Increased Detectability of Alpha Glutamate/Glutamine on Brain 1H-MRS in Neonatal Seizures Caused by Hypoxic-Ischemic Encephalopathy

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Introduction

The cerebral metabolic disturbances during seizures and hypoxic ischemic events in patients with Hypoxic-Ischemic Encephalopathy (HIE) can lead to excessive synaptic and extracellular concentration of glutamate with concomitant and subsequent neuronal cell injury or death (1). Numerous studies also suggest that increased glutamate concentration in the brain induce seizures (2). Therefore, glutamate is intimately involved in the pathogenesis of the seizures. However, there is no evidence to our knowledge that shows a relationship between the severity of neonatal seizures caused by HIE and the concentration of glutamate in the brain. In this study, we divided the patients with HIE into two groups based on the presence or absence of seizures in order to examine and evaluate the existence of any relationship between the grade of seizures in neonates caused by HIE and the Glutamate/glutamine (Glx) concentration in the brain.

Methods: Initial ^{1}H-MRS was performed in three groups of age-matched neonates (2-7 days old). Group I was a control group that consisted of 7 healthy neonates (mean gestational period of 39.3 weeks and mean birth weight of 3.386 kg). Group II consisted of 14 neonates with HIE and no seizures (mean gestational period of 37.8 weeks and mean birth weight of 2.730 kg). Group III consisted of 7 neonates with HIE and seizures (mean gestational period of 39.6 weeks and mean birth weight of 3.250 kg). The types of seizures in group III included subtle seizures in two cases, clonic seizures in two cases and generalized tonic seizures in three cases (3). The seizures were graded according to their severity and frequency (4) from grade 1 to grade 3. Grade 1 was defined as occasional and transient seizures. Grade 2 was defined as repeated seizures (< 3 times of the seizures/day) with each seizure lasting less than 3 minutes. Grade 3 was defined as repeated seizures (> 3 times of the seizures /day) with each seizure lasting more than 3 minutes. Follow-up MRS examinations were performed in 8 neonates of group II at 10-17 days of age and in 4 neonates of group III at 13-16.5 days of age. ^{1}H MRS examinations were done using a clinical MRI imager operating at 1.9 Tesla. The spectral volume of interest was placed in the center of the brain including the basal ganglia, centra semiovales, thalami and parts of the lateral & third ventricles. All of the ^{1}H MRS data was acquired using PRESS (Point Resolved Spectroscopy Sequence) and STEAM (Stimulated Echo Acquisition Mode) sequences with CHESS (chemical selective water suppression sequence) water suppression using the pre-defined tip angle, which minimized the water signal and after optimized shimming (with FWHM of the suppressed water peak <0.1 ppm). The scan parameters of the PRESS sequence were TR of 2000 ms, TE of 135 ms, with averages of 250 and a volume of interest of 18 cm^3. The scan parameters of the STEAM sequence were TR of 2000 ms, TE of 24 ms, middle time of 63 ms, averages of 250 and a volume of interest of 18 cm^3. The prominent N-acetyl-aspartate (NAA) peak at 2.0 ppm on ^{1}H MRS was used as an internal chemical shift reference for the ^{1}H MRS. We used the Glx complex instead of just glutamate because the two amino acids are structurally very similar and can not be separated by present clinical MRI/^{1}H MRS systems (5). Glx was measured at its α– proton peak at 3.75 ppm because the peaks of the β and γ– protons of Glx were complicated by complex phase modulation (6).

Results

On initial ^{1}H MRS study, the detection rate of the α-Glx at 3.75 ppm peak was significantly higher in group III (7/7) than in groups I (1/7) and II (7/14) (Fisher's exact tests, $P < 0.01$). The peaks of NAA, Cho and TCr were clearly seen in all neonates. The level of the peak-area ratio of α-Glx/TCr in group III (0.5/2.42, n=7) was significantly higher than in group I (0.00/0.12, n=7) and group II (0.00/0.33, n=14) (Unpaired Mann-Whitney U tests, both $P<0.01$). The difference of the level was not statistically significant between group I and group II. Additionally, the level of the peak-area ratio of α-Glx/TCr in group III was positively correlated with the grade of seizures (Spearman rank correlation, $r = 0.769$, $p < 0.05$, n=7). In neonates with grade 1 seizures, the ratio was 0.38-0.5 (n=3). In neonates with grade 2 seizures, the ratio was 0.5 (n=2). In neonates with grade 3 seizures, the ratio was 0.5-2.8 (n=2). On the follow-up ^{1}H MRS study, the obvious peak of the α-Glx was still visible in all 4 neonates of group III, and the level of the peak-area ratio of α-Glx/TCr in group III was still significantly higher than in group II ($P<0.01$). In 3 neonates of group III, the seizure symptoms subsided and the level of the peak area ratio of α-Glx/TCr decreased from 0.500/0.00 to 0.330/0.110 after supportive treatment. However, one neonate of group III still had seizures, a high level of the peak area ratio of α-Glx/TCr (1.0) and died 2 days after the follow-up ^{1}H MRS study. There was no significant difference in the peak-area ratios of NAA and Cho to TCr in all three groups on both the initial and follow-up ^{1}H MRS studies ($p>0.05$).

Discussion

Changes in neurotransmitter metabolism in seizures have not been reported in neonates, although an elevated glutamate concentration has been reported in surgically resected human epileptic tissue (7) and by in-vivo observation by the microdialysis technique (8). Recent ^{1}H MRS studies demonstrated that Glx increased in the ipsilateral hippocampus of patients with temporal lobe epilepsy (9) and in the frontal lobe and thalamus of patients with idiopathic generalized epilepsy (10). Our current study demonstrated for the first time that the Glx peak at its alpha region is increased in the basal ganglia, centra semiovals, thalami and part of lateral and third ventricles in all neonates with seizures caused by HIE. It also demonstarted a positive correlation between the detectability of α-Glx and the severity of the seizures. These findings imply that glutamate is involved in the pathogenesis of neonatal seizures caused by HIE. These findings are consistent with the notion that glutamate plays an important role in the pathogenesis of epilepsy that has been documented by previous studies (5, 11-12). Although the reduction of NAA was considered a main finding of ^{1}H MRS in the epiletogenic regions (13), it was not seen in this study. We think this was because we performed the ^{1}H MRS scans in neonates with seizures caused by HIE within one week of life for the initial study, and within 17 days of life for follow-up scans. Normally, ^{1}H MRS studies in medically refractory seizures are performed for pre-surgical localization of the epileptogenic zone many years after the onset of seizures (13).

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