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Validating myelin water imaging with transmission electron microscopy in a rat spinal cord injury model

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ABSTRACT

Myelin content is an important marker for neuropathology and MRI generated myelin water fraction (MWF) has been shown to correlate well with myelin content. However, because MWF is based on the amount of signal from myelin water, that is, the water trapped between the myelin lipid bilayers, the reading may depend heavily on myelin morphology. This is of special concern when there is a mix of intact myelin and myelin debris, as in the case of injury. To investigate what MWF measures in the presence of debris, we compared MWF to transmission electron microscopy (TEM) derived myelin fraction that measures the amount of compact appearing myelin. A rat spinal cord injury model was used with time points at normal (normal myelin), 3 weeks post-injury (myelin debris), and 8 weeks post-injury (myelin debris, partially cleared). The myelin period between normal and 3 or 8 weeks post-injury cords did not differ significantly, suggesting that as long as the bilayer structure is intact, myelin debris has the same water content as intact myelin. The MWF also correlated strongly with the TEM-derived myelin fraction, suggesting that MWF measures the amount of compact appearing myelin in both intact myelin and myelin debris. From the TEM images, it appears that as myelin degenerates, it tends to form large watery spaces within the myelin sheaths that are not classified as myelin water. The results presented in this study improve our understanding and allows for better interpretation of MWF in the presence of myelin debris.

Introduction

Myelin is essential for normal functioning of the nervous system by allowing efficient and rapid propagation of action potentials. Quantitative T2 based myelin water imaging (MWI) can measure myelin content in normal and diseased brain and spinal cord tissue (Kozlowski et al., 2008a; Laule et al., 2004; Minty et al., 2009). Direct imaging of myelin with MRI is difficult because the majority of the signal from protons associated with myelin has decayed by 3 ms (Henkelman et al., 2001). Therefore, quantitative T2 based MWI assesses myelin content by probing the properties of the water trapped between the myelin lipid bilayers (myelin water). Although some people argue that MWI constitutes a direct measurement method because the trapped water is an integral component of the myelin (Rosenbluth et al., 1996), such argument fails if changes in myelin morphology alter the relationship between myelin water and myelin content.

Typically, analysis of the T2 decay curves obtained with a multi-echo spin echo pulse sequence yields three distinct T2 components in the brain and spinal cord tissues. The three components are attributed to water trapped between the myelin lipid bilayers, intra/extracellular water, and cerebrospinal fluid (Whittall et al., 1997). Association of the short T2 component with myelin water is backed up by histological analysis showing good correlation between the myelin water fraction (MWF, calculated as the integral of the myelin water peak over the total integral of the T2 distribution) and myelin content, and has been successfully applied in studying the demyelination process in multiple sclerosis (Laule et al., 2004, 2008), and spinal cord injury (SCI) (Kozlowski et al., 2008b). In these studies, MWI were validated using optical microscopy, which has a resolution limit of approximately 0.2 μm. While the use of immunohistological staining can provide certain molecular information, it does not provide direct access to the fine,

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nanometer-scale structure of myelin (ultrastructural scale). Changes at the ultrastructural level can impact the relationship between MRI-based myelin water imaging technique and myelin, including quantitative T2. While studies have suggested that MWF measures both intact myelin and myelin debris (McCreary et al., 2009; Kozlowski et al., 2008a, b; Webb et al., 2003), the relationship between MWF and myelin debris was unclear.

Because the short T_2 component is related to the signal from water trapped between myelin lipid bilayers, changes in the spacing between these bilayers can influence both the myelin water pool size and its T2. If these changes in the local tissue environment occur over time, it may make the interpretation of the MWF difficult. This is especially important in acute spinal cord injuries, where large amounts of myelin debris are present throughout retrograde and Wallerian degeneration. Thus, one may expect a very different relationship between MWF and myelin content in myelin debris vs. intact myelin, due to myelin morphological changes. Myelin debris can persist for several months before being removed by macrophages (Ludwin, 1990; Stoll et al., 1989), and can potentially confound MWF measurements during that time. To test whether ultrastructural differences between intact myelin and myelin debris indeed affect the relationship between MWF and myelin content, we compared myelin measurements derived from MRI and transmission electron microscopy (TEM) in a rat spinal cord injury model. Specifically, MWF was compared to TEM-derived measurements of compact myelin content in both intact myelin and myelin debris.

There are many rat injury models available, with the most common being transection, contusion, and compression, although clinically, nearly half of SCI are caused by vertebral dislocation (Sekhon and Fehlings, 2001). More recently, dislocation and distraction models were developed to closely mimic the common mechanisms of injury in humans (Seifert et al., 2011; Choo et al., 2009; Dabney et al., 2004; Fiford et al., 2004); however, transection remains the most prevalent model in rat studies (Zhang et al., 2014), because it is easy to perform, highly reproducible, and spares non-targeted white matter. Most importantly, transection guarantees the complete interruption of the targeted white matter, which is useful for reliably creating areas of myelin debris. For this study, a C5 dorsal column transection (DC Tx) was used to study MWI in the presence of myelin debris. Based on our previous findings using this model (Kozlowski et al., 2008b), we expect mainly myelin debris (plus reactive glial cells) at 3 weeks post-injury, which is partially cleared by 8 weeks post-injury in the *fasciculus gracilis* at 5 mm cranial/distal to the injury site. The *fasciculus gracilis* was chosen because it is an ascending tract with virtually no intermingling of neuronal cell bodies at the cervical level, which allows direct comparison of contiguous sections. It is also closer to the sample's edge and therefore expected to be better fixed, especially in the injured cords, where tissue integrity is reduced. The use of separate sections enables each to be differently fixed in order to satisfy the conflicting requirements of high-resolution TEM and MR.

