Asparaginase in Combination Chemotherapy for Remission Induction of Childhood Acute Lymphocytic Leukemia

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SUMMARY

Forty-six children with acute lymphocytic leukemia in relapse were divided randomly into two groups and treated with combinations of asparaginase (L-asparagine amidohydrolase, EC 3.5.1.1), cytosine arabinoside, and 6-azauridine. The objectives were to improve the 41% incidence of remission induction obtained earlier at this hospital with asparaginase alone and to compare the efficacy and toxicity of combining cytosine arabinoside versus cytosine arabinoside plus 6-azauridine with asparaginase. The first group, 24 children, received 10,000 i.u. of asparaginase per sq m and 300 mg of cytosine arabinoside per sq m on Days 1 and 8 (Schedule A). The second group, 22 children, received identical doses of these two agents plus 15 g of 6-azauridine per sq m on Days 1 and 8 (Schedule B). Complete remission was induced in 15 of the patients on Schedule A and in 13 of those on Schedule B. The median duration of response for the first group was 48 days (range, 14 to > 365); for the second group it was 52 days (range 9 to 177). When compared retrospectively with asparaginase alone, asparaginase combined with cytosine arabinoside increased the incidence but not the duration of remission induction. As determined by simultaneous comparison, there was no advantage in the present study from adding 6-azauridine to the asparaginase-cytosine arabinoside combination.

Thymidine incorporation into DNA by leukemic lymphoblasts was severely inhibited after asparaginase and cytosine arabinoside administration, and remained on the average more than 90% inhibited for over 24 hr.

INTRODUCTION

Asparaginase (L-asparagine amidohydrolase, EC 3.5.1.1) successfully reinduces remission in patients with acute lymphocytic leukemia in relapse (6, 13, 15, 20, 35, 38). Remission frequency is 40 to 50%, and there is no cross-resistance with other oncolyctic drugs (10, 34), 2 properties that favor its continued use in leukemia therapy. Factors that limit the usefulness of asparaginase are its antigenicity (13, 15, 21, 24), the short duration of remission (29, 30), and the invariable appearance of liver dysfunction (3, 11, 13, 21, 28).

The response of childhood acute leukemia to cytosine arabinoside has been disappointing, with a remission incidence of 10% or less (17, 37). The remissions are generally brief and are accompanied by severe bone marrow depression. This poor response has been improved by combining cytosine arabinoside with asparaginase (8, 18, 23, 26). When administered sequentially, cytosine arabinoside first, the 2 drugs induced remission in 8 or 9 patients with acute lymphocytic leukemia (18) and cured mice inoculated with EARAD 1, a transplantable acute lymphocytic leukemia (2). We have presented evidence that concurrent use of cytosine arabinoside and asparaginase increases the incidence of remission of acute lymphocytic leukemia (26). Others have also reported the effectiveness of simultaneous administration of these agents (8, 23).

Although 6-azauridine reduced the leukocyte count of patients with acute leukemia, the drug failed to induce remission (14). When combined with 6-mercaptopurine and vincristine, however, 6-azauridine appeared to increase the incidence of remission in patients with acute myelocytic leukemia (36).

In attempts to increase the 41% incidence of remission reinduction obtained with asparaginase at this hospital (26, 27, 29, 30), we combined the enzyme with cytosine arabinoside or with cytosine arabinoside and 6-azauridine in a comparative study of remission induction of acute lymphocytic leukemia in relapse.

MATERIALS AND METHODS

Patients

Forty-six children in relapse with acute lymphocytic leukemia that was resistant to conventional induction therapy were admitted to this study. Patients were assigned by random card technique to 1 of 2 treatment schedules.

Dosage Schedules and Procedure of Drug Administration

Schedule A. Twenty-four patients ranging in age from 2 to 17 years received asparaginase and cytosine arabinoside. The median time from diagnosis to beginning of this treatment schedule was 514 days with a range of 21 to 2,195 days. They received asparaginase, 10,000 i.u./sq m, simul-

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SCHEDULE A.

