Manganese-enhanced MRI of severe hypoxic-ischemic injuries in neonatal rat model

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INTRODUCTION

Manganese-enhanced MRI (MEMRI) has been employed previously to study the acute adult brain ischemia by using the divalent ionized manganese (Mn^{2+}) as an analogue for calcium ion with blood-brain-barrier (BBB) transiently disrupted by intra-arterial D-mannitol administration [1]. A previous study also showed that, without any Mn^{2+} administration, the SI increase in T_1-weighted image (T1WI) in the adult cerebral ischemia are caused by deposition of endogenous paramagnetic Mn ions, which can reflect neurodegeneration mediated by the Mn-superoxide dismutase (Mn-SOD) [2]. In severe cerebral neonatal hypoxic-ischemia (H-I)**, the cascades of neurotoxic events involving energy failure, glutamate release, influx of calcium and excess oxidative stress are similar to those described for stroke in mature CNS. Moreover, neonatal brains have unique vulnerability to H-I insult because of the immaturity of vascular regulation and maturational difference in metabolic pathways [3]. This study aims to investigate the spatial-temporal MRI changes with and without systemic Mn^{2+} injection in neonatal rat with severe H-I injury.**

MATERIALS and METHOD

Animal Preparation: Neonatal rats (Sprague-Dawley, 7-day old, 12 ~16g, n =16) were divided into 3 groups. Group1 (n=10): H-I injury with Mn^{2+} administration; Group2 (n=4): H-I injury without Mn^{2+} administration; Group3 (n=2): normal controls (without H-I injury) with Mn^{2+} administration. Intraperitoneal injection of 0.1 mol/L isotonic $MnCl_2$ solution (87.5 mg/kg) was administrated 1 day before H-I insult. Severe hypoxia-ischemia was produced by unilateral carotid artery occlusion plus exposure to hypoxia for 3 hours at ambient temperatures of 36°C [4]. **MRI protocols:** T1WIs and T2WIs were acquired 1, 2, 3, 14, 21, and 28 days after MnCl2 injection on a 7T PharmaScan 70/16 with FOV = 2.5 cm, slice thickness = 0.5 mm, matrix = 256×256, 20 slices. 2D T1WIs were obtained by a RARE sequence with TR/TE= 500ms/7ms, NEX=6 or 12, while the T2WIs were acquired using a dual-echo RARE sequence with TR/TE1/TE2 =6000ms/60ms/200ms, NEX=2 or 4. **Histology:** The brains were perfuse transcardially with 10% formaldehyde and removed, then cut into two parts at approximately 2 mm posterior to the bregma and fixed in 10% formaldehyde for 24 hours, and subsequently embedded in paraffin. Coronal sections (8 μm) were cut at locations corresponding to the posterior and anterior MRI slices, and processed for hematoxylin and eosin (H&E) staining, immunohistochemical staining for Mn-SOD by avidin-biotin-peroxidase [2], and modified Perls staining for iron by intensifying the reaction product with 3, 3'-diaminobenzidine (DAB) [5].

RESULTS

Severe cerebral H-I injuries were induced in 13 out of 14 animals, showing severe cerebral atrophy with formation of a large porencephalic cyst, loss of the ipsilateral cortex and/or hippocampus (Fig. 1). Few animals exhibited contralateral hemispheric changes with ventriculomegaly and thinning of the cortex. (**a**) In H-I group with Mn^{2+} administration (Fig. 1 top row), pronounced Mn-induced SI increase in T1WIs was detected in basal ganglia, hippocampus and cortex from day 3 up to day 28 after H-I insult, while SI decrease was observed in T2WIs at the corresponding time points and locations. During the 1st 3 days, the hypointense regions in T1WIs correlated with the hyperintense regions in T2WIs. (**b**) In H-I group without Mn^{2+} administration (Fig. 1 mid row), the prominent hypointensity in T2WIs and an inconspicuous hypointensity in T1WIs were observed in the basal ganglia (4/4) and /or subcortex (2/4) from day 7 to day 28. (**c**) Good correlation was observed between Mn-enhanced regions in T1WIs and the dark regions in H&E staining (showing condensed and shrunken nuclei and hyperchromatic cytoplasm) (Fig. 2 top row). Fig. 2 shows the corresponding correlation of the hypointense regions in T2WIs with the strong and positive iron staining (bottom row). Strong Mn-SOD presence was observed in regions that were significantly hyperintense in T1WIs in Group 1 (Fig. 3). Mn-SOD presence was also found in other regions in all groups to much lesser extent, including structures such as hippocampus, which was expected [6].

