Novel lesions in the spinal cord of the EAE model of multiple sclerosis identified with SWI MRI

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Introduction Recent controversies in multiple sclerosis (MS) which postulate blocked veins as a cause, link iron with the pathophysiology of MS. Susceptibility-weighted imaging (SWI) is a non-invasive MRI tool sensitive to tissue iron, thereby allowing for visualization of physiological processes related to iron deposition[1]. MS plaques in patients have been shown to have abnormally high iron content[2]. As SWI has the ability to become part of the standard imaging protocol for MS patients, it is crucial to have utilized this tool in the study of MS using animal models, both to characterize the signal changes, and to confirm their presumed source (iron). The experimental allergic encephalomyelitis (EAE) animal model is widely used to study MS, but little is known about iron in the EAE model with respect to SWI lesion detection. We used an SWI 3D gradient echo MRI on the lumbar spinal cords of EAE mice. High resolution MRI was performed *in vivo* and *ex vivo* on fixed spinal cords to visualize lesion pathology.

Methods This study used seven mice including two controls. Animals were induced with EAE using methods previously described[3] and were scored on a 14-point grading scale for motor function and behavior daily to gauge their level of disability[4]. Imaging was done with a 9.4T MRI Bruker Biospec using a 3D gradient echo (FLASH sequence). The lumbar spinal cords of three animals (one control) were imaged *in vivo* using gradient echo without flow compensation (256x128x32 matrix, FOV=0.92x1.28x1.28 cm, TE/TR=4/50, flip angle=15°, 17 averages). Four mice (one control) were imaged *in vivo* using gradient echo with flow compensation (192x128x32 matrix). These four animals were imaged again *ex vivo* using gradient echo (192x128x32 matrix, FOV=0.92x1.28x1.50, TE/TR=4/50, flip angle=15°, voxel size=48x100x469μm). Post-imaging, using SPIN software (MRI Institute, Detroit, MI), phase images were processed using a 32x32 Hanning filter to create filtered phase images; filtered phase data was combined with magnitude data to create SWI images.

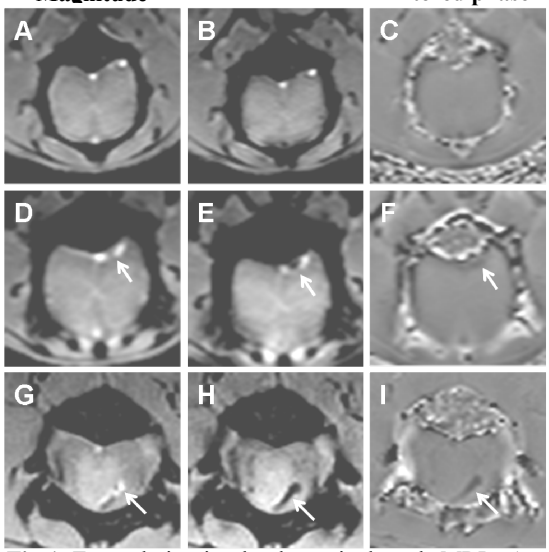

Fig 1. Example in vivo lumbar spinal cords MRIs. **A to C** show a control mouse (flow-compensated). **D to F** are from a peak EAE mouse (flow-compensated). White arrows show region of abnormality in the SWI image. **G to I** are from a chronic EAE mouse (not flow-compensated) with arrows indicating abnormalities and enhanced contrast in the SWI and phase image compared to the magnitude image.

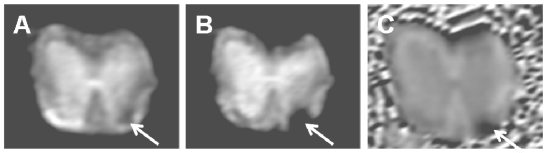

Fig 2. Ex vivo lumbar spinal cord MRI from an EAE mouse. **A, B, C** show magnitude, SWI and filtered phase images respectively for a mouse with chronic EAE. White arrows show a region of abnormality in the phase and SWI images with much greater contrast than that seen in the magnitude image.

Results *In vivo* MRI (Fig 1) showed significant differences between the magnitude and SWI images for peak and chronic EAE mice, both with and without flow-compensated acquisitions. Some white matter lesions containing a dark spot in the center of the lesion were present in the filtered phase and SWI that were not seen in the magnitude image. Also, SWI showed enhanced contrast compared to the magnitude image in instances where the magnitude image hinted at an abnormality as seen in panels G through I (Fig 1). The control mouse did not appear to have any regions of abnormalities. *Ex vivo* data (Fig 2) confirmed that the filtered phase and SWI images both contained abnormalities that appeared much more enhanced compared to the magnitude image.

Discussion Phase usually reflects changes in B_0 (from tissue susceptibility) or motion[5]. Tissue processes that can cause these changes and show contrast in the phase image include iron deposition (as iron is paramagnetic), flow, and changes to the tissue geometry and microstructure[6]. The phase changes could stem from blood flow related motion and not from pathology-based factors. However, similar changes are observed with and without flow compensation suggesting this is not the main cause of the phase changes. The signal changes are also probably too large to be caused entirely by changes to the microstructure of the tissue, suggesting iron may play the dominant role. As the EAE mouse is one of the most common models of MS, it is very useful to know that SWI and phase MRI do show abnormal lesions. It is also interesting to note that the primary induction in these animals relates to the immune response, and not to blocked draining veins.

Conclusions SWI and filtered phase images can be used to detect novel lesions and to show abnormalities within lesions that may not be visible by looking only at the magnitude image. This work shows that the EAE mouse may have abnormal iron deposition related to the development of the disorder and that blockage of major draining veins may not be required to induce the disease.

References [1]Haacke, EM et al. (2009) *J Magn Reson Imaging*. 29:537-44. [2]Eissa, A et al. (2009) *J Magn Reson Imaging*. 30:737-42. [3]Tsutsui, S et al. (2004) *J Neurosci*. 24:1521-9. [4]Giuliani, F et al. (2005) *J Neuroimmunol*. 158:213-21. [5]Haacke, EM et al. (2009) *AJNR Am J Neuroradiol*. 30:19-30. [6]He, X and Yablonskiy, DA (2009) *Proc Natl Acad Sci*. 106:13558-13563.