SWI lesion load and tissue hypoxia in multiple sclerosis: a study using the experimental autoimmune encephalomyelitis animal model at 9.4T

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Target Audience: Researchers and clinicians interested in the physiology of multiple sclerosis (MS). Specifically those interested in the potential link between pathology, venous regulation and low oxygen (hypoxia) in MS.

Purpose: To determine if venous hypoxia as detected with susceptibility weighted imaging (SWI) coincided with tissue hypoxia and inflammation in the spinal cord of the EAE animal model.

Methods: The experimental autoimmune encephalopathy (EAE) mouse model of MS were used as well as controls injected with adjuvant but no sensitizing protein (CFA). 3 CFA controls and 3 EAE animals were studied, 2 EAE at 7 days (pre-peak motor dysfunction) and 1 at 20 days (after peak). Mice were scored on a 15 point EAE behavioural deficit scale[1] and then administered pimonidazole HCl (Hypoxyprobe Inc, Burlington, USA) intraperitoneally 15 minutes before MRI at a dose of 60 mg/kg body weight. Pimonidazole administration and MRI imaging were both done in the presence of anesthesia to allow for direct comparison. After MRI, which took 1.5 hours, mice were perfused with PBS and 10% formalin, and immediately re-imaged. MRI was undertaken using a 9.4T Bruker Avance console with a 20mm surface coil and 3D GEFC (matrix=192 x128x32, FOV=0.92cmx1.28cmx 1.28cm, TE/TR/α=4ms/ 50ms/15°, NEX=17,voxel size=48x 100x400μm). SPIN software was used[2] to process images, with a 32x32 Hanning filter and by multiplying the negative phase mask into the magnitude data 4 times to create SWI images.

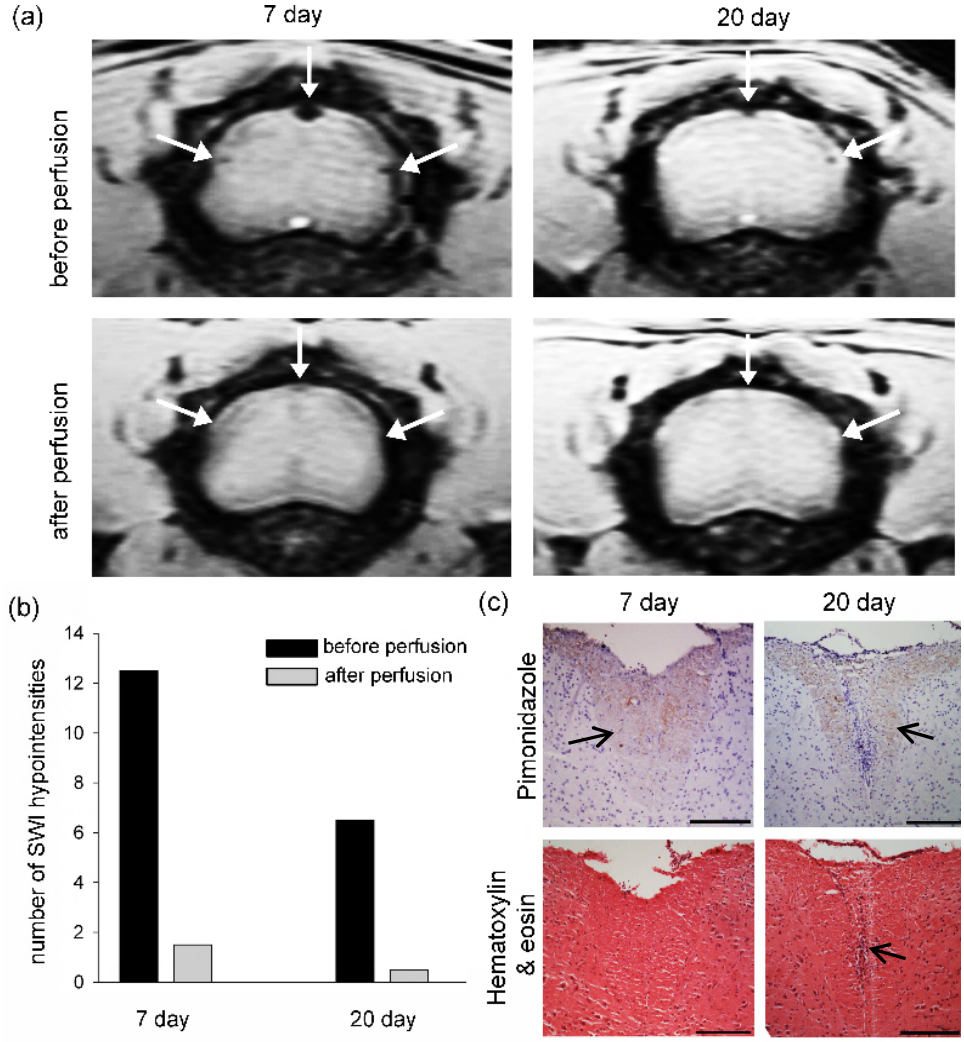

Figure 1: (a) MRIs of EAE mice at days 7 and 20 post-immunization, before and after perfusion. Arrows show SWI hypointensities that are eliminated by perfusion **(b)** Quantification of SWI hypointensities in the EAE mice shown in (a). **(c)** Pimonidazole (brown stain indicating hypoxia shown by arrow seen at both days) and hematoxylin & eosin (hypercellularity shown by arrow indicating leukocyte infiltration in day 20) staining of the lumbar dorsal funiculus of the spinal cords of the EAE mice shown in (a).

Results: Day 7 mice and the day 20 mouse had behavioural scores of 0 and 10, respectively. SWI hypointensities were more prominent in day 7 EAE mice compared to the day 20 EAE mouse. Many hypointensities seen before perfusion were not seen after perfusion in both groups (**Fig. 1a**). Additionally, day 7 EAE mice had a greater number of SWI hypointensities than the day 20 EAE mouse (**Fig. 1b**).

Pimonidazole staining of the dorsal spinal column in EAE at both days was positive for hypoxia, with the 20 day cord appearing to have stronger staining (**Fig. 1c**). Hematoxylin & eosin (H&E) staining of the two cords revealed no obvious signs of inflammation in 7 day EAE mice, but was positive in the 20 day EAE mouse (**Fig. 1c**).

Discussion: The disappearance of SWI hypointensities after the removal of blood from the vasculature indicates that they are due deoxyhemoglobin. It has previously been shown that such hypointensities are increased in EAE mice at peak and long term compared to CFA/PTX controls[3]. In this previous study, there was no direct measurement of hypoxia, making it possible only to hypothesize such a link. In this study, we confirm that were SWI vascular based lesions were present, there was also hypoxia. We also confirm that high levels of inflammation are not required for hypoxia to be present. Our data indicates that venous and tissue hypoxia occurs earlier in EAE than has previously been shown by others[4] and may occur before behavioural deficits.

Conclusion: This work supports the hypothesis that increased SWI lesion load may indicate the presence of tissue hypoxia. We show that it is possible for hypoxia to precede significant inflammatory load or behavioural deficit. To our knowledge, this is the first time hypoxia has been reported in EAE mice. The combination of SWI and hypoxia staining provides a powerful multimodal tool to study the role of SWI in monitoring hypoxia in MS.

References:

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