Reversible Axonal Injury Detected by DTI and Immnohistochemistry

H-F. Liang[1], S-W. Sun[1], P-C. Chang[2], C. Chang[2], A. H. Cross[3], and S-K. Song[1]

[1]Radiology, Washington University School of Medicine, St. Louis, MO, United States, [2]IBMS, Academia Sinica, Taipei, Taiwan, Taiwan, [3]Neurology, Washington University School of Medicine, St. Louis, MO, United States

Introduction

The axial ($\lambda_{\parallel}$) and radial diffusivity ($\lambda_{\perp}$) derived from diffusion tensor imaging (DTI) have been demonstrated as biomarkers of axon and myelin damage respectively (1-3). Previously, the axonal damage of corpus callosum (CC) resulting from cuperzone treatment was shown to be reversible according to the initially decreased and subsequently recovered $\lambda_{\parallel}$ (3). The immunohistochemical staining of nonphosphorylated neurofilament (npNF, SMI-32) and β-amyloid precursor protein (β-APP) were preciously employed to validate the DTI detected axonal injury (3), where the positive staining of npNF and β-APP were seen paralleling the degree of decrease in $\lambda_{\parallel}$. However, the injury positive markers like npNF and β-APP are not capable to distinguish between axonal loss and recovery. Our previous findings have not definitively proved the presence of reversible axonal injury in CC of cuprizone treated mice.

In this study, in vivo DTI and immunohistochemical staining were performed on three groups of mice with normal, injured and recovered axons. Both injured (npNF) and healthy axons (phosphorylated neurofilament, pNF) immunohistochemical markers were employed and the results were quantitatively analyzed. Since each mouse came with its DTI measurements and immunohistochemical staining quantities, the correlation analysis between immunohistochemical stainings and *in vivo* DTI was also performed.

Materials and Methods

Nine 8-week old male C57BL/6 mice were placed on a diet of 0.2% cuprizone thoroughly mixed into chow. At 0, 4, and 8 weeks with treatment, *in vivo* DTI was conducted on 3 randomly picked mice. Data were acquired using Oxford Instruments 200/330 (4.7 T) magnet and spin-echo diffusion weighted imaging sequence with TR 1 sec, TE 70 ms, slice thickness 0.5 mm, field-of-view 3 cm, b 850 s/mm^2, and data matrix 256 × 256. $\lambda_{\parallel}$ and $\lambda_{\perp}$ were measured in CC. At the conclusion of *in vivo* DTI examinations, mice were perfusion fixed with 4% paraformaldehyde in PBS. Three-μm-thick slices matching the DTI images were cut for different histological examinations. The healthy and injured axons were stained using primary antibodies to pNF (SMI-31) and npNF (SMI-32) respectively. Images were captured with a Photometrics CCD digital camera. The healthy (pNF positive) and injured (npNF positive) axons were counted from each tissue sections in a blinded fashion. DTI and immnohistochemistry parameters were analyzed using analysis of variance (ANOVA). For parameters that exhibited significant difference ($p < 0.05$), two-sample post hoc t tests were performed between groups. Difference was considered significant at $p < 0.05$.

