Biochemical and Pharmacological Studies with Asparaginase in Man

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SUMMARY

The biochemical and pharmacological effects of Escherichia coli asparaginase were studied in 45 patients with leukemia and solid tumors. The drug was given in three different schedules: as a single dose, daily, or weekly. After i.v. injection, the peak activity obtained was dose related; the initial clearance of the enzyme from plasma followed first-order kinetics, and a half-life was about 14 to 22 hr. Daily administration of the enzyme caused cumulation in serum. Enzyme activity was detectable 13 to 22 days after single injections. Plasma asparagine and aspartic acid levels in leukemia were compared with levels in 20 normal individuals. Three of 10 patients with acute lymphocytic leukemia had marked aberration in their amino acid levels. Patients with acute myelocytic leukemia as a group had lowered levels of asparagine. After enzyme administration, plasma asparagine fell precipitously to nearly unmeasurable levels. Asparagine reappeared in plasma 23 to 33 days after single injections. Even 0.2 i.u./kg as a skin test dose produced a substantial fall of plasma asparagine. Asparaginase produced multiple manifestations of toxicity involving brain, liver, pancreas, kidney, fingernail, and hypersensitivity reactions. Asparaginase was given as a “rescue” infusion in three patients for acute brain dysfunction with benefit. Ten of 24 patients with acute lymphocytic leukemia and 1 of 12 patients with acute myelocytic leukemia were induced to bone marrow rating 1 marrow remission, 2 after single injections. It was difficult to correlate response in vivo and a requirement of the leukemic cells for asparagine in vitro.

INTRODUCTION

Regression of transplanted mouse and rat lymphomas by guinea pig serum was first described in 1953 (25). The antitumor factor was later shown to be an enzyme, asparaginase (L-asparagine amidohydrolase, EC 3.5.1.1) (4, 5). Search for other sources of the enzyme revealed that enzymes of different origins had different rates of clearance from plasma and dissimilar biological activity (6, 31). Isolation of an active fraction of asparaginase from E. coli (11, 28, 35) provided a practical source of the enzyme for clinical study. Initial clinical trials in ALL in children with the E. coli enzyme were encouraging (24, 30).

In the initial clinical trials with E. coli enzyme, chills, fever, emesis, pyrexia (30), hypersensitivity reactions (24, 30), and fatty liver (24) were reported. In a critically ill child with ALL, hypotension, hemolysis, and intestinal hemorrhage were observed after guinea pig serum enzyme administration (14). Nevertheless, general toxicity of the host was not regarded as a critical problem (14, 24, 30), perhaps in part because of the initial assumption that the drug acted on a specific metabolic defect in sensitive tumor cells. When more enzyme became available, further studies were conducted and a wide spectrum of toxicity was recognized (2, 9, 12, 17, 18, 20–23, 26, 33, 37, 40). The present paper reports our observations on biochemical and pharmacological studies with E. coli asparaginase in patients with leukemia and solid tumors. Portions of this work have been reported in a preliminary form elsewhere (32).

MATERIALS AND METHODS

Forty-five patients were studied: 24 with ALL of whom 4 had converted from preexistent LSA, 12 with AML, 2 with malignant melanoma, 2 with osteogenic sarcoma, and 1 each with CML in the blastic phase, choriocarcinoma, neuroblastoma, multiple myeloma, and carcinoma of the colon. Five patients with AML had received no prior treatment, whereas the remaining leukemic patients were in frank relapse from prior chemotherapy at the time of the asparaginase trial. All patients with solid tumors had previously been treated; each had palpable or radiological evidence of tumor. Patients with leukemia were evaluated by the rating criteria of Acute Leukemia Group B (15). Response in solid tumors was defined by at least 25% decrease in the product of 2 diameters as a reflection of tumor size.

