Response to Highly Purified \(\text{L}\)-Asparaginase during Therapy of Acute Leukemia

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SUMMARY

Seven of nineteen patients with acute lymphatic leukemia and eight of eighteen patients with acute granulocytic leukemia received \(\text{L}\)-asparaginase according to a daily dosage schedule of 2,000 IU or more per kg for twenty-one days or more. Six of the seven acute lymphatic leukemia patients and four of the eight acute granulocytic leukemia patients underwent a complete bone marrow remission. In contrast, only two patients with acute lymphatic leukemia and none of the patients with acute granulocytic leukemia obtained a complete remission at lower dosage levels. \(\text{L}\)-Asparaginase is relatively nontoxic and does not exhibit cross-resistance with conventional agents. Further study is merited in order to determine its ultimate effectiveness either alone in higher dosages or in combination with conventional agents.

INTRODUCTION

Intravenous administration of the enzyme, \(\text{L}\)-asparaginase, used for the treatment of acute leukemia, represents a new approach to the therapy of malignant disease. In 1953, Kidd (9) observed that the administration of guinea pig serum caused regression of certain malignant tumors in rats and mice. This was followed by important discoveries by Neuman and McCoy (13), Broome (1), Mashburn and Wriston (10), Roberts et al. (18), Hill et al. (7), Old et al. (15), and Dolowy et al. (4). The work of these and other investigators set the stage for clinical trials. Dolowy et al. (5) reported a slight response in a patient with acute lymphatic leukemia treated with \(\text{L}\)-asparaginase concentrated from guinea pig serum. In 1967, Hill et al. (8) described 3 cases of acute leukemia treated with \(E. \text{coli}\) \(\text{L}\)-asparaginase. Included in this series was the first patient to achieve a complete bone marrow remission with \(\text{L}\)-asparaginase therapy. Oettgen et al. (14) have provided further evidence of the effectiveness of \(\text{L}\)-asparaginase therapy in acute leukemia.

Unlike normal cells, certain malignant cells are unable to synthesize sufficient quantities of the amino acid, \(\text{L}\)-asparagine, to meet their own metabolic demands. They must satisfy these needs by the uptake of \(\text{L}\)-asparagine which has been produced by other cells. Consequently, continuous extracellular destruction of this amino acid by the enzyme leads to death of the neoplastic cell. However, the exact intracellular mechanisms by which cell death results from asparagine deprivation are not fully understood. For a more detailed discussion of possible mechanisms see Broome (2, 3).

Tissue culture studies of the metabolic requirements of a variety of malignant cells give evidence that, for their survival, other amino acids may also be uniquely essential (6, 13). Clinical experience indicates that \(\text{L}\)-asparaginase does not possess the toxicity associated with conventional treatment (8, 14). The effectiveness of the agents of conventional chemotherapy depends on subtle kinetic differences in cellular processes common to both normal and malignant cells. Thus, they act as cellular poisons and have significant toxic effects at or near effective levels of dosage. In contrast, the action of \(\text{L}\)-asparaginase is one of selective starvation which leaves normal cells relatively unscathed. This process has been referred to as amino acid depletion therapy (8).

Early therapeutic trials of \(\text{L}\)-asparaginase were limited both in scope, by insufficient supply, and in dosage levels, by the presence of endotoxin-like impurities in the preparations of enzyme which were available. However, Roberts et al. (18) have developed practical technics by which large amounts of highly purified enzyme can be produced (18). On a pilot plan scale, they have produced sufficient quantities of this enzyme to allow acceleration of clinical trials. Daily doses of 10 to 50 times those previously reported have been administered. Comparison of these results with those obtained with lower dosage has revealed new information with regard to the speed of response, type of case susceptible, effect on central nervous system leukemia, blood levels, disappearance rate, toxicity, and effect on normal hemopoiesis.

MATERIALS AND METHODS

The first 3 patients (8) received \(\text{L}\)-asparaginase purchased from Worthington Biochemical Corporation supplemented by some enzyme produced in our laboratories. Subsequently, the
enzyme used was purified in the Wadley laboratories, from our strain of *E. coli* HAP. The cells were grown in quantity by Merck, Sharp and Dohme Research Laboratories according to growth conditions for maximum enzyme yield as described by Roberts et al. (17). Subsequently, they supplied enzyme preparations which were partially purified by alcohol precipitation (17), Southwestern Drug Company supplied similar materials.

