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Amino acid concentrations in cerebrospinal fluid in children with acute lymphoblastic leukemia undergoing chemotherapy

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Abstract

Cerebrospinal fluid (CSF) amino acid concentrations were measured in 45 children with acute lymphoblastic leukemia (ALL). Central nervous system (CNS) disease was absent in 34 and present in 11 (Groups L and M, respectively) at diagnosis. Thirty-two otherwise healthy children with febrile convulsions were studied for comparison. Results from this study show that glutamine levels at Day 0 were significantly higher in patients than in controls. Patients in Group M had elevated glutamine levels compared to Group L. In comparison, at Day 14, concentrations of glutamine and asparagine decreased, while glutamic acid amounts increased significantly in Group L. Glutamine levels fell at Day 42 in Group M, which may have resulted from more intensive treatment. From this study we hypothesise that higher baseline glutamine levels are indicative of a greater risk for CNS leukemia. Large-scale prospective trials are required to confirm increased baseline CSF glutamine levels in ALL patients, to identify glutamine as a marker for CNS disease and to clarify underlying mechanisms regulating glutamine in ALL.

Keywords: Acute lymphoblastic leukemia; Acute lymphocytic leukemia; ALL; CNS leukemia; CSF amino acids; CNS prophylaxis

1. Introduction

During the past two decades, major advances in the treatment of childhood acute lymphoblastic leukemia (ALL) have increased event-free survival to greater than 70% [1–3]. The main reasons for this remarkable improvement are multidrug chemotherapy, central nervous system (CNS)-directed therapy, and better supportive care [4]. Since the introduction of CNS-directed treatment, where drug is administered intrathecally or at high intravenous doses for methotrexate, incidence of CNS leukemia relapse in childhood ALL has declined from >50% to 10% [2,3,5,6].

Changes in amino acid composition of the cerebrospinal fluid (CSF) have been noted in several disease states (e.g., bacterial meningitis [7], traumatic brain injury [8] and epilepsy [9]). Similarly, it is of interest to know if there are changes to the levels of various amino acids in the CSF of children with ALL and, if so, whether this relates to the presence of malignant cells in the CNS. Any changes in CSF amino acid composition during treatment are important as it may enable identification of possible markers for response to therapy in ALL children. In this report, we have investigated levels of amino acids during induction and preconsolidation therapy in CSF of children with ALL with and without CNS involvement.

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2. Patients and methods

2.1. Participant selection

Between August 1992 and July 2002, 159 patients at our hospital began treatment for ALL. From this group, 45 patients (<18 years) were diagnosed for the first time with ALL, and subsequently enrolled in this study. Patients were excluded if their initial lumbar puncture was traumatic or if they were referred from other hospitals for care without having had an initial CSF examination. Of the 45 participants in the study, 34 had no evidence of CNS leukemia and were designated Group L. The 11 patients who had CNS leukemia at initial diagnosis were designated Group M. Additionally, 32 patients (<18 years) who had lumbar puncture for evaluation of febrile convulsions with resulting normal CSF findings and no history of neurological or malignant disease were recruited and designated the control group. Informed consent was obtained from the parents of each child prior to enrollment in the study and approved by the Ethics Committee of the hospital.

2.2. Chemotherapy and cerebrospinal fluid sampling

Induction therapy for both patient groups consisted of prednisolone (60 mg/m^2/day to maximum 60 mg, tapering to zero from days 28 to 35), vincristine (1.5 mg/m^2/week on days 0, 7, 14, 21), L-asparaginase (5000 IU/m^2/day 3 times a week for the first 3 weeks) and epirubicin (20 mg/m^2/week on days 0 and 7 in both groups, and continued for days 14 and 21 in Group M). In the post-remission phase, patients received consolidation treatment with etoposide and cytarabine. For patients in Group L, CNS-directed therapy was administered by cranial irradiation (18 Gy) and triple chemotherapy with methotrexate, hydrocortisone and cytarabine *via* injection on days 0, 14, and 42. Patients in Group M received an intensified triple chemotherapy course on days 0, 7, 14, 21, and 35. If CSF samples on days 21 and 35 were normal, intrathecal treatment was continued on days 42, 49, 56 and every 4 weeks thereafter (e.g., weeks 12, 16, 20, 24, 28 and 32). A higher dose of cranial irradiation (24 Gy) was administered to Group M patients between weeks 35 and 39. For patients younger than 2 years of age with initial CNS involvement, the same periodic intrathecal therapy regimen was used until age 2 years before CNS irradiation was administered.

