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Communication

Spin tagging for hyperpolarized ^{13}C metabolic studies

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ABSTRACT

In studies utilizing pre-polarized ^{13}C substrates to investigate metabolic activities *in vivo*, the metabolite signals observed in a region or a voxel contains a mixture of intracellular and extracellular components. This extracellular component arriving via perfusion may confound the measurements of metabolic flux or exchange rates. But if spin tagging is performed on the magnetization of the substrate, it may be possible to measure the signals of the metabolic products in the intracellular space that were derived from the tagged substrate spins locally. In this study, a spin tagging pulse sequence designed for acquiring data from spatially tagged longitudinal magnetization in hyperpolarized ^{13}C metabolic studies was presented and tested. Using a spectral–spatial RF pulse during the tagging preparation enabled the observation of metabolite signals derived exclusively from the tagged substrate *in vivo*.

1. Introduction

Numerous studies have demonstrated the feasibility of characterizing metabolic reactions in tissues after the injection of a hyperpolarized ^{13}C labeled substrate such as [1-^{13}C]pyruvate [1–4]. In these studies, the pre-polarized substrate was injected intravenously and taken up by various tissues/organs where enzyme-facilitated reactions that presumably occurred within the cellular cytosol and mitochondria resulted in the observation of [1-^{13}C]lactate, [1-^{13}C]alanine and ^{13}C bicarbonate resonances. It may be therefore reasonable to assume that the measured ^{13}C signals contain a large intracellular component. However, the metabolites observed in a particular region or voxel likely also include an extracellular component arriving via perfusion. This mixture of intra- and extracellular ^{13}C MR signal may confound the measurements of specific fluxes or exchange rates in the tissue of interest.

The hypothesis of this study was that sinusoidal spatial tagging of the longitudinal magnetization could be used to differentiate ^{13}C metabolic products created in the intra-cellular space from those arriving via the blood pool. Substrate spins that reside in the intra-vascular space when spin tagging is performed will be strongly suppressed in the data acquisition if a sufficient time interval is allowed between the tagging and the readout, as flow would displace these molecules. Also, if the spin tagging is performed exclusively on the ^{13}C spins of the injected substrate, then the ^{13}C signal from spatially-tagged metabolic products in the subsequent data acquisitions can be differentiated from any inflowing products, since these will not exhibit the tagging pattern. This may make it feasible to model enzymatic reaction rates with a known input function – the tagged substrate signal.

A pulse sequence which consisted of a spin tagging preparation period followed by a series of small tip readouts was designed. The pulse sequence was tested *in vitro* using pre-polarized [1-^{13}C]pyruvate in solution as the test phantom. *In vivo* studies were performed using this pulse sequence in a normal rat following injection of the same substrate.

2. Methods

2.1. Pulse sequence

A pulse sequence timing diagram of the spin-tagging pulse sequence is shown in Fig. 1. Similar to the STimulated Echo Acquisition Mode (STEAM) pulse sequence [5], the sinusoidal spatial tagging is created by a gradient pulse following the initial RF excitation (RF1 – 90°), the prepared magnetization is then stored in the longitudinal plane by another RF pulse (RF2 – 90°). Unlike the typical STEAM acquisition where the tagging preparation is performed for each repetition, in this case, the spin tagging is performed only once, then the tagged longitudinal magnetization is then accessed by consecutive small tip angle RF pulses (RF3). Each readout RF pulse is followed by a gradient pulse (same size as the tagging gradient pulse) and a data acquisition window. Both a conventional slice selective pulse and a spectral–spatial pulse that only excite

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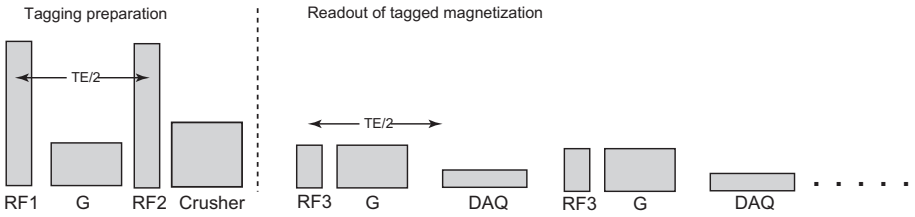