Assays of the enzyme were made as previously described (18). The specific activity of the enzyme used was approximately 250 IU per mg of protein as determined by the Biuret method. This expression of the specific activity of the enzyme gives a much lower value than is the case when protein is determined by the absorption at 260 and 280 millimicrons. This seems to be due to a low tyrosine and a very low tryptophan content of the enzyme.

The final material had a usual concentration of 10,000 to 20,000 IU per ml of solution. This material was put in vials and stored in the frozen state or freeze-dried. This therapeutic preparation as well as electrophoretically pure enzyme had from 1 to 3 percent L-glutaminase activity at pH 7.5. The effectiveness of enzyme preparations against lymphoma 6C3HED in mice was tested as previously described when any change in the method of preparation was made (18). Tests for pyrogenicity and endotoxin activity were made in rabbits and chick embryos (11, 12).

Tests for acute toxicity of L-asparaginase were made in 4 rabbits using 2 control animals. The test animals received a single dose of 10,000 IU per kg intravenously twice in one week. These animals were not sacrificed at the end of the experiment. Similarly, 5 Holtzman rats were each given 100,000 IU per kg intravenously, daily for 5 successive days. Four control rats were given only 5% dextrose in water. All rats were sacrificed at the end of 5 days, and sections were made of liver, lung, kidney, spleen, and bone marrow.

In several patients, serial determinations of serum L-asparaginase levels were made following a final intravenous dose in order to determine the rate of disappearance from the circulation.

Usually, cells from patients considered for L-asparaginase therapy were tested for sensitivity to asparaginase deprivation by the C-14 valine incorporation test (8, 19). The cells were obtained from bone marrow or biopsy and occasionally from the blood buffy coat. All determinations were performed in triplicate. The mean values were used for qualitative calculation of results. The "sensitivity" equals the counts per minute with asparagine minus counts per minute without asparagine, divided by counts per minute with asparagine. A 15% difference was considered to be the dividing line between a positive and a negative sensitivity test.

All L-asparaginase was given intravenously with the exception of a few trials of the intramuscular route. Until recently, skin tests were performed prior to enzyme administration. In early studies, very small starting doses of 25 to 100 IU were given intravenously as a biologic test for toxicity, followed by progressively doubled doses hourly until desired levels were reached. More recently, initial test doses have been larger (5,000 IU). Careful progression of the dosage from this level was observed as before. Desirable daily dosages given once daily or upon a divided basis were soon reached.

### RESULTS

A total of 65 patients have received intravenous L-asparaginase. Of these, 46 had acute leukemia. Early in the study, those leukemia patients chosen for therapy had disease which was already resistant to conventional modes of treatment. Several were nearly terminal. A few patients were given the enzyme intramuscularly with inconclusive results. Six patients received only a single dose of L-asparaginase and were deleted from the study. Three patients with acute granulocytic leukemia were considered separately because they were already in remission on conventional agents when L-asparaginase was started.

Nineteen patients with acute lymphatic leukemia and 18 patients with acute granulocytic leukemia remained. Tables 1 and 2 summarize the data in cases obtaining a complete bone marrow remission.

#### Results in Acute Lymphatic Leukemia

In acute lymphatic leukemia (ALL), there were a total of 8 complete remissions, 6 partial remissions, 2 fair responses, and 3 poor results. Of 7 patients who received L-asparaginase according to a schedule of at least 2,000 IU per kg per day for at least 21 days, there were 6 complete remissions and 1 fair response. The duration of the remissions varied from 4 to 20 weeks.

The duration of therapy to the onset of complete bone marrow remission varied from 8 to 44 days. In the 12 patients with schedules well below 2,000 IU per kg per day or who were treated for less than 21 days, there were 2 complete remissions, 5 partial remissions, 2 fair responses, and 3 poor results.

#### Results in Acute Granulocytic Leukemia

In acute granulocytic leukemia (AGL), there were a total of 4 complete remissions, 5 partial remissions, 3 fair responses, and 6 poor results. Of the 8 patients who received L-asparaginase according to a schedule of at least 2,000 IU per kg per day for at least 21 days, there were 4 complete remissions, 2 partial remissions, 1 fair response, and 1 poor result. The duration of the remissions varied from 5 to 23 weeks. Therapy of from 14 to 35 days was required to obtain a complete remission. In those patients who received L-asparaginase according to a schedule of less than 2,000 IU per kg per day or who were treated for less than 21 days, there were 3 partial remissions, 2 fair responses, and 5 poor results.

