Dynamic MRS of hyperpolarized 1-^{13}C pyruvate in brain tumor afflicted mice treated with temozolomide

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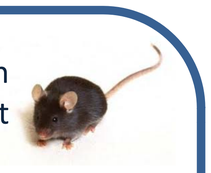
Introduction:

Hyperpolarized [1-^{13}C] pyruvate has been proved to be a promising tool in oncology, where lactate/pyruvate measured ratios have been shown to correlate with disease progression and response to therapy.[1] Most preclinical studies have focused in subcutaneous lymphoma or prostate tumors, but less work has been performed in brain tumors, especially in mice. The aim of this work was To evaluate the detection of response to therapy in a well characterized mouse brain glioma model using hyperpolarized [1-^{13}C] pyruvate.

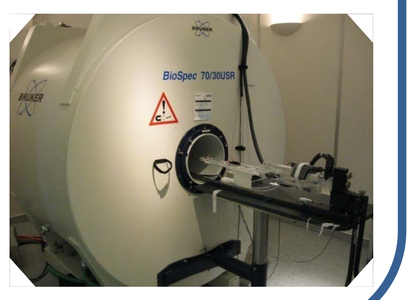
Methods:

Animal Model: fourteen C57BL6 female mice (18-22g) were used in this work. Four mice were used as controls (WT), and the remaining animals harbored a GL261 brain glioblastoma. Four of the glioma-bearing mice were treated with 60mg/Kg of temozolomide at days 11, 12, 13, and 15 post inoculation of GL261 cells. Mice underwent ^{13}C MR on day 18±1 post-inoculation.

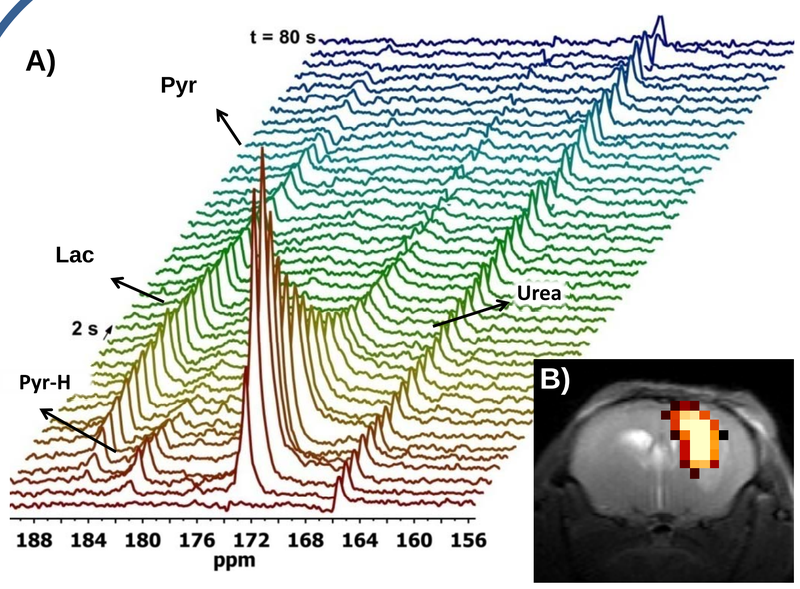


Dynamic Nuclear Polarization (DNP): A sample of [1-^{13}C] pyruvic acid, 15 mM OX63 trityl radical and 1.5 mM Dotarem was hyperpolarized using a HyperSense DNP polarizer for approximately 1 h (≈ 94.1 GHz, 100 mW). The sample was subsequently dissolved in a pressurized and heated alkaline buffer (≈ 4 mL), with a resulting polarization of 18±2% and physiological temperature and pH. Mice were injected through the tail vein 0.01mL/g of the 80 mM hyperpolarized [1-^{13}C] pyruvate solution.

Magnetic resonance (MR): ^{13}C-magnetic resonance spectroscopy studies were performed in a 7 T Bruker BioSpec 70/30 USR with a ^{1}H/^{13}C surface coil placed on top of the mouse head. ^{13}C-pulse-acquisition spectra were acquired over 3 min after the beginning of the injection (TR, 2s; excitation flip angle, 5°; sweep width, 150kHz; acquired points, 2048; frequency centered on the pyruvate resonance). Peak areas of the spectra were measured by using AMARES[2] as implemented in jMRUI[3]. ^{13}C CSI was acquired in two glioma bearing mice at 30s post hyperpolarized pyruvate injection, 1.5h after the first injection.

