

Magnetic Resonance Imaging of Delayed Administration of Manganese After Transient Focal Cerebral Ischemia

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INTRODUCTION: It has been recognized that brain regions remote to the striatum are less susceptible to oxidative stress [1] and may undergo later onset for delayed neuronal death due to ischemia and reperfusion [2]. Microglial reaction at remote sites such as the neocortex, thalamus and both hippocampi takes about 5 days to become fully apparent after transient focal cerebral ischemia [3], whereas brain macrophages are clearly visible at 3-7 days [4]. Given the previous reports on the role of manganese (Mn) as an antioxidant after manganese chloride (MnCl₂) administration to animals [5,6], this study aims to test the feasibility of mediating remote infarction by delayed administration of manganese after transient middle cerebral artery occlusion (MCAO).

MATERIALS AND METHODS: Animal Preparation: Adult C57BL/6N mice (18-22g, N=8) were divided into 2 groups (n=4 each) and were subjected to 30min-transient MCAO with procedures previously described [7]. The mice were evaluated for neurological deficits 24 hours after MCAO [7], showing no significant differences in the neurological scores between two groups (p>0.05). 2 days after MCAO, mice in Group 1 (n=4) were administered with isotonic MnCl₂ solution (45mg/kg, 100mM) intraperitoneally, while Group 2 (n=4) received no injection. MRI was performed to all animals at 3, 7, 10, 14 (n=4) and 21 (n=4) days after MCAO, whereby 2 mice from each group were sacrificed for histology after MR examinations at Day 10.

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 mouse brain quadrature resonator. 2D T1-weighted RARE sequence was acquired with FOV = 2.0 x 2.0 cm², matrix resolution = 256 x 256, slice thickness = 0.7 mm, number of slices = 10, TR/TE = 400/7.5 ms, RARE factor = 4 and NEX = 20; T2WI was performed under the same dimensions with TR/TE = 6500/120ms, RARE factor = 12 and NEX = 4; SE-EPI diffusion weighted images were acquired with FOV = 3.0 x 3.0 cm², matrix resolution = 128 x 128, TR/TE = 3000/28 ms, NEX = 4, b = 0 and 1000 s/mm², number of shots = 4 and 30 diffusion directions.

Histology: After MR examinations, the mice were transcardially perfused with 4% paraformaldehyde. The brains were then removed, cut into 10 μm sections, stained with hematoxylin and eosin (H&E) to detect general morphological abnormalities, 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: Ipsilateral Striatum: In Group 2, at Day 3 to Day 21 after MCAO, SI increased in T1WI (ANOVA p<0.01) and decreased in T2WI (ANOVA p<0.01) in the dorsolateral striatum in a pattern similar to Fujioka's study [8] suggestive of delayed ischemic striatal neurodegeneration and inflammatory response [9]. Whereas in Group 1, such pattern was also observed in T2WI (ANOVA p<0.05) but appeared less obvious in T1WI (ANOVA p = 0.17). The trace values had also dropped in the corresponding hyperintense regions in T2WI, suggestive of the presence of oedema; At Day 3, significant increase in SI in the perilesional rim was observed in T1WI compared to the ischemic core 24 hours after Mn²⁺ injection in Group 1 (paired t-test, p<0.05) but not Group 2 (p=0.18), while the Mn-enhanced MRI (MEMRI) pattern at Day 10 was in good colocalization with GFAP, MnSOD and GS immunostainings as in Figure 1. It was found that astroglial cells highly immunostained for GFAP would secrete reactive oxygen species, resulting in an upregulation of MnSOD and GS [10, 11], which are Mn dependent enzymes. It is possible that exogenous Mn injection would lead to an upregulation of MnSOD and GS leading to the enhanced MEMRI detection of oxidative stress and gliosis [5, 11, 12]. The total infarcted volumes indicated by hyperintense regions in T2WI in ipsilateral forebrain were not different between the two groups in Figure 2.

