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Original contributions

Perfusion and diffusion sensitive ^{13}C stimulated-echo MRSI for metabolic imaging of cancer

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ABSTRACT

Metabolic imaging with hyperpolarized [1-^{13}C]-pyruvate can rapidly probe tissue metabolic profiles in vivo and has been shown to provide cancer imaging biomarkers for tumor detection, progression, and response to therapy. This technique uses a bolus injection followed by imaging within 1–2 minutes. The observed metabolites include vascular components and their generation is also influenced by cellular transport. These factors complicate image interpretation, especially since [1-^{13}C]lactate, a metabolic product that is a biomarker of cancer, is also produced by red blood cells. It would be valuable to understand the distribution of metabolites between the vasculature, interstitial space, and intracellular compartments. The purpose of this study was to better understand this compartmentalization by using a perfusion and diffusion-sensitive stimulated-echo acquisition mode (STEAM) MRSI acquisition method tailored to hyperpolarized substrates. Our results in mouse models showed that among metabolites, the injected substrate ^{13}C-pyruvate had the largest vascular fraction overall while ^{13}C-alanine had the smallest vascular fraction. We observed a larger vascular fraction of pyruvate and lactate in the kidneys and liver when compared to back muscle and prostate tumor tissue. Our data suggests that ^{13}C-lactate in prostate tumor tissue voxels was the most abundant labeled metabolite intracellularly. This was shown in STEAM images that highlighted abnormal cancer cell metabolism and suppressed vascular ^{13}C metabolite signals.

1. Introduction

Using injected hyperpolarized [1-^{13}C]pyruvate for *in vivo* metabolic imaging has been recently demonstrated for feasibility and to have potential clinical value [1–15]. These studies have been made possible by the development of methods utilizing Dynamic Nuclear Polarization (DNP) and rapid dissolution techniques that provide a polarization increase of over 40,000 for [1-^{13}C]pyruvate while producing an injectable solution with physiologic pH, osmolarity, and temperature [1,2]. Following injection, the conversion of pyruvate to its metabolic products of lactate, alanine, and bicarbonate *in vivo* can be detected in sub-minute acquisition times. This is of particular value for cancer imaging in which the metabolic profile has been shown to distinguish between normal and diseased tissues in preclinical animal models [3–5,8,10–12]. This metabolic information has also been used to monitor cardiac function, and reperfusion in both the heart and lung following ischemia [6,7,9,15].

In vivo imaging with hyperpolarized substrates requires rapid and efficient MR imaging techniques because the high polarization is irreversibly lost due to T_1 relaxation to thermal equilibrium and T_2 relaxation following any RF excitation. This also requires that data be acquired within 1–2 minutes after the bolus injection, and metabolites are observed in the vasculature in addition to the tissues or organs of interest. Furthermore, the [1-^{13}C]lactate generated following a [1-^{13}C]pyruvate injection – a potential biomarker because it is elevated in tumors – is both transported out of cells and also generated in the blood by erythrocytes, which have detectable lactate dehydrogenase (LDH) activity [16,17]. High [1-^{13}C]lactate levels observed in kidneys are presumably predominantly from blood flow into the organ and excretion of lactate, which is not an accurate reflection of metabolism within the kidney. The localization of metabolites can be precisely studied in a setting where flow and perfusion can be controlled such as with cells in a bioreactor [18,19]. In vivo, the signal from flowing metabolites can confound the interpretation of metabolic imaging results. One approach proposed to address this problem is to use a spectral-spatial suppression pulse applied across the chest cavity to saturate lactate and alanine flowing through the heart and lungs [20], which provides qualitative contrast improvements but is not a quantitative measure of perfusion.

Another approach is to use a subsequent Gadolinium injection to shorten the T_1 of vascular and extracellular metabolites, which then

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isolates the ^{13}C signal to intracellular components [21]. It has also been shown that flowing metabolites can be suppressed by using the crusher gradients around spin-echo pulses [22], as done in spin-echo diffusion-weighted imaging.

The purpose of this study was to examine the compartmentalization of metabolites at the short interval after injection of hyperpolarized [1-^{13}C]pyruvate. To investigate this compartmentalization, we developed and applied a perfusion and diffusion sensitive stimulated-echo acquisition mode (STEAM) MRSI acquisition method tailored for hyperpolarized substrates. Stimulated echoes are inherently sensitive to motion, a characteristic which has been utilized for cardiac [23,24], diffusion [25,26], and spectroscopic imaging [27]. The contrast from motion sensitivity includes both perfusion processes (macroscopic scale) and molecular diffusion (microscopic scale) [28,29]. This motion sensitivity can also be achieved in diffusion-weighted spin-echo sequences, but STEAM allows for larger encoding strengths to observe a broader range of perfusion and diffusion contrast. Because the encoding strength (b-value) is proportional to γ^2, this is especially important in ^{13}C, which has a gyromagnetic ratio, γ, that is about 4-times smaller than that of ^{1}H.

Compartmentalization of metabolites has been performed on cell cultures with diffusion-weighted ^{1}H MRS [30–38]. These studies demonstrated diffusion-weighting can be used for separation of intracellular metabolites. However, their approaches cannot be directly applied to this study because they were not designed for hyperpolarized experiments, were not performed within the limitations of a clinical MRI system, and did not include imaging. The STEAM MRSI approach used in this study addresses all of these concerns.