Fig. 1. Pulse sequence timing diagram. The first two RF pulses (RF1 and RF2), with the encoding gradient (G) in-between, set up the spatial tagging pattern. The train of small tip pulses (RF3) read this tagged magnetization as it evolves over time. The rewinder gradient after each small tip creates a stimulated echo from the tagged magnetization, while suppressing signal from any un-tagged magnetization.

[1-^{13}C]pyruvate can be used for RF1, resulting in tagging that is achieved either on all resonances in the spectrum or only the injected substrate. The spectral–spatial RF pulse used in this study is similar to that described previously [6], with minimum slice thickness of 17.4 mm for ^{13}C at 3 T (see Fig. 2).

2.2. MR Hardware, polarizer and compound

All studies were performed using a GE MR750 3 T scanner with 50 mT/m, 200 mT/m/ms gradients (GE Healthcare, Waukesha, WI) equipped with the MNS (multinuclear spectroscopy) hardware package. The RF coil used in the experiments was a micro-strip dual-tuned ^{1}H–^{13}C rat coil (Magvale, San Francisco, CA). A HyperSense DNP polarizer (Oxford Instruments, Abingdon, UK) was used to polarize the substrate following previously described methods [7]. The preparation used was a mixture of neat (99% purity) [1-^{13}C]pyruvic acid (Isotec, Miamisburg, OH) and 15 mM of OX063 trityl radical (Oxford Instruments). The samples were polarized in a field of 3.35 T at approximately 1.4 K. Each ~32 μL [1-^{13}C] pyruvic acid sample was polarized for ~60 min prior to dissolution with ~5 mL of solvent consisting of 40 mM TRIS, 80 mM NaOH in distilled water, giving a nominal final pH of 7.4 [8].

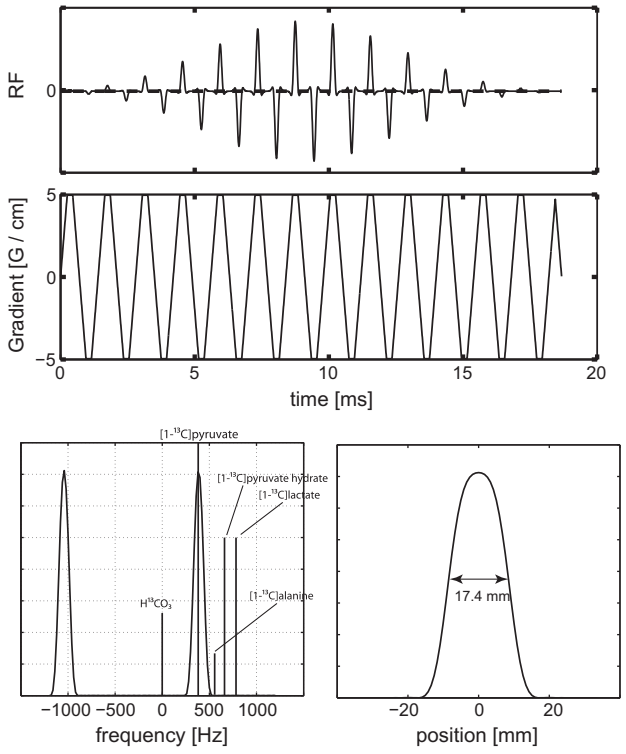

Fig. 2. Spectral–spatial pulse (top) for excitation of only the substrate spins and its simulated spectral (lower left) and spatial (lower right) profile. The duration of each RF sub-lobe was 700 ms, with 26 sub-lobes giving a net duration of 18.2 ms. The peak RF amplitude for a 90° tip angle was 16.8 mT, and the slew rate for the gradient sub-lobes was 200 mT/m/ms (the maximum supported).

