Ex-vivo MRI Measurement of Lesion Volume in Mouse Spinal Cord Contusion Injuries Correlated with Histological Examinations

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Purpose

We investigated the feasibility and reliability of using MR imaging and analysis methods to accurately measure lesion volume in the spinal cord of contusion-injured mice. Histological examination using fibronectin immunostaining is the current standard. However, it is very time consuming. High quality MRI of ex-vivo rat cords has been proven feasible [1-2]. Imaging of mouse cord is more challenging due to its smaller size. In this study, we measured the lesion volumes of injured mouse cord using a 7 Tesla MRI scanner. The results were compared to lesion volumes determined by stereological sampling of fibronectin immunostained sections.

Methods

Forty 8-week old female C57Bl/6 mice were used in this study. They were separated into 4 groups, 10 mice in each group. Three different injury severities were generated using a mild (30 kilodyne), moderate (50 kilodyne), or severe (70 kilodyne) contusion injury at the T9 vertebral level with the IH Impactor. One group serves as control. After injury, all mice were tested for functional recovery with the BBB (Basso, Beattie, Bresnahan) and BMS (Basso Mouse Scale) locomotor rating scales. Three weeks after injury, mice were euthanized and the spinal cord between the T6 and T12 spinal roots were collected, and stored in PBS at 4°C. Each cord was placed into a glass NMR tube and vacuumed to de-bubble. The MRI was performed using a 7T horizontal-bore MRI scanner equipped with a SMIS acquisition console. Nine cords were positioned into a holder and imaged together. A localization sequence was first applied to define the location of the cords. Then a 3D-SPGR (Spoiled Gradient Recall) pulse sequence was applied to obtain 128 T₂-weighted cross-sectional images, covering all 9 cord segments. The imaging parameters were 20 mm field of view (FOV); 128 x 128 matrix; repetition time [TR] = 30 msec; echo time [TE] = 3.0 msec; flip angle = 30°, and 20 excitations. The resulted in-plane resolution was 0.156 mm, and the slice thickness was 0.234 mm. The total imaging time was 164 minutes. An analysis program based on histogram analysis was developed to measure lesion volume. The 3 structures, PBS, intact cord tissue, and lesion were first segmented based on intensity histogram thresholding, then followed by morphological dilation or erosion. The lesion area on each image was determined, and then the total volume calculated. After MRI, cords were processed for histological examination. Each cord was embedded in polyester wax, and 20 μm coronal sections were cut on a rotary microtome. Every twelfth section was processed for immunocytochemistry using anti-Fibronectin. An Olympus microscope was used to stereologically measure the fibronectin positive volume and total spared white matter volume, using the Cavalieri Method. The MRI lesion volume was compared to the injury force, histological lesion volume, and the BBB and BMS functional recovery scores.

Results

Figure 1 shows images from 3 cords, with mild, moderate, and severe injury. The cord was placed in a 5 mm NMR tube filled with PBS. The outside PBS had a lower signal intensity compared to the cord tissue, and lesion area inside the cord also showed a lower signal intensity. Each cord was analyzed separately. A threshold based on signal intensity histogram was used to separate the intact cord from the injured tissue. Then the connecting tissues to the lesion may be included or excluded into the lesion category by morphological dilation or erosion. The Fibronectin immunostaining slides from three cords are also shown in Fig.1. The staining clearly demonstrates the injury border. Figure 2 shows the correlation between MRI lesion volume and Force (Spearman Rank Rho=0.805, p < .0001), and between MRI lesion volume and Fibronectin lesion volume (Spearman Rank Rho=0.749, p = .0002), both significant. The MRI lesion volume was a good predictor of lesion volume in different injury severities. The mild injury group had faster recovery and reached to higher scores 3 weeks after injury (BBB= 15, BMS= 8), and the severe group had lowest recovery and could only reach to lower scores (BBB= 8, BMS= 3).

Discussion

Histological examination has been the standard method for quantifying spinal cord injury. However, it is highly dependent on staining quality, involving subjective judgement, and very time consuming. High resolution MRI has been proven useful, however still involving subjective analysis [1-2]. In this study, we demonstrated that the image acquisition setting employed here can achieve a reasonable spatial resolution and contrast between intact and injured tissues, and 9 cords could be imaged at the same time to achieve a high throughput. The analysis still involved determination of threshold, based on intensity histogram not arbitrary operator choice. Another feature is dilation and erosion based on tissue connectivity. We have demonstrated that the lesion volume determined by MRI was correlated with injury force, histological volume, and functional recovery. Future studies are planned to further refine the speed and resolution of the MRI scanning, in order to develop optimal cost vs. time and resolution parameters to allow accurate determination of lesion volumes and tissue sparing.

References


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Figure 1: The MRI of 3 cords with mild, moderate, and severe injury, lesion area on coronal and parasagittal view outline in red, cord in green. Fibronectin immunostaining demonstrates increased lesion area with severity.

Figure 2: The MRI lesion volume was correlated with injury force (Rho=0.805, p < .0001), and Fibronectin lesion volume (Rho=0.749, p = .0002). The three injury groups are indicated by different colors. It can be seen that the variation in severe injury group was higher, which may in part due to cord shrinkage and indistinguishability between lesion and intact tissues in Fig.1.