Radical-free and metal-free hyperpolarized MRI using endogenous pyruvate analogues

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Synopsis

Using nonpersistent radicals generated by UV-irradiation of endogenous metabolite precursors for dissolution DNP avoids the need for radical filtration and may potentially lengthen the measurement window of hyperpolarized MRI measurements. Here, the endogenous pyruvate-analogues alpha-ketobutyrate and alpha-ketovalerate were proposed as nonpersistent radical precursors. Radical yields were characterized along with their performance as polarizing agents for in vitro and in vivo dDNP experiments. A ^{13}C-glucose liquid state polarization of 26.4% was attained using alpha-ketobutyrate-derived radical, while pyruvate-derived radical yielded 21.7% (compared to 18.9% reported with the persistent trityl radical). Alpha-ketobutyrate was used to hyperpolarize [1-^{13}C]butyrate and measure cardiac metabolism in vivo.

Introduction

Hyperpolarization via dynamic nuclear polarization (DNP) enables a many-fold increase in the MR signal[1] but the process requires free radicals polarizing agents. This poses two major challenges for clinical translation: 1) free radicals shorten the longitudinal relaxation time of ^{13}C nuclei after dissolution[2] and affect the already short duration of the hyperpolarized state; 2) free radicals require filtration prior to injection which is a time-consuming process, and further shortens the measurement window[3]. The use of nonpersistent radicals generated by UV irradiation such as pyruvic acid (PA)[4,5,6], phenylglyoxylic acid[7] and (d_8)-trimethylpyruvic acid[8] may address both challenges. The aim of this study was to investigate the endogenous pyruvate analogues alpha-ketobutyrate (αkB) and alpha-ketovalerate (αkV) as nonpersistent radical precursors for dissolution DNP and provide a comparison with PA.

Methods

Sample preparation: **(I) For radical characterization**, PA, αkB or αkV were mixed in 1:1 glycerol:water (GW1:1). **(II) For Solid State (SS) and Liquid State (LS) measurements**, 2M [U-^{13}C, U-^{2}H]glucose was mixed with GW1:1 and 33%, 60% and 11% volume fractions of PA, αkB or αkV were admixed (n=5, Fig.1c). **(III) For in vivo measurements**, 0.66mmol [1-^{13}C]-butyric acid (BA*) of volumetric composition αkB:GW1:1:BA*=3:4:2 was mixed.
All samples were sonicated at 50°C for 20min prior to freezing 7μl droplets in liquid nitrogen to create glassy beads and then irradiated with UV light for 200s with a DymaxBlueWave200 UV-lamp using a home-built setup[6]. Preparations were optimized to generate approximately 40mM final radical concentration.
Electron Spin Resonance (ESR): X-band ESR at 77K was used to estimate radical yield as a function of UV irradiation time. Absolute radical concentration was determined using a calibration curve with 0-100mM TEMPOL dissolved in GW1:1 (n=4, Fig.1a). Radical concentration build-up times were calculated using a mono-exponential fit (n=4, Fig.1d).
Hyperpolarization with DNP: Samples were hyperpolarized in a 7T home-built polarizer for 2hrs. Microwave (MW) frequency sweeps were conducted with and without MW frequency modulation (FM) to establish conditions for maximum DNP efficiency.
MRS: After dissolution of the hyperpolarized samples, LS and in vivo measurements were performed at 9.4T. Hyperpolarized ^{13}C spectra were acquired 3s after dissolution using a 5° RF excitation pulse. Thermal equilibrium ^{13}C spectrum was acquired using a 90° RF excitation pulse, Repetition Time (TR) of 60s with 64 averages. The enhancement ϵ was calculated as ratio of hyperpolarized and thermal signal intensity referring to carbon position C_1 and polarization as $P = \epsilon * tanh(\hbar\gamma_C B_0/2k_B T)$. Hyperpolarized in vivo experiments were performed in male Wistar rats to measure cardiac metabolism as described in [5] and were approved by the local regulatory body.

Results

ESR spectra of the UV generated radicals in αkB and αkV are similar and narrower compared with PA (Fig.2).
Following UV irradiation PA, αkB and αkV yielded radical concentrations of 55mM, 57mM and 54mM respectively. A plateau was observed after 200s (Fig 1b). The radical generation build-up time constant for UV-irradiated αkB+[U-^{13}C,U-^{2}H]glucose was 52.0±2.3s (n=4, Fig.1d). MW frequency sweeps with and without FM showed that the polarization level of the αkV-glucose sample was doubled by FM and the αkB-glucose sample gained 50% (Fig.3).
Liquid state polarization of ^{13}C glucose was 21.7±2.8% for PA, 26.4±0.6% for αkB and 14.7±4.7% for αkV. Solid state build-up times were similar for all three samples (Fig.4, with spectra for αkB).
The [1-^{13}C]butyrate-αkB samples had a polarization build-up time of t_x=3.3k±0.3k s (n=5). Cardiac metabolism resulted in ^{13}C labeling of [1-^{13}C]acetylcarnitine, [1-^{13}C]acetoacate and [1-13C]butyrylcarnitine. The natural abundance ^{13}C resonances of C_1 αkB, C_1 αkB-hydrate and C_2 αkB were also observed (Fig.5).

