Marked Elevation of Excitatory Amino Acids in Cerebrospinal Fluid Obtained From Patients With Rotavirus-Associated Encephalopathy

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Rotavirus is the most common cause of severe gastroenteritis in young children; however, its pathogenesis and immunity are not completely understood. Even less well recognized is rotavirus-induced central nervous system (CNS) involvement, which has been associated with seizure, encephalopathy and death, among others. To elucidate the host response to rotavirus infection, we retrospectively examined neurotransmitter amino acids in the cerebrospinal fluid (CSF) of 19 children with CNS involvement associated with rotavirus infection. Subjects were classified into two groups: those with encephalopathy followed by prolonged seizure (encephalopathy group) and those

who had experienced afebrile, brief cluster of seizures without encephalopathy (cluster group). The levels of glutamate, glycine, and taurine in the encephalopathy group were significantly higher than those in the cluster group. Increased levels of excitatory amino acids in the CSF may induce neurological disorders and be related to disorder severity. To the best of our knowledge, this is the first report regarding amino acids in the CSF obtained from patients with rotavirus-induced CNS involvement. *J. Clin. Lab. Anal.* 29:328–333, 2015. © 2014 Wiley Periodicals, Inc.

Key words: rotavirus infection; CNS; CSF; EAAs; IAAs

INTRODUCTION

Rotavirus infection is a frequent cause of severe gastroenteritis in children. Infection is usually localized in the intestine, but some studies have reported the extraintestinal involvement of central nervous system (CNS) complications associated with rotavirus infections (1–3). Children with rotavirus infection can suffer afebrile, brief cluster of seizures without encephalopathy (4, 5) or encephalopathy followed by prolonged seizures (6, 7).

To date, CNS involvement associated with rotavirus infection has not yet been extensively investigated. The aims of this study were to evaluate the concentrations of neurotransmitter amino acids in the cerebrospinal fluid (CSF) of 19 children with CNS involvement associated with rotavirus infection, and to establish whether these concentrations are related to the severity of encephalopathy and cluster convulsion.

MATERIALS AND METHODS

The diagnosis of encephalopathy was based on the following criteria: patients had altered consciousness or loss of consciousness after rotavirus infection. Patients with meningitis, myelitis, and febrile convulsions without prolonged unconsciousness were excluded (8). We enrolled 19 children admitted to our hospital between 1999 and 2009 in this study, and classified them into two groups of patients: those with encephalopathy followed by prolonged seizure (encephalopathy group, $n = 7$; 6 boys,

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TABLE 1. Clinical characteristics of patients

Case	Age	Sex	Clinical symptom	Seizures: no. of attacks duration	GCS	CSF: cell count pro, sug (mg/dl)	WBC (/μl)	CRP (mg/dl)	Outcome
(A) Clinical characteristics of 7 patients with encephalopathy following prolonged seizures (encephalopathy group)									
1	6Y9M	M	Vomiting, diarrhea Fever	GTCS 1 time 20 min	E2V3M2	Cell count 7/μl pro 90, sug 89	10,500	1.3	Healthy
2	1Y8M	M	Vomiting, diarrhea	GTCS 1 time 40 min	E1V1M2	Cell count 3/μl Not examined	9,800	1.0	Healthy
3	1Y3M	M	Vomiting, diarrhea Fever	GTCS 1 time 30 min	E1V2M2	Cell count 4/μl pro 22, sug 153	7,900	1.8	Healthy
4	3Y3M	F	Vomiting, diarrhea Fever	CPS 1 time 30 min	E2V3M2	Cell count 1/μl pro 13, sug 96	9,300	0.5	Healthy
5	1Y11M	M	Vomiting, diarrhea Fever, DIC	CPS 1 time 20 min	E1V1M1	Cell count 1/μl pro 15, sug 54	4,400	<0.3	Developmental Delay
6	1Y1M	M	Vomiting, diarrhea CPA, MOF	GTCS 1 time	E1V1M1	Cell count 3/μl Not examined	2,200	2.3	Died
7	8M	M	Vomiting, diarrhea DIC, RF	GTCS 1 time 30 min	E1V1M1	Cell count 2/μl pro 35, sug 56	19,900	<0.3	Developmental Delay
(B) Clinical characteristics of 12 patients with afebrile, brief cluster of seizures without encephalopathy (cluster group)									
1	1Y6M	F	Vomiting	GTCS 7 times 1 min	E4V5M6	Cell count 3/μl not examined	9,000	<0.3	Healthy
2	1Y6M	M	Vomiting	GTCS 4 times 1 min	E4V5M6	Cell count 3/μl not examined	8,000	<0.3	Healthy
3	2Y10M	M	Diarrhea	GTCS 1 time 2 min	E4V4M5	Cell count 3/μl pro 17, sug 65	4,500	<0.3	Healthy
4	1Y9M	F	Vomiting, diarrhea	GTCS 4 times 1 min to 3 min	E4V5M6	Cell count 4/μl pro 17, sug 57	10,200	<0.3	Healthy
5	1Y6M	M	Vomiting, diarrhea	GTCS 3 times 2 min to 4 min	E3V3M5	Cell count 5/μl pro 9, sug 84	11,000	1.3	Healthy
6	1Y11M	F	Vomiting, diarrhea	GTCS 2 times 10 sec to 20 sec	E4V4M6	Cell count 1/μl pro 11, sug 128	3,600	1.7	Healthy
7	2Y4M	F	Vomiting	GTCS 4 times 15 sec to 1 min	E4V4M5	Cell count 2/μl pro 12, sug 77	8,100	0.4	Healthy
8	1Y9M	F	Vomiting, diarrhea	GTCS 6 times 2 min to 3 min	E4V5M6	Cell count 11/μl pro 8, sug 70	10,000	<0.3	Healthy
9	1Y2M	F	Vomiting, diarrhea	GTCS 4 times 1 min to 3 min	E4V5M6	Cell count 1/μl pro 10, sug 63	7,300	0.4	Healthy
10	2Y1M	M	Vomiting, diarrhea	GTCS 3 times 2 min to 3 min	E4V4M5	Cell count 22/μl not examined	6,000	<0.3	Healthy
11	1Y10M	F	Vomiting, diarrhea	GTCS 3 times 1 min to 3 min	E4V5M6	Cell count 3/μl pro 10, sug 104	6,900	<0.3	Healthy
12	1Y4M	F	Vomiting, diarrhea	GTCS 3 times 1 min	E3V4M5	Cell count 17/μl pro 27, sug 54	8,000	<0.3	Healthy