Asparaginase was obtained from Merck, Sharp and Dohme Research Laboratories, West Point, Pa., through the National Cancer Institute. The sources of chemicals used were as follows: L-asparagine, anhydrous, and L-aspartic acid, Nutritional Biochemical Corp., Cleveland, Ohio; phenol loose crystals, Mallinckrodt Chemical Works, St. Louis, Mo.; sodium hypochlorite, Will Scientific, Inc., Buffalo, N. Y.; Tris and sodium nitroprusside, Fisher Scientific Co., Fairlawn, N. J.; crystals, Mallinckrodt Chemical Works, St. Louis, Mo.; sodium hypochlorite, Will Scientific, Inc., Buffalo, N. Y.; Tris and sodium nitroprusside, Fisher Scientific Co., Fairlawn, N. J.;

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α-ketoglutaric acid, Sigma Chemical Co., St. Louis, Mo.; GOT, malic dehydrogenase, and NADH, Boehringer Mannheim Co., New York, N. Y.; Ur-3H (specific activity 20.0 Ci/mmole), DL-lysine-4,5-3H (specific activity, 45.0 Ci/mmole), and L-valine-5-3H (specific activity 0.6 Ci/mmole), Schwarz Bio- research, Inc., Orangeburg, N. Y.; 6C3HED lymphoma was obtained from Dr. T. Hauschka, Roswell Park Memorial Institute, and was maintained in C3H mice in ascites form.

Asparaginase Activities in Plasma. Blood plasma was obtained either by finger prick into heparinized capillary tube or by venipuncture after i.v. administration of asparaginase. From the capillary tube, 5 to 10 µl of plasma were used directly or after appropriate dilution for the assay (31). The activity was expressed in i.u. The sensitivity of the method was such as to detect activity of 1 × 10⁻³ i.u./ml. Protein was measured by the method of Lowry et al. (27).

Assay of Plasma L-Asparagine and L-Aspartic Acid. Assays were carried out according to the method described by Cooney et al. (13). Samples of plasma were placed in a boiling water bath for 10 min to inactivate intrinsic enzymes. The congealed plasma was cooled and centrifuged at 105,000 × g at 4° for 1 hr. Clear supernatant, 0.9 ml, and 0.1 ml of reaction mixture consisting of 10 mM α-ketoglutaric acid, 2 mM NADH, 0.3 i.u. of GOT, and 500 mM Tris buffer, pH 8.0, were placed in a silica cuvet. Malic dehydrogenase, 0.72 i.u. in 0.1 ml of 100 mM Tris buffer, pH 8.0, was added, and the decrease in absorbance at 340 mµ was recorded until the reaction reached a plateau (40 to 50 min). This change in absorbance represented the plasma L-aspartic acid. Then 0.8 i.u. of asparaginase (E. coli) in 0.01 ml of 0.9%NaCl solution was added, and the further decrease in absorbance at 340 mµ was similarly recorded, which represented the amount of L-asparagine. The values of L-asparagine and L-aspartic acid were calculated from concurrent standards made from known concentrations of the amino acids.

Test of Leukemic Cells for L-Asparagine Requirement in Vitro. The white cells were separated from the peripheral blood or bone marrow aspirate by a method developed in this laboratory (16). The concentration was adjusted to 6 × 10⁸ leukemic cells/ml in Eagle's medium with Hanks' balanced salt solution containing 15% dialyzed fetal calf serum and the incorporation of Ur-3H (final activity, 1 µCi/ml; final concentration, 0.05 µM), L-valine-5-3H (final activity, 4 µCi/ml) or DL-lysine-4,5-3H (final activity, 1 µCi/ml) in the presence of L-asparagine (final concentration, 0.38 mM) or asparaginase (final activity, 0.083 i.u./ml) were carried out by the method described by Oettgen et al. (30). 6C3HED lymphoma cells in the same concentration were incubated concomitantly to ensure that the system was optimal. Percentage of incorporation was calculated by the area under the curve for uptake at several time points with asparaginase divided by that area under the curve for uptake when the cells were incubated with L-asparagine.

