In Vivo Magnetization Transfer Measurements of Experimental Spinal Cord Injury in the Rat

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Magnetization transfer (MT) imaging techniques were implemented to study a clip compression model of spinal cord injury (SCI) in the rat. The purpose of this study was to determine if the magnetization transfer ratio (MTR) could be used to classify the stage and severity of SCI. Two clip compression injuries were studied: mild SCI and severe SCI. MTRs were determined for gray matter (GM) and white matter (WM) regions and the GM–WM contrast was determined on days 1 and 7 following surgery. Despite differences in pathologic features of mild and severe SCI, the GM–WM contrast did not allow discrimination between the two degrees of severity of SCI. WM MTR allowed differentiation of mild and severe SCI on day 1. These preliminary results suggest that WM MTR may provide an indication of the severity of injury in SCI. Magn Reson Med 45: 159–163, 2001. © 2001 Wiley-Liss, Inc.

Key words: magnetization transfer ratio; spinal cord injury; magnetic resonance imaging; gray matter–white matter contrast

Traumatic spinal cord injury (SCI), a common event in North America, not only leads to a loss in mobility and sensation, but can also result in serious disturbances in normal organ function, cardiovascular control, and chronic pain (1). An important current research in this area will be the development of effective ways of monitoring the progress of a particular treatment in vivo in a noninvasive manner over a period of time. While qualitative assessments are useful, methods to quantify changes that occur in vivo in the injured area of the cord are critical. MRI is the preferred modality for visualizing spinal cord injuries. In animal models of SCI, monitoring disease progression by MRI can be linked to measurements of spinal cord pathology.

Several studies have shown that MRI is a valuable method for in vivo assessment of intact spinal cord and spinal cord pathology in animal models of SCI (2–7). Only a few groups have used MRI in vivo to study the time course of pathological changes in rats following SCI (8–12). In vivo MRI of small animals, and in particular of the spinal cord, is challenging. A major drawback is often the long imaging times required to collect high-resolution images with suitable SNR. The use of high magnetic field strengths and custom built gradient and RF coils offsets some of the difficulties. Implantable RF coils have been used to obtain high resolution, high SNR in vivo images of rat spinal cord (8,9,13). With this solution, Narayana et al. (9) successfully assessed gray matter-to-white matter (GM–WM) contrast to characterize SCI in the rat. The results of their study suggest that a loss of GM–WM contrast is closely related to loss of cholesterol throughout cord tissue and subsequent edema.

Alterations in the lipid components of nervous tissue, such as cholesterol, can also be measured using magnetization transfer (MT) (14). MT contrast is obtained by applying off-resonance RF irradiation designed to preferentially saturate the immobile, or bound, protons of macromolecules. This saturates the energy level of the bound protons, inducing energy transfer to the mobile, or free water, protons and consequently reducing their signal intensity. The relative difference in signal intensity (SI) with and without magnetization transfer can be easily and reliably measured as the magnetization transfer ratio (MTR) (15).

MT has not previously been applied to study SCI in vivo, although Berman et al. (16) showed that, in fixed tissue from cord-injured rats, MT histogram analysis was highly predictive of injury level and pathological events. The goal of our study was to determine if MTRs could be used to classify the severity and stage of SCI in a rat model of SCI. We hypothesize that the pathology of SCI would lead to a reduced MTR in spinal cord WM that would vary depending on the severity and stage of injury, thus allowing the characterization of SCI in a more quantitative manner. As a corollary, we predicted that the MTR would provide a more specific indication of spinal cord pathology than the determination of GM–WM contrast.

METHODS

Rat SCI Model

SCI was induced in Wistar rats (200–250g) by the clip compression method of Rivlin and Tator (17), which employs a spring-loaded modified aneurysm clip calibrated to close with a specific force. Two clip compression injuries were used in these studies: a mild SCI induced by a 20 g compression clip (n = 4) and a severe SCI induced by a 50 g compression clip (n = 5). All rats were given preanesthesia medications of 3.5 mg/kg diazepam ip and 0.05 mg/kg atropine sc. The rats were then anesthetized by induction with 4% halothane in 100% oxygen and maintained on 1–2% halothane throughout the surgical procedures. A dorsal laminectomy was performed and a clip compressed the spinal cord for 1 min at the T3 segment. For the purposes of comparison, one rat was subjected to only the dorsal laminectomy without the clip compression and five intact rats served as controls.

