Differences in MION-mediated detection of spinal cord lesions in two mouse models of MS

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Introduction

MRI is an essential clinical tool for diagnosing and monitoring multiple sclerosis (MS) disease progression. In animals, experimental autoimmune encephalomyelitis (EAE) is an inflammatory, demyelinating disease of the CNS that mimics many aspects of MS and has been used for over 30 years. Several groups have reported that systemically administered ultra-small (USPIO) or monocrystalline (MION) iron oxide nanoparticles accumulate in EAE brain lesions, internalized within macrophages and/or microglia involved in the inflammatory response, enabling lesion detection with MRI [1, 2]. Similar to human MS patients, clinical (motor) disease in animals with EAE is more strongly correlated with spinal cord than brain involvement [3]. We therefore examined the utility of MION-mediated MRI detection of spinal cord lesions in mice with induced EAE [4] and in transgenic mice with spontaneous EAE [5].

Materials & Methods

Induced EAE Model: Normal C57Bl6 mice were induced into a state of EAE using a subcutaneous injection of a mixture of MOG (myelin oligodendrocyte glycoprotein peptide, 100 ng) and H37RA (mycobacterium tuberculosis coat antigen, 200 ng) in complete Freund's adjuvant (100 µl total volume per mouse) [4]. Each mouse was also given two intravenous (IV) injections of pertussis toxin (200 ng), first immediately after the MOG injection, and then again two days later. Mice with induced EAE progressed to an advanced disease state (partial to complete hind limb paralysis) very rapidly at approximately 3 weeks after MOG injection.

T/R- Transgenic EAE Model: A transgenic mouse model that spontaneously develops EAE was also studied [5]. In the T/R- model, an anti-myelin basic protein (MBP 1-11) T-cell receptor (TCR) transgenic mouse line was crossed with mice deficient in the *Rag-1* gene, with the result that only the transgenic TCR is expressed in T/R- mice. All T/R- mice spontaneously develop EAE by 12 weeks of age, and progress more slowly in their disease than mice with induced EAE, typically exhibiting hind limb paralysis by 3-4 months of age.

MRI: MRI experiments were performed on a micro-imaging system (SMIS) with a 7T horizontal magnet (Magnex) with actively shielded gradients (250 mT/m max strength, Magnex). During MRI, mice were maintained in the supine position in a custom holding device, with a cylindrical surface coil fitting closely around the spinal cord and a nose-cone for isoflurane anesthesia delivery (1-1.5% in air, 2l/min flow rate). Mice were imaged with a multi-slice T2*-weighted gradient echo sequence (TE/TR = 12/1500 ms; in-plane resolution = 60 µm; slice thickness = 150 µm; acquisition time = 105 minutes), to acquire 11 contiguous axial slices covering the lumbar L1 vertebral region. For contrast-enhanced MRI, mice were injected IV with MION-46L (CMIR-MGH, Charlestown MA) at a dose of 25 mg Fe/kg body weight. Spinal cord images were acquired before and immediately after injection, and again 4 and 24 hours (h) after MION injection.

Histology: After MRI, L1 spinal cord specimens were dissected and prepared for cryosectioning (20 µm thick) and histological analysis. Luxol fast blue staining was used to identify regions of demyelination, and Prussian blue staining was used to detect iron to assess MION accumulation. At this time, no attempt has been made to perform quantitative correlation between histological and MRI results.

Results and Discussion

Assessment of MR images after MION injection demonstrated that circulating levels of contrast agent clear from the spinal cord by 24 h (Fig. 1), while vascular MION was still evident at 4 h (data not shown). Based on this result and a previous report showing that detection of rat EAE brain lesions was optimal at 24 h [2], we analyzed MR images of normal and EAE spinal cord 24 h after IV injection of MION. In mice with induced EAE (partial to complete hind limb paralysis), MION injection revealed numerous lesions, often perivascular, that were not present in pre-disease images of the same mice, or in pre-MION images at the advanced stage of EAE (N=4, Fig. 1). The patterns of induced lesion formation on MRI agreed qualitatively with those revealed by luxol fast blue (demyelination) and Prussian blue (iron) staining (data not shown). In contrast, MR images of T/R- mice at a similar stage of EAE (hind limb paralysis) demonstrated areas of signal loss most often in the peripheral spinal cord, together with peri-spinal inflammation (bright signal) on T2*-weighted MRI (N=5; Fig. 2). Furthermore, MION injection in the T/R- mice did not reveal EAE lesions, which were already apparent as a signal loss in pre-MION MR images. Further histological analysis is required to determine whether the T2*w signal loss in T/R- lesions is due to any obvious neovascularization or vasculitis, which has recently received attention in human MS studies [6]. Our results demonstrate the feasibility of detecting induced EAE lesions in the mouse spinal cord with MION-enhanced MRI. Significantly, the differences between EAE mouse models indicate potential limitations of MION-enhanced MRI for human MS, where demyelinating and inflammatory lesions develop more slowly, similar to those found in the transgenic T/R- mice.

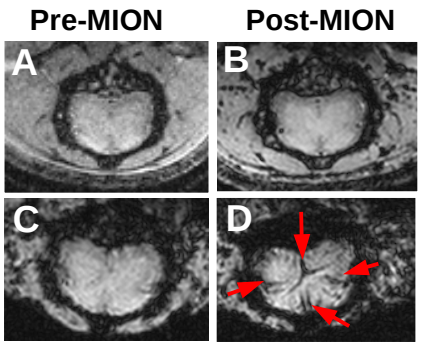

Fig 1: Before EAE, pre (A) and 24 h post-MION (B) images show no differences. In induced EAE, pre (C) vs. post-MION (D) MR images reveal numerous spinal cord lesions (arrows).

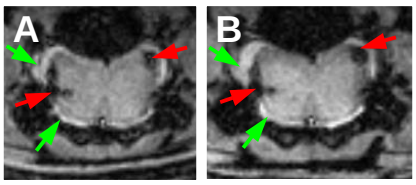

Fig 2: In T/R- mice with EAE, pre (A) and post-MION (B) images are equivalent, both showing lesions (red arrows) and peri-spinal edema (green arrows).

Acknowledgements

We thank Drs. Glaucia Furtado and Katarina Akassoglou for help with disease staging, genotyping and histological analysis. This research was funded by NIH grant NS38461.

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