Assessing Axonal Injury, Demyelination, Inflammation and Tissue Loss in Mouse Contusion Spinal Cord Injury

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Introduction

Traumatic spinal cord injury (SCI) is a devastating neurological disorder affecting 12,000 individuals every year in the USA alone. The rodent model of contusion spinal cord injury (SCI) has been widely employed to investigate the underlying pathophysiological mechanisms and as a test bed for the preclinical drug trials[1]. According to the reported mechanism and time course of dynamic cellular responses after SCI in rodent models, increased cell density at sub-acute phase (1 – 4 day post injury) due to immune cell infiltration and proliferation of resident cells is expected while neuron degeneration and oligodendrocyte apoptosis would dominante causing tissue loss at the chronic phase (2 weeks post injury)[2-5]. However, no MRI study has so far looked into inflammation associated cellularity increase or increased water content due to tissue loss and vasogenic edema. Herein, diffusion basis spectrum imaging (DBSI)[6] was employed to simultaneously quantify axon and myelin integrity as well as the extend of inflammation and tissue loss at sub-acute and chronic phase of mouse spinal cord contusion injury.

Method

Contusion injury: T10 (vertebral level) laminectomy was performed on 12 – 14 weeks old male C57BL/6J mice (n=6). After laminectomy, the mouse spine was supported and stabilized with a custom-designed holder. The contusion injury was delivered using a custom-fabricated electromagnetically driven impact device, the OSU design, on the exposed spinal cord with a speed of 0.4m/s and displacement of 0.8mm at T10 vertebral level. The detailed procedure is similar to that described previously[7]. All surgical interventions and animal care were performed in accordance with the Public Health Service Policy on Humane Care and Use of Laboratory Animals,Guide for the Care and Use of Laboratory Animals and with the approval of the Washington University Institutional Care and Use Committee. ***MRI***: Mice were subjected to intra-cardiac perfusion fixation using 0.01M phosphate-buffered saline (PBS) followed by 4% paraformaldehyde in 0.1M PBS on the 3rd (n=3) and 14th (n=3) day post injury (DPI). Mouse vertebral columns were excised, post-fixed overnight. Fixed mice cords underwent ex vivo DBSI examination on a 4.7 T scanner. A solenoid coil was used as both transmit and receive coil. Images of 3 contiguous transverse slices covering T9 through T11 vertebral segments were acquired using the following parameters: TR 1.0 sec, TE 38 ms, Δ 20 ms, δ 5 ms, slice thickness 2.0 mm, spatial resolution (78 μm x 78 μm), total data acquisition time ~ 3.0 hr, diffusion gradient were applied along 99 directions on a 3D grid with maximum b value of 3000 s/mm^2.

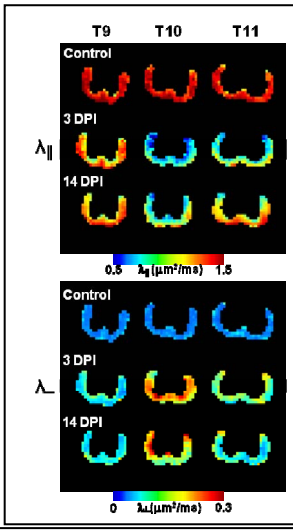

Figure 1. Representative DBSI-derived $\lambda_{\parallel}$ and $\lambda_{\perp}$ maps of spinal cords at T9, T10 (injury epicenter) and T11 vertebral level VLWM region from the control, 3 and 14 DPI mice. Both 3 and 14 DPI cords show significantly lower $\lambda_{\parallel}$ and higher $\lambda_{\perp}$. VLWM of T10 level at the 3 DPI cord has the lowest $\lambda_{\parallel}$ and highest $\lambda_{\perp}$ value.

