Original Article

Vitamin B6 in acute encephalopathy with biphasic seizures and late reduced diffusion

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Abstract

Background: The initial presentation of acute encephalopathy with biphasic seizures and late reduced diffusion (AESD) is indistinguishable from that of complex febrile seizures (FS), which poses a great diagnostic challenge for clinicians. Excitotoxicity is speculated to be the pathogenesis of AESD. Vitamin B6 (VB6) is essential for the biosynthesis of gamma-aminobutyric acid, an inhibitory neurotransmitter. The aim of this study is to investigate our hypothesis that VB6 deficiency in the brain may play a role in AESD.

Methods: We obtained cerebrospinal fluid (CSF) samples from pediatric patients with AESD after early seizures and those with FS. We measured pyridoxal 5′-phosphate (PLP) and pyridoxal (PL) concentrations in the CSF samples using high-performance liquid chromatography with fluorescence detection.

Results: The subjects were 5 patients with AESD and 17 patients with FS. Age did not differ significantly between AESD and FS. In AESD, CSF PLP concentration was marginally lower ($p = 0.0999$) and the PLP-to-PL ratio was significantly ($p = 0.0417$) reduced compared to those in FS.

Conclusions: Although it is impossible to conclude that low PLP concentration and PLP-to-PL ratio are causative of AESD, this may be a risk factor for developing AESD. When combined with other markers, this finding may be useful in distinguishing AESD from FS upon initial presentation.

Keywords: AESD, biomarker, febrile seizure, pyridoxal 5′-phosphate, pyridoxal kinase, risk factor

1. Introduction

Acute encephalopathy with biphasic seizures and late reduced diffusion (AESD) is a clinically and radiographically defined syndrome characterized by epileptic seizures (often status epilepticus) at the onset of febrile illness (early seizures), followed by transient recovery of consciousness, and subsequent seizure clustering and global cognitive regression 3 to 7 days later (late seizures). At this late stage, brain magnetic resonance imaging (MRI) often demonstrates restricted diffusion in the subcortical white matter [1]. AESD affects 100 to 200 children in Japan every year and poses a great challenge for clinicians, because its initial manifestation is indistinguishable from that of complex febrile seizure (FS). Research on inflammatory markers, neuronal/glial markers, and susceptibility genetic variants has been conducted to understand the pathogenesis of AESD [2-5]. At present, however, there remains no certain way to distinguish AESD from FS at onset and to prevent late seizures, even if AESD is suspected.

Currently there are no established treatments for AESD. There is no evidence that high-dose methylprednisolone, intravenous immunoglobulin, or hypo/normothermia therapy are efficacious. To improve the neurodevelopmental outcome of AESD, the discovery of reliable methods to identify AESD in the early stage and the development of new therapeutic strategies based on the pathogenesis of AESD are required. Imbalance between the excitatory and inhibitory systems may play a role in AESD. Excitotoxicity has been proposed for the pathogenesis of AESD based on an increase in glutamate/glutamine complex demonstrated by magnetic resonance spectroscopy [6]. Theophylline, which inhibits pyridoxal kinase (PDXK) [7], is associated with AESD [8]. PDXK activates vitamin B6 (VB6), which is required for the biosynthesis of gamma-aminobutyric acid (GABA), an inhibitory neurotransmitter [9] (Figure 1). There is a case series report in which the use of VB6 might have prevented the worsening

of AESD [10]. Another report suggested that early administration of vitamins B1, B6, and L-carnitine prevented the development of AESD [11]. Based on these reports, we hypothesized that VB6 may play a role in the pathogenesis of AESD, namely, VB6 deficiency in the brain may be an aggravating or triggering factor for AESD by downregulating the inhibitory system. To investigate this hypothesis, we conducted a study to measure VB6 compounds in cerebrospinal fluid (CSF) samples acquired from pediatric patients with AESD and FS.

2. Methods

2.1. Subjects

Subjects consisted of pediatric (<18 years old) patients with AESD after early seizures and those with FS who were admitted to Okayama University Hospital or other hospitals in Japan that were participating in this study, and who underwent lumbar puncture for diagnostic purposes. The diagnostic criteria of AESD in this study were 1) seizure onset at the beginning of presumptive infection, especially with status epilepticus (early seizure) and either 2) seizure(s) 3 to 7 days after seizure onset (late seizure) or 3) restricted diffusion involving the subcortical white matter and/or cortex (bright tree appearance) by MRI 3 to 14 days after seizure onset. Patients receiving vitamin B6 therapy were excluded. The collected CSF samples were aliquoted and used for routine laboratory tests (e.g., cell count, protein, and glucose), cultures, and polymerase-chain reaction tests to determine causative organisms. The remaining CSF samples were protected from light and frozen below –70°C within 1 h for this study. Samples collected outside Okayama University Hospital were shipped on dry ice to our laboratory.

