Experimental Neurobiology
Vol. 18, pages 57~61, June 2009

Manganese-Enhanced Magnetic Resonance Imaging of the Spinal Cord in Rats

Myeoung Hoon Cha[1], Chulhyun Lee[2], Jee Hyun Cho[2], Myung-Ae Chung[3], Jin-Hun Sohn[4], Chaejoon Cheong[2], Hye-Jung Lee[5] and Bae Hwan Lee[1]*

[1]Department of Physiology and Brain Korea 21 Project for Medical Science, Yonsei University College of Medicine, Seoul 120-752, [2]Korea Basic Science Institute, Daejeon 350-333, [3]Electronics and Telecommunications Research Institute, Daejeon 305-350, [4]Department of Psychology, Chungnam Nat'l University, Daejeon 305-764, [5]Acupuncture and Meridian Science Research Center, Kyung Hee University, Seoul 130-701, Korea

ABSTRACT

Manganese-enhanced magnetic resonance imaging (MEMRI) offers a novel neuroimaging method in visualizing the activity patterns of neural circuits. MEMRI is using the divalent manganese ion, which has been used as a cellular contrast agent. The present study was conducted to determine the contrast-enhancing effects of manganese ion administered into the spinal cord of rats. Manganese ion was administered into the spinal cord by lumbar puncture. Ex vivo magnetic resonance images were obtained at 6, 12, 24, and 48 hours after manganese ion injection. Although the highly contrasted images were not observed 6 or 12 hr after manganese injection, the distinctive manganese-enhanced images began to appear at 24 hours after manganese ion injection. These results suggest that the gray matter is the foci of intense paramagnetic signals and MEMRI may provide an effective technique to visualize the activity-dependent patterns in the spinal cord.

Key words: manganese, magnetic resonance imaging, spinal cord, rat

INTRODUCTION

The magnetic resonance imaging (MRI) enables to visualize the nervous tissues of humans or animals non-invasively. Among the various contrast agents of MRI, the manganese ion has been shown to be a very useful MRI contrast agent for studying the brain. Due to the ability of manganese ion to enter the cells through voltage-gated calcium channels, manganese ion can contribute to the functional study of the nervous system by providing contrast enhancing effects. This MRI technique is called manganese-enhanced MRI (MEMRI) and offers a novel neuroimaging method in visualizing the activity patterns of neural circuits in different animal models.

Manganese ion has been known to show strong contrast-enhancing effects in T1-weighted MRI as a

*To whom correspondence should be addressed.
TEL: 82-2-2228-1711, FAX: 82-2-393-0203
e-mail: bhlee@yuhs.ac
Received January 14, 2009
Accepted for publication June 10, 2009

paramagnetic ion (Lin and Koretsky, 1997). MEMRI can be used to observe the activity-related functions of the nervous cells (Lin and Koretsky, 1997; Van der Linden et al., 2002). By injecting manganese ion into the specific regions in the central nervous system or peripheral sites, on the other hand, MEMRI tract tracing has been demonstrated in many systems in animals such as olfactory bulb (Chen et al., 2007), visual system (Chan et al., 2008), somatosensory pathway in rats (Weng et al., 2007), and song control pathway in birds (Van der Linden et al., 2002). These various MEMRI studies were based on that manganese ion is a divalent ion which has chemical property similar to calcium ion (Ca^{2+}) and enters into neural cells through the voltage-gated calcium channel (Simpson et al., 1995).

The spinal cord consists of the gray and white matters. The somata of neurons are contained in the gray matter which is located in inner part of the spinal cord. The white matter is consisted of ascending and descending fiber tracts and surrounds the gray matter. These gray and white matters may be differentiated by an MR contrast agent such as manganese ions. The present study was conducted to determine the contrast-enhancing effects of manganese ions administered into the spinal cord of rats by lumbar puncture.

MATERIALS AND METHODS

Animals

The animals used were male Sprague-Dawley rats that weighted between 220～250 g at beginning of the experiment. All animal experiments were approved by the Institutional Animal Care and Use Committee of Yonsei University Health System.

