TEMPERATURE AND FIXATION EFFECTS ON WATER DIFFUSION IN CELL GHOSTS

P.E. Thelwall (Newcastle U, England), T.M. Shepherd (UF, Neuroscience), G.J. Stanisz (U Toronto, Canada), S.J. Blackband (UF, Neuroscience)

Introduction

Studies of pathological samples can be used to complement in vivo studies. Usually higher resolution data can be acquired under more controlled conditions and used to interpret usually poorer quality in vivo data. We have been investigating a variety of ex vivo models towards this goal, including brain tissue slices and isolated single neurons. The data are collectively being used to help develop working mathematical models of MR signals in tissues, which are then tested in perturbation studies and to simulate disease process. These systems are still complicated, so we have also been developing a simpler model for MR studies, namely the erythrocyte cell ghost. In this models human red blood cells are treated so that their insides are removed and the cell membrane resealed to leave an 'empty cell' (1). Since ex vivo studies are typically performed at room temperature, and samples are frequently preserved by fixation, it is important to understand how environmental and chemical changes dictated by ex vivo studies alter the physical and MR properties of a sample. MR measurements were used to assess the effects of temperature change and aldehyde fixation on the MR properties of ghosts suspended in buffer or agarose gel.

Experimental

Data were collected on a Bruker 600 MHz system at UF. Ghosts were prepared as described previously. Diffusion and releaction time measurements were made as a function of temperature, with the sample temperature was varied between 10 and 37 degrees C. The effect of three commonly used tissue fixatives, glutaraldehyde, paraformaldehyde and Karnovskys solution, were also examined. Diffusion MRI data were analyzed with a biophysically appropriate two-compartment exchange model.

Results and Discussion

Temperature change resulted in a complex alteration of water diffusion properties due to the compartmental nature of tissues and alteration in membrane permeability. Formaldehyde, Karnovsky's solution, and glutaraldehyde all caused statistically significant changes to the biophysical and MR properties of the samples. For an example, see figure 1. Fixation also caused large decreases in water proton T2, which was restored to near prefixation values by washing free fixative from the samples. Water membrane permeability was also significantly altered by fixation.

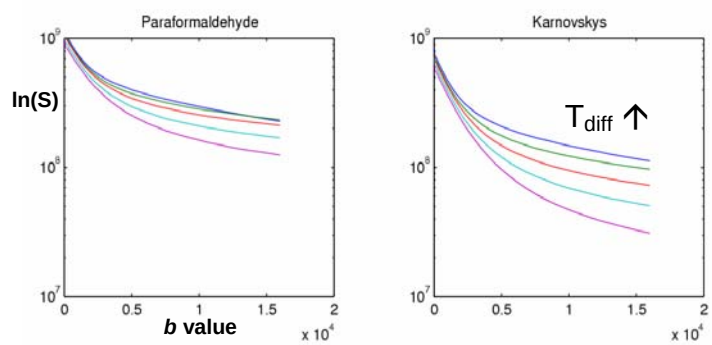

Figure 1. Diffusion curves from ghosts in two fixatives as a function of the diffusion time, T_{diff}.

Conclusions

This study demonstrates that relating in vivo MR data to chemically fixed ex vivo data requires an understanding of the effects of sample preparation. Controlled data of this type aids in the development of working mathematical models of MR signals in tissues. Similar studies are underway in other fixed tissues.

References.

(1) Thelwall PE et al., Magn Reson Med. 2002 Oct;48(4):649-57
(2) Thelwall PE et al., Magn Reson Med. 2006 Aug;56(2):282-9.

Acknowledgements

Support was provided by NIH grant R01-NS36992, P41 RR 16105 and the National High Magnetic Field Laboratory.