Twenty-two patients, age 1 to 17 years, received asparaginase, cytosine arabinoside, and 6-azauridine. Median duration of prior disease was 284 days with a range from 31 to 1388 days. The doses of asparaginase and cytosine arabinoside were the same as for Schedule A; 6-azauridine was administered at a dosage of 15 g/sq m. The 3 drugs were given simultaneously on Days 1 and 8.

Amorphous Escherichia coli asparaginase (MSD C-7632) with a specific activity of 363 i.u./mg of protein was supplied by the Cancer Therapy Evaluation Branch of the National Cancer Institute. Immediately before administration, 5 ml of water were added to 10,000 i.u. of asparaginase. The calculated dose was further diluted with 5
dextrose to a total volume of 12 ml and was administered i.v. over a 5-min period.

Cytosine arabinoside (Cytosar, The Upjohn Co., Kalamazoo, Mich.) was supplied by the Cancer Therapy Evaluation Branch of the National Cancer Institute in vials containing 500 mg of dry powder. The drug was dissolved in the furnished diluent (bacteriostatic water for injection with benzyl alcohol, 0.9% w/v) to a concentration of 50 mg/ml and was administered by direct i.v. injection after asparaginase.

6-Azauridine, formulated by the hospital pharmacy, was administered by intravenous infusion over a 1-hr period. This drug was given after asparaginase and cytosine arabinoside.

Evaluation of Patients

Hemograms, coagulation studies, and tests of renal and hepatic function were made before treatment and at least twice weekly thereafter. Bone marrow was aspirated from all patients before and after treatment, and in 29 of the 46 patients marrow was also aspirated before the second treatment. Blood or blood components, antibiotics, and i.v. fluids were given as required.

Criteria of Response

Marrow response was evaluated by the following criteria: (a) complete remission marrow (M-1): 5% or fewer blast cells and 40% or fewer lymphocytes and blast cells; (b) partial remission marrow (M-2): 5 to 25% blast cells or more than 70% lymphocytes plus blast cells; (c) no response (M-3): more than 25% blast cells or more than 70% lymphocytes plus blast cells.

Metabolic Studies

Blood specimens were taken just before the drugs were administered and at regular intervals thereafter. Two to 5 ml of blood were collected with 12 mg disodium EDTA, and each volume was diluted with 20 or 40 volumes of Eagle’s minimal essential medium containing glutamine, 10 times the usual phosphate content, no CaCl₂, and 10% fetal calf serum. If the leukocyte count exceeded 100,000 cells/cu mm, 40 volumes of medium were used for dilution to prevent later clogging of the filters. The diluted blood was kept at room temperature for the shortest possible time, during which the cells were kept in suspension with a slowly rotating magnetic stirring bar.

Two ml of diluted blood were added to a 15- x 100-mm tube, and 0.5 μCi in 25 μl of tritium-labeled precursor was added on a carefully timed schedule that permitted filtration at the end of incubation. The cells were incubated with 1.25 μM thymidine-methyl-³H or uridine-5-³H or 12.5 μM leucine-4, 5-³H. At the end of a 1-hr incubation at 37°, 2 ml of 0.9% NaCl solution and 1 ml saponin (0.5 g/liter of 0.9% NaCl solution) preheated to 37° were added to each tube. Ten sec after the addition of saponin the contents were washed from the tube with prewarmed 0.9% NaCl solution and collected on a Whatman GF/A filter. The filters were washed 4 times with approximately 5 ml of 37° 0.9% NaCl solution and twice with cold 5% trichloroacetic acid. The filter with its contents was placed in 10 ml of liquid scintillation counting medium, and, after a lapse of 10 min or more, the contents of the vial were mixed with the aid of a Vortex mixer. The liquid scintillation medium was prepared by the Amersham/Searle recipe: 12 g of 2, 5-bis[(5-tert-butylbenzoxazolyl)]thiophene, 204 g of naphthalene, 1800 ml of toluene, and 1200 ml of ethylene glycol monomethyl ether.

This technique avoided the preliminary preparation required for studies with the isolated white blood cell fraction (33). Saponin treatment selectively lyses red blood cells and should be initiated within 10 sec of filtration. If the preparation is not immediately collected on a filter, protein coagulates and plugs the filter. To avoid possible microbial contamination, sterile 0.9% NaCl solution and saponin, frozen immediately after preparation, were used only on the day they were opened or thawed.