Remission maintenance therapy consisted of 6-mercaptopurine, methotrexate, dexamethasone, vincristine, epirubicine, cyclophosphamide and cytarabine for 18 months for children without initial CNS disease (Group L) and for 24 months for the children with initial CNS disease (Group M).

Lumbar punctures were performed for diagnostic and therapeutic purposes as indicated clinically for the control group and as dictated by the treatment protocol for ALL patients. The total volume of CSF sampled from each subject was limited to 5 ml unless an intrathecal medication was given simultaneously. In such instances, the volume of CSF removed was equal to the volume of medication given. The investigators who performed the CSF amino acid analysis were blinded to all patient information until completion of study.

For both patient groups (L and M), CSF samples were obtained from lumbar punctures performed for intrathecal drug administration on day 0 (L0, M0), day 14 during induction therapy (L14, M14) and day 42 at the start of consolidation therapy (L42, M42). For patients without initial CNS disease (Group L), each intrathecal drug dose for CNS-directed therapy consisted of methotrexate (8–12 mg based on patient age), cytarabine (16–24 mg), and hydrocortisone (8–12 mg). For patients with initial CNS involvement (Group M), each dose of intrathecal medication consisted of methotrexate (15 mg/m^2, maximum 15 mg), cytarabine (30 mg/m^2, maximum 30 mg) and hydrocortisone (15 mg/m^2, maximum 15 mg).

3. Cerebrospinal fluid analysis

Cell counts, protein levels, glucose levels, and cytology were determined immediately for all CSF samples collected. Approximately 2 ml CSF specimen from each patient was stored at −60 °C for 4–8 weeks until amino acid analysis was performed. Stored CSF specimens were thawed and deproteinised using an equal volume of 15% (W/V) sulfosalicylic acid (Sigma Chemical Co, St Louis, MO, USA). Standard amino acid solutions were diluted with LiS buffer (Beckman Instruments Inc, Palo Alto, CA, USA) to generate standard curves ranging from 1.25 to 2000 μM. It has been shown that the analyzer detection limit is 1 μM for each amino acid including asparagine; the imprecision (CVs) of standards were within 10% using the standard analysis method listed below. Samples (50 μl) were auto injected into a 10-cm cation ion exchange column integrated into a Beckman Model 6300 amino acid analyzer (Beckman). The solvent flow rate (2:1 water/ninhydrin) was maintained at a constant 0.5 ml/min. Column temperature was maintained at 33 °C. Absorbance was measured at 570 nm following post-column color development with ninhydrin RX (Beckman) at 131 °C. Beckman System Gold software was used for data acquisition and management.

4. Statistical analysis

The Kruskal–Wallis test was used throughout the study, followed by the Dunn procedure for comparisons of specific groups. The underlying distributions of

amino acid concentrations were found to be Gaussian and data is presented in tables as mean ± standard deviation (SD). A P value of less than 0.05 was considered statistically significant.

5. Results

All patients with leukemia (Groups L and M, $n = 45$) achieved remission after induction therapy. However, three patients without initial CNS disease (Group L, $n = 34$) had bone marrow relapse between 10 and 24 months after initial diagnosis while on maintenance treatment, and two patients with initial CNS disease (Group M, $n = 11$) developed refractory CNS leukemia and died of severe infection or CNS leukemia at 11 and 13 months respectively. Demographic characteristics of patients and controls are shown in Table 1. No significant differences were found among the three groups.

Table 1
Baseline characteristics of patients and controls

Characteristic	ALL (no CNS leukemia, Group L)	ALL (CNS leukemia, Group M)	Controls
Total (n)	34	11	32
Male/female (n/n)	21/13	7/4	18/14
Age at diagnosis (yr)			
Median	6.2	4.2	3.7
Range	0.5–14.8	0.3–11.9	0.2–5.8

A total of 129 CSF samples were collected from the 45 patients in Groups L and M, and 32 CSF samples were collected from control children. There were no significant differences in CSF amino acid concentrations between the control group and the two patient groups at diagnosis (L0 and M0, respectively), except for glutamine, which was significantly higher in both patient groups ($P < 0.05$) (Table 2). In addition, glutamine concentration among patients with CNS disease (Group M) was significantly higher than in children without CNS disease (Group L) ($P < 0.05$ for M0 compared with L0). For patients without initial CNS disease (Group L), glutamine concentration levels decreased to be comparable with those of the control group by Day 14 (L14) ($P < 0.05$) and were low at the end of the CNS-directed treatment period (Table 3). A similar trend, but not statistically significant, was observed for Group M from initial diagnosis (M0) into induction therapy (M14). However, the glutamine concentration during induction (M14) for these children was still significantly higher than the value obtained pre-consolidation (M42), showing that the decrease in glutamine was delayed in these children ($P < 0.05$) (Table 4).