CPA, cardiopulmonary arrest; MOF, multiple organ failure; DIC, disseminated intravascular coagulation; RF, renal failure; GTCS, generalized tonic-clonic seizures; CPS, clonic partial seizure; GCS, Glasgow coma scale, pro, protein, sug; sugar.

1 girl) and those with afebrile, a brief cluster of seizures without encephalopathy (cluster group, $n = 12$; 4 boys, 8 girls). The age was presented as the mean $\pm$ SD values, 28 ± 25 months (range, 8 to 81 months) in the encephalopathy group and 22 ± 6 months (range, 14 to 34 months) in the cluster group. All patients had been given a diagnosis of rotavirus-induced CNS involvement. All rectal swab specimens were positive for the rotavirus antigen using the latex agglutination test. CSF samples were collected from patients for exclusive diagnosis after the parents of the children gave written informed consent. Rectal swab specimens and CSF samples were obtained at the time of

admission. Patient profiles are shown in Table 1. We used 15 CSF samples from 15 patients (6 boys, 9 girls) with fever of unknown etiology, with pneumonia or urinary tract infection, but with no other abnormal findings as controls. The mean age was 22 ± 8 months (range, 10 to 36 months). It is difficult to perform CSF examination in healthy child as control because CSF examination is invasive. They had no CNS events and no patient showed abnormal findings on routine CSF examination. Therefore, we used them as controls.

All CSF samples were kept frozen at -80°C immediately after collection until final analysis. Analysis of amino

TABLE 2. Concentrations of Amino Acids in CSF

Amino acids	Encephalopathy group (nmol/ml) (<i>n</i> = 7)	Cluster group (<i>n</i> = 12)	Control (<i>n</i> = 15)
Age (months)	28 ± 25	22 ± 6	22 ± 8
Phosphoethanolamine	6.09 ± 2.72 ^a	2.45 ± 2.35	3.76 ± 2.37
Urea	5335.17 ± 4656.52	2562.72 ± 860.27	1970.55 ± 667.17
Threonine	36.67 ± 25.38 ^a	12.33 ± 2.27	22.56 ± 33.26
Serine	70.81 ± 66.05 ^a	23.72 ± 5.85	31.57 ± 11.96
Glutamine	329.71 ± 202.48	223.48 ± 61.24	325.51 ± 75.06
Alanine	111.34 ± 135.91 ^a	16.88 ± 2.82	28.67 ± 33.84
Valine	74.56 ± 92.14 ^a	16.63 ± 5.0	17.48 ± 11.77
Isoleucine	25.19 ± 31.48 ^a	6.0 ± 2.40	5.28 ± 3.83
Leucine	60.83 ± 76.97 ^a	11.94 ± 4.21	12.66 ± 5.61
Tyrosine	24.64 ± 19.25 ^a	7.66 ± 4.41	8.63 ± 6.73
Phenylalanine	73.81 ± 90.77 ^a	11.79 ± 6.52	21.42 ± 14.12
Histidine	26.2 ± 25.2 ^a	7.59 ± 3.06	6.46 ± 3.80
Ornithine	19.97 ± 25.96 ^a	3.95 ± 3.22	9.11 ± 12.89
Lysine	50.09 ± 52.85 ^a	13.03 ± 4.65	13.8 ± 13.60
Arginine	50.83 ± 59.03 ^a	12.62 ± 3.09	14.31 ± 11.68

