Temporal brain metabolite changes in preterm infants with normal development

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Abstract

Objective: Preterm infants are at high risk for developmental delay, epilepsy, and autism spectrum disorders. Some reports have described associations between these conditions and gamma-aminobutyric acid (GABA) dysfunction; however, no study has evaluated temporal changes in GABA in preterm infants. Therefore, we assessed temporal changes in brain metabolites including GABA using single-voxel 3-Tesla (T) proton magnetic resonance spectroscopy (^{1}H-MRS) in preterm infants with normal development.

Methods: We performed 3T ^{1}H-MRS at 37–46 postmenstrual weeks (PMWs, period A) and 64–73 PMWs (period B). GABA was assessed with the MEGA-PRESS method. N-acetyl aspartate (NAA), glutamate–glutamine complex (Glx), creatine (Cr), choline (Cho), and myo-inositol (Ins) were assessed with the PRESS method. Metabolite concentrations were automatically calculated using LCModel.

Results: Data were collected from 20 preterm infants for periods A and B (medians [ranges], 30 [24–34] gestational weeks, 1281 [486–2030] g birth weight). GABA/Cr ratio decreased significantly in period B ($p = 0.03$), but there was no significant difference in GABA/Cho ratios ($p = 0.58$) between the two periods. In period B, NAA/Cr, Glx/Cr, NAA/Cho, and Glx/Cho ratios were significantly increased ($p < 0.01$), whereas Cho/Cr, Ins/Cr, and Ins/Cho ratios were significantly decreased ($p < 0.01$). There was no significant difference for GABA or Cho concentrations ($p = 0.52$, $p = 0.22$, respectively). NAA, Glx, and Cr concentrations were significantly increased ($p < 0.01$), whereas Ins was significantly decreased ($p < 0.01$).

Conclusions: Our results provide new information on normative values of brain metabolites in preterm infants.

Keywords: Brain metabolites; Magnetic resonance spectroscopy; Preterm infants

1. Introduction

Recent clinical advances in perinatal care have increased the survival rate of preterm infants. However, preterm infants still have higher risks of cerebral palsy, cognitive deficiency, behavioral or psychological problems, blindness, hearing loss, epilepsy, and autism spectrum disorders [1,2]. Diagnoses of intraventricular hemorrhage or periventricular leukomalacia made using cranial ultrasonography (US) or conventional magnetic resonance imaging (MRI) show sufficient sensitivity to detect delayed complications such as spastic diplegia or quadriparesis and are usually the first imaging modalities used in preterm infants [3]. However, they cannot completely exclude later adverse neurologic outcomes: for example, preterm infants can appear normal on neonatal US and exhibit no white matter lesions on MRI but later develop cerebral palsy and suffer cognitive delays.

Proton magnetic resonance spectroscopy (^{1}H-MRS) can noninvasively measure various brain metabolites that are known to be altered during rapid brain development in the first year of life [4]. MRS may provide additional diagnostic value to cranial US and MRI in infants. The use of 3 Tesla (T) MRS offers better quality spectra in shorter acquisition times than 1.5T MRS, and 3T MRS can also discriminate gamma-aminobutyric acid (GABA) spectrum. An immunohistochemical study showed GABAergic neuron loss following perinatal brain injury and revealed that white matter lesions affect cortical development and GABA receptor expression in cortical layers; the authors suggested that this may contribute to the pathogenesis of neurologic deficits [5]. GABA abnormalities have also been reported in the settings of childhood autism spectrum disorders and epilepsy [6,7].

The purpose of this study was to assess temporal changes in brain metabolites including GABA with single-voxel 3T ^{1}H-MRS in preterm infants with normal development until 64–73 postmenstrual weeks (PMWs, the gestational weeks plus the weeks after birth).

