Manganese enhanced MRI tracing for spinal cord injury

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Introduction. Spinal cord injury (SCI) remains a clinical challenge, despite multiple technologies to treat these injuries. Much of this difficulty lies in a lack of understanding of the factors required for neural regeneration once the cord is damaged or severed. Nonetheless, many techniques are currently being developed to promote neural regrowth after spinal cord injury, and animal models are used to test them. In animal models of SCI, regeneration is usually evaluated histologically, requiring tissue biopsy. Thus there is a need for a technique to detect changes in SCI noninvasively over time. Manganese enhanced MRI (MEMRI) can be used to assess neuronal tissue regeneration, and can allow real-time assessment of the rate of axonal transport through the spinal cord before and after injury. Mn^{2+} acts as a paramagnetic neuronal tract tracer due to its transport through voltage-gated calcium channels in neurons (1, 2). Mn^{2+} causes a shortened T_1, and appears bright in MRI. It is known that Mn^{2+} can be delivered to the spinal cord by direct or intraventricular injection, and that it is predominantly transported through gray matter (3, 4). The goal of this work was to quantify the amount of Mn^{2+} transport through the spinal cord with or without a spinal cord transsection using mass spectrometry, and to compare this to MRI signal enhancement with MEMRI of the same spinal regions. This establishes the repeatability of MEMRI of the spinal cord during injury, and makes it possible to compare changes in axonal transport rates through the spine after neuronal regeneration *in vivo*. This may lead to a better understanding of factors required to promote spinal cord regeneration.

Materials and Methods. *Spinal cord injury model:* Ten female Sprague-Dawley rats were used for this study. Animals were anesthetized with a Ketamine-Xylazine-Acepromazine cocktail. 5 rats were with SCI. 5 rats served as control. A single rat received neither SCI nor manganese, as a sham control. A T9-level total spinal cord transsection was made, approximately 2 mm in length. *Manganese-enhanced MRI:* For 3 rats from SCI group and for 2 rats from control group, 2 microliters of 0,2M $MnCl_2$ saline solution was stereotactically injected after 5 weeks after the SCI by intracerebroventricular injection of both ventricles . Coordinates (from bregma) for injection: 2mm caudal, 2mm lateral, and 3mm dewo. 60 hours after Mn injection, MRI was performed using a 3T whole-body scanner, with a wrist birdcage RF coil. A T_1-weighted spoiled gradient-echo pulse sequence was used, with 30° FA, TE/TR = 6/100 ms, with a 7 mm FOV, 256 x 256 matrix, and a 1 mm slice thickness (0.5 mm separation). For analysis, the signal intensity of the spinal cord on different levels was measured and normalized to the intensity of the perivertebral muscles. *Histology:* Immediately after MRI, animals were transcardially perfused with 4% paraformaldehyde. Spinal cords from Mn-injected rats and one rat without Mn injection were divided into three sections: cervical level, thoracic level above SCI, and thoracolumbar level below SCI. The sections were analyzed by inductively-coupled plasma mass spec (ICP-MS , Bodycote Testing Group, CA) for total manganese content. The results of ICP were compared to MRI.

Results. The animals were divided into four groups: I. Rats with SCI + Mn^{2+} injection (n=3), II. Rats with SCI and no Mn^{2+} injection (n=2), III. Healthy rats with Mn^{2+} (n=2), IV. Healthy rats without Mn^{2+}. MR images of the spinal cords in these groups are shown in Fig 1. Signal intensity in both groups with manganese injection was enhanced, except in lumbar images of rats with SCI. The average signal intensity in Mn-injected groups decreased from cervical to lumbar level (Fig 2), and dropped sharply in the lumbar images of rats with SCI. This is consistent with a loss of Mn^{2+}, transport down the spine with SCI. In rats with SCI+ Mn^{2+}, the signal intensity was higher above the injury than below. ICP-MS data were consistent with the MRI results (Fig 3). In rats with SCI+ Mn^{2+}, Mn^{2+} concentration decreased from cervical to thoracic level and also from thoracic to lumbar level (below injury). In healthy rats with no Mn^{2+}, the Mn^{2+} concentration decreased along the entire spinal cord. When no Mn^{2+} was injected, it was not detected with ICP-MS.

Conclusions The results of this study confirm Mn^{2+} uptake in the spinal cord after intraventricular injection, and that the MRI signal intensity correlates with spinal Mn^{2+} concentration as measured with ICP-MS, consistent with a model of Mn^{2+} as a neuronal tract tracer. MEMRI is thus useful tool for studying SCI, and for characterizing materials to promote spinal cord regenerataion *in vivo*.

References: 1) Pautler RG et al. Magn Reson Med. 1998; 40(5): 740-8. 2) Stieltjes B et al. Magn Reson Med. 2006 May;55(5):1124-31. 3) Walder N et al. Invest Radiol. 2008 May;43(5):277-83.4) Bonny JM et al. Neuroimage. 2008 May 1;40(4):1542-51.

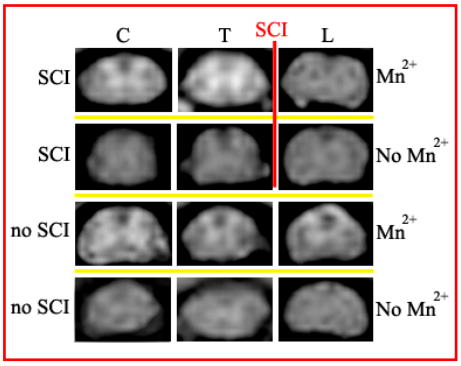

Figure 1: Typical T1-weighted images of the spinal cord after intraventricular Mn2+ injection, at cervical (C), thoracic (T) and lumbar (L) levels, in healthy animals (No SCI) and animals with spinal cord injury (SCI).

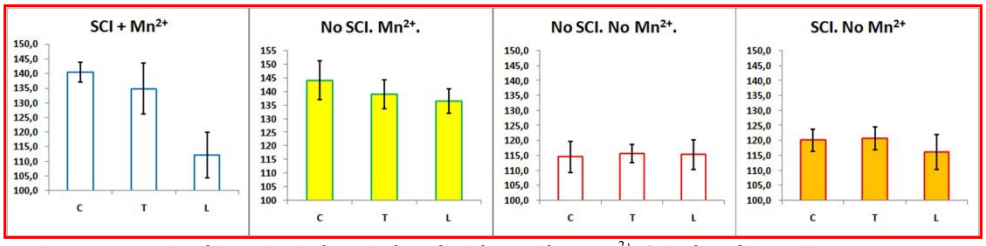

Figure 2: MRI signal intensity in the spinal cord, with or without Mn^{2+}. Spinal cord intensities were normalized to the intensity of the perivertebral muscle, as shown on the y-axis . Error bars are ±1 SD. Slices were taken from cervical (C), lumbar (L), and thoracic (T) levels.

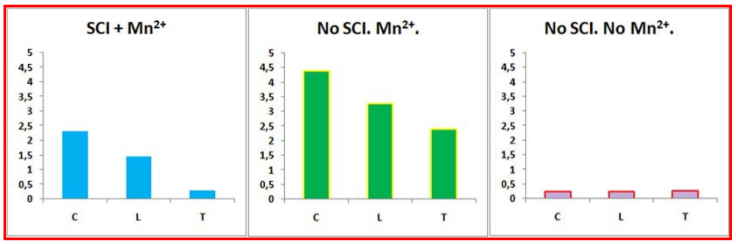

Figure 3: Typical ICP-MS –measured manganese concentration (µg/g) in the spinal cord. from cervical (C), lumbar (L), and thoracic (T) levels.