Not surprisingly, CSF asparagine concentration decreased significantly from diagnosis (L0) into induction therapy (L14) ($P < 0.05$) among the patients without initial CNS disease (Group L). The decline in Group M, a much smaller population of children, was not statistically significant. There was a small but significant increase in CSF glutamic acid concentration in Group L from diagnosis (L0) into induction therapy (L14)

Table 2
CSF amino acid concentrations in control group and Groups L and M at time of diagnosis (L0, M0)

Amino acid	Concentration (μmol/l)		
	Control ($n = 32$)	L0 (no initial CNS disease) ($n = 34$)	M0 (initial CNS disease) ($n = 11$)
Taurine	13.4 ± 2.7	8.0 ± 4.3	10.4 ± 11.1
Threonine	45.9 ± 12.3	41.0 ± 15.9	30.2 ± 9.8
Serine	42.4 ± 15.1	38.3 ± 15.5	31.9 ± 8.8
Asparagine	19.0 ± 11.1	11.1 ± 6.7	7.9 ± 6.4
Glutamic acid	32.6 ± 6.9	26.8 ± 5.9	23.6 ± 8.5
Glutamine	623.6 ± 71.6	1061.0 ± 230.2*	1875.3 ± 285.8*,**
Glycine	9.5 ± 4.7	9.7 ± 4.9	13.4 ± 6.4
Alanine	37.8 ± 6.8	34.8 ± 7.8	46.1 ± 13.5
Valine	23.8 ± 4.5	17.9 ± 6.7	21.8 ± 7.2
Methionine	6.1 ± 3.1	6.0 ± 5.3	3.5 ± 0.4
Isoleucine	8.4 ± 2.6	5.9 ± 4.0	7.1 ± 4.2
Leucine	14.5 ± 3.7	13.1 ± 5.6	16.5 ± 7.0
Tyrosine	24.5 ± 8.9	13.0 ± 4.1	12.6 ± 3.3
Phenylalanine	18.0 ± 7.2	16.4 ± 6.3	18.6 ± 5.2
Tryptophan	20.4 ± 8.2	13.0 ± 5.2	12.8 ± 3.5
Ornithine	8.3 ± 4.7	7.6 ± 3.1	6.9 ± 3.6
Lysine	27.7 ± 8.0	26.9 ± 11.1	21.9 ± 6.2
Histidine	20.7 ± 7.5	21.4 ± 6.6	10.3 ± 3.7
Arginine	20.2 ± 6.3	21.5 ± 4.8	16.6 ± 6.3

Data expressed as mean ± SD (standard deviation).
* $P < 0.05$ Group L and Group M compared with control group.
** $P < 0.05$ between values at L0 and M0.

Table 3
CSF amino acid concentrations in Group L during prophylactic treatment (time points L0, L14, L42)

Amino acid	Concentration (μmol/l)		
	L0 (n = 34)	L14 (n = 33)	L42 (n = 31)
Taurine	8.0 ± 4.3	3.4 ± 0.7	5.5 ± 2.3
Threonine	41.0 ± 15.9	33.9 ± 13.0	33.1 ± 6.3
Serine	38.3 ± 15.5	29.0 ± 6.4	35.2 ± 4.7
Asparagine	11.1 ± 6.7	2.3 ± 1.2*	5.2 ± 2.7
Glutamic acid	26.8 ± 5.9	35.8 ± 7.3*	30.6 ± 5.8
Glutamine	1061.0 ± 230.2	558.4 ± 66.3*	705.6 ± 98.4
Glycine	9.7 ± 4.9	5.0 ± 3.0	7.0 ± 4.0
Alanine	34.8 ± 7.8	30.4 ± 5.7	42.8 ± 7.3
Valine	17.9 ± 6.7	13.6 ± 6.0	20.5 ± 4.2
Methionine	6.0 ± 5.3	2.7 ± 1.9	6.7 ± 5.1
Isoleucine	5.9 ± 4.0	4.6 ± 3.1	8.7 ± 2.9
Leucine	13.1 ± 5.6	11.4 ± 5.3	15.5 ± 3.3
Tyrosine	13.0 ± 4.1	8.4 ± 4.3	11.9 4.9
Phenylalanine	16.4 ± 6.3	16.3 ± 3.2	11.9 ± 4.0
Tryptophan	13.0 ± 5.2	14.4 ± 3.9	9.6 ± 5.2
Ornithine	7.6 ± 3.1	6.1 ± 4.1	9.5 ± 4.4
Lysine	26.9 ± 11.1	22.7 ± 4.6	32.2 ± 6.6
Histidine	21.4 ± 6.6	16.1 ± 5.1	18.8 ± 4.8
Arginine	21.5 ± 4.8	16.2 ± 4.6	21.1 ± 5.1

Data expressed as mean ± SD (standard deviation).
L0: at diagnosis; L14: at Day 14 (induction); L42: at Day 42 (pre-consolidation).
* $P < 0.05$ between L0 and L14.

