Detection of Neural Damage in the Hippocampus CA1 Region using Manganese Enhanced MRI (MEMRI) in a Cardiac Arrest Model

I. Aoki[1], Y. Mori[2], M. Umeda[1], T. Ebisu[3], M. Fukunaga[4], S. Naruse[5], C. Tanaka[1,2]

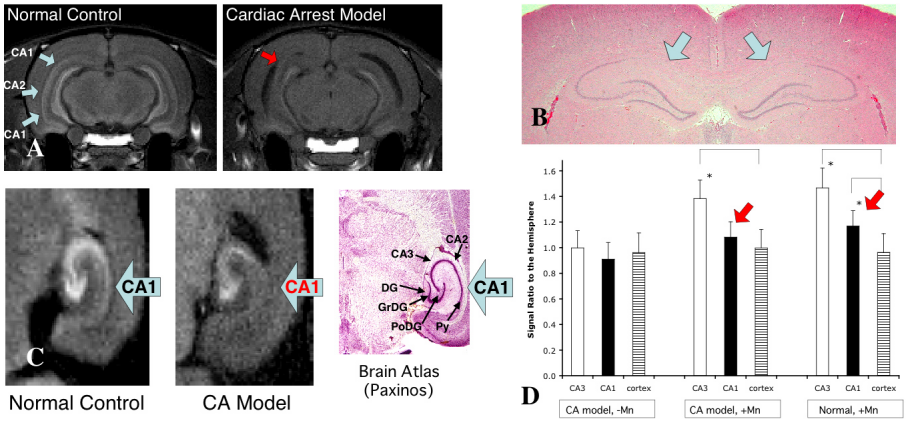
[1]Department of Medical Informatics, Meiji University of Oriental Medicine, Hiyoshi, Kyoto, Japan, [2]Department of Neurosurgery, Meiji University of Oriental Medicine, Hiyoshi, Kyoto, Japan, [3]Department of Neurosurgery, Nantan Hospital, Yagi, Kyoto, Japan, [4]Laboratory of Functional and Molecular Imaging, National Institute of Neurological Disorders and Stroke, NIH, Bethesda, MD, United States, [5]Department of Radiology, Kyoto Prefectural University of Medicine, Kyoto, Kyoto, Japan

Introduction

It is known that global ischemia causes neuronal death in the selectively vulnerable CA1 region of the hippocampus. This histopathologic neural damage is explained by the combined effects of glutamate over-excitation and free radical toxicity (1). Functional and morphological in-vivo evaluation of hippocampus is important to estimate memory disturbance after ischemic disease such as stroke or myocardial infarction. However, hippocampal function has been difficult to visualize in-vivo because the structure is complicated, and there are no specific contrast enhancements on MRI, CT, and PET. Recently, it has been reported that manganese-enhanced MRI (MEMRI) can enhanced the hippocampal CA1-3 formation and dentate gyrus (DG) selectively at least one day after systemic intravenous $MnCl_2$ administration (2, 3). Manganese is known that it can shorten T_1 relaxation time and enhance T_1-weighted MRI. Blood-CSF barrier can uptake manganese via choroid plexus and transport it to the DG and hippocampus directly. Therefore, MEMRI can distinguish the hippocampal formation in the intact rat brain. However, there is as yet no report demonstrating whether MEMRI can observe the neruonal damage in the hippocampus. Accordingly, we investigated the hypothesis that transient cardiac arrest using a short-acting ß-blocker in the rat would alter MEMRI enhancement due to neuronal damage in the hippocampus. Furthermore, we improved the cardiac arrest method of Liachenko et al (4) by using only autologous blood without the need for donor's blood.

Materials and Methods

12 male Wistar rats (300-350 g) were used. Two groups of rats were used including sham control (n = 4) and rats 4 weeks after cardiac arrest (n = 4). The cardiac arrest model was prepared using a modified technique of the model described by Liachencko et al (4). Rats were initially anesthetized with 4.0 % isoflurane, and then anesthesia was maintained with 1.5-2.0 % isoflurane mixed with a 1:2 O_2 / room air gas mixture using a rodent ventilator. Rectal temperature was maintained at 37.5 ± 0.2 ºC using an auto-controlled heart pad. Polyethylene catheters (PE-50, Becton Dickinson) were placed in both the femoral artery and left femoral vein for drug administration, blood pressure monitoring and blood gas measurements. After collection of arterial blood (5 ml/kg), the muscle relaxant vecuronium (1 mg/kg, Sankyo) was administrated to suppress any spontaneous respiration. Then, 25 mg/kg esmolol (short-acting ß-blocker, Baxter) was administrated IV to induce cardiac arrest. After confirmation that the blood pressure decreased and the heart beat stopped, time keeping was started. Artificial ventilation and heat pad were shut off during cardiac arrest. Arterial blood withdrawn from the animal was re-administered with added epinephrine (0.024 mg/kg), sodium bicarbonate (17.64 mg/kg), and heparine (5 U/ml) 3 minutes after starting the timekeeping. Typically, blood pressure and heart beat were recovered 5 minutes after the esmolol induced cardiac arrest.