Values are expressed as mean ± SD.

^a*P* < 0.05 Statistically significant when compared to the cluster group.

acids was performed using high-performance liquid chromatography (HPLC). In this method, amino acids which were isolated using a cation exchange resin and added with ninhydrin yielded Ruhemann purple. It was measured that corresponding UV-visible spectra had absorption maxima at 570 and 440 nm.

The differences between groups were analyzed using the Mann–Whitney *U*-test. A *P*-value less than 0.05 was considered to indicate a statistically significant difference. Statistical analysis was performed using Statcel software (OMS, Saitama, Japan).

RESULTS

Patients (Table 1): In the encephalopathy group, seizure occurred once and lasted for more than 20 min, with a depressed level of consciousness. Clinical symptoms other than seizures included vomiting, diarrhea, and fever. No patient had abnormal findings on routine CSF examination. Stool bacterial culture tests were all negative. Three patients (cases 5, 6, and 7) were severely ill and required intensive care due to disseminated intravenous coagulation, cardiopulmonary arrest, multiple organ failure (MOF), or renal failure. Developmental delay was noted in two patients (cases 5 and 7) and one patient (case 6) died. All patients except cases 5, 6, and 7 were discharged without any neurological sequelae.

In the cluster group, all patients except case 3 suffered 2 or more seizures. Clinical symptoms other than seizures included vomiting and diarrhea. No patient had fever during the seizures, although some had fever at other times

during the clinical course of the illness. No patient showed abnormal findings on routine CSF examination. Stool bacterial culture tests were all negative. All patients were discharged without any neurological sequelae.

Amino acids (Table 2, Fig. 1A and B): Concentrations of amino acids in the CSF obtained from patients with CNS involvement associated with rotavirus infection and in the CSF of controls are shown in Table 2. The level of the excitatory amino acid (EAA) glutamate in the encephalopathy group was significantly higher than those in the cluster and control groups (Fig. 1A). The levels of the inhibitory amino acids (IAAs) glycine and taurine in the encephalopathy group were also significantly higher than those in the cluster group (Fig. 1B).

Moreover, the levels of phosphoethanolamine, threonine, serine, alanine, tyrosine, phenylalanine, histidine, ornithine, lysine, valine, isoleucine, leucine, and arginine in the encephalopathy group were higher than those in the cluster group. Aspartate and γ -amino butyric acid (GABA) were not detected in any patients. CSF examination is invasive in children, so we did not measure any amino acid levels in the convalescent stage.

DISCUSSION

Rotavirus is the most common cause of severe gastroenteritis in young children; however, its pathogenesis and immunity are not completely understood. Less well recognized is the association of rotavirus-induced CNS involvement, which has been associated with seizure, encephalopathy, and death. Therefore, in this study, we set

Fig. 1. (A) The glutamate level in the encephalopathy group was significantly higher than those in the cluster and control groups. Each point represents the actual value of each patient. The black bar shows the mean of each group. The number in parentheses is the total number of patients in each group. $*P < 0.05$. (B) The glycine and taurine levels in the encephalopathy group were significantly higher than those in the cluster group. Each point represents the actual value of each patient. The black bar shows the mean of each group. The number in parentheses is the total number of patients in each group. $*P < 0.05$.

out to elucidate the host response to rotavirus infection by retrospectively examining amino acids in the CSF of 19 children with CNS involvement associated with rotavirus infection.

