* using the same pulse sequence following infusion of ~15 ml/83mM of pre-polarized [1,2-$^{13}C_2$] pyruvate (t=0 at the start of infusion, TR=2R-R or ~1.3s). In two of the animals, data was also acquired using only the hard pulse (TR=4R-R, 10°) in separate studies.

Results and Discussion: Representative spectra from hyperpolarized $H^{13}CO_3^-$ phantom experiments, which were acquired using the interleaved excitation pulse sequence, are shown in Fig. 1. The average pH obtained using the interleaved pulse sequence (using $^{13}CO_2$ signal from the selective pulse) was 7.42 (n=4, stdev.=0.02), and differed slightly from the pH meter by an average value of 0.07 (pH meter values were lower in all runs, stdev.=0.02). *In vivo* hyperpolarized $H^{13}CO_3^-$ and $^{13}CO_2$ data from the pig heart following infusion of pre-polarized [1,2-$^{13}C_2$] pyruvate are shown in Fig. 2 (left). An increase in the intracellular pH values from ~7.1 to 7.5 was observed over the period that $^{13}CO_2$ was measurable (SNR > 3) in the spectra from ~20 s to 50 s after the start of tracer infusion (Fig. 2 right). The same pattern was observed in all three animals studied. This observation was not likely the result of RF saturation on the $^{13}CO_2$ resonance as similar trend was observed when only a small tip hard pulse was used, but further investigation is needed to elucidate the mechanism of this increase in $H^{13}CO_3^-/^{13}CO_2$ ratio over time. It is worth noting that in order to obtain an accurate $H^{13}CO_3^-/^{13}CO_2$ ratio using the method proposed here, a good knowledge of the B1 is required for tip angle correction. However, in the small tip angle regime, error in the B1 would not influence this correction substantially. For example, for the 10°/40° pulses used here, +/- 3dB in transmit power would change the correction by less than 10%. A 10% error in the $H^{13}CO_3^-/^{13}CO_2$ ratio would only represent a difference in measured pH of ~0.05 units in the physiological pH range. In conclusion, this study has demonstrated a method that yields higher SNR for the $^{13}CO_2$ pool when hyperpolarized $H^{13}CO_3^-$ and $^{13}CO_2$ are used to estimate pH *in vivo*.

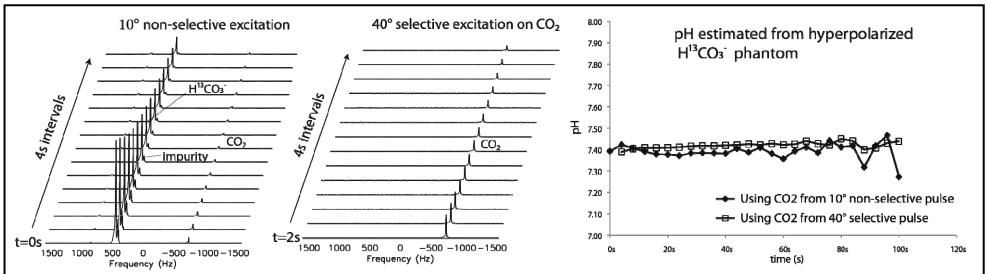

Figure 1. Hyperpolarized ^{13}C MRS data acquried from pre-polarized $H^{13}CO_3^-$ and CA enzyme in buffer using an interleaved RF excitation scheme (left). Stable pH values were obtained temporally (right, range: 7.38 - 7.43) that agrees well with the pH meter measurement (7.35).

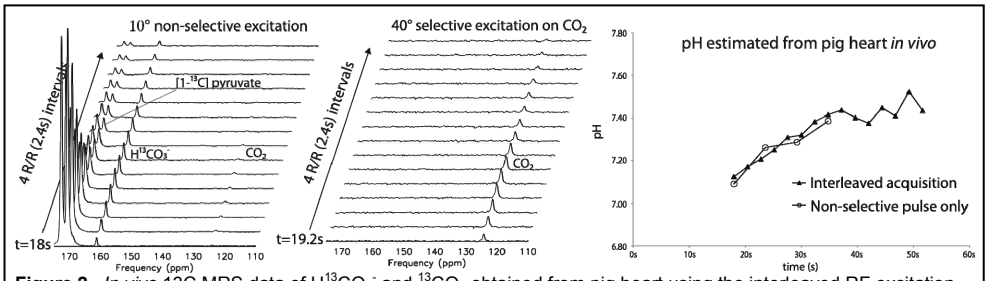

Figure 2. *In vivo* 13C MRS data of $H^{13}CO_3^-$ and $^{13}CO_2$ obtained from pig heart using the interleaved RF excitation scheme following infusion pre-polarized of [1,2-$^{13}C_2$] pyruvate (left). pH estimated from this data and from data obtained using only hard pulse (in a separate study) are shown plotted.

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