Experimentally Measured Intracellular Water at Very Short Diffusion Times

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Introduction

The apparent diffusion coefficient (ADC) as measured by diffusion-weighted MRI (DWMRI) has proved useful as a quantitative measure of water movement in tissue. It has also been shown to be sensitive to cellular properties and tissue integrity. However, despite its clinical utility, a complete understanding of the biophysical tissue properties affecting the ADC is not in hand. Many models have been developed to interpret DWMRI results and calculated ADC based on relevant physiological parameters like cell size, membrane permeability, intrinsic T2 relaxation times, and intrinsic intracellular diffusion. However, these models rely on assumed values of a number of parameters. In this work, we present, for the first time, experimentally measured diffusion coefficients of intracellular water at very short diffusion times. Using oscillating gradient diffusion methodology, combined with novel hollow fiber bioreactor (HFBR) cell cultures, we are able to measure the diffusion coefficient of intracellular water at diffusion times as short as 1.5 ms.

Methods

As described previously [1], C6 cells were grown at 37 °C in a HFBR perfused cell culture system and studied via MRI and MRS at 9.4T on a Bruker DRX400 spectrometer with a 27 mm dual tuned ¹H/³¹P birdcage RF coil. With the introduction of 5 mM Gd-DTPA to the perfusing media, the water signal was separated into three spectral peaks corresponding to the intraluminal/extracellular water (+181 Hz), water residing in the porous fiber wall (+125 Hz), and intracellular water (+30 Hz) [1]. Stejskal-Tanner pulsed gradient spin-echo (PGSE) and oscillating gradient spin-echo (OGSE) diffusion preparation [2] were incorporated into a volume localized ( 8 x 8 x 8 mm³) spectroscopy sequence with TE = 56 ms. DWMRS experiments were carried out with diffusion weighting factors b = 50, 100, 150, 200, 250, 300, and 350 mm²/s at diffusion times Δ = 1.5, 2.0, 3.0, 6.0, 12.0 (OGSE) and 15, 20, 25, 30, 35 ms (PGSE). Spectra were individually phased and fit to three Lorentzian line shapes, corresponding to the three identified spectral peaks. Intracellular ADC (iADC) values were calculated by fitting the intracellular water peak to an exponential decay $S = S_0 e^{-\frac{D_{iADC}}{2}}$.

Results and Discussion

A representative localized water spectrum from the HFBR cell culture is shown in Fig. 1. The three peaks are assigned as previously described [1] and indicated in the figure. The spectral separation allows individual investigation of intracellular water. Calculated iADC values are shown in Fig. 2 as a function of diffusion time. There is an increase in the iADC with decreasing diffusion times due to reduced restrictions of the intracellular water. Experiments carried out in a tetradecane phantom showed no such diffusion time dependence as expected from unrestricted diffusion (data not shown). These data allow, for the first time, the unrestricted diffusion coefficient of intracellular water to be estimated to be in the range of 2.0 to 2.4 μm²/ms. This value is significantly higher than the value of 1.0 μm²/ms, often assumed in diffusion models.

Conclusion

By using novel HFBR cell culture systems and oscillating gradient experiments, the diffusion of water in the intracellular space of mammalian cells has been measured at very short diffusion times for the first time. This has allowed accurate estimation of the intrinsic, i.e. unrestricted, diffusion coefficient of the intracellular space. Experimental determination of this parameter will now allow a more accurate assessment of DWMRI by the various models currently being used.

References


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