TOWARDS IN VIVO BRAIN TUMOR PHENOTYPING WITH PROTON CSI PATTERN PERTURBATION

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INTRODUCTION: After describing PRESS-CSI (Chemical Shift Imaging with PRESS localization) in routine high field MR studies with mouse models [1,2] and proposing MRS pattern perturbation as a potential tool for improving brain tumor phenotyping *in vivo* (single-voxel studies using a glioma mouse model) [3], we now advance one step further towards this objective by merging these two approaches. PRESS-CSI has been used to monitor the effects of acute hyperglycemia in a glioma mouse model. This should provide us with a first insight into brain tumor heterogeneity as inspected from a dynamic response perspective to a defined metabolic challenge.

PURPOSE: To monitor the effects of acute hyperglycemia in a mouse model of brain glioma using multi-voxel proton spectroscopy (PRESS-CSI) at 7 Tesla.

METHODS: Tumors were induced in three C57BL6 mice (20-27g) by intracranial sterotactic injection of GL261 cells in the caudate nucleus, as in [3]. MR studies, conducted one month after this procedure, were carried out in a 7 Tesla 16 cm *PharmaScan* (Bruker BioSpin, Ettlingen, Germany) equipped with a B-GA9S gradient coil and a 23 mm birdcage resonator. Anesthesia was kept with 1-2.5% isoflurane in O_2, maintaining the respiratory frequency between 40-60 breaths/min, while animal body temperature was controlled with a heated water blanket. Before entering the magnet animals were cannulated *i.p.* with a 26G catheter for acute hyperglycemia induction while conducting the experiments. This was achieved by a bolus injection of 10 ml/Kg D-Glucose at 25% m/v (1.4 M). Tumors were first localized with T2-W MRI (RARE, TR/TE: 4200/36 ms, 8 echoes). For CSI studies, linear and second order shims were automatically adjusted with FASTMAP. CSI was performed with the PRESS localization method [4], positioning the selection VOI (4.7x4.7x1.0 mm) in a way that included both tumor and healthy tissue; other parameters were 2.0x2.0 cm FOV, 500 scans (acquisition-weighted: Hanning window) and 6 µl nominal resolution. Water suppression was performed with VAPOR [5]. Studies were carried out with 2500 ms TR (21 min total acquisition time), 12 and 136 ms TE, and the signal was sampled in the time domain with 2 k points. For each study, a reference CSI image (T2-W) was initially acquired, as well as 12 and 136 ms TE control CSIs (euglycemia). After inducing hyperglycemia, from six to ten repeated 12ms TE CSI acquisitions were performed. These were interleaved with one 136ms TE CSI, which was acquired at 65 min post-injection of glucose ($PI_{glucose}$). CSI spectra, visualized with *TopSpin* after acquisition, were post-processed with *CSIapo* software [6] with 4.0 Hz line broadening and zero filed to 8 k points. First order phase correction was performed in one spectrum of the grid (central) and then automatically applied to the rest. Metabolite maps were obtained by AMARES fitting of selected resonances [7]. These were further processed (*R* scripts) for generating maps of the percentage increase of glucose (GPI maps), using CSI at euglycemia as reference

RESULTS: The FASTMAP procedure consistently produced a 16-21 Hz line width for the water resonance in the CSI-PRESS selection VOI. GL261 glioma CSI spectral patterns observed (Figure 1-A) reproduced the perturbation profiles previously described by single-voxel studies in response to hyperglycemia [3]. Quantification of the CSI data collected (Figures 1-B) and translation into GPI maps provided a time-course visualization of MR-detectable extracellular glucose accumulation caused by the simultaneous contribution of tumor glucose uptake, consumption and washout in its different regions (Figure 1-C). Our results suggest heterogeneity in tumor response to hyperglycemia, not only in each glioma microenvironment but also among the three tumor cases studied (results not shown here).