2.3. Phantom experiment

Phantom study was performed to test the pulse sequence and to verify that the spatial tags persisted over a sufficient duration to observe metabolic conversion *in vivo*. ~1 mL of hyperpolarized solution [1-^{13}C]pyruvate was drawn into a 1 mL syringe and placed within the coil. A 2 ms, 0.5 G/cm encoding gradient for spin tagging was applied along the axis of the syringe (Z), creating tags with a 1 cm period. The spectral–spatial excitation pulse was not used in the phantom study. A 10° RF3 pulse (Fig. 1) was used in the phantom studies with an interval of 3 s between each RF3. The pulse sequence was run with a spatial encoding gradient during the data readout after each 10° RF pulse so that the tags could be visualized.

2.4. In vivo MRS experiment

All animal experiments followed a protocol approved by the local institutional animal research committee. *In vivo* data were acquired from a healthy nude rat (Harlan Laboratories, Mississauga, Ontario, Canada). For each study, 2 mL of hyperpolarized [1-^{13}C]pyruvate in solution was injected through a tail-vein catheter over a period of 10 s, with the data acquisition started at the end of the injection. The encoding gradient for spin tagging was 1.1 G/cm with 2 ms duration applied along the z-axis (tags with 0.45 cm period). Two studies were performed, the first with a 2 cm × 2 cm × 2 cm voxel centered on one of the kidney and the second with a voxel of the same size centered on the rat heart (^{1}H T2 weighted FSE images were acquired in coronal and axial planes for voxel prescription). The spectral–spatial excitation pulse designed to tip only [1-^{13}C]pyruvate and not its metabolites was used for RF1 during the spin tagging. TE was 45 ms, and an interval of 1 s between each RF3 (20°) was used. The delay between RF2 and the first RF3 was 14 ms. Data acquisition filter of 5000 Hz/2048 pts was used during readout.

3. Results

Using hyperpolarized [1-^{13}C]pyruvate in a syringe as a phantom, the data acquired with the spin-tagging pulse sequence with an imaging readout at the same direction as the tagging gradient (Z) demonstrated the sinusoidal spatial tagging pattern created by the spin-tagging preparation (Fig. 3, left). The decay of the pre-polarized [1-^{13}C]pyruvate has a time constant of 50 s (Fig. 3, right, not corrected for RF tipping, 67 s if corrected for RF tipping) using the sum signal from all the tags. The signal in some region appeared to decay faster which was presumably due to mixing of $+z$ and $-z$ magnetization resulted from small convection currents

