Temperature Dependent Changes in the Diffusion Properties of Water in Biological Tissues

P. E. Thelwall[1], G. J. Stanisz[2], T. M. Shepherd[1], S. J. Blackband[1]

[1]Department of Neuroscience, McKnight Brain Institute, University of Florida, Gainesville, Florida, United States, [2]Imaging Research, Sunnybrook and Women's CHS, University of Toronto, Toronto, Ontario, Canada

Introduction

The relationship between tissue microstructure and the diffusion properties of water has been widely employed, such as for visualisation of cerebral ischemia in the clinic and mapping of white matter fiber tracts with Diffusion Tensor Imaging (1). A more quantitative approach to determining tissue microstructure from water diffusion measurements has relied on measuring diffusion properties at a range of diffusion times and to very high *b*-values and analysing data with appropriate mathematical models. As diffusion measurements are performed on *in vivo* living systems and *ex vivo* samples (sometimes histologically fixed), measurements are made at a range of temperatures. The diffusion coefficient of unrestricted water is temperature dependent. As biological tissues are compartmental and complex, the relationship between temperature and the diffusion properties of water in a tissue is non-trivial. Investigating and understanding this relationship will aid interpretation of diffusion data in terms of tissue microstructure.

In this study we employed a simple, highly controllable tissue model composed of erythrocyte ghosts, in which the diffusion properties of water were measured at temperatures between 10 and 37°C. The ghost model is well suited to MR studies of tissue microstructure as it allows independent control of cell size, density, intra- and extracellular viscosity and membrane permeability (2). MR data were analysed with a two compartment exchange model (3).

Methods

Human erythrocyte ghosts were prepared and cell density assayed as previously described (2). Three samples of ghosts were diluted to 50% intracellular fraction, 100 µl aliquots were placed in 2.5 mm NMR tubes. All MR data were acquired using a 14 T Oxford magnet and Bruker Spectrometer equipped with triple axis 300 G/cm shielded gradients. Sample temperature was controlled with a Bruker probe variable temperature unit that was calibrated using the chemical shift of methanol. Pulsed Gradient Spin Echo (PGSE) experiments were performed at six diffusion times between 10 and 50ms (δ = 3ms), gradients were linearly incremented in 32 steps to produce *b*-values between 0 and 13000 s mm^{-2} for all diffusion times. MR data were analysed with a two compartment model incorporating exchange between compartments, intracellular restriction and extracellular tortuosity (2,3). The analysis provided an index of cell size, extracellular apparent diffusion coefficient, intracellular fraction and the rate of exchange between intra and extracellular water. (3)

Results and Discussion

Figure 1 shows data from PGSE experiments at 10, 20, 30 and 37°C, diffusion times of 10, 17, 25, 35 and 50ms are shown at each temperature. Non-monoexponential diffusion was observed in all datasets. The changing initial slope, signal at high *b*-value, and effect of increasing diffusion time is apparent with increasing temperature. The two compartment exchange model was fitted to the data, calculated cell size, intracellular volume fraction and tortuosity of the extracellular space remained constant with increasing temperature. However, the ADC of extracellular water and the rate of exchange between compartments increased with increasing temperature. Figure 2 plots calculated extracellular ADC against temperature, Figure 3 plots exchange rate against temperature; both show a linear increase with temperature. The increase in exchange rate with temperature is greater than would be predicted from the increase in extracellular ADC, indicating that membrane permeability increases with temperature – a similar observation has been made in erythrocyte suspensions (3). The increase in water exchange rate with temperature was similar to that observed with T_2-based measurements of erythrocyte ghost water exchange (4).

The results of this analysis support our previous studies on water diffusion in the erythrocyte ghost model, which proposed that intracellular water diffusion is dominated by restriction by the cell membrane, extracellular diffusion is moderated by the tortuosity of the extracellular compartment, and water exchange between these two compartments is significant for experimentally attainable diffusion times.

Conclusions

This study investigated the effects of temperature change on the diffusion properties of water in a biological tissue, over the range of temperatures frequently employed in MR measurements of water diffusion. The temperature dependence of water diffusion in biological tissues is more complex than that of pure water due to the compartmental nature of biological tissues. In the model system studied, the ADC of water in the extracellular compartment increased with temperature whereas the ADC of intracellular water did not. The extent of exchange between intra- and extracellular compartments during the diffusion time increased with temperature. The data demonstrate that comparison of diffusion data acquired from *in vivo* and *ex vivo* samples may be compromised if temperature is not taken into account, and that sophisticated analytical models such as the one employed here can provide an accurate description of tissue microstructure.

Acknowledgements and References

Grant sponsor: NIH - RO1 NS36992, P41 RR16105. Thanks to Dan Plant for technical assistance. References: (1) D LeBihan, Nat Rev Neurosci. 4:469-80 (2003). (2) Thelwall *et al.* Magn Reson Med. 48:649-657 (2002). (3) Li *et al*. Magn Reson Med. 40:79-88 (1998). (4) G Benga *et al*. Biochim Biophys Acta 905:339-348 (1987).

Figure 1

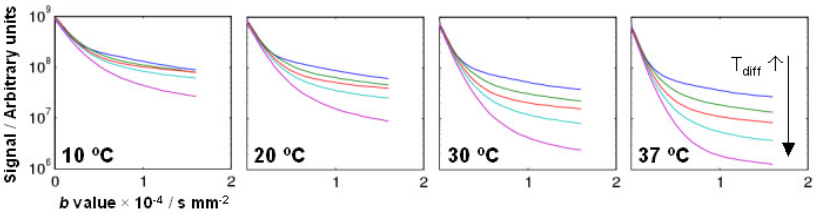

Figure 2

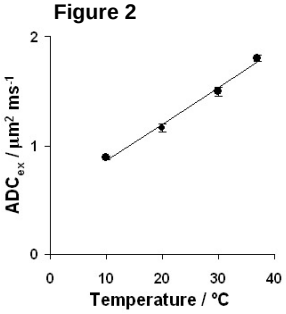

Figure 3

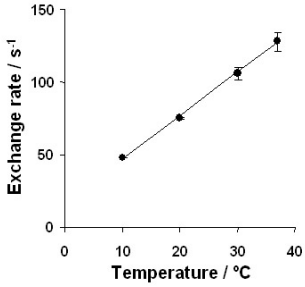

Figure 1 - Plots of log(Signal) against *b* value for a ghost sample at 10, 20, 30 and 37 °C. Diffusion times of 10, 17, 25, 35 and 50 ms were employed. The data demonstrate an increase in extracellular water ADC and membrane permeability as temperature rises.

Figure 2 – Extracellular water ADC (mean +/- SD) vs temperature, calculated using the two compartment exchange model.

Figure 3 – mean exchange rate of intracellular water (mean +/- SD) plotted against temperature.