In vivo lactate T_1 and T_2 relaxation measurements in ER-positive breast tumors using SS-SelMQC editing sequence

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Introduction

Multiple quantum (MQ) editing techniques have been developed for lactate (Lac) detection with complete suppression of water and lipid (Lip) resonances in a single scan (1-3). In malignant tumors, due to elevated glycolysis, Lac may likely to be a marker for tumor diagnosis. A recent study (3) also reported that different tumor models exhibit varying average Lac concentrations at different tumor volumes with low Lac levels at smaller tumor volumes. In our previous work (SS-SelMQC) (1), we have reported 2-3 fold increase in the Lac signal to noise than SelMQC using invivo rat prostate tumors. This method has high potential for studying breast tumors implanted on mice at smaller tumor volumes. Absolute quantification of Lac requires the measurement of correction factors due to J-coupling evolution, molecular diffusion, T_1 and T_2 relaxation factors. Though we can analytically calculate the effects of J-couplings and molecular diffusion effects, one needs to measure T_1 and T_2 for absolute quantification. Hence we report a modified T_1 and T_2 variants of SS-SelMQC sequence (1) to measure Lac T_1 and T_2 values with increased signal-to-noise as seen in the original sequence **(Fig.1)**(1). We measured T_1 and T_2 accurately using different phantom conditions as well as *in vivo* ER-positive breast tumors.

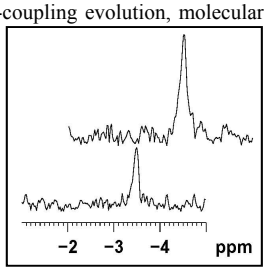

Fig 1. Lactate spectra of SS-Selmqc(top) and SelMQC(bottom); Signal enhancement > 2 times in SS-SelMQC was observed. MCF-7 with tumor volume 230mm^3

Materials and methods

Animals: MCF-7 and BT-474 cells were prepared with Matrigel. 4 to 6 weeks old Balb/c female nude mice were used and two days before the cell inoculation, estrogen pellet was induced in the mice body. 5 * 10^6 cells were inoculated on the mammary fat pad of the mice and tumor growth was started after one week of cell inoculation and tumor growth was monitored every week **(Fig.2).** The tumor volume was calculated by measuring the length (l) breadth (b) and height (h) of the tumor using the formula π*(l*b*h)/6. Animal studies were conducted in compliance with protocols approved by our institutional committee.

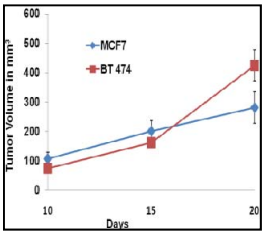

Fig 2: Plot of tumor volume of MCF-7 and BT-474 induced breast tumors in mice.

MR experiments: All experiments were performed on a 4.7 T Bruker Biospin spectrometer (40 cm horizontal bore). Studies were verified using Lac phantoms and demonstrated using a MCF-7 and BT-474 breast cancer tumor, subcutaneously implanted on mammary fat pad region of nude mice. The mice were anesthetized using a mixture of isoflurane and air (20% O_2) and placed in the animal holder. The tumor was placed inside a 2 turn home built 15 mm diameter tuned coil. MR spectroscopy acquisition parameters were same as our previous study (1,4) included a 45° and 90° pulse flip angle three-lobe sinc shaped pulses with 200µs and 400µs pulse duration, a pulse repetition of 3 s and spectral width of 12.5 ppm. Transmitter is set at CH frequency and all other experimental parameters are chosen from ref (1). The ZQ →DQ coherence transfer pathway is selected with the G_{sel} gradients in a ratio of 0:-1:2; Non-localized spectra were obtained with 16 transients for T_1 and 32 transients for T_2 measurements. In the T_1-SS-SelMQC, T_1 measurement of Lac was performed with insertion of inversion 'mao4' shaped pulse with 2000 µs pulse width, similar to (4) and varied the inversion time before applying the SS-SelMQC. In T_2-SS-SelMQC, Lac T_2 relaxation was measured by incorporating CH_3 selective 15ms single lobe 'sinc' pulse, during the MQ-preparation period of SS-SelMQC. This allows inserting a variable delay time TE' to measure Lac T_2 decay. The T_1 measurement was done by subtraction method (5) .

