Less severe spinal cord injury in dysmyelinated mice evaluated using DTI and locomotion

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Introduction

In contusion spinal cord injury (SCI), the secondary neuron degeneration post mechanical damage plays a crucial role in the progressive spinal cord degeneration[1]. The secondary degenerative processes involve microglial and macrophage infiltration followed by the clearance of myelin debris. However, the myelin debris in SCI has been known to inhibit the axonal growth and regeneration due to the inhibition of axonal regeneration by myelin constituents proteins such as Nogo and MAG[2]. Thus, the lack of myelin sheaths in shiverer mice may provide a more hospitable environment for axonal regrowth after SCI. Diffusion tensor imaging (DTI) has emerged as a sensitive noninvasive diagnostic tool to examine the white matter integrity *in vivo*[3,4]. The objective of this study is to evaluate the impact of myelin sheath deficiency on SCI progression with *in vivo* DTI.

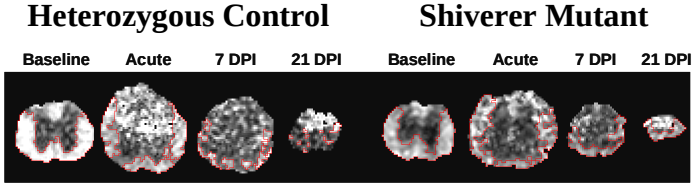

Fig. 1 Relative anisotropy (RA) maps are shown from the images taken in the progression of SCI. The regions of normal appearing ventral white matter, i.e. spared ventral white matter, were segmented by applying the threshold values between 0.6~1.3 in RA maps to indicate the extent of spared white matter.

Materials and Methods

Severe contusion SCI were generated in four 10-12 week-old female shiverer mutant MBP^{shi}/MBP^{shi} (myelin-deficient) mice and four heterozygous $MBP^{shi}/+$ (normal myelin sheath) controls littermate, weighting 19-22 g, using previously reported method[4]. On the day before injury, DTI of spinal cord were performed for each mice to acquire the baseline data. After severe contusion SCI at the T9 vertebral level, a sequential DTI were performed on each mouse at the hyper-acute (~3hrs), sub-acute (~7 DPI), and chronic phase (~21 DPI). The hind limb motor function was assessed daily by using Basso Mouse Scale (BMS).

In vivo DTI was performed by using a diffusion weighted spin-echo sequence applying diffusion sensitizing gradients in six directions, i.e. (Gx,Gy,Gz) = (1,1,0), (1,0,1), (0,1,1), (-1,1,0), (0,-1,1), (1,0,-1), (0,1,1), (1,0,0). The other acquisition parameters were: TE, 38 ms; Δ, 21 ms; δ, 7 ms; b-value, 0 and 1000 s/mm^2; FOV, 1 × 1 cm^2, data matrix, 128 × 128 zero-filled to 256×256; number of average, 4.

For both baseline and injured cords, the regions of spared ventral white matter was segmented by threshold of mean±2SD derived from the RA value in the ventral white matter of baseline controls. The region of interest (ROI) depicted the region of spared ventral white matter in each phase. (Fig. 1) The volume of spared ventral white matter was then normalized to the baseline white matter volume to take into account the effect of atrophy of the injured cords.

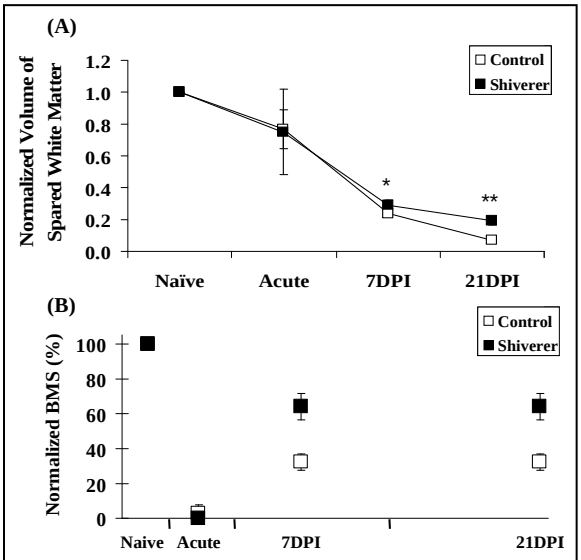

Fig. 2 (A) The normalized volumes of spared ventral white matter determined by RA show difference between the control and shiverer mice after 7DPI. (B) The BMS scores of hind limb motor function show two different groups of recovery from contused SCI. (*P<0.05, **P<0.001, significant differences between control and shiverer)