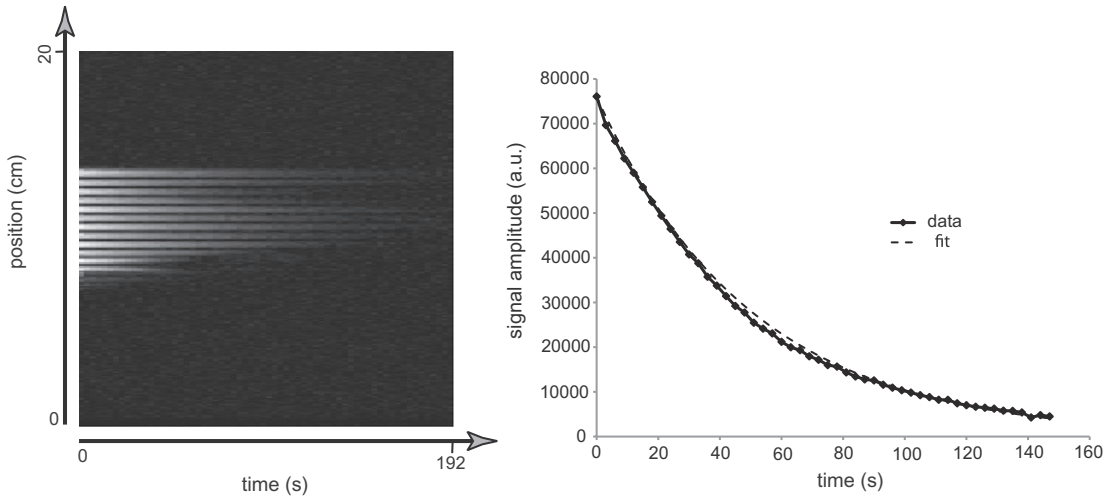

Fig. 3. Data from the phantom study showing the persistence of the spatial tags over time (left). Fitting of the global decay curve (all tags summed) yielded an apparent T1 decay of 50 s (right, not corrected for RF tipping). Note that some regions appear to decay faster than others, which is presumably due to mixing of $+z$ and $-z$ magnetization resulted from small convection currents within the liquid.

within the liquid. But the tagged spins persisted for periods approaching the T1 of [1-^{13}C]pyruvate at 3 T.

^{13}C MRS data acquired from a rat *in vivo* using the spin-tagging pulse sequence with the spectral–spatial excitation pulse following intravenous injections of hyperpolarized [1-^{13}C]pyruvate are shown in Fig. 4. Because the spin-tagging preparation requires two RF pulses and another RF pulse is used for data readout, a single voxel localized by the three orthogonal slice-selective pulse (with RF1 being a spectral–spatial) can be prescribed in this study. Data from both a rat kidney and the rat heart showed that the 1st spectra acquired immediately after the spin-tagging preparation have signal from only the injected substrate. [1-^{13}C]lactate appeared in subsequent readouts in both studies but the signal from this metabolite appeared later in time and also disappeared more quickly in the heart. [1-^{13}C]alanine was observed from the rat kidney. The [1-^{13}C]lactate peak was approximately -80° out of phase from that of the substrate, measured from summed spectrum from 0 to 10 s of the data from the kidney (low SNR for [1-^{13}C]alanine and [1-^{13}C]lactate in the heart prevented measurement of phase shifts in those cases).

4. Discussion

Time resolved ^{13}C MRS or MRI data utilizing pre-polarized substrates may allow estimation of metabolic conversion rates non-invasively *in vivo* [9,10]. However, even with data that are time

