Characterizing white matter damage in rat spinal cord with quantitative MRI and histology

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Introduction

The most significant effect of the spinal cord injury (SCI) is the loss of function resulting from white matter damage. Most of the therapeutic procedures in SCI are oriented towards regeneration and restoration of the interrupted nerve fibre tracts. Clinical evaluation of these procedures requires non-invasive imaging technology that can assess changes in white matter following therapy, while in the preclinical setting the availability of such technology would greatly facilitate the *in vivo* analysis of the treated spinal cord injury site and minimize the number of required animals. In this study we evaluated the ability of two MRI based techniques: Diffusion Tensor Imaging (DTI) and myelin water imaging to characterize white matter (WM) damage in a rat model of SCI. The MRI results were validated against histology.

Methods

Ten Sprague-Dawley rats (250-280g) underwent dorsal column transection (DC Tx) injury at C5 [1]. MRI was carried out ex vivo on the control (n=6) and injured spinal cords at 3 weeks (n=6) and 8 weeks (n=4) post-injury. Rats were perfusion fixed with paraformaldehyde (4%) solution and the spinal cords excised prior to MRI. All MRI experiments were carried out on a 7T animal scanner (Bruker, Germany). The excised spinal cords were positioned in a 4.5 mm inner-diameter plastic tube filled with the fixation solution. A small plastic rod was positioned alongside the cord to prevent it from bending. A four-turn, 13 mm inner-diameter and 20 mm long solenoid coil was used for pulse transmission and signal reception. Myelin water measurements were carried out using a single slice, multi-echo CPMG sequence [2] (256x256 matrix, FOV=2cm, slice thickness=1mm, TR/TE=1500/6.7ms, NA=6, 32 echoes) and DTI data was acquired using a multi-slice spin-echo sequence (128x128 matrix, FOV=1.28cm, slice thickness=1mm, TR/TE=2000/21.3ms, b values of 0 and 750mm^2/s, 6 non-collinear directions using icosahedral encoding scheme [3], NA=4). 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.

Following MRI examination the spinal cords were cryoprotected in 24% sucrose and tissues prepared for histological analysis. Luxol Fast Blue (LFB) and Myelin Basic Protein (MBP) staining was used to assess myelin damage and neurofilament-H in combination with neuron specific β-III-tubulin (NF/Tub) staining was used to assess axonal damage. Average values of MWF, fractional anisotropy (FA), longitudinal diffusivity (D_{long}), transverse diffusivity (D_{trans}), and average diffusivity (D_{ave}) were calculated in the fasciculus gracilis, fasciculus cuneatus and the dorsal corticospinal tract (CST) 5 mm cranial as well as 5 mm and 10 mm caudal to injury and correlated with histology.

Results and discussion

DC Tx injury results in damage to fasciculus gracilis (ascending sensory tract) cranial to injury and to CST (descending motor tract) caudal to injury. Axonal damage is clearly seen on FA and D_{long} parametric maps (magenta arrows on Figure 1), as well as NF/Tub sections (not shown). Damage to myelin can be identified as low MWF and high D_{trans} values on the parametric maps, as well as low levels of optical density on LFB sections (red arrows on Figure 1). Myelin debris that is formed in DC WM cranial and caudal to injury can be identified as high intensity areas on MWF maps and LFB sections, and low intensity areas on D_{trans} maps (cyan arrows on Figure 1).