2. Materials and methods

2.1. Patients

From April 2014 to March 2015, we prospectively recruited 79 preterm infants (gestational age 6 34 weeks) who were admitted to our neonatal intensive care unit. Cranial US, conventional MRI, and ^{1}H-MRS were performed at 37–46 PMWs (period A). Follow-up MRI and 1H-MRS scans were performed at 64–73 PMWs (period B). The analysis included infants with data for both periods who were determined to have normal development at period B. Of 79 preterm infants, we excluded 59 due to early

death (n = 2), major anomaly (n = 1), respiratory failure (n = 1), clip ligation of patent ductus arteriosus (n = 6), hospital transfer before examination (n = 14), no written informed consent (n = 6), acute disease (n = 16), motion artifacts (n = 4), technical problems with ^{1}H-MRS (n = 6), and MRI abnormalities and/or developmental delay at period B (n = 3).We ultimately included 20 preterm infants in the analysis.

The developmental quotient (DQ) was assessed using the Enjoji Scale of Infant Analytical Development in period B. A DQ score <70 indicated developmental delay [8]. The Denver Development Screening Test II adopted for Japanese children (DDST II) was also administered. Failure of ≥2 items out of 28 total items indicated abnormal development [9].

This study was approved by the ethics committee of Iwate Medical University (approval number H24-37).

2.2. MRS examination

1H-MRS examinations were conducted using a 3T scanner (Discovery MR750 3.0T DV24.0, GE Healthcare, Milwaukee, WI, USA) equipped with an eightchannel head coil. All subjects were sedated with oral triclofos sodium (80 mg/kg) 30 min before the examination. Heart rate and transcutaneous oxygen saturation were monitored during examinations. Single-voxel ^{1}H-MRS was performed on the volume of interest (VOI), a 25 × 25 × 25 mm volume in the right basal ganglia (BG) on the axial section through the BG under the guidance of T2-weighted images (Fig. 1). Since the VOI was larger than that in our previous report using a 1.5T multivoxel MRS [10], the frontal lobe was not acquired in this study. GABA was assessed with the Meschier-Garwood point-resolved echo spectroscopy (MEGA-PRESS) method. We used the differenceediting technique for optional measurement of GABA. N-acetyl aspartate (NAA), glutamate–glutamine complex (Glx), creatine (Cr), choline (Cho), and myoinositol (Ins) were assessed with the PRESS method. The acquisition parameters were as follows: reception time = 1500 ms, echo time = 68 ms, number of excitations = 256, and acquisition time = 6 min 54 s.
High-order global shimming and optimization of radio frequency power for water suppression were automatically performed.

Brain metabolite concentrations in the BG were automatically calculated using LCModel version 6.1 (S-provencher Inc., Oakville, ON, Canada). Only results with a standard deviation ≤30% were included in the analysis, where the % standard deviation reflected the Cramer-Rao lower bound.

2.3. Statistical analyses

Wilcoxon signed-rank tests were used to compare temporal changes in brain metabolite ratios and raw brain metabolite concentrations between the two periods.

Statistical analyses were performed using SPSS Ver. 22 (IBM Inc., Armonk, NY, USA). A p-value less than 0.05 was considered statistically significant.

3. Results

Table 1 shows the clinical characteristics of the study population. Data are expressed as numbers (%) or medians (ranges) unless otherwise indicated. They were born at 30 (24–34) gestational weeks of age with a birth weight of 1281 (486–2030) g, 7 (35%) of which were extremely low birth weight infants (<1000 g). Sixteen (80%) were appropriate for dates (AFD) infants and 4 (20%) were asymmetric small for dates (SFD) infants. SFD was defined as both birth weight and height less than the 10th percentile of the normal values at each gestational age according to published new norms for Japanese infants [11]. Head circumference of AFD infants was 27.1 (22.5–32.2) cm, and that of SFD infants was 25.0 (22.4–27.5) cm, indicating a non-significant difference between the two groups ($p = 0.17$, Mann– Whitney test). The DQ score was 99 (81–116), and the DDST II score was 28 (27–28).