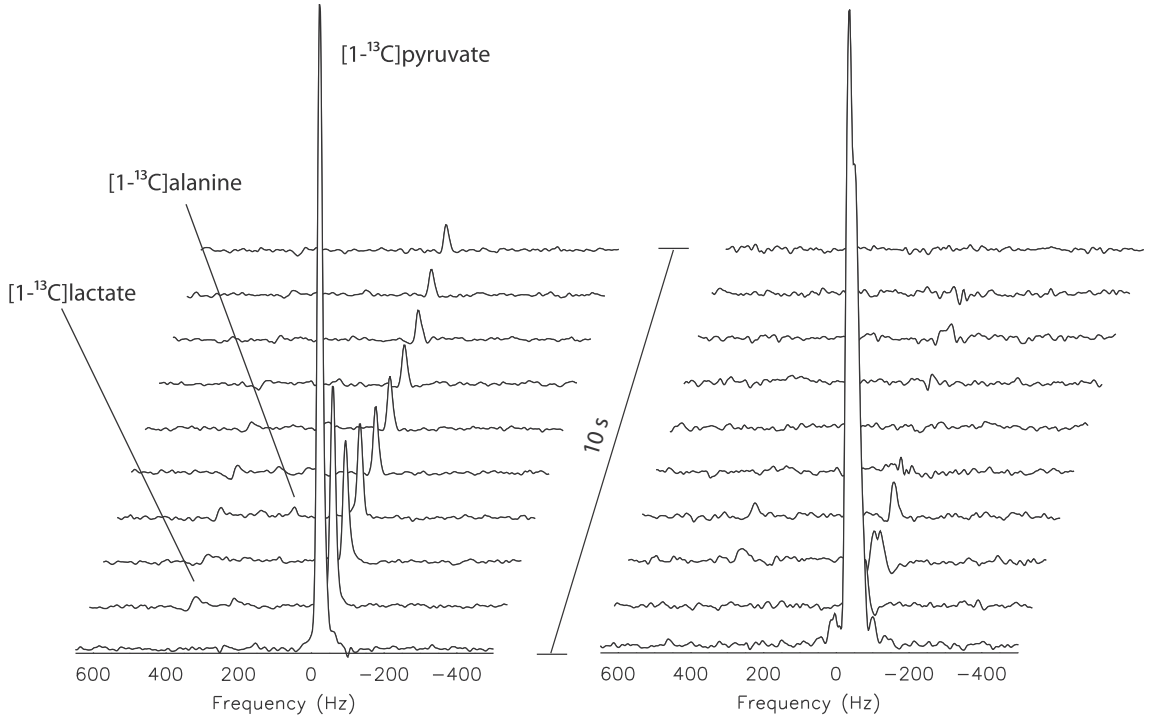

Fig. 4. ^{13}C MRS data acquired using the spin-tagging pulse sequence from a 2 cm × 2 cm × 2 cm voxel centered on a rat kidney (left) and the rat heart (right). Note that, as expected, the 1st spectrum acquired immediately after the spin-tagging with the spectral–spatial excitation pulse contained only the tagged substrate signal. [1-^{13}C]lactate signal appeared in subsequent readout periods in both studies, while [1-^{13}C]alanine was observed only in the kidney voxel.

and spatially resolved, it is still difficult to exclude metabolites converted outside the tissue of interest and then subsequently delivered to the voxel via perfusion. Utilizing ^{13}C metabolic imaging data from regions that are primarily vascular may offer a way to estimate the portion of the ^{13}C lactate signal within the tissue of interest that was contributed by inflow metabolites [10]. And outer volume suppression or selective RF saturation of the downstream metabolites may reduce this vascular component of the signal [11,12]. However, neither method allows direct observation of just the metabolites that were converted from the substrate within the tissue of interest. Using the spin-tagging pulse sequence presented in this study and by tagging only the substrate magnetization that resides in the targeted voxel, the metabolite signals that are observed in the following data readouts can be deemed to result exclusively from local conversion of the tagged substrate. The fact that lactate (tagged) appears in the second temporal frame of Fig. 4 therefore strongly supports the conclusion that this lactate signal is created in the kidney tissue, and is not arriving by perfusion.

In this study, the feasibility of using tagged pre-polarized magnetization for metabolic studies was confirmed first in a phantom study showing that the decay of the tagged spins was similar to that of T1. In the *in vivo* studies, the phase shift between [1-^{13}C]pyruvate and [1-^{13}C]lactate estimated from the kidney voxel further confirms that the metabolite signals indeed came from the tagged substrate (frequency shift of modulated spins occurred during the mixing time is observed as a TE dependent phase shift) [13]. Although the minimum TE of this pulse sequence is longer than that of the pulse-acquire method due to the long spectral–spatial excitation pulse and time required for the tagging and refocusing gradients, the TE used in this study was similar to what had been applied in spin-echo based sequences for hyperpolarized ^{13}C studies [14]. Since the *in vivo* T2 relaxation times of ^{13}C labeled metabolites being investigated are quite long (approximately 0.5 s for [1-^{13}C]lactate in normal rat liver) [15], the SNR lost due to T2 decay during the echo time utilized in this study is likely to be small.

