Analytical Model of Water Diffusion in Rat Isocortex Slices

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INTRODUCTION

Diffusion MRI provides a clinically important surrogate marker of acute brain injury. The biophysical basis for water diffusion in nervous tissue remains uncertain, complicated and difficult to investigate without detailed measurements using high-field magnets equipped with powerful imaging gradients. These requirements limit investigations in animal or human subjects. Alternatively, rat brain slices model the MRI properties of nervous tissue well [1] and can be imaged for 10-12 hrs without artifacts from motion or perfusion. This approach has been used successfully in studies of perfused hippocampal brain slices (REF) but are limited in turn by the complex tissue heterogeneity in that tissue section. Subregions give significantly different results and can be analyzed separately, but are then greatly limited with respect to SNR. Alternatively a larger more homogeneous brain slice section may have greater utility, such as cortical sections. In this study, we completed T_1, T_2 and water diffusion measurements in rat cortical slices. This data was analyzed using a two-compartment model incorporating exchange between compartments, intracellular restriction and extracellular tortuosity [2]. This analysis provided an index of cell size, extracellular apparent diffusion coefficient (ADC), intracellular fraction and the rate of exchange between intra- and extracellular water (intracellular residence time).

METHODS

Vibratome-cut slices of rat isocortex (500-µm thick) were procured from male P30 Long-Evans rats, then imaged using a multislice perfusion chamber [3] inside a 600 MHz spectrometer with a 10-mm birdcage coil. The imaging protocol, consisting of diffusion measurements at 3 diffusion times (T_d) along with T_1 and T_2 measurements. MR images were of limited in-plane resolution (128 x 64 matrix, 1.5 cm FOV) to improve signal-to-noise while reducing the time required per measurement. Water diffusion in isocortex slices was measured with a pulsed-gradient spin-echo multislice sequence with 12 diffusion-weighted images using diffusion gradients aligned with the read gradient (0-940 mT/m) and T_d's of 10, 20 and 35 ms. These experiments had a 1.5s repetition time while echo time was minimized (23.5, 33.5 and 48.5 ms respectively). T_1 and T_2 values were measured in slices with saturation recovery (TR = 150 ms – 10 s) and fast spin-echo sequences (TE = 10 ms, 30 echos) respectively. To assess diffusion anisotropy in the isocortex, diffusion tensor data of perfusion-fixed rat isocortex were also collected at similar resolution. A two-pool diffusion model with exchange [2] assumes restricted diffusion in the intracellular space (that is dependent on diffusion time) and extracellular water diffusion mediated by tortuosity. The model allows for water exchange between tissue compartments. The model estimates the apparent diffusion coefficient in the extracellular space, the average cell dimension, the mean intracellular residence time and the intracellular volume fraction

RESULTS

Water diffusion, T_1 and T_2 data were collected from 18 rat cortical slices. Figure 1 illustrates typical data for saturation recovery experiments (A), CPMG experiments (B) and diffusion weighted signal attenuation curves at 3 diffusion times (C). Using this data, the two-compartment model determined the intracellular free diffusion coefficient (1.6 ± 0.1 µm^2/ms), extracellular ADC (0.5 ± 0.1 µm^2/ms), restriction diameter (1.9 ± 0.2 µm), intracellular residence time (46 ± 4. ms) and the intracellular fraction (0.47 ± 0.04) [mean ± standard deviation] with chi^2 values 1.5. The T_1 and T_2 values for rat cortical slices at 600 MHz were 2.02 ± 0.05 s and 98 ± 6 ms respectively. Diffusion tensor data indicates that rat cortex can be considered essentially isotropic at the resolution used for the present experiments (data not shown).

DISCUSSION

This study uses a relatively homogeneous piece of cortical tissue to improve SNR, enabling better data collection and more sophisticated modeling techniques to be developed and tested.

The average residence time is much lower than that previously reported [4], but comparable to that reported by others for the glial and axonal cells of approximately similar cellular dimensions [5]. This indicates that the avergage residence time inside the cell is mainly mediated by the cellular size and that the permeability of cell membranes is weakly dependent on cellular size. The low estimate of intracellular volume fraction Vin, can be associated with lower density of water in the intracellular space. Surprisingly, the ADC of extracellular water is lower that that of red blood cell ghosts with similar cellular concentration [6]. This phenomena can be possibly explained by either reduced mobility (free diffusion coefficient) of water of the etxracellular water, or more likely that the "model" extracellular space represents larger cells in which the effects of restrictions at this limited diffusion times are not evident.

REFERENCES

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