

# Detection of Transient Changes in Photothrombotic Cortical Injury by Manganese-enhanced Magnetic Resonance Imaging

K. C. Chan<sup>1,2</sup>, H-X. Su<sup>3</sup>, K. X. Cai<sup>1,2</sup>, C-T. Chiu<sup>1</sup>, M. M. Cheung<sup>1,2</sup>, W-T. Wu<sup>3</sup>, and E. X. Wu<sup>1,2</sup>

<sup>1</sup>Laboratory of Biomedical Imaging and Signal Processing, The University of Hong Kong, Pokfulam, Hong Kong, <sup>2</sup>Department of Electrical and Electronic Engineering, The University of Hong Kong, Pokfulam, Hong Kong, <sup>3</sup>Department of Anatomy, The University of Hong Kong, Pokfulam, Hong Kong

**INTRODUCTION:** Manganese (Mn) is an essential constituent of two important metalloproteins involved in the pathophysiology of cerebral hypoxia and ischemia. Mn binds to the mitochondrial Mn-superoxide dismutase (MnSOD) enzyme which acts against cellular oxidative stress. It also binds to glutamine synthetase (GS) which is a glia specific enzyme for regulating extracellular glutamate and reducing glutamate excitotoxicity. In experimental animals of stroke, delayed hyperintensity was found in T1WI due to MnSOD accumulation at the ischemic core [1], whereas upon exogenous Mn<sup>2+</sup> injection to neonatal rat models of hypoxic-ischemic encephalopathy, upregulation of MnSOD and GS activities was observed, leading to Mn-enhanced MRI (MEMRI) detection of lesions undetectable by other MR modalities [2,3]. Given a previous study indicating a transient increase of MnSOD in remote brain areas after focal photothrombotic cortical injury (PCI) [4], a relatively non-invasive and reproducible model for stroke, this study aims to employ *in vivo* MEMRI to detect transient changes in adult rat model of PCI in different brain regions.

**MATERIALS AND METHODS: Animal Preparation:** Sprague-Dawley male rats (200-250 g, N=15) were divided into 4 groups. The first 3 groups were subjected to focal cortical photothrombotic lesions in the center of motor cortex of one side of the brain using the rose bengal techniques previously described [4]. 2 and 6 days after surgery, the rats in Group 1 (n = 4) and Group 2 (n = 4) were respectively administered with an intraperitoneal injection of MnCl<sub>2</sub> solution (45mg/kg, 100mM), while Group 3 (n = 4) and Group 4 (n = 3) received no Mn<sup>2+</sup> injection. MRI was performed to the PCI animals at 3, 7, 14 and 21 days after PCI, whereby 2 rats from Group 1 and 3 each were sacrificed for histology after MR examinations at Day 7, and another 2 rats from Group 2 and Group 3 at Day 14. For the normal rats in Group 4, MEMRI was performed before, 1 day and 5 days after Mn<sup>2+</sup> injection to compare with the PCI groups at the corresponding time points with and without Mn<sup>2+</sup> administration.

**MRI Protocols:** All MRI measurements were acquired utilizing a 7 T Bruker scanner. Under inhaled isoflurane anaesthesia (3% induction and 1.5% maintenance), animals were kept warm under circulating water at 37°C and were imaged using a receive-only surface coil. 2D T1-weighted RARE sequence was acquired with FOV = 3.2 x 3.2 cm<sup>2</sup>, matrix resolution = 256 x 256, slice thickness = 1 mm, number of slices = 10, TR/TE = 400/7.5 ms, RARE factor = 4 and NEX = 16; T2WI was performed under the same dimensions with TR/TE = 6500/120ms, RARE factor = 12 and NEX = 2; Signal intensities (SI) were normalized with a saline phantom placed beside the rat; SE-EPI diffusion weighted images (DWI) were acquired with FOV = 3.2 x 3.2 cm<sup>2</sup>, matrix resolution = 128 x 128, TR/TE = 3000/28 ms, NEX = 4, b = 0 and 1000 s/mm<sup>2</sup>, number of shots = 4 and 30 diffusion directions.

**Histology:** After MR examinations, the rats were transcardially perfused with 4% paraformaldehyde. The brains were then removed, cut into 10 μm sections, and immunostained for glial fibrillary acidic protein (GFAP), manganese superoxide dismutase (MnSOD) and glutamine synthetase (GS), which are markers for gliosis, oxidative stress and glutamate excitotoxicity, respectively.

## RESULTS AND DISCUSSION:

**PCI without Mn<sup>2+</sup> injection (Group 3):** The dynamic changes of T2WI and DWI abnormalities in PCI generally followed as previously described [5]. At day 3 after photothrombosis, no T1 signal enhancement was observed in the PCI model at the also ipsilateral perilesional rim without Mn<sup>2+</sup> application (p>0.05) as in Figure 1. Yet at day 7 when the MnSOD activity was supposed to peak in the perilesional rim [4], an apparent T1 signal enhancement was observed at the perilesional rim upon windowing. However, such increase was not statistically significant when compared to the same regions in the normal rats in Group 4 before Mn<sup>2+</sup> injection (p>0.05), even though MnSOD and GS immunoreactivities were observed in the ipsilateral cortex as in Figure 2, while astroglial cells highly immunostained for GFAP in Figure 2 would also secrete reactive oxygen species, causing an upregulation of MnSOD and GS [6]. The apparent T1 signals then dropped as time went by.

