L-Asparaginase Pharmacokinetics and Asparagine Levels in Cerebrospinal Fluid of Rhesus Monkeys and Humans

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ABSTRACT

L-Asparaginase has been widely used for the treatment of acute lymphoblastic leukemia. Therapeutic and toxic effects in the central nervous system have been noted with systemic treatment. In order to better define the relationship between L-asparaginase administration and cerebrospinal fluid (CSF) asparagine levels, L-asparaginase and asparagine were measured in the CSF of rhesus monkeys following intrathecal and i.v. administration.

Following intrathecal injection, the enzyme activity of Escherichia coli L-asparaginase in the CSF demonstrated a more rapid terminal half-life than that of '**In-labeled diethylentriaminepentaacetic acid, a marker of CSF bulk flow [4 ± 0.7 (S.D.) hr versus 5.8 ± 0.2 hr]. Intrathecal injection of E. coli asparaginase resulted in complete depletion of CSF asparagine for at least 5 days. A similar period of CSF asparagine depletion was observed following i.v. administration of L-asparaginase. Similar results were found in seven patients undergoing systemic L-asparaginase therapy. The minimal plasma level of L-asparaginase necessary to deplete CSF asparagine in both species was 0.1 IU/ml.

Two other enzymes, Erwinia L-asparaginase and succinylated Acinetobacter glutaminase-asparaginase, were cleared from the CSF at the same rate as bulk flow.

These data indicate that systemic L-asparaginase therapy may be a feasible means of treating central nervous system involvement in patients with acute lymphoblastic leukemia and that there is no therapeutic advantage to using intrathecal L-asparaginase.

INTRODUCTION

The antitumor activity of the enzyme L-asparaginase is well established, and this agent is currently being utilized in the treatment of patients with acute lymphoblastic leukemia (5, 9, 13, 18).

L-Asparaginase exerts its antitumor activity by hydrolysis of asparagine to aspartic acid and ammonia. The depletion of asparagine selectively affects those leukemic cells which possess low levels of asparagine synthetase (16). In the absence of asparagine, a decrease of protein and glycoprotein synthesis occurs which ultimately leads to leukemic cell death (3, 14).

Although the pharmacology of Escherichia coli L-asparagi-nase has been studied extensively, the CNS pharmacokinetics of this enzyme has not been defined. CSF L-asparaginase activity has been reported to range from less than 0.1 to 0.8% of the corresponding plasma activity (8, 23, 24). Nevertheless, both neurotoxicity and remission of CNS leukemia have been reported after systemic administration of E. coli L-asparaginase (6, 7, 12, 24-26). In addition, i.t. administration of E. coli L-asparaginase in patients with acute lymphoblastic leukemia has produced CSF responses, and in the study of one such patient the enzyme was found to be rapidly cleared from the CSF (23-25).

The current study was undertaken to better define the relationship between systemic and CSF L-asparaginase and corresponding CSF asparagine levels and to investigate the pharmacokinetics of i.t. administered L-asparaginase. These pharmacological studies were pursued both in a subhuman primate model and in humans.

MATERIALS AND METHODS

Five adult male rhesus monkeys (Macaca mulatta) weighing between 6.5 and 8.1 kg were obtained from the NIH Primate Center. Each animal was kept in a separate cage and fed Purina monkey chow (Ralston, St. Louis, Mo.) and water ad libitum. In each animal, a silicone Pudenz catheter was surgically placed into the fourth ventricle and attached to a s.c.-implanted Ommaya CSF reservoir. This system permits sampling of ventricular CSF over extended periods of time without requiring chronic immobilization and provides mixing of injected drugs with lateral ventricular CSF (20, 27, 28). All injections and sampling were performed on conscious animals sitting in primate chairs. Venous blood samples were obtained from a heparinized indwelling femoral vein catheter. CSF samples were collected from the s.c. Ommaya reservoir using sterile technique. Aliquots of plasma and CSF were deproteinized with sulfosalicylic acid, adjusted to pH 2.6 with lithium hydroxide, frozen, and later thawed for amino acid analysis as described previously (11). All amino acids prepared in this manner are stable frozen for at least 21 months.4

The amino acids were analyzed on a Joel 5AH with 0.3 M lithium citrate buffer, pH 2.88, and Durrum resin 1A at 39° (Palo Alto, Calif.). Other aliquots were rapidly frozen at −20° and later assayed for L-asparaginase activity by 3 methods: (a) release of ammonia and direct nesslerization; (b) release of ammonia, distillation, and assay with a phenol-hypochlorite reagent; or (c) ['C]aspartate formation and separation on AG 1-X8 columns (BioRad Laboratories, Richmond, Calif.). These assays are described in detail in our previous publications (10, 11, 21). In general, the first assay was used for samples with enzyme activity greater than 1 IU/ml, the second assay was used for activity greater than 0.1 IU/ml, and the third assay was used for lower activi-

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4 The abbreviations used are: CNS, central nervous system; CSF, cerebrospinal fluid; i.t., intrathecal; DTPA, diethylentriaminepentaacetic acid.

D. S. Spackman, unpublished data.
ties. The limit of sensitivity of the third method was 0.0005 IU/ml. There was excellent agreement between the 3 assays. The enzyme activity in plasma and CSF appeared to be stable for at least 6 months when frozen.

