Towards the assessment of intracellular viscosity: diffusion spectroscopy at ultra short diffusion time in the rat brain

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Introduction

Whereas water molecules diffuse everywhere and can exchange between the extra- and intracellular compartments, cerebral metabolites are specifically located in the intracellular compartment. Diffusion-weighted (DW) spectroscopy is therefore a unique tool to probe the intracellular space of the brain *in vivo*, as compared to DW-MRI [1]. Given the diffusion time T_d traditionally used (from 15 ms to 250 ms for published works), the apparent diffusion coefficient (ADC) of metabolites is expected to exhibit little dependence on the free (i.e. unrestricted) diffusion coefficient, but to mostly reflect restriction effects [2]. While diffusion measurements governed by restriction allow assessing variations in cell size, organelle content or intracellular membrane permeability, accessing the free diffusion coefficient in the intracellular space, and thus the intracellular viscosity, may open a new window in the study of brain biophysics under normal and pathological conditions. Reaching a short T_d requires strong gradients and a long echo time TE. While signal loss associated with long TE may not be critical for DW-MRI, where signal is abundant, it is often deleterious for spectroscopy. This might explain why diffusion measurements for ultra short T_d (~1 ms) have only been performed for water [3]. In the present work, we introduce a new DW-spectroscopy sequence combining oscillating gradients with a LASER localization scheme. It is demonstrated in the rat brain that this sequence allows measuring brain metabolite ADC at ultra short T_d. It is also shown that, at ultra short T_d, metabolite ADC is extremely sensitive to variations in T_d, suggesting that we observe the transition from a restricted diffusion regime to a free diffusion regime.

Theory

Oscillating gradients offer a convenient way to perform short T_d diffusion measurements while mitigating gradient strength requirement, as compared to pulsed gradients. In the present work, double sine-modulated diffusion gradients as described in [3] were used. Each period (duration τ) of the double sine-modulated gradient yields an attenuation factor $b=\gamma^2(G\tau/\pi)^2(3\tau/8)$, γ being the gyromagnetic ratio and G the peak amplitude of the gradient. The effective diffusion time is therefore $T_d=3\tau/8$.

Method

NMR setup: Experiments were performed on a Varian 7 T scanner equipped with a rodent gradient coil reaching 600 mT/m along each axis. RF transmission was performed using a birdcage coil, while reception was achieved with a 4-channel array surface coil.

DW-spectroscopy sequence: Double-sine gradients were inserted in a LASER sequence [4] on the 3 axes simultaneously, as shown in Fig. 1. In addition to the traditional advantages of LASER localization (namely insensitivity to B_1 inhomogeneity and large pulse bandwidth allowing reduced localization error), we took advantage of the increased T_2 associated with the train of 180° pulses in LASER to increase TE while retaining signal, in order to repeat the double-sine a large number of times. TE=147 ms was chosen, the total duration of diffusion gradients during TE being 120 ms. Three different periods were used: τ=10 ms, 5 ms and 3.33 ms, corresponding to T_d=3.75 ms, 1.88 ms and 1.25 ms.

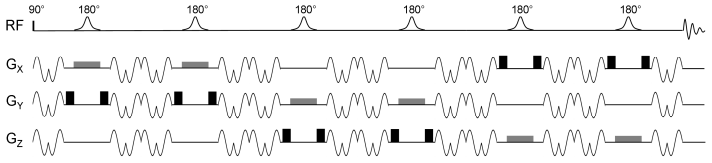

Fig. 1: *DW-LASER sequence with double-sine gradient waveforms to perform metabolite ADC measurements at ultra short diffusion time.*

Experiments: All experiments consisted in acquiring a reference spectrum at b=0, followed by spectra acquisitions at b=1500 s/mm^2 for each of the three different T_d. First, water ADC was measured in a water phantom at 20°C. Then, *in vivo* experiment was performed in a large 450 µL voxel in the rat brain. Water ADC was measured. Metabolite spectra were also acquired (256 averages, TR=2 s). No individual scan phasing was required, as shown by additional experiments on water, which did not exhibit phase variation between scans. Spectra were quantified using LCModel [5]. ADC was quantified for NAA, total creatine (tCr) and total choline (tCho).

