MRI ANALYSIS OF BRAIN LESIONS IN A NOVEL MOUSE MODEL OF MULTIPLE SCLEROSIS

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Introduction: Multiple sclerosis (MS) is a chronic autoimmune disease of the central nervous system (CNS) that leads to pathologic changes in the white matter (WM) and may result in neuronal death and muscle paralysis. In recent years, magnetic resonance imaging (MRI) has emerged as a noninvasive imaging tool for diagnosing and characterizing MS pathology[1]. Gadolinium-enhanced T1-weighted (T1-Gd) technique, as well as advanced MR methods such as diffusion-tensor imaging (DTI), are highly sensitive in detecting MS plaques and provide a quantitative assessment of inflammatory activity and lesion load[2]. Experimental allergic encephalomyelitis (EAE) mouse model is commonly used to study disease pathogenesis and to test new therapeutic approaches. However, in most MS mouse models[3], the neurologic damage mostly affects the spinal cord with limited damage to the brain, which cannot be monitored by MRI as used for humans. Hence, it is critical to develop a therapeutic intervention for an EAE mouse model that shows all features of MS and can be monitored by MRI. In this study, we used MRI to characterize the development of brain lesion of non-obese diabetic (NOD) mice which are known to develop spontaneous autoimmune diabetes, thus makes it a perfect candidate for investigating the development of other autoimmune diseases. We suggest using NOD mice as a suitable model for studying MS using MRI towards future diagnostic and drug development.

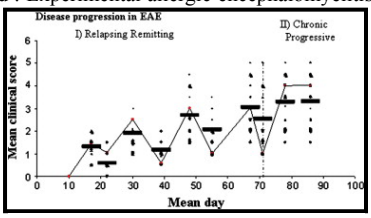

Figure 1: An avaraged clinical score of EAE induced NOD mice.

Methods: 10-week-old NOD and 8-week-old C57BL/6J mice were induced with EAE by subcutaneous immunization of MOG peptide (n=10). Clinical signs of the disease were assessed on a daily basis as following: 0,no disease; 1, loss of tone in the tail; 2, hind limb weakness; 3, hind limb paralysis; 4, hind limb plus forelimb paralysis; and 5, moribund state. NOD mice without MOG served as control group ($n = 4$). MRI experiments were performed on a 7T Bruker scanner 90 days following EAE induction and included T1-Gd and standard DTI scan. T1-Gd images were acquired 10 min after intraperitoneal injection of Gd, using FLASH sequence with the following parameters: TR/TE = 1000/7.3 ms, 12 coronal slices (750 µm thickness) with a matrix size of 256 × 256 and in-plane resolution of 70µm isotropic. For DTI acquisition, we used DW-EPI pulse sequence with the following parameters: TR/TE = 2800/21 ms, Δ/δ = 10/4.5 ms, 4 shot EPI, b-value of 1000 s/mm^2 at 16 non-collinear gradient directions. DTI was acquired in 12 coronal slices with a resolution of 140 × 140 × 750 µm^3.

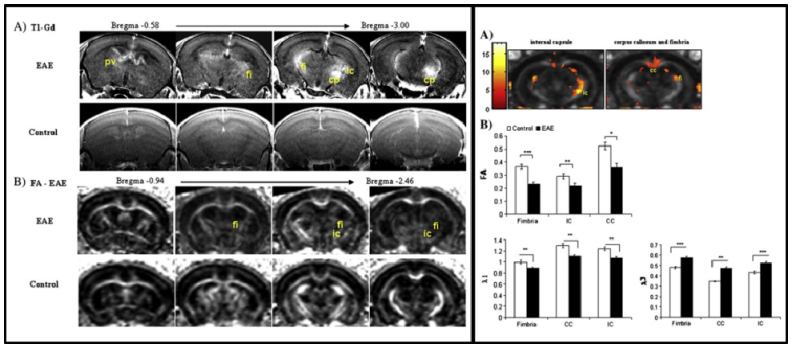

Figure 2 Left: Development of brain lesion in EAE induced NOD mice as compared to control at T1-Gd and FA images. Right: (A) statistical parametric maps of FA analysis ($p<0.01$) (B) VBA of FA, λ1 and λ3 maps.

