PROTON MRS IN THE LATE STAGE OF NEONATAL HYPOXIC-ISCHEMIC CEREBRAL INJURY

A. M. Chow[1,2], I. Y. Zhou[1,2], S. J. Fan[1,2], K. W. Chan[1,2], K. C. Chan[1,2], and E. X. Wu[1,2]

[1]**Laboratory of Biomedical Imaging and Signal Processing, The University of Hong Kong, Pokfulam, Hong Kong SAR, China, People's Republic of,**

[2]**Department of Electrical and Electronic Engineering, The University of Hong Kong, Pokfulam, Hong Kong SAR, China, People's Republic of**

INTRODUCTION

Neonatal hypoxic-ischemic (HI) cerebral injury is a major cause of permanent damage to neuronal cells which associated with high morbidity and mortality in the perinatal period, leading to cerebral palsy or impaired cognition[1,2]. After undergoing massive cell death and atrophy at the first week after injury, the neonatal brain undergoes regenerative processes toward the functional restitution which continues late after the HI injury when the infarct lesion stabilizes and lasts for at least 6 months[3,4]. While proton magnetic resonance spectroscopy (^{1}H MRS) has been employed to investigate metabolic changes during the acute-phase of neonatal HI cerebral injury[5], roles of major neurochemicals as markers for neurodegeneration and neuroprotection at late stage are also important for studying the neurophysiological changes. In this study, we aim to characterize metabolic changes at late stage of HI cerebral injury using an experimental rat model with ^{1}H MRS at 7 T.

METHODS

Animal Preparation: Sprague-Dawley rats (12-16 g, $N = 10$) were prepared and were divided into 2 groups. The HI-injured group ($N = 5$) underwent unilateral ligation of the left common carotid artery at postnatal day (P) 7 under isoflurane anaesthesia, followed by hypoxia in 8% oxygen and 92% nitrogen at 36-37 °C for 2 hours, as previously described[2]. The normal group ($N = 5$) was untreated and served as a control. At the age of 3 months, ^{1}H MRS was performed to all animals.

MRI: All MR measurements performed on a 7 T Bruker MRI scanner using a 72-mm birdcage transmit-only RF coil with an actively decoupled receive-only quadrature surface coil. Under inhaled isoflurane anaesthesia, the animal was kept warm under circulating water at 37 °C. Scout images were first acquired in three orthogonal planes with a FLASH sequence. T_2-weighted anatomical images were acquired using 2D RARE sequence. For ^{1}H MRS, a $0.8 \times 2.8 \times 2.8$ mm^3 voxel was placed over the posterior cortex and another $2.8 \times 2.8 \times 2.8$ mm^3 voxel over the thalamus contralateral to the injury. The volume of interest was maximized to obtain higher signal-to-noise ratios and to cover the gray matter predominantly, while avoiding the margins of the white matter structures, which were clearly distinguishable in T_2-weighted images. After first- and second-order localized voxel shimming with field map based shimming technique, a full-width half-maximum linewidth of water signal of ≤ 20 Hz would be achieved. The water signal was suppressed by variable power RF pulses with optimized relaxation delays (VAPOR). Outer volume suppression (OVS) combined with point-resolved spectroscopy (PRESS) sequence was used for signal acquisition using TR = 2500 ms, TE = 20 ms, spectral bandwidth = 3 kHz, 2048 data points and 512 averages.

Data Analysis: MR spectra were processed using the jMRUI software[6]. The raw data were apodized with a 15-Hz Gaussian filter. In addition, the signal of residual water was filtered with Hackel–Lanczos Singular Value Decomposition (HLSVD) algorithm preprocessing with 25 spectral components for modeling. Spectral peaks were assigned in the references of the singlet peak of NAA (CH_3-group). Metabolite areas were estimated using the quantitation based on quantum estimation (QUEST) method combined with subtraction approach for background modeling[7]. To reduce systematic variations among studied animals and to accurately extract the dominating metabolite changes, a relative quantification method using creatine (Cr) peak as the internal spectral reference was applied given that concentration of Cr remains relatively constant *in vivo*[8]. The numerical time-domain modal functions of 10 metabolites [acetate (Ace), alanine (Ala), aspartate (Asp), N-acetylaspartate (NAA), Cho, Cr, taurine (Tau), glutamate (Glu), lactate (Lac) and myo-inositol (m-Ins)] were used as prior knowledge in QUEST. These metabolite model signals were quantum mechanically simulated in NMR spectra calculation using operators (NMRSCOPE) for the *in vivo* experimental protocol. NAA:Cr, Cho:Cr, Glu:Cr, Lac:Cr, and m-Ins:Cr ratios were statistically evaluated. The reliability of metabolite quantitation was assessed using the Cramer-Rao lower bounds (CRLB)[7]. An estimate was considered as relevant when the corresponding bound was found below 25% of the estimate. Mann-Whitney test was employed between normal and HI-injured animals of all measurements with $p < 0.05$ considered as statistically significant.

