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Bio-interfacial magnetic resonance imaging of hyperpolarized contrast agents for metabolic flux interrogation *in vivo*

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ABSTRACT

We utilized dynamic nuclear polarization to hyperpolarize three metabolic imaging agents as single agents and together (co-polarization with two different organic radicals; OX63 and BDPA). In addition, we describe our initial two slice selective spectroscopy *in vivo* experiments in mice. These techniques along with advances by other laboratories will allow for multiple metabolic pathways to be imaged in real-time and *in vivo*.

Introduction

Since the discovery of Warburg effect in 1956, changes in metabolism during tumor growth has been widely studied and, metabolomics is used to discover biomarkers both for diagnosis and characterization of cancers [1]. Over the past few decades, various magnetic resonance (MR) imaging and spectroscopy techniques have been utilized in cancer detection and treatment [2,3]. Among them, hyperpolarization, in particular dynamic nuclear polarization (DNP), is one of the most promising emerging techniques in the field of MR [4–6]. DNP allows for over 10,000 fold sensitivity improvement compared to conventional magnetic resonance which allows for real-time *in vivo* metabolism of the imaging agent to be measured. The mostly widely utilized hyperpolarized agent is 1-^{13}C pyruvic acid which is utilized to determine glycolytic flux (conversion to lactate, alanine, and bicarbonate) *in vivo* and *in vitro* [7–10]. High conversion rate or high lactate production has been revealed as a biomarker for various cancers. Proof-of-concept experiments have been extensively demonstrated in numerous cancer animal models, and the first clinical trial with the 1-^{13}C pyruvic acid was recently reported [11,12]. However, the single metabolic conversion cannot interrogate more detailed information about cancers, such as aggressiveness, hypoxic character, and multiple enzyme activities, and others.

In this regard, new imaging agents or multiple agents used concurrently would allow for multiple metabolic pathways and states of the tumor (pH, reductive/oxidative potential) to be measured and would expand the utility of hyperpolarized MR significantly [13,14]. In this work, we tested two potential imaging agents (1,4-$^{13}C_2$ diethyl succinate (DES) and 1-^{13}C glycine) for multi-step metabolisms, namely citric acid cycle and glycine/serine metabolism, respectively. In combination with the 1-^{13}C pyruvic acid, we expect that metabolic profiles and metabolic flux rates of the new agents to substantially increase our current understanding of metabolism in cancer. Here, we intensively investigated DNP polarization processes in solid state with three metabolic imaging agents to maximize signal enhancements. We optimized radical concentration, microwave frequency, concentration of relaxation inducing agent, and glassing agent, for each single polarization. We further tested co-polarization methods by mixing two different imaging agents. In addition, we tried to test a slice-selective spectroscopic approach for the study of *in vivo* cancer animal models.

Experimental

For the solid state polarizations, neat solution of 1-^{13}C pyruvic acid was mixed with 15 mM tris{8-carboxyl-2,2,6,6-tetra[2-(1-hydroxymethyl)]-benzo (1,2-d:4,5-d′) bis (1,3) dithiole-4-yl}

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methyl sodium salt ("OX63"; Oxford Instruments, Tubney Woods, UK). 1-^{13}C-glycine and the organic free radical were mixed in a glass-forming solvent (60% glycerol and 40% water (vol/vol)). Synthesized 1,4-$^{13}C_2$ diethyl succinate containing 40 mM free radical α,γ-bisdiphenylene-β-phenylallyl (BDPA; Sigma–Aldrich, St. Louis, MO) was prepared in a glassing agent with sulfolane [15]. 1 mM of gadolinium (III) compound (ProHance, Bracco Diagnostic Inc.) was also added into these samples. All of the ^{13}C-labeled chemicals and solvents were purchased from Sigma–Aldrich, St. Louis, MO.

For the microwave sweep, 80 μL of 1-^{13}C pyruvic acid and 1,4-$^{13}C_2$ diethyl succinate were used to find optimal microwave frequencies for OX63 and BDPA, respectively. Hyperpolarization was accomplished in the HyperSense DNP polarizer (Oxford Instruments, Tubney Woods, UK) where a 100 mW power of the optimal microwave frequency was irradiated at a temperature of 1.4 K. The polarized sample was dissolved by 4 mL pre-heated buffer solution (40 mM Tris, 50 mM NaCl, and 100 mg/L ethylenediaminetetraacetic acid (EDTA), pH 7.5), and transferred to a sample reactor or mice. 1.5–3.0 mL of the resulting sample was transferred to a 5 mL phantom syringe for magnetic resonance spectroscopy and imaging. The final concentration of the hyperpolarized agents in the phantom after dissolution was determined by using 1D ^{1}H NMR spectra (500 MHz Bruker AVANCE III HD NMR equipped with a Prodigy BBO cryoprobe). All of the hyperpolarized spectra and spectroscopic imaging were acquired at 7T Bruker MR scanner, B-GA12 imaging gradients, and a dual-tuned actively decoupled ^{1}H/^{13}C volume resonator (Bruker Biospin Corp, Billerica, MA). Dynamic and slice selective spectroscopic data were processed using the TOPSPIN 3.1 program (Bruker Biospin), and Matlab (MathWorks, Natick, MA). Chemical shifts of ^{13}C were calibrated with 8 M ^{13}C urea phantom (set to 0 ppm).

