Quantification of evolving white matter injury in mouse spinal cord

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Introduction

Each year in the U.S., approximately 11,000 people sustain a severe traumatic spinal cord injury (SCI). As new therapeutic interventions are developed, it would be exceptionally useful to have an indicator of white matter injury as a surrogate end point. By detecting the incoherent displacement motion of water molecules, magnetic resonance diffusion imaging has evolved into an important research tool for examining the microstructure of neurological pathology in the central nervous system (CNS). Recently, an analytical approach using diffusion tensor imaging (DTI) derived parameters has been developed to quantitatively assess CNS white matter injury (1). We conducted longitudinal DTI over 7 days *in vivo* of mouse spinal cord that underwent mild contusion injury. Additional DTI measurements of the excised cord and histological quantification were performed at the conclusion of longitudinal *in vivo* assessment. Histological findings confirmed that directional diffusivities, i.e., axial diffusivity ($\lambda_{\|} = \lambda_1$), and radial diffusivity ($\lambda_{\perp} = (\lambda_2 + \lambda_3)/2$), serve as markers for axonal and myelin degeneration following SCI.

Methods

A mild contusion injury (0.5 Newton force, 0.3 mm displacement, total impact time 20 msec) was applied with an electromagnetic force driven impactor at vertebral segment L2 of a 10-week-old female C57BL/6 mouse. *In vivo* DTI data were acquired serially at 1, 3, and 7 days after injury. An inductively coupled surface coil (20 mm × 8 mm) used as the receiver covered the lumbar cord L1 through L3. A 9 cm i.d. Helmholtz coil was employed as the RF transmitter. A Stejskal-Tanner spin-echo diffusion-weighted sequence was modified to acquire images under respiratory gating (2). All images were acquired with acquisition parameters of: TR 1 sec (gated acquisition), TE 43 msec, Δ 25 msec, δ 10 msec, slice thickness 0.75 mm, number of slices 12, field-of-view 1×1 cm^2, data matrix 128×128 (zero filled to 256×256), total data acquisition time 90 min. (Gx,Gy,Gz) = (1,1,0), (1,0,1), (0,1,1), (-1,1,0), (0,-1,1), and (1,0,-1), and b = 0 and .785 μm^2/ms. Image resolution was $78 \times 78 \times 750$ μm^3. Locomotor function was assessed using Basso, Beattie and Bresnahan (BBB) scores (3) immediately before each DTI measurement. The spine was harvested with bone for *ex vivo* DTI measurement and histology after *in vivo* DTI measurement at day 7. Skeletal landmarks were used for both *in vivo* and *ex vivo* DTI measurements. The cord was carefully sectioned at L2 and assessed for myelin injury and axonal integrity using a luxol fast blue stain and a stain for phosphorylated neurofilament (SMI-31), respectively. Region of interest (ROI) analysis were performed on both *in vivo* and *ex vivo* DTI maps of the injured mouse spinal cord.

Results and Discussion

The BBB scores show close agreement with previously reported results following T9 contusion with mice using a similar impactor, i.e., 11, 15, and 18 at 1, 3, and 7 days after injury (4). Figure 1 compares the acquired dorsal and ventral white matter directional diffusivities at 1 and 7days after injury with the summary data of live normal "non-injured" mice (n=5). In the dorsal white matter, axial diffusivity decreases almost 50% at 1 day after injury and sustains the decreased value at 7 days after injury (Fig. 1A). In contrast, relatively modest drop (25%) of axial diffusivity is observed in ventral white matter (Fig. 1B). Radial diffusivity shows a different trend. There is little or no change of radial diffusivity in dorsal white matter 7 days after injury (Fig. 1C). A 30% increase of radial diffusivity was observed in ventral white matter (Fig. 1D). The comparison of *in vivo* and *ex vivo* DTI parameters on the same cord at 7days after injury is shown in Fig. 2. Relative anisotropy (data not shown) and trace normalized directional diffusivities are indistinguishable between *in vivo* and *ex vivo* measurements.