Administration, Dosage, and Schedule. The lyophilized enzyme was dissolved with 0.9% NaCl solution (5 ml/vial) and injected i.v. directly or through the side arm of a running infusion within 0.5 hr after being dissolved. The drug was given as single, daily, or weekly doses. Most patients on the daily schedule received a single injection as a test dose 4 to 14 days prior to 5 daily injections of the drug. One patient received 5 additional doses every other day after he reached M1 marrow, another received the enzyme daily for 27 doses and a 3rd patient with ALL received a 5-day course after weekly treatment had been shown to be ineffective. The total dose range varied from 200 to 40,000 i.u./kg. The highest total dose given was 2,200,000 i.u.

Immunological Study. The first 34 patients received 10 or 20 i.u. of asparaginase intradermally as a skin test prior to the commencement of treatment. This was later abandoned because initial skin tests were never positive and anaphylactoid reactions occurred in patients despite negative skin test.

In some patients, presence of antibody against asparaginase in the serum was determined by the method described by Campbell et al. (10).

RESULTS

Asparaginase Activity in Plasma. Asparaginase vials of different lots were randomly selected, and the activity was measured. These enzymes were dissolved as long as 10 months
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prior to the testing date and stored at -20°. Frozen storage of this duration was found not to cause any loss of activity. Eleven vials from 5 different lots labeled as containing 10,000 i.u. were found to have activity ranging from 6,100 to 7,900 i.u./vial and specific activity of 116 to 182 i.u/mg of protein.
The doses in this paper are expressed in terms of label potency to afford comparison with the data of others. The activity of asparaginase found in plasma, however, is reported as such.

The clearance of plasma asparaginase is shown in Chart 1. It may be seen that the peak activity lasted for about 3 hr after the injection, it was dose related, and the clearance of the activity from plasma followed first-order kinetics with plasma half-times of 14 to 22 hr. When blood samples were obtained by finger prick, the activity in the 2-hr samples was consistently higher than the preceding samples, but this finding was not confirmed in the samples obtained by venipuncture. In 2 patients, the plasma enzyme activity was measured serially for a longer period. The activity was detectable 13 and 22 days after single injection of 1000 and 5000 i.u. /kg, respectively, but not after 18 and 25 days, respectively. Daily administration of 400 or 1000 i.u./kg of the enzyme had a cumulative effect (Chart 2).

Development of immunological reactions were associated with rapid clearance of plasma enzyme. Thus, in one patient who developed arthralgia, erythematous skin eruption, and positive intradermal test to 10 i.u. of the enzyme of Day 15, 5 days after the end of a 5-day course of 1000 i.u./kg/day, the plasma asparaginase level fell rapidly and became undetectable 4 days after the last injection. In another patient whose plasma enzyme activity became undetectable 8 days after a 2nd dose of 5000 i.u./kg of enzyme, the plasma was found to have a positive precipitation reaction to asparaginase.

Plasma L-Asparagine and L-Aspartic Acid. Plasma L-asparagine and L-aspartic acid values in 10 healthy men, 10 healthy women, 10 patients from 7 to 44 years with ALL, and 8 patients from 12 to 72 years with AML were plotted in Chart 3. In healthy adult controls, L-asparagine values were 60 ± 13 (mean ± 1 S.D.) and 54 ± 16 nmoles/ml in men and in women, respectively. L-Aspartic acid concentrations were 11 ± 4 and 10 ± 4 nmoles/ml in men and women, respectively.

Among 10 patients with ALL, there appear to be 2 groups: 1 group with L-asparagine concentration within or near the normal range and with normal L-aspartic acid, the 2nd group with low L-asparagine and reciprocally elevated L-aspartic acid. This subgroup of 3 patients was unique in that 2 patients died within 48 hr of injection. The 3rd patient was induced into complete remission. Patients with AML had lower levels of L-asparaginase with normal L-aspartic acid concentration.

After enzyme administration, plasma L-asparagine fell precipitously, with a reciprocal rise in L-aspartic acid. Even 0.2 i.u./kg of the enzyme, which is in fact the skin test done, caused substantial fall of plasma L-asparagine levels by the following day. In 6 measurements, L-asparagine fell from 31 ± 7 (mean ± S.E.) to 4 ± 1, and L-aspartic acid rose from 26 ± 6 to 43 ± 3 nmoles/ml. After a single injection of 1000 i.u./kg i.v. in 1 patient, 16 measurements in 32 days revealed that
L-asparagine levels remained unmeasurable until Day 21 but returned by Day 23. In another patient, 14 measurements in 33 days after a single injection of 5000 i.u./kg showed that L-asparagine was unmeasurable until Day 29 but returned by Day 33. In both cases, it took about 10 days after the enzyme was no longer detectable by the techniques used for L-asparagine levels to rise. These results indicate the prolonged activity of exogenous enzyme in man.