This study was performed in accordance with the Declaration of Helsinki and approved by the Research Ethics Board at Okayama University Hospital. Written informed consent was

obtained from patients' parents or guardians.

2.2. Vitamin B6 assay

We measured the CSF concentrations of pyridoxal 5′-phosphate (PLP) and pyridoxal (PL) using the previously reported method [12]. In brief, 100 μL of CSF samples were derivatized by semicarbazide hydrochloride. Thereafter, PLP and PL were separated and their concentrations were determined by high-performance liquid chromatography with fluorescence detection.

2.3. Statistical analysis

Statistical analysis was conducted by R 3.4.2 (https://cran.r-project.org/). Group comparison was performed by Wilcoxon's rank sum test. The significance level was set at 0.05.

3. Results

3.1. Subject characteristics

There were 5 patients with AESD and 17 patients with FS. Most FS patients had complex FS, that is, prolonged and/or clustering seizures. The age of onset was 2 months to 5 years 2 months (median, 1 year 0 months) in AESD and 8 months to 4 years 11 months (median, 1 year 5 months) in FS, and there were no significant differences between AESD and FS (p = 0.271). The timing of CSF sample collection was 0 to 1 day (median, 0 days) in AESD and 0 to 2 days (median, 0 days) in FS after seizure onset, which were not significantly different (p = 1.0). Clinical characteristics are presented in Table 1.

3.2. Vitamin B6 assay

PLP and PL concentrations and PLP-to-PL ratios (PLP/PL) are presented in Figure 2. Most

patients with FS had PLP and PL concentrations and PLP-to-PL ratios within the reference range. Three out of five (60%) patients with AESD had low PLP concentrations, and four out of five (80%) patients had PLP-to-PL ratios that were low or around the lower limit of the reference range. One patient with AESD had a PLP concentration (54.9 nmol/L) and a PLP-to-PL ratio (1.3) that slightly exceeded the upper limit of the reference range. The CSF PLP concentration was <3.5 (below the low limit of quantitation) to 54.9 nmol/L (median 11.3 nmol/L) and 16.2 to 53.7 nmol/L (31.5 nmol/L) in AESD and FS, respectively. The CSF PLP concentration in AESD tended to be lower than that in FS ($p = 0.0999$). The PL concentration was 22.0 to 42.4 nmol/L (30.0 nmol/L) and 11.5 to 58.5 nmol/L (28.5 nmol/L) in AESD and FS, respectively. There was no significant difference in CSF PL concentration between AESD and FS ($p = 1.0$). The PLP-to-PL ratio was 0.1 to 1.3 (0.5) and 0.5 to 2.5 (1.0) in AESD and FS, respectively. The PLP-to-PL ratio in AESD was significantly lower than that in FS ($p = 0.0417$). There were no significant differences in CSF PLP concentration ($p = 0.778$) and PLP-to-PL ratio ($p = 0.438$) between patients with known causative organisms and patients with unknown etiologies.

4. Discussion

The analysis of CSF VB6 demonstrated marginally lower PLP concentration and significantly lower PLP-to-PL ratio in AESD compared with FS. Although we cannot conclude that reduced PLP is the cause of AESD, we speculate that low CSF PLP may be a risk factor for developing AESD. Whether PLP reduction occurred acutely at the onset of AESD or baseline PLP concentration was low before AESD onset is unclear. There was one patient with AESD who had a CSF PLP concentration and a PLP-to-PL ratio that were slightly higher than the upper limit of the reference range. This suggests that there are factors other than PLP contributing to the development of AESD in this patient.

Because PLP cannot cross the blood–brain and blood–CSF barriers directly, PLP in the CSF is mainly produced within the choroid plexus, where PL is taken up from plasma and converted to PLP by intracellular PDXK [13] (Figure 1). Therefore, the reduced PLP-to-PL ratio in the CSF may reflect reduced PDXK activity within the choroid plexus epithelial cells. Low PLP-to-PL ratios in the serum and CSF have been reported in poisoning by gingkotoxin, a PDXK inhibitor [14]. Another possibility is increased activity of tissue-nonspecific alkaline phosphatase (TNSALP) expressed at the membrane of ependymal cells, which converts PLP to PL so that CSF PLP can be taken up into brain parenchyma (Figure 1). There is a genome-wide association study that suggested a common single nucleotide polymorphism of the *ALPL* gene coding TNSALP affected the PLP-to-PL ratio in plasma and CSF [15].

