Establishment of the *In Vivo* Tracing of Spinal Pathway Using Diffusion Tensor Tractography in Nonhuman Primates

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Introduction: We previously established a reproducible spinal cord injury (SCI) model in adult common marmosets and demonstrated that transplantation of human neural stem/progenitor cells into the injured spinal cord promoted functional recovery [1]. Although the evaluation of axonal fibers is essential to assess the severity of injury and efficacy of any treatment protocol, conventional methods such as tracer injection in brain parenchyma are highly invasive and technically demanding. Furthermore, since histological examinations are required to evaluate tracer studies, it has been impossible to evaluate axonal fibers in vivo and follow the sequential growth of axonal fibers in the same animal. We therefore sought to establish a non-invasive method to evaluate axonal fibers *in vivo* using diffusion tensor tractography (DTT), a new magnetic resonance imaging technique that makes in vivo tracing of axonal fibers possible. The properties and clinical applications of DTT in the brain have been reported, but technical difficulties have limited DTT studies of the spinal cord. In this study, we report the effective use of DTT to visualize both intact and surgically disrupted spinal long tracts in adult common marmosets**.**

Materials and Methods: A total of nine adult common marmosets were used. One group (n=3) received a hemisection SCI at the C5/6 level, and the other group recived contusion SCI at C5 using modified NYU impactor (17g)(n=3) and the remaining animal was used as a non-operate naïve control (n=3). The spinal cord was imaged in a 7.0 Tesla MRI at 2 weeks after operation in the hemisection group. In contusion model, the spinal cord was imaged in MRI at 2, 4 weeks after injury . Diffusion tensor MRI data and fiber tracking were analysed with software Volume One and dTV IISR [2]. To verify the feasibility of spinal cord DTT, we first performed DTT of postmortem marmosets and then performed the DTT in live models. Histological examination using Hematoxylin-Eosin, Luxol fast blue (to detect white matter) and calmodulin dependent protein kinase staining to detect the corticospinal tract was conducted and confirmed the DTT findings in both control and injured groups.

Magnetic resonance imaging: MRI was performed using a 7.0 Tesla-MRI, PharmaScan 70/16 (Bruker BioSpin, Germany) with a coil dedicated for small animals. In the studies using postmortem animals (control, hemisection, contusion = 2 each), conventional T2WI were first obtained, followed by intracardiac perfusion with 4% paraformaldehyde (PFA; pH 7.4) and diffusion Fig: DTT of the Hemisected cervical cordtensor MRI. T2WI and diffusion tensor MRI of the hemisected animals were conducted 2 weeks after injury. DTI data sets were acquired with a spin-echo sequence based on the Stejskal-Tanner diffusion preparation. Scanning parameters were as follows; repetition time (TR) = 15000ms, echo time (TE) = 40ms, flip angle = 90deg, field of view (FOV) = 55 × 55mm, acquisition data matrix = 256 × 256, reconstructed image resolution = 0.215mm (with zero-filling interpolation), slice thickness = 0.85mm, b-value = 1000sec/mm^2, and motion probing gradient (MPG) orientations = 12 axes, number of avaraging (NA) = 1. In the studies using live animals (control and hemisection group n = 1 each), conventional and diffusion tensor MRI were performed under the general anesthesia as mentioned above. MRI scans of the hemisected animal were conducted 2 weeks after injury. In live animals, to reduce motion artifacts from the blood flow and cerebrospinal fluid flow, animals were immobilized on an acrylic bed with a specially designed head positioner and electrocardiogram (ECG) probe (SA instruments, U.S.A) for gated imaging was attached to the animal's front thorax. DTI data sets in live animals were acquired with an ECG gated standard diffusion weighted spin-echo pulse sequence based on the Stejskal-Tanner diffusion preparation (Stejskal and Tanner, 1965). Scanning parameters were as follows; repetition time (TR) = 3500ms, echo time (TE) = 40ms, flip angle = 90deg, field of view (FOV) = 40 × 40mm, acquisition data matrix = 128 × 128, reconstructed image resolution = 0.31 × 0.31mm, slice thickness = 0.94mm, b-value = 1000sec/mm^2, and motion probing gradient (MPG) orientations = 12 axes, NA = 1.

