TOWARDS HYPERPOLARIZED 13C SUCCINATE IMAGING OF BRAIN CANCER

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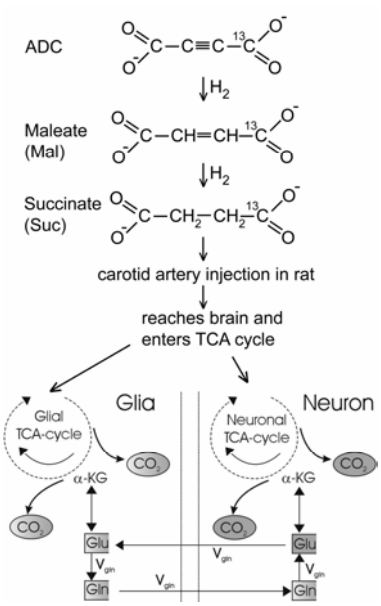
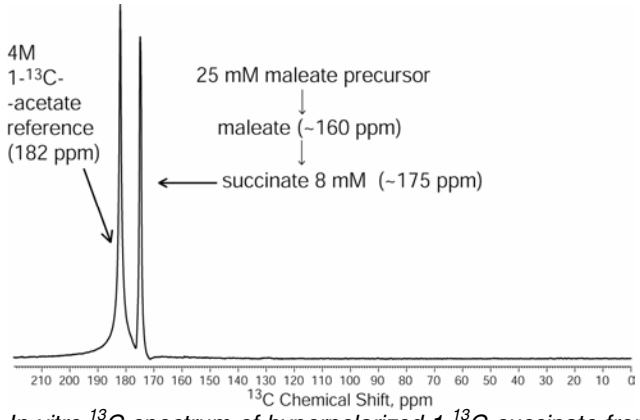
Introduction: *In vivo* ^{13}C MRS of human brain defines concentrations of important fuels and neurotransmitters between 1-10 mM and reaction rates of 1-5 mmoles/min/gram. Parahydrogen and synthesis allow dramatically enhanced nuclear alignment (PASADENA) provides signal enhancement for ^{13}C in excess of 100,000 on currently utilized MRI scanners. The hyperpolarization techniques have shown utility in fast *in vivo* ^{13}C imaging and spectroscopy. With achievable ^{13}C signal enhancement of ^{13}C *in vivo* imaging and spectroscopy of ^{13}C metabolites should permit contrast agent concentrations to be reduced to 1-10 mM corresponding to physiological levels, providing the most useful metabolic information.

Aim: The purpose of this work is to demonstrate that ^{13}C-enriched succinate and maleate, which can be hyperpolarized by PASADENA, cross the blood brain barrier into the tumor where they are metabolized to glutamate and glutamine and act as metabolic biomarkers.

Methods: We used disodium acetylenedicarboxylate (ADC) as the unsaturated precursor for the molecular addition of dihydrogen in PASADENA. The chemical goal was to achieve this reaction in a timescale which is small compared to spin lattice relaxation times. In order to break the symmetry to achieve PASADENA hyperpolarization on ADC, the ^{13}C label is confined to only one carbon nucleus (C1) in this symmetric molecule. The choice of carbonyl C1 label is also to maximize the T_1 relaxation for the hyperpolarized species. ADC is converted to maleate in the first hydrogenation step and then to succinate after addition of a second parahydrogen molecule. The figure on the left demonstrates the ^{13}C spectrum of hyperpolarized 1-^{13}C-succinate at 175 ppm with respect to a reference sample of 4M 1-^{13}C-acetate at 182 ppm, obtained *in vitro* with a 4.7T in Bruker Avance animal scanner. In a separate experiment, the resulting mixture (16 mM of 1-^{13}C-maleate and 8 mM 1-^{13}C-succinate) is passed through an ion-exchange filter to remove Rh-based catalyst and 3 ml was injected in the carotid artery of a 9L tumor- bearing rat. We anticipate that 1-^{13}C-succinate is taken up differently by brain and brain tumor and would then be metabolized primarily in TCA cycle of glia and neurons. One hour was allowed to metabolize succinate before brain and tumor tissues were collected for *ex vivo* high resolution NMR.





In vitro ^{13}C spectrum of hyperpolarized 1-^{13}C-succinate from ADC precursor. The spectrum is acquired using 40 mm solenoid based ^{13}C coil at 4.7T.

Results: Using *ex vivo* ^{13}C spectroscopy at 11.7T we find that glutamine and glutamate C1 and C5 carbons are enriched with ^{13}C label from C1 of succinate in tumors. Our results suggest that hyperpolarized 1-^{13}C-succinate could be a highly potent molecular imaging agent to assess the differential metabolism of normal and diseased tissue by non-invasive NMR and MRI methods. We find that a few mM succinate when injected in carotid artery of 9L brain tumor bearing rat undergoes much different metabolic transport, pathways and/or kinetics in tumor and normal brain tissues. Most likely, succinate and maleate both enter the tumor through the dysfunctional blood brain barrier, but are substantially excluded by the normal brain tissue.

Conclusions: These experiments indicate that it may be possible to perform *in vivo* dynamic MR spectral-spatial imaging and spectroscopy of tumor metabolism following injection of millimolar concentrations of hyperpolarized 1-^{13}C-succinate with high contrast and high SNR (experiments in progress).

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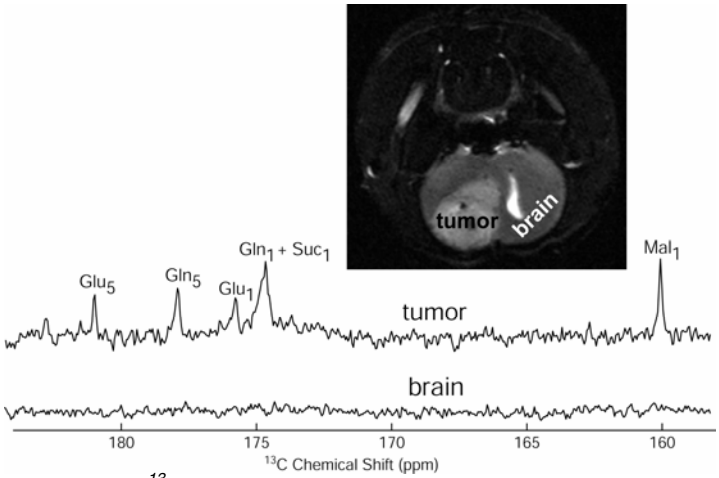

Ex vivo MAS ^{13}C spectra of brain and brain tumor tissues. 80 mg of tissue was used in each experiment. Spectra were acquired using 11.7T Bruker NMR console at 4°C and using ^{1}H decoupling.