2*Complete remission*, clearing of leukemic blasts from marrow and blood that persists at least two weeks; patient well by standard clinical criteria. *Partial remission*, clearing of blasts in peripheral blood without complete clearing of marrow; improvement in clinical status. *Fair remission*, significant improvement in laboratory and clinical findings not qualifying as partial remission. *Poor results*, failure to reach above criteria.

3Several cases averaged less than 2,000 IU per kg but over 1,000 IU per kg because of smaller doses on some days, usually at the start of treatment.
Table 1

<table>
<thead>
<tr>
<th>Patient</th>
<th>Case No.</th>
<th>Sex</th>
<th>Age (yr.)</th>
<th>Total dose (IU)</th>
<th>Dose to induce remission (IU/kg/day)</th>
<th>Days to obtain remission</th>
<th>Remission length in weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. L. G.</td>
<td>66 ALL 323</td>
<td>M</td>
<td>4</td>
<td>3,470,930</td>
<td>3,325</td>
<td>44</td>
<td>15a</td>
</tr>
<tr>
<td>F. H.</td>
<td>66 ALL 300</td>
<td>M</td>
<td>9</td>
<td>213,000</td>
<td>171</td>
<td>39</td>
<td>4b</td>
</tr>
<tr>
<td>D. B.</td>
<td>66 ALL 302</td>
<td>M</td>
<td>13</td>
<td>302,424</td>
<td>236</td>
<td>13</td>
<td>6c</td>
</tr>
<tr>
<td>L. W.</td>
<td>68 ALL 316</td>
<td>F</td>
<td>7</td>
<td>3,715,750</td>
<td>2,057</td>
<td>18</td>
<td>12d</td>
</tr>
<tr>
<td>S. F.</td>
<td>68 ALL 320</td>
<td>F</td>
<td>2</td>
<td>3,806,000</td>
<td>1,080</td>
<td>9</td>
<td>18e</td>
</tr>
<tr>
<td>W. M. H.</td>
<td>68 ALL 319</td>
<td>M</td>
<td>24</td>
<td>3,055,000</td>
<td>1,405</td>
<td>30</td>
<td>10.5b</td>
</tr>
<tr>
<td>D. C.</td>
<td>67 ALL 305</td>
<td>F</td>
<td>4</td>
<td>2,133,000</td>
<td>1,503</td>
<td>8</td>
<td>20a</td>
</tr>
<tr>
<td>D. H.</td>
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<td>M</td>
<td>17</td>
<td>4,220,000</td>
<td>1,483</td>
<td>19</td>
<td>5c</td>
</tr>
</tbody>
</table>

1-Asparaginase therapy in acute lymphatic leukemia (ALL). Cases obtaining complete remission.
aIn remission, living.
bOut of remission, living.
cDead.

dMajor erythroleukemia component.

Results in Central Nervous System Leukemia

Intravenous L-asparaginase was administered to 2 patients with central nervous system involvement. In both, there was complete clearing of leukemic cells from the spinal fluid with a return of the cell count to less than 10 cells per cubic mm. Chart 1 illustrates the findings in one of these cases. Paradoxically, in this patient, bone marrow relapse was observed as the spinal fluid was clearing.

Valine-14C Incorporation Test

The sensitivity of cells to L-asparagine deprivation was determined in 15 of the 19 cases of acute lymphatic leukemia. In 12 of these patients, the sensitivity was over 15% and ranged as high as 48%. However, in 6 of the 15 patients tested, the amount of enzyme given was substantially below 200 IU per kg per day, a level arbitrarily considered to be adequate, or treatment was of substantially less than 21 days duration. Of the 9 adequately treated patients, 7 obtained complete remissions. In all 7, the sensitivity of their bone marrow cells was 21% or greater. The remaining 2 obtained only partial remissions and had sensitivity tests of 3% and 5% respectively.

Sensitivity tests were done on cells in 12 of the 18 patients with acute granulocytic leukemia. In 5 of these patients, the sensitivity was 15% or greater and ranged as high as 45%. However, sensitivity tests were not obtained in the 4 acute granulocytic leukemia patients who achieved a complete bone marrow remission.