Toxicity. Toxicity observed after asparaginase administration is summarized in Table 1. Chart 4 shows the relation between dose range and composite mean score for toxicity ratings of 0 to 4+, implying none, mild, moderate, severe, and life threatening. Thirteen of 15 patients who received a single dose were scheduled to receive additional drug in weekly courses, but because of death from advanced disease in 8, and because of toxicity in 5, no further drug was given. It is thus not unanticipated that the mean toxicity ratings for the group that received only single doses would be higher than for the other regimens. In the other dosage regimens, mean toxicity score seems not to be dose dependent. This suggests difference in the susceptibility of host cells of different individuals to L-asparagine starvation.

Immediate reactions usually occurred 0.5 to 1 hr after injection and lasted approximately another hr. These reactions included nausea and vomiting, fever, and chills. In some patients, nausea or emesis lasted a few days. In patients who received weekly injections, premedication with antihistaminics and hydrocortisone often modified or eliminated these immediate reactions.

Hepatic toxicity was observed more frequently than any other. Increase in bilirubin, GOT, LDH, and alkaline phosphatase and, decrease in albumin, cholesterol, fibrinogen, and other coagulation factors occurred. At death, severe fatty metamorphosis was seen (Fig. 1). Microscopic observation has been completed on 16 patients who died after asparaginase treatment. With the exception of 2 patients, 1 who died on the day of asparaginase administration, and 1 who died 10 days after receiving 200 i.u./kg, livers of all patients showed severe fatty metamorphosis. Albumin decreased an average of 1.8 g/100 ml during the first 2 weeks and then reached a plateau. Seven of 8 evaluable patients showed hypocholesterolemia with average decrease of 62 mg/100 ml. Eight of 10 evaluable patients had a fall of fibrinogen level averaging 214 mg/100 ml. In 3 of those 10 patients, there was a concomitant deficiency in Factor V, Factor VII, and Factor X. Bleeding, observed frequently in patients with leukemia, could not usually be ascribed only to fibrinopenenia or other coagulation protein deficiencies, since thrombocytopenia often existed also. In contrast to the decrease in his serum albumin, one patient with IgA multiple myeloma showed no changes in immunoglobulin levels with serial determinations after 5000 i.u./kg of the enzyme was administered.

Mild azotemia was commonly noted at about Day 7 of the study. We did not establish whether this was renal or prerenal in origin.

The most striking side effect of treatment, when it occurred, was cerebral dysfunction. Mild depression and drowsiness were found in about 25% of the patients. Severe brain dysfunction, manifested as disorientation and confusion, was seen in 7 patients. In 2, it was noted within 24 hr; in the other 5, it did not appear until after 1 week. In 2 of the delayed dysfunction patients, disorientation and confusion were

