Characterization of Neurodegeneration in Experimental Autoimmune Encephalomyelitis Induced Rat Using High Resolution DTI

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Introduction

Multiple sclerosis (MS) is a common neurological disease with the characteristic of widespread demyelination of central nervous system (CNS) axons caused by degeneration of oligodendrocytes [1]. Degeneration of oligodendrocytes not only results in demyelination but also causes degeneration of axons and neurons in the late stage of MS [2]. With the availability of a widely accepted animal model of MS, experimental autoimmune encephalomyelitis (EAE), directional diffusivities, such as axial diffusivity ($\lambda_{//}$) and radial diffusivity ($\lambda_{\perp}$), obtained from DTI can then be correlated to the pathology underlying the disease. However, it is still uncertain whether the current analysis of the DTI parameters is accurate and reliable for the diagnosis of the disease. Furthermore, there is no report on the quantitative characterization of demyelination using DTI. We therefore performed high resolution *ex vivo* DTI to evaluate the degree of demyelination of the chronic EAE-induced rats, and to evaluate the directional diffusivity analysis for EAE diagnosis.

Materials and Methods

EAE Induction: EAE was induced in eight adult female SD rats (220-280g) by injecting intradermally at the base of the tail with a total volume of 100µl mixed solution containing the following components: 50µl of complete Freund's adjuvant (Chondrex Inc.) containing 200µg heat-inactivated Mycobaterium tuberculosis (strain H 37 RA; Difco Laboratories) and 50µl of 50µg myelin oligodendrocyte glycoprotein (provided by Biogen, Idec.) in saline (1:1). Animals' motor function behavior was assessed daily using behavioral EAE score. Animals developed EAE signs 9-15 days after the immunization. Animals were sacrificed and perfusion-fixed 35 days after the EAE induction. L2-L3 of the spinal cord (SC) was excised and placed in a plastic phantom tube containing 4% paraformaldehyde solution for DTI experiments, whereas slice adjacent to L3 was taken for myelin staining using Toluidine Blue.

Diffusion Tensor Imaging: All experiments were performed using a 7-T Bruker scanner (70/16 Bruker PharmaScan, Germany). Diffusion weighted (DW) images were acquired with a diffusion-weighted spin echo imaging sequence. A rotationally invariant icosahedral encoding scheme with 6 encoding directions was used to acquire DW images [3]. The imaging parameters were: TR/TE = 1600/29.0ms, Δ=20ms, δ=3ms, slice thickness = 2mm (interslice gap = 0.2mm), FOV = 27mm, data matrix = 256 x 256 (zero filled to 512 x 512), image resolution = 105 x 105 µm^2 and two b-values were used (0 and 1000 s/mm^2). The sequence was repeated three times for signal averaging, resulting in an acquisition time of 145 minutes. The diffusion tensors were extracted and diagonalized using an in-house Matlab program interfaced to a software for CNLS estimation for DTI (by Dr. CG Koay, STBB/LIMB/NICHD, NIH). $\lambda_{//}$, $\lambda_{\perp}$, fractional anisotropy (FA) and trace were measured in the ventral, dorsal, left lateral and right lateral column of the L2 and L3 segment of the SC. ROIs were drawn based on $\lambda_{//}$, $\lambda_{\perp}$ and T2-weighted images to avoid covering gray matter. Volume fraction of demyelination (% demyelination) was also determined by drawing ROI around the demyelinated region in the histological images and was measured using this area over the entire area of SC. Correlations between $\lambda_{\perp}$ and % demyelination were performed using Spearman's rho with Prism (GraphPad Software, Inc).

