Q-space analysis of intracellular water diffusion in cultured cells

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Introduction

The values of apparent diffusion coefficients (ADCs) in biological tissues and their changes to patho-physiological conditions have been the object of numerous theoretical and experimental studies [1-5]. A still much debated question is to how to interpret the measured ADCs in terms of physiological parameters such as compartment fractions, exchange/permeability and inherent compartment diffusivity. In a previous communication we described a methodology for measuring the intracellular water signal in cultured rat brain cells (C6) by taking advantage of a Gd-DTPA induced magnetic susceptibility shifts of the resonance frequency of extracellular water [6]. In this report we present a q-space analysis of the intracellular water diffusion in C6 cells using the signal originating exclusively from 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 cellulose acetate/cellulose nitrate copolymer microporous hollow fibers (0.32 mm ID) with a pore size of 0.2 microns. *Cell Culture.* Rat glioma cells (C6) 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. *Diffusion-weighted MRS*, DWMRS experiments were carried out using 34 linearly spaced gradient strengths ranging from 0 to 850 mT/m and 16 diffusion times (20-160 ms) using a standard diffusion-weighted stimulated-echo pulse sequence. The maximum q-value was 0.164 υm^{-1}. Other parameters included δ = 7ms, TE = 20ms, and TR = 2.5 sec.

Results and Discussion

Figure 1 shows a fully relaxed proton spectra collected from a confluent HFBR after introduction of 5 mM Gd-DTPA to the perfusate. As previously shown [6], the two upfield peaks originates from the extracellular water including the intra and extra fiber space (at +185 Hz) and from the water located within the fiber walls at (+130 Hz) [6]. The intracellular water signal is relatively broadened and minimally shifted (+ 20 Hz) from the initial water resonance.

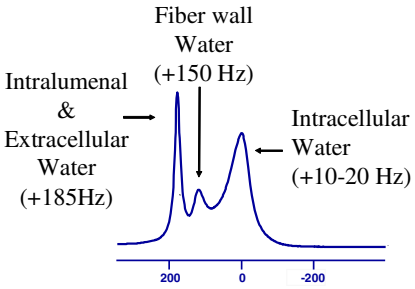

Figure 1: *^{1}H spectrum in presence of 5mM Gad-DTPA*

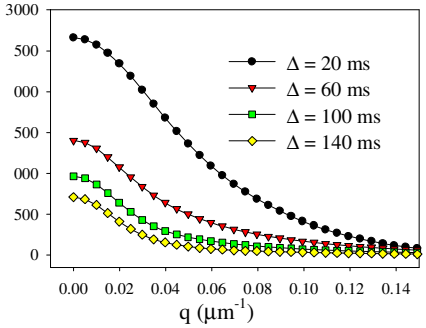

Figure 2: *Intracellular signal decays*

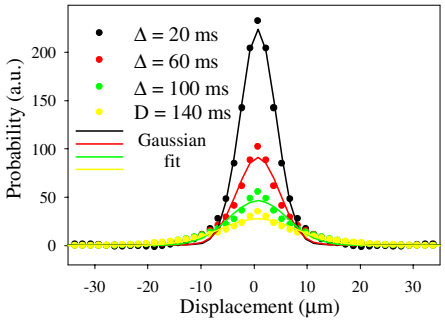

Figure 3: *Displacement distributions*

Figure 2 shows the signal decays for the intracellular water signal as a function of *q* at four selected diffusion times. These signal decay curves were Fourier transformed to obtain the displacements distributions shown in Figure 3. The displacement distributions could not be fit to a single Gaussian distribution model (solid lines in Figure 3), particularly at longer diffusion times. Non-Gaussian behavior of water diffusion has been observed and predicted for a single compartment with boundaries or in presence of a combination of restriction and exchange [5].

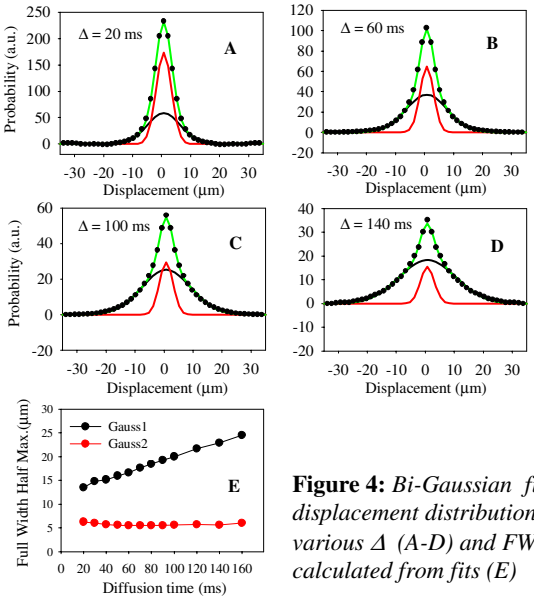

Figure 4: *Bi-Gaussian fit to displacement distributions at various Δ (A-D) and FWHM calculated from fits (E)*

In contrast, bi-Gaussian distributions were found to adequately fit the displacement distributions (Figure 4, A-D). For each displacement distribution (●), Figure 4 shows the two individual Gaussian distributions (▬ and ▬) and their sum (▬). The full width at half maximum (FWHM) computed from the individual Gaussian distributions are plotted versus diffusion time in Figure 4E and show the existence of a narrow component that is independent of diffusion time and a broader component whose width increases with diffusion time. These results would be consistent with the existence of a highly restricted pool of water within the cells coexisting with a pool of water both restricted and at exchange with the extracellular water. However, the bi-Gaussian fit used in this study is essentially phenomenological. We are currently developing non-analytical models using finite-difference methods to better describe our diffusion data in terms of structural and physiological parameters.

References: [1]Sehy et al, *MRM* 48:765 (2002), [2]Latour et al, *PNAS* 91:1229 (1994), [3]Pfeuffer et al, *NMR Biomed* 11:19 (1998), [4]Szafer et al, *MRM* 33:697 (1995), [5] Stanisz, *IsrJChem*; 43:33 (2003), [6]Galons et al. *MRM* 54:79 (2005)

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