The metabolite ratios are shown in Fig. 2 and Table 2. The GABA/Cr ratio decreased significantly in period B ($p = 0.03$). There was no significant difference in the GABA/Cho ratios ($p = 0.58$) between the two time points. In period B, NAA/Cr, Glx/Cr, NAA/Cho, and Glx/Cho ratios were significantly increased ($p < 0.01$), whereas Cho/Cr, Ins/Cr, and Ins/Cho ratios were significantly decreased ($p < 0.01$). The raw metabolites concentrations are listed in Table 3. There was no significant difference for GABA or Cho concentration ($p = 0.52$ and $p = 0.22$, respectively). NAA, Glx, and Cr concentrations were significantly increased ($p < 0.01$), whereas Ins was significantly decreased ($p < 0.01$).

4. Discussion

Our results show brain metabolite alterations measured with 3T ^{1}H-MRS during early infancy in preterm infants with normal psychomotor development. In a comparison of measurements taken during periods A and B, the GABA/Cr ratio was significantly decreased, but there were no significant differences in the GABA/Cho ratios and GABA concentrations. The changes in NAA/Cr, NAA/Cho, Cho/Cr, Ins/Cr, and Ins/Cho ratios are consistent with findings from our previous study using 1.5T ^{1}H-MRS [10] and those of another group [12]. We also observed increases in the Glx/Cr and Glx/Cho ratios over time.

Two MRS studies have reported GABA concentrations in preterm infants [13,14]. Kreis et al. measured brain metabolites including GABA in 21 infants (32.7 ± 1.6

gestational weeks, mean ± SD) by using 1.5T MRS in three cerebral locations; centrum semiovale, thalamus, and occipital gray matter. In their report, they speculated that some of the findings concerning the smaller signals such as GABA in the ^{1}HMR spectrum might be biased by the limited resolution potential of spectra obtained at 1.5 T and by the model fitting analysis considerably extending the range of observable metabolites [13]. Kwon et al. measured GABA in 17 preterm infants (27 ± 1.8 gestational weeks, mean ± SD) at term equivalent age collected from a single 20 × 30 × 30 mm region of interest localized over the right frontal lobe in a 3T MRS. They compared GABA concentration and its relationship to functional connectivity in the brains of these infants and term infants [14]. To our knowledge, the present paper is the first study to investigate temporal changes in GABA in preterm infants using 3T ^{1}H-MRS.

GABA is the major inhibitory neurotransmitter in adult brain but has multimodal functions in the developing brain. The active chloride (Cl^-) homeostasis hypothesis states that GABA acts as an excitatory neurotransmitter in the immature brain [15,16]. GABA activation of the $GABA_A$ receptor, a ligand-gated chloride channel, mediates a depolarizing response due to high intracellular Cl^- concentrations in neurons of the immature brain. High intracellular Cl^- results from the balance of the Na–K–Cl cotransporter NKCC1 and the K–Cl cotransporter KCC2. The excitatory action of GABA is considered to be a key factor for numerous developmental phenomena including neuronal proliferation, neuronal migration, and synapse organization [15]. The observed decrease in the GABA/Cr ratio in the present cohort may be influenced by increasing Cr concentrations. Unaltered GABA/Cho ratios suggest that brain GABA concentrations may remain constant during early development to facilitate brain development.

GABAergic neurons migrate into the cortex and BG between 15 and 26 weeks of gestation, and their movement from the ganglionic eminence to the thalamus only occurs in the human brain [17]. GABAergic neuron migration is completed by term gestation. Theoretically, patients with cystic periventricular leukomalacia with a reduced thalamic volume and GABAergic interneuron damage may have decreased GABA concentrations in the BG. Future studies should be performed to assess brain GABA concentrations in preterm infants with MRI abnormalities or developmental delay.

We only observed development until 64–73 PMWs. In the rodent brain, GABA concentrations in the BG decrease in the first 7 postnatal days and then increase [18]. Follow-up studies in these preterm infants over a longer time period will clarify the role

of GABA in the premature brain.