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Asparaginase toxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>The percentage in each category was calculated on the basis of patients with adequate observations to determine toxicity in question.</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>1. Immediate</th>
<th>Nausea, vomiting, and chills</th>
<th>27</th>
<th>38</th>
<th>71</th>
</tr>
</thead>
<tbody>
<tr>
<td>2. Delayed</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Hepatic</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Increase in bilirubin, GOT, LDH, alkaline phosphatase, decrease in albumin, cholesterol, fibrinogen, and other hepatocogulation factors—fatty metamorphosis.</td>
<td>32</td>
<td>33</td>
<td>97</td>
<td></td>
</tr>
<tr>
<td>2. Loss of weight (more than 5% of pretreatment level)</td>
<td>24</td>
<td>30</td>
<td>80</td>
<td></td>
</tr>
<tr>
<td>3. Azotemia</td>
<td></td>
<td>25</td>
<td>38</td>
<td>68</td>
</tr>
<tr>
<td>4. Neurological:</td>
<td></td>
<td>13</td>
<td>39</td>
<td>33</td>
</tr>
<tr>
<td>Headache, drowsiness, depression, disorientation, confusion</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. Pancreatic:</td>
<td></td>
<td>6</td>
<td>39</td>
<td>15</td>
</tr>
<tr>
<td>Abdominal pains, increase in amylase or lipase, hyperglycemia, hypoinsulinemia, malabsorption syndrome</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6. Immunological:</td>
<td>Positive skin test, anaphylactoid reaction</td>
<td>5</td>
<td>39</td>
<td>13</td>
</tr>
<tr>
<td>7. Miscellaneous:</td>
<td>Transverse banding of the fingernails, hypotension</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Chart 3. Plasma L-asparagine (ASN) and L-aspartic acid (ASP) values in normal healthy men and women, and in patients with ALL and AML. o, ◦, v, +, v, * , and ◦, 3 patients with reverse correlation of the amino acids.
Chart 4. Composite mean toxicity score versus dose and schedule of asparaginase. The first 7 patients receiving weekly treatment were given 1000 i.u./kg/week, for example, and the next 3 patients received 4000 i.u./kg. Under daily treatment, gaps in dose schedule schema represent observation intervals of 4 to 14 days. Three patients from the single injection group and 1 patient from the daily injection group were omitted because of inability to evaluate toxicity scores. Toxicity score: 0, none; 1, mild; 2, moderate; 3, severe; 4, life-threatening toxicity.

accompanied by loss of recent memory, confabulation, and easy suggestibility which resembled Korsakow's psychosis. The syndrome lasted nearly 1 month in each. In one case, even a single dose of 1000 i.u./kg of asparaginase produced late-appearing dysfunction (33).

Pancreatic toxicity was manifest in varying degree in 4 patients as abdominal pain, upper abdominal tenderness, diarrhea, increase in serum and urinary amylase, hyperglycemia, hypoinsulinemia and hypocalcemia. Development of a malabsorption syndrome was suggested in 2 of these patients by a positive Schilling test done with and without intrinsic factor.

Nonketotic hyperglycemia occurred in 3 adults and 2 children, of whom 2 showed no clinical evidence of pancreatitis. Blood glucose rose as high as 955 mg/100 ml in 1 patient. In 2 patients, the hyperglycemia was accompanied by polyuria of 11,000 and 8,000 ml, respectively, with 4+ urine sugar tests. Polyuria responded to insulin but not to anti-diuretic hormone. Blood sugar levels fell back to normal range in approximately 2 weeks. The response of hyperglycemia to insulin was appropriate, showing neither unusual sensitivity nor resistance. Hyperglycemia occurred in one man only after the 1st dose and was not seen after the 2nd, 3rd, or 4th weekly dose of asparaginase, possibly because of depletion of hepatic glycogen, as suggested by negative periodic acid-Schiff stain of the liver at autopsy.

Positive skin test or anaphylactoid reaction were not observed at the first exposure to the enzyme. Intradermal injection of 10 i.u. evoked a positive response 18 hr later in a 44-year-old man who received a single injection of 1000 i.u./kg 1 week previously. He had no evidence of clinical allergy but was not treated. A similar reaction in a 16-year-old girl 4 days after a 5-day course of 1000 i.u./kg/day was followed by an illness resembling serum sickness with generalized arthralgia and paravenous erythema near vein sites where asparaginase had been injected. Three other patients developed anaphylactoid reactions. The reaction includes shaking chills, hypotension, stridor, numbness, cyanosis and, in one patient, coma, beginning within 2 min. All 3 patients had had negative skin tests before their repeat weekly injections: the 2nd dose of 5000 i.u./kg, and the 3rd and 4th, respectively, of 1000 i.u./kg. These patients were treated with epinephrine, antihistaminics, and steroid and recovered without complication.

