Ghosts, Phantoms and Spectra: Water Diffusion and Cell Swelling in Diffusion Weighted Imaging

Peter E. Thelwall[1], Stephen J. Blackband[1]
[1]University of Florida Brain Institute, Department of Neuroscience, P.O. Box 100244, Gainesville, Florida, United States;

Introduction

Biological tissues are multicompartmental and heterogeneous. A change in the MR properties of a tissue component can indicate a pathology *in vivo*, for example ischemia in the brain results in a rapid decrease in the ADC of water in the tissue (1). This change is most commonly attributed to cell swelling increasing the fraction of water with low ADC. Theoretical models have been developed to describe compartmental effects on water diffusion, (2,3) yet an understanding of the effects of tissue structure and microstructure on water diffusion remains elusive. The common goal of research in this field is to comprehend the observed changes in clinical MRI in terms of tissue structure, and relate this to tissue function in a quantitative manner.

We have developed a model tissue composed of erythrocyte ghosts to study the effects of compartmentalisation on water diffusion. The cell density, cell size, and membrane permeability to water can be modulated. The constituents (and therefore biophysical properties) of both the intracellular and extracellular compartments can be controlled. The simplicity of this model system aids interpretation of the data and allows descriptive mathematical models of this system to be generated, which we hope to develop in future studies.

We have observed non-monoexponential water diffusion in this model tissue. To aid in a preliminary interpretation, data were fitted to a biexponential function.

Methods

Erythrocyte ghosts were prepared from human blood by a hypotonic gel filtration method, as described by PG Wood (4). Ghosts were resealed by restoration of isotonicity, producing saline filled ghosts free from visible contamination by haemoglobin. 75 ul of packed ghosts were suspended in 75 ul of isotonic or +/- 25% and +/- 12.5% hypo- and hypertonic pH buffered solutions, to effect cell swelling and shrinkage. MR data were acquired from erythrocyte ghost suspensions using a 5 mm imaging probe interfaced to a Bruker 750 MHz widebore instrument and console. A pulsed gradient spin-echo (pgse) sequence was used to obtain the data, gradient strengths of up to 1480 mT/m were employed to produce b-values up to 7000 s/mm^2. The diffusion time was 7 ms, the TE was 15 ms, the TR was 3 s and 4 averages were taken at each b-value. Signal intensity was determined by calculating the integral of the water peak. Biexponential analysis fit the data to the function:

$$S = S_0 \cdot (f_1 \cdot e^{(-b \cdot D_1)} + (1 - f_1) \cdot e^{(-b \cdot D_2)})$$

Where S_0 is the signal intensity in the absence of diffusion weighting gradients, F_1 the fraction of water with fast ADC, D_1 and D_2 the ADCs of the fast and slow diffusing water components respectively.

Results

Figure 1 shows a plot of log (Signal intensity) against b-value for the hypo-, iso- and hypertonic cell suspensions. Ghost samples under hypotonic conditions show increased signal at high b-values, as expected for an increase in intracellular volume. Biexponential analysis demonstrated a decrease in F_1, D_1 and D_2 on cell swelling, as shown in Table 1. The trends in F_1, D_1 and D_2 on cell volume increase are consistent with the water diffusion trends observed in rat brain *in vivo* on ischemic insult (1).

The fraction F_1 from ghosts suspended in isotonic solution at 50% cell density is 0.784 +/- 0.007. Experiments altering cell density (under isotonic conditions) have suggested that the fast diffusing component of the biexponential fit is composed of extracellular and intracellular fast-diffusing water, the fraction F_1 is proportional to, but not equal to the fraction of extracellular water (data not shown). This is consistent with our studies of single isolates neurons, which demonstrated fast and slow diffusing water populations within the cytoplasm alone (5).

Acknowledgements

Grant Sponsor: National Institutes of Health; Grant number RO1 NS36992.

SJB is affiliated with the National High Magnetic Field Laboratory, Tallahassee, Florida.

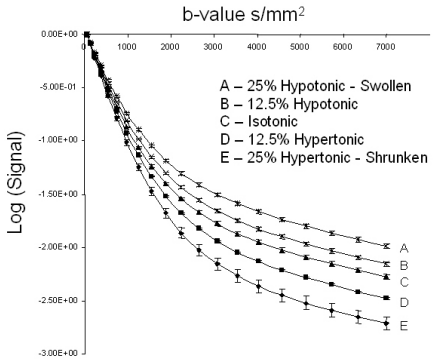

Figure 1

Ghost Sample	F_1	D_1 / mm^{2}s^{-1}	D_2 / mm^{2}s^{-1}
25% Hypotonic	0.85	0.00144	0.000131
12.5% Hypotonic	0.81	0.00139	0.000127
Isotonic	0.78	0.00137	0.000118
12.5% Hypertonic	0.76	0.00134	0.000118
25% Hypertonic	0.72	0.00130	0.000114

Table 1

Discussion

Several groups have used model systems composed of cell suspensions to study water diffusion in biological systems, such as the studies of Stanisz *et al* (6), developing an analytical model of human blood describing relaxation and restricted diffusion, studying compartmental dynamics by diffusion and relaxation. Other studies have applied analytical models to cultured neuronal cells (7,8), a tissue model physiologically more relevant to the brain *in vivo*, which may be used to assess the effects of physiologically relevant insults on the diffusion properties of water. Our studies of perfused rat hippocampal slices provided a physiologically relevant sample with the controllable environment of an *in vitro* study. Biexponential analysis of water diffusion was used to follow cell volume change on insult (9,10).

The development of the erythrocyte ghost model allows us to study water diffusion in a highly controllable system, where tissue structure, intra- and extracellular diffusion coefficients and membrane permeability are known. This will aid the development of an accurate mathematical model of the system. Although the erythrocyte ghost model differs from brain tissue - simplistic structure, smaller cell size, higher membrane permeability to water - the data presented here show changes in the diffusion profile on cell swelling analogous to the effects of cell swelling observed *in vivo* (1). Our goal is to understand the biophysical mechanisms that determine MR signal contrast in *in vivo* models and in the clinic. Non-monoexponential water diffusion was recently observed in human brain (11) (albeit at long diffusion times, allowing for water exchange between compartments). A better understanding of the effects of compartmentation and exchange will aid analysis of such data and increase the clinical potential of such data.

References

1) Niendorf *et al*, Magn Reson Med 36:847-857 (1996)
2) Szafer *et al*, Magn Reson Med 33:697-712 (1995)
3) Stanisz *et al*, Magn Reson Med 37:103-11 (1997)
4) Wood, Methods in Enzymology 173:346-355 (1989)
5) Grant *et al*, Submitted to Magn Reson Med (2000)
6) Stanisz *et al*, Magn Reson Med 39:223-33 (1998)
7) Pfeuffer *et al*, NMR Biomed 11:11-18 (1998)
8) Pfeuffer *et al*, NMR Biomed 11:19-31 (1998)
9) Blackband *et al*, Magn Reson Med 38:1012-1015 (1997)
10) Buckley *et al*, Magn Reson Med 41:137-142 (1999)
11) Mulkern *et al*, Magn Reson Med 44:292-300 (2000)