Results

The white and gray matter of spinal cord could be clearly differentiated based on the RA maps of shiverer and control mice in the baseline images (Fig. 1). After SCI, the total spinal cord volume decreased progressively from acute phase to 21DPI in both groups (data not shown). However, the normalized volume of spared white matter of shiverer mice was 7% higher than that of control mice at 7DPI, and was 12% higher than that of control mice at 21DPI (Fig. 2A). Correspondingly, shiverer mice showed better hind limb motor function than control mice from 7DPI to 21 DPI (Fig. 2B).

Fig. 3 shows the longitudinal changes of DTI derived $\lambda\perp$, reflecting myelin integrity and $\lambda\parallel$, reflecting axonal integrity[3], in the spared white matter of control and shivere mice. $\lambda\perp$ showed no significant changes on either group before 7DPI. However, higher $\lambda\perp$ was observed in control mice (184% compared to baseline) than that of shiverer mice (119% compared to baseline), suggesting more severe myelin damage in control mice than that in shiverer mice (Fig. 3A). The changes of $\lambda\parallel$ of two groups of mice exhibited similar longitudinal changes of except at 7DPI (Fig. 3B).

Discussions and Conclusions

The effect of dysmyelination on *in vivo* DTI parameters as well as the hindlimb locomotion was examined in control and dysmyelinated shiverer mice. The increase of normalized $\lambda\perp$ at 21DPI was substantially lower in shiverer mice, suggesting less extent of myelin degeneration post SCI in shiverer mice. $\lambda\perp$ not changing over time implied the reach of upper limit of $\lambda\perp$ in dysmyelinated shiverer mice. The DTI detected higher volume of spared white matter and the better hindlimb locomotion in shiverer mice suggested that the lack of myelin debris may indeed have a significant impact in axonal regeneration in SCI. Furthermore, the long-term functional outcome depends on both the initial damage and also the extent of secondary injury[1]. Our data suggest that when the initial injury is similar between the two groups, the different degrees of recovery are probably due to the lack of myelin debris for the shiverer mice.

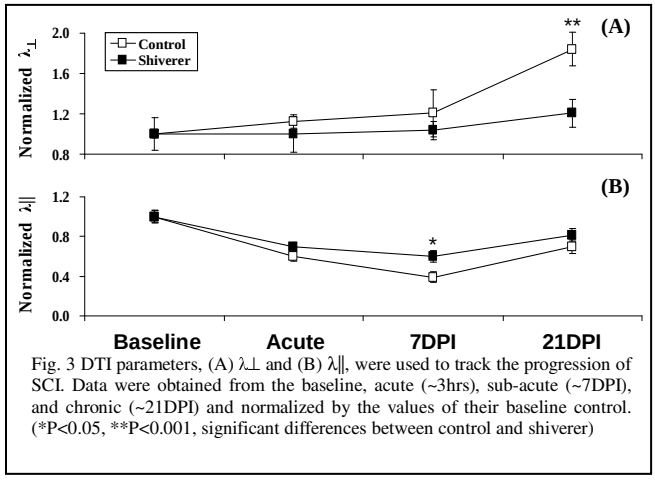

Fig. 3 DTI parameters, (A) $\lambda\perp$ and (B) $\lambda\parallel$, were used to track the progression of SCI. Data were obtained from the baseline, acute (~3hrs), sub-acute (~7DPI), and chronic (~21DPI) and normalized by the values of their baseline control. (*P<0.05, **P<0.001, significant differences between control and shiverer)

References

1. Schwartz et al., Prog. in Brain Res., 2002, 137: 177-190; **2.** Xie et al., Exp Neurol, 2008, 209: 302-312; **3.** Song, et al., J. Neuroimage 2002, 17: 1429-1436. **4.** Kim, et al., MRM 2007, 58: 253-260.