Clearance of L-Asparaginase from Blood

In Chart 2, the disappearance curve of L-asparaginase in the serum of 5 patients is depicted. Each received a final dose of 330 to 3,500 IU per kg. The mean half-life was approximately 18 hours with a wide variation from the mean, but no relationship to the disease status of the patient was apparent. The examples given included one case in complete remission (66 ALL 496), two cases in partial remission (67 AGL 522, 67 AGL 512) and a case (67 ALL 300) at the seventh day of asparaginase therapy four weeks prior to complete remission. The case numbered 67-374 was a malignant melanoma showing slight regression with therapy. Chart 3 shows the clearance rate after a single, very large dose of asparaginase in a patient who was in complete remission from acute granulocytic leukemia at the start of the study.

Biologic Toxicity Test

No ill effect was noted in the 4 rabbits which were subjected to 10,000 IU per kg twice in a week or in the rats receiving 100,000 IU per kg on each of 5 successive days. Autopsy of
L-Asparaginase Therapy of Acute Leukemia

Chart 1. Effect of L-asparaginase on leukemia of meninges.

Chart 2. L-Asparaginase clearance from blood.
the rats revealed no significant differences between the control and test animals except for mild to moderate cloudy swelling of hepatic parenchymal cells in 3 of the 5 test animals.

Clinical Toxicity

As enzyme preparations of progressively greater purity became available from our laboratories, toxic effects diminished and the safe administration of much greater doses became possible. As small a dose as 100 IU of enzyme, when given to some of our earliest patients, was followed by a shock-like response with a drop in blood pressure, cyanosis, chills, fever, nausea, or vomiting. Desensitization with frequent doses of increasing strength was utilized to reach therapeutic levels safely. For example, the maximum daily dose which was given to the first patient to obtain a complete remission (Case #66 ALL 300) was 560 IU per kg. By contrast, recently a patient (Case #68 AGL 552) has received up to 17,000 IU per kg in a single dose. This dosage was well tolerated. Nevertheless, clinical pyrogenicity and endotoxin-like reactions of mild to moderate degree were found with occasional batches of “high purity” L-asparaginase. Neither the rabbit nor chick embryo tests consistently gave assurance of the complete absence of “toxins” in these batches although the latter test reliably detected as little as 0.0001 micrograms of endotoxin.

Side Effects

Anorexia, weight loss, decreased hemoglobin levels, and mild leukocyte depression were observed in some patients. Only 1 of 15 patients receiving large doses lost any noticeable amount of weight. In this patient (Case #66 ALL 324), who initially lost 17 pounds during the induction of remission, no weight loss occurred during the subsequent 9 days of therapy at the same dosage. Anorexia has been variable. Very high doses have been associated with nausea. Production of normal bone marrow elements appeared relatively unimpaired by high dosage L-asparaginase treatment. Normal granulocytic and erythrocytic elements appeared in the bone marrow within two to four weeks after the marrow was cleared of leukemic cells. In addition, the peripheral platelet count usually returned to normal during this time. Furthermore, in 1 patient with a solid tumor, there was no significant impairment of his normal hemopoiesis with doses which ranged from 2,000 to 9,000 IU per kg per day over a 21-day period.

A gradual decrease of serum albumin and globulin was commonly noted. Clinically, bleeding was not a problem in any leukemia patients in this series who were treated with “higher purity” L-asparaginase. However, in general, fibrinogen declined during intensive therapy; in some of our patients, to levels as low as 50 milligrams percent without clinical bleeding. On continuing maintenance at high dosage, such as 2,000 IU per kg per day given twice weekly, the fibrinogen slowly increased to near normal levels. No consistent pattern of abnormalities in other coagulation factors was found, even in the higher dosage group. Specific coagulation studies will be presented in a subsequent communication.

Possible central nervous system toxicity was difficult to evaluate. Patients on very high dosages did not complain of headache, vertigo, paresthesias, or paralysis. A few were drowsy or
slightly confused for brief periods. Some degree of nausea and vomiting, apparently unrelated to these observations, has been noted.

DISCUSSION

Intravenous L-asparaginase has been found to be highly effective in the induction of remission in acute lymphatic and acute granulocytic leukemia. Its usefulness has also been demonstrated in clearing the cerebrospinal fluid of cells in cases of central nervous system leukemia. Dosages in the range of approximately 2,000 IU per kg per day appear to be more effective in the induction of remission than do lower dosages, particularly in acute granulocytic leukemia. The effectiveness of even higher dosages on a continuous basis has not been evaluated.