LCModel automatically quantitates brain metabolite concentrations using water peaks obtained from the VOI as an internal reference by comparing the default basis set based on the adult brain. However, these metabolite concentrations may not be accurate for infants given the water content and changes during brain development [19]. Therefore, we used reference metabolites to evaluate changes in metabolites of interest. We also showed raw brain metabolite concentrations as reference values. Cr is a relatively stable metabolite in the adult brain and is usually used as a reference compound for semi-quantitative analyses. However, brain Cr concentrations reportedly increase with postmenstrual age [20]. Indeed, our data showed a significant increase in Cr concentrations. Cho concentrations were relatively stable in the assessed periods; therefore, we also calculated metabolite ratios with Cho. However, it is important to note that we were able to identify dynamic changes in brain metabolite ratios using either Cr or Cho as a reference.

NAA levels increase gradually from 24 weeks gestation [20] and rapidly during infancy [12], reflecting neuronal development and axon elongation. NAA is synthesized by and mainly stored in mature neurons and axons. Its rapid increase in early brain development is a surrogate measure of neuronal maturation and axonal growth [21]. Cho is a membrane metabolite and indicator for de novo myelin and cell membrane synthesis, and reportedly remains close to constant during the first 3 months [22]. Indeed, we found that Cho remained almost constant during the early brain development of preterm infants. Finally, we observed decreases in Ins/Cr and Ins/Cho, which are markers of glial cells [23], suggesting a relative decrease in glial process volume during brain maturation.

Glutamate (Glu) and glutamine (Gln) are typically more easily resolved at 3T than at 1.5T [12], but we found that theMRS spectra of Glu and Gln are complex and overlapped. We therefore present Glx data, which is a robust and general identification in complex mixtures. Future studies should determine whether Glu and Gln can be resolved with high precision in the infant brain. We did determine that Glx/Cr and Glx/Cho significantly increased over the periods examined. Glu is the most abundant amino acid in brain metabolism [23] and is also the major excitatory neurotransmitter in the brain. Glu furthermore plays an important role in GABA metabolism [24]. Therefore, increases in Glx/Cr and Glx/Cho ratios in infancy may reflect the development of both excitatory and inhibitory neurons.

There were some limitations of this study. First, the exclusion of 59 out of 79 infants may have resulted in subject selection bias. Second, our subjects may not have been

neurologically normal since developmental outcome was not determined after 6 months of age. Further studies with larger sample sizes to evaluate long-term outcome are needed. Third, six infants were excluded from analysis due to shimming failure during ^{1}HMRS. This modality is more sensitive than MRI to nonuniformities in the magnetic field. Poor shimming decreases signal-to-noise ratio and also affects the line shape. Finally, a previous study reported that brain GABA concentrations vary between sexes in adulthood [25], but we did not consider this effect in our study.

In conclusion, our data provide new information about the normative values of brain metabolites in preterm infants with normal development. A future investigation will be performed to evaluate their levels in preterm infants with abnormal development. Further research is also needed to identify accurate imaging markers to predict psychomotor development in preterm infants.

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Figure Legends

Fig. 1. (a and b) Typical spectra of the right basal ganglia of a female infant born at 34 weeks of gestation weighing 1628 g. 1H-MRS was performed at 44 postmenstrual weeks. GABA was assessed with the Meschier-Garwood point-resolved echo spectroscopy (MEGAPRESS) method. NAA, Glx, Cr, Cho, and Ins were assessed with the PRESS method. (c) Location of the measurement voxel in the right basal ganglia. Voxel dimensions: 25 × 25 × 25 mm. GABA, gamma-aminobutyric acid; NAA, N-acetyl aspartate; Glx, glutamate–glutamine complex; Cr, creatine; Cho, choline; Ins, myo-inositol.

Fig. 2. Temporal changes in brain metabolite ratios in the right basal ganglia on 3-Tesla 1H-MRS in preterm infants with normal development. Data are shown in double boxplots. The vertical and horizontal axes show brain metabolite ratios and postmenstrual weeks, respectively. The vertical and horizontal lines inside the box are the medians. The bottom and top of the boxes represent the first and third quartiles, respectively. The ends of the whiskers represent the minimums and maximums. Values more and less than 1.5 times of the upper and lower quartiles are plotted as outliers. Abbreviations for metabolites are the same as those in Fig. 1.