RESULTS AND DISCUSSIONS

Figure 1 illustrates the voxel placements to the posterior cortex and thalamus contralateral to the injury, and the typical ^{1}H MRS spectra of the posterior cortex and thalamus in HI-injured and normal animals. Figure 2 shows the estimated metabolite ratios in normal and HI-injured animals. It was consistently observed that all HI-injured animals showed a marked decrease in Glu signal with respect to Cr signal as compared with normal animals, in both posterior cortex and thalamus by 29% and 14% respectively ($p < 0.01$). Glu is an amino acid that is excitatory neurotransmitters in the brain[9] and its role as an excitotoxin is well studied[10,11]. The significant decrease observed in Glu:Cr ratio may result from the neuroprotective effect of reduced Glu in the posterior cortex and thalamus contraleteral to the injury in response to reinnervation by neurons of the remaining intact hemisphere[12].

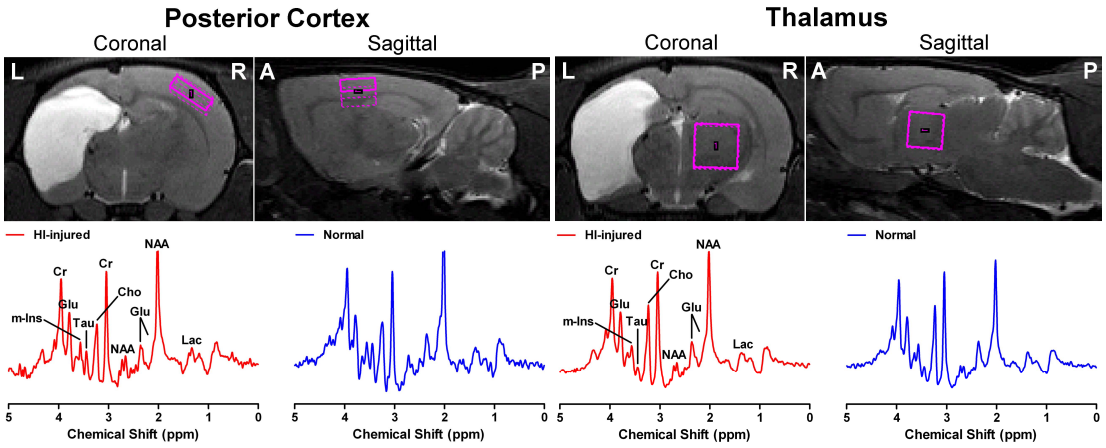

FIG. 1 (Top row) Illustraion of voxel placements (solid-line boxes) in the posterior cortex and thalamus contralateral to the injury for ^{1}H MRS. (Bottom row) Typical ^{1}H MRS spectra on each side of the posterior cortex and thalamus in HI-injured and normal animals. Note the lower Glu signal in HI-injured animal then that in normal animals for both posterior cortex and thalamus. (L: left; R: right; A: anterior; P: posterior)

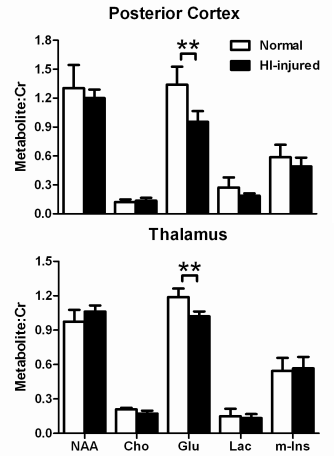

FIG. 2 Metabolite ratios at the posterior cortex (Top) and thalamus (Bottom) contralateral to the injury. Mann-Whitney test was performed with ** for $p < 0.01$.

CONCLUSIONS

The experimental results of this study showed that alteration in the metabolism at late stage in the posterior cortex and thalamus contralateral to injury is associated with neonatal HI cerebral injury. The decrease in Glu signal with respect to Cr signal in the HI-injured animals may result from the neuroprotective effect of reduced Glu, likely caused by the response to reinnervation. This may provide insights into the changes in plasticity and adaptive and compensatory modifications within the brain following neonatal HI cerebral injury.

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