Inflammation may also play a role in the reduced PLP-to-PL ratio in AESD. Decreased plasma PLP has been reported in inflammation, and alteration in PLP distribution reflecting the tissue-specific mobilization of PLP to the sites of inflammation is hypothesized [16]. In addition, PLP has recently been suggested as a scavenger of reactive oxygen species [16]. Whether similar phenomenon is happening in the brain with AESD is an open question.

It is uncertain whether low CSF PLP is a surrogate marker of low PLP in the brain. Although there is no direct way to estimate intracellular PLP status in the brain, an investigation of functional VB6 biomarkers in the CSF may be worthwhile. For example, CSF 3-O-methyldopa and 5-hydroxytriptophan become elevated when PLP is deficient in the brain, because of the reduced activity of PLP-dependent aromatic L-amino acid decarboxylase [17].

This study is significantly limited by its very small sample size. It was difficult to control the

condition of CSF collection in the emergency setting, although we tried to standardize sample handling protocol after collection. Future directions include confirmation by studies with larger sample size, functional VB6 biomarker assays in the CSF using targeted metabolome analysis, and investigation of factors affecting PDXK and TNSALP activity, such as polymorphisms of the *PDXK* and *ALPL* genes.

5. Conclusion

AESD patients after early seizures had marginally lower CSF PLP concentration and significantly reduced CSF PLP-to-PL ratio than in FS patients. Although this mechanism is uncertain, these findings may be useful to distinguish AESD from FS upon initial presentation, when combined with other markers. The therapeutic effect of VB6 for AESD remains to be elucidated.

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Conflict of Interest

The authors have no conflicts of interest to disclose.

References

[1] Takanashi J, Oba H, Barkovich AJ, Tada H, Tanabe Y, Yamanouchi H, et al. Diffusion MRI abnormalities after prolonged febrile seizures with encephalopathy. Neurology 2006;66:1304-9.

[2] Ichiyama T, Suenaga N, Kajimoto M, Tohyama J, Isumi H, Kubota M, et al. Serum and CSF levels of cytokines in acute encephalopathy following prolonged febrile seizures. Brain Dev 2008;30:47-52.

[3] Shiihara T, Miyake T, Izumi S, Watanabe M, Kamayachi K, Kodama K, et al. Serum and cerebrospinal fluid S100B, neuron-specific enolase, and total tau protein in acute encephalopathy with biphasic seizures and late reduced diffusion: a diagnostic validity. Pediatr Int 2012;54:52-5.

[4] Shinohara M, Saitoh M, Takanashi J, Yamanouchi H, Kubota M, Goto T, et al. Carnitine palmitoyl transferase II polymorphism is associated with multiple syndromes of acute encephalopathy with various infectious diseases. Brain Dev 2011;33:512-7.

[5] Shinohara M, Saitoh M, Nishizawa D, Ikeda K, Hirose S, Takanashi J, et al. ADORA2A polymorphism predisposes children to encephalopathy with febrile status epilepticus. Neurology 2013;80:1571-6.

[6] Takanashi J, Tada H, Terada H, Barkovich AJ. Excitotoxicity in acute encephalopathy with biphasic seizures and late reduced diffusion. AJNR Am J Neuroradiol 2009;30:132-5.

[7] Gandhi AK, Desai JV, Ghatge MS, di Salvo ML, Di Biase S, Danso-Danquah R, et al. Crystal structures of human pyridoxal kinase in complex with the neurotoxins, ginkgotoxin and theophylline: insights into pyridoxal kinase inhibition. PLoS One 2012;7:e40954.

[8] Mizuguchi M, Yamanouchi H, Ichiyama T, Shiomi M. Acute encephalopathy associated with influenza and other viral infections. Acta Neurol Scand Suppl 2007;186:45-56.

[9] Wilson MP, Plecko B, Mills PB, Clayton PT. Disorders affecting vitamin B6 metabolism. J Inherit Metab Dis 2019;42:629-46.

[10] Ishii C, Noda M, Oda A, Ishikawa R, Oba K, Narui K, et al. Trial administration of pyridoxal phosphate to 9 AEFCSE cases (in Japanese). J Jpn Soc Emerg Pediatr 2009;8:35-41.

[11] Fukui KO, Kubota M, Terashima H, Ishiguro A, Kashii H. Early administration of vitamins B1 and B6 and l-carnitine prevents a second attack of acute encephalopathy with biphasic seizures and late reduced diffusion: A case control study. Brain Dev 2019;41:618-24.

[12] Akiyama T, Akiyama M, Hayashi Y, Shibata T, Hanaoka Y, Toda S, et al. Measurement of pyridoxal 5'-phosphate, pyridoxal, and 4-pyridoxic acid in the cerebrospinal fluid of children. Clin Chim Acta 2017;466:1-5.