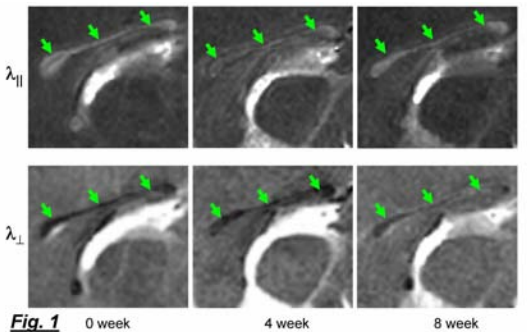

Fig. 1

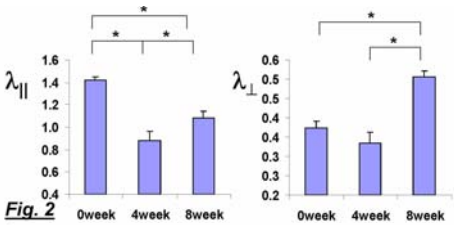

Fig. 2

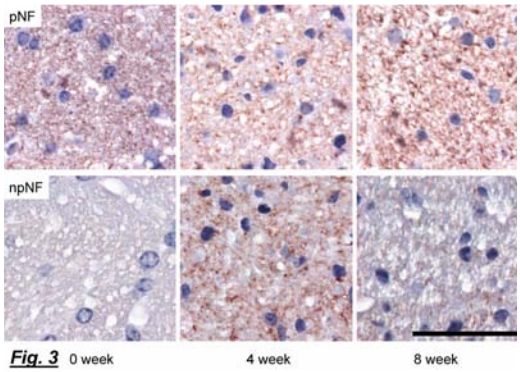

Fig. 3

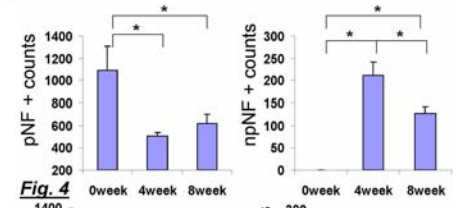

Fig. 4

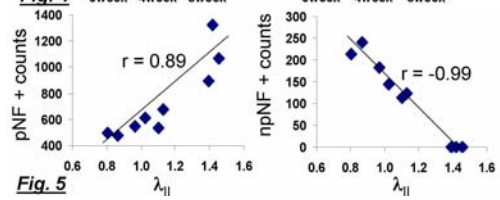

Fig. 5

Results

Examples of $\lambda_{\parallel}$ and $\lambda_{\perp}$ from mice treated with cuprizone for 0, 4, and 8 weeks are shown in Fig. 1 (arrows point to CC), and the measurements are summarized in Fig. 2. $\lambda_{\parallel}$ decreased to 38% of 0 week level ($p < 0.05$) at 4 weeks and recovered to the sub-normal level at 8 weeks suggestive of incomplete recovery of axonal damage from 4 to 8 weeks; a significant 51% increase in $\lambda_{\perp}$ comparing with that of 0 week was seen at 8 weeks suggestive of demyelination. The representative pictures of immunostaining of pNF and npNF at 0, 4, and 8 weeks are shown in Fig. 3, where axons are stained in brown and the scale bar represents 20 μm. At 0 week, significant positive pNF without npNF staining indicated the integrity of axons in CC. In contrast, less pNF staining with increasing npNF staining indicated the presence of axonal damage at 4 weeks. Interestingly, increased pNF and decease npNF were observed at the 8 weeks suggestive of incomplete recovery of axonal pathology. By counting pNF and npNF positive axons, as shown in Fig. 4, the number of healthy axons decreased significantly by 54% from 0 to 4 weeks and increased insignificantly from 4 to 8 weeks ($p = 0.08$) suggestive of the incomplete axonal recovery. As for npNF staining, the injured axons were not detected at 0 week. A significant count at 4 weeks is apparent. From 4 to 8 weeks, 40% decrease in the number of injured axons confirming the axonal recovery. The scatter plots of healthy and injured axon counts vs. $\lambda_{\parallel}$ exhibits the high correlation ($p < 0.05$, Fig. 5).

Discussion and Conclusion

In this study, both DTI and pNF and npNF immunostaining were applied to verify the presence of a reversible axonal damage in CC from mice undergoing cuprizone treatment. The correlation between immunostaining and $\lambda_{\parallel}$ demonstrated that (1) cuprizone treatment resulting in a reversible axonal injury in CC, and (2) $\lambda_{\parallel}$ can serve the biomarker of axonal injury to dynamically monitor the axonal integrity non-invasively.

References *1. Song SK, et al. Neuroimage 2002;17(3):1429-1436.* *2. Song SK, et al. Neuroimage 2003;20(3):1714-1722.* *3. Sun SW, et al. Magn Reson Med 2006;55(2):302-308.*

Acknowledgement: NMSS: RG 3864, CA 1012-A-13; NIH: R01 NS 047592, R01 NS 054194.