Effect of the Monocarboxylate Transporter Inhibitor α-cyano-4-hydroxy-cinnamate on *In Vivo* Hyperpolarized MR Spectroscopic Imaging with [1-^{13}C]Pyruvate

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Introduction: Development of hyperpolarized technology utilizing dynamic nuclear polarization has enabled the measurement of ^{13}C metabolism *in vivo* at very high SNR [1]. Thus far, the most researched agent for *in vivo* applications has been [1-^{13}C]pyruvate. In this work, the role of cell membrane transport on the conversion of [1-^{13}C]pyruvate to [1-^{13}C]lactate and to [1-^{13}C]alanine *in vivo* was investigated by utilizing the monocarboxylate transporter (MCT) inhibitor α-cyano-4-hydroxy-cinnamate [2]. Reduced hyperpolarized alanine and lactate were detected after α-cyano-4-hydroxy-cinnamate administration, which is in agreement with recent *in vitro* work where decreased hyperpolarized lactate was detected after breast cancer cells were given an MCT inhibitor [3].

Methods: All studies were performed on a GE 3T scanner with a custom ^{1}H/^{13}C mouse coil. ^{13}C 3D-MRSI data (TE/TR = 140ms/215ms, 0.034 cm^3 voxel size, 16 second acquisition time) were acquired with a double spin-echo compressed sensing pulse sequence [4] after injection of 0.35 mL of 80mM hyperpolarized $^{13}C_1$-pyruvate. Metabolite areas were derived from the spectral arrays over regions of interest identified from the co-registered anatomical images. For each animal studied, a baseline hyperpolarized acquisition was first acquired. Then the acquisition was repeated 30 minutes after an intraperitoneal injection of 90 mg/kg of 4-CIN (Sigma Aldrich) dissolved in a pH 7.5 phosphate buffer. Healthy and liver cancer mice (Tet-o-MYC/LAP-tTA double-transgenic mice in which the human MYC proto-oncogene is selectively overexpressed in the liver [5]) were used.

Results: Figure 1 shows a comparison of hyperpolarized spectra from a liver tumor region before and after 4-CIN administration. The representative voxels in Figure 1 show somewhat lowered lactate and dramatically decreased alanine. Table 1 and Figure 2 show data from all mice studied. The percentage change values in Table 1 and Figure 2 were computed over all liver tumor voxels for the MYC mice and over all normal liver voxels for healthy mice. The liver was chosen as the region of interest because of the high hyperpolarized alanine levels present.

Discussion: These results show that MCT inhibition had a significant impact on conversion of [1-^{13}C]pyruvate *in vivo*, which is in agreement with previous studies performed *in vitro* [3]. As shown by the data, lactate and alanine dropped after 4-CIN administration, suggesting that hyperpolarized conversion occurs intracellularly, i.e. after uptake of pyruvate. In conclusion, these initial data show that this MCT inhibitor approach can be used to investigate the role of cell membrane transport of pyruvate on the resulting hyperpolarized spectra *in vivo*.

References: [1] Ardenkjaer-Larsen et al. PNAS (2003) 100:10158 [2] Schurr et al. Brain Research (2001) 895:268. [3] Harris et al. PNAS (2009) 106:18131. [4] Hu et al. 3D Compressed Sensing for Highly Accelerated Hyperpolarized ^{13}C MRSI with *In Vivo* Applications to Transgenic Mouse Models of Cancer. MRM (2009) In Press [5] Shachaf et al. Nature (2004) 431:1112.

Acknowledgments: Funding from NIH EB007588 & CA137298.

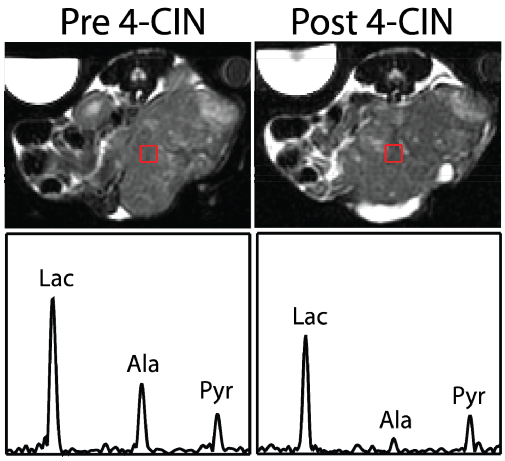

Figure 1: Effect of MCT inhibitor 4-CIN on hyperpolarized spectra from a tumor region. In this example, lactate levels dropped slightly, but alanine levels dropped dramatically after 4-CIN administration.

% Change after MCT Inhibition				
	Lactate	Alanine	Pyruvate	Total Carbon
Mouse 1 MYC Tumor	-42.7%	-61.8%	-68.0%	-50.3%
Mouse 2 MYC Tumor	-19.5%	-71.0%	+20.7%	-24.1%
Mouse 3 Healthy	-34.9%	-70.1%	+33.6%	-6.3%

Table 1: Quantitation over all tumor (for MYC) and liver (for healthy) voxels for mice in which MCT inhibition experiments were performed. Note: the pyruvate category includes pyruvate-hydrate. As shown by the data above, in all cases, alanine and lactate decreased after 4-CIN administration.

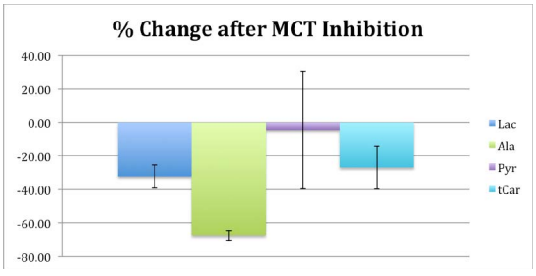

Figure 2: The mean percent changes (derived from Table 1) with standard errors shown.