Results and Discussions

Different parameter maps obtained from high resolution DTI clearly distinguished the demyelinated region (as shown in Fig.1(a)-(d)). It should however be noted that if demyelination occurred in one region at one slice, the same location in the adjacent slice might not be demyelinated. This highlighted the nature of spatial inhomogeneity of neurodegeneration in EAE. Fig.2(a) shows the behavioral EAE score for each animal. It was found that $\lambda_{\perp}$ significantly correlated with the % demyelination determined by histology (Fig.2(b)), suggesting that $\lambda_{\perp}$ is sensitive to the extent of demyelination or myelin quality, and can be potentially be used to quantify demyelination. Fig. 2(c) plots the distribution of $\lambda_{//}$ and $\lambda_{\perp}$ among all animal studied. The lower right regime with low $\lambda_{//}$ and high $\lambda_{\perp}$ mostly belonged to animals with severe functional deficit, (animal number 5 and 8 with EAE score 3.0 and 3.5 respectively). In the upper left regime, there existed wide variation in both $\lambda_{//}$ and $\lambda_{\perp}$. It is worthwhile to notice that animal number 6 with EAE score 3.5 had relatively high $\lambda_{//}$, low $\lambda_{\perp}$ and low % demyelination, likely because severely degenerated region was not located in the L2 and L3 segments studied. More comprehensive histological analysis is being performed.

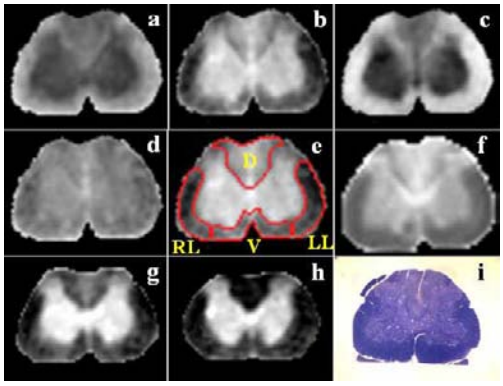

Figure 1. L3 segment of the spinal cord (SC) of an EAE-induced rat with severe demyelination in dorsal (D) area. **(a-d)** shows the $\lambda_{//}$, $\lambda_{\perp}$, FA and trace map of the SC respectively. **(e)** illustrates the ROI placement for ventral (V), dorsal (D), left lateral (LL) and right lateral (RL) column in (b). **(f)** T2-weighted image. **(g-h)** shows two $\lambda_{\perp}$ maps of L2 segment that are adjacent to L3 in (b). Slice adjacent to L3 stained for myelin using Toluidine Blue **(i)**

Conclusions

$\lambda_{\perp}$ is shown to be sensitive and useful for demyelination or myelin quantification, and thus for evaluation of the disease severity. Relatively large variation of radial and axial diffusivities was observed. In addition, SC degeneration was found to be spatially inhomogeneous. These results underscore the necessity of using volumetric DTI analysis to characterize neurodegeneration in both EAE and possibly MS, and that careful interpretation of all DTI parameters has to be implemented in order to guarantee sensitivity and specificity.

References

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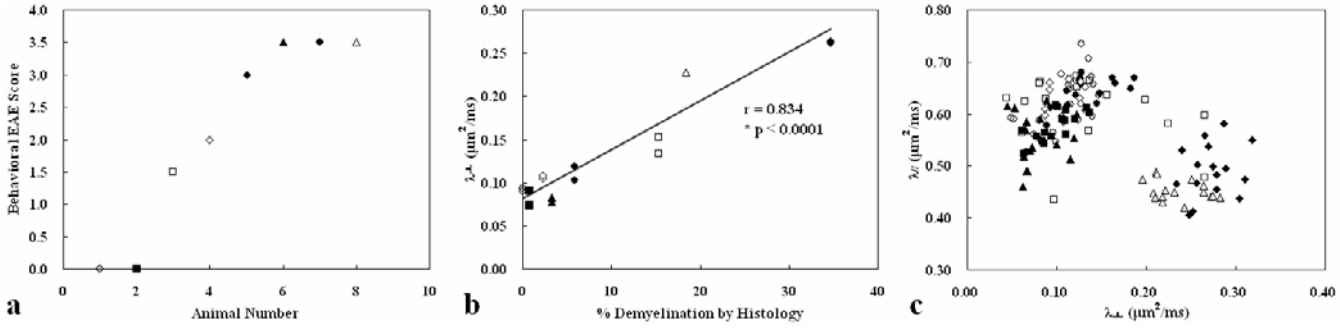

Figure 2. (a) EAE score for each animal.**(b)** Correlation between $\lambda_{\perp}$ in the white matter of L3 segment of SC and % demyelination in the adjacent slice. **(c)** Diffusivities ($\lambda_{//}$ and $\lambda_{\perp}$) distribution plot for EAE diagnosis. Each data point was taken from each ROI in all slices of L2 and L3 of all animals.