Determining the Biophysical Mechanisms of Intracellular Water Diffusion and its Response to Ischemia in Perfused Cell Cultures

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Introduction

It is known that the apparent diffusion coefficient (ADC) of water measured by DWMRI decreases 30-50% within minutes of the onset of clinical ischemic stroke. Although this finding was initially reported nearly two decades ago, there is still active debate over the biophysical mechanisms responsible. Many parameters have been proposed to play a key role including the intrinsic diffusion coefficient of the intracellular space, D_{int}, cell volume fraction and membrane permeability. In this work, we have employed oscillating gradient spin-echo (OGSE) [1] and pulsed gradient spin-echo (PGSE) diffusion spectroscopy experiments in cell cultures to monitor the diffusion time dependence of the ADC of intracellular water (iADC) before and after ischemia. The OGSE experiments allowed measurement of iADC values down to sub-millisecond diffusion times. Through model fitting, the iADC measured at multiple diffusion times can be parameterized into D_{int} and restriction length (i.e. cell size). The results obtained help answer the long standing questions regarding the changes in ADC that are measured after clinical stroke.

Methods

All MR experiments were carried out on a 9.4T Bruker AVANCE spectrometer. C6 rat glimoa cells were inoculated into a HFBR cell culture system [2], and the temperature was maintained at 37ºC by flowing heated water through the MRI gradient coils surrounding the HFBR. The infusion of 5mM Gd-DTPA into this cell culture system is known to split the spectrum of water into 3 peaks, corresponding to the extracellular water + water within the lumen of the fiber, water present within the fiber walls, and intracellular water [2]. OGSE and PGSE diffusion-weighted PRESS (TE=56ms, TR=2500ms) experiments were used to measure water diffusion within an 8x8x8 mm^3 voxel at the center of the reactor. The spectrum is fit to the sum of three Lorenzian peaks, and the signal decay of the intracellular peak as a function of b-value is fit to an exponential decay to quantify the iADC. By using a combination of OGSE and PGSE experiments, diffusion is measured at Δ_{eff} = 0.83, 1.25, 2.5, 5 (OGSE), 10, 15, 20, 30, and 40 (PGSE) ms. iADC vs. Δ_{eff} curves were fit to a model of restricted diffusion [3] to provide estimates of D_{int} and the restriction length.

Results and Conclusions

The iADC vs. time after the onset of ischemia is plotted in **Fig. 1** at three diffusion times, Δ_{eff} = 0.83, 5 and 30 ms. Changes in the iADC values take place within the first 50 min post-ischemia, after which, they appear stable. iADC vs Δ_{eff} curves pre- and 50 min post-ischemia are shown in **Fig. 2**. **Figs. 1 and 2** both indicate that the iADC measured at longer Δ_{eff} increases after ischemia, which is consistent with previous measurements [2]. However, at short Δ_{eff}, the iADC decreases after ischemia. Fits of the iADC vs. Δ_{eff} data to the Balinov et al. model of restricted diffusion [3] are also included in **Fig. 2**. The parameters extracted from these fits, D_{int} and restriction length, are plotted vs. time post-ischemia in **Fig. 3**. The model fitting indicates that the increased iADC at long diffusion times is sensitive to an increase in the cell size, which is consistent with cell swelling – a known cellular response to ischemia. Further, the fits indicate that the decrease in the iADC at shorter Δ_{eff} is caused by a decrease in the D_{int}, and the decrease in D_{int} is highly correlated with a decrease in ATP within the cells measured via ^{31}P spectroscopy [2]. The decrease in D_{int} could be caused by either an increase in intracellular viscosity due to protein dissociation, or a decrease in the energy dependent movement of water within the cell, so called cytoplasmic streaming. Within the context of clinical ischemia, these results indicate that the biophysical mechanisms responsible for the drop in ADC post-ischemia are diffusion time dependent. At short diffusion times, the ADC should be sensitive to the intrinsic diffusivity of intracellular water, while at longer diffusion times the ADC would be more sensitive to the cell size and intracellular volume fraction.

References

1) Does et al. MRM 49:206-215 (2003)
2) Trouard et al. MRM, 60, 258-264 (2008)
3) Balinov et al. JMRA 104: 17-25 (1993)

Acknowledgements

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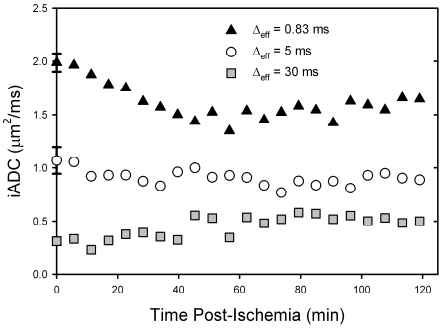

Fig. 1: Intracellular ADC (iADC) vs. Time Post-Ischemia at Δ_{eff} = 0.83, 5, and 30 ms.

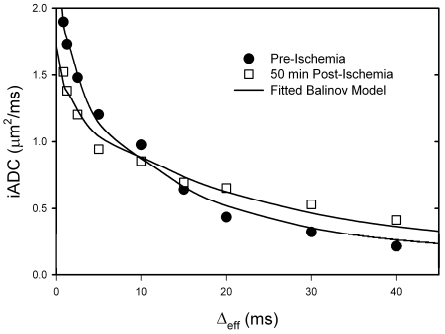

Fig 2: iADc vs. Δ_{eff} at pre- and post-ischemic conditions. Curves have been fit to the Balinov et al. model [3].

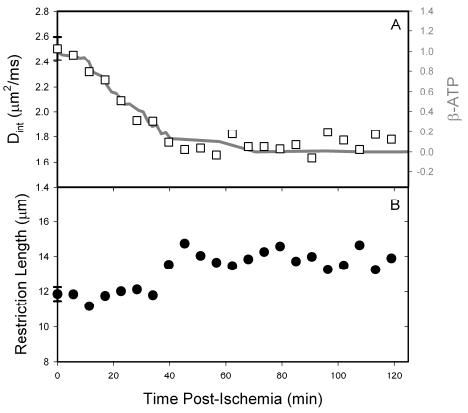

Fig 3: D_{int} and restriction length from Balinov et al. model [3] fitting vs. time post-ischemia. The gray line represents the signal of ß-ATP from ^{31}P spectroscopy.