Oral Manganese as an MRI Contrast Agent for the Detection of Nociceptive Activity

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Background: Chronic pain is a widespread, serious disease, but its management suffers from diagnostic and therapeutic limitations. Manganese-enhanced magnetic resonance imaging (MEMRI) is a potential diagnostic method that would allow physicians to identify active neural regions of nociception for image-guided interventions and facilitate individualized therapy. The ability of divalent manganese ion (Mn^{2+}) to enter cells via calcium channels and potentially other cation channels makes Mn^{2+} an excellent magnetic resonance imaging (MRI) contrast agent for imaging nociceptive activity (1). There is growing evidence that pain sensing neurons have increased expression and activity of calcium channels, which would allow for selective accumulation of manganese at these sites (2).

Objective: We hypothesize that non-toxic oral dosaging of manganese chloride leads to enhancement of neural pain centers in MRI. Oral administration is an easier, safer alternative to intravenous administration with obvious clinical application. Using the spared nerve injury (SNI) rat model of neuropathic pain, we show that oral manganese can successfully enhance nerve sources of pain.

Methods: Manganese has been given safely to human subjects at oral doses up to 1.6g per ~70kg individual. Manganese chloride was given in addition to alanine and vitamin D3, chosen to enhance absorption of manganese from the intestinal tract (3). We used this precedent as a starting point for dosing in our animals experiments. All animal experiments were approved by the AALAC. Utilizing a serial dosaging strategy, we gave two groups of rats (both n=3)—one with pain, having undergone sciatic SNI and one without pain (sham)—increasing doses of manganese plus enhancers by oral gavage (Figure 1). Animals were fasted with water ad libitum for 12 hours before each dose and 6 hours following administration to maximize intestinal absorption of manganese. Animals were imaged in a 7T small-animal MR (Magnex Scientific, Oxford, UK) using a T1-weighted fast-spin echo sequence (TR/TE 800/7.7 ms, in-plane resolution 234x234 µm, ETL=8, 31.1 kHz bandwith, 1mm thick slices) 24 and 48 hours following each dose, then sacrificed 72 hours following the final dose. Pain behaviors were measured using the Von Frey filament test for allodynia on each day of imaging to ensure that rats had consistent pain thresholds over the time period. RT Image software was used to analyze MR data; ROIs were drawn about the sciatic nerve in axial images and all nerve signal intensities normalized to surrounding air signal. Finally, nerve tissue was analyzed by inductively coupled plasma spectrometer (ICP) for manganese content to confirm ion uptake into nerves. All statistical analysis was performed using Excel.

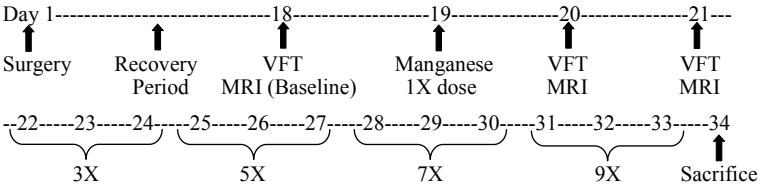

Figure 1. Experiment Timeline. Animals were imaged 24 and 48 hours following oral manganese at either 1, 3, 5, 7, or 9X doses. Doses are shown as multiples of the initial 1X dose=1.6g MnCl2, 1g alanine, and 1600 IU Vitamin D3 per 70kg. Von Frey Testing (VFT) was done prior to each MRI session.