DISSCUSION AND CONCLUSION

Previous MEMRI study by Aoki et al [1] showed that BBB opening by mannitol could lead to immediate Mn enhancement in the focal ischemia in adult rat brain within 30 min. In our study, no Mn enhancement was observed in edema area despite that BBB is disrupted in acute phase of neonatal rat H-I injury [7]. Likely, absence of such early Mn enhancement in our study was caused by the dysfunction of microtubule-associated protein that occurs transiently during the first 48 hrs after the H-I insult [8]. T2WI hypointensity in basal ganglia was observed in all H-I animals, with and without Mn injection. It was caused by iron accumulation in the regions, as shown by the positive iron staining in our study and other previous histological studies [5]. Mn-SOD production is an indicator of oxidative stress in cerebral ischemia [2, 6]. Our study suggests that T1WI enhancement by exogenous Mn may be associated with increased Mn-SOD production (Fig. 1 top and mid row, and Fig. 3) though more analysis is needed to address this issue. In summary, MEMRI by systemic **Mn^{2+} injection was examined in neonatal rat model of severe cerebral H-I injuries. It can enhance the detection of damages in cortical,** hippocampus **and basal ganglia regions during the middle and later phase, and may be used to understand the pathophysiological cascades (e.g., microtubule dysfunction, Mn-SOD and iron deposition) involved in the severe H-I injuries.**

REFREENCES

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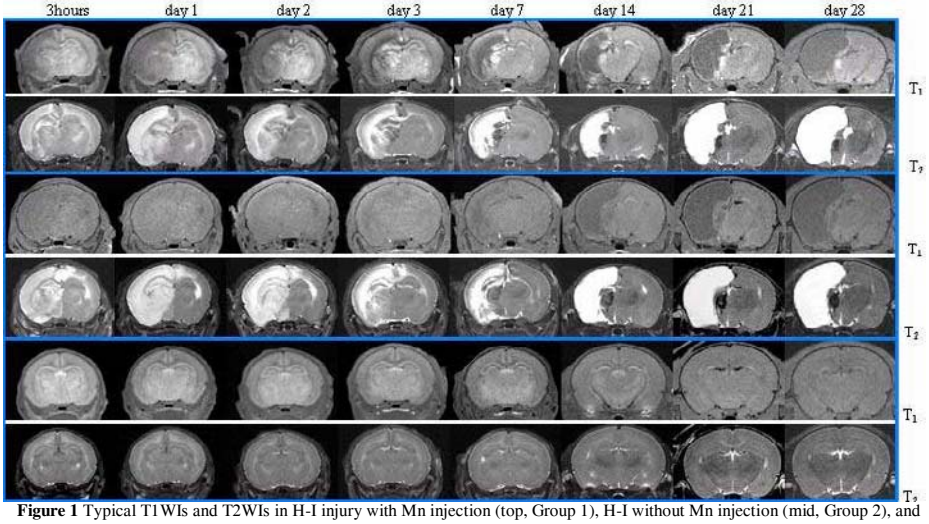

Figure 1 Typical T1WIs and T2WIs in H-I injury with Mn injection (top, Group 1), H-I without Mn injection (mid, Group 2), and normal control rats with Mn injection (bottom, Group 3 controls).

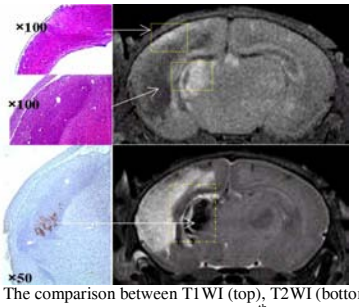

Figure 2 The comparison between T1WI (top), T2WI (bottom), and corresponding H&E, modified Perls stain at 7th day after H-I insult.

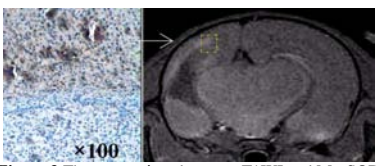

Figure 3 The comparison between T1WI and Mn-SOD immunohistochemical stain at the 28th days after H-I insult.