Manganese injection by lumbar puncture

Eight rats were anesthetized with 4% enflurane in oxygen via nose cone. The lumbar region was shaved, prepared with betadine solution, and the vertebral column was flexed around L3-L5 levels, widening these intervertebral spaces. Using the anterior part of the iliac crest as a tactile landmark for the L5-L6 intervertebral level, a 2 cm longitudinal incision was made with a scalpel rostral to this point. A curved 29-gauge syringe needle which filled with $MnCl_2$ (25 mM, Sigma-Aldrich, St. Louis, MO, USA) dissolved in saline was introduced. The solution (50 μl bolus injection) was injected slowly over a few minutes. To prevent backflow, the needle was left in place for several minutes prior to withdrawal. After injection, the needle was removed. Immediately, the skin was sutured tightly and the animals were then left to recover in its cage.

Ex vivo magnetic resonance imaging

In order to get MR images from the thoracic spinal cord, rats were euthanized at 6, 12, 24 and 48 hours after $MnCl_2$ injection (Fig. 1). Animals were reanesthetized with urethane (1.25 g/kg) and were transcardially perfused with 200 ml of saline, followed by 200 ml 4 % paraformaldehyde solution in sodium phosphate buffer. The vertebral column was excised from the first thoracic to the first lumbar vertebra. Samples were stored in 4 % paraformaldehyde solution for further processing.

All experiments were performed on a Biospec 4.7 T MRI system (Bruker BioSpin, Ettlingen, Germany). Coronal and sagittal scouts were acquired using a RARE T2-weighted sequence. For analyzing the distribution of manganese ions, a set of noncontiguous T1-weighted (T1W) images were acquired. It consisted of a spin-echo sequence using the following imaging parameters: TR=500 ms, TE=10 ms, 32 averages, slice thickness=2 mm, field of view=16×16 mm^2, matrix=128×128, leading to a voxel size of 0.03 mm^3. Each slice was manually adjusted in the axial orientation versus its corres-

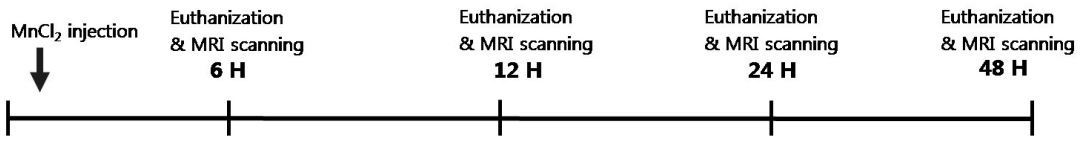

Fig. 1. Experimental procedure of ex vivo MEMRI. At each time point, all animals were euthanized and ex vivo MRI scanning was performed.

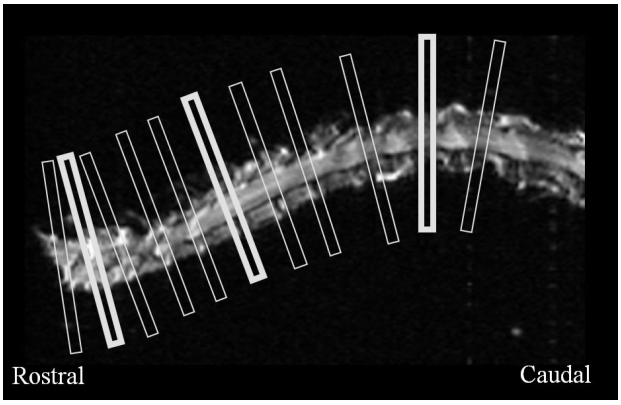

Fig. 2. A typical sagittal scout image showing positions of the 11 axial slices acquired for MEMRI. $MnCl_2$ was injected by lumbar puncture. Afterward, MEMRI was performed with 4.7 T scanner (TR/TE=500/10 ms thickness=2 mm, FOV=16×16 mm^2) from the thoracic vertebrae. The images from rostral, middle, and caudal thoracic levels depicted by thick solid lines were compared.

ponding metamer according to the scout images.

