Anatomic and Functional Organizations in Developing Neonatal Rat Brain Revealed by Manganese Enhanced MRI and ^{1}H-MRS

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Introduction

Manganese ion (Mn^{2+}) is an intracellular T_1 relaxation agent and a calcium analogue that accumulates in functionally active cells in the living organs [1, 2]. Manganese enhanced MRI (MEMRI) is a newly emerging technique that can be used for mapping anatomic and functional organizations of the brain and for measuring functional neural circuits and activities [3, 4]. For neonatal brains, conventional imaging technologies such as MRI CT, and US have limitations to image the fine anatomic structures due to incomplete differentiation of gray matters and white matters during neonatal period. In this study, we employed MEMRI 1) to investigate the formation of the anatomic structures as a function of time and 2) to monitor functionally active cells during the neonatal period. ^{1}H-MR Spectroscopy was employed in conjunction with MEMRI to monitor the potential toxicity of Mn^{2+} in the brain that might alter the normal metabolic changes occurring during this period.

Methods

Animal Preparation: Neonatal rats (Sprague-Dawley, 7days olds, 15-20g) were anesthetized with intraperitoneal ketamine (50mg/kg). 120mM isotonic $MnCl_2$ solution was administered at the left ventricle by intracerebroventricular (ICV) injection. The rectal temperature was carefully monitored and maintained at 36 ± 1 □. $MnCl_2$ was administered at two different doses: high dose (n=7, 5 mg/Kg) and low dose (n=7, 2.5mg/kg). In all cases the solution was infused at a rate of 1 □/min. After MRI and 1H-MRS, animals were recovered from anesthesia and returned to their cage for the next MR examinations.

MEMRI: All MR experiments were performed in a horizontal 4.7T/30 cm magnet (Bruker, Fllanden, Switzerland) and 25 mT/m active shielded gradients interfaced with ASPECT3000 computer with TOMIKON console (Bruker, Fllanden, Switzerland). A custom holder and 15 mm (i.d.) saddle-backed surface coil for transmitting and receiving was used. MEMRI was acquired using a multi-slice T_1-weighted spin-echo sequence (TR/TE=400/13 ms) with in-plane resolution of 100x100 □ and slice thickness of 750 mm. MEMRI were acquired 1, 6, 12, 24 hours, 5 days and 1week after $MnCl_2$ administration on the same animal. The value of SNR in MEMRI was calculated using the air signal as a reference [5].

^{1}H-MR Spectroscopy: Spectra were acquired 1, 12, 24, 72 hours, 1week, and 2weeks after $MnCl_2$ administered, immediately after MEMRI. Water suppressed ^{1}H MR spectra were acquired using VOSY sequence with detection of the double-refocused spin echo signal from the selected voxel (3x3x3 mm^3, 27 □. Fig. 2) using the following acquisition parameters: SW=5000 Hz, SI=4096 pts, NS=64, TR/TE=3000/30 and 135 msec. To identify the peak at 1.3 ppm, the spectra were acquired at two echo times of TE = 30 and 135 msec. to differentiate the lactate peak from the lipid peak. All spectroscopic raw were processed using the TOMIKON software. The values of [Lipid/Cr] and [NAA/Cr] were calculated.

Results

Figure 2 shows a location of the voxel for ^{1}H-MRS, which was placed in the hippocampus region. At 1hour, the Mn^{2+} injected at the left ventricle was diffused into the next neighboring tissues and the cerebellum was highly enhanced. At 6 hours, both sides of the hemisphere were enhanced, and the structure of the hippocampus was clearly visualized that remained enhanced until 5 days. At 1week, the Mn^{2+} seemed to be cleared out from the brain tissues. The hippocampus and cerebellum showed well visualized structures as shown by other investigator (ref, 6). The SNR measurement showed that the contrast was not significantly different between high and low doses of Mn^{2+}, 14.85±1.66 *vs.* 14.48±2.13, for high and low doses, respectively, measured at 24 hours. However, with high Mn^{2+}dose, the significant peak at 1.3 ppm was observed in the ^{1}H-MR spectrum in contrary to the spectra with low dose of Mn^{2+} (Figure 4). The interesting finding was that the peak at 1.3 ppm observed at high dose spectra gradually disappeared in time although other metabolite peaks such as NAA, Cr, Cho showed the normal developments. The peak at 1.3ppm is clearly related to the higher dose of Mn^{2+}, which doesn't seem to affect the normal development of other metabolites. The exact identification as well as the function of the peak shown at 1.3ppm associated with high dose of Mn^{2+} in MEMRI is subjected to the further investigation.

Discussion

In this study, we demonstrated that MEMRI could successfully image the anatomic structures in the neonatal brain, which cannot readily be obtained by using conventional imaging techniques. The low dose (2.5mg/kg) for MEMRI seems to be an optimal dose for ICV injection without noticeable damages in the brain metabolites. MEMRI should be performed between 12-24 hours after Mn^{2+} injection to maximize the contrast. MEMRI with ^{1}H-MRS seems to be a useful method to investigate various neurological diseases involved with neonatal brain.

References

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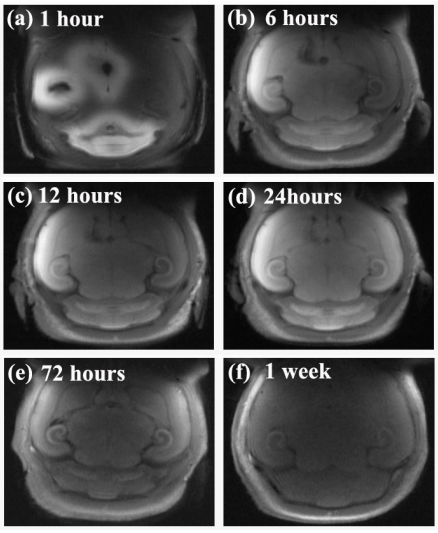

Figure 1. The course of T_1 weighted coronal images obtained after ICV injection of $MnCl_2$ (2.5 mg/kg).

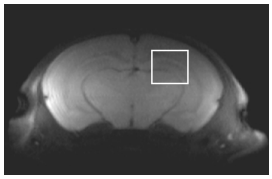

Figure 2. Localization of a selected voxel for ^{1}H-MRS.

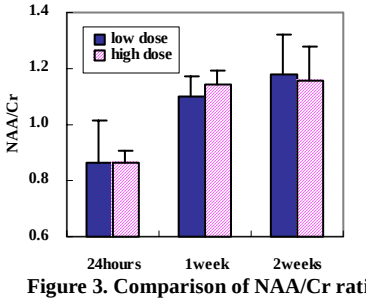

Figure 3. Comparison of NAA/Cr ratios.

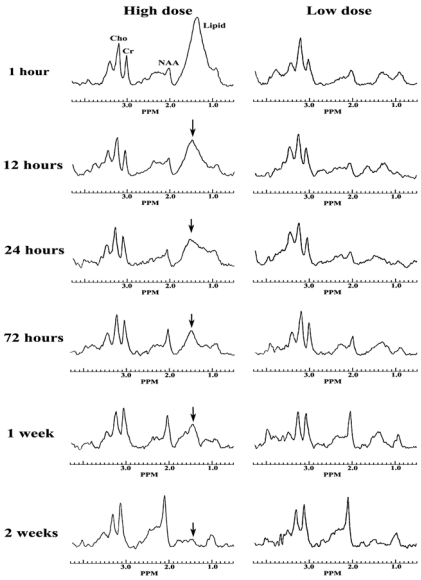

Figure 4. Comparison of ^{1}H-MR spectra.