RESULTS

Therapeutic Effects. Fifteen of 24 patients receiving asparaginase and cytosine arabinoside (Schedule A) responded with complete remission marrows. An additional patient had a partial remission marrow after 2 weeks of treatment. The median duration of response for patients who received 2 agents was 48 days; the range was from 14 to 365 days.

Thirteen of 22 patients receiving asparaginase, cytosine arabinoside, and 6-azauridine (Schedule B) achieved complete marrow remission. The median duration of remission was 52 days with a range of 9 to 177 days.

Drugs singly or in combination for maintenance of remission included methotrexate, mercaptopurine, cyclophosphamide, and cytosine arabinoside. All patients, with 2 exceptions, had been treated earlier with the 1st 3 agents; none had received cytosine arabinoside. There was no correlation between duration of remission and the selected maintenance therapy. The 2 patients who had not received prior maintenance therapy were placed on methotrexate, mercaptopurine, and cyclophosphamide continuation therapy.
Bone marrow cellularity was graded for 29 patients (16 on Schedule A, 13 on Schedule B) whose marrow was aspirated 1 week after the 1st treatment. Eighteen smears were hypocellular; 11 were normal. Two weeks after the start of therapy, 22 patients had normal marrow cellularity and 21 had hypocellular or dilute marrow specimens. Whether a patient was on Schedule A or B had no apparent effect on marrow cellularity. Organomegaly decreased in all patients attaining marrow remission.

**Adverse Reactions.** Nausea and vomiting occurred in 23 of the 24 patients receiving asparaginase and cytosine arabinoside (Table 1). Thirteen of 21 evaluable patients on Schedule B had a significantly lower incidence of nausea and vomiting, \( p < 0.01 \). Fever occurred infrequently after drug administration by either schedule, and anaphylactoid reactions were not encountered. Anorexia and weight loss were more frequent in patients who received 2 agents.

Frequency of hepatic dysfunction was the same in both treatment groups (Table 1). Most patients developed significant elevations of plasma ammonia on the day following drug administration; these levels returned to normal range within 4 days. Many patients developed significantly increased levels of serum glutamic-oxaloacetic transaminase and decreased serum cholesterol and albumin levels. An elevation of serum bilirubin was associated with acute hepatitis in 1 patient with *Pseudomonas* septicemia. Elevations of serum amylase and serum glucose were also encountered in 1 patient with acute hepatitis.

Azotemia and hyperuricemia occurred in more than one-half of the patients on each schedule (Table 1), probably because of increased destruction of leukemic cells and impairment of renal function. Levels returned to normal in 3 to 4 days.

Lethargy was occasionally noted within 24 hr after the 1st treatment and did not persist beyond that period. Patterns on posttreatment electroencephalograms, obtained 24 hr following drug administration, showed slight diffuse slowing for 3 of 19 patients receiving asparaginase plus cytosine arabinoside and for 5 of 16 patients receiving asparaginase, cytosine arabinoside, and 6-azauridine.

The most frequently encountered coagulation abnormality in both treatment groups was prolongation of partial thromboplastin time (Table 1). Abnormal decreases in serum fibrinogen were infrequent, and bleeding was not associated with changes of coagulation factors.

Two patients on Schedule A died during the study period after asparaginase and cytosine arabinoside administration; 1 patient died with *Pseudomonas* septicemia and 1 with varicella pneumonia. One patient on Schedule B died with *E. coli* sepsis after perforation of the cecum.

Intercurrent diseases and requirements for blood or blood components were greater for Group A patients. How-

