Effect of Osmolarity on Myelin Water Fraction Measurement in Aldehyde Fixed Spinal Cord Tissue

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Introduction

Myelin content is an important marker for central nervous system pathology. Quantitative T2 based myelin water imaging has been shown to measure myelin content in normal and diseased brain and spinal cord tissues [1,2]. Because myelin is difficult to image directly with MR due to very short T2 relaxation times, this technique focuses on indirect measurement of myelin by probing the properties of the surrounding water. Therefore it is important to understand how changes in tissue morphology affect the water environments. Rat models are widely used for the study of spinal cord injuries and associate repair therapies, but due to the challenges in obtaining *in vivo* images, *ex vivo* aldehyde fixed spinal cord samples are often used as an interim solution for the validation of MR techniques versus histological measurements. However, fixation procedure may affect tissue properties and, subsequently, the measured myelin water fraction (MWF). In this study we looked at the impact of osmolarity on MR measurements of MWF by varying the concentration of the phosphate buffer (PB) used in the fixative solution and correlating it to the changes in water environment measured by the tunneling electron microscopy (TEM).

Methods

One Fischer 344 rat was perfused intracardially with paraformaldehyde in 0.1 M sodium PB for 30 minutes followed by a 3 minute long PBS flush. The cervical spinal cord was then extracted and cut into six sections alternating between 3 mm and 1 mm thick, with adjacent 3mm and 1mm sections constituting a pair. The three pairs were fixed separately overnight in glutaraldehyde solutions which varied in their PB concentration (0.17 M, 0.1 M, and 0.05 M).

MR experiments were carried out on the 3 mm cord sections on a 7T animal scanner (Bruker, Germany) using a 5 turn, 13 mm i.d. solenoid coil. Quantitative T2 data were acquired using a single slice multi-echo CPMG sequence placed at C5 level (256 × 256 matrix, TE/TR = 1500/6.738 ms, 32 echoes, 1.79 cm FOV, 1 mm slice, NA = 12, 70 μm in-plane resolution) [3]. CPMG data were processed using a non-negative least square analysis technique [4]. MWF maps were generated by integrating the 7.75-20 ms range and dividing by the total integral of the T2 distribution in each pixel.

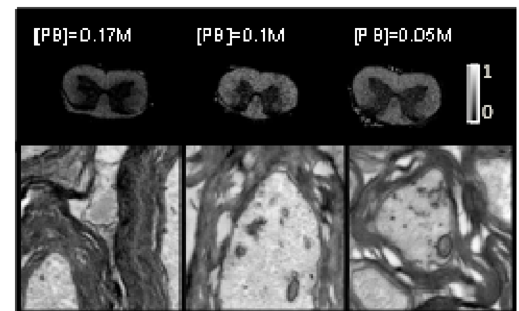

Figure 1. MR generated MWF map and selected TEM micrographs of rat spinal cord samples prepared at different phosphorous buffer concentrations. There is a noticeable decrease in MWF at [PB] = 0.17 M.

The 1 mm sections were fixed in an osmium tetraoxide and potassium ferrocyanide solution then embedded in spur resin. Approximately 100 nm thick sections were cut on an ultra-microtome, mounted on a copper grid, and stained with uranyl acetate. TEM images of the *fasciculus gracilis* were taken at 25,000× magnification at fixed intervals. Myelin sheaths were manually traced and the myelin water space and total water area were measured by intensity thresholding. The myelin water space was divided by the total water area to produce a MWF measurement for each image.

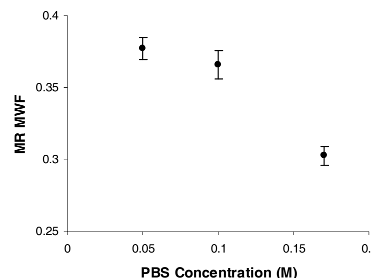

Figure 2. MWF measurement in the *fascicules gracilis* versus the phosphate buffer concentration. The MWF decreases with increased PB concentration.

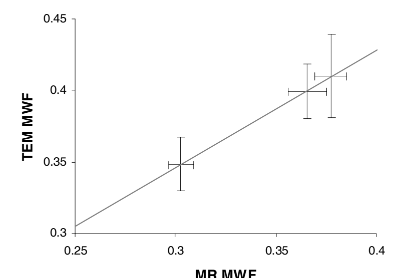

Figure 3. MR MWF measurement TEM MWF measurement. Error bars are standard error. The two different methods of MWF measurement correlates well.

Results and Discussion

Figure 1 shows the MR generated MWF maps with example TEM micrographs; Figure 2 shows the MR measured MWF of the *fasciculus gracilis* over the different PB concentrations. The MR MWF measurements are plotted against the TEM MWF results in Figure 3. The *fasciculus gracilis* was chosen for the analyses because there is little intermingling of axons at the cervical level and it provides a consistent myelin content through all sections studied here. There is a trend of decreasing MWF with increasing PB concentration. This is somewhat surprising as one may expect that high concentration PB will pull water out of the extra/intracellular space resulting in the increase in MR MWF values; however, because the intra/extracellular space do not form sealed compartments (extracellular space is contiguous with the surrounding fluid, and the axons have been severed at both ends) the PB is expected to freely permeate these spaces. On the other hand, the myelin sheaths are intact so generally impermeable to phosphate ions. This means that the osmotic gradient from the increased concentration preferentially removed myelin water, leading to a lower MWF reading in both methods. The MR vs. TEM MWF comparison (Fig. 3) suggests very strong correlation between the two methods; however more data points are needed to achieve statistical significance.

Acknowledgment

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