Table 1. Characteristics of the study population (n=20)

Antenatal steroids	18	(90)
Multiple birth	4	(20)
Gestational age (weeks)	30	(24–34)
Birth weight (g)	1281	(486–2030)
Extreamely low birth weight <1000 g	7	(35)
Small for dates	4	(20)
Gender (male)	7	(35)
Apgar score 1 min	4	(2–8)
5 min	7	(4–9)
10 min	8	(7–9)
Respiratory distress syndrome	12	(60)
Received indomethacin	8	(40)
Postnatal steroids	10	(50)
Chronic lung desease	6	(30)
DQ at period B	99	(81–116)
DDST II at period B	28	(27–28)

Data are shown as numbers (%) or medians (ranges).
DQ, developmental quotient; DDST II, Denver Developmental Screening Test II; period B, 64–73 postmenstrual weeks.

Table2. Temporal changes in brain metabolite ratios in the basal ganglia

	period A	period B	*p* value
GABA/Cr	0.15 (0.13–0.18)	0.13 (0.10–0.17)	0.03
NAA/Cr	0.75 (0.69–0.85)	1.03 (1.00–1.06)	< 0.01
Glx/Cr	0.52 (0.49–0.61)	0.70 (0.61–0.75)	< 0.01
Cho/Cr	0.40 (0.39–0.42)	0.32 (0.31–0.33)	< 0.01
Ins/Cr	1.40 (1.27–1.56)	0.79 (0.72–0.87)	< 0.01
GABA/Cho	0.37 (0.35–0.45)	0.40 (0.34–0.53)	0.58
NAA/Cho	1.83 (1.74–2.21)	3.25 (3.11–3.46)	< 0.01
Glx/Cho	1.29 (1.21–1.52)	2.13 (1.81–2.35)	< 0.01
Ins/Cho	3.53 (3.13–3.86)	2.53 (2.31–2.67)	< 0.01

Data are shown as medians (interquartile ranges).
Period A, 37–46 postmenstrual weeks (PMWs); period B, 64–73 PMWs.
Abbreviations for metabolites are the same as those in Figure 1.

Table3. Temporal changes in brain metabolite concentrations in the basal ganglia

	period A	period B	*p* value
GABA	1.06 (1.00– 1.41)	1.13 (0.93– 1.55)	0.52
NAA	5.67 (5.13– 6.21)	9.82 (9.05–10.54)	< 0.01
Glx	3.70 (3.54– 4.66)	6.74 (5.91– 7.26)	< 0.01
Cr	7.34 (6.95– 7.79)	9.64 (8.77–10.22)	< 0.01
Cho	3.02 (2.73– 3.10)	3.02 (2.74– 3.35)	0.22
Ins	10.31 (9.72–11.13)	7.72 (6.97– 8.21)	< 0.01

Data are shown as medians (interquartile ranges).
Period A, 37–46 postmenstrual weeks (PMWs); period B, 64–73 PMWs.
Abbreviations for metabolites are the same as those in Figure 1.