The highly variable time interval required to obtain remission indicates that treatment should be continued as long as the hematologic status of the patient is improving. A definitive statement about the length of remissions obtained cannot yet be made. However, it is clear that some remissions are of short duration. Whether the combination of L-asparaginase with conventional modes of chemotherapy or the use of this enzyme alone in much higher doses will achieve a greater response awaits further evaluation. However, it is already clear that cross-resistance between L-asparaginase and conventional agents is not a problem.

The sensitivity tests in acute lymphatic leukemia suggest a high incidence of responsiveness. The clinical results indicate that a high percentage of these cases can obtain complete remissions from L-asparaginase therapy. Positive sensitivity tests were also found in a substantial proportion of the cases of acute granulocytic leukemia. However, the test was not done on the cells of the 4 patients in this group who obtained a complete bone marrow remission. Thus, further tests are needed in this group in order to define the relationship of the sensitivity test to clinical responsiveness.

Since the electrophoretically pure enzyme has an L-glutaminase activity which is from 1 to 3 percent as great as that of its L-asparaginase activity, it is possible that L-glutamine deprivation is of importance either alone or in combination with the L-asparaginase activity. At the daily dosage level of 2,000 IU per kg, as much as 60 IU of glutaminase activity per kg may also have been effectively administered.

The role of combination therapy has also not undergone adequate evaluation. Such studies are under way. However, since the toxicity of L-asparaginase is entirely different than that of the conventional modes of chemotherapy, it is expected that these agents can be used at or near their known effective dosages either simultaneously or in sequence with the administration of high doses of L-asparaginase.

L-Asparaginase is of special interest because it does not appear to be an immunosuppressive agent. In animals it has been shown that curative doses of L-asparaginase in mice with Gardner lymphosarcoma resulted in the development of long-term immunity preventing reimplantation of the tumor (16). In contrast, when L-asparaginase as well as 6-mercaptopurine were administered, the animals were cured of their tumor, but reimplantation of the tumor was possible.

The toxicity of L-asparaginase has been related to endotoxin-like properties of materials which are difficult to separate from the enzyme. The purification technics reported by Roberts et al. (18) have yielded a product which can be given in very high dosages.

The fibrinogen levels have declined during therapy. Nevertheless, in our experience, bleeding has not been a clinical problem. Indeed, our patients, even in the presence of low platelets, did not tend to hemorrhage after the onset of L-asparaginase therapy.

The intravascular destruction of amino acids by their specific enzymes appears to be a very promising area of research in the therapy of malignant disease. Comprehensive studies of the nutritional requirements of a broad number of tumor cell types and attempts to exploit these nutritional requirements by destruction of circulating nutrients certainly merit extensive trials. Hopefully, such studies will contribute significantly to the therapy of malignancies in man.

ADDENDUM

Recent clinical observations since submission of this paper for publication seem worthy of mention. Two adults with acute leukemia who developed a possible toxic syndrome on high dosages of L-asparaginase were seen within a ten-day period. Each developed a toxic syndrome consisting of nausea, vomiting, dehydration and hyperglycemia, drowsiness, and laboratory and clinical findings of acute pancreatitis. A woman with acute lymphatic leukemia received a total of 400,000 IU in four days with a maximum of 2,000 IU per kg per day. In a 40-year-old man with acute granulocytic leukemia, these symptoms developed more rapidly and more severely after he received a total of 941,000 IU in four days with an initial daily dose of 2,500 IU per kg and a maximum of 5,000 IU per kg. Both patients survived and are living today.

One additional example of pancreatitis was seen earlier in our series. The diagnosis was made 8 hours before death by the elevated serum amylase. Hemorrhagic pancreatitis was demonstrated at autopsy. Many other patients with similar higher dosages have not exhibited the above-mentioned findings.

The possible role of concomitant mild to moderate viral pancreatitis or of minute but undetectable amounts of endotoxin in the asparaginase preparations, and other factors such as preexisting conditions predisposing to pancreatitis, need to be considered. Nevertheless, it would seem wise to observe patients treated with high dosages of asparaginase carefully for the possible development of pancreatitis, especially when high dosage is reached abruptly. The above cases will be reported in other communications.

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