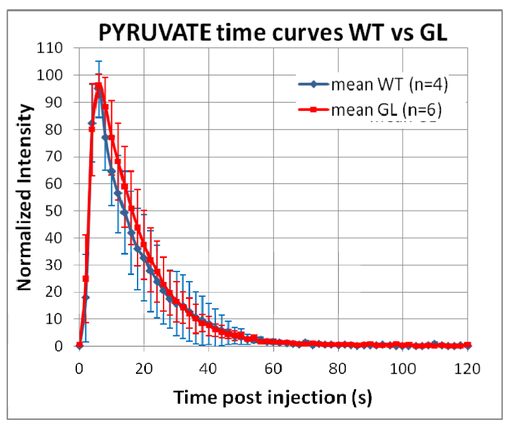


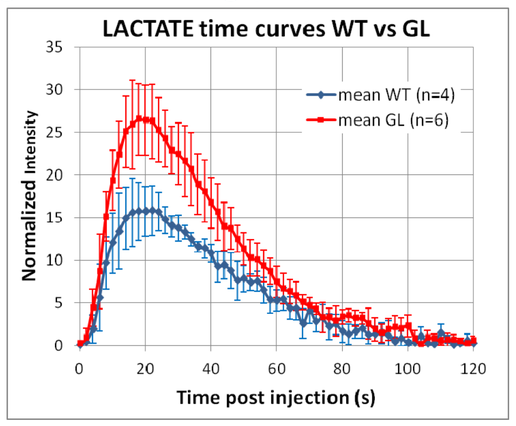
Results:

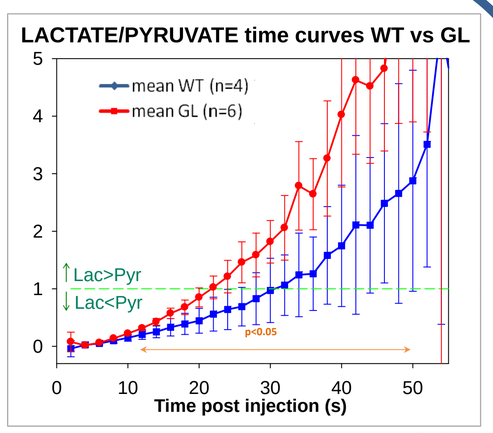


Representative time-course ^{13}C spectra of the glioma-bearing mouse brain following hyperpolarized pyruvate injection (A). Peaks were assigned to pyruvate (Pyr), lactate (Lac), pyruvate hydrate (Pyr-H), and urea from a 8M ^{13}C-urea phantom located over the mouse head which was used for chemical shift reference and calibration purposes. **^{13}C MRSI mapping lactate signal (B)** at 30s post-injection, overlaid on a T_{2w} image, showed increased lactate signal from within the tumor region.

Wild type (WT) versus untreated glioma-bearing (GL) mice:

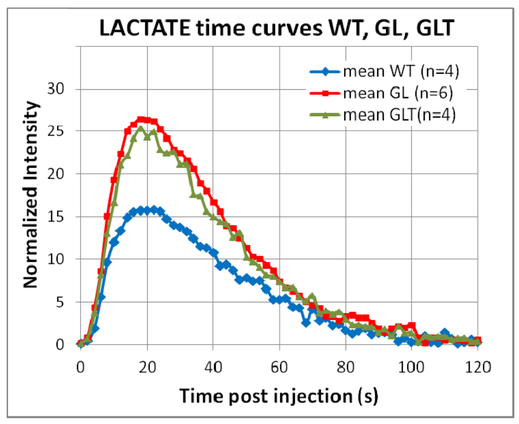




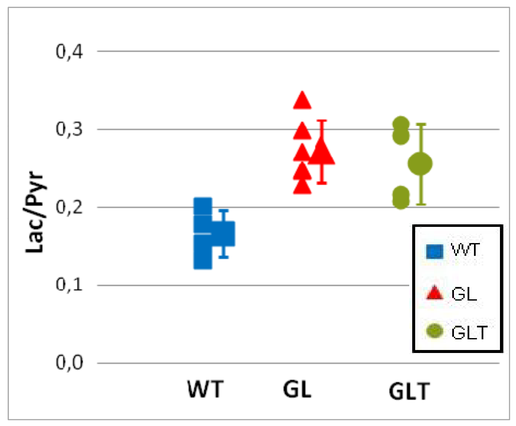


Comparing WT versus GLT, no-significant differences were observed in the pyruvate mean time curves of the fitted spectra, however significant differences ($p<0.05$) were detected in the lactate signals and in the lactate/pyruvate ratios from 12s to 50s post-injection.

Temozolomide treated glioma-bearing (GLT) mice:



Non-significant differences were found between treated and untreated mice in the mean lactate time curves (left) nor in the mean total Lac/Pyr ratios (right). Nevertheless, Total Lac/Pyr ratios of the individual GLT mice (left) seemed to have two separate patterns, one with increased ratios and the other with smaller values, which could indicate different behavior with respect to therapy response variability.



Conclusions:

Our experimental protocol, using ^{13}C MRS with hyperpolarized [1-^{13}C] pyruvate in a mouse model of brain glioblastoma, was able to discriminate wild type from glioma bearing mice but was not able to detect differences between temozolomide treated and untreated mice. Longitudinal studies, where Lac/Pyr ratios can be normalized to individual initial time points, may be necessary to fully corroborate our preliminary results. Additionally, exploring TMZ treated mice later in the therapy protocol (>21 days p.i.) may produce better Lac/Pyr differential profile changes

References: [1] Day, *et al*., *Nat Med*. 2007, 13: 1382. [2] Vanhamme, *et al*., J Magn Reson 1997, 129: 35. [3] Stefan, *et al.,* Meas Sci Technol 2009, 20:104035

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