Results and discussion

For DNP, the carbon-13 labeled imaging agent(s) is mixed with an appropriate organic radical and a glassing agent. The mixture is then placed in the DNP polarizer (Fig. 1) where it is cooled to around 1.4 K and microwave irradiation is performed for a given amount of time (30 min to several hours). After solid state polarization plateaus, the hyperpolarized agent is rapidly dissolved in heated media. We determined the optimal frequencies of two organic radicals (OX63 and BDPA) in our DNP polarizer to achieve optimum solid state enhancement. For the purpose of microwave sweep processes, 1-^{13}C pyruvic acid/OX63 and 1,4-$^{13}C_2$ diethyl succinate/BDPA mixtures were tested. Sweep width was selected from 94.060 GHz to 94.180 GHz, with 4 ~ 5 MHz sweep interval.

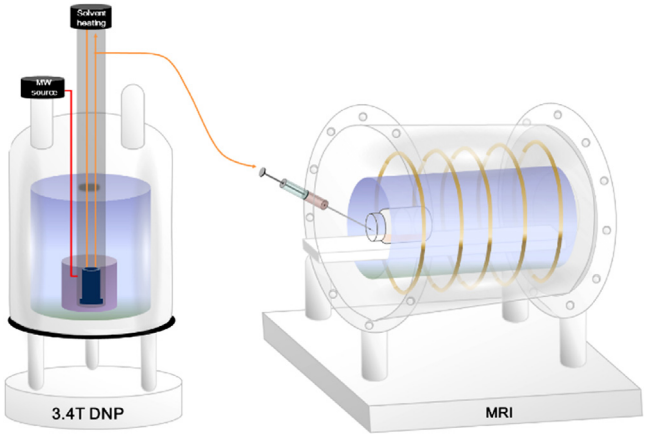

Fig. 1. Schematic diagram of dynamic nuclear polarization enhanced MRI set-up.

For each step, certain frequency of microwave was irradiated to the sample for 1 min and a single 90° excitation pulse was applied to acquire ^{13}C spectrum in the solid state. Within the sweep, prior to changing to a new microwave frequency a destroying pulse was applied to remove any remained polarization. From the sweep processes, optimal polarization microwave frequencies were determined to be 94.130 GHz and 94.100 GHz for OX63 and BDPA radical, respectively (Fig. 2).

With the optimal microwave frequency, solid state build-up curves of these metabolic imaging agents were acquired (Fig. 3a). The polarization build-up in solid state is measured with small pulses every 5 min. For the purpose of the efficient polarization enhancement, small amount of gadolinium (III) relaxation agent (ProHance, Bracco Diagnostic Inc.) was added into each sample. Generally, 1 mM of the relaxation agent showed the best solid state polarization enhancement [16]. After the polarization build up plateaus, build-up time constant of solid state polarization for each compound was determined with a single exponential fit function. 1-^{13}C pyruvic acid and 1,4-$^{13}C_2$ diethyl succinate have relatively fast build-up time constants (~700 s), while 1-^{13}C glycine is ~1400 s.

When solid state polarization plateaued, the hyperpolarized agents were rapidly dissolved in a stream of heated buffered solution (~200 °C under pressure). After the dissolution process, 3 mL of the hyperpolarized sample was transferred to an empty phantom, and then time-resolved ^{13}C spectrum was acquired at 7T Bruker MR scanner. The spectra were obtained for 64 s through a series of 10° flip angle excitations (Fig. 3b). From the time-resolved spectra, the spin-lattice relaxation time (Fig. 3c) was determined using exponential decay of the integrate signal (highlighted in red Fig. 3b) over time. Signal intensities of these agents at time zero were normalized to unit intensity. In addition to the solid state polarization, the level in liquid state right after the dissolution process (refer to time zero) were calculated by comparing signal intensity with an 8 M ^{13}C urea phantom as a standard. They showed relatively long T_1 relaxation times and high polarization levels, potentially applicable for *in vivo* hyperpolarized magnetic resonance applications. Spin-lattice relaxation times and polarization levels (P level) of these agents are summarized in Table 1.

Along with the single agent polarization, we tested simultaneous multi-component polarizations. With co-polarization, multiple imaging agents can be used concurrently to probe different metabolic cycles or physiological states (such as pH)

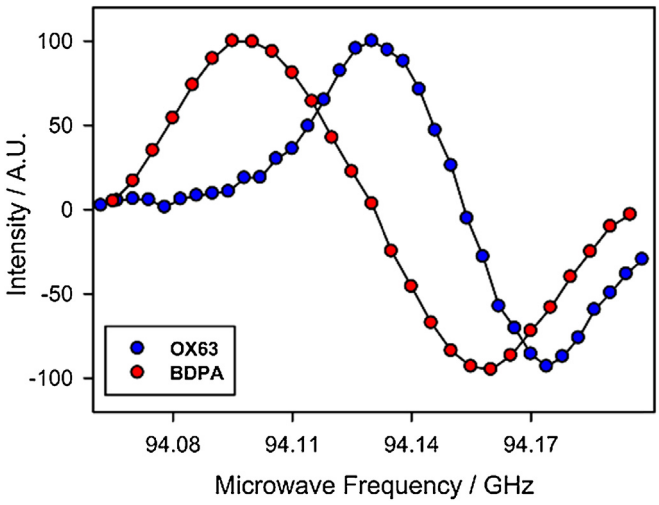

Fig. 2. Microwave frequency dependence of nuclear spin polarization levels (Microwave Sweep) for OX63 and BDPA radicals. Maximum enhancements were achieved near frequencies of $\omega_e \pm \omega_N$.