### Table 1

<table>
<thead>
<tr>
<th>Toxicity</th>
<th>Criteria</th>
<th>Incidence after treatment with:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Asp + ara-C</td>
</tr>
<tr>
<td>Nausea, vomiting</td>
<td></td>
<td>23/24</td>
</tr>
<tr>
<td>Fever</td>
<td></td>
<td>2/24</td>
</tr>
<tr>
<td>Anaphylactoid reaction</td>
<td></td>
<td>0/24</td>
</tr>
<tr>
<td>Abnormal liver function</td>
<td>Ammonia &gt; 48 mg/100 ml</td>
<td>23/23</td>
</tr>
<tr>
<td></td>
<td>SGOT &gt; 40 units/ml (Karmen)</td>
<td>10/22</td>
</tr>
<tr>
<td></td>
<td>Alkaline phosphatase &gt; 12 units/ml (Bodansky)</td>
<td>4/21</td>
</tr>
<tr>
<td></td>
<td>BSP retention &gt; 5% retension</td>
<td>4/15</td>
</tr>
<tr>
<td></td>
<td>Bilirubin, total &gt; 1 mg/100 ml</td>
<td>7/21</td>
</tr>
<tr>
<td></td>
<td>Albumin &gt; 10% decrease</td>
<td>16/23</td>
</tr>
<tr>
<td></td>
<td>Cholesterol &gt; 25% decrease</td>
<td>16/22</td>
</tr>
<tr>
<td>Abnormal kidney function</td>
<td>Blood urea nitrogen &gt; 20 mg/100 ml</td>
<td>14/21</td>
</tr>
<tr>
<td></td>
<td>Uric acid &gt; 5.9 mg/100 ml</td>
<td>17/23</td>
</tr>
<tr>
<td>Abnormal pancreatic function</td>
<td>Hyperamylasemia &gt; 160 units/100 ml (Caraway)</td>
<td>1/23</td>
</tr>
<tr>
<td></td>
<td>Hyperglycemia, Fasting blood glucose &lt; 80 mg/100 ml</td>
<td>1/23</td>
</tr>
<tr>
<td>Central nervous system dysfunction</td>
<td>Depression, lethargy</td>
<td>5/24</td>
</tr>
<tr>
<td>Abnormal coagulation tests</td>
<td>Fibrinogen &gt; 25% decrease</td>
<td>4/23</td>
</tr>
<tr>
<td></td>
<td>Partial thromboplastin time &gt; 35 sec</td>
<td>14/23</td>
</tr>
<tr>
<td></td>
<td>Prothrombin time &gt; 13 sec</td>
<td>10/23</td>
</tr>
<tr>
<td></td>
<td>Wt loss &gt; 5% pretreatment wt</td>
<td>13/24</td>
</tr>
<tr>
<td>Deaths during study</td>
<td></td>
<td>2/24</td>
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</table>

- The abbreviations used are: Asp, asparaginase; ara-C, cytosine arabinoside; 6-azaU, 6-azauridine; SGOT, serum glutamic-oxaloacetic transaminase; BSP, Bromsulphalein.
- Data obtained from Refs. 27, 29, and 30.
- Denominators in all fractions represent the number of patients for whom observations were made.
ever, these patients had a longer median duration of leukemia prior to the study. Two patients in Group A had subarachnoid hemorrhages, and 1 each had *Pseudomonas* cellulitis and septicemia, pneumonia, impetigo, Asian influenza, oral candidiasis, and fever of undetermined origin. In Group B, 2 patients had perioral herpes simplex infection and upper respiratory infection. Patients in Group A received a total of 34 units of blood and 134 units of platelets while Group B patients required 21 units of blood and 54 units of platelets.

**Cellular Pharmacology Studies.** The effect of Schedule A on thymidine incorporation into DNA, uridine incorporation into nucleic acids, and leucine incorporation into protein by leukocytes was monitored for 6 patients (Table 2). With the exception of 1 patient lacking blast cells in the peripheral blood, the white blood cell count of these 6 ranged from 9,800 to 175,000 cells/cu mm and contained from 40 to 80% blast cells. The white blood cell counts of these 5 patients dropped a minimum of 40% within 24 hr after the 1st treatment and ranged between 6 and 60% of the initial count.

Within 1 hr after injection, the combination of cytosine arabinoside and asparaginase almost completely inhibited thymidine incorporation into DNA of leukemic cells. By 24 hr the mean rate of thymidine incorporation was 9% of the pretreatment rate. Pretreatment incorporation ranged from 0.41 to 6.36 pmoles/hr/million leukocytes with a mean of 2.69 ± 2.68. This incorporation, as previously observed, did not correlate with the white blood count or percentage of blast cells in the population (33).