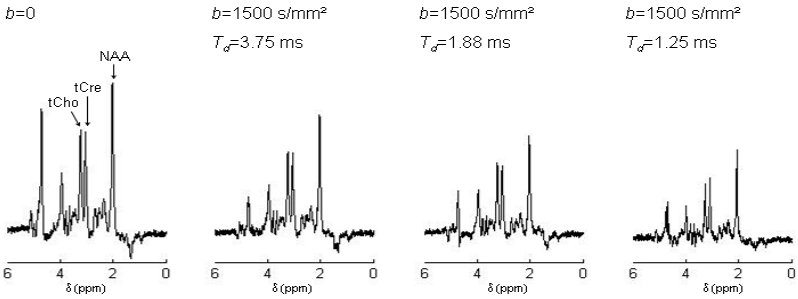

Fig. 2: *Spectra acquired in the rat brain at b=0 and b=1500 s/mm^2, for three different diffusion times. Signal attenuation becomes stronger as T_d decreases, demonstrating decreased restriction effects.*

Results and Discussion

Water ADC in phantom showed great stability despite variations in T_d (2.07±0.02 µm^2/ms over the three T_d), which is expected for free diffusion. In contrast, water ADC *in vivo* increased from 0.95 to 1.33 µm^2/ms when T_d varied from 3.75 to 1.25 ms. High quality metabolite spectra could be obtained despite the long TE, as shown in Fig. 2. An increased signal loss could be readily observed on spectra as T_d decreased, demonstrating decreased restriction effects. Metabolite ADC at T_d=3.75 ms were close to (though slightly higher than) values reported in the literature for longer T_d: ADC_{NAA}=0.18 µm^2/ms, ADC_{tCr}=0.17 µm^2/ms and ADC_{tCho}=0.15 µm^2/ms. Increased ADC was consistently observed for the three metabolites at T_d=1.88 ms. Finally, at T_d=1.25 ms, a dramatic 3- to 4-fold increase was observed (ADC_{NAA}=0.53 µm^2/ms, ADC_{tCr}=0.57 µm^2/ms and ADC_{tCho}=0.65 µm^2/ms), suggesting a rapid transition from a restricted regime to a free diffusion regime. All results are displayed in Fig. 3.

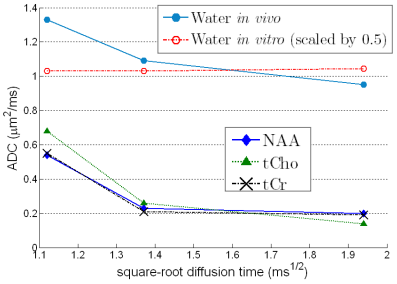

Fig.3: *Water and metabolite ADC as a function of T_d.*

The fact that the relative ADC increase is much stronger for metabolites than for water is consistent with the fact that restriction effects are presumably stronger for metabolites (due the higher membrane permeability for water compared to metabolites). It is also consistent with the fact that, due to their larger hydrodynamic radius, the distance traveled by diffusing metabolites is smaller than for water during a given T_d, so that metabolites will approach a free diffusion regime quicker than water as T_d is decreased. Regarding absolute values, metabolite ADC has already been measured in the past along the direction of white matter fibers [6] or nerves [7], yielding ADC between 0.3-0.45 µm^2/ms. However, long T_d (>20 ms) were used in these studies, so that restriction effects inside the intracellular space (such as caused by cytoskeleton or organelles) could still be significant. Our ADC values at T_d=1.25 ms are higher than these previously reported values, and actually tend to approach values measured for metabolites in free water at 37°C (D_{free}=0.78 µm^2/ms was measured for NAA in [6]). This suggests that intracellular viscosity is not much higher than that of free water.

Conclusion

Using an original LASER sequence incorporating oscillating diffusion gradients, we were able to measure metabolite ADC in the rat brain at ultra short diffusion time. The dramatic increase of ADC as T_d is decreased reveals that restriction effects are significantly reduced for T_d approaching 1 ms. This work opens new perspectives for probing intracellular viscosity using DW-spectroscopy.

[1] Nicolay *NMR Biomed* 2001; [2] Valette *JCBFM* 2007; [3] Does *MRM* 2003; [4] Garwood *JMR* 2001; [5] Provencher *MRM* 1993 ; [6] Kroenke *MRM* 2004 ; [7] Ellegood *JMR* 2007.