Probing the in vivo compartmentalization of hyperpolarized pyruvate using Gadodiamide induced T1 relaxation

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Introduction: Conventional Gadodiamide chelate (Gd) contrast agent is known to remain extra cellular and intra vascular following administration but prior to renal elimination [1]. In this work Gd induced ^{13}C relaxation is used to probe the in vivo biodistribution of hyperpolarized (HP) ^{13}C C1 labeled pyruvate. This work operates on the hypothesis that if paramagnetic-induced ^{13}C relaxation is observed in vivo, then this strongly suggests that pyruvate has a significant extra cellular or intra vascular components. Conversely, if an increase in relaxation does not occur, this suggests that the agent may be located in an intracellular environment. Therefore, this study is directed at using quantitative relaxation time (T_1) measurements to probe the *in vivo* compartmentalization of intravenously administered pyruvate and pyruvate hydrate. The differentiation of the compartmental components of each species is vital for metabolic quantitation because only the intracellular components are actively being metabolized, and are therefore the more functionally interesting components. *In vitro* relaxivity measurements were also performed in order to gauge the relaxivity of pyruvate to varying concentrations of Gd.

Methods: Two sets of experiments were performed using C1 labeled pyruvic acid, which was hyperpolarized at 1.4K using an Oxford Instruments DNP polarizer (HyperSense®, Tubney Woods, Abingdon, Oxfordshire, UK). For the *in vivo* experiments, HP pyruvate (Cambridge Isotope Laboratories, Andover, MA, USA) and Gd (Novation, Irving, TX, USA) (0.5mmol/kg) were administered via a cannulated tail vein to ICR mice (n=5, weighing 30.3 ± 1.6 g) anesthetized with isoflurane. ^{13}C spectra were obtained using a Varian 4.7T imaging and spectroscopy system utilizing a homebuilt dual tuned concentric ^{1}H (5 cm diameter) and ^{13}C (3.8 cm diameter) surface coil setup. For the in vivo experiments, anesthetized animals were positioned over the surface coil with their livers placed immediately over the center of the ^{13}C coil. Spectra were acquired using a global pulse and FID acquire spectroscopy sequence. Quantitative T_1 measurements were obtained by fitting the pyruvate spectral decay curve to an exponential model of T_1 and taking into account the flip angles by fitting all flip angles used. The spectral decay curves were acquired both before and after the Gd injection, a minimum of 1 time before and 3 times after, stopping after approximately 2 times the T_1, when the SNR dropped too low to reliably fit the data. The *in vitro* relaxivity quantitation was performed by measuring the relaxivity of pure solutions of pyruvate, which was measured using hyperpolarized solutions of pyruvate containing between 0 and 0.5 mM Gd utilizing a similar fitting procedure as for the in vivo data.

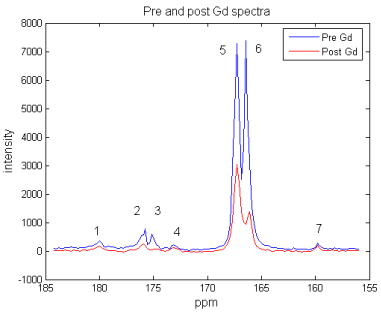

Figure 1: Immediate pre- and post-Gd spectra (Δt=20s). The peaks from left to right are consistent with 1. intracellular lactate, 2. extracellular pyruvate hydrate, 3. intravascular pyruvate hydrate, 4. intracellular alanine, 5. extracellular pyruvate (the reference peak), 6. intravascular pyruvate, and 7. *ex vivo* urea.

Results: In vitro relaxation time measurements of hyperpolarized pyruvate at a pH of ~7 yielded a relaxivity value of 0.05 $mmol^{-1}s^{-1}$ which indicates a high concentration of Gd is required to significantly decrease the T1 of HP pyruvate under physiological circumstances. In vivo studies show split peaks for pyruvate and pyruvate hydrate, as seen in **Fig. 1**, likely indicating differing pH compartments. The in vivo pyruvate and pyruvate hydrate ^{13}C T1 times decreased following administration of Gd from an average of 29s to 22s indicating all 4 peaks are affected by the injected Gd. Qualitative observation of the change in the peak heights indicates the downfield pyruvate and pyruvate hydrate peaks (peaks 2 and 5) decay extremely rapidly during the Gd injection, but the decay is very similar before and after injection. This indicates that the downfield pyruvate and pyruvate hydrate peaks see the Gd immediately, which are likely intravascular components, and the Gd enters the extra cellular compartment relatively quickly after injection. By plotting the peak heights from a single experiment as percent differences from the reference peak (defined to be the upfield pyruvate peak, believed to be extra cellular), as seen in **Fig. 2**, one can see how the low intensity peaks change relative to the reference peak. The downfield pyruvate hydrate, similar to the intravascular pyruvate (peak 6) decays dramatically relative to the reference peak during injection, but remains relatively constant after injection, indicating that it resides primarily in an intra vascular compartment. The upfield pyruvate hydrate remains at nearly constant intensity relative to the reference peak, indicating it resides in the same compartment, most likely the extra cellular compartment. Both the lactate (peak 1) and alanine (peak 4) peak heights are constant before Gd injection, then increase relative to the reference peak post Gd injection, indicating they are not influenced by the Gd, likely because they are intra cellular components. Note that the a random spectral noise point is plotted to indicate that while the SNR is low after 40 seconds, none of the observed peaks has fallen below the noise floor. Further experiments, while not shown, show similar spectral dynamics during and after Gd injection.

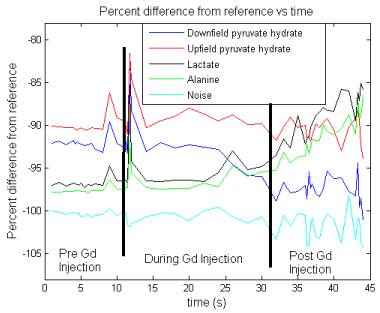

Figure 2: Shows the percent difference from the presumed extra cellular pyruvate reference peak. This shows the 3 likely compartments, the intravascular hydrate, extracellular hydrate, and the intracellular lactate and alanine. The spikes at around 10s are believed to be physiological noise.

Conclusion: Paramagnetic induced changes in ^{13}C pyruvate signal intensity, following Gd administration, demonstrate signal change that are consistent with the compartmental components of the ^{13}C spectral signal. Quantitative T_1 measurements confirm that the T_1 decreases significantly after the Gd injection, affecting intra vascular and extra cellular compartments containing primarily pyruvate and pyruvate hydrate, whereas the lactate and alanine peaks appear to be unaffected by the Gd injection. Future work will include spectroscopic imaging to spatially localize the spectra in a volume coil, more quantitative modeling to account for compartmental exchange and multiple decay constants, as well as optimizing the timing and concentration of the Gd injection for maximum differentiation between the compartments.

References: [1] Krestin, G.P., et al., European Journal of Radiology, 1992. **2**: p. 16-23.

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