Discussion

Two promising endogenous polarizing agents were studied for radical-free dissolution DNP. The use of αkB increased the polarization of ^{13}C glucose (26.4%) compared with PA (21.7%), and was 40% higher than previously reported using the persistent trityl radical Ox063[9]. Although UV-irradiated αkB and αkV demonstrated similar ESR lineshapes and radical yield in a neat GW1:1 matrix, adding glucose or butyric acid required a unique sample composition for each. Unexpectedly, αkV, which is self-glassing, performed worse than PA, although the ESR linewidth was narrower. This illustrates that sample formulation requires a careful optimization in terms of UV-generated radical yield and polarization level for each ^{13}C labelled metabolic substrate, which is still a largely empirical and nontrivial process. Compared to previous work using ^{13}C-butyrate[5], a different UV source, polarizing agent and the use of microwave modulation contributed to improved polarization levels leading to the detection of an increased number of metabolites.

Conclusion

The pyruvate analogues αkB and αkV were proposed as endogenous polarizing agents for dissolution DNP. αkB generated 26.4% liquid state polarization on ^{13}C glucose and was successfully used in vivo to measure cardiac metabolism of [1-^{13}C]butyrate. αkB and αkV are promising alternatives for radical-free and metal-free translational clinical hyperpolarized MRI with high polarization and no need for radical removal via filtration.

Acknowledgements

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Figures

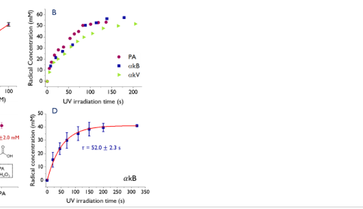

Figure 1: Radical concentration calibrations obtained from X-band ESR at 77K. **A)** Concentration calibration curve of TEMPOL + GW1:1 and linear fit (R^2 = 0.999, red) for n = 4. **B)** Radical generation rates for precursor:G:W = 2:1:1 volumetric ratios. **C)** [U-^{13}C, U-^{2}H]glucose samples optimized to the target radical concentration of 40 mM upon 200s UV irradiation, n = 5. **D)** Radical concentration rate of αkB + GW1:1 (n = 4) with mono-exponential fit (R^2 = 0.999, red) yields radical generation build-up time 52 ± 2.3 s.

Figure 2: X-band ESR spectra of three endogenous metabolites at 77 K after 200 s of irradiation with a UV source of 40 Wcm^{-2} surface power density. αkV and αkB are more narrow linewidth radicals compared with PA. The ESR lineshapes of αkV and αkB were found to overlap largely.

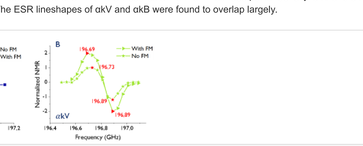

Figure 3: Hyperpolarized ^{13}C signal as a function of microwave frequency with and without frequency modulation at 7 T and 3.6 K. FM was set to 40 MHz modulation amplitude at a frequency of 5 kHz. Samples contained [U-^{13}C, U-^{2}H]glucose + GW1:1. MW frequencies for DNP maxima and minima are reported in red. Hyperpolarization was achieved using the UV radicals **A)** αkB and **B)** αkV.

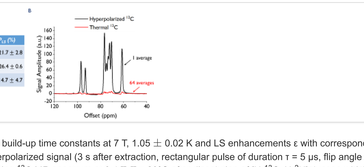

Figure 4: Characterization of [U-^{13}C, U-^{2}H]glucose samples. **A)** Polarization build-up time constants at 7 T, 1.05 ± 0.02 K and LS enhancements ε with corresponding polarization levels P_{LS} at 9.4 T, 20°C (N = 3). ε was calculated as a ratio of hyperpolarized signal (3 s after extraction, rectangular pulse of duration τ = 5 µs, flip angle α = 5°) and thermal signal (64 averages of α = 90° with τ = 90 s, TR = 60 s). **B)** Resulting ^{13}C MR spectrum at 9.4 T, T = 20°C after dissolution of [U-^{13}C, U-^{2}H]glucose which was hyperpolarized using αkB. Hyperpolarized spectrum acquired 3 s post-dissolution.

Figure 5: In vivo spectrum of cardiac metabolism following the injection of radical-free hyperpolarized [1-^{13}C]butyrate. The UV-generated nonpersistent radical αkB was used to polarize the sample. Resonances around the chemical shift of [5-^{13}C]glutamate and [1-^{13}C]hydroxybutyrate were also present but not well resolved due to vicinity of [1-^{13}C]butyrate.