In Vivo Localized Sensitivity Enhanced ^{13}C MRS of [1,6-$^{13}C_2$]-Glucose Metabolism of an Intracerebral Glioma in Mice.

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Introduction The first identified biochemical hallmark of tumours compared to healthy tissue was their shift in glucose metabolism from oxidative phosphorylation to aerobic glycolysis [1]. Unlike their healthy counterparts, tumour cells utilize glycolysis instead of oxidative phosphorylation for glucose metabolism even when in oxygen rich conditions. Metabolic conversions involved in cancer are controlled by complex pathways involving all kinds of enzymes and proteins [2]. Until now most of the knowledge about tumour metabolism was obtained by in vitro studies, histology or extract studies. In this study we aim to realize conditions to examine tumour glucose metabolism *in vivo* by using localized sensitivity enhanced ^{13}C MR spectroscopy [3]. We show that polarization transfer techniques in combination with efficient RF coils enable the detection of glucose metabolism in an intra cerebral tumour bearing mouse.

Materials and methods BALB/c nu/nu mice were injected with a U87 glioma cell suspension (20 µl suspension of 100,000 cells), 2 mm from the midline in the right parieto-occipital region and at a depth of 3 mm from the skin, 21 days before MR measurements. To enable contrast agent and glucose perfusion the mouse tail vein was cannulated prior to the MR experiments. For tumour localization, a ^{1}H 12 mm brain surface coil was used for excitation and detection. Gradient echo images were acquired in three perpendicular directions for anatomical localization of the mouse brain. Immediately after Gd-DTPA perfusion (0.2 mmol / kg), T_1 weighted gradient echo images (TE = 6 ms, TR = 400 ms, FOV 35 x 35 mm, 256 x 256) were acquired for tumour localization (fig. 2a). For ^{13}C MRS a birdcage coil was used for homogeneous ^{1}H excitation and a 12 mm surface coil was used for ^{13}C detection. A semi adiabatic version of Distortion Enhanced Polarization Transfer (DEPT) using BIR4 pulses (0.5 ms segments at ^{13}C) was used for ^{13}C sensitivity enhanced spectroscopy [4]. Localization was performed by ISIS at the ^{1}H frequency (bandwidth 6.5 kHz). This was combined with broadband ^{1}H decoupling using WALTZ16 during ^{13}C detection (60 ms). The ^{13}C bandwidth was about 80 ppm, sufficient for glucose metabolism detection. The voxel positioned in the tumour was only 6 x 4.5 x 4.5 mm^3 (fig. 2b), other scan parameters TR = 1000ms, 256 averages. A phantom containing alanine was positioned above the mouse head to verify decoupling conditions. A first bolus of 20 µl 1.075M [1,6-$^{13}C_2$]-glucose dissolved in water was followed by a constant infusion rate of 2.5 µl / min during the rest of the measurement. Spectra were analyzed using jMRUI 3.0, the results were compared with a control data set of a non-tumour bearing mouse being subject to the same infusion and MR protocol.

Results The use of [1,6-$^{13}C_2$]-glucose in combination with local sensitivity enhanced polarization transfer allowed the detection of glucose and its converted metabolites in a very small volume (122 µl) of an intra cerebral tumour in the mouse. The acquired ^{13}C MR spectrum (fig. 1) shows signals originating from several metabolites involved in brain (tumour) metabolism. Glucose carbon-13 labelled at both 1 and 6 locations ($Glc_{1,6}$), lactate (Lac), fatty acids (Fat), glutamate (Glu), glutamine (Gln), aspartate (Asp) and gamma amino butyric acid (GABA) can be distinguished in the spectra. Compared to control spectra several resonances show differences such as those of glucose and glutamine. Furthermore, faster ^{13}C label incorporation into Lac_3 and Glu_4 is observed (fig. 3).

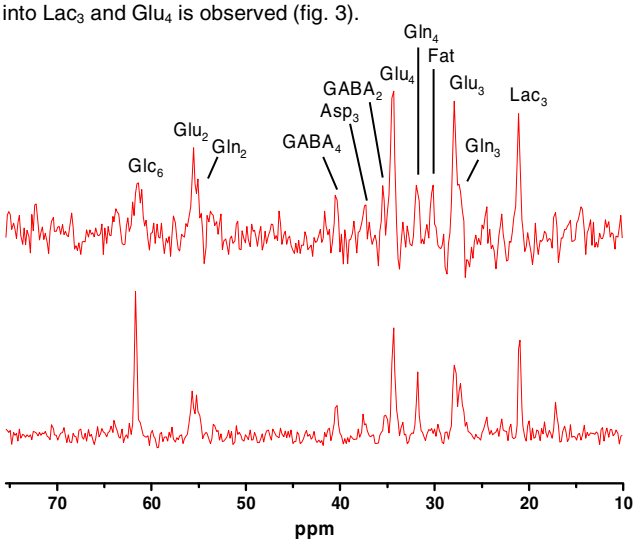

Figure 1: In vivo ^{13}C MR spectra of the U87 glioma (top) and non-tumour bearing control (bottom). Voxel size were 6 x 4.5 x 4.5 mm^3 and 7 x 5 x 5 mm^3 respectively. Spectra were acquired after 2 hours of continuous [1,6-$^{13}C_2$]-glucose infusion, TR=1000ms, 4096 averages.

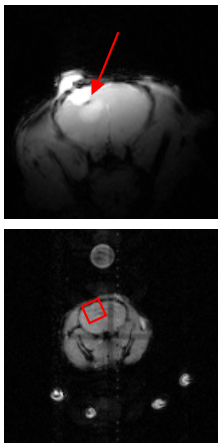

Figure 2: T_1 weighted GE image (top) highlighting the tumour (red arrow), Gradient echo image (bottom) with projection of the ISIS localized voxel (red box) The alanine phantom is positioned immediately above the brain.

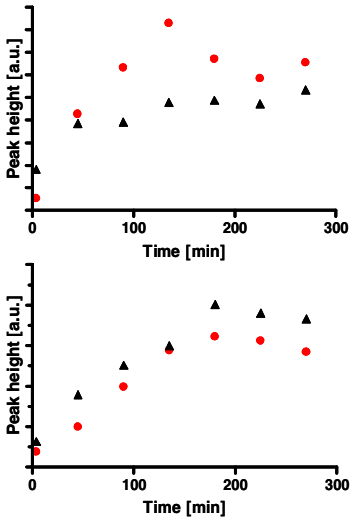

Figure 3: Dynamic ^{13}C label incorporation into Lac_3, (●) and Glu_4 (▲) in the tumour (top) and in control (bottom) presented as peak heights vs time.

Discussion and Conclusion Here we show the first *in vivo* localized sensitivity enhanced ^{13}C MRS of glucose uptake and metabolism of an intra cerebral tumour in the mouse. SNR challenges presented by very small volumes were overcome by the use of DEPT and [1,6-$^{13}C_2$]-glucose, enabling sufficient signal to noise ratio to observe label incorporation in glucose and its metabolized compounds. Dynamic representation of Lac_3 and Glu_4 signals indicated a faster incorporation of ^{13}C label in tumour tissue compared to normal brain tissue, suggesting faster metabolite turnover in the glioma as compared to control. The relatively low Gln_x enrichments suggest that Glu-Gln conversions are reduced in the tumour. The remaining glutamine signals could originate from contributions of Glu-Gln conversions of normal brain tissue included in the ISIS voxel. If performed in larger tumours *in vivo* ^{13}C MRS would enable dynamic determination of metabolites involved in glucose metabolism of intra cerebral tumours in the mouse.

References

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