The major fraction of intracellular metabolites in the primate brain is localized in long fibers rather than in cell bodies, as shown by diffusion-weighted spectroscopy at long and ultra-long diffusion times

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Introduction

In vivo ^{1}H diffusion-weighted NMR spectroscopy (DW-MRS) allows probing the intracellular environment [1]. Due to membrane impermeability to brain metabolites and their pure intracellular compartmentation, metabolite diffusion is expected to be much more restricted than water diffusion. An overview of past studies, carried out for relatively long t_d (from 13 ms [2] to 224 ms [3]) in the rat, monkey and human brains, reveals no strong dependence of the apparent diffusion coefficient ADC on t_d, which is surprising in a context of restricted diffusion, although comparisons between studies are always subject to caution due to inter-species and methodological differences. In contrast, *in vivo* DW-MRS performed at ultra-short t_d (from 1 ms to 13 ms) in rodents showed a strong decrease of brain metabolites ADC as t_d increases [Marchadour *et al.*, this symposium]. In an effort to reconcile these two different behaviors over different time-scales, we decided to explore metabolite diffusion at long and ultra-long t_d. Metabolite ADC in the monkey brain was measured for t_d varying from ~100 ms to ~1 s. No dramatic dependence of the ADC on t_d could be observed over this large time-window, confirming the stability of the ADC suggested by the literature. In an attempt to explain this stability and relate it to plausible cell geometries, two geometrical models were tested to fit the experimental data: a "neurite" model, describing diffusion in long fibers such as axons and dendrites, and a "cell body" model, accounting for diffusion into spherical cell bodies.

Materials and Methods

Experiments: Experiments were performed on a Varian/Agilent primate 7 T system (gradient coil reaching 100 mT/m along each axis in 325 µs). A ^{1}H quadrature surface coil was used for RF transmission and reception. A 5.8 mL voxel was positioned as shown in Fig. 1. Shimming was performed with Fastmap (~12 Hz *in vivo*). Spectra were acquired using a modified STEAM sequence (TR/TE=2350/50 ms), for b=0 and 5000 s/mm², at different t_d reached by varying mixing time TM. TE=50 ms was chosen to minimize the macromolecule signal while retaining singlet resonances. Since at long t_d cross-terms between diffusion gradients and other gradients may become significant and lead to biased ADC [4], additional DW-spectra were acquired with diffusion gradients of opposite polarity for each t_d [5]. First, water ADC was measured in an agarose gel phantom (c_{agar}=3%). Then, two *in vivo* experiments were carried out on two healthy macaque monkeys. Primary anesthesia was induced by an i.m. ketamine-xylazine injection (0.7 mL). Anesthesia was maintained by an i.v. propofol infusion. Animals were monitored using a MR-compatible Maglife system. Physiological parameters remained stable within normal range during the experiments. The animals were held in the sphinx position and their head was positioned in a stereotaxic frame. Spectra were acquired for water and metabolites (128 repetitions for each b, gradient polarity and t_d) for t_d=97, 272, 522, 772, 1022 ms.

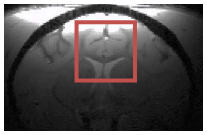

Fig.1: *Position of the 5.8 mL voxel in the monkey brain.*

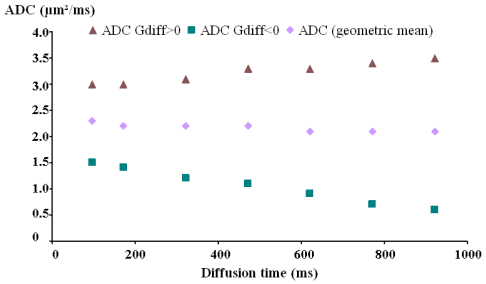

Fig.2: *In vitro water ADC as a function of t_d for diffusion gradients with positive and negative polarities. It is critical to derive ADC from signal's geometric mean to get stable ADC.*

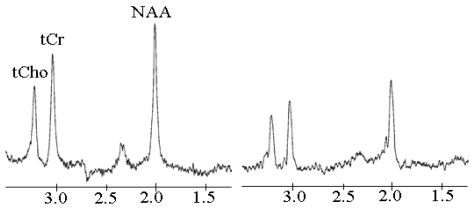

Fig.3: *Examples of in vivo spectra acquired at b=0 and 5000 s/mm² for t_d=552 ms.*

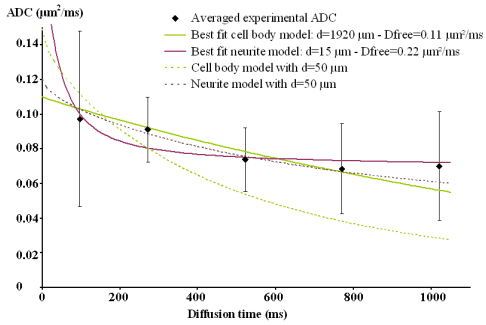

Fig.4: *Modeling of metabolite ADC averaged for NAA, tCho and tCr. The cell body model cannot account for the fairly stable ADC with reasonable values for cell diameter.*

Processing: Scan-to-scan phasing and eddy current correction using water reference were performed. Spectra were analyzed with LCModel [6] with a different basis-set for each TM. At b=5000 s/mm², the geometric mean of the signal measured with both diffusion gradient polarities was calculated. The ADC was finally quantified for water and three brain metabolites: NAA, total creatine (tCr), total Cho (tCho).