One patient with AML, who after 1,000 i.u./kg of the enzyme developed acute brain dysfunction, pancreatitis, malabsorption, and muscle wasting, was later found to have transverse banding of the nails (33). Another patient with AML developed hypotension of 70 mm Hg systolic and 45 mm Hg diastolic occurring 3 days after 10,000 i.u./kg of the enzyme. He received metaraminol and steroid for 3 days and recovered.

Bone marrow aplasia was not observed in nonleukemic patients.

Although we have seen a variety of unusual toxic effects, the drug was fairly well tolerated by most of the patients and the toxicities were essentially reversible. Two patients died on Day 1 who had far-advanced neoplasms with overwhelming infections, and causal relation to asparaginase was not found at autopsy. Another patient with ALL who received 1000 i.u./kg died on Day 2 with disorientation, confusion, fever, and increase of blood urea nitrogen from 4 to 40 mg/100 ml. At autopsy, bronchopneumonia was seen.

L-Asparagine “Rescue” Infusion. L-Asparagine rescue infusion was given to 3 patients for the acute brain dysfunctional syndrome in hopes of reversing the abnormalities. The dose of L-asparagine was chosen from a previous human experiment (38). Pertinent data are summarized in Table 2. No adverse reaction was observed from the amino acid infusion. In 2 patients, definite improvement ensued, and in 1, probable...
N. Y.], and infused in 24 hr.

One day's dose of the amino acid was dissolved in 2 liters of 5% glucose, sterilized by Nalgene Filter Unit (Nalgene Labware Division, Rochester, N. Y.), and infused in 24 hr.

Table 2

<table>
<thead>
<tr>
<th>Patient</th>
<th>Diagnosis</th>
<th>Dose of asparaginase (i.u./kg × 1)</th>
<th>Plasma L-asparagine before infusion (mmoles/ml)</th>
<th>Serum albumin before infusion</th>
<th>Dose of L-asparaginase (mmoles/kg/24 hr)</th>
<th>Days given</th>
<th>Maximum L-asparaginase during infusion (mmoles/ml)</th>
<th>Serum albumin after infusion</th>
<th>Improvement in mental status</th>
</tr>
</thead>
<tbody>
<tr>
<td>D. W.</td>
<td>AML</td>
<td>1,000</td>
<td>2.6 (Day 7)</td>
<td>1</td>
<td>12–17</td>
<td>16</td>
<td>2.3 (Day 18)</td>
<td></td>
<td>Definite</td>
</tr>
<tr>
<td>J. C.</td>
<td>CML (blastic)</td>
<td>5,000</td>
<td>1.7 (Day 9)</td>
<td>2</td>
<td>9–14</td>
<td>0</td>
<td>2.1 (Day 15)</td>
<td></td>
<td>Probable</td>
</tr>
<tr>
<td>D. D.</td>
<td>AML</td>
<td>10,000</td>
<td>0.9 (Day 27)</td>
<td>1</td>
<td>38–44</td>
<td>16</td>
<td>2.0 (Day 48)</td>
<td></td>
<td>Definite</td>
</tr>
</tbody>
</table>

improvement was noted. The relation to infusion was such that clinical observers considered the phenomena to be causally related (33).

Therapeutic Effect. The response to asparaginase in patients with leukemia is summarized in Table 3. We have frequently seen definite decrease or disappearance of leukemic cells from periphery and/or rise in platelets after asparaginase with bone marrow changes within the same classification. These patients were classified as "hematological effect only." Decrease in peripheral leukocytes alone was not included. The enzyme is most effective against ALL in children but has some value in AML of adults as well. Although the numbers are small, there appear to be no differences in the response rate in children with ALL between those who received daily injections (M1/total, 5/8) and those with weekly treatment (M1/total, 4/8). One patient with AML was induced into complete remission after a single dose of 1000 i.u./kg (33). Another woman with AML whose marrow was induced to M2 died from massive pulmonary embolism. All the 10 ALL patients who were induced into M1 with asparaginase had had extensive prior chemotherapy and had failed to remit again on vincristine and/or Daunorubicin with prednisone. Among these 10 ALL patients who were induced into M1, 3 relapsed on Days 14, 15, and 21; 1 died from empyema despite the M1 marrow; and 6 went into a Phase II maintenance program on other drugs. No patient with solid tumor responded to asparaginase.

