In vivo Staining of Apoptosis by Manganase-Enhanced Magnetic Resonance Imaging(MEMRI) in the Hypoxic-Ischemic Newborn Rat

J. T. Kim[1], K. H. Lim[2], H. J. Cho[2], J. H. Lee[3], K. S. Kim[1]

[1]University of Ulsan College of Medicine, seoul, Korea, Republic of, [2]Asan Institute for Life Sciences, seoul, Korea, Republic of, [3]Sungkyunkwan University School of Medicine, seoul, Korea, Republic of

Purpose

It is well known that apoptosis is a major pathophysiology of hypoxic-ischemic brain injury in newborn rats [1]. Calcium influx to intracellular space is an important mechanism for apoptosis during hypoxic brain injury. Based on the notion that manganase (Mn^{2+}) is an analogue for calcium ion (Ca^{2+}) in many biological systems [2, 3], we hypothesized that Mn^{2+} may enter the intracellular space through the calcium channel during the active stage of apoptosis and that those apoptotic cells may be detectable by MEMRI [4]. This study was purposed to see whether MEMRI can detect apoptosis for the hypoxic-ischemic newborn rat brain *in vivo*.

Methods

Animal Preparation: Neonatal rats (Sprague-Dawley, 7 day olds, 12-16g, n=18) were divided into 3 groups: Group 1 (n=6), hypoxic-ischemia with Mn^{2+} administered; Group 2 (n=6), hypoxic-ischemia without Mn^{2+} administered; and Group 3 (n=6), controls with Mn^{2+} administered. Hypoxic- ischemia was induced by ligation of right common carotid artery under anesthesia with 2% isoflurane followed by a short recovery for 2 hours under normal air. Hypoxia was induced by exposer to 8% oxygen for 3 hours after the recovery period. Intraperitoneal injection of 120mM isotonic $MnCl_2$ solution (87.5mg/kg) was given to the animals 24 hours before the hypoxic-ischemic injury.

MEMRI: All MRI experiments were performed on a horizontal 4.7T/30 cm magnet interfaced to a Bruker Avance MRI console (Bruker Biospin, Ettlingen, Germany), and equipped with a 20 cm gradient set capable of supplying up to 100 mT/m. A birdcage coil (72mm i.d.) for transmitting and a saddle-backed surface coil (15mm i.d.) for receiving the signal were used. MEMRI was acquired using 3D T_1-weighted spin-echo sequence (TR/TE=300/12 ms) with in-plane resolution of 140x140x140 ㎛. DWI sequence (TR/TE=2000/60 ms, b-value=1500 s/mm^2) was used to mark the injured area. A serial MEMRI was acquired 3, 24, 72 hours and 1week after hypoxic-ischemic injury for the same animals (n=6) under anesthesia with 2% isoflurane with careful monitoring and maintaining of the rectal temperature at 36±1□ using a water bath. The SNR of the 3 selected regions of interest (ROI) were calculated [5].

Histologic examination: The rat brains were prepared for histologic evaluation after MEMRI. The terminal deoxynucleotidyltransferase-mediated dUTP-biotin nick end labeling (TUNEL) stains were done and TUNEL positive cells were evaluated as apoptotic markers on 24 hours, 72 hours and 1week after hypoxic injury. The apoptotic areas were compared with MEMRI images.

Results

The hippocampus, basal ganglia and cortex showed strongly increased signal intensities from 72 hours up to 1 week for the hypoxic-ischemic injured brain (Fig. 1 and 2). The signal intensities gradually increased up to 1 week as shown in the graph (Fig. 1). Interestingly, the signal enhancement on those regions was not observed at 24 hours after the injury when the TUNEL staining showed the most number of TUNEL positive cells. Figure 3 showed a good correlation of MEMRI with TUNEL staining for marking apoptosis.

Discussion and Conclusion

MEMRI seems to detect apoptosis 72 hours after the hypoxic ischemic injury for newborn rat brain. However, early detection of apoptosis was not possible on 24 hours after the injury when the most number of TUNEL positive cells were detected. The result of the current study may be explained by 1) Mn^{2+} accumulated in the apoptotic cells could be detected when Mn^{2+} in the uninjured normal areas start being washed out, or 2) Mn^{2+} keep being accumulated in the apoptotic cells so that the considerable concentration of intracellular Mn^{2+} might be necessary to be detectacted by MEMRI.

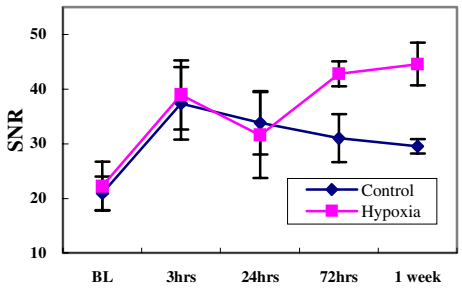

Figure 1. The changing pattern of SNR at basal ganglia.

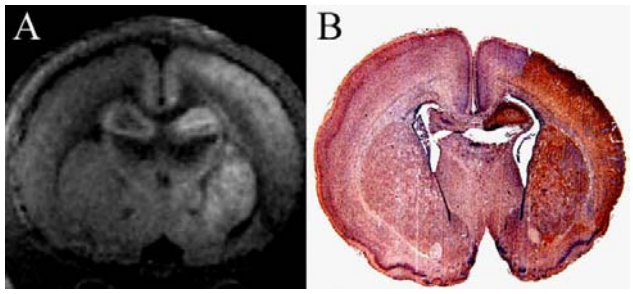

Figure 3. The comparison with coronal T1 image(A) and histology (B) at 72hrs (POD conversion of TUNEL for apoptosis).

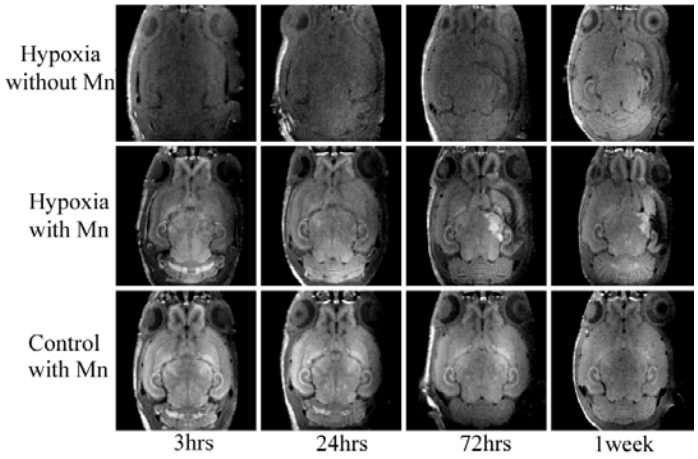

Figure 2. Horizontal T1 image of hypoxic-ischemic injured and control brain.

References

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