Representative *in vivo* and *ex vivo* maps of axial and radial diffusivity and histology of the cord at L2 level from the same animal are displayed in Fig. 3. The ventral white matter is outlined in red, while the dorsal white matter is outlined with yellow or blue. The ventral white matter shows a less intense LFB staining than the dorsal white matter (Fig. 3E), suggesting more demyelination in ventral white matter at the epicenter 7 days after mild impact. The result of LFB staining is consistent with significant elevation of radial diffusivity in ventral white matter (Fig. 1 D). The decrease in SMI-31 staining in dorsal white matter at the epicenter 7 days after impact (Fig. 3F) shows severe axonal degeneration and is in good agreement with the significant decrease in axial diffusivity (Fig. 1A). These histology findings are consistent with previously reported studies demonstrating that axial and radial diffusivities may be surrogate markers of axonal and myelin degeneration, respectively (1).

In this study the capability of directional diffusivity as a surrogate marker for axonal and myelin degeneration was validated with histology in a mouse model of spinal cord contusion injury. Interestingly, there is a significant elevation of radial diffusivity in ventral but not dorsal white matter, suggesting selective demyelination of ventral but not dorsal white matter. It is not clear why such a different pattern of injury occurs in different regions of the cord. However, the capability of performing noninvasive DTI on spinal cords from live mice reported herein offers a unique opportunity to investigate the underlying mechanism of spinal cord injury.

References

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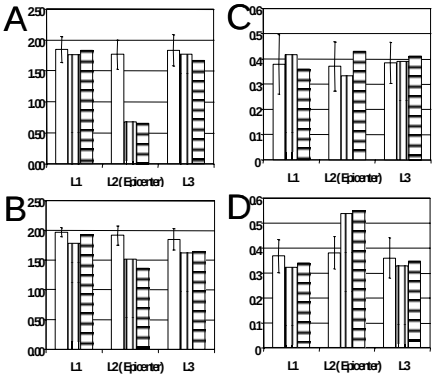

Figure 1. The DTI parameter changes for dorsal (A, C) and ventral (B, D) white matter at L1, L2 (epicenter) and L3 from an injured mouse. A and B show axial diffusivities in μm/msec while C and D show radial diffusivities. White bars are the average of 5 normal cords expressed as mean ± SD. Vertically-hashed bars = day 1; horizontally-hashed bars = day 7.

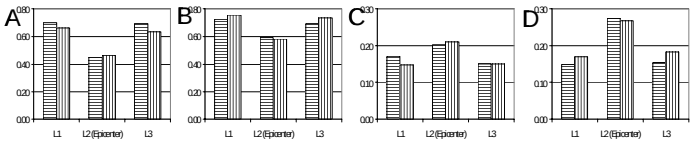

Figure 2. The trace normalized directional diffusivities at 7days after impact. A (dorsal) and B (ventral) show normalized axial diffusivities while C (dorsal) and D (ventral) show normalized radial diffusivities. Horizontally-hashed bars = *in vivo*; vertically-hashed bars = *ex vivo.*

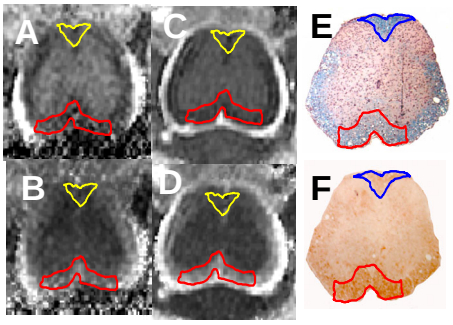

Figure 3. Images of cords from the L2 level in injured mouse: (A) *in vivo* $\lambda_{\perp}$, (B) *in vivo* $\lambda_{\|}$, (C) *ex vivo* $\lambda_{\perp}$, (D) *ex vivo* $\lambda_{\|}$, (E) LFB (myelin), and (F) SMI-31 (phosphorylated neufofilament for intact axons). The hyper-intense rim outlining the cord in DTI maps, incomplete *in vivo* and complete *ex vivo*, is cerebrospinal fluid (CSF).