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Imaging Protocol

Imaging was performed at 4.0 T on a Varian/Siemens UNITY INOVA using a custom-built solenoid RF coil (5 cm diameter, 4 cm length). Rats were placed in the supine position with the epicenter of injury centered in the RF coil. Anesthesia was induced with 2% isoflurane in oxygen and maintained during the MRI experiment with 1% isoflurane administered into a Plexiglas chamber that housed the RF coil. All SCI animals were scanned on days 1 and 7 following surgery. A multislice proton density-weighted spin echo (SE) sequence was used to identify the axial spinal cord image slice to be used for subsequent MT experiments.

MT imaging was performed with a proton density-weighted SE single-slice acquisition. The single-slice mode was chosen to avoid incidental MT effects. The imaging parameters were as follows: repetition time (TR) of 5 sec, echo time (TE) of 20 msec, slice thickness of 3 mm, 5 cm field of view, and image matrix of 256 × 256. The in-plane spatial resolution was 195 μm². The characteristics of the saturating pulse train (pulse shape, pulse width, average power (B₁), frequency offset, number of saturation pulses, and time between pulses) were tested and optimized previously (18). A distilled water phantom was included in the field of view as a reference to estimate the direct saturation of the pulse train. The optimized parameters were: MT saturation produced by a train of 30 15 ms Gaussian RF pulses with average B₁ of 4.2 μT, 1.5 kHz off resonance. The time between pulses was 3.75 ms, resulting in a duty cycle of 80% for the saturating RF pulse train. The mean square saturating power (Pₛₑₑ) and effective flip angle (θₑₑ), calculated as described by Berry et al. (19), were 20.1 μT² and 961°, respectively. The amount of direct saturation, estimated from SI measurements in the distilled water phantom, was found to be less than 7% for the optimized MT sequence.

Image Analysis

The MTR was calculated as:

\[ \text{MTR} = \left(1 - \frac{M_s}{M_0}\right) \times 100\% \]

where M₀ is the mean intensity of pixels within a region of interest (ROI) on an image without saturation and Mₛ is the mean intensity of pixels corresponding to the same ROI on an image with saturation. ROIs were measured in GM and WM in the spinal cord. An average MTR was determined for each group. The GM–WM contrast was measured in the proton density-weighted SE images obtained without saturation and calculated as the ratio of the average SI of GM and WM in the spinal cord image slice. For SCI rats, measurements were obtained in spinal cord sections rostral to the injury epicenter. Data are presented as the mean plus or minus the standard error. Statistical analysis consisted of one-way ANOVA with the Student-Newman-Keuls method for multiple comparisons. Statistical significance was defined with \( P < 0.05 \).

Histopathology

After MR imaging on day 7 all rats were anesthetized with urethane (3 g/kg ip) and perfused transcardially with 250 ml of oxygenated tissue culture medium at pH 7.4 followed by 500 ml of 4% formaldehyde fixative in 0.1 M phosphate buffer (pH 7.4). The thoracic spinal cords were removed. Following overnight fixation, the spinal cords were cut into horizontal sections (50 μm) on a cryostat, mounted on slides, and stained with cresyl violet using standard procedures.

RESULTS

Magnetization Transfer Ratios

Representative axial SE images of intact rat spinal cord, obtained with and without saturation pulses are shown in Fig. 1a,b. The mean SNR in the pre-MT images was 61.6 for WM and 72 for GM. The in-plane spatial resolution for all images was 195 μm². The average MTR for intact spinal cord WM and GM were 45.6% ± 3.21% and 40.4% ± 2.29%, respectively. The MTRs for all groups are presented in Fig. 2. The average WM MTRs measured on day 1 and 7, after mild SCI, were not significantly different from those in intact cords. After severe SCI, the average WM MTR measured at day 1 was significantly reduced in comparison to values in intact cords or after mild SCI on day 1. In addition, the WM MTRs measured at day 7 were significantly greater than those at day 1. The average MTR for GM in all SCI rats was not significantly different from that in intact rats.

GM–WM Contrast Measurements

The average GM–WM contrast measured in intact rat spinal cord was 1.35 ± 0.08. Proton density-weighted spinal cord images for mild SCI and severe SCI are shown in Fig.
3. A loss in the GM–WM contrast, as compared to the intact spinal cord (Fig. 1b) is apparent in all of the images. The spinal cord images in the severe SCI group typically demonstrated gross abnormalities in structure that were not evident in the images of cord after mild SCI. For both groups, and at each time point, the GM–WM contrast was significantly less in injured cords than that measured in intact spinal cord. However, no statistically significant differences were found between groups or between measurements made on day 1 or day 7. These results are presented in Fig. 4.

