Diffusion-weighted spectroscopy in the healthy and U87 glioblastoma-induced mouse brain

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Introduction

Diffusion-weighted (DW) spectroscopy is a unique tool for probing the intracellular compartment *in vivo* [1]. Indeed, because cerebral metabolites are almost exclusively present in the intracellular compartment, their diffusion properties are more likely to specifically reflect the intracellular geometry, membrane content and viscosity than the diffusion properties of water, which is present in the extracellular space as well. DW-spectroscopy has already been performed in the healthy and diseased brain, mostly in rats (e.g. [2-5]), but also in macaque monkeys (e.g. [6]) and in humans (e.g. [7,8]). As far as we know, the apparent diffusion coefficient (ADC) of metabolites has never been reported in the mouse brain. In this preliminary work, the ADC of six metabolites is measured in a mouse brain for the first time, using an original DW-LASER sequence. In addition, measurements are performed in a Human U87-MG glioblastoma induced in the same animal, showing a dramatic increase in the ADC of choline compounds, which might be ascribed to lactacidosis-induced cell swelling.

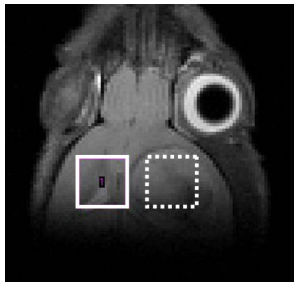

Fig.1: *Voxel position in the healthy tissue (left, plain box) and in the tumor (right, dotted box).*

Methods

Human U87-MG glioblastoma cells were cultured in Dulbecco's Modified Eagle Medium. For implantation, an immuno-depressed "nude" mouse was anesthetized with pentobarbital and positioned in a sterotaxic frame. After exposure of the skull, a hole was drilled through the skull and the medium was injected (2.8 µL, ~10^5 cells) in the right hemisphere. Two weeks after implantation, NMR experiments were performed on a Bruker 7 T system. The mouse was positioned with bite-bar and ear rods and anesthetized with 1.5% isoflurane. A quadrature surface coil was positioned on the top of the head and used for radiofrequency transmission and reception. Measurements were performed in a 2.5×2.5×2.5 mm^3 voxel, in the left hemisphere that contained no tumor tissue and in the right hemisphere where the tumor had developed (Fig. 1). Shimming was performed using Fastmap [9], yielding a 12-Hz linewidth in the control voxel and an 18-Hz linewidth in the tumor. DW-spectroscopy was then performed using a new DW-sequence, close to the LASER (Localization by Adiabatic SElective Refocusing) sequence (Fig. 2), as detailed elsewhere [submitted, this symposium]. Briefly, this sequence allows the measurement of the trace of the diffusion tensor in a single scan, without bias due to cross-terms between diffusion gradients and other gradients, yielding a measurement of the ADC which is independent of the cellular orientation within the scanner. Sequence parameters were TE/TR=48/2500 ms, diffusion gradient duration δ=2 ms, diffusion gradient separation Δ=13.8 ms. Hyperbolic secant (HS1) pulses were used for refocusing (R=20, 1 ms duration). Water was suppressed using a VAPOR scheme, and metabolite signal was measured at b=0 and 1000 s/mm^2 (360 repetitions). A water reference signal was acquired at both b-values to estimate water ADC and to correct for eddy-currents on metabolite spectra. In order to correct for movement artifact, spectra were individually phased before being summed and analyzed using LCModel [10]. The ADC was finally evaluated by ADC=-1/b×log(S/S_0) for the most reliably quantified metabolites (Cramér-Rao lower bounds <10% at both b-values), i.e. total N-acetyl-aspartate (tNAA), total creatine (tCr), total choline (tCho), glutamate (Glu), myo-inositol (Ins) and taurine (Tau).

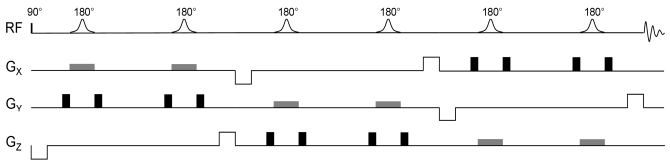

Fig.2: *Volume selective DW-LASER sequence used for the measurement of the ADC in a single scan.*

