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In vivo longitudinal Myelin Water Imaging in rat spinal cord following dorsal column transection injury☆

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ABSTRACT

Longitudinal Myelin Water Imaging was carried out in vivo to characterize white matter damage following dorsal column transection (DC Tx) injury at the lumbar level L1 of rat spinal cords. A transmit–receive implantable coil system was used to acquire multiple spin-echo (MSE) quantitative T2 data from the lumbar spinal cords of 16 rats at one week pre-injury as well as 3 and 8 weeks post-injury (117 microns in-plane resolution and 1.5 mm slice thickness). In addition, ex vivo MSE and DTI data were acquired from cords fixed and excised at 3 or 8 weeks post injury using a solenoid coil. The MSE data were used to generate Myelin Water Fractions (MWFs) as a surrogate measure of myelin content, while DTI data were acquired to study damage to the axons. Myelin damage was assessed histologically with Eriochrome cyanine (EC) and Myelin Basic Protein in degenerated myelin (dgen-MBP) staining, and axonal damage was assessed by neurofilament-H in combination with neuron specific beta-III-tubulin (NF/Tub) staining. These MRI and histological measures of injury were studied in the dorsal column at 5 mm cranial and 5 mm caudal to injury epicenter. MWF increased significantly at 3 weeks post-injury at both the cranial and caudal sites, relative to baseline. The values on the cranial side of injury returned to baseline at 8 weeks post-injury but remained elevated on the caudal side. This trend was found in both in vivo and ex vivo data. This MWF increase was likely due to the presence of myelin debris, which were cleared by 8 weeks on the cranial, but not the caudal, side. Both EC and dgen-MBP stains displayed similar trends. MWF showed significant correlation with EC staining ($R = 0.63$, $p = 0.005$ in vivo and $R = 0.74$, $p = 0.0001$ ex vivo). MWF also correlated strongly with the dgen-MBP stain, but only on the cranial side ($R = 0.64$, $p = 0.05$ in vivo; $R = 0.63$, $p = 0.038$ ex vivo). This study demonstrates that longitudinal MWI in vivo can accurately characterize white matter damage in DC Tx model of injury in the rat spinal cord.

1. Introduction

Functional loss following spinal cord injury (SCI) is largely caused by the interruption or demyelination of axonal tracts in the white matter. In particular, many axons that remain intact following injury lose their function due to myelin degradation as a result of oligodendrocytes undergoing cell death [1,2]. Myelin is essential for the conduction of nervous signals [3], and the initial loss of myelin and subsequent chronic demyelination process have been proposed to play a major role in the loss of motor and sensory function and poor recovery following SCI [4]. Myelin repair has therefore been identified as a major goal in many experimental therapies for SCI. In some ways, re-establishing myelin around existing axons appears easier than reconstructing neurons and re-growing their connections [5,6]. Therefore, significant effort has been directed to designing restoration therapies that rebuild myelin at the injury site by cellular transplantation [7]. Pre-clinical assessment of the efficacy of such therapies would strongly benefit from a non-invasive technique capable of measuring myelin content repetitively over prolonged periods of time.

MRI is currently the most effective radiological method for assessing SCI. However, standard MRI techniques are unable to directly measure the myelin content in white matter tracts, since most of NMR signal from myelin protons has completely decayed

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before it can be recorded (since the T_2 of these protons is typically much shorter than 1 ms [8]). Several methods have been developed to identify myelin indirectly by studying properties of water associated with myelin sheaths and its interaction with protons associated with proteins and lipids that form myelin sheaths. One such technique, called Myelin Water Imaging (MWI), exploits the differences in T_2 relaxation times between various water compartments in the Central Nervous System (CNS), including water trapped in between myelin sheaths, intra-/extra-axonal water, and free water in CSF [9]. MWI provides a surrogate measure of myelin content by calculating the Myelin Water Fraction (MWF), which is the fractional amount of water trapped between myelin bilayers, as identified by the relative amplitude of the short T2 components of multi-exponential decay curves extracted from the multiple spin echo images [10]. Histological analysis has shown good correlation between myelin content and MWF in normal and diseased brain and spinal cord tissue [11,12].

In a previous work, we have successfully applied MWI to measurements of myelin content in excised rat spinal cords [12,13]. Specifically, in a dorsal column transection (DC Tx) model we have shown that MWF correlates better with the myelin content assessed by histology than the more commonly used measure of transverse diffusivity. Recently, we applied ex vivo MWI to characterize the efficacy of transplant skin-progenitor cell-derived Schwann cells (SKP-SC) therapy in neuroprotection and white matter repair following a contusion injury in rat spinal cord [14]. Our results showed good correlation with histology and demonstrated that the structural effect of SKP-SC therapy in rat spinal cords is measurable by examining lesion size, myelin water fraction, and longitudinal diffusivity ex vivo. These and other results thus strongly suggest that MWI is a good candidate for non-invasive longitudinal assessment of re-myelination therapies in the pre-clinical setting.