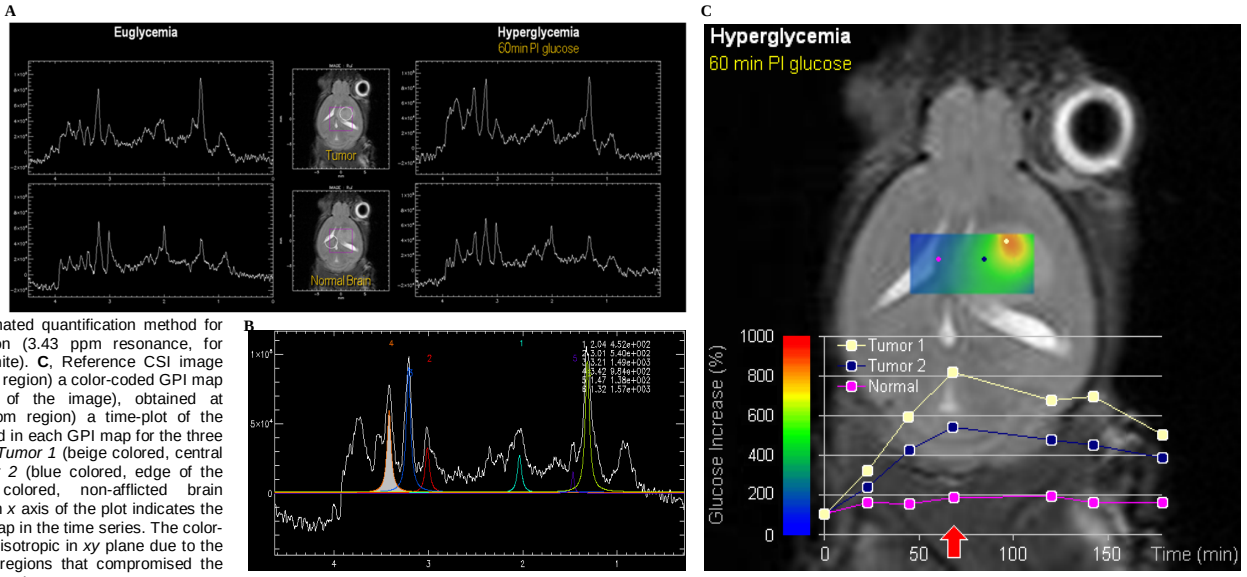

Figure 1 – Example of one CSI study. **A**, CSI spectra collected during euglycemia (left column) and during hyperglycemia (right column), either in GL261 tumor (top) and normal brain parenchyma (bottom); reference CSI images (central column) show the PRESS-CSI selection VOI (in pink) and the position of each spectrum over the FOV (white marker), in both cases. **B**, AMARES automated quantification method for metabolite map generation (3.43 ppm resonance, for glucose, highlighted in white). **C**, Reference CSI image superimposed with (central region) a color-coded GPI map (scale on the left side of the image), obtained at 60min_$PI_{glucose}$, and (bottom region) a time-plot of the glucose variations observed in each GPI map for the three pixels positions indicated: *Tumor 1* (beige colored, central part of the tumor); *Tumor 2* (blue colored, edge of the tumor); *Normal* (pink colored, non-afflicted brain parenchyma). Red arrow in *x* axis of the plot indicates the position of the displayed map in the time series. The color-coded map is displayed anisotropic in *xy* plane due to the cut-off of artifact-affected regions that compromised the AMARES quantification procedure.

CONCLUSIONS: These results show the potential of both MRS pattern perturbation protocols and CSI proton spectroscopy to increase the dynamic range for *in vivo* brain tumor classification, as different tumor types and grades may, in principle, respond differently to a comparable metabolite perturbation. Thus, this methodology should be of interest to study other animal models of human brain tumors (i.e., genetically engineered mouse models). Furthermore, other metabolite perturbations should be tested in order to establish a "metabolite toolbox", optimized for MRS pattern perturbation studies of brain tumors.

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