Fig. 2 shows the anatomical picture of thoracic vertebrae in rats and the positions which MR images were obtained. In Fig. 2, the images from the rostral, middle, and caudal thoracic levels depicted by thick solid lines were compared.

RESULTS

Microinjection of $MnCl_2$ into the spinal cord resulted in the uptake of paramagnetic manganese ions and manganese-enhanced T1-weighted MR images over a distance covering almost the thoracic level of the spine. Signal enhancement was detected from the thoracic spinal cord. Fig. 3 shows T1-weighted images of time dependent manganese-enhanced patterns which were obtained at 6 (A～C), 12 (D～F), 24 (G～I) and 48 hours (J～L) after lumbar puncture injection of $MnCl_2$. The images from rostral (A, D, G, J), middle (B, E, H, K), and caudal (C, F, I, L) thoracic levels were displayed in column. From these images, the time-dependently increased manganese-enhanced signals were observed in the gray matter of the spinal cord. As shown in Fig. 3, the distinctive manganese- enhanced images began to appear from 24 hours after manganese injection, although the highly contrasted images were not observed 6 or 12 hours after manganese injection. In general, the gray matter was the foci of intense paramagnetic signals and showed much higher manganese-enhanced images than outer white matter at 24 and 48 hours after manganese injection.

DISCUSSION

In the present study, we observed using MEMRI that the distinctive manganese-enhanced images began to appear from 24 hours after $MnCl_2$ injection, although the highly contrasted images were not observed at 6 or 12 hours after manganese injection. In MEMRI of the spinal cord, the gray matter showed intense paramagnetic signals but not the white matter. These results suggest that MEMRI may provide an effective technique to visualize the spinal cord.

Manganese ion exerts three actions in the nervous system. Firstly, it acts as a contrast-enhancing agent. When manganese ion is injected systemically, the specific uptake pattern of manganese ions can be observed. Manganese ions can be absorbed in the gray matters of the brain (Watanabe et al., 2002; Aoki et al., 2004). Secondly, manganese ions can be used as an analog of calcium ions. Therefore, it can be used to visualize the function of neural cells which is related to activity (Lin and Koretsky, 1997; Van der Linden et al., 2002). In this condition, the amount of absorbed manganese ions depends on the level of activity of neurons. Thirdly, manganese ions can be used to depict connections between neural cells. For example, imaging of the tract of the optic nerve can be made when manganese ions are injected into the eye of mice (Pautler et al., 1998). Once manganese ions enter the axons of neurons, they can be transported through microtubules either anterogradely or retrogradely (Pautler et al., 1998). Furthermore, these manganese ions can move transsynaptically (Pautler et al., 2003).

The present study was designed to use the ex vivo MEMRI to delineate the spinal cord. This is useful for analyzing the spinal cord images without motion artifact. On the other hand, MEMRI can be used to study activity-dependent patterns of the spinal cord. For example, Walder et al. (2008) studied relationship between the images of MEMRI and motor functions in rats with spinal cord injury. Using MEMRI, Stieltjes et al. (2006) found that