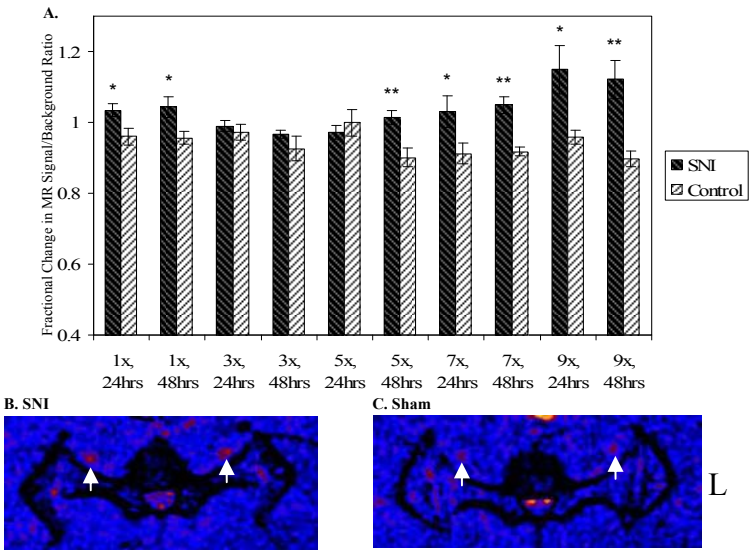

Figure 2. MRI of SNI and control animals. **A.** Fractional change in nerve MR signal/air background ratio at each time point. The x-axis shows the dose given and time point following indicated dose. The y-axis indicates the fractional change in MR signal/background ratios as compared to pre-manganese baseline. There was a statistically significant difference between the SNI (n=3) and sham control group (n=3) at the 1X time points and all time points after 5X 48 hours. Bars depict s.e. of the mean. (two-sample t-test, * $p \leq 0.05$ ** $p \leq 0.005$). **B.** T1-weighted FSE MRI of representative sham control and **C.** SNI rat 48 hours after 9X oral manganese dose administration. Sciatic nerves (white arrows) are better visualized in the SNI rat.

Results: Von Frey testing confirmed the presence of allodynia in all three SNI rats and the lack of allodynia in sham rats. At 1X, 5X, 7X, and 9X doses, nerve MR signal was significantly higher in the SNI group compared to sham group (figure 2). This difference was also seen in ICP measurements of nerve tissue manganese (figure 3A), with a strong correlation between MR signal and manganese content (figure 3B).

Discussion: An increase in MR nerve signal at both 24 and 48 hours for multiple dose levels in SNI rats, with increasing statistical significance at 48 hours brings two conclusions: nerves associated with pain take up more manganese to start and are more likely to retain that manganese over time. An increase in manganese in nerves as measured by ICP confirms our MR findings. Therefore, giving oral manganese is a viable method for enhancing nerves involved in nociception. We will next assess the toxicities, if any, of a minimal single dose of oral manganese. While there is a significant body of research on the toxicities of chronic exposure to manganese, very little research has been done on acute exposure. We hope to show that a single dose of oral manganese is a safe MR imaging agent for application in clinic.

References:
1. Silva and Koretsky et al. *NMR Biomed.* 2004 17 (4): 532-43.
2. Cao Y. *Pain* 2006; 126: 5-9.
3. Thomsen et al. *Academic Radiology* 2004; 11: 630-636.

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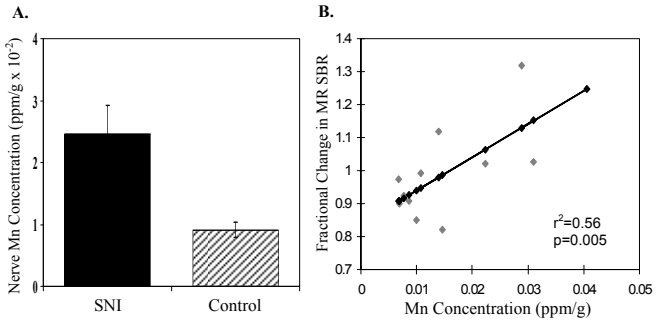

Figure 3. **A**. Mn concentration in nerves ($p<0.05$; s.e. shown). ICP concentrations (in ppm) were adjusted for nerve tissue weights (in grams). **B**. Regression Analysis. X-axis is Mn concentration in nerves as measured by ICP. Y-axis is the fractional change in MR signal to background ratios (SBR) for the final time point (9X, 48 hours).