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 while other studies have found that BBB permeability occurs during inflammation. 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. Weight and functional impairment using a 0 to 14 scoring scale 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 O₂/N₂O (30/70) and maintained during imaging using 1.5-2% halothane. Interleaved multi-slice multi-echo T₁-weighted imaging (TE=26.8ms, TR=2300ms, matrix size=256x256, FOV=2.5x2.5cm, slice thickness=1.5mm, interslice distance=1.5mm, 8 echos, 2 averages) and T₁-enhanced T₁-weighted images were obtained, starting immediately following the Gd-DTPA administration. Groups of mice were imaged at the onset of disease signs 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 T₁-weighted images were obtained, starting immediately following the Gd-DTPA administration. The role of BBB permeability in the initial development and during the progression of the disease is unclear.

Results
A ring of distribution. 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, 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.

Image Analysis: Contrast enhancement was quantified by calculating the percent intensity change following Gd-DTPA injection, as: (Δpost contrast image – pre contrast image)/pre-contrast image)*100%. Regions of interest (ROIs) outlining the spinal cord were determined from T₁-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).

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 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.

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