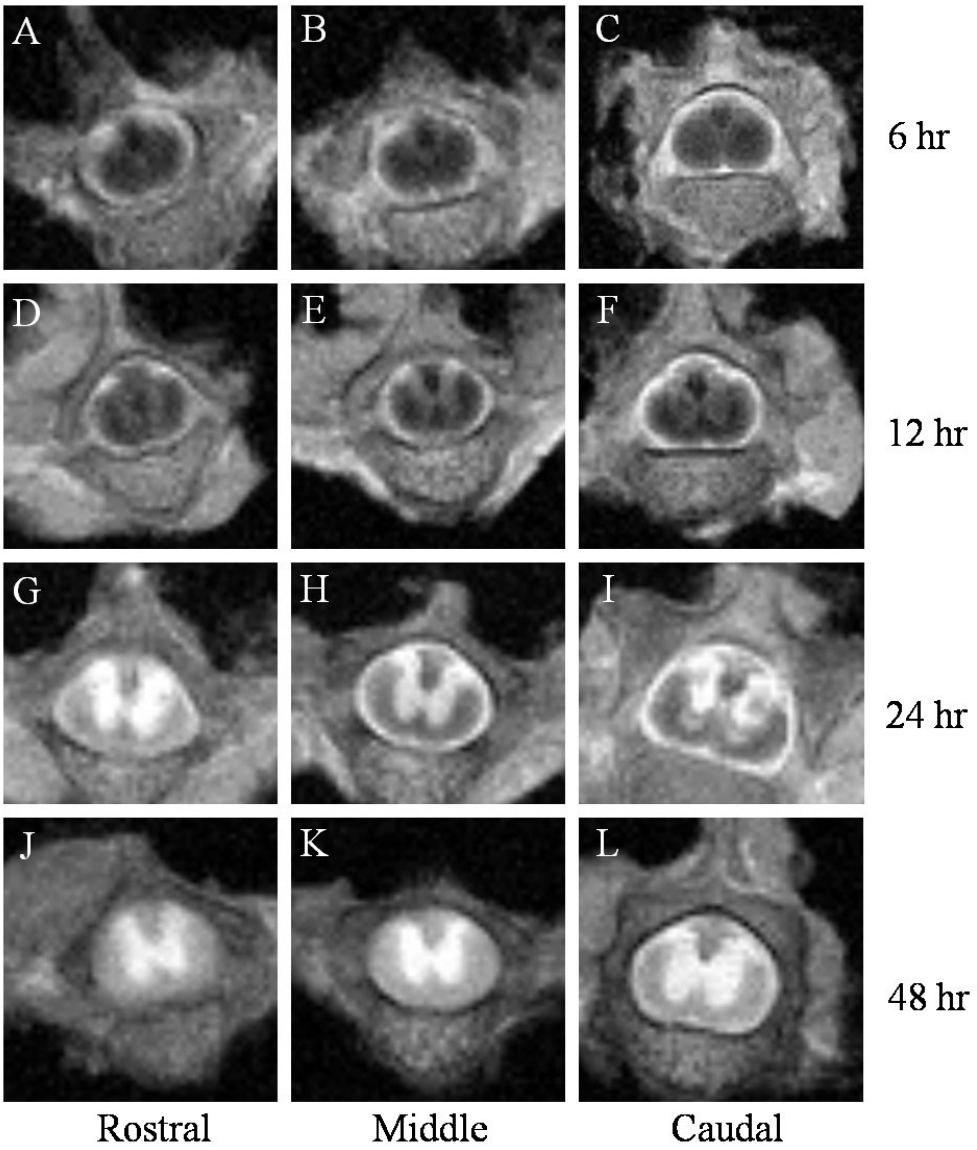

Fig. 3. T1-weighted images of time-dependent manganese-enhanced patterns. MR images were obtained at 6 (A~C), 12 (D~F), 24 (G~I) and 48 hours (J~L) after lumbar puncture injection of $MnCl_2$. The images from rostral (A, D, G, J), middle (B, E, H, K), and caudal (C, F, I, L) thoracic levels were displayed in column. Note the distinctive manganese-enhanced images began to appear from 24 hours after manganese injection. From these images, the time-dependently increased manganese-enhanced signals were observed in the gray matter of the spinal cord.

Manganese-enhancement is reduced after spinal cord injury and the uptake of manganese ions correlates with functional recovery following spinal cord injury. Bonny et al. (2008) produced complete or partial injury to the spinal cord of rats and found that MEMRI can determine laminar-specific activity and that the manganese contrast profile along the spinal cord axis accurately reflects the type of spinal cord injury. While the studies of the spinal cord using MEMRI are mainly limited to spinal cord injury up to date, these studies suggest that manganese ions can be used to reveal the function of the spinal cord.

