In vivo longitudinal Myelin Water Imaging in rat spinal cord following Dorsal Column transection injury

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Introduction

After the traumatic spinal cord incident, the acute impact causes the primary injury (within a few hours) and the long lasting process of secondary damage and myelin clearance which can occur over weeks to months later. Previous studies have shown applicability of Myelin Water Imaging (MWI) [1] to follow white matter degeneration over time in injured rat spinal cords ex vivo [2]. The present work aimed to determine applicability of MWI in an in vivo model of Dorsal Column transection (DCTx) to assess temporal changes of myelin content in the rat spinal cord following injury.

Methods

The dorsal column of the spinal cord was transacted in 10 adult male Sprague-Dawley rats at the level of the 1st lumbar vertebra. MRI was carried out on a 7T animal scanner (Bruker, Biospin Gmbh, Germany) using transmit/receive implantable RF coils [3] and a 5-turn 15 mm i.d. solenoid for the in vivo and ex vivo measurements respectively. MWI measurements were performed using a single slice, multi echo CPMG sequence (FOV=30x30 mm in vivo/25.6x2.56 mm ex vivo, matrix size 256x256, TR/TE= 1500/6.378 ms, slice thickness=1.5 mm in vivo/1.0 mm ex vivo, six averages). The slice was positioned at 5 mm cranial to injury. Data were collected at 3 time points: before injury (control scan), 3 weeks, and 8 weeks after the injury. Five animals were sacrificed at the 3 weeks time point, and remaining animals were sacrificed at 8 weeks post-injury. All sacrificed animals were perfusion fixed with 4% paraformaldehyde and the cords harvested. The ex vivo MWI was carried out one day following fixation. For histological assessment spinal cords were stained with Eriochrome Cyanine (EC), a lipophilic dye staining myelin lipids/lipoproteins, and antibody to degenerating Myelin Basic Protein (dgen-MBP), recognizing an epitope in degenerated myelin. T_2 distributions were calculated from the multi-echo data using non-negative least squares analysis [4]. Myelin Water Fraction (MWF) maps were generated by integrating the 7.75-20 ms range and divided by the total integral of the T_2 distribution in each pixel. ROIs encompassing fasciculus gracilis were manually outlined on the MWF maps and the average values within ROIs calculated. For histological evaluation optical density measurements were measured from digitized histology sections. ROIs encompassing fasciculus gracilis were manually outlined and average values of optical density calculated. To minimize variability due to tissue processing average values of the optical density were normalized to the average normal left ventral white matter.

Results and Discussion

Figure 1 shows the average MWF values for the in vivo and ex vivo data. Average MWF in vivo was significantly higher at 3 weeks post-injury than in the control group (LSD, p = 0.028). However at 8 weeks post injury this trend was reversed and the average MWF value in vivo was closer to the control value. The trend was similar ex vivo where the average MWF was smaller at 8 weeks than at 3 weeks post-injury (LSD, p = 0.0004). Figure 2 shows average optical density values for the EC and dgen-MBP stains at 3 and 8 weeks post-injury. The average EC optical density was significantly higher at 3 weeks than at 8 weeks post-injury (LSD, p = 0.00024), while the dgen-MBP stain showed opposite trend with the average optical density at 3 weeks post-injury being significantly lower than at 8 weeks (LSD, p = 0.029). Quantitative analysis showed statistically significant correlation between MWF values and EC optical density for all data points (Pearson, r = 0.72, p = 0.031). No correlation between MWF and dgen-MBP was observed.

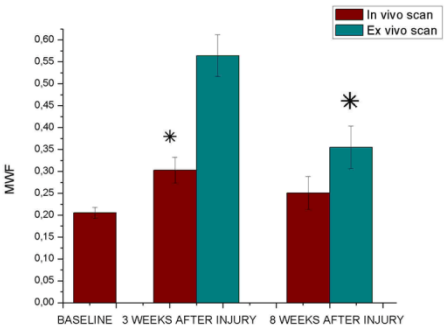

Figure 1. Average MWF values calculated from the ROIs encompassing fasciculus gracilis on MWF maps acquire in vivo and ex vivo at different time points following injury.

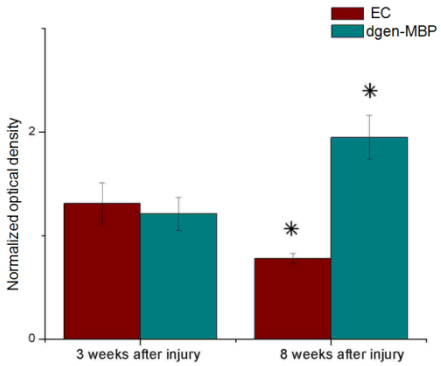

Figure 2. Normalized average values of optical density calculated from the ROIs encompassing fasciculus gracilis on histology sections stained with EC and dgen-MBP at 3 and 8 weeks post-injury.

Higher values of MWF and EC stain optical densities in fasciculus gracilis at 3 weeks post-injury reflect the processes of axonal loss and degeneration of myelin (Wallerian degeneration). The increase in MWF signal as a result of Wallerian degeneration is consistent with our previous reports [1,2] and is likely caused by an increased myelin water compartment. The subsequent decrease in MWF at 8 weeks post-injury is also consistent with previous results [2] and likely reflects further deterioration of myelin membranes and the resultant decrease in myelin water compartment. This is consistent with changes in EC optical density. The initial increased value at 3 weeks post-injury likely reflects increased accessibility of the myelin lipid constituents to the stain and/or their spatial enlargement. The subsequent decrease in the level of EC staining at 8 weeks reflects decreased amount of myelin membranes likely caused by the clearing of myelin debris by macrophages. On the other hand, the increase in dgen-MBP staining between 3 and 8 weeks post-injury likely reflects increased amount of exposed antigen (degeneration specific epitope) for degenerating myelin protein, suggesting continuing presence of significant amounts of myelin debris. Ultimately, electron microscopy is required to accurately assess the amount of debris present in the tissue.

Acknowledgments

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References: [1] Kozlowski P, et al., *Magn Reson Med*, 2008, **59**, 796; [2] Kozlowski P, et al., *J Neurotrauma*, 2008, **25**, 653; [3] Yung AC, et al. *Magn Reson Imaging*, 2007, **25**, 1215; [4] Whittall KP, et al. *Magn Reson Med*, 1997, **37**, 34;