Vascular Expansion and Blood-Brain-Barrier Permeability: A Comparative Volumetric Study in Acute Japanese Macaque Encephalomyelitis

Ian Tagge[1,2], Steven Kohama[3], Jim Pollaro[1], Lawrence Sherman[3], Dennis Bourdette[4], Randy Woltjer[4], Scott Wong[3], and William Rooney[1,2]

[1]Advanced Imaging Research Center, Oregon Health & Science University, Portland, Oregon, United States, [2]Biomedical Engineering, Oregon Health & Science University, Portland, OR, United States, [3]Oregon National Primate Research Center, Oregon Health & Science University, OR, United States, [4]Neurology, Oregon Health & Science University, Portland, Oregon, United States

Introduction: Inflammation, blood brain barrier (BBB) compromise, and impaired microvascular function are common in multiple sclerosis (MS).[1,2] BBB permeability can be visualized with contrast-enhanced MRI using gadolinium based contrast agents (GBCA). Quantifying blood volume fractions in brain areas with a broken BBB requires sophisticated correction algorithms or use of a blood-pool contrast agent that does not readily extravasate. Japanese Macaque Encephalomyelitis (JME) is a spontaneous demyelinating disease that has many similarities to MS including intense focal neuroinflammation, macrophage infiltration, demyelination, and BBB compromise.[3,4] Acute JME lesions are commonly found in the brainstem, cerebellum (CBLL), internal capsule (IC), cerebral white matter, and corpus callosum (CC). Here we use bolus injection of ferumoxytol (FeO), an ultrasmall superparamagnetic iron oxide, to investigate vascular expansion during acute episodes of JME. Enhancement volume and location is compared between FeO and GBCA.

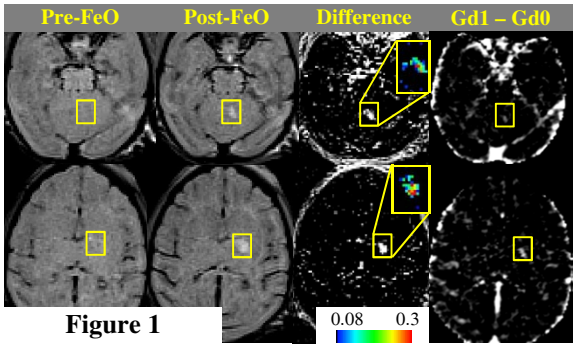

Figure 1

Methods: Five animals experiencing an acute episode of JME were identified and selected for MRI examination. All MRI data were acquired on a whole-body Siemens 3 Tesla (T) MRI instrument (Erlangen, Germany) using a quadrature radiofrequency (RF) coil with inner diameter of 15 cm. Animals were initially sedated with Telazol, intubated and maintained on 1% isoflurane in 100% O_2 and were continuously monitored by pulse oximetry, respiration, and end tidal CO_2 levels during the study. Quantitative R_1 ($\equiv 1/T_1$) mapping was performed with a multiple-inversion recovery experiment employing 3D T_1-weighted magnetic prepared rapid acquisition gradient echo (MPRAGE) sequence (TR: 2500 ms; TE: 3.49 ms; FA: 8°; FOV 130 mm x 97.5 mm x 96 mm, matrix: 192x144x96; TI: 200, 900, 2000ms, no inversion). R_1 maps were acquired before and after bolus injection of 0.2mmol/kg GBCA (Prohance). A bolus injection of 0.4mg/kg FeO was administered approximately 60 minutes after GBCA. Axial 2D T_1-weighted TSE sequence (TR:800 ms; TE 14 ms; FOV 160 mm x 120 mm, matrix 192x144, ST 1.0mm) was acquired before GBCA injection and after FeO injection on all animals. T_1-weighted images were acquired immediately before FeO injection on two animals. Relative blood volume maps were created for these two animals as a ratio of signal intensity between the pre- and post-FeO T_1 TSE images: $(S_{post\text{-}FeO} - S_{pre\text{-}FeO})/S_{pre\text{-}FeO}$. Difference maps were created by subtracting pre-contrast images from post-contrast images for both R_1 and T_1-weighted image sets to determine GBCA and FeO enhancement volumes, respectively, and lesions were identified manually. Two animals were returned to MRI for follow-up examination one or three days after FeO injection. R_1 maps and T_1-weighted images were acquired without additional exogenous contrast administration. Follow-up images were coregistered to corresponding images from the initial visit and difference maps were created to evaluate extent of FeO accumulation in tissue ($\Delta R_1 = R_{1Day3} - R_{1Day0}$).

