MEMRI Study of Neonatal Hypoxic-ischemic Injury in the Late Stage

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INTRODUCTION:

Divalent manganese ion (Mn^{2+}), as a calcium analog, has been introduced as a valuable cellular contrast agent for tracing neuronal pathways, for the enhancement of neural architecture and for brain function [1]. Several previous studies have reported the use of manganese-enhanced MRI (MEMRI) to detect neurodegeneration during the acute-phase of neonatal hypoxic-ischemic (H-I) cerebral insult [2, 3]. However, significant recovery is known to occur weeks after the initial H-I insult and last for at least 6 months [4], indicating that the regenerative processes toward the functional restitution continues late after the H-I insult when the infarct lesion stabilizes. These processes include hyper proliferation of astrocytes [5], neurogenesis [6] and regeneration of synapses [7]. This study aims to employ *in vivo* MEMRI to investigate the cellular alterations at the late stage after H-I insult.

MATERIALS AND METHODS:

Animal Preparation: Adult Sprague-Dawley rats (300-400 g, 5-12 months old, N=12) were divided into 2 groups. Group 1 (N=6): H-I injury with Mn^{2+} administration; Group 2 (N=6): normal controls (without H-I injury) with Mn^{2+} administration. $MnCl_2$ solution (60 mg/kg, 100 mM) was slowly infused intraperitoneally at a rate of 15 µl/min. **MRI Protocols:** All MRI experiments were conducted using a 7 T Bruker scanner with a 38 mm volume coil. During the MRI scan, rats were anaesthetized with isoflurane (3% induction and 1.5% maintenance) with respiratory monitoring and kept warm under circulating water at 37 °C. 2D T1-weighted images and 2D T2-weighted images were acquired prior to Mn^{2+} administration and at 1, 7 days after the injection. T1WIs were collected with a RARE sequence using FOV = 3.2 x 3.2 cm^2, 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; T2WIs were acquired using the same voxel dimensions and slice geometry with TR/TE = 4200/38.7ms, RARE factor = 8 and NEX = 2. **Analysis:** SPM5 was utilized for coregistration of brain images at different time points within the same animal. Percentage change maps were computed using the coregistrated image sets for visualization and quantification of the signal intensity (SI) changes in the brains. ROIs were manually defined and signal intensity was measured using ImageJ. **Histology:** In addition, one H-I rat was sacrificed for preliminary histological analysis. It was transcardially perfused with 4% paraformaldehyde. 10 µm sections were prepared and stained with glial fibrillary acidic protein (GFAP) as markers for gliosis and with hematoxylin and eosin (H&E) to detect general morphological abnormalities.

RESULTS AND DISCUSSION:

(a) 1 day after Mn^{2+} injection, conspicuous Mn-induced SI enhancement in T1WIs was observed in the perilesional regions in Group 1 (H-I), especially in the remaining thalamus and cingulate cortex (Fig.1 top row - yellow arrows; Fig.2 top row - white arrows) while neither the contra-lateral brain nor the normal brain exhibited the same contrast enhancement in the same area. These Mn-induced SI changes in the ipsilateral perilesional area were quantified among the two animal groups (Fig.3). Such MRI enhancement spatially correlated with the hyperproliferation of astrocytes as observed in GFAP staining (Fig.4), indicating the existence of reactive gliosis in the late phase after H-I injury. Note that other cellular alterations in the perilesional areas, such as subventricular zone neurogenesis [7] and the regeneration of synapses [8], may also contribute to the increased Mn^{2+} uptake observed in MRI. **(b)** 7 days after Mn^{2+} injection, SI decreased significantly in the perilesional rim, likely arising from the gradual Mn^{2+} clearance. (**c**) Contralesional thalamus exhibited a slower clearance of Mn^{2+} enhancement (as calculated by SI at day 7 over that at day 1) with marginal significance p=0.09 (Fig.1 top row - red arrows; Fig.2 top row - black arrows). This might result from the sustained Mn^{2+} accumulation in contralesional thalamus as a result of reinnervation after injury [9]. In summary, our experimental findings above provide information regarding the cellular alterations after ischemic injury. Such MEMRI approach may be useful in investigation of post-injury cellular events and functional reorganization.