($P < 0.05$) and a similar, but statistically insignificant, change in Group M (Tables 3 and 4). The only significant difference in CSF amino acid concentration between the two patient groups on day 14 was for glutamine (Tables 3 and 4). Glutamine was significantly higher in patients with initial CNS leukemia (M14) than in patients without initial CNS disease (L14). The Group M patients had both a higher baseline level and a lesser degree of reduction in concentration between baseline and day 14 (M0–M14).

Table 4
CSF amino acid concentrations in Group M during treatment (time points M0, M14, M42)

Amino acid	Concentration (μmol/l)		
	M0 (n = 11)	M14 (n = 11)	M42 (n = 9)
Taurine	10.4 ± 11.1	5.9 ± 2.1	3.8 ± 2.5
Threonine	30.2 ± 9.8	44.4 ± 9.4	37.3 ± 8.8
Serine	31.9 ± 8.8	37.2 ± 9.5	34.6 ± 8.0
Asparagine	7.9 ± 6.4	4.4 ± 2.3	5.7 ± 2.9
Glutamic acid	23.6 ± 8.5	29.4 ± 8.1	21.5 ± 6.7
Glutamine	1875.3 ± 285.8	1273.8 ± 147.9	843.2 ± 153.1*
Glycine	13.4 ± 6.4	7. 2 ± 2.6	8.1 ± 2.2
Alanine	46.1 ± 13.5	33.7 ± 4.2	52.0 ± 6.0
Valine	21.8 ± 7.2	17.9 ± 5.4	25.5 ± 10.1
Methionine	3.5 ± 0.4	2.8 ± 0.7	5.4 ± 0.6
Isoleucine	7.1 ± 4.2	3.8 ± 0.6	8.3 ± 1.1
Leucine	16.5 ± 7.0	13.0 ± 2.1	20.1 ± 6.4
Tyrosine	12.6 ± 3.3	11.7 ± 3.5	14.0 ± 2.6
Phenylalanine	18.6 ± 5.2	23.3 ± 6.7	24.6 ± 7.9
Tryptophan	12.8 ± 3.5	14.1 ± 5.2	13.9 ± 4.9
Ornithine	6.9 ± 3.6	11.4 ± 3.7	7.8 ± 2.0
Lysine	21.9 ± 6.2	14.7 ± 4.3	27.4 ± 3.3
Histidine	10.3 ± 3.7	5.5 ± 1.6	9.4 ± 2.4
Arginine	16.6 ± 6.3	16. 2 ± 3.5	20.1 ± 3.0

Data expressed as mean ± SD (standard deviation).
M0: at diagnosis; M14: at Day 14 (induction); M42: at Day 42 (pre-consolidation).
* $P < 0.05$ compared with M0 and M14.

6. Discussion

Information on amino acid concentrations in CSF of ALL patients and changes in these values during therapy are limited. Korinthenberg and colleagues [10] have conducted biochemical analysis on CSF of pediatric hematology patients treated with L-asparaginase. In that study, baseline amino acid composition for children with ALL was within normal range. However after induction therapy, CSF glutamine concentrations had significantly decreased ($P < 0.01$) while glutamic acid levels increased ($P < 0.05$). Neither asparagine or aspartic acid could be detected. The authors concluded that the amino acid changes were consistent with L-asparaginase activity in the CNS. Noting a correlation between elevated glutamine levels and electroencephalographic (EEG) abnormalities, they conducted additional research and found that a significant proportion of EEG abnormalities were present before chemotherapy was started. They concluded that the EEG changes reflected underlying disease rather than altered CSF composition due to chemotherapy [11]. Our results are different from those previously reported. Most strikingly, in the present study, glutamine concentrations at diagnosis were significantly higher in ALL patients than in the control children. In addition, patients with initial CNS involvement had significantly higher glutamine levels than patients without CNS involvement.