A stimulated-echo approach with frequency-selective tagging of the hyperpolarized [1-^{13}C]pyruvate substrate has been used to isolate [1-^{13}C]lactate and [1-^{13}C]alanine signals derived exclusively from the substrate [39]. We have also presented initial results using a perfusion-sensitive super stimulated-echo preparation approach with hyperpolarized [1-^{13}C]pyruvate in normal ($N=4$) and transgenic prostate tumor bearing mice (TRAMP, $N=4$) [40]. This study showed improved tumor lactate delineation as reflected by a significant increase in both the [1-^{13}C]lactate to [1-^{13}C]pyruvate ratio within the tumors and the ratio of peak tumor [1-^{13}C]lactate to peak kidney/liver [1-^{13}C]lactate, indicating that the [1-^{13}C]lactate observed in kidney and liver tissue had a larger vascular fraction than the [1-^{13}C]lactate within tumor tissue. Based on these promising results, we acquired STEAM MRSI with varying encoding strengths (b-values) in mice ($N=6$ normal and $N=8$ prostate tumor) in this study to better characterize the metabolite compartmentalization in the liver, kidneys, muscle, and prostate tumor tissue.

2. Theory

The motion sensitivity of stimulated-echoes is due to the gradient encoding between the initial 90–90 pulses, which are refocused after the mixing time (TM) but only for stationary spins. The sensitivity to motion is determined by the b-value [25,26]. With the rapid STEAM pulse sequence [41] (Fig. 1A), phase encodes are acquired at different mixing times because a single encoding step is sampled repeatedly. This sequence has a b-value that varies across k-space:

$$b\left(\vec{k}\right) = (\gamma G_{STEAM}\delta)^2 \left(\Delta\left(\vec{k}\right) - \delta/3\right) \quad (1)$$

$$= (\gamma G_{STEAM}\delta)^2 \left(TM + n\left(\vec{k}\right) TR + 2\delta/3\right), \quad (2)$$

where G_{STEAM} and δ are the STEAM encoding gradient amplitude and duration, respectively (neglecting gradient ramps), $\Delta(k)$ is the separation between the dephasing and rephasing diffusion gradients, and $n(k)$ represents the phase encode ordering. For the concentric ordering used in this study, intravoxel motion during the

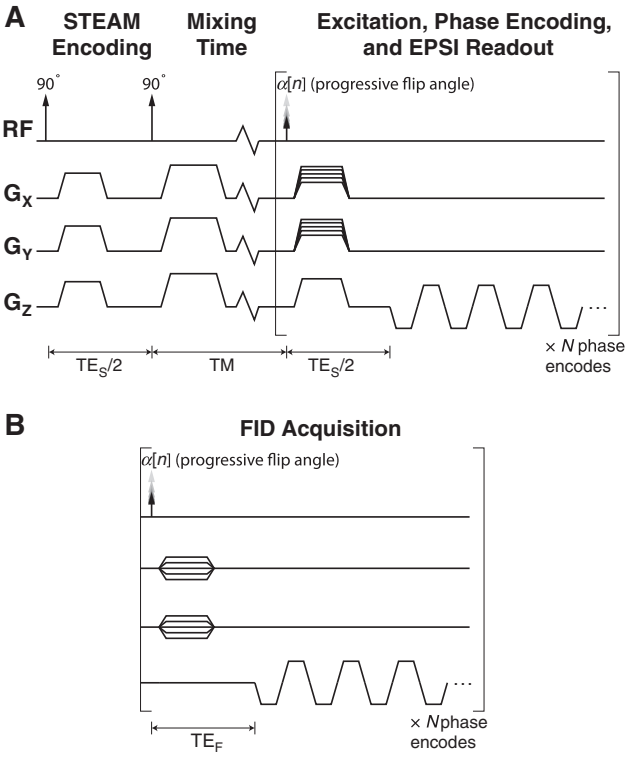

Fig. 1. (A) 3D STEAM MRSI pulse sequence for hyperpolarized substrates. A single stimulated-echo encoding step is used, followed by a series of excitations and readouts to acquire a 3D MRSI. (B) FID MRSI pulse sequence used for Control studies. The excitation flip angle, $\alpha[n]$, is incremented to efficiently utilize all of the magnetization [42].

$n(k)$ phase encodes will lead to isotropic spatial blurring. We characterized the encoding strength for this sequence using point spread function (PSF) analysis by first computing

$$PSF\left(\vec{x}, D\right) = F\left(exp\left(-b\left(\vec{k}\right) D\right)\right) \quad (3)$$

for the concentric phase encode ordering, where D is the apparent diffusion coefficient (ADC). The PSFs in Fig. 2 show resulting losses in

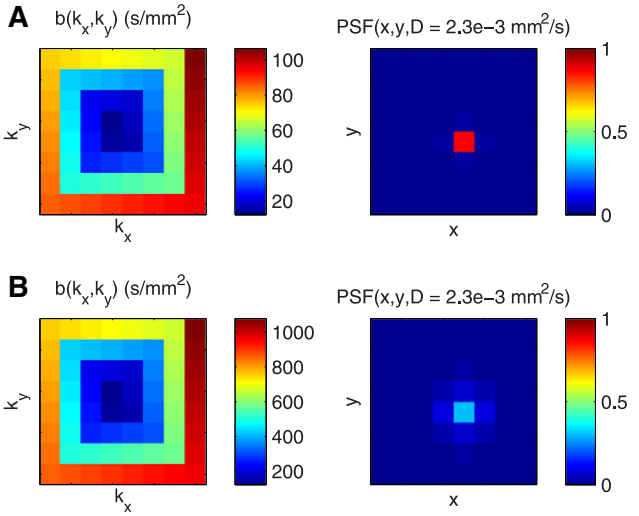

Fig. 2. Point spread function (PSF) analysis of b(k) for concentric phase encode ordering used to characterize the diffusion-weighting. The ideal PSF is a delta-function with an amplitude of 1, which the diffusion-weighting decreases. (A) Smaller encoding strength weighting, for which the effective b-value is $\hat{b}=58.4$. (B) Larger encoding strength weighting, for which the effective b-value is $\hat{b}=513.3$.