Real-time measurement of substrate uptake and metabolism in heart using Dynamic Nuclear Polarization-Magnetic Resonance (DNP-MR)

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Introduction

DNP-MR is a molecular imaging method which combines the technique of enhancing nuclear polarization in the solid state known as Dynamic Nuclear Polarization[1], with Ardenkjær-Larsen's method of dissolving the solid state polarized spins whilst maintaining high levels of polarization[2]. The resulting hyperpolarized solution is then used as a contrast agent in MRI or MRS. Using DNP-MR, signal associated with traditionally low MR sensitivity nuclei such as ^{13}C are enhanced to the extent that real-time metabolism can be monitored *in-vivo* on a sub-second timescale, and even used to create metabolic maps. Molecular Imaging technologies such as DNP-MR will revolutionize our understanding, detection, diagnosis and treatment of disease.

Here we have used DNP[1] coupled with Ardenkjær-Larsen's dissolution method[2] to produce hyperpolarized $^{13}C_1$-pyruvate for *in-vivo* MRS. Surface coil localised spectroscopy has been used monitor the uptake and conversion of hyperpolarized ^{13}C labelled pyruvate in healthy rat heart.

Methods

In this study $^{13}C_1$-pyruvic acid (isotope enriched to 99%) was polarized at 1.2 K using microwaves in the GHz range. Polarization build-up was monitored until steady-state polarization was reached. Hyperpolarized $^{13}C_1$-pyruvic acid was dissolved with sufficient aqueous NaOH and TRIS buffer to yield a 79 mM solution of $^{13}C_1$-pyruvate with pH 7.6 at approximately 38°C. The dissolved solution was injected into an empty glass vessel inside the magnet for set-up experiments, and via a rat tail vein cannula to study uptake and metabolism *in-vivo*. Spectroscopy was performed in a 7T horizontal bore magnet interfaced to a Varian Inova console (Varian Inc., Palo Alto, CA) using a butterfly RF transmit and receive coil.

For set-up experiments, spectra were acquired with one 12° pulse every 2 seconds for 2 minutes from the beginning of injection. For *in-vivo* experiments, spectra were acquired with variable flip-angles from 3.7° to 90° to maximise signal available over experimental time course, of the heart and chest region of five female Wistar rats. Studies were repeated on two occasions to investigate intra- and inter-subject variation of metabolism in age and weight matched rats. All investigations conformed to Home Office Guidance on the Operation of the Animals (Scientific Procedures) Act, 1986 (HMSO) and to institutional guidelines.

Results

Hyperpolarized $^{13}C_1$-pyruvate was injected into a glass vial to observe the hyperpolarized signal, followed by a multi-averaged acquisition to determine the signal available when the sample had returned to thermal equilibrium. Figure 1 shows the 22100 times signal enhancement achieved after dissolution, compared with thermal equilibrium polarization. This corresponds to 0.0006% thermal polarization, and 13.3% hyperpolarized polarization.

Dissolution, transport and injection were achieved within 25 seconds, including 10 second injection duration. Accumulation of hyperpolarized tracer and subsequent decay of signal was observed by real-time acquisition of spectra. Figure 2 shows the arrival (during injection) and decay of the pyruvate signal injected into a glass vial. Figure 3 is an *in-vivo* example which was generated with constant flip-angle to illustrate the bolus of polarized pyruvate arriving at the rat heart and its subsequent metabolism into lactate, alanine and bicarbonate.

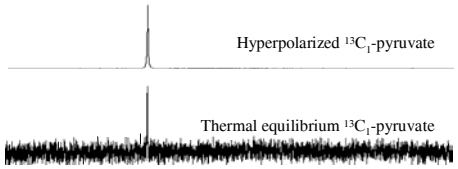

Figure 1: Top: 13.3% hyperpolarized spectrum SNR 480 in 0.5 seconds Bottom: 3 hour thermal equilibrium spectrum (0.0006% polarization; SNR 0.025 after correction for 2048 averages).

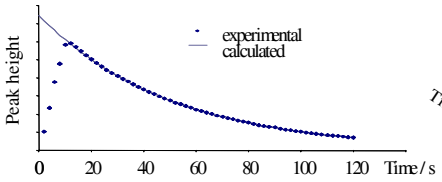

Figure 2: 12° pulse, 0.5 second acquisition (2048 complex points) repeated every 2 seconds for 2 minutes. Injection duration was 10 seconds.

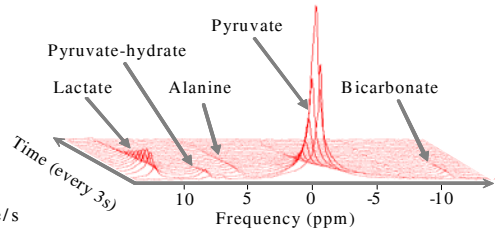

Figure 3: example heart and chest ^{13}C NMR spectra, 5° pulse, 0.5second acquisition repeated every 3 seconds for 1 minute. Injection of 1 cm^3 over 8 seconds into the tail vein of a female Wistar rat.

Conclusions

We have demonstrated high levels of polarization, which enables us to visualise the uptake of polarized pyruvate *in-vivo* and its metabolism to lactate, alanine and bicarbonate in real-time. Future work will focus on spectroscopic imaging, kinetic modelling of metabolic processes demonstrated in Figure 3, and detecting differences in real-time metabolism between normal and diseased heart.

References

1. Jeffries. Phys Rev. 106, 164-165 (1957).
2. Ardenkjaer-Larsen, J.H., et al. Proc Natl Acad Sci USA. 100(18), 10158-63 (2003).

Acknowledgements

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