Axial diffusivity correlates with the degree of neurological disability in a mouse model of Multiple Sclerosis

M. D. Budde[1], H-F. Liang[1], A. H. Cross[1], S-K. Song[1]
[1]Washington University, St Louis, MO, United States

Introduction

The pathologic characteristics of Multiple Sclerosis (MS) and experimental autoimmune encephalomyelitis (EAE), a mouse model of MS, include varying degrees of demyelination, inflammation, and axonal damage[1]. The directional diffusivities derived from diffusion tensor imaging (DTI), axial and radial diffusivity, have proven useful in detecting axonal and myelin damage, respectively[2,3]. We applied DTI in addition to contrast enhancement imaging in order to detect the pathological processes in the spinal cord white matter of mice with acute and chronic EAE. The work has implications to the detection of pathology in MS and the use of DTI as a tool to monitor disease progression and therapeutics.

Methods

Thirteen 10-week old C57Bl/6 mice were immunized with 50 μg MOG_{35-55} in complete Freund's adjuvant. Clinical scores were assessed daily. I*n vivo* DTI was performed at either the peak of the acute (n=6) or chronic (n=7) phase of the disease. Two age-matched mice served as controls. 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 $ms/\mu m^2$. DTI parameter maps were calculated for $\lambda_{||}$, $\lambda_{\perp}$, and relative anisotropy (RA). T1-weighted spin echo images (TR=700ms, TE=18ms, signal averages=4) were collected immediately before and 6 minutes after the administration of 0.2 ml/kg (0.3 mmoles/kg) gadopentetate dimeglumine (Magnevist) through the tail vein. Enhancement maps were computed using the equation $(S_{post}-S_{pre})/S_{pre} * 100$. Manually drawn regions of interest encompassing the ventrolateral white matter were used for subsequent analyses. Group differences were assessed with a Student's t-test. Correlations between EAE clinical scores and MR parameters were assessed using Spearman's rho.

Spinal cords were perfusion fixed in 4% paraformaldehyde immediately following imaging. Adjacent sections were stained with Luxol fast blue-Periodic acid-Schiff (LFB-PAS), hematoxylin-and-eosin (H&E), and for phosphorylated neurofilaments (SMI-31, Sternberger Monoclonals Inc.).

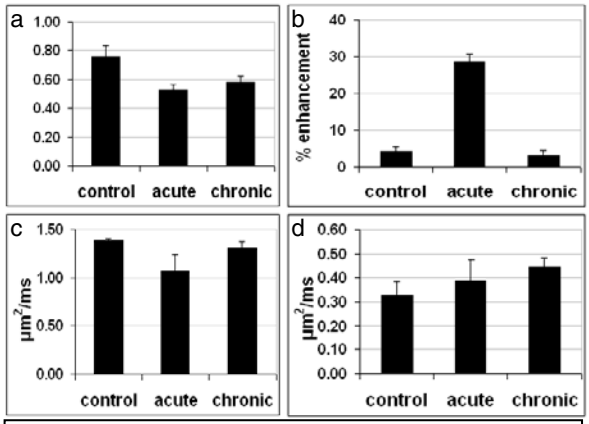

Figure 1. Ventrolateral white matter values from control (n=2), acute (n=6) and chronic (n=7) mice with EAE for RA (a), contrast enhancement (b), axial diffusivity (c), and radial diffusivity(d).

Results

The ventrolateral white matter in the spinal cord of mice with acute and chronic EAE had significantly decreased RA compared to controls (Figure 1). The degree of contrast enhancement was significantly greater in mice with acute EAE than chronic EAE or control mice. Axial diffusivity was significantly decreased in mice with acute EAE compared to chronic EAE or control mice. Radial diffusivity increased in mice with acute EAE compared to control mice and further increased in mice with chronic EAE, but these changes were not significant.

Representative histological sections from the spinal cord white matter of mice with acute EAE demonstrated increased cellular infiltration compared to control mice, whereas cellular infiltrates reduced to nearly control levels in mice with chronic EAE (Figure 2). Mice with acute and chronic EAE demonstrated a loss of staining for phosphorylated neurofilaments compared to control. Mice with acute EAE had decreased staining for myelin compared to control mice, and mice with chronic EAE demonstrated an even greater decrease in staining for myelin compared to control mice.

EAE clinical scores were significantly correlated with axial diffusivity, the percentage of contrast enhancement, and RA (Table 1). Radial diffusivity did not correlate with EAE clinical score.

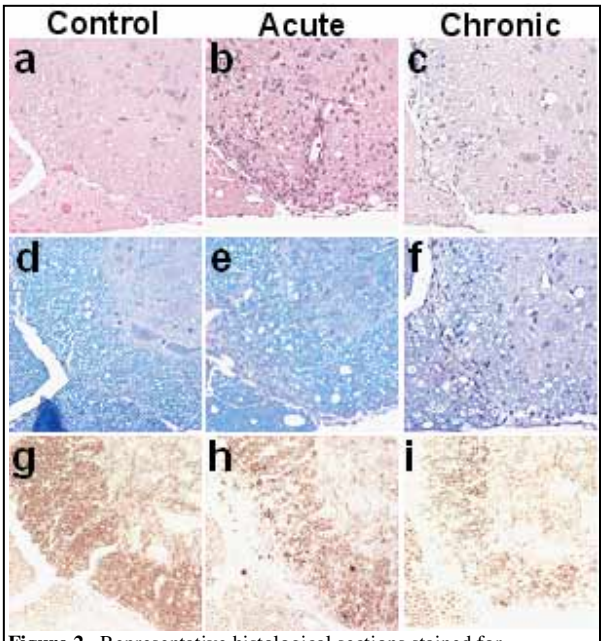

Figure 2. Representative histological sections stained for inflammation with H&E (a, b, & c), myelin content using Luxol fast blue (d, e, & f), and intact axons using primary antibody for phosphorylated neurofilaments (g, h, & i).

Discussion

Pathologically, the acute phase of EAE is associated with a significant amount of inflammation and this is detected with MRI as a substantial increase in the degree of contrast enhancement. Cellular infiltration is reduced in chronic mice, and the return of contrast enhancement to control levels mirrors the pathology. An increase radial diffusivity in the acute phase reflects the loss of myelin and the additional increase in radial diffusivity in the chronic phase mirrors the even greater loss in myelin staining in the chronic phase. Axonal loss appears to worsen in the chronic phase of the disease according to the histological staining for intact axons even though axial diffusivity returns nearly to control levels in the chronic phase. However, staining for intact axons does not give a full indication of axonal pathology and it remains to be determined how axial diffusivity changes with axonal loss, damage, or both.

Both axial diffusivity and contrast enhancement were highly correlated with EAE clinical scoring, which suggests that axial diffusivity truly reflects axonal dysfunction.

Table 1 Correlation with EAE clinical score

Parameter	*rho*
Axial diffusivity	-0.93*
Radial diffusivity	-0.35
Relative Anisotropy	-0.70*
Contrast Enhancement	0.91*

* = $p < 0.001$

Conclusions

The use of axial and radial diffusivity in combination with contrast enhancement allows the investigation of the pathological features of axon damage, myelin damage, and inflammation that underlie EAE. Adding DTI to the diagnostic repertoire could therefore become useful in the diagnosis and monitoring of treatment effects in MS.

References

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3. Song, S. K. et al. Neuroimage 17, 1429-36 (2002).