Analysis: DTI analysis was done by DiVa software[4]. FA maps of all rats were normalized to FA brain template using a 12-parameter affine nonlinear transformation. Voxel-based analysis[5] (VBA) was performed using SPM2 (UCL, London, UK) between the EAE mice and controls. Post hoc ROI analysis was performed on selected regions: hippocampus, fimbria, and corpus callosum. Following imaging, histology was performed for validation. Coronal brain sections and lumbar spinal cord were prepared from EAE-induced NOD and C57BL/6J mice during the chronic phase of the disease using cryostat and were compared to control mice ($n = 6$). Brain sections were stained with Luxol fast blue (LFB) and hematoxylin and visualized by light microscopy for evaluation of CNS demyelination and cell infiltration. Furthermore, the brain sections were stained for fibrinogen, GFAP, and Iba1 staining and analyzed using Odyssey scanner.

Results: Figure 1 shows an average clinical score of every relapse of each EAE-induced NOD mouse, and was calculated for average day. The line represents the disease progression of representative mouse. Note the relapse and remitting pattern which is followed by chronic progressive stage similar to MS human pattern disease. Figure 2 (left) shows a development of brain lesions in EAE-induced NOD mice as compared to control mice at T1-Gd and FA maps. An enhancement was found at T1-Gd images specifically in the fimbria (fi), internal capsule (ic), perivascular zones (pv), and cerebral peduncle (cp). Reduction of FA values was found on the same regions. Figure 2A (right) shows statistical parametric maps superimposed on a template mouse brain, where significant reduction of FA are emphasized. VBA analysis (Fig 2 right) of FA maps revealed a significant reduction mainly at the fimbria, internal capsule and corpus callosum. VBA of λ1 analysis revealed a significant reduction in the same regions. We discovered an increase in λ3 value, which relates to the demyelination process in the same brain regions. Figure 3 depicts histopathology of brain and spinal cord lesions in EAE-induced NOD mice, where representative staining sections of LFB and hematoxylin during the chronic phase of the disease are given as compared to control and C57BL/6J EAE mice. LFB staining of brain slices of EAE-induced NOD mice showed demyelination in the brain WM specifically in the fimbria (I), in parallel to massive cell infiltration (hematoxylin staining) (II), as compared to control mice brains and C57BL/6J EAE mice brains (Fig. 3A). Spinal cord sections (Fig. 3B) of same staining methods showed demyelination and elevation in cell infiltration in the WM of both the C57BL/6J and EAE mice as compared to control. Fig 4 shows immunohistologic analysis of brain lesions in EAE mice. Brain sections from EAE induced NOD mice, C57BL/6J EAE mice and control mice were examined for signal intensity using an Odyssey scanner.

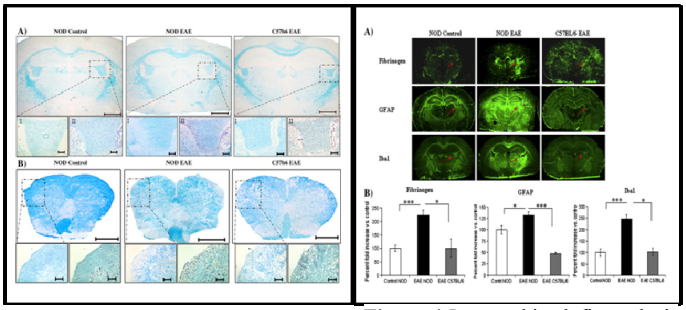

Figure 3: LFB and hematoxylin staining of brain and spinal cord sections of EAE induced NOD mice as compared to C57BL/6J EAE and NOD control mice.

Figure 4: Immunohistolofic analysis of brain lesions using Odeyssey scanner at EAE mice, C57BL/6J EAE and NOD control mice.

(A) Representative images of fibrinogen, GFAP, and Iba1 staining. Elevation in signal intensity was observed specifically in the fimbria (arrows). (B) The signal intensity of a specific area containing the fimbria and the internal capsule was analyzed using the Odyssey software. Statistical analysis was performed by the Student t-test.

Discussion: We have characterized brain lesion development in EAE-induced NOD mice, and found that the disease pathology can be diagnosed and analyzed *in-vivo* using MRI[6]. Disease progression in NOD mice has a relapse and remitting phase that develops into a chronic progressive phase that resembles different disease phases in humans. In this model, MRI lesions have been detected both in WM and in gray matter similar to the findings of MS in humans. By developing a chronic stage following the formation of brain lesions, the NOD model of MS could contribute to the understanding of pathologic processes in the MS brain and the development of new therapeutic approaches that would address the chronic progressive stage, demyelination processes, and axonal injury in the brain, which can be monitored by MRI as done in humans.

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