High Resolution 13C HR-MAS Spectroscopy analysis of different brain regions from rats bearing C6 implanted gliomas

V. Righi[1,2], P. Lopez-Larrubia[2], L. Schenetti[3], V. Tugnoli[4], M-L. Garcia-Martin[2,5], and S. Cerdan[2]

[1]Department of Surgery, NMR Surgical Laboratory,MGH Harvard Medical School, Boston, Massachussetts, United States, [2]Instituto de Investigacion Biomedicas "Alberto Sols", Madrid, Spain, [3]Departement of Chemistry, Università di Modena, Modena, Italy, [4]Departement of Biochemistry, Università di Bologna, Bologna, Italy, [5]RM Ntra. Sra. del Rosario, Madrid, Spain

Introduction– Cerebral tissue heterogeneity plays a fundamental role in physiological and pathological conditions. Previously, ^{13}C Nuclear Magnetic Resonance (NMR) approaches in rodents allowed the *in vivo* investigation of sufficiently large tissue regions (approaching the size of the whole brain) or the *in vitro* analysis of extracts derived from abundant tissue biopsies (> 1g) (1, 2). These limitations precluded a detailed ^{13}C NMR analysis of regional cerebral metabolism in smaller regions. To overcome these limitations, and opening the way to investigate cerebral tissue heterogeneity within the microliter range, High Resolution Magic Angle Spinning (HR-MAS) NMR approaches were suggested. Recently, by using HR-MAS NMR high quality ^{13}C spectra from small tissue biopsies (ca. 10 mg) were obtained. Here, we report for the first time to our knowledge, a ^{13}C HR MAS study of normal and diseased brain regions of rats bearing C6 gliomas implanted. The expression of the genes involved in the glycolytic metabolism was investigated in brain biopsies from the same cerebral regions. The analysis of the expression of glycolytic metabolism genes could provide complementary information to interpret the observed ^{13}C HR-MAS alterations.

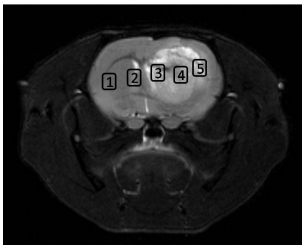

Figure 1. T_1-weighted spin-echo axial MR images of rat brain after the injection Gd(III)DTPA. Regions labeled 1 to 5 indicate the areas where biopsies were taken.

Materials and Methods– C6 gliomas were induced Wistar rats (180-250 g) by stereotaxic injection of C6 cells in the left caudate nucleus. Tumor growth was evaluated *in vivo* using T_1 and T_2 weighted MRI (Bruker Pharmascan 7 Tesla). Three weeks after implantation, rats were anesthetized with isoflurane and infused with [1-^{13}C] glucose (8 µmol/100g, 45 minutes). After the infusion cerebral metabolism was arrested using a high-power focused microwaves (5 kW), the fixed brain removed from the skull and five biopsies taken from different brain regions (Figure 1); 1: contralateral brain, 2: normal brain region limiting to the edema limits of the tumour, 3: peripheral (vascularised) tumour zone, 4: tumour central (necrotic) zone and 5: ipsilateral normal hemisphere. Biopsies were analyzed by ^{1}H and ^{13}C HR-MAS (4 KHz spinning, 4° C, 11.7 Tesla Bruker AVANCE500 WB spectrometer). Before HR-MAS analysis, each sample (15-20 mg) was flushed with D_2O to remove the residual blood and water in order to improve water signal suppression. The sample was introduced in a HR-MAS zirconium rotor (4mm OD) fitted with a 50 µl cylindrical insert to increase sample homogeneity and then transferred into the MAS probe, previously cooled to 4 °C. The quantification of the tumor metabolites detectable in the *ex vivo* spectra was performed using the software program LCModel, a package for the automatic quantification ^{1}H HR-MAS NMR spectra (Linear Combination of Model Spectra, http://s-provencher.com/pages/lcmodel.shtml, (3)). ^{13}C HR-MAS metabolites data were analyzed by Student's test. The expression of the glycolitic pathway genes was assayed using the individual probes for each gene commercialized as TaqMan® (Applied Biosystems, Foster City, CA, USA). Genes data were analyzed by ANOVA. The level of significance was set at $p<0.05$.

