In vivo high Resolution q-space imaging of the spinal cord injury in Nonhuman primates

K. Hikishima[1,2], M. Nakamura[3], K. Fujiyoshi[3,4], M. Yamada[2,5], K. Kitamura[3,4], S. Momoshima[6], K. Yagi[7], N. Tamaoki[2], Y. Toyama[3], and H. Okano[4]

[1]Center of Integrated Medical Research, Keio University School of Medicine, Sinjuku, Tokyo, Japan, [2]Central Institute for Experimental Animals, Kawasaki, Kanagawa, Japan, [3]Department of Orthopaedic Surgery, Keio University School of Medicine, [4]Department of Physiology, Keio University School of Medicine, [5]School of Health Sciences, Fujita Health University, [6]Department of Radiology, Keio University School of Medicine, [7]Department of Radiological Science, Tokyo Metropolitan University Graduate School of Health Science

Introduction Diffusion Weighted Image (DWI) enables us to evaluate more directly the biological structures than other MR methodologies. In particular, Diffusion Tensor Imaging (DTI) is a powerful tool to evaluate fibers of white matter in the central nervous system. We previously established reproducible spinal cord injury (SCI) model in adult common marmosets [1] and have reported that DTT enables *in vivo* tracing of neural tracts in intact and injured spinal cord of marmosets [2]. On the other hand, q-space imaging (QSI) which has been used to detect the size of microstructure using higher b-value, is one of the latest methodologies of DWI. There are some reports about *ex vivo* QSI of the spinal cord after injury in adult rats [3], but no report about *in vivo* QSI of the spinal cord in nonhuman primates. To evaluate the structural changes of the injured spinal cord in the same animal longitudinally. In this study, we performed *in vivo* high resolution QSI of both intact and injured spinal cords in common marmosets and confirmed the accuracy of QSI through histology.

Materials and Methods **MRI experiments:** MRI was performed on a 7T PharmaScan 70/16 System (Bruker BioSpin, Germany). A 60mm (i.d.) volume coil tuned 300.5MHz for proton resonance was used. DW SE sequence parameters were 3500ms/37.8ms (TR/TE), matrix of 256×128, FOV of 4.1 $(cm)^2$, slice thickness of 1.5mm, NA of 1. Diffusion experiments were carried out with different 12 b-values from 0 to 12200s/mm^2 (corresponding to 0~1034cm^{-1} in q-value). MPG duration time (δ) and separation time (Δ) were kept at 12ms and 20ms. MPG direction was perpendicular to the long axis in the spinal cord. **Subjects:** Eight adult female common marmosets were used in the present study. All surgeries were performed under general anesthesia induced by intramuscular injection of ketamine and maintained by isoflurane. After laminectomy at the C5 level, contusive SCI (17g) was induced in common marmoset using modified NYU device. Then we performed QSI at 3day, 1, 2, 4, and 10 weeks after injury. Two animals were used as a naive control. **Post image processing:** After linear interpolation of the DW images to the matrix of 256×256, the normalized diffusion curve $E_\Delta(b)$ was rearrangement to q-space $E_\Delta(q)$. The displacement profile P(R) was calculated from the FFT of the $E_\Delta(q)$. $P(R) = FFT(E_\Delta(q))$. We used the 0.425 FWHM of the P(R) as the displacement value [4]. **Histological analysis:** After QSI at 3days, 1, 2, 4, and 10 weeks after injury each animal was perfused intracardially with 4% paraformaldehyde and then the spinal cord tissues were removed. Tissue preparation were stained with hematoxylin-eosin (HE) for general histological examinations and Luxol fast blue (LFB) for evaluation of the myelinated area. Immunostaining with anti-glial fibrillary acidic protein (GFAP) antibody was performed to examine the glial scar formation. Two animals (one is intact and another is injured spinal cord at 2weeks after injury) were used for electron microscopic evaluation.

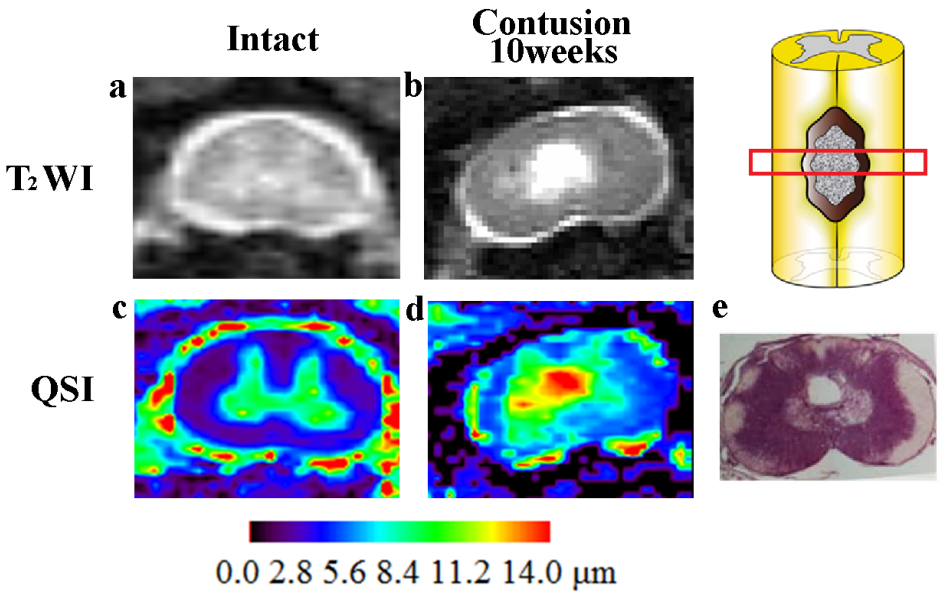

Fig.1 In-vivo high resolution q-space displacement map of spinal cord: T_2WI of (a) intact and (b) injury subject. QSI maps of (c) an intact subject and (d) an injury subject. And (e) HE stain figure.

Results and Discussions At 3day after injury, the microstructural size of inner white matter increased, and the contrast of white and gray matter of the map lost gradually. At 1week after injury, the displacement further increased in localized area to the epicenter. These changes were similar to the region of demyelination in the white matter and infiltration of inflammatory cells in the gray matter. At 10 weeks after injury, the larger size area which is coded in green to yellow-red color, see Fig.1(c, d) was expanded. Histological examinations revealed that these changes of QSI were consistent with the histological findings such as glial scar and cavity formation. Conventional T2WI showed the existences of SCI with high intensity, but didn't give us any specific information about the histological changes. We succeed in getting *in vivo* high resolution QSI of common marmoset and revealed that QSI map of SCI model depicted the smaller structural change such as infiltration of inflammatory cells, neuronal loss, glial scar formation, which was never detected by conventional MRI, DTI and DTT.

Conclusion We succeeded in *in vivo* high resolution QSI map longitudinally of the injured spinal cord in nonhuman primates. High Resolution QSI map reflect the histological changes of the injured spinal cord and is useful for its evaluation. QSI of the spinal cord will be 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] *Fujiyoshi K., and Yamada M. ,et al:J Neurosci..2007;27:11991-8.* [3] *Nossin RM, et al:J of Neurotrauma.2007; 24(3): 481-91.* [4]*Assaf, et al:NMR Biomed..2002; 15:516-42.*