Materials and methods

Animal Preparation

All experimental procedures were carried out in compliance with the guidelines of the Canadian Council on Animal Care and approved by the local Animal Care Committee prior to conducting the study.

Eighteen male Sprague-Dawley rats (250–280 g) were randomly divided into three groups: injury group studied 3 weeks post-injury (six animals), injury group studied 8 weeks post-injury (six animals), and control group (six animals). The animals were housed in a standard rodent care environment with food and water freely available.

Spinal cord DC Tx was performed as described previously (Chan et al., 2005). In brief, the rats were deeply anesthetized, and placed in a stereotaxic surgical frame in the prone position with their skin on the neck shaved and disinfected. A longitudinal midline incision of the neck was followed by a midline split of the neck muscles to expose the cervical vertebrae. A laminectomy of the C5 lamina was performed with a small rongeur. A hypodermic needle was used to puncture two holes on each side of the DC and then a pair of micro-scissors was inserted to a depth of 1.8 mm (depth marked on scissors) to perform the DC Tx.

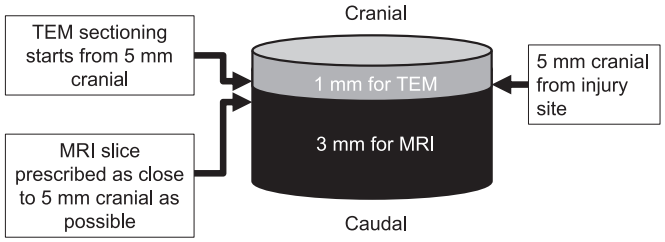

Fig. 1. Division of spinal cord samples for MRI and TEM. The 3 mm section length for MRI allows easier sample orientation in the sample holder. The MRI slice was 0.5 mm thick and positioned as close as possible to the top edge of the sample, which coincided with the bottom edge of the sample used for TEM.

To excise the spinal cords, rats were deeply anesthetized and perfused intracardially with phosphate buffered saline (PBS) for 3 min, followed by freshly hydrolyzed paraformaldehyde (4%) and glutaraldehyde (1%) in 0.1 M sodium phosphate buffer at pH 7.4. Spinal cords were then harvested and placed in the same fixative for 30 min on ice before being divided for TEM and MRI as shown in Fig. 1.

MRI experiments

The 3 mm spinal cord sections were further postfixed overnight in 2% glutaraldehyde before scanning. All MRI experiments were carried out on a 7-Tesla preclinical scanner (Bruker BioSpin GmbH, Ettlingen, Germany) using an in-house built, 13 mm inner-diameter and 25 mm long, five-turn, transmit/receive solenoid coil. Each of the excised cord section was transferred into a 4.5 mm inner diameter plastic tube filled with 2% glutaraldehyde fixative solution for scanning. Two plastic rods were used to prevent in-plane and vertical movements of the cord samples.

A single slice multi-echo spin-echo sequence (Poon and Henkelman, 1992) was used to acquire quantitative T2 data with the following parameters: 1500 ms repetition time (TR), 6.738 ms echo time (TE) and echo spacing, 256×256 matrix size, 32 echoes, 1.79 cm field-of-view, 0.5 mm slice, and 12 averages, giving an in-plane resolution of 70 μm and a scan time of 77 min. Composite pulses were used for refocusing and were bracketed by alternating and descending crusher gradients. Slice location was prescribed as close to 5 mm cranial to injury as practical (Fig. 1), ensuring that the MRI and TEM slices are no more than a millimeter apart.

TEM experiments

The 1 mm spinal cord sections were postfixed in 2% glutaraldehyde for protein fixation for 1 h at 4 °C. The sections were then washed in cacodylate buffer for 10 min (at 4 °C) and placed in 1% osmium tetroxide and 1.5% potassium ferrocyanide in 0.1 M cacodylate buffer for 1 h for lipid fixation (at 4 °C). Afterward, the sections were rinsed in Milli-Q water for 10 min before being stained with 2% uranyl acetate in 30% ethanol for 30 min (4 °C). Finally, the sections were dehydrated by washing them in successively higher concentrations of ethanol (2×5 min in 50%, 2×5 mi in 70%, 2×5 min in 90%, 3×10 min in 95%, and 3×10 min in 100%) at 4 °C. Before embedding, the sections were rinsed at room temperature in propylene oxide (3×20 min), infiltrated with 1:1 propylene oxide and Spurr's resin (4 to 5 h), infiltrated with 1:3 propylene oxide and Spurr's resin (15–16 h), and finally infiltrated with 100% Spurr's resin for 5–6 h. The resin was