Some limitations exist with this approach. At the time that the spin tagging is performed, a sufficient amount of the injected substrate must be present in the tissue to allow later observation of the metabolite with adequate SNR. In the *in vivo* studies presented in this work, the spin tagging was performed at the end of the bolus when the substrate signal reaches its peak in well perfused organs such as heart and kidneys, based on prior studies [4,8]. However, it is possible that only a portion of the substrate tagged at this time was within the tissue or subsequently taken up by the tissue, thus the conversion rate may be underestimated. The spatial tagging pattern on the intravascular magnetization is almost certainly destroyed by the variations in flow rate for each spin as it moves throughout the circulatory system, thus the vascular substrate signal was expected to decay below the noise within a few seconds. This makes signal contamination by re-circulated, tagged magnetization extremely unlikely. In the *in vivo* data acquired from the rat heart using the spin tagging pulse sequence, very little metabolite signal was observed as compared to previous studies using the pulse-acquire method [4]. It is likely that the majority of the tagged [1-^{13}C]pyruvate spins were intravascular at the time of tagging, and only a small percentage of the tagged spins were taken up by the myocardium. It is also possible that the rapid cardiac motion in a rat heart makes the tag refocusing much less efficiently than in stationary tissue, especially since cardiac gating was not used. These hypotheses are also supported by the faster decay of the pyruvate signal in the heart relative to that in the kidney.

The other limitation is that metabolites that were converted from the substrate tagged within the tissue in the voxel may be excreted or diffuse out of the cells and may no longer be refocused by the rewinder gradient and not observable during readout. It is possible that some of the metabolites that were transported out of the cells remain in the extracellular/extravascular space during the data acquisition and were refocused. Thus the amount of metabolite signal observed will likely be a combination of intracellular and extracellular–extravascular components. The relative contributions from either may depend on the tissue of interest and its disease state. The potential effect of these mechanisms may be reduced or enhanced by changing the size of the tagging gradient as well as the tip angle of the readout RF pulse. Using larger tagging/refocusing gradients would result in smaller tagging periods, making it easier for moving spins to become "untagged" thus not observed in the readout. Larger gradients would also improve the suppression of the untagged spins in the FID component from the readout pulse; although even with the modest gradient strength and duration used in the these experiments, the residual FID component after the gradient dephasing is around the level of the second side lobe of a sinc function (greater than 15 fold suppression, assuming a uniform slice profile). Larger gradients would also increase the diffusion weighting on the data, and accordingly the STEAM pulse sequence has previously been used in diffusion studies [16] because of its ability to achieve high *b*-values at a limit in TE and G. In our case, maximizing *b*-value is not the purpose. Instead we use the T1-dependent mixing interval to provide sufficient time for metabolic reaction, with limited loss of the non-recoverable hyperpolarized magnetization, and with moderate *b*-value discrimination of tagged spins flowing in and out of our voxel of interest. Using larger tip angle and/or shorter mixing times would on the other hand suppress the effects of molecular diffusions because more of the metabolite data could be acquired earlier after spin-tagging. A system with well controlled perfusion/diffusion characteristics such as cells in a bio-reactor may be suitable for investigation of these tradeoffs in future studies.

The SNR of this method is lower as compared to commonly used pulse-acquire or spin-echo based methods. STEAM based pulse sequences utilize only half of the magnetization accessible by the initial RF pulse. Also, the acquisition scheme presented in this study only performs the tagging preparation once after the infusion of the hyperpolarized substrate, thus substrate arriving in the voxel either before or after the tagging were left unutilized. However, the method could be modified to use fewer data readout pulses (with larger tip-angle), making it feasible to repeat the sequence many times throughout the window that hyperpolarized spins can be observed. It may also be investigated in future studies whether this technique can provide greater specificity for changes in tissue metabolism notwithstanding the lower sensitivity as compared to other techniques.

5. Conclusions

The feasibility of using a spin tagging pulse sequence for hyperpolarized metabolic MR studies *in vivo* was demonstrated in this study. The use of a spectral–spatial RF pulse during the tagging preparation enabled the observation of metabolite signals derived exclusively from the tagged substrate. This method may allow a more direct estimate of local metabolic conversion rates *in vivo*.

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