Decreased axial diffusivity as a biomarker of axonal injury in the spinal cord white matter of mice with EAE validated with immunofluorescence

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Introduction

An accumulating body of work has shown that a decrease in axial diffusivity derived from DTI reflects axonal injury in white matter[1,2]. It has previously been shown that axial diffusivity of the spinal cord white matter, but not radial diffusivity or relative anisotropy, correlated with the degree of neurological impairment a mouse model of Multiple Sclerosis[3]. We performed *in vivo* DTI of the spinal cord of mice with experimental autoimmune encephalomyelitis (EAE) and compared maps of axial diffusivity to immunofluorescence stains to demonstrate the relationship between neurological impairment, axial diffusivity, and histological markers of axonal damage.

Methods

EAE was induced in ten 10-week old C57Bl/6 mice with 50 μg MOG_{35-55} in complete Freund's adjuvant with five age-matched mice serving as controls. Clinical scores were assessed daily. I*n vivo* DTI was performed at the chronic phase of the disease (> 21 dpi). Mice were placed in a custom MR compatible holder and RF coil. *In vivo* DTI of the spinal cord was performed with the following parameters: TR=1.5s, TE=49 ms, Δ=25 ms, δ=10 ms, signal averages=4, slice thickness=1.0 mm, FOV=1 cm^2, data matrix 128 x 128 (zero filled to 256 x 256), 6 diffusion encoding directions, b-values of 0 and 0.785 ms/μm^2. DTI parameter maps were calculated for $\lambda_{\parallel}$, $\lambda_{\perp}$, and relative anisotropy (RA).

Immediately following imaging, mice were perfusion fixed with 4% paraformaldehyde. Spinal cords were excised and placed in fixative overnight. Sections were cut at a thickness of 3μm and adjacent sections were stained with primary antibodies for either non-phosphorylated neurofilaments (SMI-32, Sternberger Monoclonals Inc.) to indicate degenerating axons, phosphorylated neurofilaments (SMI-31, Sternberger Monoclonals Inc.) to indicate normal axons, or myelin basic protein to indicate normal myelin. Fluorescence images were digitized using identical intensity and exposure time settings for each of the primary antibodies.

Results

The spinal cord white matter of mice with moderate EAE (CS2) displays SMI-32 positive axons along the perimeter of the ventrolateral white matter, whereas mice with severe EAE (CS4) display a greater number of SMI-32 positive axons. The pattern of injury appears to coincide with the decrease in axial diffusivity on the DTI maps. The white matter of mice without EAE (CS0) does not contain any SMI-32 positive axons. In the SMI-31 stained sections, the white matter of mice without EAE shows small punctuate, normal appearing axons. In both mice with moderate and severe EAE, significant numbers of swollen axons are visible along the perimeter of the white matter, which is a further indication of axonal damage. The SMI-31 swollen axons and SMI-32 positive axons overlap in the same regions.

Discussion

The current results confirm previous reports showing that axonal damage as detected by histology correlates with neurological impairment in chronic EAE. These results also provide further evidence that the decrease in axial diffusivity with worsening severity in EAE corresponds to an increase in the amount of axonal damage. Furthermore, the pattern of axonal damage on the histological sections coincides with the pattern of axial diffusivity decreases detected *in vivo*.

Conclusions

The results further support the use of axial diffusivity as a noninvasive measure of axonal damage in white matter. The application of the directional diffusivities to Multiple Sclerosis may provide the highly sought link between pathology and neurological impairment, which could ultimately lead to more accurate diagnosis and better patient management.

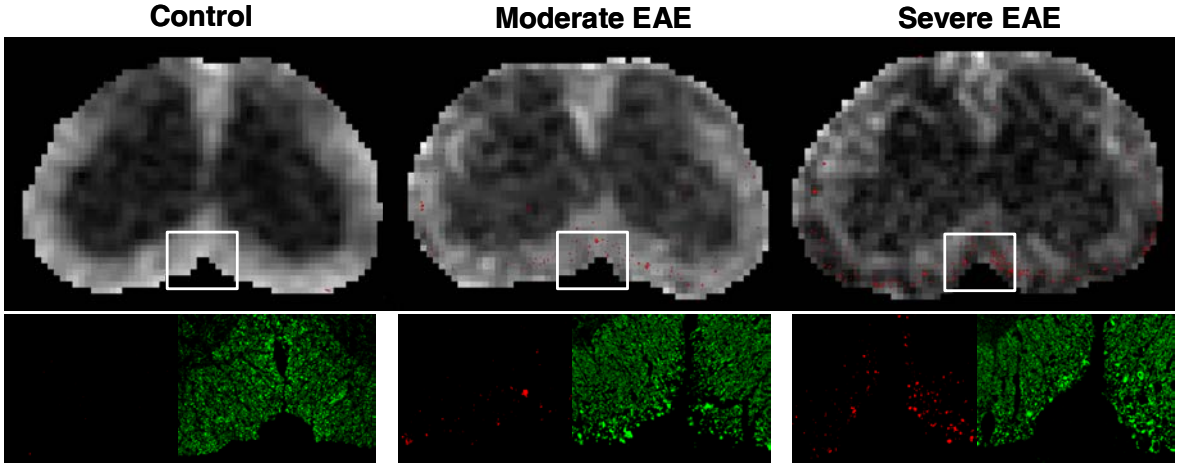

Figure 1. Axial diffusivity (top) decreases in the spinal cord white matter with worsening severity of EAE. Immunofluorescence for non-phosphorylated neurofilaments (red) were superimposed on the axial diffusivity maps and demonstrates the regional correspondence between axonal damage and decreases in axial diffusivity. The magnified regions (bottom) that were stained for non-phosphorylated neurofilaments (red) and phosphorylated neurofilaments (green) further demonstrate the increase in axonal damage that occurs with worsening disease

References

1. Song, S.K. et al. Neuroimage 20, (2003) 2. Song, S.K. et al. Neuroimage 17, (2002) 3. Budde, M.D. et al. ISMRM #27 (2006)