Lesion Location	FeO Ratio (± SD)	FeO Volume (mm^3)	Gd Volume (mm^3)
CBLL	0.12 (± 0.07)	84	81
IC	0.15 (± 0.08)	37	48

Results: Fourteen lesions were identified in five animals, with at most 5 lesions in an individual animal. Ten of the fourteen lesions exhibited larger FeO enhancement volume than GBCA. The remaining four lesions (one animal) showed FeO volumes equal (n=2) to or less (n=2) than GBCA. On Average FeO apparent lesion volume was 37% (+/- 25%) larger than GBCA lesion volume, but a wide variance was observed. **Figure 1** shows axial T_1-weighted slices through the CBLL (top row) and IC (bottom row). Pre-FeO images were acquired immediately prior to FeO injection, thus difference maps reflect only FeO enhancement. False-color maps of FeO enhancement ratio are shown in the inset. FeO enhancement appears fairly even, but ratio maps demonstrate lesion heterogeneity. Panels on the far right demonstrate GBCA enhancement ($=R_{1post} - R_{1pre}$). Lesion volumes for this animal are shown in the **Table**. Regions of interest selected in contralateral normal appearing brain tissue (NABT) showed zero average enhancement. **Figure 2** shows CBLL lesions in a second animal. Multi-focal enhancement is observed post-GBCA R_1 maps (top row). FeO accumulation after 3 days is visualized in R_1 difference maps (bottom row). Diffuse FeO accumulation is evident in CBLL after 3 days (**Figure 2**), and extends far beyond focal GBCA enhancement as evident in **Figure 2**, top row. Focal accumulation of FeO was observed in IC and CBLL of separate animal after 24 hrs (data not shown).

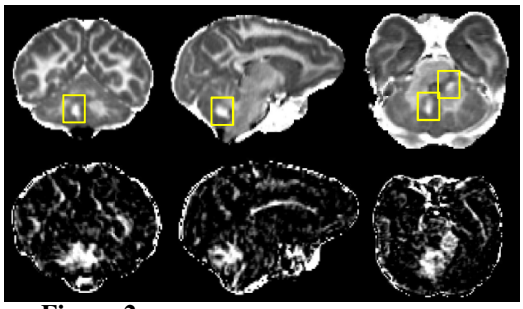

Figure 2

Discussion: We observe two types of FeO enhancement in JME; i) early, determined within minutes after FeO administration when plasma levels are high, and ii) late, typically 24-72 hrs post FeO administration when FeO plasma levels are low. FeO does not show significant extravasation at early times after administration, even in leaky glioblastoma tumors.[5] Taken as a surrogate for rCBV, early FeO enhancement ratio demonstrates heterogeneous blood volume increase within acute lesions. Based on previous estimations of white matter blood volume in Japanese Macaque NABT (2.7%),[6] FeO relative enhancements suggest lesion blood volumes ranging from 3-8%. JME lesions are frequently centered on venules or small veins; vascular dilation or alternatively, new blood vessel formation, may account for large blood volumes near the core of the lesions seen in **Figure 1** inlays. GBCA and FeO enhancements are colocalized, with the FeO volume generally exceeding that of GBCA. These findings suggest that vascular expansion extends well beyond the area of BBB compromise. FeO accumulation in tissue over 24-72 hours indicates heavy macrophage activity in and around acute lesions in JME, and extends well beyond areas of BBB breakdown visualized by GBCA. That there is a relationship between inflammation and BBB compromise is well accepted, but the temporal nature of the relationship is unknown. Lesions with FeO enhancement volumes equal to or less than GBCA may be pathologically distinct or represent a different disease stage than those shown here. Longitudinal studies combining FeO and GBCA may reveal a causal relationship between expanded vascular space and BBB compromise throughout lesion evolution.

Grant Support: OHSU/Vertex SRA-05-07; Oregon Opportunity, DOD CDMRP-00486280; NIH: P51-RR00163-49; UL1-RR024140-S1; S10 OD018224 01

References: 1. Popescu, *et al*, *Continuum* **19**:901-21 (2013). **2.** Ge, *et al*, *AJNR* **26**:1539:1547 (2005). **3.** Axthelm, *et al*, *Ann Neurol* **70**:362-73 (2011). **4.** Rooney, *et al*, *PISMRM* **13**:6973 (2005). **5.** Weinstein, *et al*, *J Cereb Blood Flow Metab* **30:**15-25 (2010). **6.** Njus, *et al*, *PISMRM* **17**:1480 (2009).