Interrogating Tricarboxylic Acid Cycle: A Comparative Study by Hyperpolarized Succinic Acid and its Diethylester

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Purpose: The goal of this work is to interrogate the succinate level in the cytosol by hyperpolarization of succinate, a key metabolite of Tricarboxylic Acid Cycle (TCA) and its diethyl ester derivate. Succinate Dehydrogenase (SDH)-catalyzes the oxidation of succinate to fumarate with the reduction of ubiquinone to ubiquinol and is an oncogene defined in many cancers. Here we demonstrate different metabolic profiles *in vivo* using two molecules with slight variations but with very different uptake rates. Hyperpolarized diethyl ester of succinic acid (diethylsuccinate) is taken up rapidly *in vivo* in both normal and tumor bearing mice, while hyperpolarized succinic acid is apparently taken up only in some tumor bearing animals.

Materials and Methods: Two molecules were hyperpolarized by Parahydrogen Induced Polarization (PHIP) a) Succinic acid and b) Diethylsuccinate before injecting via tail vein (10 micromoles) into control and tumor-bearing mice (renal cancer RENCA and Lymphoma A20). ^{13}C spectra were acquired with following ^{13}C MRI.

Results and Discussion: After injection of hyperpolarized 1-^{13}C diethylsuccinate hyperpolarized metabolic products were detected with 20,000 fold increased sensitivity for *in vivo* ^{13}C imaging and spectroscopy over 3 – 5 minutes in control animals (Fig.1). No metabolites from 1-^{13}C succinate were observed in control mice; however hyperpolarized metabolic products were observed in tumor bearing mice (Fig. 3). Furthermore, the ratio of signal intensities of hyperpolarized diethylsuccinate and its products (metabolites marked in blue in Fig. 3) versus hyperpolarized succinate and its downstream metabolites (metabolites marked in red in Fig. 3) reflect the higher uptake rate of diethylester in the cells leading to the formation of succinate in the cytosol by esterases. The differences in metabolic profile in control and cancer mice could be assigned to the hypoxia inducing factor HIF1α in tumor bearing mice where SDH was impaired upstream (Fig. 4). Hyperpolarized diethylsuccinate, a neutral molecule was injected *in vivo* at physiological pH (7.2) while hyperpolarized succinate was injected at either high (8.5) or low (2.9) pH as constrained by the conditions of PHIP hyperpolarization.

Fig. 1: Metabolites from Diethylsuccinate in normal mouse

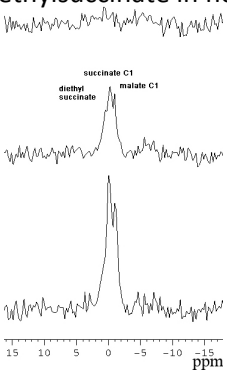


Fig. 2: TCA Cycle

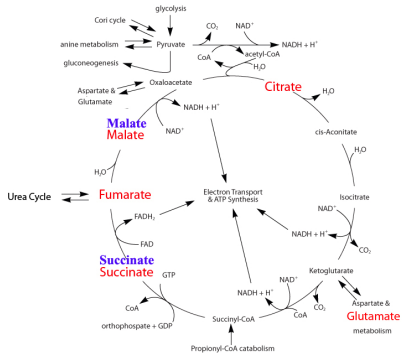


Fig. 3: Metabolites from Succinate in RENCA

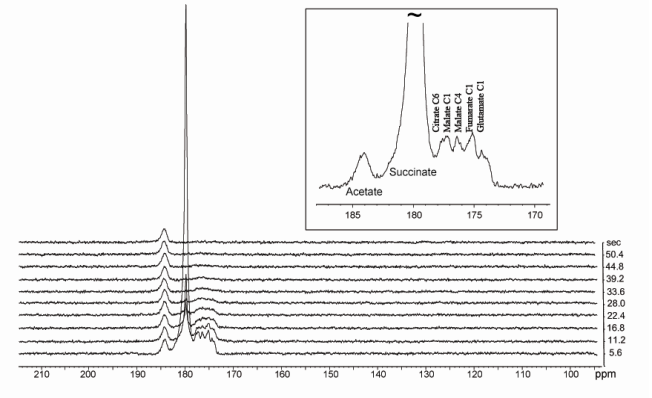
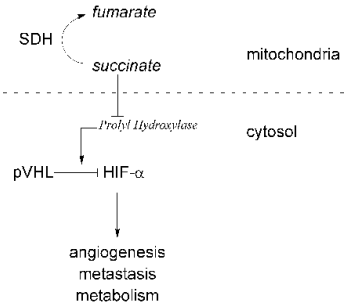


Fig. 4: Role of succinate in the mitochondrion-to-cytosol signaling pathway



Conclusion: Hyperpolarized MR effectively images ^{13}C intermediates of the Krebs TCA cycle using parahydrogen induced hyperpolarization (PHIP) with potential for preclinical and clinical application in cancer molecular imaging. The results underscore the importance of chemical modification in choosing molecular targets to achieve desired metabolic imaging using hyperpolarization.

References: 1. Bhattacharya P et al. Imaging Cancer TCA Cycle Metabolism by PHIP Hyperpolarization of ^{13}C Succinate *In Vivo*. Chem. & Biol. 2010; submitted. 2. Bhattacharya P et al Towards Hyperpolarized ^{13}C Succinate Imaging of Brain Cancer. J Magn Reson 2007; 186:108-113. 3. Chekmenev EY et al. PASADENA Hyperpolarization of Succinic Acid for MRI and NMR. J Am Chem Soc 2008; 130:212-4213. 4. Selak MA et al. Succinate links TCA cycle dysfunction to oncogenesis by inhibiting HIF1α prolyl hydroxylase . Cancer Cell 2005; 7: 77-85.