Histopathology

In Fig. 5, representative cresyl violet-stained spinal cord sections are presented for a laminectomy control rat (5a,b), a mild SCI rat (5c,d) and a severe SCI rat (5e,f). For each example a low-magnification view of the section, with the corresponding MR image inset, and a high-magnification view of an outlined region of interest are shown. Normal gross morphology and cellular architecture was observed in the laminectomy control spinal cord sections (5a,b). Neurons appeared to be of normal size and morphology (arrows, 5b). The MR images of the laminectomy control spinal cord appear similar to the images obtained from intact rats. After mild SCI, localized regions of intense inflammation were visible under low magnification. The inflammatory infiltrate was most intense in the GM and appeared to expand into the WM. Under high-power magnification, the infiltrate can be seen to consist mostly of macrophages. The absence of staining in a portion of the cord WM (arrow) may represent the loss of cellular integ-

![MT of Rat Spinal Cord Injury](image_url)
rity in this region. Severe SCI was characterized by cavitation and vacuolization of GM especially, which contributed to a total loss of the normal gross architecture and GM–WM discrimination in the histologic section. Inflammation after severe SCI was more widespread than after mild SCI. The pathologic features of severe SCI are reflected in the MR image in which compression of the spinal cord is visible as well as a complete loss of GM–WM contrast. In both the mild and severe SCI, neurons in areas of inflammation appeared shrunken in size and were more rounded.

**DISCUSSION**

SCI has not previously been studied in vivo using MTR. In these experiments, significant reductions in the mean WM MTR were measured in the rat spinal cord on day 1 after severe SCI. By day 7 the average MTR for this group had increased by approximately 80% and was no longer statistically different from the average MTR for intact spinal cord WM. The average WM MTR for mild SCI at day 1 or day 7 was not different from the control value.

After the initial injury the cord undergoes sequential pathologic changes, including edema, inflammation, hemorrhage, and necrosis followed by infarction and demyelination. The severity of these pathologic features is much greater after severe SCI than after mild SCI (20). This was confirmed in our histological examination and is a possible explanation for reduced WM MTRs in severe SCI but not in mild SCI. The increase in the WM MTR over time after severe SCI may signify changes in the characteristics of the pathophysiology between days 1 and 7. After the initial injury, edema and hemorrhage diminish as inflammatory infiltrates arise and gliosis and cell death occur. It is important to note that although the MTR increased from day 1 to day 7 after severe SCI, approaching values for intact cord, this change was not reflected in the MR images of the spinal cord and the GM–WM contrast was not different from day 1 to day 7.

Of additional interest is the relative insensitivity of MTR to inflammatory changes in the cord GM. In our previous MT studies, using experimental allergic encephalomyelitis brain of guinea pigs, we found that the MTR was sensitive to inflammatory-related changes to the structure of normal-appearing WM (18). Within regions of intense inflammation in normal-appearing GM, however, the MTR was unchanged. The composition and structure of WM and GM govern their relative MT effects and may also determine the extent to which inflammatory processes modulate the degree of intermolecular interaction between macromolecules and water.

The GM–WM contrast measurement was abnormal for all SCI subgroups. We did not observe any transition in the GM–WM contrast over the 7-day examination period. This is in agreement with Narayana et al. (9), who reported that a temporal recovery of the GM–WM contrast occurs only in spinal cord sections caudal to the epicenter of the injury. Clip compression causes injury to spinal cord tissue both caudal and rostral to the epicenter of the injury. Since our primary interest was the characterization of SCI using MT, and not the recovery of GM–WM contrast in SCI, we chose to examine SCI in sections that were located approximately 2 mm rostral to the site of injury. At this position we were able to acquire high-quality images without the need for respiratory gating to compensate for motion-generated image artifact, which we commonly observed in images caudal to the injury.

Despite the differences in the pathophysiologic features of mild and severe SCI, the proton density-weighted GM–WM contrast did not allow discrimination between the two degrees of severity of SCI. On the other hand, using WM MTR allowed differentiation of mild and severe SCI at day 1. Because the MT behavior of many general pathologic processes overlap, additional studies are required to elucidate the specific underlying pathology related to the reduction in the MTR. GM–WM contrast was only examined in proton-density weighted SE images. This type of contrast ratio, measured from other images, for example,
$T_1^*$, $T_2^*$, or $T_2$-weighted images, might also provide a measure of the extent of injury in SCI. These preliminary results suggest that WM MTR may provide an indication of pathology in SCI. More advanced MR methods, perhaps the determination of $T_1$ sat and MT rate constants, may generate additional information as to the state of the injured cord tissue in vivo.

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