High Dose of Mn^{2+} Injected Intracerebrally Causes Neuronal Losses in the Ventral Tegmental Area of Rat

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Introduction Manganese-enhanced magnetic resonance imaging (MEMRI) has been used extensively to trace neuronal tracts, study brain functions and reveal fine neuroarchetectures in experimental animals[1-3]. In MEMRI, exogenous Mn^{2+} is often introduced by intracerebral injection of concentrated $MnCl_2$ solution (i.e., 100 mM-1 M). However, Mn^{2+} is known to be neurotoxic, and injection of large amount of Mn^{2+} directly into a small brain region might result in very high local Mn^{2+} concentration and consequently neuronal injuries, affecting the results of MEMRI studies[4]. In this work, we tried to address the question: at which concentration and dosage $MnCl_2$ solution injected intracerebrally will not cause significant toxicity to the neurons near the injection site? In the experiments, we injected $MnCl_2$ solution of different concentrations into the ventral tegmental area (VTA) of rat, which is a midbrain region implicated in the rewarding circuit[5,6], and assessed the neuronal injuries near the injection site with hematoxylin and eosin (H&E) staining.

Materials and Methods Male Sprague-Dawley (SD) rats, weighing 250-300g, were randomly divided into 4 groups, and anesthetized by intraperitioneal injection of chloral hydrate (5%, 0.5mL/100g body weight). According to the group they were assigned, the rats received stereotaxic injection of 100 nL solution of 200 mM $MnCl_2$, 400 mM $MnCl_2$, 300 mM NaCl or 600 mM NaCl into the right VTA (coordinates determined from Swanson's rat brain atlas: 5.1 mm posterior from bregma, 0.1 mm lateral from midline, and 8.1 mm ventral to skull surface). The concentration of NaCl solution was chosen so that it had equivalent osmotic pressure to the corresponding $MnCl_2$ solution (i.e., 300 mM NaCl vs. 200 mM $MnCl_2$). The solution injected was prepared by dissolving $MnCl_2$ or NaCl crystals in the 100 mM bicine solution, and had its pH adjusted to 7.4 with NaOH[7]. At 24 hrs after injection, the rats were anesthetized and perfused transcardially. The brains were removed, cut into 5 μm-thick sections and stained with H&E. For each rat, the numbers of normal-appearing neurons (i.e., with clearly visible nuclei) were counted in five non-continuous visual fields near the needle tract and in the mirror positions from the contralateral hemisphere under ×200 magnification. The average numbers of the normal-appearing neurons in the two hemispheres and the ipsilateral/contralateral ratio were calculated. Statistical analysis was carried out using one-way ANOVA.

Results Figure 1 shows the photographs of H&E stained VTA ipsilateral to injection of 100 nL solution of 300 mM NaCl (a), 600 mM NaCl (b), 200 mM $MnCl_2$ (c) or 400 mM $MnCl_2$ (d). In the VTA injected with 300 mM NaCl solution (Fig. 1a), only few vacuoles were observed and most neurons appeared normal (showing homogeneous stain of the cytoplasm). In the VTA injected with 600 mM NaCl solution and 200 mM $MnCl_2$ solution (Fig. 1b and c), scattered degenerated (inhomogeneous stain of the cytoplasm) and/or dead neurons were observed, along with more vacuoles. The VTA injected with 400 mM $MnCl_2$ solution showed extensive neuronal loss, infiltration of inflammatory cells and lots of vacuoles (Fig 1d). Corresponding to the photographs shown in Fig. 1a-d, the ipsilateral/contralateral ratios of the number of normal-appearing neurons in the four groups were 0.99±0.16, 0.85±0.23, 0.65±0.25 and 0.10±0.10, respectively (Fig. 2). The ratio in the group injected with 400 mM $MnCl_2$ solution was the lowest and statistically significantly different from to the results obtained from the other three groups.

Discussion Theoretically the sensitivity of MEMRI in revealing neuronal tracts and fine-neuroarchitacture increases with the amount of exogenous Mn^{2+} applied. However, high concentration of Mn^{2+} may cause acute neurotoxicity. In this study, we compared histologically the neuronal injuries caused by stereotaxic injection of hyperosmotic $MnCl_2$ and NaCl solution into the VTA of rat. The results showed that intracerebral injection of 100 nL 400 mM $MnCl_2$ solution induces extensive neuronal loss near the injection site, and lowering the injecting $MnCl_2$ concentration to 200 mM will decrease the extent of neuronal injuries near the injection site significantly. Due to the neurotoxicity of Mn^{2+}, intracerebrally-injected $MnCl_2$ solution causes more injuries to the VTA neurons than the NaCl solution having equivalent osmotic pressure. Therefore Mn^{2+}-induced neurotoxicity and neuronal loss should be taken into account when interpreting the results of MEMRI studies, especially when exogenous Mn^{2+} is induced by intracerebral injection of concentrated (i.e., ≥400 mM) $MnCl_2$ solution.

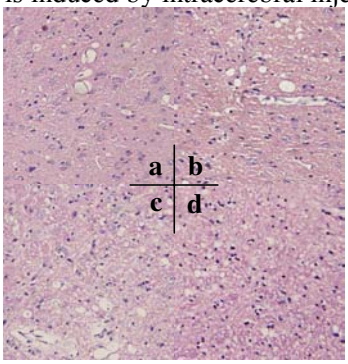

Fig. 1 The VTA on H&E stained rat brain sections (×400) ipsilateral to injection of 100 nL solution of 300 mM NaCl (a), 600 mM NaCl (b), 200 mM $MnCl_2$ (c) and 400 mM $MnCl_2$ (d). Histological assessment was done at 24 hrs after injection.

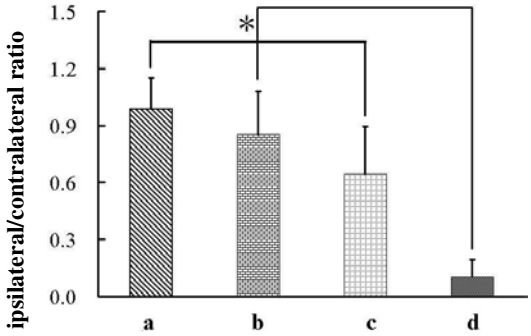

Fig. 2 The ipsilateral/contralateral ratio of the number of normal-appearing neurons in the VTA of rats injected with 300 mM NaCl (a), 600 mM NaCl (b), 200 mM $MnCl_2$ (c) and 400 mM $MnCl_2$ (d). *p< 0.05, compared to a, b and c by one-way ANOVA.

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