Correlating the decreased axial diffusivity with morphological changes after axonal injury

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Introduction

Axial ($\lambda_{\parallel}$) and radial diffusivities ($\lambda_{\perp}$) derived from Diffusion Tensor Imaging (DTI) have been demonstrated as potential biomarkers of axonal and myelin damage in the central nervous system (1, 2). In the contusion Spinal Cord Injury (SCI), the decreased ventrolateral white matter (VLWM) axial diffusivity has been reported in the hyper-acute phase (3-5). However, the mechanism underlying the decreased axial diffusivity in contusive SCI is still unclear. In this study, the spinal cords of transgenic thy1-YFP-16 mice undergoing a moderate (0.8 mm displacement) T9 contusive injury were examined using *in vivo* DTI followed by the *ex vivo* confocal microscopic examination. The result reveals the morphological change resulting from axonal damage, which suggests that axial diffusivity is a sensitive and specific biomarker to detect axonal damage.

Materials and Methods

Ten 10-12 week-old female transgenic thy1-YFP-16 mice, weighing 19-22 g, were first undergoing naïve *in vivo* scans as its own control. After sham operation or contusive SCI, mice were scanned again in the hyper-acute phase (~3hrs) to evaluate the VLWM integrity.

Spinal Cord Injury

All mice were anesthetized by the inhalation of isoflurane/oxygen mixture. After dorsal laminectomy at the T9 vertebral level, mice received 0.8 mm displacement at 0.2 m/sec impact speed utilizing a modified OSU impactor. The surgical site was closed in layers with 4-0 Vicryl and nylon sutures. Injection of lactated ringers was administered subcutaneously after the surgery.

Diffusion Tensor Imaging

Data were acquired pre- and post-SCI by using spin-echo sequence modified by adding *Stejskal-Tanner* diffusion-weighting gradient. The spin echo time (TE) = 38 msec, time between application of gradient pulses (Δ) = 21 msec, and diffusion gradient on time (δ) = 7 msec for all measurements. The repetition time (TR~1.5 sec) was varied dependent on the period of respiratory cycle (~270 msec). Three different image slices were collected during every breath. Images were obtained with diffusion sensitizing gradients applied in six-directions: (Gx,Gy,Gz) = (1,1,0), (1,0, 1), (0, 1, 1), (-1, 1,0), (0,- 1, 1), and (1,0,- 1). Two diffusion-sensitizing b values, 0 and 1.02 ms/μm^2 were used. Eight scans were averaged per k-space line with field of view (FOV) = 1 × 1 cm^2 and data matrix = 128 × 256 (zero-filled to 256×256).

Confocal Microscopy

A Zeiss (Oberkochen, Germany) LSM510-META laser scanning confocal microscope was used for imaging YFP^+ axons of ventrolateral white matter using the Z-stack mode (5 optical sections in the z-axis) and a 40× (water immersion; numerical aperture, 1.2) with a 488 nm argon laser.

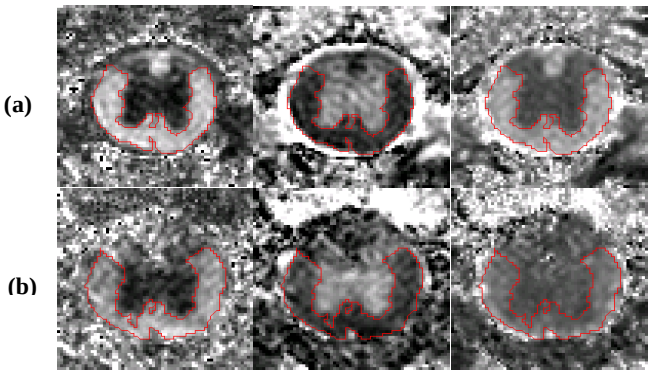

Fig. 1 DTI index maps of a (a) naïve and (b) injured (acute) mouse spinal cord.

Results

RA and radial diffusivity of DTI index maps provide good contrast between gray and white matter both in the naïve and injured cords at T9 vertebrae level (Fig. 1). The decrease in gray to white matter contrast in the axial diffusivity map after injury is consistent with a significant decrease in axial diffusivity after cord injury. From the region of interest (ROI) analysis of DTI maps (Fig. 1), the measured DTI index values are summarized (Fig. 2). No significant difference of DTI parameters in VLWM was observed between naïve and sham operated animals enabling the use of the naïve animal as its own control. The decreased RA is primarily caused by the decreased axial diffusivity, while the radial diffusivity does not change significantly between the naïve and injured cords. A series of confocal images on the YFP^+ axons were then captured from the impact center, 1.0, and 2.0 mm caudal to the impact site. Results show that axial diffusivity correlate with axonal damage in SCI (Fig.3).

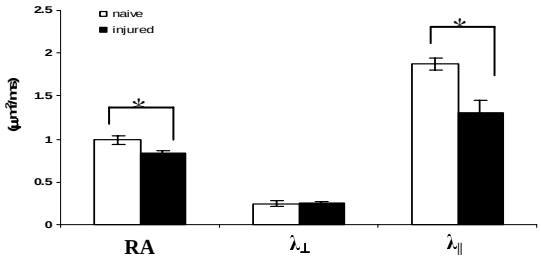

Fig. 2 Comparison of measured DTI indexes - RA, $\lambda_{\perp}$ and $\lambda_{\parallel}$ between naïve and injured VLWM in hyper-acute phase. *(RA is the dimensionless index.)*

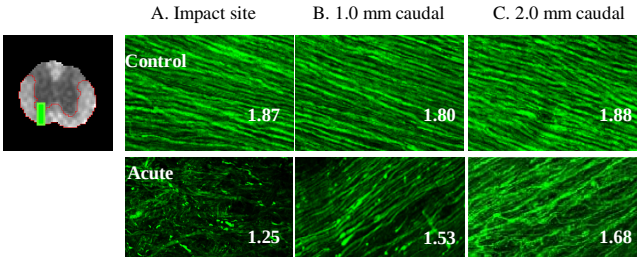

Fig. 3 Confocal images of VLWM show axonal damage after contusion SCI at T9 vertebrae level. A: at impact site, B: 1.0 mm caudal to the impact site C: 2.0 mm caudal to the impact site. The top row is for the control, the bottom row is for the hyper-acute. The numbers indicate the axial diffusivity.

Discussions and Conclusions

Axonal injury is evidenced by axial diffusivity and microscopic findings of transgenic YFP mice. Swelling, beading, and compressed fragmenting YFP+ axons were observed in the injured cord, parallels decreased axial diffusivity. Moreover, the further the axon fibers are away from the impact site, the better the fiber morphology remains. The correspondent axial diffusivity strongly correlates with the axonal fiber integrity, which also indicates the degree of axonal injury. The current findings suggest that axial diffusivity is a sensitive biomarker to detect axonal injury non-invasively in the mouse model of contusive SCI.

References

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