Limits of Detection of Hyperpolarized ^{13}C *in vitro* and *in vivo*

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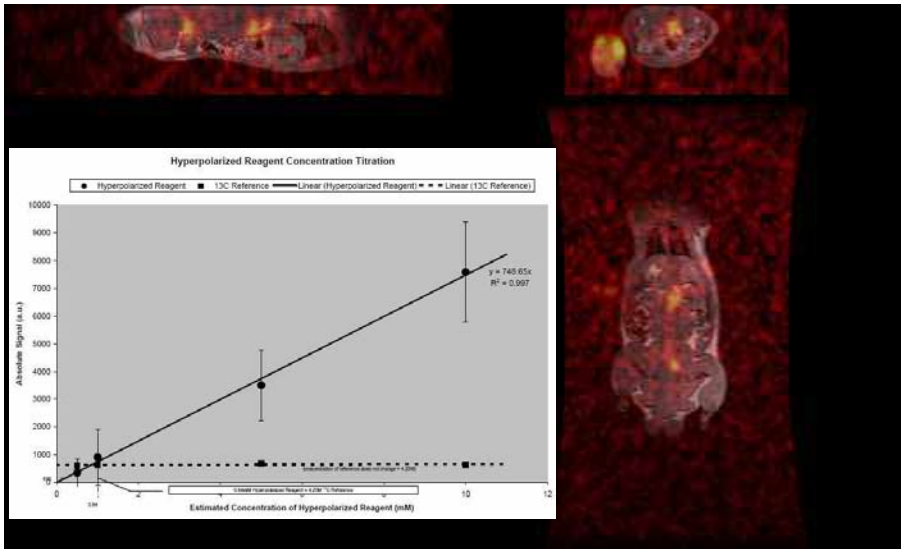
Objective: Fast ^{13}C imaging at safe, physiological concentrations of water-soluble, non-toxic, hyperpolarized 13C contrast agents.

Background: *In vivo* ^{13}C MRS of human brain defines concentrations of important fuels and neurotransmitters between 1-10 mM[1] and reaction rates of 1-5 μmoles/min/gram[2]. Two novel methods of hyperpolarization of ^{13}C, dynamic nuclear polarization (DNP[3]) and parahydrogen and synthesis allow dramatically enhanced nuclear alignment (PASADENA[4]); which provide signal enhancement for ^{13}C in excess of 10,000 (even reaching 40,000 in recent studies in this Laboratory[5]). Both techniques have shown utility in *in vivo* fast ^{13}C imaging and spectroscopy and DNP is poised for clinical trials. However, even after removal of toxic constituents and sterilization, the concentration of ^{13}C pyruvate contrast agent employed (0.5M) will be 5000 times higher than physiological, presenting unacceptable biochemical and osmotic stress. Similarly, PASADENA (a.k.a. parahydrogen induced polarization: PHIP) ^{13}C imaging, which was demonstrated initially with acetone-solutions, and later with aqueous solutions of a ^{13}C reagent, employed a concentration (0.3M) 250 times higher than the known lethal dose (LD_{50} = 1.3mM[6]). With achievable ^{13}C signal enhancement of ^{13}C of 40,000 fold, *in vivo* images of ^{13}C metabolites comparable to those available from proton of water (80M) should permit contrast agent concentrations to be reduced to 10mM. We demonstrate with PASADENA, that ^{13}C imaging can be achieved as concentrations as low as 0.5 mM *in vitro* and 9 mM *in vivo*.

Materials and Methods: Hyperpolarized ^{13}C reagents were generated using 98% parahydrogen, norbornadiene catalyst and ^{13}C hydroxyethyl propionate exposed to low-field ^{13}C spin-transfer pulse sequence in a custom-built polarizer. Product was collected every 2–5 minutes and transferred rapidly to a GE 1.5 Tesla clinical MR scanner for acquisition of ^{13}C images using a dual-tuned surface coil. 3D FIESTA, ^{13}C MRS or ^{13}C CSI sequences developed in this Laboratory were employed as appropriate. For *in vivo* studies, rats, anesthetized with i.p Nembutal received rapid injection of hyperpolarized contrast agent through indwelling jugular or femoral vein catheters. Hyperpolarized ^{13}C signal intensity was quantified with comparison to an internal ^{13}C standard containing 4.25M ^{13}C acetate.

Results: Hyperpolarization of ^{13}C PASADENA reagent ranged between 10,000 and 37,000. Intensity of ^{13}C images was linear and proportional to concentration (Fig 1, inset). As predicted, *in vitro* ^{13}C images were readily produced at ^{13}C concentrations as low as 0.5 mM. *In vivo* proton images of rats in which ^{13}C contrast accumulated in the inferior vena cava at a concentration of 9 mM are illustrated (Fig 1).



Discussion: PASADENA lends itself to rapid generation of hyperpolarized ^{13}C reagents for studies necessary to establish dose-response curves in vitro and in vivo. As was theoretically anticipated, the signal enhancement achieved by routine hyperpolarization was sufficient to permit detection of ^{13}C concentrations as low as 0.5 mM. Somewhat higher ^{13}C concentration, 9 mM, necessary for *in vivo* imaging are in part a function of the additional time taken for injection. Allowing a dilution factor of 4 – 12 fold when contrast agent is mixed with circulating blood, results *in vivo* and *vitro* were comparable.

Conclusion: *In vivo* PASADENA ^{13}C imaging with hyperpolarized contrast agents can be achieved using near-physiological concentrations of naturally occurring metabolites (DNP), and relevant C=C molecules, yet to be described.

References:

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