The rate of uridine incorporation into the nucleic acids, primarily RNA, was less sensitive to these 2 drugs than was the rate of thymidine incorporation. Sixty % of the original rate of incorporation remained after 1 hr, and the lowest mean rate was observed 24 hr after cytosine arabinoside and asparaginase administration. The pretreatment rate of uridine incorporation ranged from 0.18 to 3.91 pmoles/hr/million leukocytes with a mean value of 1.55 ± 1.62.

The rate of leucine incorporation into protein dropped soon after cytosine arabinoside and asparaginase administration and remained at this level 24 hr after drug administration. The initial rate of leucine incorporation ranged from 31.8 to 170 pmoles/hr/million cells with a mean value of 74.6 ± 57.5.

**DISCUSSION**

The present treatment schedule with asparaginase, cytosine arabinoside, and 6-azauridine was selected from our information on response to the individual agents. The administration of 10,000 i.u./sq m doses of asparaginase 1 week apart was equivalent to other dosage schedules for remission reinduction (27). To permit maximum combined action by the 3 drugs, we administered cytosine arabinoside and 6-azauridine immediately after asparaginase injection.

In addition to the oncolytic activity of each of the agents used in this study, other pharmacological characteristics were considered to favor their use in combination therapy.

### Table 2

*In vitro precursor incorporation by lymphoblasts from patients treated with asparaginase and cytosine arabinoside*

<table>
<thead>
<tr>
<th>Patient</th>
<th>Time (hr)</th>
<th>Leukocytes 10⁹ (cells/cu mm)</th>
<th>Lymphoblasts (%)</th>
<th>Incorporation of precursor (pmoles/hr/million cells)</th>
<th>Clinical response</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Thymidine</td>
<td>Uridine</td>
</tr>
<tr>
<td>R. K.</td>
<td>0</td>
<td>120</td>
<td>54</td>
<td>1.95</td>
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<tr>
<td></td>
<td>3</td>
<td>151</td>
<td></td>
<td>T⁰</td>
<td>0.22</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>52</td>
<td></td>
<td>T</td>
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<tr>
<td>C. R.</td>
<td>0</td>
<td>9.8</td>
<td>39</td>
<td>0.42</td>
<td>0.18</td>
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<tr>
<td></td>
<td>4</td>
<td>16.9</td>
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<tr>
<td></td>
<td>24</td>
<td>5.9</td>
<td>47</td>
<td>T</td>
<td>T</td>
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<tr>
<td>M. C.</td>
<td>0</td>
<td>175</td>
<td>79</td>
<td>6.36</td>
<td>3.91</td>
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<tr>
<td></td>
<td>1</td>
<td>200</td>
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<td>0.06</td>
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<td></td>
<td>4</td>
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<td>0.03</td>
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<td>22</td>
<td>10.5</td>
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<tr>
<td>E. R.</td>
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<td>0.12</td>
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<tr>
<td>M. C.</td>
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<td>143</td>
<td>70</td>
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<tr>
<td></td>
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<td>4</td>
<td></td>
<td></td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td></td>
<td>22</td>
<td>54</td>
<td>22</td>
<td>0.11</td>
<td></td>
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<tr>
<td>M. H.</td>
<td>0</td>
<td>78.5</td>
<td>74</td>
<td>5.80</td>
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<tr>
<td></td>
<td>2</td>
<td>83.5</td>
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<td>0.23</td>
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<td></td>
<td>25</td>
<td>10.1</td>
<td>81</td>
<td>0.19</td>
<td>1.11</td>
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<tr>
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<td>49</td>
<td>4.1</td>
<td>19</td>
<td>1.04</td>
<td>0.61</td>
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</table>

* T, less than 1% of the pretreatment incorporation rate.
The immunosuppressive action of cytosine arabinoside (19) could complement the antitumor activity of asparaginase (5, 22, 25) and possibly reduce immunological response to the enzyme. The S-phase specificity (7) and rapid action (32) and disappearance of cytosine arabinoside from the blood contrasts with the activity of asparaginase, which disappears slowly from the blood and presumably maintains an asparagine deficiency for 24 hr or longer (4, 16).