Figure 1

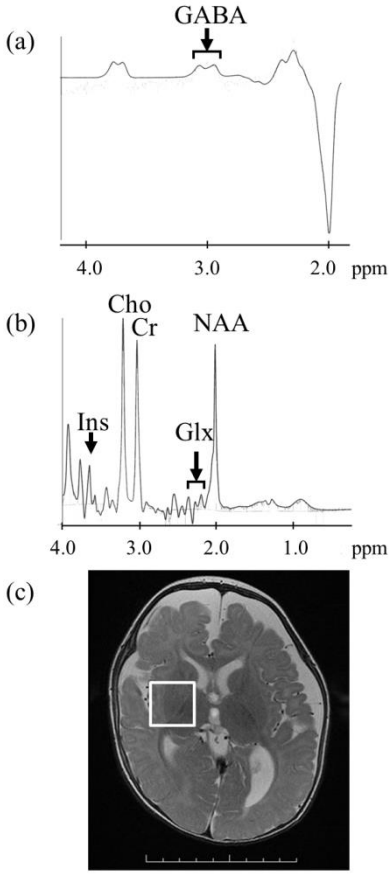
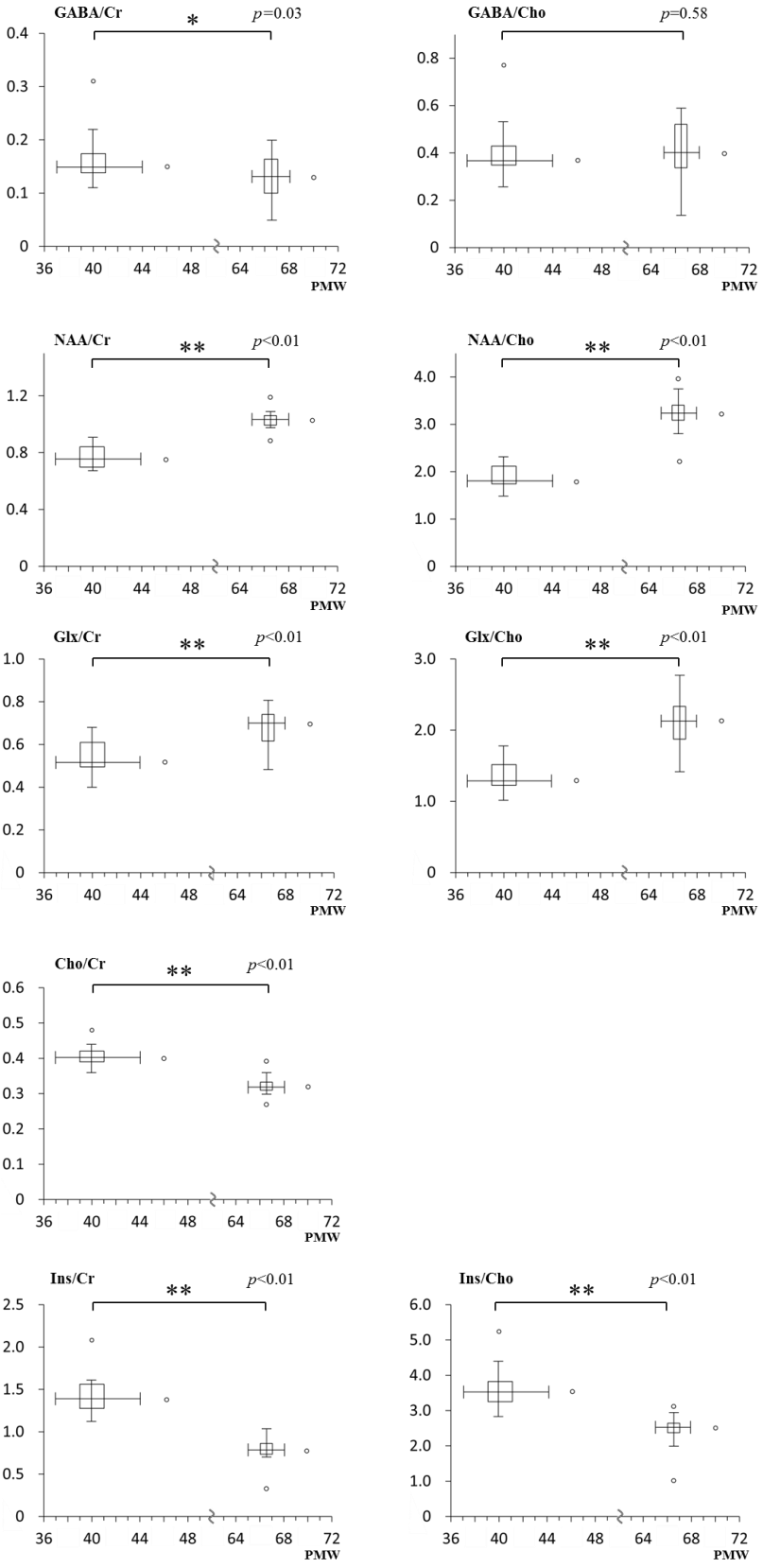


Figure 2


GABA/Cr
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p=0.03
GABA/Cho
p=0.58
NAA/Cr
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p<0.01
NAA/Cho
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PMW