Contrast-enhanced ex vivo MR reveals inflammatory zone in X-linked adrenoleukodystrohpy

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Background: X-linked adrenoleukodystrophy (ALD) is a genetic disorder characterized by progressive brain inflammation [1]. Plasma and other tissues reveal an increase of very long chain fatty acids, lysophosphatidylcholine (C26:0) in particular [2]. Luxol fast blue (LFB) selectively binds lipids and was used as a white matter-selective magnetic resonance (MR) imaging contrast agent for human *ex vivo* brain tissue. Tissue blocks from four ALD occipital lobes were imaged at 14T before and after LFB staining; image contrast and relaxation rates were compared.

Methods: Brain tissue was stained with LFB, following a novel protocol [3], and imaged at 14T. Longitudinal and transverse relaxation rates were measured with inversion recovery-prepared spin echo, multi-echo spin echo, and gradient-recalled echo sequences. After imaging, samples were bisected and one half used for immunohistochemistry (IHC) and electron microscopy; the other half was dried and weighed, followed by lipid analysis and LC-MSMS [4]. Identical lipid analyses were also performed on non-ALD control tissue from occipital lobes.

Results: LFB staining revealed an additional anatomical zone in high-resolution MR images. Although previous work suggests T1 weighting produces optimal LFB contrast enhancement in normal tissues [3], T2*-weighted images produced the largest LFB contrast enhancement in ALD samples, with contrast-to-noise increases as high as four-fold over unstained samples. IHC methods verified the classification of four regions in the LFB-stained MR image as (1) core lesion, (2) inflammatory zone, (3) normal-appearing white matter (NAWM), and (4) cortex (see Fig. B). As seen in the bar graphs below, the newly MR-identified inflammatory zone exhibited the largest increase in R2* (Fig. C) and also contained an abundance of microglia and macrophages (IBA-1 positive, Fig. D). Whereas NAWM displayed the largest increase in R1 (Fig. E) and myelin basic protein (MBP) density (Fig. F). R2* increases correlated more with IBA-1 positive cells, while R1 increases correlated more with myelin basic protein density. Electron micrographs showed macrophages to be laden with lipids and myelin debris. Furthermore, in LFB-stained tissues electro-dense particulates were found attached to intact and degenerating myelin engulfed by macrophages (Fig. G). Lipid analysis revealed a 10.4-fold increase in the total C26:0 lysophosphatidylcholine compared to non-ALD samples.

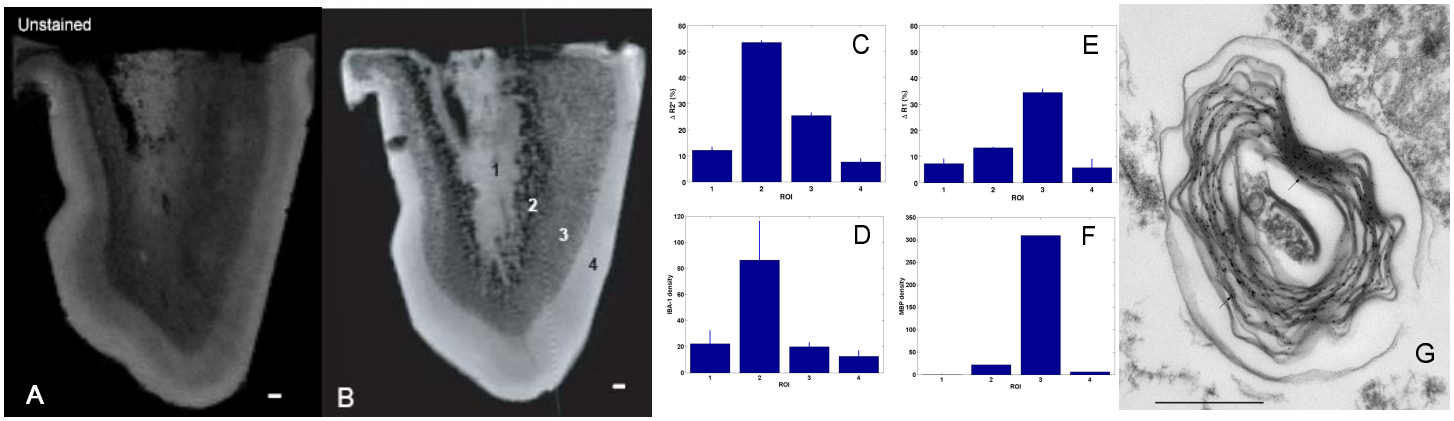

Figure. MR images of unstained ALD samples resemble myelin basic protein stains, which correspond to myelin density; whereas LFB-MR images superimpose additional information about macrophage and microglia density atop myelin distribution. Images (A) and (B) are the GRE acquisitions pre- and post-LFB-staining; scale bar equals1 mm. Increases in R2* (C) and R1 (E) were calculated for each of the four regions shown in (B). A mirror-image tissue sample was used to obtain the IBA-1 (D) and myelin basic protein (F) densities. LFB particles are localized within myelin sheaths on electron micrograph (G); scale bar equals 500 nm.

Conclusions: We demonstrate with LFB-MR microscopy a zone of macrophages and myelin debris at the leading edge of active demyelination. Both immunohistopathology and biochemistry helped to explain the distinct contrasts seen in LFB-enhanced MR images: inflammation on T2*-weighting and NAWM on T1-weighting. Thus, a single contrast agent is able to measure different biochemical parameters. Electron microscopy of LFB-stained samples localized electro-dense particulates within myelin sheaths and within macrophages, bound to myelin breakdown products and cytoplasmic inclusions. These were presumed to be accumulations of LFB dye molecules, whose family of chemicals are prone to aggregation by van der Waals' attractive forces between their phthalocyanine rings [5]. Our findings support the conclusion that enhanced susceptibility contrast in ALD samples is partly due to compartmentalization of LFB. The increased total C26:0 lysophosphatidylcholine found in this region may promote additional LFB binding. Our technique of contrast-enhanced ex vivo MR microscopy permits the investigation of lipids and inflammation in the process of demyelination.

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