The oncolytic action of 6-azauridine has been attributed to an inhibition of de novo pyrimidine biosynthesis (12). This effect is thought to reduce the deoxycytidine nucleotide pools and consequently to reduce deoxycytidine interference with cytosine arabinoside. In our preliminary studies, 2 patients with acute myelocytic leukemia were treated by a 4- or 6-hr infusion of 15 g of 6-azauridine per sq m. A 40 to 55% inhibition of in vitro thymidine incorporation by the isolated leukocytes was observed during the period of drug infusion. Recovery of the pretreatment rate for thymidine incorporation was incomplete for 1 patient at 24 hr. At the extracellular thymidine concentration the rate-limiting reaction for thymidine incorporation was saturated and, on the basis of the proposed mechanism for drug action, the inhibition is presumed to have resulted from a decrease in the availability of dCTP.

The remission reinduction incidence of acute lymphocytic leukemia was apparently increased by combining asparaginase with cytosine arabinoside. Fifteen of 24 patients treated with Schedule A achieved complete remission. The administration of 6-azauridine with these 2 drugs did not increase the incidence of remission. Thirteen of 22 patients achieved complete remission with 3-drug treatment. In our previous studies, various asparaginase dosages induced complete remission for 28 of 68 patients (41%) (27, 29, 30). The incidence of remission induction for the 2 groups in this study was 61% (28 of 46 patients). By χ² analysis of the present pooled data and our previous experience with asparaginase alone, a statistically significant increased incidence of remission was demonstrated for multiple-drug therapy (p < 0.025).

The course of treatment was short, and the response was evaluable in 2 weeks. Even patients not achieving complete marrow remission experienced a pronounced decrease in white blood cell count and in percentage of blast cells in the marrow and blood. Remission duration was brief, however, and combining cytosine arabinoside with asparaginase for remission induction did not increase the duration of remissions beyond that achieved with asparaginase alone in past experience.

Although 3 patients died during the study, none of the data suggested drug intoxication as the cause of death. Except for variations in nausea, vomiting, and weight loss, there were no significant differences in toxicity between the 2 schedules. The lower incidence of nausea and vomiting among patients on Schedule B presumably contributed to better maintenance of body weight in this group. Also, the incidence of changes in coagulation factors was lower for multiple therapy than for asparaginase alone in past studies.

The pronounced inhibition of in vitro thymidine incorporation into the peripheral white blood cells of 6 of the patients for 24 hr indicates that DNA synthesis was inhibited without repeated doses or prolonged infusion of the agents. There was no correlation between the marrow responses and thymidine incorporation by patient’s peripheral white blood cells. This pronounced effect on thymidine incorporation contrasts with the poor inhibition of uridine and leucine incorporation at a time when the blood leukocyte population was decreasing and changing (Table 2). In these studies, a correlation was not observed between the changes in leukocyte count, percentage of lymphoblasts, thymidine incorporation for the 1st 24 hr and the eventual clinical evaluation of marrow status. The marked decrease in lymphoblasts in the blood and marrow of all patients and the pronounced inhibition of thymidine incorporation by lymphoblasts after drug administration again poses the question of assaying pharmacological response by clinical criteria for remission when large numbers of lymphoblasts are presumed to remain after induction of remission (9, 31).

Preclinical studies with these 3 drugs indicated a combined action by cytosine arabinoside and asparaginase that resulted in a synergistic therapeutic response against L1210 and L5178Y murine transplantable tumors (1). The addition of 6-azauridine to treatment with cytosine arabinoside plus asparaginase made response much more dependent on cytosine arabinoside dosage in mice and reduced the effectiveness of the 2-drug combination. In the present study, 6-azauridine made no apparent contribution to therapeutic effect. The low weekly dosage of 6-azauridine was equivalent to the daily dosage used in earlier studies as a 6-hr infusion for 10 consecutive days (36). The present sequence of drug administration and the schedule of 6-azauridine administration may have reduced its oncolytic effectiveness. However, in the preclinical studies 6-azauridine interfered with response to cytosine arabinoside plus asparaginase suggesting the need for caution in future studies of the 3-drug combination.

The present treatment schedule with asparaginase plus cytosine arabinoside appears to offer advantages over treatment with either drug alone.

REFERENCES


Asparaginase in Combination Chemotherapy


Asparaginase in Combination Chemotherapy for Remission Induction of Childhood Acute Lymphocytic Leukemia


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