Table 3

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Adult</th>
<th>Child</th>
<th>LSA→ALL</th>
<th>Adult</th>
<th>Child</th>
<th>LSA→ALL</th>
<th>Adult</th>
<th>Child</th>
<th>AML</th>
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<tbody>
<tr>
<td>M1</td>
<td>0</td>
<td>0</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>M2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Hematological effect only</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>No response</td>
<td>3</td>
<td>6</td>
<td>0</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>4</td>
<td>16</td>
<td>1</td>
<td>3</td>
<td>10</td>
<td>2</td>
<td></td>
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</table>

L-Asparagine Requirement by Leukemic Cells In Vitro. Chart 5 shows the 36 studies of in vitro incorporation of Ur-3H and L-valine-5-3H (or DL-lysine-4,5-3H) by leukocytes in 20 patients as an attempt at predictive behavior. These values are arranged on the abscissa according to order of decreasing antileukemic effect and were compared with the values of a sensitive mouse lymphoma. In the presence of asparaginase, incorporation of uridine and lysine was always less than 30% of controls in the mouse lymphoma. The inhibitions in human leukemic cells were never this pronounced. Furthermore, incorporation of precursors by control human culture was always less than the mouse lymphoma cells ranging from 0.45 to 20.8%, with an average of 6.6%. Biochemical prediction of therapeutic outcome was not reproducibly successful, since indistinguishable biochemical results were found in ALL and AML, and within diseases no good correlation with hematological response occurred.

DISCUSSION

Serial measurements of asparaginase after a single injection of the enzyme has shown prolonged clearance rates of the enzyme. Systematic increase in enzyme levels for the first 2 hr in finger prick blood, as contrasted to blood taken by venipuncture, suggests that tissue fluids were squeezed from the finger pad in obtaining the capillary samples. This implies an early and appreciable extravascular space of distribution. Initial peak activities of 5, 12, and 60 i.u./ml obtained after injections of 400, 1000, and 5000 i.u./kg, respectively, are reasonably close to estimated values of 5.6, 14, and 70 i.u./ml which are calculated from the actual dose given being 70% of labeled dose, and distribution in the plasma volume estimated as 5% of body weight. After asparaginase administration, sustained depression of L-asparagine concentration was observed. These findings are in contrast to the results in mice where the E. coli enzyme is cleared from plasma within 24 hr and where depressed L-asparagine returned to normal in 48 hr (7, 8). We have previously shown, by using avian and guinea pig enzymes, an inverse correlation between the rate of clearance of exogenous enzyme in the plasma and the therapeutic effects in mouse tumors (31). From this point of view, the prolonged clearance of E. coli enzyme in man may be regarded as a favorable factor in its effectiveness. Prolonged half-life of E. coli enzyme in man [8 to 30 hr (E. Frei, III, personal com...
increase in endotoxin dose exceeded the pyrogenic threshold in man but might have been tested as a negative in rabbits based on dose concentrations alone. Bacterial endotoxin is known to cause chills, fever, vomiting, hypotension, fibrinogenopenia, hepatic injuries (39), depletion of liver glycogen, renal failure, coagulopathies, and delayed hypersensitivity reaction (41). These effects resemble many of the toxicities observed after asparaginase. Probably the most intriguing finding to explain much of the toxicity is the deficiency in protein biosynthesis produced by L-asparagine depletion. Thus, after asparaginase, incorporation of L-methionine-35S into albumin was markedly inhibited (J. F. Holland, L. Walter, and T. Ohnuma, unpublished data). Clinical findings suggest that synthesis of albumin, insulin, fibrinogen, and fingernail protein were defective, whereas that of globulin was apparently unaffected. The failure to find hypoglobulinemia suggests some systematic differential requirement for L-asparagine between liver cell and plasma cell. Since immunoglobulin contains the same general amounts of L-asparagine as most other proteins, the plasma cell presumptively has a well-developed system to supply its own L-asparagine. Together with the evidence of patients who developed immunological reactions, these findings suggest that asparaginase treatment is not immunosuppressive, a situation dissimilar to data from the mouse (36). Since skin testing did not indicate the patients who sustained anaphylactic response, we have abandoned it. We are aware of 1 fatal instance of anaphylaxis encountered by a colleague, despite immediate and vigorous therapeutic measures. L-Asparagine has been shown to protect against ethionine- or carbon tetrachloride-induced fatty liver, which presumably resulted from defective removal of fat because of inhibition of protein biosynthesis (1). The similarity of pathological changes in the liver after these chemical toxins and after asparaginase suggests that the hepatic injury is indeed due to inhibition of protein synthesis because of L-asparagine deficiency.