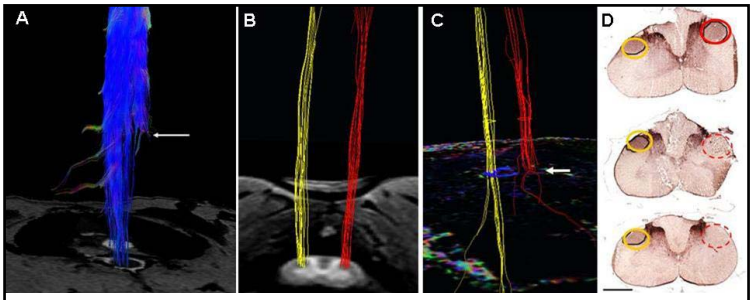

Fig 1. DTT of the white matter fibers in the hemisection SCI of marmoset (A), DTT of the corticospinal tract in intact (B) and hemisected (C) cervical cord of marmoset. CaMK Ⅱ α staining of the hemisected cervical cord (D).

Results and Discussions: DTT clearly illustrated spinal projections such as the corticospinal tract and afferent fibers in control animals and depicted the severed long tracts in the injured animals (Fig 1, 2). Furthermore we succeed in demonstrating the pyramidal decussation of the marmoset. To the best of our knowledge, there is no report about DTT of the pyramidal decussation (Fig 3). Histology of the spinal cords in both control and injured groups were consistent with DTT findings, verifying the accuracy of DTT. We also conducted DTT in live marmosets and demonstrated that DTT can be performed in live animals to reveal in vivo nerve fiber tracing images, providing an essential tool to evaluate axonal conditions in the injured spinal cord. Taken together, these findings demonstrate the feasibility of applying DTT to preclinical and clinical studies of spinal cord injury.

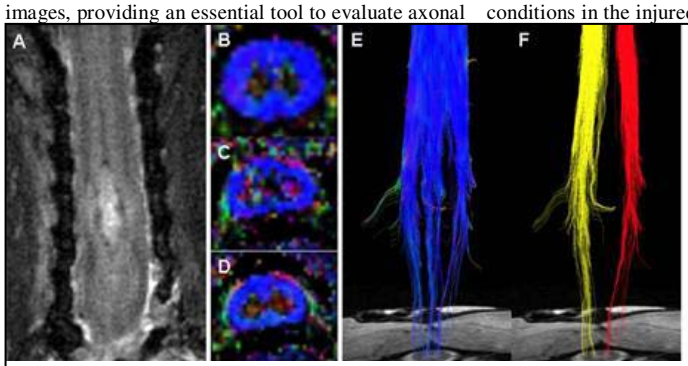

Fig 2. Conventional T2WI (A), color coded FA map (B,C,D), DTT of the white matter (E) and the corticospinal tract in the contusive SCI of marmoset.

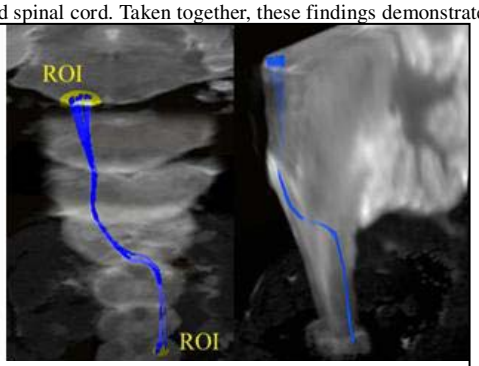

Fig 3. Pyramidal decussation of marmoset.

Conclusion: Our results revealed that DTT can depict the course and the disruption of specific neural pathways even in live animals, demonstrating the possible contribution of DTT to the clinical studies of SCI therapy. DTT of the spinal cord is a powerful tool with tremendous potential if its properties and limitations are fully understood and correctly applied.

References

[1] *Iwanami A., and Yamane J., et al:J Neuro. Res. 2005; 80:172-81.* [2] *Masutani Y., et al: Eur J Radiol 2003; 46:53-66.*