For all groups, 252.7 µmol/kg $MnCl_2$ (Sigma) was given by infusing 75 ml/kg of a 50 mM $MnCl_2$ at a rate of 2.5 ml/hour through the tail vein (2). During $MnCl_2$ infusion, the anesthesia was kept light between 0.8-1.5 %. After the $MnCl_2$ administration, animals were kept in an incubator (30-32 ºC) for 24 hours to maintain body temperature. $MnCl_2$ was administered four weeks after cardiac arrest was performed. The MRI acquisitions were performed 3 days after the Manganese administration on a 4.7-T horizontal MRI (CSI-II-Omega, Bruker). A 30 mm Litz coil (Doty Scientific) was used. The measurements were performed in the following order: T_1-weighted (T1W) MRI, DWI (b = 1000), and T_2-weighted imaging. Multi-slice T1W coronal and horizontal images were obtained using a spin-echo sequence with the following parameters: pulse repetition time (TR) = 250 ms, echo time (TE) = 10 ms, matrix size = 256 × 256, field of view (FOV) = 32 mm, slice thickness (ST) = 1.2 mm, number of acquisitions (NA) = 18, number of slices = 8, and acquisition time for one set = 19.2 minutes. One complete set of T1W measurements consisted of two T1W scans with the offsets of 0mm and 1mm to maintain continuity of slices and to cover entire volume. T2W and DWI were obtained using a spin-echo sequence with the same slice orientation as the MEMRI with the following parameters: TR = 2500 ms, TE = 80 ms, matrix size = 256 × 256, FOV = 32 mm, ST = 1.2 mm, NA = 2, number of slices = 8, and acquisition time for one set = 46 minutes.

Results and Discussion

There were two major findings in this study: 1) Signal was not enhanced significantly in the hippocampal CA1 region in the cardiac arrest group on the T1W MRI (Fig. A). Signal intensity in CA1 was decreased in the cardiac arrest model in comparison with CA3 (Fig. B). In contrast, significant signal enhancement was observed in the CA1 region in the sham control group and CA3 region in both the sham and cardiac arrest group ($P < 0.05$, Bonferroni/Dunn test, Fig. C). 2) Histopathologic neural damage was observed at CA1 region in the cardiac arrest group (Fig. D). An improved cardiac arrest model using only autologous blood provided transient global ischemia and selective neuronal damage in the hippocampal CA1 region. These results confirm that MEMRI can provide visualization of the hippocampus and furthermore MEMRI is sensitive to hippocampal neuronal damage even four weeks after a short (5 min) ischemic event. We speculate that the changes in hippocampal enhancement detected with MEMRI reflects a functional deficit. In addition, we have improved the cardiac arrest model using only autologous blood enabling investigation of the relationship between ischemic disease and blood proteins such as neuro-protective protein, tolerance, and apoptosis.

References

1. White BC, Grossman LI, O'Neil BJ, DeGracia DJ, Neumar RW, Rafols JA, Krause GS. Global brain ischemia and reperfusion. Ann Emerg Med. 1996 May;27(5):588-94.
2. Aoki, I., Lin Wu, Y. J., Silva, A. C., Koretsky, A. P. Cortical Layers Revealed by Manganese Enhanced Magnetic Resonance Imaging (MEMRI) in the Rat Brain after Systemic Administration, Proc Intl Soc Mag Reson Med. 2002 (10): 715
3. Watanabe T, Natt O, Boretius S, Frahm J, Michaelis T. In vivo 3D MRI staining of mouse brain after subcutaneous application of MnCl2.Magn Reson Med. 2002 Nov;48(5):852-9.
4. Liachenko S, Tang P, Hamilton RL, Xu Y. Regional dependence of cerebral reperfusion after circulatory arrest in rats. J Cereb Blood Flow Metab. 2001 Nov;21(11):1320-9.