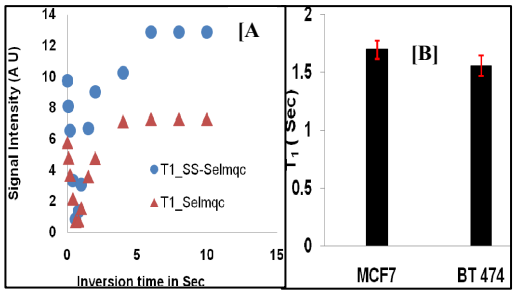

Fig 3:[A] The Lac signal recovery with variable recovery delay (0.1 to 1s) with 0.2 s increments and (1s to 10s) in steps of 2s. The signal intensity in T_1-SS-SelMQC (•) and T_1- SelMQC(Δ). Signal to noise increase observed in T_1-variant of SS-SelMQC [B]: The Lac T_1-values in MCF-7 and BT-474 tumors using SS-SelMQC variant.

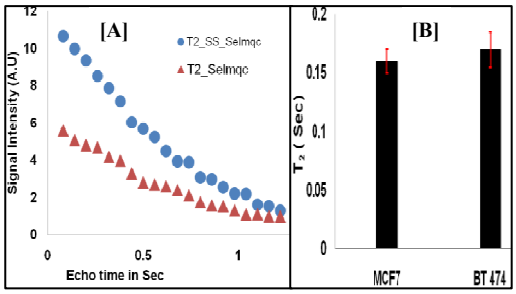

Fig 4:[A] The Lac signal decay with 2*TE' (0.01 to 1s) with 0.02 s increments. The signal intensity in T_2- SS-SelMQC (•) and T_2- SelMQC(Δ). Signal to noise increase observed in T_2-variant of SS-SelMQC [B]: The Lac T_2-values in MCF-7 and BT-474 tumors using SS-SelMQC variant.

Results and Discussion:

The in-vitro T_1 and T_2 of Lac CH3 signal was also measured in phantom using different Lac concentrations (5, 15, 30 mM) and in different chemical environment (in absence of Lip, in presence of Lip and in presence of contrast agents) all the solutions were prepared in physiological saline. The respective T_1 and T_2 of Lac –CH3 signal is presented in the **Table 1**.

In vivo T_1 and T_2 measurements **were done** using MCF-7 and BT-474 breast tumors in mice (N=3 in each group). The experimental recovery of Lac signal using T_1 variants of SS-SelMQC and SelMQC is shown in (**Fig.3A**). The T_1 relaxation times of Lac in MCF-7 and BT-474 breast tumors were found to be 1.7±0.16s and 1.56±0.17s respectively(**Fig.3B**). Similarly the T_2 measurements were done using T_2- variant of SS-SelMQC and compared with T_2- SelMQC **(Fig.4).** The exponential decay of Lac signal using T_2 variants of SS-SelMQC and Sel-MQC (**Fig 4A**). The increased signal enhancement was observed in T_2-SS-SelMQC, which facilitates to measure accurate T_2 of Lac signal in tumors with special advantage for tumors with low Lac levels. The T_2 relaxation times of Lac in MCF-7 and BT-474 tumors were found to be 0.16±0.02s and 0.17±0.03s respectively. Using phantoms of 5mM concentration, we observed marginal difference in T_1 value of Lac measured using both T_1- variants. But in T_2 measurements, increased signal to noise in T_2 of SS-SelMQC has a advantage of measuring the Lac peak areas more accurately than T_2- SelMQC due to shorter total echo time duration.

Table 1. T_1 and T_2 measurements of Lac solutions with Lip and Gd-DTPA doping

Lac Concentration (mM)	T_1 (sec)	T_2 (sec)
5	1.34±0.05	0.60±0.02
15	1.21±0.02	0.51±0.03
30	1.13±0.03	0.50±0.03
15 with lipid	1.2±0.01	0.55±0.02
30 with lipid	1.10±0.08	0.54±0.03
15 with 25 µMol Gd-DPTA	0.66±0.01	0.35±0.03
30 with 25 µMol Gd-DPTA	0.74±0.02	0.36±0.01
30 with 50 µMol Gd-DPTA	0.67±0.01	0.31±0.01

Conclusion: Using T_1- and T_2-variants of SS-SelMQC sequence, measured T_1 and T_2 values facilitates the absolute quantification of Lac concentrations *in vivo*.

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References: (1) Thakur SB, et al., Magn Reson Med 2009; 62: 591-598. (2) He Q, et al., J. Magn. Reson. B 1995; 106: 203-211. (3) Yaligar J, et al., PISMRM 2008: 369. (4) Muruganandham M, et al., Magn Reson Med 2004; 52: 902-906. (5) Kim S, et al., Magn Reson Med 1994; 31: 445-449.