Measurement of Ischemia-Induced Changes of Intracellular Water Diffusion in Rat Glioma Cell Cultures

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Introduction

Diffusion-weighted MRI is commonly used in the diagnosis and evaluation of ischemic stroke. Because of rapid decreases in the apparent diffusion coefficient (ADC) of tissue water following ischemia, regions of tissue affected by the stroke show up as hyper-intensity in a diffusion-weighted image. Although this observation has been clinically useful over the last several years, the biophysical mechanisms underlying the reduction of tissue ADC are still unknown. To help elucidate these mechanisms, we have employed a novel hollow-fiber perfused cell culture system (hollow fiber bioreactor, HFBR) that allows for cells to be grown to high density and studied via MRI and MRS. A particularly useful feature of HFBRs is that, with the addition of Gadolinium (Gd) based contrast agent to the perfusate, water in the intracellular and extracellular spaces are chemically separated, allowing the intracellular space to be studied individually [1]. In the present work, we investigate the effects of ischemia on the behavior of intracellular water in rat glioma cells.

Methods

The HFBR consists of a polycarbonate casing containing approximately 450 microporous hollow fibers with a pore size of 0.2 microns. Cells are grown in the extrafiber spaces and are supplied with nutrients by continuous flowing of oxygenated media through the lumen of the fibers at a flow rate of 150 ml/min. Rat glioma (C6) cells were obtained from ATCC and routinely cultured in Dulbecco's modified Eagles medium (DMEM) supplemented with 10% fetal bovine serum (FBS). Approximately 4×10^8 C6 cells were infused into the extrafiber space at the beginning of the experiment through an inoculation port in the side of the HFBR casing. Cell growth within the HFBR was monitored by ^{31}P MRS (levels of ATP) and diffusion-weighted ^{1}H MRI (increasing signal at high b-value) at 9.4T. After approximately 250 hrs of growth, the entire extrafiber space was filled with cells as the culture reached confluence. Gd-DTPA (5mM) was added to the media which effectively splits the water resonance of the HFBR into three separate signals corresponding to intracellular water (unshifted), intralumenal/extracellular water (shifted +185 Hz) and water within the fiber wall (shifted +135 Hz) [1]. To study the effects of ischemia, the pump supplying media to the reactor was shut off, and repetitive ^{31}P and ^{1}H MRS acquisitions were initiated. Culture temperature was monitored via a fiber optic temperature probe and maintained throughout the entire experiment by running warm water through the gradients coils surrounding the HFBR.

Results

Pre-ischemia ^{31}P spectra indicated stable culture at 250 hrs after inoculation and ^{1}H spectra indicated a cellular volume fraction of approximately 0.8 in the extra-fiber space. Following the onset of ischemia, ^{31}P signals corresponding to ATP dropped to zero within approximately 60 minutes (see Fig. 1 for spectra and Fig. 2A for plot vs. time). ^{1}H MR spectra before and after the onset of ischemia showed direct evidence of cell swelling. This can be seen in Fig.1 as an increase in the intracellular water peak with a simultaneous decrease in the extracellular water peak. At approximately 2 hours after the onset of ischemia, cells became permeable to Gd-DTPA which caused a decrease in the intracellular water signal and a coalescence of the intracellular and extracellular water resonances (Fig.1 and Fig. 2A). The ADC of the intracellular water resonance measured at two diffusion times (Δ = 10 and 25 ms) before and after ischemia are plotted in Fig. 2B. After one hour of ischemia there was a noticeable increase in the ADC at both diffusion times. The ADC was stable for the next two hours after which another increase was observed. These results are consistent with a simple dilution of the intracellular space followed by increase in membrane permeability. The increase in ADC after ischemia argues against the presence of energy dependent cytosolic streaming of water within these cells.

Fig. 1

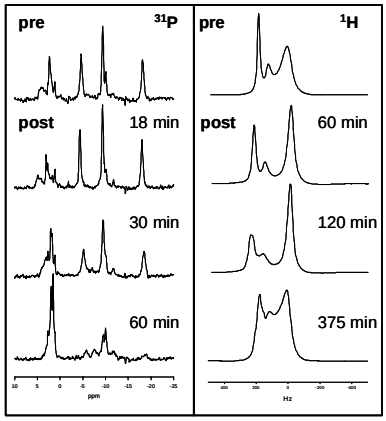
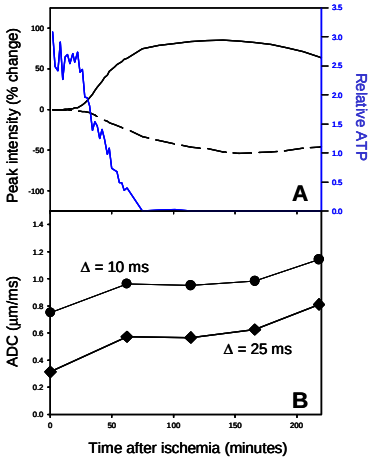


Fig. 2



Conclusion

The HFBR provides a unique system for which to study diffusion properties of water within cultured cells. Because the resonance from intra- and extracellular water can be spectroscopically resolved, the diffusion properties of the intracellular space can be studied directly. The ADC of intracellular water, before and after ischemia, has been directly measured for the first time and was found to increase after the onset of ischemia and maintain its dependence on diffusion time.

References

1. Galons et al. submitted to MRM (2004)

Acknowledgment

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Fig. 1. ^{31}P and ^{1}H MR spectra from the HFBR C6 cell culture pre and post ischemia. Time after ischemia is indicated for each spectrum in the figure.
Fig. 2. (A) Change in the signal intensity of intracellular water (solid line) and extracellular/intralumenal water (dashed line) as a function of time after ischemia. (B) ADC calculated at low b-value (0-1000 s/mm^2) and two diffusion times assuming single exponential signal decay.