Alanine Signal and T_2 Relaxation: A Potential Hyperpolarized ^{13}C Metabolic Marker for Hepatocellular Carcinoma

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Introduction

Elevated lactate signal in malignant tumors has been characterized by proton spectroscopic imaging, as well as by hyperpolarized ^{13}C technique[1-4]. Recent studies showed increasing ^{13}C-lactate signal correlates with disease progression of prostate tumors in TRAMP model[2,3]. Another study[4] demonstrated that the TRAMP mice responding to hormone treatment exhibit reduction of ^{13}C-lactate signal, whereas those not responding to treatment continue having high ^{13}C-lactate signal. No differences in alanine signal have been observed previously in any tumor studies. In this work, we investigated potential metabolic markers of hepatocellular carcinoma by using hyperpolarized ^{13}C technique. The signal level and T_2 relaxation time of ^{13}C-labeled metabolites were measured and compared between liver tumors and normal livers in rats.

Method

***Animal Preparation*:** The experiment was performed on four rats (283 to 459g) with liver tumors prepared by using Morris hepatocellular carcinoma (HCC) model[5], and three healthy Wistar rats (499 to 503 g) as controls. All HCC rats had a tumor larger than 1cm in diameter and were fasted more than 12 hours prior to ^{13}C scans. Control rats were fasted 20 hours prior to ^{13}C scans. The ^{13}C scans were repeated three times on two of the control rats to assess intra-subject reproducibility. Respiration, rectal temperature, heart rate, and oxygen saturation were monitored throughout the experiment. Animal was kept warm by using a water blanket regulated at 37ºC. Animal preparation and physiological monitoring followed a protocol approved by the Stanford University Institutional Animal Care and Use Committee.

Hyperpolarized 1-[^{13}C]-pyruvate solution: A typical dose consists of 40mg pyruvic acid/EPA mixture, prepolarized by DNP technique and dissolved[6] in 4.6g of TRIS/EDTA NaOH solution using a HyperSense™ polarizer (Oxford Instruments Molecular Biotools, Oxford, UK). Each rat was injected 3mL of 100mM ^{13}C-pyruvate solution two to three times at 2-hour intervals.

MR Hardware: A custom-built dual-tuned ($^{1}H/^{13}C$) quadrature rat coil[7] was used for both RF excitation and signal reception. All experiments were performed on a 3T Signa™ MR Scanner (GE Healthcare, Waukesha, WI) equipped with self-shielded gradients (40mT/m, 150mT/m/ms).

***Pulse Sequence*[8]:** The voxel selective excitation is achieved by an SLR[9] 90° slice-selective excitation pulse (1.75kHz BW), followed by two pairs of SLR 180° slice-selective pulses (5kHz BW) with quadratic phase to excite the second and third dimensions. The minimum voxel size is 1.1cm × 1.2cm × 1.2cm (1.6cc). A CPMG echo-train of non-selective 180° refocusing pulses (1.74kHz BW) was employed to measure T_2 decay signal of the voxel. A 19.2kHz-bandwidth spectrum of 604 points was acquired on spin echo every 42 ms. Total scan time was 8s.

Data Analysis: The spectral domain was apodized with a 60Hz Gaussian filter and zerofilled twice. T_2 decay curve was derived from magnitude peak height of each metabolite plotted as a function of time. A baseline was determined from the magnitude signal at a frequency away from any metabolite peaks, averaged over all echoes. The baseline was subtracted from the T_2 decay curves prior to mono-exponential fitting. For each of the tumor group and control group, a mean spectrum and ± one standard deviation spectra were derived from the first spin-echo spectra normalized to the individual total carbon signal (sum of four ^{13}C-metabolite peak areas) in order to evaluate relative metabolic production between tumor group and control group.

Results

Figure 1 shows that alanine in liver tumors has longer T_2 (mean±std T_2 =1.2 ± 0.1 s) than that in normal livers (T_2 =0.6 ± 0.1 s). Two-sample one-tail t-Test of the T_2 values yielded $p < 2\times10^{-5}$. All tumors have higher total carbon signal than normal livers (not shown). After normalized to total carbon signal, the mean and standard deviation spectra in Fig.2 show the relative production of alanine in liver tumors is higher than that in normal livers ($p < 3\times10^{-4}$). No significant difference in the relative production of other metabolites between tumors and normal livers. The ratio of alanine in tumors to alanine in normal livers is 2.2 ± 0.6 at the first spin-echo time. After correcting for the respective T_2 decays between the 90° excitation and the first spin-echo (78ms), the alanine signal in tumors is 2.1 ± 0.5 times of the alanine signal in normal livers. Intra-subject variation of alanine signal normalized to total carbon is 15% from the three repeated runs in one control rat and 17% from the repeated runs of a second control rat. The inter-subject variation of alanine is 24%.

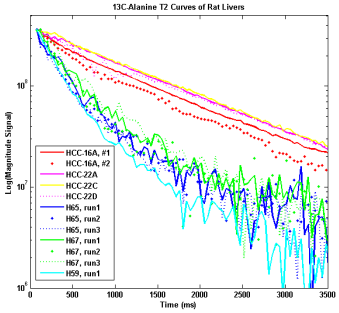

Figure 1: Logarithmic plot of T_2 decay curves of ^{13}C-alanine in liver tumors and normal livers.

Discussion and Conclusion

We report a technique to measure localized T_2 relaxation time of ^{13}C-labeled metabolites *in vivo*, and the comparisons between HCC rat model and normal rat livers. The inter- and intra-subject reproducibilities are good. HCC lesions in rats have a longer T_2 and a higher alanine production than normal rat livers. The long T_2 in HCC lesions may be due to leaky vessels in the fast growing tumors. The reported[10] high Alanine Transaminase (ALT) level in the Morris HCC rat model may be related to the high alanine production observed in this work. To make this technique more robust, further sequence development is needed to design an echo train less sensitive to B1 inhomogeneity, such as the application of composite pulses. This work demonstrates that ^{13}C-alanine may be a new marker for HCC tumors. Its diagnostic values in cancer detection and treatment monitoring are yet to be explored.

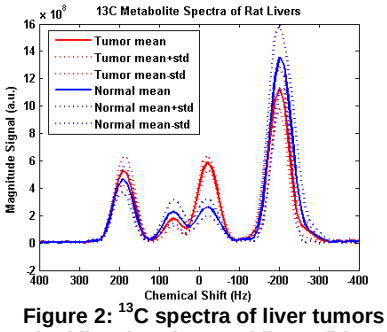

Figure 2: ^{13}C spectra of liver tumors (red lines) and normal livers (blue lines). The solid lines are the means and dotted lines depict the +/- standard deviations.

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