Quantitative Analysis of Experimental Allergic Encephalomyelitis in Rat Using 3D MR Microscopy

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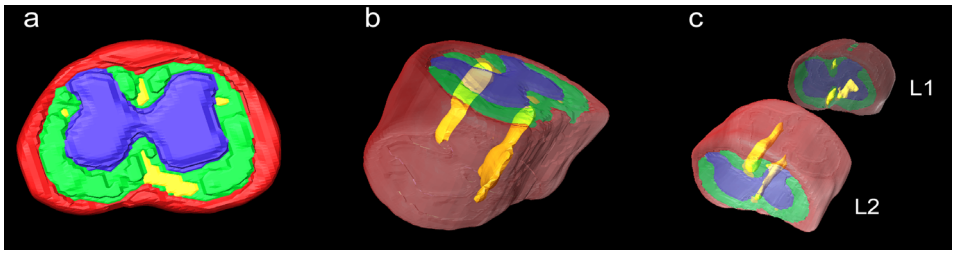
INTRODUCTION - Studies are underway to improve the speed and accuracy of methods used to assay the neurological effects of new generations of multiple sclerosis (MS) therapeutics in animal models. Towards this goal, we are exploring 3D MR microscopy (MRM) as an alternative to conventional histology. Using MRM data one can rapidly perform 3D segmentation and quantification of the central nervous system (CNS) anatomy. The experimental allergic encephalomyelitis (EAE) DA rat model [1] of MS was used for these studies. Our initial studies were in intact, fixed, spinal cord tissues from EAE and control rats. We acquired volumetric MRM data at ~50 µm isotropic resolution using an 11.7 T MRM system, and we examined the neuroanatomical changes due to EAE over the entire length of the spinal column using a quantitative segmentation analysis. From the segmented data the volumes of grey matter, white matter, spinal nerves and apparent EAE lesion load were extracted, and then mean values of the volumetric measurements were calculated for each vertebra. In the same tissues, conventional histological analysis for inflammation, demyelination, and cell loss was used to compare with the MRM results. Collectively, these data are an excellent baseline for future neuropharmacological studies in the EAE model.

METHODS - Female DA rats were used from Harlan UK (160-180 g). EAE was induced using syngeneic spinal cord homogenate [1]. Complete Freund's adjuvant (CFA) was prepared by mixing 100 mg of *Mycobacterium tuberculosis* (Difco, DF3114-33-8) into 10 ml of incomplete Freund's adjuvant (Difco, DF0639-60-6). Portions of frozen DA rat spinal cord were weighed (0.25 mg per rat). The spinal cords were homogenized in ice-cold sterile CFA (0.2 ml per rat) using an Ultra-Turrax dispersing tool. A homogenous thick fluid was obtained (spinal cord homogenate, SCH). Rats were anesthetized using metaphane, the tail base was shaved, and the rats were given a subcutaneous injection at the tail base with 0.2 ml of SCH. Rats were then housed in the vivarium in the Pittsburgh NMR Center. Animals were fed and watered freely. Rats were weighed and monitored daily for clinical symptoms. Animals were sacrificed at day 32, where all showed significant manifestations of the disease (e.g. hind limb paralysis), although some recovery was often noted. Tissues were preserved by anesthetizing the animal, followed by a transcardial perfusion with phosphate buffered solution (PBS) and then fixative (4% paraformaldehyde). The spinal columns were then dissected, with the vertebral bones intact. The columns were cut into segments corresponding to the cervical, thoracic, and lumbar regions. The sacral region was omitted from this study. Specimens were immersed in PBS in 10 mm sealed NMR tubes. Intact segments were imaged in 3D using a Bruker 11.7 Tesla MRM system at the Pittsburgh NMR Center for Biomedical Research. Data were acquired at approximately ~50 µm isotropic resolution using a T_2-weighted 3D spin-echo sequence with TR/TE=900/45 ms. Digital segmentation was performed in a semi-automated fashion using the software package Amira (TGS Inc., San Diego, CA). Regions of interest were segmented by tracing their boundaries slice-by-slice using a digitizing tablet. White matter, grey matter, spinal nerves, apparent EAE lesions, and total spinal cord were segmented. In the T_2-weighted data EAE lesions appear hyperintense. The 3D data sets were further subdivided into the specific volumes for each vertebra, and these were appropriately enumerated (*e.g.* L1 and L2 for lumber 1 and 2, etc.). The vertebral boundaries were demarcated halfway between consecutive descending spinal nerve roots. Mean volumes were calculated for all rats studied (n=5), along with the standard deviations of the mean.

RESULTS - Figure 1 shows a lumbar vertebra from a typical EAE rat and clearly shows the 3D topology of several segmented lesions. Figures 2 show exemplary results of this quantitative segmentation analysis of the mean lesion load along the spinal axis (n=5). Lesion volumes were found to be prominent in the upper lumbar (L1 and L2) region and the upper thoracic/cervical (C7, T2 and T3) regions (Fig. 2). After MRM, the same tissues were prepared for conventional histological analysis, and the MRM observations were correlated with semi-quantitative analysis of the stained histology sections.

DISCUSSION- These methodologies represent a novel approach to rapidly assay the pathologic state of fixed CNS tissues containing demyelinating/inflammatory lesions. MRM in fixed tissues represents a 'high-throughput' alternative to conventional histology and yields more accurate quantitative results than *in vivo* MRI approaches.

FIGURE 1. Three-dimensional rendering of segmented upper lumbar vertebrae. Here, spinal nerves (red), white matter (green), and grey matter (blue) are shown translucent against opaque lesions (yellow). In (b) and (c) oblique views show that lesions can be followed throughout the 3D volumes.



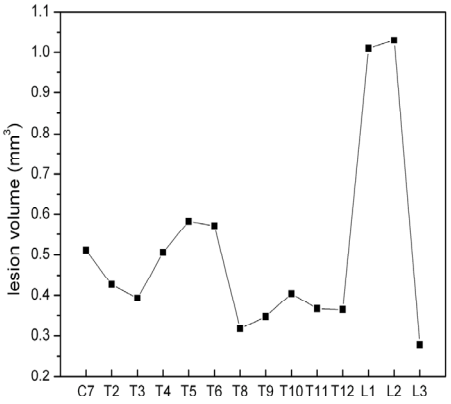


FIGURE 2. Mean lesion volume in EAE rats of segmented vertebrae (n=5).

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REFERENCES – 1. J.C. Lorentzen et al., *J. Neuroimmunol.* **63**, 193-205 (1995).