Results and discussion

DW-spectroscopy in the mouse brain: Spectra measured in the healthy tissue for b=0 and b=1000 s/mm^2 are shown in Fig. 3A. ADC for metabolites ranged from 0.12 µm^2/ms for Glu to 0.35 µm^2/ms for Tau, with tNAA (0.15 µm^2/ms), Ins (0.18 µm^2/ms), tCr (0.24 µm^2/ms) and tCho (0.24 µm^2/ms) ranging in-between. Water ADC was 0.83 µm^2/ms. Values are summarized in Table 1. Generally speaking, measured ADC are in good agreement with literature values for other species, although in the upper range, which might be ascribed to the short diffusion time used in the present study. The high ADC for Tau relative to other metabolites is consistent with other works [4]. The low Glu ADC may possibly result from a bias due to macromolecules in the baseline signal (in this preliminary work MM were estimated by LCModel).

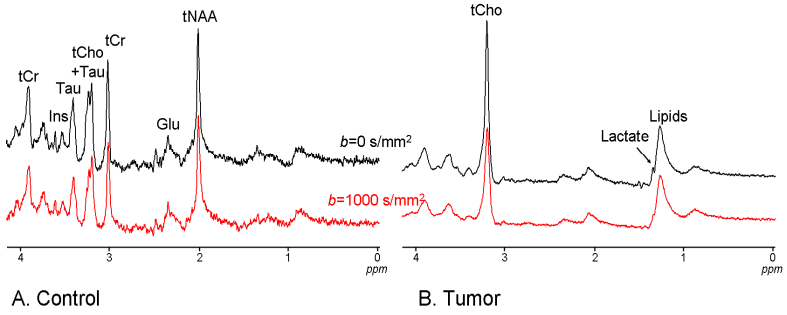

Fig.3: *Spectra acquired with the DW-LASER sequence at b=0 and b=1000 s/mm^2 in (A) healthy tissue and (B) tumor in the mouse brain.*

Diffusion in U87 glioblastoma: Tumor spectra are shown in Fig. 3B. The spectral signature of tumor tissues can be clearly recognized, with a dramatic increase of the tCho peak relative to other metabolites, and the appearance of lipid signal around 1.3 ppm, which can presumably be ascribed to lipid droplets [11]. In addition, a strong lactate signal was visible at 1.33 ppm, although hardly quantifiable because on the very edge of the lipid peak. The signal of other metabolites was too low for reliable quantification. The ADC calculated for tCho (0.51 µm^2/ms) was more than doubled compared to healthy tissues. It is tempting to ascribe this dramatic increase to lactacidosis-induced cell swelling [12]. A previous study reported an increase in ADC for tCho in human brain tumors [8], while another one reported no change in the rat [5]. These discrepancies may arise from different sequence parameters (e.g. diffusion time) or different tumor status. Note that water ADC in the tumor was slightly lower than in healthy tissue (0.80 µm^2/ms), which might be due the fact that water is also present in the extracellular space (in contrast with tCho), or simply to the lower cerebrospinal fluid content in the tumor (a significant fraction of the control voxel was ventricle).

	Glu	tNAA	Ins	tCr	**tCho**	Tau	Water
Control	0.12	0.15	0.18	0.24	**0.24**	0.35	0.83
Tumor	×	×	×	×	**0.51**	×	0.80

Table 1: *ADC of metabolites (in µm^2/ms) for healthy and U87 glioblastoma tissues measured in one mouse brain.*

Conclusion

This preliminary work reports the first measurement of metabolite ADC in the mouse brain, using an original DW-LASER sequence weighting the signal by the trace of the diffusion tensor. In addition, the ADC of choline compounds was measured in a Human U87-MG glioblastoma, exhibiting a dramatic two-fold increase compared to control. Considering the strong lactate peak in the tumor voxel, this increase might be ascribed to lactacidosis-induced cell swelling. This study argues in favor of DW-spectroscopy being a very sensitive tool to monitor tumor status. Additional experiments are now underway to confirm these results.

[1] Nicolay *et al.*, NMR Biomed 2001; [2] Merboldt *et al.*, MRM 1993; [3] Pfeuffer *et al.*, JCBFM 2000; [4] Dreher *et al.*, MRM 2001; [5] Hakumaki *et al.*, Cancer Res 1998; [6] Valette *et al.*, JCBFM 2007; [7] Posse *et al.*, Radiology 1993; [8] Harada *et al.*, NMR Biomed 2002; [9] Gruetter, MRM 1993; [10] Provencher, MRM 1993; [11] Barba *et al.*, Cancer Res 1999; [12] Staub *et al.*, JCBFM 1990.