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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