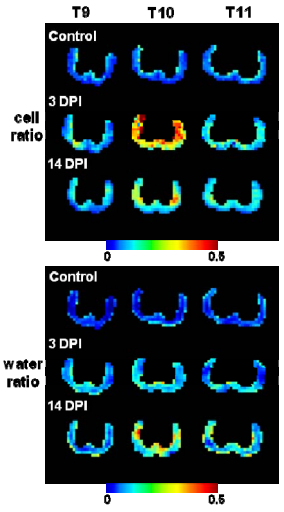

Figure 2. Representative DBSI-derived cell and water ratio maps of T9, T10 (injury epicenter) and T11 vertebral level VLWM region from control, 3 and 14 DPI mice show increased cell and water ratio after injury. VLWM of T10 level of the 3 DPI cord has the highest cell ratio while VLWM of T10 level of the 14 DPI cord has the highest water ratio

Results and Discussions

Regions of interest of ventro-lateral white mater (VLWM) area were manually drawn on each slice. $\lambda_{\parallel}$, $\lambda_{\perp}$, cell ratio, and water ratio of VLWM were calculated using DBSI. As demonstrated in DBSI-derived $\lambda_{\parallel}$ and $\lambda_{\perp}$ maps (Fig. 1), decreased $\lambda_{\parallel}$ and increased $\lambda_{\perp}$ can be clearly observed at injury epicenter (T10), indicating axon injury and demyelination respectively. Compared to the control cords, mean $\lambda_{\parallel}$ of T9 – T11 levels of the 3 DPI cords decreased 12%, 35% and 23%, while mean $\lambda_{\perp}$ increased 36%, 105% and 61% respectively. On the other hand, 15%, 28% and 17% lower $\lambda_{\parallel}$ as well as 33%, 114% and 35% higher $\lambda_{\perp}$ were seen in T9 – T11 level at 14 DPI, respectively. The information of two other critical components of SCI pathology: cell infiltration/proliferation and edema/tissue loss, can also be extracted by DBSI, as cell ratio and water ratio. The 3 DPI cords exhibited 48%, 170%, and 29% higher cell ratio compare to that of control at T9 – T10 level respectively. This result stands in line with the fact that at sub-acute phase (1 – 4 DPI) of SCI, the affected spinal cord reaches the peak of cell proliferation and infiltration. At the same time, 93%, 86% and 41% increased water ratio can also be seen in the 3 DPI cords probably as a result of vasogenic edema. For the 14 DPI cords, cell ratio of the VLWM region are 65%, 75% and 61% higher than that of control cords while water ratio are 96%, 149% and 75% higher, likely due to chronic immune cell invasion/activation and tissue loss, respectively.

	$\lambda_{\parallel}$	$\lambda_{\perp}$	cell %	water%
		T9		
Control	1.40 ± 0.01	0.080 ± 0.002	10.4 ± 0.8	6.2 ± 1.4
3DPI	1.23 ± 0.02	0.109 ± 0.008	15.4 ± 1.7	12.0 ± 1.9
14DPI	1.18 ± 0.06	0.107 ± 0.002	17.1 ± 1.8	18.0 ± 0.3
		T10		
Control	1.39 ± 0.01	0.082 ± 0.007	11.5 ± 1.4	7.8 ± 1.1
3DPI	0.90 ± 0.18	0.168 ± 0.034	31.1 ± 14.0	14.6 ± 1.8
14DPI	1.00 ± 0.05	0.175 ± 0.023	20.1 ± 2.7	19.4 ± 3.0
		T11		
Control	1.36 ± 0.02	0.087 ± 0.007	12.1 ± 1.2	8.5 ± 3.2
3DPI	1.05 ± 0.05	0.139 ± 0.014	15.7 ± 2.9	12.0 ± 1.9
14DPI	1.13 ± 0.05	0.117 ± 0.004	19.5 ± 1.4	15.0 ± 0.6

Table 1. DBSI parameters ($\lambda_{\parallel}$, $\lambda_{\perp}$, cell ratio, and water ratio) of VLWM from T9 – T11 vertebral segments of spinal cords from control, 3 and 14 DPI mice (Mean ± STD).

Conclusion

The DBSI results in current study are in agreement with well documented time course of cellular responses after SCI in rodent model. This indicates that DBSI not only provides more accurate diffusivity estimates but also, for the first time as an MRI method estimating cell and water ratio, which is equally critical to progonosis and evaluating new drugs for SCI.

References

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