USPIO Contrast Enhanced MRI Study Monitoring Inflammatory Lesions in Brain of the Relapsing-Remitting Model of EAE in SJL/J mice

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Purpose

Multiple sclerosis (MS) is a chronic autoimmune, inflammatory, neurodegenerative disease of the central nerve system (CNS) [1]. Experimental autoimmune encephalomyelitis (EAE) is a well characterized and reproducible animal model for MS. Ultrasmall superparamagnetic iron oxide (USPIO) nanoparticle enhanced MRI has been used for in vivo tracking of macrophages in chronic relapsing and other EAE models in rats [2-3]. The goal of the present study is to assess MS-like inflammatory lesions and examine treatment effect in a Relapsing-Remitting model of EAE (RR-EAE) in SJL/J mice using the USPIO enhanced MRI technique.

Methods

Mouse Model: The pathogenesis of the RR-EAE mouse model involves the presentation of antigens to T cells, and the migration of activated T cells into the brain and spinal cord results in the progression of EAE disease and development of inflammation and/or demyelination upon recognition of these antigens. To induce EAE, SJL/J female mice (Harlan, 9-11 weeks) were injected subcutaneously with 50µg of mouse $PLP_{139-151}$ in complete Freund's adjuvant (CFA) containing 2 mg/mL mycobacterium tuberculosis H37Ra (Difco, Detroit, MI) at 2 sites (0.1ml/site) on the back. Pertussis toxin (300 ng) was injected intraperitoneally two hours later and two days later. Clinical scoring and body weight were monitored 3 times per week throughout the duration of study.

Experiment Design: Three groups were studied, 1) naïve group with no disease induction (n=5); 2) EAE group without treatment (vehicle, n=6); and 3) EAE group with treatment of fingolimod, 1 mg/kg/day *per os* (n=6). MRI acquisition was performed at 2, 3, 5, and 6 weeks post disease induction which present 1st relapsing, 1st remitting, 2nd relapsing, and 3rd relapsing, respectively. USPIO (Molday Ion, BioPAL, Inc.) at 0.3mmol Fe/kg dose was injected through the tail vein 24 hours prior to imaging to allow for uptake in inflammatory lesions in the brain. Brain samples were collected upon imaging completion and immuno-histology was performed.

MRI Method: MRI Experiments were performed on a Bruker Biospec 7.0 T 20-cm horizontal bore system (Bruker, Billerica, MA) with a 38mm (ID) volume coil, using a RARE sequence with TR=4.5s, RARE factor = 8, two echoes with TE_Eff1/TE_Eff2 = 14ms/70ms, in-plane resolution = 100×100 μm^2, slice thickness=0.7 mm, and 12 averages. Continuous axial slices were acquired covering the entire brain. A custom MATLAB (Mathworks, Natick, MA) based image analysis toolkit was developed to perform the pixel-wise R2 calculations and initiate semi-automated brain segmentation and co-registration, to align and register (AFNI [4]) the brain volumes of the naïve group to the brain of each individual animal. Pixel-wise R2 maps were calculated using:

$$R2 = \log(S(TE\_\ Eff1)/S(TE\ \_Eff2))/(TE\_\ Eff2\text{-}TE\_\ Eff1).$$

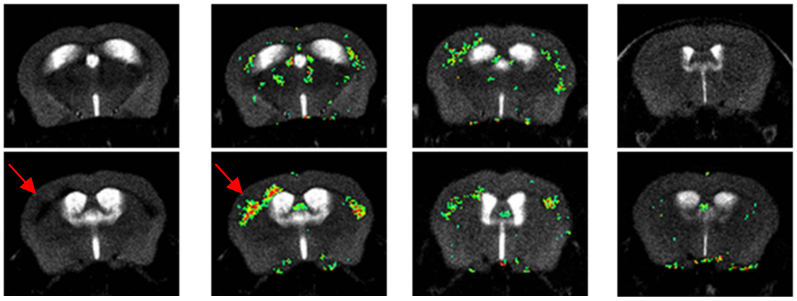
Change of R2 (ΔR2) maps were estimated using the mean R2 map of the naïve group as baseline mapping, and histograms of ΔR2 maps were generated to evaluate the USPIO uptake in brain lesions at each of the four disease stages. The number of voxels associated with the USPIO uptake was calculated based on the thresholding of ΔR2 maps, with a threshold of 5 1/s determined empirically. Cluster of less than 5 connected voxels were excluded. The MS-like lesion load was assessed by the total volume of selected voxels.