In this work, we present results of an in vivo longitudinal Myelin Water Imaging measurement in the DC Tx model. This model is particularly suitable to evaluate the sensitivity of MWI to spinal cord pathology because of the well defined injury pattern, which shows degeneration in the fasciculus gracilis cranial to injury, and the cortico-spinal tract (CST) caudal to injury. The main objective of this study was to determine the applicability of MWI in assessing temporal changes of myelin content in the in vivo rat spinal cord following injury. Axonal damage was assessed with DTI data, which were acquired ex vivo only to minimize the in vivo experiment time.

2. Materials and methods

2.1. Animal procedures

All experimental procedures were carried out in compliance with guidelines of the Canadian Council for Animal Care and were approved by the institutional Animal Care Committee. Sixteen adult male Sprague–Dawley rats (250–300 g) were obtained from a breeding facility at the University of British Columbia and acclimatized for seven days prior to the beginning of the study. The animals were randomly divided into 2 groups: 8 animals were scanned at two time points (2 days pre-injury and 3 weeks post-injury), and the remaining animals were scanned at 3 time points (2 days pre-injury, 3 and 8 weeks post-injury).

9 days before dorsal column transection, rectangular coils 22 × 19 mm were surgically implanted over the lower thoracic and lumbar spine (T12/L1) as described previously [15]. The coils were secured by suturing the arches to the closest rib, i.e. cranial arch was sutured to the 11th rib, and the caudal arch to the 13th rib. A baseline MRI scan was performed 7 days later (2 days pre-injury) as described below.

Dorsal column transection was carried out as described before [13]. Briefly, animals were anesthetized with 2% isofluorane and placed in prone position within a stereotaxic surgical frame; the skin on the neck was then shaved and disinfected. A laminectomy of the T12 lamina was performed with a pair of small rongeurs. The dorsal column transection was performed with a pair of micro-scissors. Inflicting injury one week following the RF coil implantation required opening the wound to re-expose the spinal column.

For MRI experiments, animals were anaesthetized with isofluorane (5% induction, 2% maintenance) mixed with medical air and positioned supine in a specially designed holder. Respiratory rate and body temperature were monitored using an MRI compatible monitoring system (SA Instruments, Stony Brook, NY). Heated circulating water was used to maintain the body temperature at 37 °C.

At three weeks post-injury, the 8 animals in the first group were deeply anaesthetized by an overdose of chlorohydrate (100 mg/kg i.p., BDH Chemicals, Toronto ON, Canada) and perfused intracardially with phosphate buffered saline (PBS) for 3 min followed by freshly hydrolyzed paraformaldehyde (4%) in 0.1 M sodium phosphate buffer at pH 7.4. Spinal cords were then harvested and post-fixed overnight in the same fixative. Ex vivo MRI measurements were carried out one day after the excision and post-fixation of the cords. The remaining animals were sacrificed at eight weeks post-injury in the same manner. The detailed timeline of the study is shown in Fig. 1.

2.2. MRI experiments

MRI was carried out on a 7 T animal scanner (Bruker, Biospin Gmbh, Germany). All rats were scanned in vivo with the MWI technique 2 days before injury (1 week after coil implantation) and 3 weeks after injury. The cords from one half of the rats were excised after the 3 week post-injury scans and scanned ex vivo using MWI and DTI techniques. The remaining rats were maintained and scanned in vivo using MWI at 8 weeks post-injury, with subsequent cord excision and ex vivo MWI and DTI scans one day later.

For the in vivo experiments, an implantable coil system was used for pulse transmission and signal reception. The coil system consisted of a rectangular loop (22 × 19 mm), surgically implanted over the lumbar spinal cord and inductively coupled to an external 3 cm diameter pick up coil. The ex vivo experiments used a five-turn solenoid coil with 15 mm inner diameter and 20 mm length. The excised spinal cords were placed in a plastic tube filled with the fixative, alongside a plastic rod to prevent them from bending.

Myelin Water Imaging was carried out using a single slice, multi echo sequence [16] with the following parameters: FOV = 3 cm in vivo/2.56 cm ex vivo, matrix size 256 × 256, TR/TE = 1500/

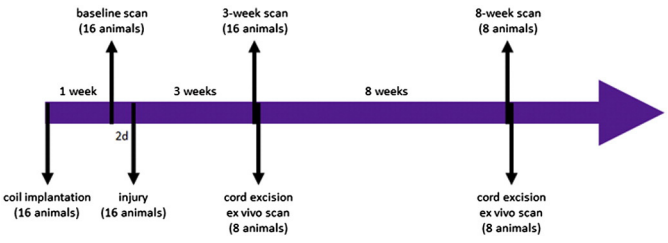

Fig. 1. Timeline of the study: 16 animals underwent baseline scan one week following the coil implantation procedure; two days after the baseline scans all animals underwent DC Tx injury; 3 weeks post injury all animals underwent in vivo scan; 8 animals were sacrificed immediately following the scan, their cords excised and scanned ex vivo 24 h later; the remaining 8 animals were scanned in vivo at 8 weeks post injury, sacrificed immediately following the scan, and their cords excised and imaged ex vivo 24 h later.