Brain Regions Remote to Ischemic Core: Along the time course, hyperintensity was observed in the hippocampus, thalamus, midbrain and superior and inferior colliculi in all the mice in Group 2, while mild infarction could only be found in the thalamus of one of the mice in Group 1. As shown in Figure 3, total infarcted volumes in the posterior parts of the ipsilateral brain had significantly reduced after Mn²⁺ injection. Previous MEMRI studies have shown that Mn²⁺ accumulated and gave peak enhancements in T1WI in the posterior parts of the mouse brain within 24 hours after systemic MnCl₂ administration [13]. Given the fact that Mn²⁺ triggered the scavenging of superoxide and hydroxyl radicals [5], whereby MnSOD and GS activities increased upon MnCl₂ administration to normal and stroke animals [5, 6, 11, 12], it is possible that the reduction in infarcted volumes in T2WI may be associated with neuroprotection of Mn²⁺ at remote sites before apparent delayed secondary damage took place, though further analyses are needed to address this issue. More histological and neurobehavioural tests are currently undergoing after Mn²⁺ injection. Recent studies have shown marked neuroprotective effects of manganese complexes against focal ischemic insults up to 6 hours after ischemia [14,15]. The results of this study illustrated the potential neuroprotective effects of manganese after administration at a longer posts ischemic delay.

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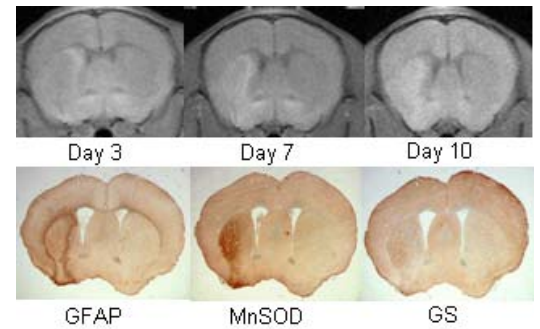


Fig. 1: Mn-enhanced MRI of the striatum at 3, 7 and 10 days following MCAO, and the corresponding GFAP, MnSOD and GS immunohistochemical stains at Day 10.

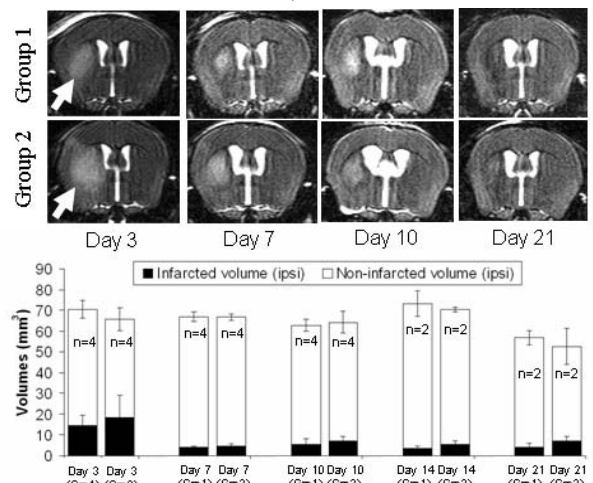


Fig 2: (Top) Typical T2WI at Bregma -0.58mm, showing hyperintensity in the ipsilateral dorsolateral striatum (arrows) with (Group 1) and without (Group 2) Mn²⁺ injection. (Bottom) Total infarcted volumes indicated by T2 hyperintensity between Bregma -1.70mm and 1.54 mm in ipsilateral forebrain were not different between the groups (unpaired t-test, p>0.05).

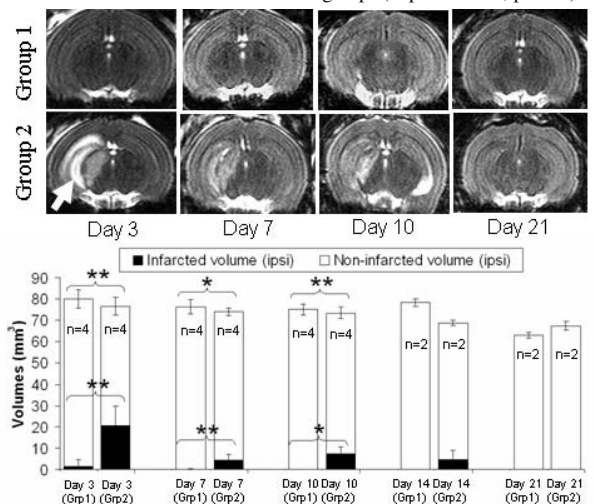


Fig 3: (Top) Typical T2WI at Bregma -3.08mm, showing hyperintensity in the ipsilateral midbrain and hippocampus (arrow) in Group 2 but not in Group 1. (Bottom) Total ipsilateral infarcted volumes in the posterior part of the brain between Bregma -4.96 mm and -1.70 mm had significantly reduced after Mn²⁺ injection (unpaired t-tests between two groups on infarcted volumes (black) or non-infarcted volumes (white), ** p<0.01, * p<0.05).