Neuroprotective Effects of Growth Hormone against Hypoxic-Ischemic Brain Injury in Neonatal Rats: ^{1}H Magnetic Resonance Spectroscopic Study

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Introduction

The exact mechanism of hypoxic ischemic brain injury for neonates has been controversial. There have been numerous speculations focusing on apoptosis as a major mechanism of cell death due to hypoxic ischemic brain injury in adults, not specifically in neonates. Caspases are believed to play a key role in the delayed neuronal cell death observed in the rat brain after hypoxic-ischemic insult. For hypoxic ischemic injury, one of our authors has previously reported that the lipid peak in ^{1}H MR spectrum can be a marker for apoptosis. This study was purposed to evaluate the effects of growth hormone as caspase inhibitors on hypoxic-ischemic injury in neonatal rat brain with 1H-MR spectroscopy.

Methods

Animal preparation: Seven-day old Sprague-Dawley rats (mean weight=13.3 gms) were used. Right common carotid artery was ligated under halothane anesthesia. After a recovery period of 3 hours, they were exposed to 8% oxygen at 37□ for about 120 minutes. Growth hormone (Eutrophin, LGPhD, Korea) were given just prior to hypoxic ischemic insult and growth hormone pretreatment animals were divided into four groups: control (same amount of distilled water only, N=29), intracerebroventricular (ICV, 10ug growth hormone in 10uL distilled water, N=23), intracerebroventricular/intraperitoneal (ICV+IP, N=21), and intraperitoneal (IP, 10mg/kg growth hormone in distilled water, N=23) pretreatment group.

^{1}H-MR Spectroscopy: Localized *in vivo* ^{1}H MR spectroscopy were performed on a Bruker Biospec 4.7T MRI/MRS System equipped with active shielded gradients and ASPECT3000 computer with TOMIKON hardware and software (Bruker, Fällanden, Switzerland). Spectra were acquired in the right cerebral hemisphere of rats 24 hours after the onset of hypoxic ischemic injury. Water suppressed ^{1}H MR spectra were acquired using VOSY sequence with detection of the double-refocused spin echo signal from the selected voxel (3x2x2 mm^3, 12 l) using the following acquisition parameters: SW=5000 Hz, SI=4096 pts, NS=128, 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. Peak areas were measured and the values of [Lipid/NAA] and [Lipid/Cr] were used as apoptotic markers.

Apoptotic Cell Counting: After the ^{1}H-MRS examinations at the 1st day, 6 brains from each group were perfused with 0.9% saline solution mixed with 2 units/ml of heparin, and the perfusion was repeated with 4% paraformaldehyde in PBS solution. The brain was isolated, and the TUNEL staining was performed according to the method by Garrieli *et al*. using an *in situ* Cell Death Detection Kit, POD (Boehringer Mannheim, Germany). The apoptotic cells were viewed and counted in the parietal area of the brain for 3 times using x200 lens, and the mean apoptotic cell counts were calculated by using Image Analyzer software.

Gross morphological score: The gross morphologic changes scored at 2 weeks were used for evaluating the drug effects. The scale is 5 point grading system; 0 point means no change and 4 point means the most severe injury.

Results

The Lip/NAA ratios were significantly lower in the intracerebroventricular and intracerebroventricular/ intraperitoneal group than those of the control group. Lip/Cr ratios were significantly lower in the intracerebroventricular group than those of the control group. (Fig.1, 2) TUNEL positive cells were relatively decreased in the intracerebroventricular group and intracerebroventricular/ intraperitoneal group. The degrees of morphological changes of the brain injury on day 14 were significantly lower in the intracerebroventricular group and lower in the intracerebroventricular/ intraperitoneal group, but not significant. (Fig.3)

Discussion

This result suggests growth hormone exerts neuroprotective effect in cerebral hypoxic-ischemic injury probably by inhibiting apoptosis especially in early stage after insult. Growth hormone as a caspase inhibitor can be therapeutic value to prevent the hypoxic ischemic brain injury

References

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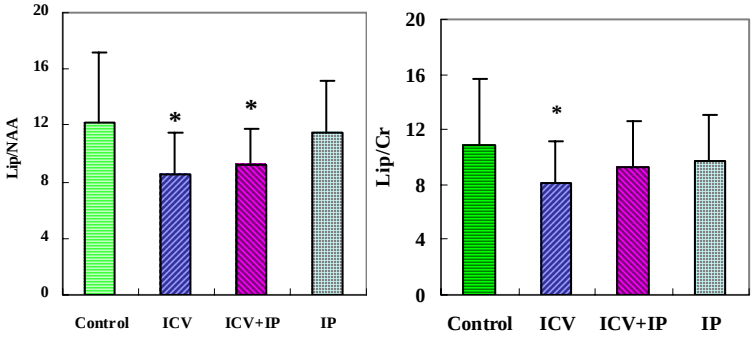

Fig. 1. Lip/NAA and Lip/Cr ratios among control and growth hormone pretreated groups.

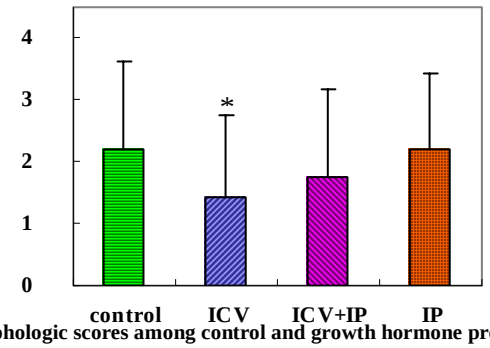

Fig. 3. Morphologic scores among control and growth hormone pretreated groups. (* p<0.05: significantly different from control).

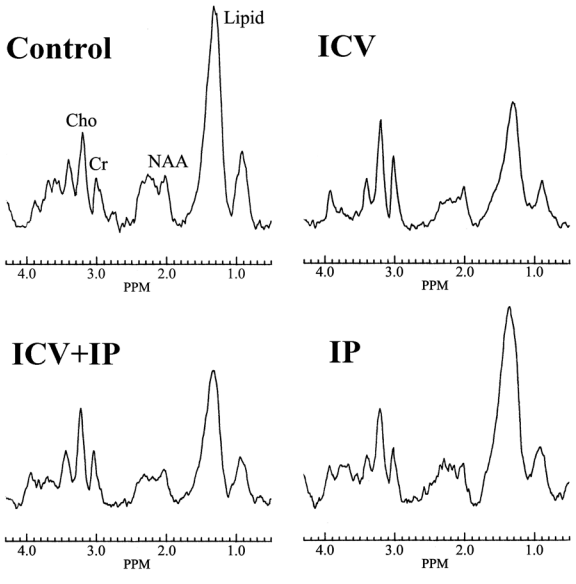

Fig. 2. Comparison of ^{1}H MR spectra