Water Diffusion in Perfused Human Glioma Cells

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Introduction

The attractiveness of diffusion-weighted magnetic resonance spectroscopy (DWMRS) resides in its ability to detect local microstructural changes associated with treatment long before their effects are translated into effective size changes. However, interpreting the changes of the measured parameters (apparent diffusivities and their volume fractions) in terms of morphologic and physiologic parameters remains a challenge. In this study we used a human glioma cell line (U251) grown in a hollow fiber bioreactor to characterize water diffusion and relaxation in a stable and well-controlled environment. The effect of restriction, relaxation and compartmental exchange on apparent diffusivities and their volume fractions are discussed.

Materials and Methods

Cell culture: Individual Fibracel® disks were cut into eighths and sandwiched between two porous Teflon screens contained within a standard 10 mm NMR tube. Human glioma U251Cells were loaded onto the disks by using an infusion pump to push a 50 mL suspension of freshly harvested cells at a density of $1x10^6$ cells/ml through the reactor bed. Once loaded, cultures were perfused at a rate of 4 mL/min using a reactor circuit incorporating a membrane oxygenator equilibrated with 95% air/5% CO_2. DMMP (dimethylmethylphosphonate, 10 mM) and 3-APP (3-aminopropylphosphonate, 10mM) were loaded as ^{31}P MRS markers of total water space and extracellular water space respectively [1].

DWMRS: All experiments were performed on a Bruker AVANCE spectrometer at 9.4 Tesla equipped with microimaging gradients (100G/cm). ^{1}H DWMRS was used to determine intracellular volume and water exchange as previously described by Pfeuffer et al [2]. A complete set of diffusion-weighted MRS data were recorded including; (1) Constant time (*ct*) experiments acquired with 32 b-values ranging from 0 to 20,000 mm^2/s for 16 different diffusion times (20ms $<t_d<$ 500 ms), (2) Constant gradient (*cg*) experiments acquired with 16 diffusion times ranging from 0 to 500ms for 32 gradient strengths, (3) T2 relaxation- diffusion correlation experiments conducted with 32 b values (0-20000mm2/s) for 16 different TE (0-250 ms) at a constant diffusion time (Δ) of 40 ms, (4) T1 relaxation-diffusion correlation experiments conducted with 32 b values (0-20000mm^2/s) for 16 different inversion recovery times (0-5sec) at three different Gadolinium-DTPA concentrations[0-4 mM]. For each *ct* and *cg* experiments, the intensity of the water peak, normalized to the total water signal without diffusion weighting, was fit to a double exponential decay for the full range of b values. Volume fractions and associated apparent diffusion coefficients were derived at each diffusion time (Δ) for *ct* experiments and at each gradient strength (G) for *cg* experiments.

Results and Discussion.

A bi-exponential function was found to adequately fit the data yielding two apparent diffusivities ADC_{slow} and ADC_{fast} and their respective volume fraction V_{slow} and V_{fast}. Consistent with previous studies [3] the two compartments also differed in their relaxation properties as determined by the relaxo-diffusography experiments. The water pool associated with ADC_{fast} had a long apparent T1 relaxation constant (2.5 sec) and a long apparent T2 relaxation constant (250ms) while the ADC_{slow} compartment had a shorter T1 and T2 relaxation constants (500ms and 80 ms, respectively).

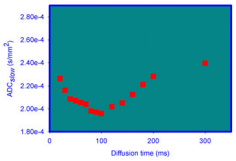

Fig.1: ADC_{slow} vs diffusion time.

ADC_{slow} showed a dependence on diffusion time indicating restriction at short diffusion time (Δ <100ms) and exchange at longer diffusion time and consistent with intracellular water (Fig.1). However the volume fractions obtained from the bi-exponential analysis did not correlate with the volume fractions directly estimated from the DMMP/3-APP ratio obtained from the fully relaxed ^{31}P NMR spectrum. This discrepancy reflects the influence of exchange and relaxation on the apparent volume fractions obtained from the bi-exponential analysis.

Fig.2 shows the *cg* experiments used to estimate the mean intracellular residence time τ_{intra} . Linear regression was performed in the range of diffusion time Δ = [25-100]ms (Fig.2 left) and yielded τ_{intra} (Fig.2 right) at various values of gradient strength (*G* = (20-600 mT/m). The measured time constants decay towards τ_{intra} with increasing gradient strength amplitude as the system move into the slow exchange rate [4]. The value of 45 +/- 2.0 ms for τ_{intra} is similar to previously measured values in isolated cells [2]. The effect of exchange is seen in the T1 relaxation- diffusion correlation experiment (Fig3.)

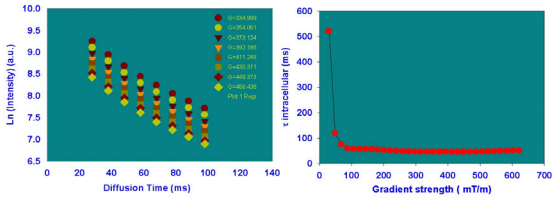

Fig.2: Mean intracellular residence time τ_{intra} (right) measured from *cg* experiments (left). Regressions are shown only for the range [335-468 mT/m].

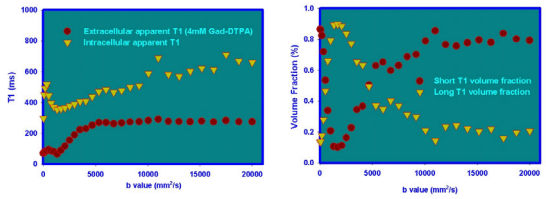

Fig.3: T1 relaxation-diffusion correlation experiment in presence of 4mM Gad-DTPA. (Left), T1 constant values obtained from a bi-exponential analysis for various b values, (right), corresponding volume fractions.

In presence of 4mM gadolinium, the apparent T1 of extracellular water becomes very short (< 80ms). Fig 3 shows that the short T1 apparent volume fraction decays rapidly with increased b values as expected for extracellular water. However at high b value, the bi-exponential analysis reveals than an important fraction of intracellular water have "seen" the gadolinium and display a shorter apparent T1 consistent with exchange with relaxed extracellular water

Conclusion: While the bi-exponential function can adequately fit the data in our bioreactor, the extracted volume fractions and ADC values are not directly correlated to the physical volume fractions. The determination of exchange rates and true relaxation constants should allow a correct estimation of intracellular and extracellular volume fractions.

References: [1] Bhujwalla Z, et al, *Brit. J. Cancer* 78:606 (1998), [2] Pfeuffer et al, *NMR Biomed* 11:19 (1998), [3] Smouha E. et al. *MRM* 46:68 (2001), [4] Meier et al, *MRM* 50:500 (2003).

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