Modeling: The "neurite" and "cell body" models were implemented on Matlab, using Stepisnik's formalism to calculate the motion spectrum [7], and computing the ADC as the integral of the motion spectrum multiplied by the square of the gradient modulation spectrum for each t_d. In the neurite model, diffusion occurs in hollow cylinders of diameter d isotropically oriented in the 3 dimensions. With this model, the ADC decreases from D_{free} to $D_{free}/3$ at long t_d (regardless of d) where D_{free} is the intracellular diffusion coefficient. In the cell body model, diffusion occurs within cells modeled by spheres of diameter d, the ADC decreases from D_{free} to 0 at long t_d. D_{free} accounts for potential intracellular tortuosity over short time scales. Fit was performed on the ADC averaged for the three metabolites.

Results and Discussion

Cross-terms suppression: Cross-terms induce an increase of the ADC quantified *in vitro* on spectra acquired with G_{diff}>0 and a decrease of the ADC quantified on spectra acquired with G_{diff}<0. When averaging the water signal measured with both polarities, ADC becomes reasonably stable around the expected water ADC at 20°C (2.03±0.05 µm²/ms), independently of t_d, as shown in Fig. 2. This demonstrates that the effect of cross-terms is cancelled out, which appears to be critical when exploring diffusion for ultra-long t_d. Note however that cross-terms with spatially varying microscopic gradients would not be compensated for by the geometric mean method.

Metabolites and water diffusion in vivo: High quality spectra could be obtained for all t_d, as exemplified on Fig. 3 for t_d=522 ms. *In vivo* water and brain metabolites ADC exhibited no strong dependency on t_d (ADC_{water}=0.51±0.01 µm²/ms, ADC_{NAA}=0.10±0.02 µm²/ms, ADC_{tCr}=0.08±0.01 µm²/ms, ADC_{tCho}=0.06±0.02 µm²/ms). The ADC averaged over the three metabolites is displayed in Fig. 4 as function of t_d. Note that metabolite ADC measured in the past in the rat, monkey and human brain were slightly higher than our values, which may be due to the scan-to-scan phase correction and to the high b-value used here, both resulting in a lower ADC [8].

Diffusion modeling: For the neurite model, best fit yields D_{free}=0.22 µm²/ms and d=15 µm. Note that, since no strong curvature is observed on data, the model is not accurate on the determination of fiber diameter d, so that larger (e.g. 50 µm, see Fig. 4) and of course smaller d are still consistent with the data. Accurate determination of d would actually require going at ultra-short t_d (~1 ms) to observe ADC's curvature [Marchadour *et al.*, this symposium]. However, the important point is that the neurite model explains very well the stability observed up to t_d=1 s. In contrast, the cell body model yields D_{free}=0.11 µm²/ms and d=1920 µm, which is a totally unrealistic cell diameter. This is due to the fact that the model has to increase cell diameter to account for ADC stability. When imposing a more realistic cell size, e.g. d=50 µm, it appears that the cell body model is not able to account for experimental ADC (Fig. 4). We must conclude that, in contrast with the neurite model, the cell body model is not consistent with current experimental ADC.

Conclusion

In the present work, brain metabolite ADC was measured in the monkey brain at long and ultra-long diffusion times. No strong dependence of the ADC on t_d was observed, consistently with a vast fraction of brain metabolites diffusing in long fibers. This suggests that the volume fraction occupied by neurites and other fibers such as astrocytic processes is very large compared to cell bodies in the brain. This study opens new perspectives for interpreting diffusion measurements, for metabolites but also for water. For example, this work strongly supports explanations for water ADC drop after ischemic stroke based on neurite beading [9].

[1] Nicolay *NMR in Biomed* 2001; [2] Dreher *MRM* 2001; [3] Posse *Radiology* 1993; [4] Tanner *J. Chem. Phys.* 1970; [5] Neeman *MRM* 1991; [6] Provencher *MRM* 1993; [7] Stepišnik *Physica* 1993; [8] Valette NMR *in Biomed* 2005; [9] Budde *PNAS* 2010.