MRI of Blood-Spinal Cord Barrier Disruption in Mice with Experimental Autoimmune Encephalomyelitis

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Introduction

Experimental autoimmune encephalomyelitis (EAE), an animal model of demyelinating disease, is associated with infiltration of macrophages and T cells into the central nervous system and the breakdown of the blood-brain barrier (BBB) and blood-spinal cord barrier (BSB). Contrast-enhanced MRI can detect changes in the extent of BBB and BSB disruption during disease progression. The role of BBB permeability in the initial development and during the progression of the disease is unclear. Some studies have indicated that vascular permeability is a distinct event that precedes cellular infiltration[1] while other studies have found that BBB permeability occurs during inflammation.[2,3] In this study we performed gadolinium diethylenetriaminepentaacetate (Gd-DTPA) enhanced imaging of the spinal cord in a mouse model of EAE. A quantitative analysis of BSB breakdown was performed to determine the extent of disruption during disease progression.

Methods

Animal Model: All animal procedures were approved by the Institutional Animal Care Committee at the University of Manitoba. EAE was induced in 21 adult female C57BL/6 mice by injecting subcutaneously 50μg myelin oligodendrocyte glycoprotein (MOG) mixed with complete Freund's adjuvant (CFA) and injecting intraperitoneally pertussis toxin (PTx, 300ng) on days 0 and 2.[4] Weight and functional impairment using a 0 to 14 scoring scale[5] were measured daily. Control animals received saline (n=2) or CFA and PTx (n=3).

Imaging: MR images of the lumbar spinal cord were obtained using a Bruker Biospec 7T/21cm spectrometer with a 20x30mm quadrature surface coil. Anesthesia was induced by ventilating with 5% halothane in O_2/N_2O (30/70) and maintained during imaging using 1.5-2% halothane. Interleaved multi-slice multi-echo T_2-weighted imaging (TE=26.8ms, TR=2300ms, matrix size=256x256, FOV=2.5x2.5cm^2, slice thickness=0.75mm, interslice distance= 1.5mm, 8 echos, 2 averages) and T_1-weighted imaging (TE=13ms, TR=600ms, matrix size=256x256, FOV=2.5x2.5cm^2, slice thickness=0.75mm, interslice distance=1.5mm, 4 averages) was performed, followed by an intravenous bolus injection of 0.4mmol/kg Gd-DTPA through a tail vein cannulation while the mouse remained in the magnet. Two sets of Gd-enhanced T_1-weighted images were obtained, starting immediately following the Gd-DTPA administration. Groups of mice were imaged at the onset of disease signs (n=7), at peak disease (n=5), and at remission (n=10).

Histology: After final imaging the animals were perfusion-fixed and the spinal column was decalcified to allow examination of the spinal cord *in situ*. Tissue sections were examined using hematoxylin & eosin stain for general features of inflammation, anti-IgG was used to look for areas of BSB leakage, and activated microglia/macrophages were detected with GS lectin.

Image Analysis: Contrast enhancement was quantified by calculating the percent intensity change following Gd-DTPA injection, as: ((post contrast image – pre contrast image)/pre-contrast image)*100%. Regions of interest (ROIs) outlining the spinal cord were determined from T_2-weighted images. These ROIs were then superimposed onto the calculated percent difference images to obtain the average percent intensity within the region of the spinal cord (Figure 1).

Results

A ring of enhancement within the lumbar spinal cord in MR images was observed in mice exhibiting initial signs of disease (Figures 1B, 3A). Spinal cord enhancement was greatest at onset of disease signs with an average intensity increase 60.8% (Figure 2). The intensity of enhancement was found to decrease significantly at peak disease, to close to half the enhancement seen at the onset of disease. Animals in remission showed minimal enhancement within the region of the spinal cord (Figure 2). Mice sacrificed at the initial stage of disease exhibited foci of dense inflammatory infiltrates at the periphery of the cord in regions surrounding vessels. These focal regions of peripheral inflammatory cells corresponded to the regions of greatest contrast enhancement at initial stage of disease (Figure 3A, 3B). Spinal cords of animals at peak disease appeared more vacuolated in the white matter suggesting an edematous state, while the inflammatory cells were more dispersed (Figure 3D). At remission, the amount of inflammation present was found to be significantly reduced. Weak labeling of IgG was found in a wedge-like peripheral distribution in only three of the four mice at initial stages of EAE and these areas corresponded to regions of dense cellular infiltrates. No positive staining for IgG was observed in spinal cord tissue at peak disease or remission.

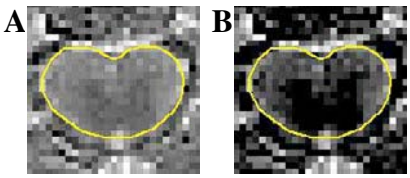

Figure 1. (A) ROI superimposed onto percent difference image. (B) An image selecting only pixels above 60% intensity exhibits a peripheral ring of distribution.

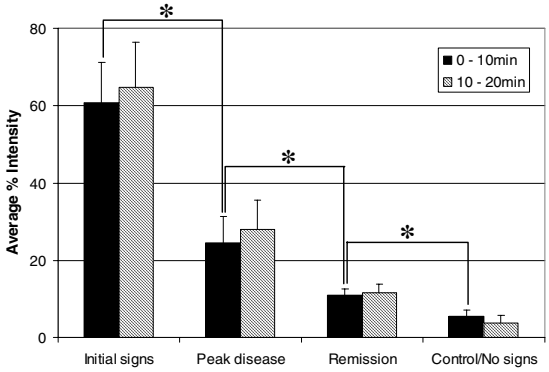

Figure 2. The average percent intensity within the spinal cord at initial signs (n=7), peak disease (n=5), remission (n=10), and control/no signs (n=8) from percent difference images for the first and second sets of images obtained post-Gd-DTPA.

Discussion

Regions on MR images showing contrast enhancement corresponded to regions of BSB disruption. The pattern of contrast enhancement was observed as a ring of pixels of highest intensity, suggesting that the BSB breakdown occurred predominantly in the peripheral white matter of the spinal cord. At the initial stage of EAE there was a simultaneous opening of the BSB and the infiltration of mononuclear cells. MR images at peak disease showed reduced enhancement on percent difference images suggesting that the disruption of the BSB was a transient occurrence at initial stages and at peak disease the BSB was more intact.

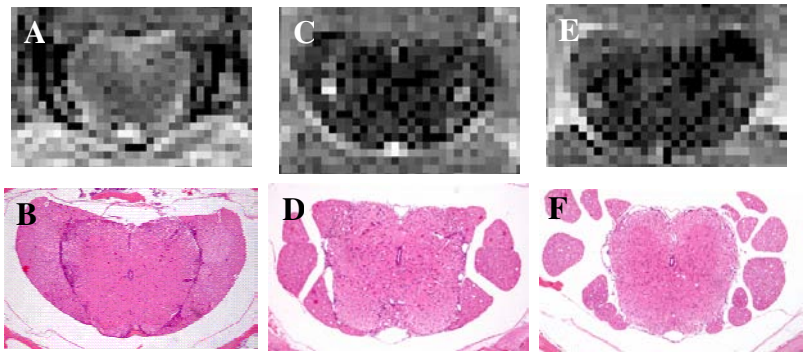

Figure 3. MR images and corresponding histological sections at the initial stage of disease (A, B), peak disease (C, D), and remission (E, F).

References

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