EAAAs are known as neurotransmitters of the CNS, and the most widely studied EAAAs are glutamate and aspartate. These EAAAs play a critical role in the excitotoxic responses of the CNS to pathological insults. Glutamate is widely distributed throughout the CNS and spinal cord, and these areas have higher concentrations than the cerebral cortex, hippocampus or cerebellum. Experimental studies in rodents and clinical studies in humans have shown that excessively high extracellular concentrations of ischemic insult-induced EAAAs in the CNS can be toxic to neurons (9). Gücüyener et al. (10) reported that the

concentrations of aspartate, glutamate, and taurine were significantly elevated in the CSF of asphyxiated infants. Levels of CSF EAAAs are increased in infants and children with severe traumatic brain injury (11) and encephalopathy (12). Tucci et al. (13) reported that CSF glutamate concentration was higher in children with bacterial meningitis. A prolonged increase in glutamate levels in the CSF can indicate poor clinical outcome in patients with bacterial meningitis, possibly because of the sustained neurotoxic effects of glutamate (14).

In the present study, the glutamate level in the encephalopathy group was significantly higher than that in the cluster or control group (Fig. 1A). All seven patients in the encephalopathy group had experienced prolonged seizures for about 20 min or more. On the other hand, in

the cluster group, patients had experienced several convulsions, but each time the duration was less than 2 min. The patients with rotavirus-associated encephalopathy were suspected to have severe ischemia.

Glutamate plays a central role in nitrogen metabolism and participates in multiple biochemical pathways. Released glutamate is taken up by the glia, where it is converted to glutamine, transported back to the presynaptic neuron, and reconverted to glutamate (15). It appears that this glutamate-glutamine cycle plays a role in neuron-glia communication in the synapse. An elevated glutamine/glutamate ratio in the CSF is found in several diseases such as schizophrenia (16) and influenza-associated encephalopathy (12). Impairment of the glutamate-glutamine cycle in the CSF may be implicated, and the glia may be activated by some viral factors and cytokines in the pathophysiology of influenza-associated encephalopathy. However, in the present study there was no statistically significant difference in the glutamine/glutamate ratio between the encephalopathy group and the cluster group (unpublished data). It has been reported that the influenza virus in the CNS could not be detected in influenza-associated encephalopathy; on the other hand, in some cases, rotavirus RNA of CSF was detected in subjects with rotavirus-associated seizures, including encephalopathy (17). Although we did not examine antirotavirus antibody titer because of the nonavailability of specimens, direct rotavirus RNA invasion in the CSF may be implicated in the pathophysiology of rotavirus-associated encephalopathy.

On the other hand, in the present study, there were convulsion accumulative type (encephalopathy group case 1, 2, 3, 4, and 7), Reye like syndrome (encephalopathy group case 5), hemorrhagic shock and encephalopathy syndrome (HSES) (encephalopathy group case 6) and no clinically mild encephalitis/encephalopathy with a reversible splenial lesion (MERS). This different frequency of acute encephalopathy syndromes in each virus encephalopathy may depend on difference of glutamate concentration in CSF.

In case 6 (encephalopathy group), the patient died due to MOF. The glutamate level of the patient was not elevated (7.6 nmol/ml). This suggests that an elevated amino acid level alone does not necessarily indicate clinical outcome.

Shen et al. (18) reported that the levels of CSF IAAs—glycine, GABA, and taurine—increased in patients with encephalitis. In addition to being the primary inhibitory neurotransmitter in the spinal cord and brain stem, glycine has also been shown to be a coagonist of the N-methyl-D-aspartate subtype of excitatory glutamate receptors (19). In the present study, aspartate and GABA were not detected in any case. The reason why we could not detect aspartate levels in CSF was unclear. However,

GABA is generally low in other type of samples such as blood plasma and urine in this HPLC method. Therefore it was suspected that this HPLC method might be not appropriate only for the detection of GABA in CSF.

However, as shown in Fig. 1B, glycine and taurine levels in the encephalopathy group were significantly higher than those in the cluster group. This suggests that glycine and taurine may be associated with severity of encephalopathy. In the acute phase, all seven patients in the encephalopathy group had experienced prolonged seizures for about 20 min or more. They were suspected to have severe ischemia at the time of admission. Therefore, we suspected that ischemic induced EAA in the CNS might work in the initial stage of infection, and IAAs might work in the second stage to depress excitation.

There were some limitations in this study. First, the number of patients in all groups was small. Second, subjects were enrolled in a single institution in a single area. Finally, subjects were ethnically homogeneous (East Asian), and therefore extrapolation of the results of the study to other countries may be limited.

However, to the best of our knowledge, this is the first report regarding amino acids in the CSF obtained from patients with rotavirus-induced CNS involvement. Further study is necessary to determine whether CSF amino acid levels have an important role in rotavirus-induced CNS involvement.

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ABBREVIATIONS

CNS	= central nervous system
CSF	= cerebrospinal fluid
EAA	= excitatory amino acids
HPLC	= high-performance liquid chromatography
IAA	= inhibitory amino acids
NMDA	= N-methyl-D-aspartate

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