Diffusion tensor imaging detects axonal injury and demyelination in the spinal cord and brain of a murine model of globoid cell leukodystrophy.

J. H. Kim[1], A. A. Hofling[1], M. S. Sands[2], and S-K. Song[1]

[1]Radiology, Washington University, St. Louis, MO, United States, [2]Divisions of Hematology and Oncology, Washington University, St. Louis, MO, United States

Introduction

Globoid cell leukodystrophy is an inherited neurodegenerative disorder caused by a deficiency of the lysosomal enzyme galactosylceramidase (1, 2). In both human patients and the authentic murine Twitcher model, pathological findings include demyelination as well as axonal damage in both the central and peripheral nervous system. Diffusion tensor imaging (DTI) has emerged as a powerful noninvasive technique that is sensitive to these white matter disease processes. In this study, DTI was applied to Nondairy evaluate the integrity of Twitcher mice CNS white matter integrity. Preliminary results showed a statistically significant decrease in axial (λ||) but increased radial (λ⊥) diffusivity in Twitchier mice CNS white matter relative to those seen in normal controls. These results were consistent with immunofluorescent evidence of axonal damage and demyelination. The application of spinal cord DTI in the setting of GLD holds potential as a noninvasive, quantitative assay of therapeutic efficacy in future treatment studies.

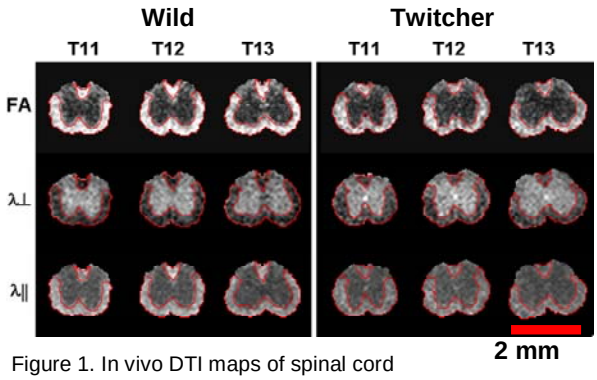

Figure 1. In vivo DTI maps of spinal cord

Methods

Mice homozygous for the twitcher mutation *(twi/twi)* as well as normal control mice (+/+), genotypes determined in newborn mice by a polymerase chain reaction method specific for the twitcher mutation, were employed for in vivo DTI examination on a 4.7 T magnet utilizing respiratory gated spin-echo diffusion-weighted sequence with actively decoupled volume (6-cm inner diameter, RF excitation) and surface coil (16 mm x 9mm , signal receiver). The overall set up is similar to that described previously (3). All images were obtained with acquisition parameters of TR 1.2 sec (gated acquisition), TE 38 ms, Δ 18 ms, δ 7 ms, slice thickness (1.0 mm) , zero filled spatial resolution (38 μm x 38 μm), total data acquisition time ~ 1.0 hr, (Gx,Gy,Gz) = (1,1,0), (1,0,1), (0,1,1), (-1,1,0), (0,-1,1), and (1,0,-1), and b = 0 and 1.0 ms/μm^2. The axonal damage and myelin integrity was examined by staining with antibodies against dephosphorylated neurofilament (SMI-32) and myelin basic protein (MBP).

Dorsal White Matter (n = 5, Mean ± SD)			
	Wild	Twitcher	*P*
FA	0.88 ± 0.05	0.75 ± 0.07	0.01
λ⊥ (μm²/ms)	0.24 ± 0.02	0.29 ± 0.05	0.04
λ|| (μm²/ms)	1.65 ± 0.13	1.38 ± 0.05	0.001

Ventrolateral White Matter (n = 5, Mean ± SD)			
	Wild	Twitcher	*P*
FA	0.85 ± 0.05	0.73 ± 0.04	0.02
λ⊥ (μm²/ms)	0.28 ± 0.03	0.32 ± 0.03	0.052
λ|| (μm²/ms)	1.69 ± 0.12	1.45 ± 0.02	0.001

Table 1. The quantified DTI parameters from spinal cord (n = 5, for each group).

Results and Discussion

Representative *in vivo* DTI maps of the spinal cord at the T11, T12, and T13 levels clearly demonstrated a distinctive gray to white matter contrast in both control and Twitchier mice (Fig. 1). DTI parameters from the manually delineated dorsal and ventrolateral white matter ROIs (Fig. 1) of the spinal cord are shown in Table 1. Combined means ± SD from the T11, T12, and T13 levels were compared between Twitcher mice (n=5) and age-matched wild-type littermates (n=5). In the dorsal and ventrolateral white matter, statistically significant decrease in FA and λ||was detected in Twitcher mice compared to wild-type. In contrast, the twitcher mice dorsal white matter λ⊥ increased statistically significantly in relative to that of littermates, but not in the ventrolateral white matter (p=0.052). Immunofluorescent detection of SMI32 and MBP in the dorsal white matter (DWM) and ventrolateral white matter (VWM) of the spinal cord show extensive increase in SMI-32 positive axons (green color, Fig. 3a) consistent with axonal damage in both the DWM and VWM of the Twitcher cord. Although a mildly generalized decrease in MBP staining is seen in the twitcher cord relative to the control cord (red color, Fig. 3b), the majority of Twitcher axons continue to display myelin sheaths. Focal areas of further decreased MBP staining are identified in the DWM and VWM of the twitcher mouse but not the normal control indicating mild, patchy demyelination (Fig. 3b, insets).

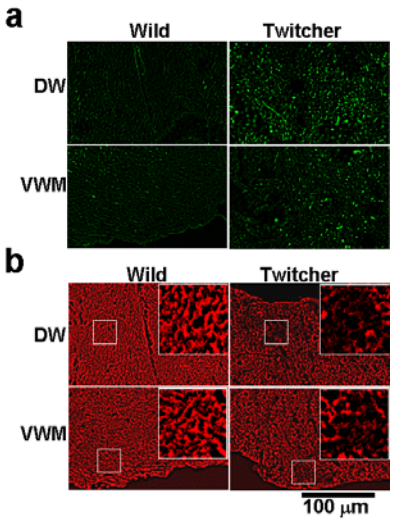

Figure 2. SMI-32 (a) and MBP stain of spinal cord white matter. DW: dorsal white matter and VWM: ventral white matter.

Conclusion

The statistically significant DTI abnormalities in spinal cord of Twitcher mice establish this imaging method as a noninvasive, quantitative assay for the therapeutic efficacy of treatment strategies. This hypothesis will be especially interesting to test in the spinal cord, particularly with treatments such as intrathecal delivery of therapeutic agents as has been performed in other mouse models of lysosomal storage disease.

References

1. Suzuki *et al.,*PNAS, 1970. 2. Wenger *et al.,*Hum Muata, 1997. 3. Kim *et al.,* Neurobio. Dis., 2006.

Acknowledgements: This study was supported by NIH: R01 NS 047592 and R01 NS 054194.