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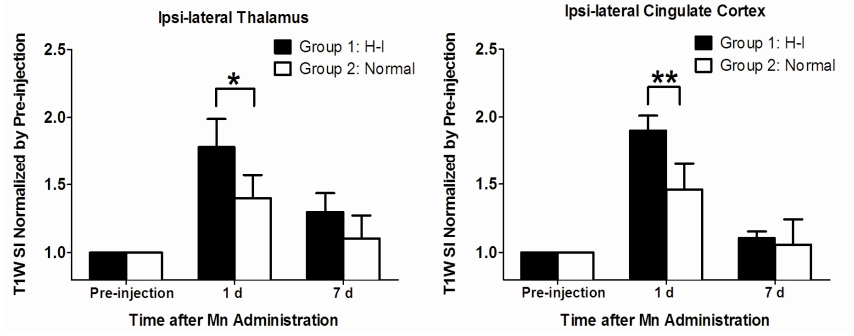

Fig.3 Mean ± SD illustrates SI changes in different regions after Mn^{2+} administration in perilesional area: ipsilateral thalamus (left) and ipsilateral cingulate cortex (right). All the post-injection SI was normalized by the pre-injection SI within the same animal, respectively, before calculating mean±SD. T-tests between Group 1: H-I (N=6) and Group 2: normal (N=6), *p<0.05, **p<0.01.

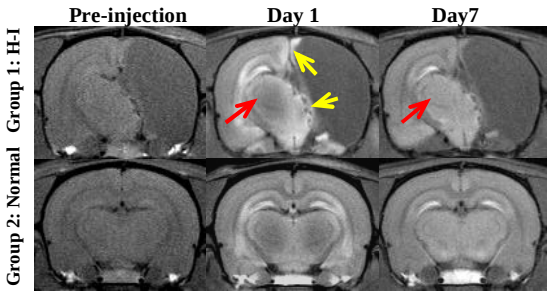

Fig.1 T1WIs of group 1: H-I (top row) and group 2: normal control (bottom row) before and after systemic Mn^{2+} administration. In group 1, perilesional rim exhibited Mn-induced SI increase at day 1 (yellow arrows) and contralesional thalamus demonstrated a delayed clearance (red arrows), compared to normal brain.

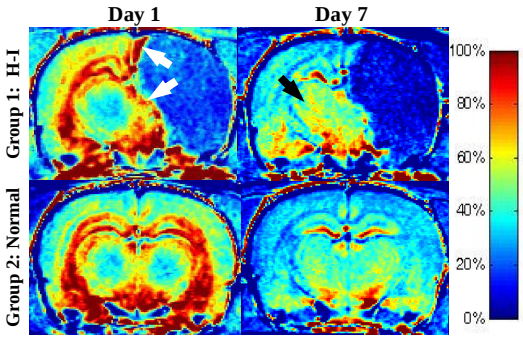

Fig.2 Percentage change maps (from pre-injection) at day 1 (left column) and day 7 (right column) computed from images with SPM coregistration, directly illustrating the significant SI increase in perilesional region at day 1 (white arrows) and relatively slow clearance in contralesional thalamus (black arrow).

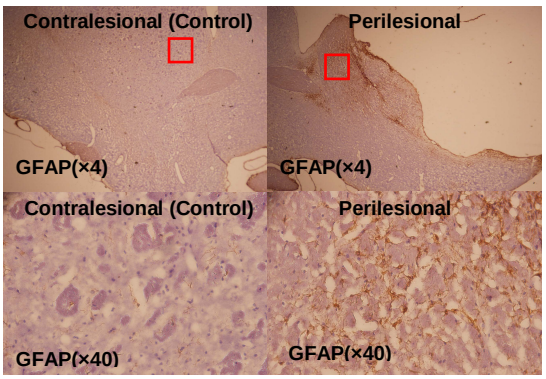

Fig.4 GFAP staining, illustrating the hyper cellular density of astrocytes in the perilesional region in thalamus as compared to the contralesional hemisphere. Similar hyper proliferation of astrocytes was also observed in cingulate cortex.