Time-course Assessment of Pathology in a Mouse Spinal Cord Model of Multiple Sclerosis using Ex Vivo 3D MR Microscopy

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INTRODUCTION: Local injection of lysolecithin into the cervical spinal cord of mice provides a model of demyelination with spontaneous remyelination [1]. Serial sectioning and histology has been used to show that there is a peak in demyelination after 1 week [2, 3] but the lesion volumes are variable [3]. The aim of this study was to determine if lesions could be identified using high resolution 3D gradient echo MR microscopy, and to characterize the lesion size over 4 weeks.

METHODS: Male mice (C57/Bl6) were anaesthetized and microinjected with 1% lysolecithin (1 µL) into the spinal cord dorsal funiculi between cervical vertebrae 5 and 6. Immediately following in vivo MR imaging, mice were cardioperfused with 4% paraformaldehyde and the spinal cord was removed. Cords were immersion fixed for at least 48 h in 4% paraformaldehyde. Prior to imaging, the samples were immersed in 0.01mmol/mL Magnevist solution for 1 h. They were then rinsed and placed in perfluorocarbon solution (Flurinert 77, 3M) for imaging. 3D gradient echo images (TR/TE=50/5 ms, NA=3, FOV = 1.28-1.92x1.28x1.28cm, matrix=256x256x256, resolution=50-75x50x50µm) were acquired on a 9.4T Bruker Avance system at 1, 2, 3, and 4 wk following injection. ROIs were manually drawn measuring the area of the dorsal funiculus and lesion for each slice covering the extent of visible lesion. 3D rendering was undertaken with ResolutionMD™ 3D reconstruction and analysis software.

RESULTS: 2D axial views from the 3D acquisitions in figure 1 show the lesion in the dorsal funiculus as well as an example of a saline-injected spinal cord. Saline injected animals showed small regions of abnormal signal around the site of injection. Figure 2 shows the 3D rendering where the extent and quality of the lesion can clearly be seen. Figure 3 shows the time-course of lesion volume changes.

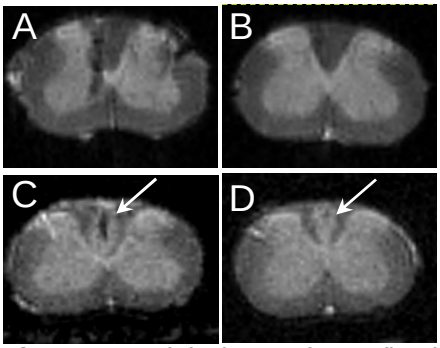

Figure 1. Axial views of two fixed mouse spinal cords: saline-injection (A,B) and lysolecithin-injected (C,D). The site of injection (A,C) and 0.75mm rostral from the injection site (B,D) are shown. Arrows indicate the lesion.

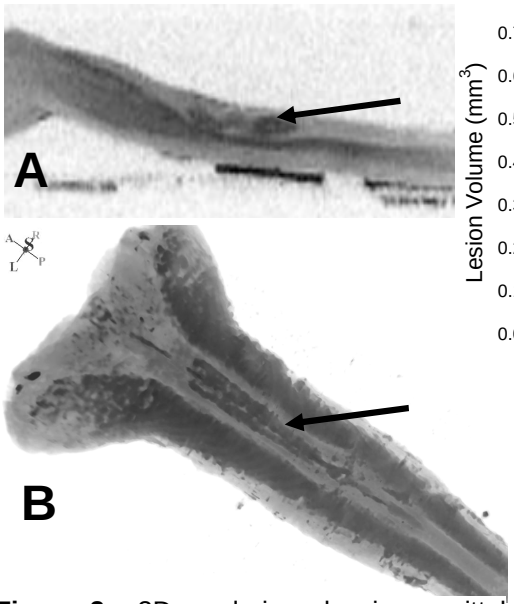

Figure 2. 3D rendering showing sagittal (A) and coronal (B) views. The full aspect of the lesion can be observed as well as apparent compression of the gray matter in A. The lesion and gray matter appear dark.

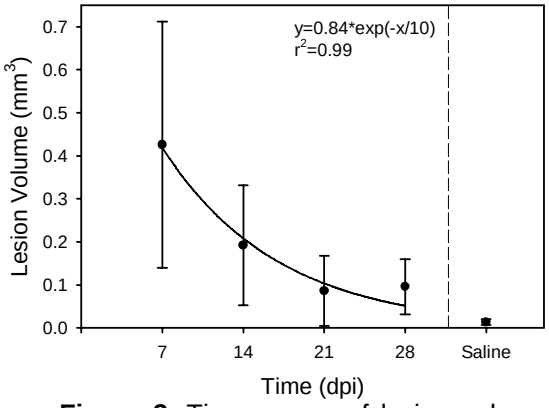

Figure 3. Time-course of lesion volume changes (mean ± SD). n=3, 4, 6, 10,and 4 for 7, 14, 21, 28 dpi and saline groups respectively. Saline-injected controls are shown but were not included in the fit. Data were fit to a single exponential decay which indicated that the recovery time constant is 10 days.

DISCUSSION: Lesion areas were clearly visible in the 3D gradient echo images. These were analysed with single slice ROI analysis, but the contrast is acceptable for 3D volumetric thresholding and automated analysis. The data are consistent with previous work indicating that the time point of the most extensive damage is 1 week after injection [2,3] The variability between cords is significant and shows the importance of being able to measure volumes in individual cords, as well as being able to render in 3D to identify other unusual features such as grey matter compression.

REFERENCES:

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