Results

As shown in Fig 1, ΔR2 maps with a threshold of 5 1/s were color coded and superimposed on the T2 weighted MR images. MS-like lesions detected in the ΔR2 maps (Fig 1b top) may not be easily observed in the T2 weighted images (Fig 1a top), especially in areas with a subtle signal decrease associated with a small amount of regional USPIO uptake. USPIO uptake indicating macrophage infiltration in the brain parenchyma (arrows) as well as ventricle enlargement were observed for the EAE group at 2 weeks post disease induction (1st relapse, Fig 1b). The clinical scores confirmed the 1st disease relapse (score 3) and remission (score 1.7) for the EAE group. USPIO uptake peaked at week 2, and was significantly reduced ($p < 0.05$) at 3 weeks (1st remission) for the EAE group (Fig 1C). ΔR2 map of naïve group showed minimal USPIO uptake (Fig 1d top). Fingolimod treated mice showed significant reduction ($p < 0.05$) of USPIO uptake (Fig 1d bottom) at 2 weeks compared to the EAE group. The profile and peak offset of the ΔR2 histogram illustrated in Fig. 2a indicates the variation of USPIO uptake at different disease stages for the EAE group (vehicle). Fig2b showed a trend of increased USPIO uptake during the 2nd and 3rd relapses in the vehicle group, which corresponded to the clinical scores at week 5 (score 2.5) and week 6 (score 2.5).

Discussion and Conclusions

USPIO enhanced MRI is a sensitive imaging approach to assess inflammatory lesion changes as well as visualize MS-like lesions in the Relapsing-Remitting model of EAE mice. MS-like lesions were detected in ΔR2 maps, which may not be easily identified in the T2 weighted anatomical images. Our results showed that USPIO enhanced MRI can be used to assess changes in the inflammatory lesion to examine treatment effect in a MS mouse model. Further validation of the semi-automatic and quantitative data analysis method is warranted.



a. T2 weighted MRI of an EAE mouse at week 2 | **b. ΔR2 overlay (≥5 1/s) on the EAE at week 2** | **c. ΔR2 overlay (≥5 1/s) on the EAE at week 3** | **d. ΔR2 overlay (≥5 1/s) on a naïve (top) and a fingolimod treated at week 2 (bottom)**

Fig 1. ΔR2 maps (with a threshold of 5 1/s) overlay for an EAE (a-c, top and bottom images are from two different slice locations), a naïve (d, top), and a fingolimod treated EAE mouse (d, bottom)

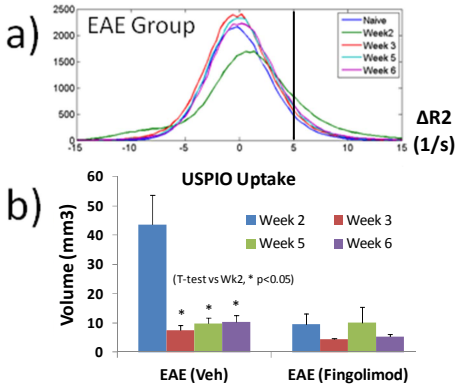


Fig 2. Mean ΔR2 histogram for EAE group (a) and the lesion load (USPIO uptake) calculated based on the ΔR2 map with a threshold of 5 1/s (b)

References: 1. Ewing C, Bernard CC, Immunol. Cell Biol. (1998); 76(1): 47-54; 2. Rausch M et. al., JMRI (2004): 10: 16-24; 3. Chih-Liang C, Madhavi P, et.al, Journal of Neuroimmunology (2009); 211:49–55. 4. Cox RW, *Comput Biomed Res* (1996); 29**:**162–173.