[13] Spector R, Johanson CE. Vitamin transport and homeostasis in mammalian brain: focus on Vitamins B and E. J Neurochem 2007;103:425-38.

[14] Sado T, Nakata S, Tsuno T, Sato M, Misawa Y, Yamauchi S, et al. Concentrations of various forms of vitamin B6 in ginkgo seed poisoning. Brain Dev 2019;41:292-5.

[15] Loohuis LM, Albersen M, de Jong S, Wu T, Luykx JJ, Jans JJM, et al. The alkaline phosphatase (ALPL) locus is associated with B6 vitamer levels in CSF and plasma. Genes (Basel) 2018;10:8.

[16] Ueland PM, Ulvik A, Rios-Avila L, Midttun O, Gregory JF. Direct and functional biomarkers of vitamin B6 status. Annu Rev Nutr 2015;35:33-70.

[17] Mills PB, Surtees RA, Champion MP, Beesley CE, Dalton N, Scambler PJ, et al. Neonatal epileptic encephalopathy caused by mutations in the PNPO gene encoding pyridox(am)ine 5'-phosphate oxidase. Hum Mol Genet 2005;14:1077-86.

Figure 1

Transport and metabolism of PLP and PL

GABA, gamma-aminobutyric acid; GAD, glutamate decarboxylase; PDXK, pyridoxal kinase; PL, pyridoxal; PLP, pyridoxal 5′-phosphate; TNSALP, tissue-nonspecific alkaline phosphatase

Figure 2

Results of PLP and PL assays in AESD and FS

Panels A, B, and C: PLP and PL concentrations and PLP-to-PL ratios vs. age. Dashed lines indicate the upper and lower limits of our reference values [12]. Panels D, E, and F: PLP and PL concentrations and PLP-to-PL ratios in AESD and FS. Solid lines indicate median values. AESD, acute encephalopathy with biphasic seizures and late reduced diffusion; FS, febrile seizure; PL, pyridoxal; PLP, pyridoxal 5′-phosphate

Table 1

Subject characteristics

Case ID	Group	Age	Sex	Causative organism	Duration of seizure	Day of CSF collection	CSF			MRI	CT	PCPC
							Total protein (mg/dL)	Cell count (/μL)	Glucose (mg/dL)			
1	AESD	5y 2m	F	Unknown	>1 h	0	32	<1	184	BTA		4
2	AESD	1y 0m	F	*E. coli*	>24 h (cluster)	1	23	1	63	N		2
3	AESD	0y 2m	M	Unknown	Cluster	0	53	3	63	BTA		1
4	AESD	0y 11m	F	Influenza A	44 min	0	22	<1	93	BTA		3
5	AESD	1y 3m	F	HHV-6	Cluster	0	22	1	133	BTA		1
6	FS	1y 10m	F	Unknown	40 min	0	9	<1	82		N	1
7	FS	1y 5m	M	Unknown	35 min	0	15	1	142		N	1
8	FS	1y 3m	F	Unknown	15 min	0	13	<1	88		N	1
9	FS	4y 11m	F	Unknown	2 h (cluster)	0	19	<1	97		N	1
10	FS	0y 9m	F	HHV-6	10 min	0	17	<1	99		N	1
11	FS	1y 7m	M	Adenovirus	5 min	2	16	<1	68	N		1
12	FS	1y 0m	F	HHV-6	25 min	0	18	<1	95	N		1
13	FS	1y 4m	M	Unknown	>3h (cluster)	0	9	1	84		N	1
14	FS	0y 8m	F	Unknown	Cluster	0	33	1	144	N		1
15	FS	2y 3m	F	Unknown	26 min (cluster)	1	13	<1	126	N		1
16	FS	1y 11m	F	Unknown	8 min	0	16	1	69			1
17	FS	1y 0m	F	Unknown	Cluster	0	12	3	84			1
18	FS	1y 11m	F	Unknown	Cluster	0	19	1	73			1
19	FS	1y 6m	F	Unknown	45 min	1	12	5	72	N		1
20	FS	1y 11m	M	Influenza A	14 min	0	16	<1	97		N	1
21	FS	1y 0m	M	Unknown	1.5 h	0	23	<1	90	N		1
22	FS	1y 5m	M	Unknown	1.5 h (cluster)	0	24	2	82	N		1

AESD, acute encephalopathy with biphasic seizures and late reduced diffusion; BTA, bright tree appearance; CSF, cerebrospinal fluid; CT, computed tomography; FS, febrile seizure; MRI, magnetic resonance imaging; N, normal; PCPC, pediatric cerebral performance category