There are a number of possible reasons for this discrepancy:

(i) *Patient population.* Korinthenberg and colleagues' study group [10] was more diverse, including 17 children with acute myeloid leukemia (AML) and 14 with non-Hodgkin lymphoma (NHL), as well as the 97 patients with ALL. Also, the age distribution may have differed between the two studies. Heiblim and colleagues [12] found higher values for glutamine in newborn infants, and Gerrits and colleagues [13] reported higher levels in infants aged 3 days to 12 months than in children aged 3–18 years.

(ii) *Assay used.* The column chromatography method of Moore and Stein [14] used by Korinthenberg and colleagues was probably less sensitive than the newer method employed in this study.

(iii) *Analysis of data.* Korinthenberg and colleagues compared values at diagnosis with normal values reported in the literature [12] but did not statistically analyze the values obtained against those for the control group.

(iv) *Selection of control group.* In this study, data was compiled for ALL patients without subsequent CNS relapse after completion of treatment. However, due to small sample size (22 patients) the group could not be as control. Similar to this study, other researchers including Korinthenberg and colleagues [10] had also used control groups other than ALL patients without CNS relapse, including children with febrile seizures. In this respect, control group selection for this study appears to be comparable with those of others, although it is not possible to assess whether the ages of the children recruited might have been associated with some physiological (age-related) differences in amino acid CSF composition.

Allowing that our observation of elevated glutamine concentration in the CSF of ALL patients, and particularly in those with CNS involvement, can be verified, what is the significance of this finding? There is no known mechanism whereby lymphoid cells produce and release glutamine into the milieu. On the other hand, glutamine is an essential component of lymphocyte cell division in culture and probably has a similar role *in vivo* [15]. It has been shown that, at least in neurosurgery patients, glutamine enters the CSF from brain tissue [16]. It may be that the brain is stimulated (perhaps by some substance produced by lymphoblasts outside the CNS) to overproduce glutamine, which may then enable the survival of lymphoid cells within the CNS. Obviously, much more work needs to be done to confirm or reject this hypothesis. If it is confirmed, a high glutamine value in the CSF of ALL patients without current CNS involvement might serve as an indicator of a greater propensity for CNS disease and the need for more intensive treatment.

Over the course of chemotherapy, we found a clear pattern of change in glutamine concentration. In children without initial CNS disease (Group L), glutamine decreased to control levels by Day 14 of chemotherapy and remained in that range. In the children with CNS disease (Group M), who had a significantly higher average baseline glutamine concentration, there was also a drop by Day 14, but it was not statistically significant. A significant decrease in glutamine occurred between Day 14 and Day 42 ($P < 0.05$). This pattern of concentration reduction during induction matches the finding of Korinthenberg and colleagues [10]. In addition to changes in glutamine concentration with chemotherapy, we found significant change in two other amino acids: in Group L, asparagine decreased significantly by Day 14 while glutamic acid had a small but significant increase. Similar changes were observed in Korinthenberg's research, with asparagine becoming not detectable and glutamic acid increasing in concentration during induction. In their case, the trend was observed only in children receiving L-asparaginase and attributed changes in amino acid levels to L-asparaginase activity in the CNS [10]. In our study, a similar trend was seen in children with initial CNS disease (Group M), but it did not reach statistical significance. The changes from day 0 to day 14 in both groups are best explained as the action of asparaginase. Asparaginase not only acts to break down asparagine but also deaminates glutamine to glutamate [17]. With the cessation of asparaginase treatment on day 21 in both groups, asparagine levels began to rise. In Group L, glutamine also began rising, while in Group M it continued to fall. This was probably due to the more intensive chemotherapy administered in the latter group. The fact that glutamic acid also fell shows that the decline in glutamine was not due to deamination to glutamate.

In this study, data were not available to do a subgroup analysis on the two patients with initial CNS disease (Group M, $n = 11$) who developed refractory CNS leukemia and died of severe infection or CNS leukemia. It would have been interesting to see if their glutamine levels rose at some point during treatment and/or remained elevated as unsuccessful treatments were given for their refractory CNS disease. Certainly, the capacity to follow the CSF composition of children over the longest possible time course will be very important in future study designs.

In conclusion, this study has perhaps raised more questions than it has supplied answers. Clearly, a large-scale prospective study in which patients can be better stratified and results analysed according to age, initial WBC count, and treatment outcome is required. Fundamental studies are also called for to clarify details of the sources and fates of relevant amino acids in the CSF, including during therapy with various agents. If the observations of this study, and the resulting hypotheses, can be verified, they may force a fundamental rethinking of the natural history of ALL and its treatment.

Conflict of interest statement

None declared.

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