Both MWF and D_{trans} showed very good correlation with Luxol Fast Blue staining, with MWF showing higher values of the Pearson correlation coefficients than D_{trans} (Table 1). The higher values of the correlation coefficient at 8 weeks, as compared to 3 weeks, post-injury are likely due to myelin debris that is largely cleared by 8 weeks post-injury. MBP was not a reliable measure of myelin during early stages of Wallerian degeneration, as can be seen from lack of significant correlation with MWF and D_{trans} values (Table 1). Both D_{long} and FA showed significant correlation with the axonal staining NF/Tub at 3 weeks post-injury, while only D_{long} showed significant correlation at 8 weeks post-injury (Table 1). Weaker correlation between FA and NF/Tub, as compared to D_{long} vs. NF/Tub, is due to the fact that FA contains information on both D_{long} and D_{trans}. It has been shown that D_{long} decreases with the loss of intact axons, while D_{trans} increases with the loss of myelin. Since DC Tx results in loss of both intact axons and myelin, changes in FA due to lower D_{long} are mitigated by the increased D_{trans} in the same area. Weaker correlation between D_{long} and NF/Tub at 8 weeks, as compared to 3 weeks post-injury, are likely due to a partial volume effect. At 8 weeks post-injury axons in the major WM tracts (especially in fasciculus gracilis) largely disappear, and as a result the tracts collapse. Consequently, the 100 µm in-plane resolution in DTI data is likely not sufficient to reliably identify the collapsed tracts.

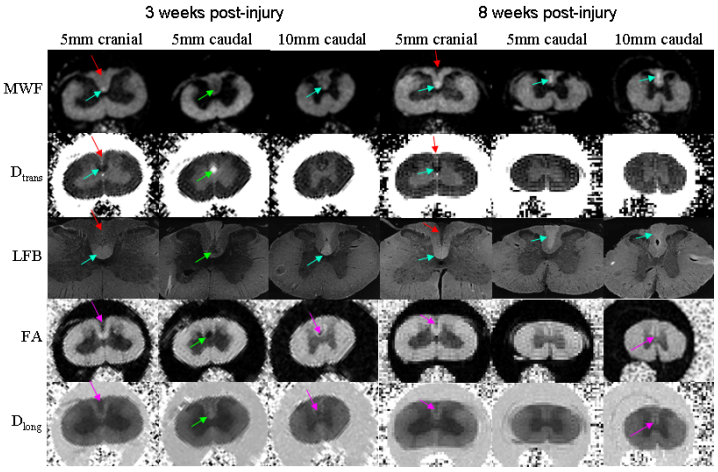

Figure 1. Reconstructed parametric maps (MWF, D_{trans}, FA, D_{long}) and LFB histological sections from representative spinal cords excised 3 weeks and 8 weeks following DC Tx injury. The arrows mark the areas of myelin damage (red), myelin debris (cyan), extension of the injury cavity (green) and axonal damage (magenta) in major DC WM tracts.

	3 weeks post-injury			8 weeks post-injury		
	LFB	MBP	NF/Tub	LFB	MBP	NF/Tub
MWF	0.64 (p<0.001)	-0.28 (p=0.07)	-0.35 (p=0.02)	0.88 (p<0.001)	0.15 (p=0.40)	0.17 (p=0.32)
FA	0.33 (p=0.02)	-0.66 (p<0.001)	0.64 (p<0.001)	0.72 (p<0.001)	0.22 (p=0.19)	0.33 (p=0.056)
D_{long}	-0.27 (p=0.07)	-0.35 (p=0.02)	0.78 (p<0.001)	0.24 (p=0.16)	0.03 (p=0.88)	0.58 (p<0.001)
D_{trans}	-0.49 (p<0.001)	0.51 (p<0.001)	-0.38 (p=0.009)	-0.71 (p<0.001)	-0.21 (p=0.22)	-0.15 (p=0.38)
D_{ave}	-0.60 (p<0.001)	0.26 (p=0.08)	0.11 (p=0.49)	-0.57 (p<0.001)	-0.19 (p=0.26)	0.20 (p=0.24)

Table 1. Correlation between MRI parameters and histology. Numbers represent Pearson correlation coefficients R.

Conclusions

The results of this study demonstrate that quantitative MRI can accurately characterize WM damage in DC Tx model of injury in rat spinal cord *ex vivo*. We were able to generate, for the first time, high quality MWF maps with in plane resolution sufficient enough to allow quantitative analysis of myelin damage in major DC WM tracts. We showed that in a traumatic spinal cord injury, like dorsal column transection, MWF is a more sensitive measure of myelin than D_{trans}.

Acknowledgments

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