Uncovering Intracellular Water in Cultured Cells

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Introduction

The complexity of biological tissues, with multiple compartments each with its own diffusion and relaxation properties require complex formalisms to model the water signal emanating from MRI or MRS experiments [1-2]. In this communication we describe a magnetic susceptibility-induced shift in the resonance frequency of extracellular water, by the introduction of a gadolinium contrast agent to media perfusing a geometrically-defined hollow fiber bioreactor containing rat gliomas cells (C6). The frequency shift of the extracellular water (+185Hz at 9.4 Tesla), uncovered the intracellular water and allowed direct measurement of motional and relaxation properties of the intracellular space.

Materials and Methods

Hollow-Fiber Bioreactor (HFBR). The HFBR system was constructed by Microgon (Laguna Hills, CA, USA). It consists of a 27mm O.D. polycarbonate casing containing approximately 450, 0.32 mm I.D. cellulose acetate/cellulose nitrate copolymer microporous hollow fibers with a pore size of 0.2 microns [3]. In this system, cells are grown to tissue density in the interfiber spaces and are supplied with nutrients by continuous flowing of oxygenated media through the lumen of the fibers at a stabilized flow rate of 150ml/min. *Cell Culture.* C6 cells were obtained from ATC and routinely cultured in Dulbecco's modified Eagles medium (DMEM) supplemented with 10% fetal bovine serum (FBS). An inoculum of ~4 x 10^8 cells was infused into the extrafiber space at the beginning of the experiment through an inoculation port.

Results and Discussion.

Figure 1 shows a series of fully relaxed proton spectra collected from a confluent HFBR after introduction of 5 mM Gd-DTPA to the perfusate. Within 35 sec, Gd-DTPA induced a splitting of the water resonance which resulted in three spectrally stable and resolved components within 300 s. There was one upfield peak at +185 Hz (relative to the starting chemical shift), a smaller intermediate resonance at +130 Hz, and one relatively broadened and minimally shifted resonance (~20 Hz). Figure 2 shows the ^{1}H spectra obtained after 30 minutes in the Gd-DTPA-containing media at 5 different stages of growth. During cell growth, a decrease in the amplitude of the +185 Hz resonance and a concomitant increase in the area of the unshifted peak were observed. After stabilization of the signals in the presence of Gd-DTPA, the diffusion and relaxation properties of these resonances were investigated by MRS.

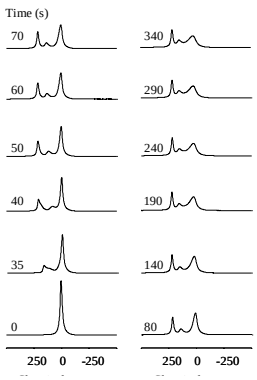

Figure 1: Kinetics of a 5mM Gd-DTPA infusion by ^{1}H MRS

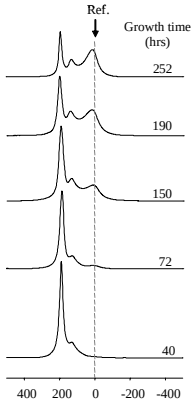

Figure 2: ^{1}H spectrum in the presence of 5mM Gd-DTPA at various stages of growth

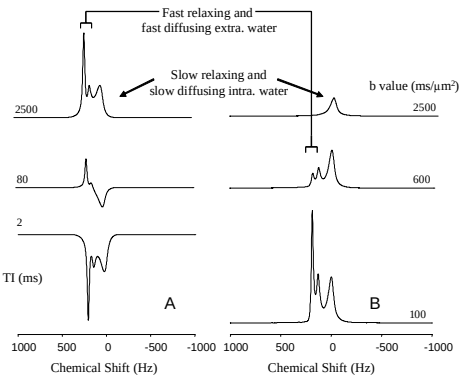

Figure 3: Representative spectra from (A) inversion recovery and (B) diffusion-weighted MRS in presence of 5mM Gd-DTPA

Figure 3A shows a series of spectra from an inversion recovery experiment obtained at 3 different inversion recovery times (TI = 2, 80 and 2500ms) in presence of 5mM Gd-DTPA The T_1 values for the shifted resonances were similar (62 ± and 68 ± ms for the +185 and +130 Hz resonances, respectively) whereas the T_1 of the unshifted resonance was longer, at 251 ± 5 ms. Figure 3B shows a series of spectra obtained at 3 different b values (100, 600, 2500 ms/μm^2) at a diffusion time Δ = 30 ms. The signal decay as a function of b values for the unshifted peak was bi-exponential revealing two components with diffusion coefficients of 0.1 and 0.8 μm^2/ms. The most shifted upfield peak also showed a non-exponential behavior, comprised of a flow component which was identified by turning off perfusion during acquisition and a rapidly diffusing pool with an ADC ~ 2.9 μm^2/ms. The +130 Hz peak exhibited a mono-exponential decay with a diffusion coefficient of 2.1 μm^2/ms. From the above observations, we propose the following assignments for the three observed resonances: The +185 Hz resonance originates from water within the lumen of the fibers (flowing media) and from water located in the extrafiber/extracellular space. This is consistent with the fast appearance of this peak after the addition of contrast, the decrease in the relative magnitude of this peak with cell growth, the observed decrease in T_1 after addition of Gd-DTPA and the presence of a non-flowing but fast diffusing component. The intermediate resonance (+130 Hz) arises from the water within the fiber walls. This component is seen in the absence of cells and its relative magnitude remains constant during the course of the experiment. Finally, the unshifted resonance arises from water in the intracellular space. Its magnitude increases with cell growth, it has a long relaxation time and slow diffusion coefficients. The shifts are consistent with changes in the bulk magnetic susceptibility caused by the presence of Gd-DTPA in the cylindrical geometries of the HFBR [4-5]. The HFBR can be considered filled with an effective susceptibility given by the volume fraction of cells to media (with Gd-DTPA). Further studies will characterize the mechanisms underlining the observed frequency shifts and behavior of intracellular water.

Conclusions: We believe that HBFR technology, with its ability to maintain high density cell cultures and its unique cylindrical geometry have the potential to become an important tool to study intracellular water diffusion /relaxation in the context of ischemia and/or chemotherapeutic response. These avenues of research are currently pursued in our laboratory.

References: [1] Chu SC *Magn Reson Med* ;13(2):239 (1990), [2] Philp DJ et al. *Magn Reson Med* 51(3):441. (2004), [3] Gillies, RJ. et al. *NMR Biomed*. 6:95 (1993), [4] Stanisz, G., et al., *Magn Reson Med* 27:103 (1997),[5] Szafer A, et al,. *Magn Reson Med* 33:697(1995)

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