We have observed 2 forms of diffuse brain dysfunctional syndrome: one occurred within 24 hr and then cleared rapidly; the other occurred insidiously about 1 week later and lasted approximately 1 month. In all instances, central nervous system leukemia, massive central nervous system bleeding, meningitis, and electrolyte imbalance and other iatrogenic causes were excluded. We have not measured ammonia on each occasion, but liberation of large amounts of ammonia by enzyme were estimated from the increases in L-aspartic acid values or "blank" ammonia values in asparaginase determination. Methionine sulfoximine-induced seizures in cats are related to inhibition of L-glutamine synthesis and administration of L-glutamine and L-asparagine to epileptic patients resulted in improvement in electroencephalographic records (38). It may be speculated that acute brain disease is related to ammonia or L-aspartic acid intoxication while the delayed brain syndrome is associated with defective protein synthesis in the brain produced fundamentally by L-asparagine deficiency.

Clinical observation of improvement in mental status after L-asparagine rescue infusion in all 3 patients studied is of interest. Mental abnormalities did not apparently increase after interruption of L-asparagine infusion in 2 patients. In the 3rd
patient (J. C.), recrudescence of brain dysfunction followed after discontinuation of L-asparaginase infusion. Serum albumin levels did not correlate well with abnormal mentality, suggesting differences in chemical abnormalities of liver and nervous system. The close correlation of L-asparaginase infusion with benefit suggests that the relation is causal and not coincidental to spontaneous recovery. It should be added, however, that rescue effect would not be expected in the days immediately after asparaginase administration because endogenous L-asparagine may be rapidly catalyzed by large amounts of the enzyme still circulating. Even 12 days after asparaginase administration in Patient D. W., abnormally high L-aspartic acid levels resulted from L-asparaginase infusion (33). Because L-asparagine given early may thus give rise to toxic levels of ammonia, its use is contraindicated when asparaginase levels are high. A nontoxic inhibitor of asparaginase would be of value to allow restitution of L-asparagine levels.

The in vitro estimation of RNA and protein biosynthesis and its inhibition by L-asparagine deficiency evolved by asparaginase did not provide reliable prognostication. Possible factors which might influence clinical results are the development of an antibody that rapidly clears the enzyme from plasma. We observed this on one occasion. When patients developed anaphylactoid responses or positive skin reaction to the drug, we terminated treatment. The derepression or induction of an L-asparagine-synthesizing system in leukemic cells after asparaginase administration has been reported (19). The rapid selection of a variant animal tumor cell population which does not require L-asparagine can occur in vitro even in the presence of L-asparagine (34). All these factors may contribute to the discrepancy of in vitro estimation and therapeutic effect.

One of the most difficult clinical problems is that the toxicity is apparently not dose related and some manifestations such as anaphylaxis, pancreatitis, brain dysfunctional syndrome, and coagulopathies can be lethal. Proper and close clinical observation of patients receiving the enzyme is therefore essential.

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REFERENCES


Fig. 1. Fatty metamorphosis of the liver. This severe fatty metamorphosis was seen in a patient whose liver function 5 days prior to death showed only slight abnormalities (bilirubin, 2.0 mg/100 ml; serum GOT, 32 units; LDH, 137 units; alkaline phosphatase, 4.6 Bessey-Lowry units; total protein, 8.0 g/100 ml). H & E.
Biochemical and Pharmacological Studies with Asparaginase in Man

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