PERTURBATION OF MOUSE GLIOMA MRS PATTERN IN VIVO AT 7.0 TESLA BY INDUCED ACUTE HYPERGLYCEMIA

R. V. Simoes[1,2], M. L. García-Martín[3], S. Cerdán[3], and C. Arús[1]

[1]Bioquímica i Biologia Molecular, Universitat Autònoma de Barcelona, Cerdanyola del Vallès, Spain, [2]Centro de Neurociências de Coimbra, Universidade de Coimbra, Coimbra, Portugal, [3]LISMAR, Instituto de Investigaciones Biomédicas "Alberto Sols" UAM-CSIC, Madrid, Spain

INTRODUCTION: ^{1}H MRS is evolving into an invaluable tool for tumor classification in human patients based on pattern recognition analysis [1] but there is still room for improvement. Here we propose a new approach in this direction: to challenge tumor metabolism *in vivo* by a defined perturbation and monitor MR spectral pattern changes.

PURPOSE: To monitor tumor ^{1}H MRS pattern changes in mice bearing a stereotactically induced brain glioma during a period of induced acute hyperglycemia.

METHODS: A total of 28 C57BL/6 mice, 25-30g weight, 16 male and 11 female were used in this study. Tumors were induced in 16 mice by intracranial sterotactic injection of GL261 cells in the caudate nucleus essentially as in [2]. After the studies were completed, animals were sacrificed (Pentobarbital i.p. 200 mg/kg, 60mg/ml) and the brains were dissected, fixed in 4% formaldehyde and, one week later, embedded in paraffin for histopathological analysis by classical Hematoxylin-Eosin (H&E) staining. Acute hyperglycemia was induced in animals by *i.p.* bolus injection of 250 µl Dextrose (Sigma, G-5000) at 25% m/v (1.4 M) [3]. Efficiency of the technique was first tested with 2 groups, with 3 animals each (group 1, control; group 2, given an *i.p.* glucose bolus) measuring their blood glucose levels with a glucometer (Glucocard G Meter, Arkray Inc., Kyoto, Japan) at different time points, mimicking the timing of posterior MR experiments. MR studies were conducted at 7 Tesla (*PharmaScan*, Bruker BioSpin, Ettlingen, Germany) with 300mT/m actively shielded gradients and a 23mm birdcage resonator. Anesthesia was carried out with 1-2.5% isoflurane in O_2 and keeping the respiratory frequency between 40-60 breaths/min. Animals were also thermostatized using a heated water blanket. Tumor growth was initially monitored *in vivo* in 6 animals at different days post-injection of GL261 cells (PI_{cells}), by both T2-W MRI and single-voxel proton spectroscopy (^{1}H-MRS) at three echo times (PRESS TE: 12, 30 and 136 ms). Dynamic ^{1}H-MRS (MRS_{dyn}) was conducted in 16 mice divided in 4 groups (A, B, C and D) at both 12 and 136 ms TE with 12 min time resolution, 3x3x3mm (27µl) voxels and 128 scans. Three control experiments (A, B and C, n = 3 animals each) were conducted first. Unlike groups C and D (n=7), mice in groups A and B were not injected with glioma cells, thus the voxel was positioned essentially in the same brain region where tumors were observed in glioma baring mice. Before entering the magnet, group B and D animals were cannulated *i.p.* with a 26G catheter for acute hyperglycemia induction during MRS_{dyn}. Diffusion Weighted Imaging was also performed in one glioma bearing animal in order to obtain additional information about the tumor microenvironment. Additionally, D-Glucose and Glucose-6-Phosphate (G-6-P) phantoms, prepared each at 20 mM in PBS, were examined by ^{1}H-MRS at 12 ms echo time. MRI processing was performed using *Image-J* software (NIH, USA) and diffusion images processed with *IDL* home written software (RSI, France) to generate ADC maps. ^{1}H-MRS spectra were processed using *MestRec* software (Mestrelab R., Spain) and further analyzed in two ways: to depict peak height variations (absolute intensity changes) at certain ppm values and generate time-course curves; to obtain the respective ASCII files and use them to generate (1) mean spectra with standard deviations superimposed and (2) difference spectra as in [4]. ASCII processing was performed with *R* software, v2.3.0, using home written scripts (GNU P., USA). When comparing data from different animals (tumors), the ASCII files were first normalized to unit length, before generating the mean spectrum.

