Comparative Metabolic Fingerprinting Employing Hyperpolarized Diethylsuccinate in Two Cancer Models In Vivo.

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Background:. Parahydrogen Induced Polarization (PHIP) can offer 50,000 fold increase in MR signal under the right conditions. Diethyl 1-^{13}C 2,3-d_2 succinate is generated through the hydrogenation of diethyl 1-^{13}C 2,3-d_2 fumarate and hyperpolarization increases the ^{13}C signal by 5000 fold. We have previously employed hyperpolarized diethyl succinate to image and observe real time metabolism in normal mice. Using ^{13}C MRS, metabolism of diethyl succinate in the Tricarboxylic Acid Cycle (TCA cycle) was observed in normal animals 5 s after a 10 µmol tail vein injection [1]. The biodistribution of the compound can be observed using ^{13}C FISP imaging. We have furthered our work by injecting 10-20 µmol of hyperpolarized diethyl succinate via tail vein injection into subcutaneous tumor bearing mice. Hyperpolarized succinate and diethyl succinate are biomedically interesting as the compounds can potentially assess the *in vivo* activity of succinate dehydrogenase (SDH), the enzyme that was recently tagged as an oncogene due to its crucial role in cell energetics [2] and its role in modulating the Hypoxic Inducing Factor (HIF1α) protein [3].

Purpose: The goal of the research is to apply hyperpolarized diethyl succinate for imaging cancer in two different subcutaneous tumor models in mice and compare their fingerprinting of the downstream metabolites in the TCA cycle in real time *in vivo*.

Methods: We utilized PHIP to hyperpolarize diethyl 1-^{13}C 2,3-d_2 succinate in a home-built polarizer and the hyperpolarized solution in near physiological concentrations (10-20 µmol) is injected via the tail vein of a BALB/c mice bearing a breast (4T1) (N=5) or renal tumor (RENCA) (N=9). A ^{1}H/^{13}C dual resonance volume coil (Doty Scientific, Inc., Columbia, SC) is utilized for ^{13}C hyperpolarized *in vivo* imaging and CSI spectroscopy. ^{13}C FISP with a flip angle of 60°, FOV 6 or 7 cm, and slice thickness of 15.2 mm was used. ^{13}C CSI (1 ms gauss pulse, 200 ms TR, 8 x 8 or 16 x 16 matrix, FOV ranging from 2.64 cm to 4 cm, slice thickness of 8 to 12 mm). CSI was processed using 3DiCSI software (Columbia University, Qui Zhao). The flux rate of the compound within the tumor was determined using a 4 cm ID solenoid volume coil and a simple pulse and acquire ^{13}C sequence. All ^{13}C imaging and spectroscopy was done on a horizontal bore Bruker Avance 4.7T animal scanner.

Results: A different biodistribution of the diethyl succinate is observed in the two types of tumors. The compound is taken up in the RENCA tumor within 10 s after injection in some animals (Figure 1). While in the breast tumor containing animals, the majority of the hyperpolarized signal is not within the tumor (Figure 2). In addition, the CSI in both tumor models reveal different biodistribution of the compound within the tumor in both animal models (Figure 3). We are in the process of determining if this difference in biodistribution between the two tumors and within the tumor tissue is because of blood flow or intrinsic issues with the tumor tissue like necrosis. The difference in biodistribution within the tumor could be due to the heterogeneity of tissue. Metabolism of diethyl succinate is observed in both tumors using ^{13}C CSI (Figure 4). Pulse and data acquisition was used with the volume coverage of 64 cm^3 to measure the flux rate of the compound using our dual tuned 4 cm ID solenoid volume coil. Preliminary data revealed different flux rates of diethyl succinate in the two different tumor types.

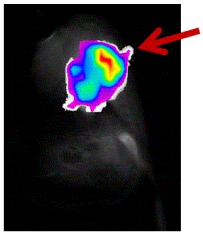


Figure 1: RENCA tumor ^{13}C FISP image in false color overlaid on ^{1}H image

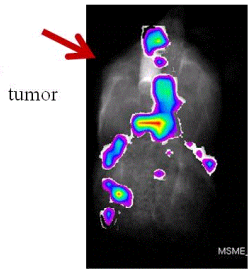
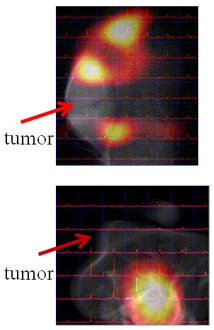


Figure 2: Breast tumor ^{13}C FISP image in false color overlaid on ^{1}H image



RENCA CSI with a heat map revealing majority of hyperpolarized signal in the image

Breast CSI with a heat map revealing majority of hyperpolarized signal in the image

Figure 3

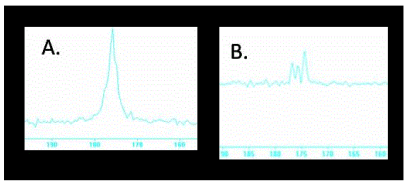


Figure 4: Average spectrum of 4 voxels in tumor A: RENCA B: Breast

Conclusions: Diethyl succinate is taken up by the RENCA mice model of cancer while it's not as specific in 4T1 breast cancer model as revealed in sub-second ^{13}C FISP images. The distribution of the hyperpolarized molecule reveals the regions of tumor heterogeneity in renal tumor like regions of necrosis and metabolically active domains. Real time ^{13}C MRS and ^{13}C CSI of the hyperpolarized metabolites *in vivo* show different ratios of the metabolites corresponding to the two different tumor types. Measurement of the relative flux-rates of the downstream metabolites as well as the ratios of the metabolites to the hyperpolarized diethyl succinate signal *in vivo* will be crucial in establishing the dynamics of the TCA cycle in these cancer models as well as their variability. Immuno-histopathological and Western blot correlation are underway to better understand the biochemical basis of the different metabolic fingerprinting as expressed in renal and breast tumors .

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References:

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