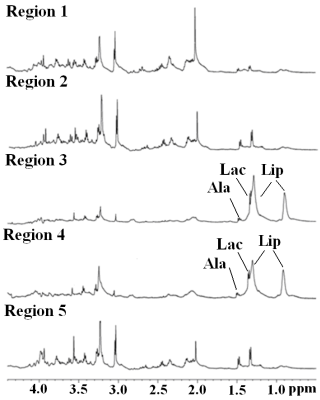

Figure 2. Representative CPMG *ex vivo* ^{1}H HR-MAS (TE:144ms) spectra of from the five cerebral regions selected in Figure 1.

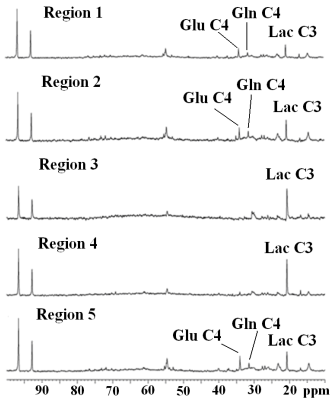

Figure 3. Representative ^{13}C *ex vivo* HR-MAS spectra of the five cerebral regions indicated in Figure 1.

Results– Figure 2 illustrates the different ^{1}H HR-MAS spectra obtained. It is remarkable the increase in lactate and mobile lipids in the tumor biopsies (regions 3 and 4). Figure 3 shows the ^{13}C labeling patterns obtained by ^{13}C HR-MAS of the same tissue regions. In the tumor biopsies, it is possible to detect significant increases of (3-^{13}C) lactate and decreases of (4-^{13}C) glutamate and (4-^{13}C) glutamine, revealing a marked increase in glycolitic metabolism in the tumor. These changes were determined using ^{1}H HR-MAS (LC model analysis) and obtained the (3-^{13}C) lactate concentration. We used this concentration to quantify the remaining ^{13}C HR-MAS observable glutamine and glutamate metabolites (Figure 4).

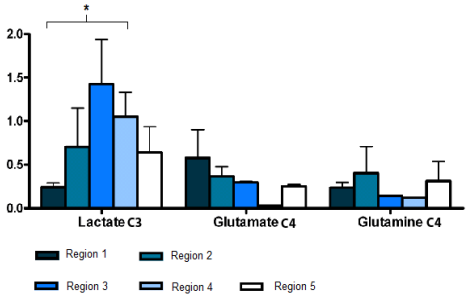

Figure 4. Relative ^{13}C amounts (mM) of Lactate, Glutamine and Glutamate to Taurine in the five regions selected in Figure 1. The ^{13}C satellite resonance of the ^{1}H HRMAS spectrum was quantified using LCModel using Taurine as reference and the ^{13}C resonances expressed relative to the lactate C3. *p< 0.05.

Discussion– *Ex vivo* ^{13}C HR-MAS spectroscopy is an analytical tool that allows for noninvasive detection of selectively enriched metabolites. In our study ^{13}C HR-MAS reveals important metabolic heterogeneity changes in different regions of the brain of rats bearing C6 gliomas, previously not detectable by *in vivo* or *in vitro* ^{13}C NMR. The glycolysis Genes studied in this work improved our understanding of the metabolic profile observed by ^{13}C HR-MAS spectroscopy in different brain regions of rats bearing C6 gliomas.

References.

1. Bouzier AK, Quesson B, Valeins H, Canioni P, Merle M. [1-^{13}C]Glucose Metabolism in the Tumoral and Nontumoral Cerebral Tissue of a Glioma-Bearing Rat. *Journal of Neurochemistry* 1999; **72**: 2445-2455.
2. Bouzier AK, Thiaudiere E, Biran M, Rouland R, Canioni P, Merle M. The Metabolism of [3-^{13}C]Lactate in the Rat Brain Is Specific of a Pyruvate Carboxylase-Deprived Compartment. *Journal of Neurochemistry* 2000; **75**: 480-486.
3. Provencher SW. Estimation of metabolite concentrations from localized in vivo proton NMR spectra. *Magn Reson Med* 1993; **30**: 672-679.