RESULTS: Tumors were observed in all animals implanted with GL261 cells by MRI/MRS (Fig. 1-A) and classified histopathologically as grade IV gliomas (GBM) with no necrosis found (coherent with low mobile lipid signal *in vivo*), well vascularized and poorly cellular (confirms Diffusion data - not shown). In MRS_{dyn} time-course curves $PI_{glucose}$ from 12 ms TE spectra, the 3.43 ppm peak (^{4}CH triplet in *α* D-glucose) had always the highest relative intensity increment (Fig. 1-B) and therefore was selected to monitor brain glucose variations as in [4]. Glucose, once given *i.p.* to mice bearing gliomas (Group D), reached the tumor region through the blood pool and showed a slow entrance and washout: about one hour to reach the maximum concentration with a complete episode of baseline pattern recovery occurring in approximately four hours (Fig. 1-C). During its residence time in the tumor microenvironment, glucose, possibly detected as an extracellular pool (D-Glucose *in vitro* spectra fitted the pattern of the difference spectrum while G-6-P *in vitro* spectra did not) with some lactate production detected at 1.32 ppm, produced significant and specific MRS pattern changes between euglycemia and hyperglycemia states (Fig. 1-D).

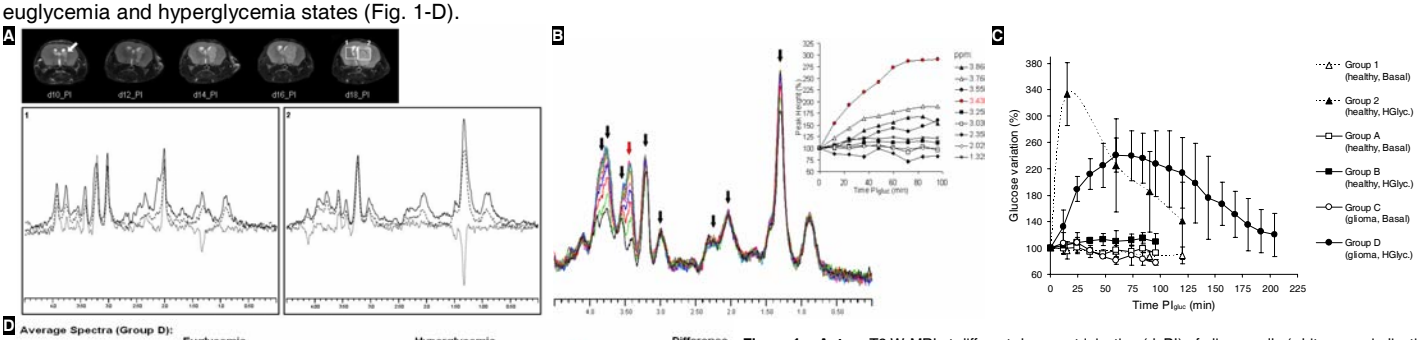

Figure 1 – **A**, **top**, T2-W MRI at different days post-injection (d_PI) of glioma cells (white arrow indicating tumor first detection at d10_PI) and, **bottom**, MRS inspection at d18_PI in the two voxels shown in the respective image (1, contralateral parenchyma; 2, tumor) at 3 TE: 12 (solid), 30 (dashed) and 136 ms (grey). **B**, MRS_{dyn} study of a single group D case at 12 ms TE (MRS spectra in different colors shown from 0, black, to 96 min $PI_{glucose}$, pink) with time-course curves for selected peaks (arrows) in top right position insert. **C**, Summary of MRS-detected acute hyperglycemia effect (black dots) detected by direct blood glucose measurements (triangles) and in brain by MRS_{dyn} (squares and circles) in healthy and glioma bearing mice, as well as control experiments for each case (empty symbols). **D**, Average spectra at TE 12 ms (black) with SD range for each spectral point (grey) for Group D mice (n=7): left, Euglycemia (0 min $PI_{glucose}$); center, Hyperglycemia (60 min $PI_{glucose}$); right, Difference spectra between the two glycemic states with *in vitro* D-Glucose phantom spectrum superimposed (red).

CONCLUSIONS: Altogether, these observations are in agreement with an extracellular accumulation of glucose, which suggests that glucose transport and/or metabolism are working close to their maximum capacity in GL261 tumors, although a certain response to systemic hyperglycemia (lactate increase) is detected. This study opens the way for other metabolites to be tested *in vivo* as potential candidates to differentially perturb tumor MRS patterns, especially those that play important roles in tumor metabolism and progression (*e.g.*, choline, taurine, myo-inositol, etc.), which could be of help in enhancing MRS-based discrimination between tumor types and grades.

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