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. 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 dominate causing tissue loss at the chronic phase (2 weeks post injury). 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) was employed to simultaneously quantify axon and myelin integrity as well as the extent 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. 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/mm².

Results and Discussions

Regions of interest of ventro-lateral white mater (VLWM) area were manually drawn on each slice. λ₁, λ₂, λ₃, cell ratio, and water ratio of VLWM were calculated using DBSI. As demonstrated in DBSI-derived λ₁ and λ₃ maps (Fig. 1), decreased λ₂ and increased λ₃ can be clearly observed at injury epicenter (T10), indicating axon injury and demyelination respectively. Compared to the control cords, mean λ₁ of T9 – T11 levels of the 3 DPI cords decreased 12%, 35% and 23%, while mean λ₃ increased 36%, 105% and 61% respectively. On the other hand, 15%, 28% and 17% lower cell content compared to the control cords at T9 – T11 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 is 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.

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