**PCI with Mn<sup>2+</sup> injection (Groups 1 and 2):** After Mn<sup>2+</sup> injection, T1 hyperintensity was observed at the perilesional rims in all animals. At day 7 after photothrombosis, animals in Group 2 gave a significant increase in signal intensities at the rims compared to the same location in both the Group 3 animals without Mn<sup>2+</sup> application (p<0.01), and the normal rats in Group 4 at 24 hours after Mn<sup>2+</sup> administration (p<0.05). Enhancement could also be found in the ipsilateral cortex distinct from the ischemic core (p<0.05) in Figures 3c and 3d (arrows) and occasionally the subcortical regions as in Figures 2 and 3d (arrowheads) compared to the normal brains after Mn<sup>2+</sup> administration.

**CONCLUSION:** Given the distribution of T1 hyperintensity was similar to that of MnSOD and GS immunoreactivities reported [2-4], it is likely that exogenous Mn<sup>2+</sup> injection may provide enhanced MEMRI detection of oxidative stress and gliosis early in the rat model of PCI.

**REFERENCES:** 1. Fujioka M, et al. *Ann Neurol* 2003;54(6):732-747; 2. Yang J, Wu EX *Neuroimage* 2007. 3. Yang J, et al. *Proc. Intl. Soc. Mag. Reson. Med.* 2007;16:313. 4. Bidmon HJ, et al. *Stroke* 1998;29(1):203-210; discussion 211; 5. Schroeter M, et al. *Acta Neuropathol (Berl)* 2001;101(2):114-122; 6. Pinteaux E, et al. *Glia* 1998;22(4):408-414.

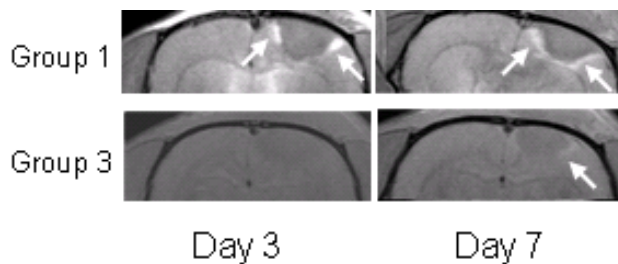


Fig 1: Typical T1WI at center of ischemic core at 7 days after photothrombosis, showing hyperintensity in the perilesional rims (arrows) with (Group 1) and without (Group 3) Mn<sup>2+</sup> injection. Hyperintensity in the perilesional rims in Group 3 at Day 7 but was less obvious.

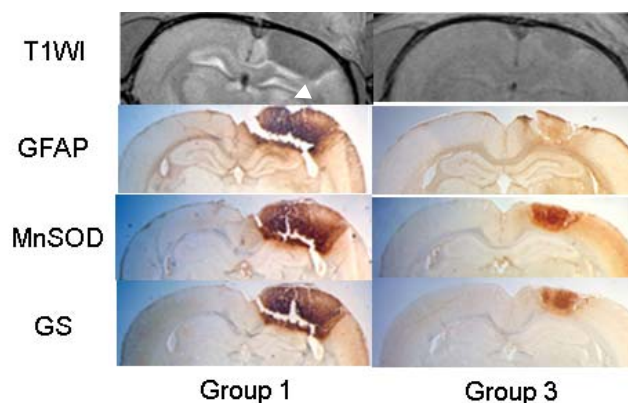


Fig 2: T1WI at the ischemic core at 7 days following photothrombosis, with (Group 1) and without (Group 3) Mn<sup>2+</sup> injection, and the corresponding GFAP, MnSOD and GS immunohistochemical stains at Day 7. Note also the enhancement in the ipsilateral thalamus in Group 1 (arrowhead).

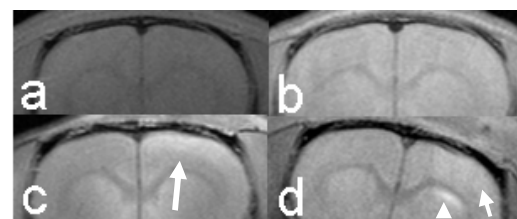


Fig 3: (Top) Typical T1WI of a normal rat (a) before and (b) 24 hours after Mn<sup>2+</sup> injection. (Bottom) Typical T1WI at about 2 mm anterior to ischemic core at 7 days after photothrombosis, showing hyperintensity in the ipsilateral cortex (solid arrows) (c) 1 day (Group 2) and (d) 5 days (Group 1) after Mn<sup>2+</sup> injection.