A Longitudinal Study Of Myelin Oligodendryocyte Glycoprotein (MOG)-Induced EAE Using Non-Invasive Imaging Techniques

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Introduction

Multiple Sclerosis (MS) is often associated with detrimental changes in the visual pathway. Some patients first present with loss of visual acuity and many have subclinical alterations of the visual system[1,2]. The prevalence of these pathologies is not certain although 68 - 100% of cases showed alterations in their visually evoked potentials (VEPs)[3,4]. The pathological basis of these changes is complex and not well understood but inflammation, demyelination and axonal damage have all been described[5,6]. Experimental Autoimmune Encephalomyelitis (EAE) is as an animal model for MS and shares many of its features. In this study, we used MOG-induced EAE as a mouse model for MS, particularly MS with early optic neuritis. We conducted non-invasive longitudinal studies *in vivo* to find correlations between lesions and evoked potentials.

Methods

Two groups of mice were studied. EAE was induced in 6-8 (group 1) and 11 (group 2) week old C57Bl/6J mice by immunizing with a MOG (35-55) peptide in Freud's adjuvant[7]. After the booster injection the mice were observed daily for clinical signs of disease: 1-tail drop, 2-ataxia and 3 hind limb paralysis. Mice from group 1 were sacrificed at stage 3 in compliance with our IACUC protocol, while mice in group 2 were sacrificed after remission to stage 0, in compliance with our CACC protocol. Mice in group 1 had VEPs prior to immunization and at various intervals after immunization using previously described techniques[8]. Mice that developed clinical symptoms were scanned using μMRI, T_2-weighted for group 1 and DWI for group 2.

Group 1: In vivo μMRIs (8 averages, 15x15x7.5 cm^3 FOV, 128x128x64 matrix size, T_R 1000 ms, T_E 10 ms, 2 hour acquisition time) were collected with isotropic resolution of 117 μm on a 9.4 T horizontal Bruker MRI scanner running Paravision 3. Fixed tissue μMRIs (8 averages, 16x12x12 cm^3 FOV, 256x192x192 matrix size reconstructed to 256^3, T_R 2s, T_E 6 ms, RARE factor 32, 307 minute acquisition time) were collected with 62.5 μm isotropic resolution on an 11.7 T Bruker MRI scanner running Paravision 3. Both types of images were collected using a T_2-weighted RARE imaging sequence[9]. Group 2: 2D T_2-weighted images (8 echoes, 2 averages, $(2.5\ cm)^2$ FOV, 256^2 matrix size (98 x 98 x 750 μm^3 resolution), T_R 2.5 s, T_E 27 ms, acquisition time 21 minutes) and DWI with SNAPSHOT-FLASH readout[10] sequence imaging (32x2 averages, $(4\ cm)^2$ FOV, 128^2 matrix size 312.5 x 312.5 x 1000 μm^3 resolution, T_R 3.0 s, T_E 3.2 ms, acquisition time 32 minutes, δ = 18 ms Δ = 40 ms, 8 g values ranging from 0.805 to 6.444 G/cm along x, y and z) from an axial slice containing the optic chiasm were acquired from the mice on a 7 T Bruker Avance spectrometer running Paravision 2.

Results

Most EAE mice showed a delayed latency after clinical symptoms appeared in the mice (Figure 1 left), indicating that demyelination occurred in the optic pathway of these mice at the same time as the motor pathway. Some mice showed a delayed latency before clinical symptoms indicating that demyelination in the optic tract can occur before demyelination occurs in the motor pathway. Some mice never exhibited delayed VEP latency (Figure 1 right), even after appearance of clinical symptoms, indicating that no demyelination occurred in the optic pathway while demyelination occurred in the motor pathway. Some of the mice showing clinical symptoms were imaged live on the 7T (Figure 2) or the 9.4T (Figure 3) and then fixed on the 11.7T for higher resolution (Figure 3). The mice in each trial were all induced at the same time using the same method, but went through different disease courses and had varying symptoms. This confirms the need for an *in vivo* assay to follow real time changes in lesions throughout the disease course.

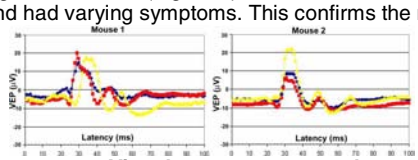

Figure 1. Visual symptoms and motor symptoms are not correlated in MOG-EAE mice. Some stage 3 mice had a delayed latency, indicating demyelination of the optic nerve, (Mouse 1, yellow) while some did not (Mouse 2, yellow). VEPs were also measured prior to (blue) and after immunization (red).

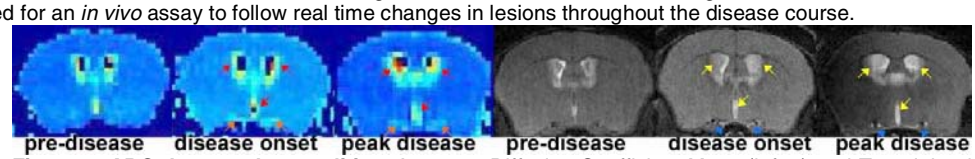

Figure 2. ADC detects abnormalities. Apparent Diffusion Coefficient Maps (left 3) and T_2-weighted Images (right 3) of EAE mice show enlarged ventricles (red & yellow arrows), visible lesions (orange & blue arrows), and a global change in diffusivity (bright blue at disease onset and dark blue at peak of disease).

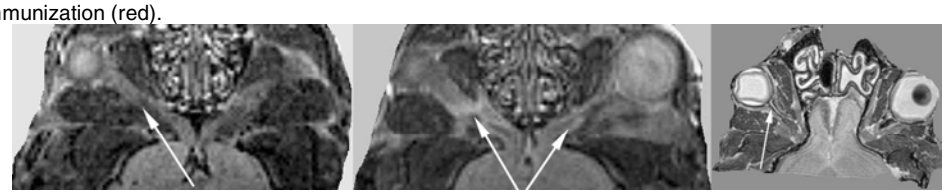

Figure 3. Possible lesions detected in the optic nerve. *In vivo* images, with 117 μm resolution, of the optic nerve of a control (left) and EAE mouse at stage 2/3 (middle) are shown. Also shown is an *ex vivo* image from a mouse perfused and fixed with 4% paraformaldehyde (right) The optic nerve of the control mouse is homogeneous as compared to that of the EAE mouse, which exhibits hyperintense regions suggesting edema (indicated by the arrow). The image of the optic nerve obtained from the fixed EAE mouse is relatively hyperintense with respect to the surrounding tissues (right) indicating possible lesions in the optic nerve.

Discussion and Conclusions

We have developed a non-invasive assay to monitor changes in the optic nerve as a result of MOG-EAE. After immunization, individual mice were followed throughout the course of their disease by observation of clinical symptoms, VEPs and *in vivo* μMRI. At the end of the experiment mice were euthanized and higher resolution MRIs were taken of the optic nerves. The optic nerves were then prepared for histology. Results indicated there are clear variations in the disease even though it is induced in inbred mice using the same peptide and injection regime. This highlights the importance of longitudinal studies where individual animals can be followed throughout the course of their disease and changes can be confirmed at the end of the experiment by high resolution MRI of fixed tissue and immuohistochemistry. This model potentially can be used to test cell-based therapies[11].

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