Simultaneous 13C MR spectroscopy measurements of hyperpolarized [1-13C] pyruvate in two cancer bearing mice

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INTRODUCTION: Hyperpolarized ^{13}C magnetic resonance spectroscopy and imaging is a rapidly expanding field with many applications, in particular in the field of cancer. Polarization of a number of enzyme substrates enabled the studies of key steps in glycolysis and TCA cycling [1]. [1-^{13}C]-pyruvate has been extensively used in this context. Due to a variety of reasons preclinical cancer studies are commonly carried out in nude mice. Using mice puts limits to the total amount of solution that can be administered intravenously, typically to about 0.2ml. The amount of compounds polarized by commonly used polarizers however is significantly larger. To make more efficient use of the amount of hyperpolarized substrates we therefore propose a scheme involving parallel acquisition of spectroscopy data from multiple animals. A similar scheme has been introduced for high throughput phenotyping with anatomical MRI [2]. In this study we have demonstrated proof-of-principle of this concept by monitoring pyruvate metabolism simultaneously in two animals using a single MR transceiver coil in combination with slice selective excitation.

MATERIAL and METHODS: *Animals*: 4 nude female mice at 7 weeks of age have been used. The mice were anesthetized using isoflurane (2 - 2.5%) in an oxygen - air mixture (1/4) administered via a face mask. The body temperature was maintained at 36.5±0.5°C and the respiration was monitored. All animal experiments were performed in strict adherence to the Swiss Law for Animal Protection. Tumor: The animals were injected 6 days before the experiments with 100ul containing 10^6 colon cancer cells (C51) in the right flank. At the day of the experiment the tumor size was approximately $(4mm)^3$. Sample production: Samples of [1-^{13}C] pyruvic acid with 15 mM trityl radical and 1.5mM Gd(DOTA) were hyperpolarized at 1.4K using a homemade DNP hyperpolarizer [3]. The sample was rapidly dissolved with Tris/NaOH/EDTA buffer solution to a concentration of 90mM, and a bolus of 0.2ml was injected intravenously to each animal simultaneously. The pH of the injected solution was 7. *MRS experiment:* All in vivo MRS measurements were performed on a Bruker BioSpec 94/30 (Bruker BioSpin MRI, Ettlingen, Germany) horizontal bore MR system using a combination of volume resonant coil, for proton imaging and a custom build 2-turn 16mm diameter surface coil for ^{13}C measurements. A FLASH sequence was used for anatomical reference images, which were used to accurately position the slices for the spectroscopy experiments in order to avoid signal contributions from organs characterized by high blood volumes. The MRS experiments were carried out using a slice selective spectroscopy sequence with the following parameters: 2 slices (one per animal), each slice was 7.2mm thick with sufficient interslice gap to avoid interference between the slices, echo/repetition time TE/TR = 0.5712ms/3000ms, NA = 1, Gaussian shape excitation pulse with 0.1ms duration and 15 degree angle, band width = 2740 Hz, number of sampling points = 4096. The spectra acquisition scan lasted 10 minutes. *Analysis of MRS data:* All spectroscopy data were processed using AMARES fitting of jMRUI (Version 4, http://www.mrui.uab.es/mrui/). *Calculation:* Peak assignments were based on published data [4].

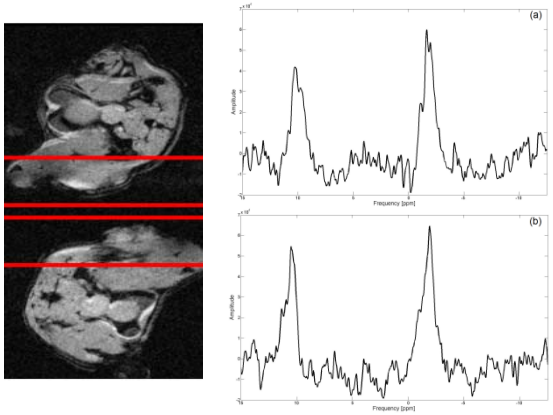

Figure 1: Reference images with slice positions indicated and spectra of pyruvate and lactate recorded 20sec after injection for slice 2 (a) and slice 1 (b).

RESULTS: As expected significant conversion of hyperpolarized [1-^{13}C] pyruvate to lactate could be observed. The spectrum quality is identical for both slices (animals) as revealed by comparable SNR values and linewidths of the metabolite signals (Fig. 1, right panels) and not significantly inferior to conventional MRS using one mouse only. In the case of the two animals the SNR value is reduced by 5% in comparison with the SNR of the conventional one animal only MRS. Fig. 2 reveals differences in pyruvate to lactate conversion in the two animals which reflects tumor heterogeneity. In both cases we observed a time delay from the appearance of the pyruvate signal to the appearance of the lactate signal. The sum of the two signals was found to be similar in the two temporal profiles (Fig. 3), indicating that the total amount of label reaching the tumor site is comparable in the two cases and that the signal is essentially due to the tumor with little contribution from adjacent tissue.

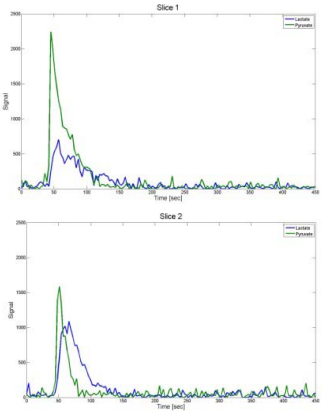

Figure 2: AMARES fitted data for time evolution of the lactate and the pyruvate peak for the two slices for the first 7.5min of the scan.

DISCUSSION: This study showed the feasibility of parallel ^{13}C MR spectroscopy measurements in two mice. The quality of the two spectra is similar both with regard to SNR values and linewidth and only slightly inferior to spectra recorded with a single mouse setup. This difference is attributed to differences in magnetic field homogeneity for the two conditions: while the linewidth in a single animal study was typically 0.6 – 0.8ppm, we measured 1.2ppm for the two-mouse setup. Optimization of the shimming routine will allow addressing this issue. Simultaneous compound administration to two animals does not appear to be a major problem as reflected by the traces of the sum of pyruvate and lactate signals, which is similar in the example shown. Parallel MRS of mice using hyperpolarized substrates is attractive as it enables more efficient use of the polarized compound and enhances throughput.

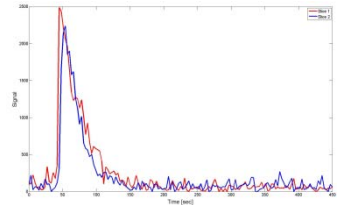

Figure 3: Time evolution of the sum of lactate and pyruvate signals for the two slices for the first 7.5min.

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