Our results show that manganese ions may be useful in visualizing the spinal cord as a contrast agent and the intensity and distribution of manganese-enhanced signals in spinal cord are time-dependent. As MEMRI can be used to study the activity-related functions of the nervous cells (Lin and Koretsky, 1997; Van der Linden et al., 2002), it

would be extended to the functional study of the spinal cord, including somatosensory, motor, and autonomic functions.

ACKNOWLEDGMENTS

This research was supported by Korea Basic Science Institute, KIKOS & MEST of Korea through GPP (Global Partnership Program), and Basic Science Research Program through the National Research Foundation (NRF) funded by the Ministry of Education, Science and Technology (R11-2005- 014).

REFERENCES

Aoki I, Wu YJ, Silva AC, Lynch RM and Koretsky AP (2004) In vivo detection of neuroarchitecture in the rodent brain using manganese-enhanced MRI. *Neuroimage* 22:1046-1059.

Bonny JM, Mailly P, Renou JP, Orsal D, Benmoussa A and Stettler O (2008) Analysis of laminar activity in normal and injured rat spinal cord by manganese enhanced MRI. *Neuroimage* 40:1542-1551.

Chan KC, Fu QL, Hui ES, So KF and Wu EX (2008) Evaluation of the retina and optic nerve in a rat model of chronic glaucoma using in vivo manganese-enhanced magnetic resonance imaging. *Neuroimage* 40:1166-1174.

Chen W, Tenney J, Kulkarni P and King JA (2007) Imaging unconditioned fear response with manganese-enhanced MRI (MEMRI). *Neuroimage* 37:221-229.

Lin YJ and Koretsky AP (1997) Manganese ion enhances T1-weighted MRI during brain activation: an approach to direct imaging of brain function. *Magn Reson Med* 38: 378-388.

Pautler RG, Mongeau R and Jacobs RE (2003) In vivo trans-synaptic tract tracing from the murine striatum and amygdala utilizing manganese enhanced MRI (MEMRI). *Magn Reson Med* 50:33-39.

Pautler RG, Silva AC and Koretsky AP (1998) In vivo neuronal tract tracing using manganeseenhanced magnetic resonance imaging. *Magn Reson Med* 40:740-748.

Simpson PB, Challiss RA and Nahorski SR (1995) Divalent cation entry in cultured rat cerebellar granule cells measured using Mn^{2+} quench of fura 2 fluorescence. *Eur J Neurosci* 7:831-840.

Stieltjes B, Klussmann S, Bock M, Umathum R, Mangalathu J, Letellier E, Rittgen W, Edler L, Krammer PH, Kauczor HU, Martin-Villalba A and Essig M (2006) Manganese-enhanced magnetic resonance imaging for in vivo assessment of damage and functional improvement following spinal cord injury in mice. *Magn Reson Med* 55:1124-1131.

Van der Linden A, Verhoye M, Van Meir V, Tindemans I, Eens M, Absil P and Balthazart J (2002) In vivo manganese-enhanced magnetic resonance imaging reveals connections and functional properties of the songbird vocal control system. *Neuroscience* 112:467-474.

Walder N, Petter-Puchner AH, Brejnikow M, Redl H, Essig M and Stieltjes B (2008) Manganese enhanced magnetic resonance imaging in a contusion model of spinal cord injury in rats: correlation with motor function. *Invest Radiol* 43:277-283.

Watanabe T, Natt O, Boretius S, Frahm J and Michaelis T (2002) In vivo 3D MRI staining of mouse brain after subcutaneous application of $MnCl_2$. *Magn Reson Med* 48: 852-859.

Weng JC, Chen JH, Yang PF and Tseng WY (2007) Functional mapping of rat barrel activation following whisker stimulation using activity-induced manganese-dependent contrast. *Neuroimage* 36:1179-1188.