E. coli L-asparaginase (Elspar-Merck, Rahway, N. J.) was radiolabeled with $^{125}$I by a lactoperoxidase-catalyzed reaction (22). Before and after iodination, the enzyme had a specific activity of 370 IU/mg protein. The iodination preparation had 8000 cpm/IU of enzyme activity; 92% of these counts were precipitable with cold sulfosalicylic acid. CSF bulk flow was measured by i.t. administration of 20 μCi of $^{111}$In-DTPA (Mediphysics, Emeryville, Calif. (1). Erwinia L-asparaginase was prepared by the PHLS Center for Applied Microbiology and Research, Salisbury, England. Acinetobacter glutaminase-asparaginase was purified and succinylated in our laboratories as described previously (10).

Curves of enzyme activity and radioactivity versus time were analyzed by ESTRIP, a basic program for analyzing pharmacokinetics data into a set of simple exponential decay curves (4).

RESULTS

Pharmacokinetics of i.t. Administered L-Asparaginase. Chart 1 compares the disappearance of DTPA, a measure of CSF bulk flow, with that of E. coli L-asparaginase following i.t. administration. Both materials were cleared rapidly from the CSF. The α phase of elimination of DTPA and E. coli L-asparaginase were similar. However, E. coli L-asparaginase demonstrated a significantly shorter ($p < 0.005$) terminal half-life compared to that of $^{111}$In-DTPA (Table 1).

The mechanism of the rapid CSF clearance of E. coli L-asparaginase was investigated with $^{125}$I-labeled enzyme. Chart 2 shows the disappearance of both enzyme activity and acid-precipitable radioactivity from the CSF and their appearance in the plasma. The ratio of acid-precipitable radioactivity to enzyme activity for all the CSF samples was 6960 ± 1420 (S.D.) cpm/IU; 92 ± 2% of the radioactivity was precipitable by acid. The ratios or percentage precipitable by acid did not change in a statistically significant manner with time. The $^{125}$I-labeled E. coli L-asparaginase injected had a ratio of acid-precipitable counts to enzyme activity of 7350 cpm/IU; 92% was precipitable by acid. Therefore, no appreciable degradation of enzyme occurred in the CSF. In contrast, only 68% of the plasma radioactivity was precipitable by acid, indicating that the enzyme was degraded in plasma.

The terminal plasma half-life of enzyme activity and acid-precipitable radioactivity were very similar at 17.5 and 21 hr, respectively. In another series of experiments, both Erwinia caratavora L-asparaginase and succinylated Acinetobacter glutaminase-asparaginase were administered. These results are shown in Table 1. The pharmacokinetics of these 2 enzymes was similar to that of DTPA, suggesting that they are cleared from CSF largely by bulk flow mechanisms.

L-Asparaginase Pharmacokinetics following i.v. Administration. Chart 3 plots the L-asparaginase level achieved in the plasma and in the CSF in 3 monkeys following 6000 IU/sq m of L-asparaginase given as a daily rapid i.v. infusion for 3 consecutive days. The disappearance half-time of L-asparaginase ranged from 15 to 30 hr in the different animals. The amount of L-asparaginase present in the CSF never exceeded 0.19% of the level present in the plasma.

CSF Asparagine Concentration following i.t. L-Asparaginase Administration. The CSF concentration of asparagine in 3 different monkeys following a single i.t. injection of L-asparaginase (1000 IU/sq m) fell from 3 to 4 μM to under the limit of sensitivity of our analysis (0.2 μM) for at least 5 days following intraventricular injection. Two of 3 monkeys had no asparagine detectable at Day 7 (Chart 4). Following i.t. injection of the same dose of succinylated Acinetobacter glutaminase-asparaginase, the concentration of asparagine was <0.2 μM for 3 days and returned to normal by Day 7. In the monkeys treated with Erwinia L-asparaginase, the concentration of CSF asparaginase was undetectable for 2 days.

Glutamine was depleted from a normal concentration of 310 to 460 μM to <5 μM at 1 hr after a dose of E. coli, but the levels returned to near normal by 24 hr. After injection of the Acinetobacter enzyme, the glutamine concentration was <5 μM for 24 hr in one monkey and 48 hr in the other. After injection of Erwinia L-asparaginase, glutamine was depleted for 24 hr in one monkey but was normal in the other animal. When the CSF glutamine was depleted, the CSF glutamate level was elevated. No changes were noted in the levels of aspartate, threonine, serine, glycine, or alanine.

CSF and Plasma Asparagine Concentration following i.v. L-Asparaginase Administration. Three different monkeys (4...
Table 1
CSF pharmacokinetics
All compounds were injected through an Ommaya reservoir. Asparaginase dose was 1000 IU/sq m. DTPA radioactivity was 20 μCi.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Monkey</th>
<th>α</th>
<th>β</th>
</tr>
</thead>
<tbody>
<tr>
<td>111m-DTPA</td>
<td>914</td>
<td>0.78</td>
<td>5.8</td>
</tr>
<tr>
<td></td>
<td>914</td>
<td>0.84</td>
<td>5.9a</td>
</tr>
<tr>
<td>1965</td>
<td>1.03</td>
<td>5.5</td>
<td></td>
</tr>
<tr>
<td>1965</td>
<td>1.05</td>
<td>6.0a</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.9</td>
<td>± 0.1b</td>
<td>5.8 ± 0.2b</td>
</tr>
<tr>
<td>E. coli L-asparaginase</td>
<td>914</td>
<td>1.0</td>
<td>3.8</td>
</tr>
<tr>
<td></td>
<td>915</td>
<td>0.6</td>
<td>3.0</td>
</tr>
<tr>
<td></td>
<td>765</td>
<td>0.6</td>
<td>4.7</td>
</tr>
<tr>
<td></td>
<td>255G</td>
<td>0.5</td>
<td>4.4c</td>
</tr>
<tr>
<td></td>
<td>0.7</td>
<td>± 0.2b</td>
<td>4.0 ± 0.7b,d</td>
</tr>
<tr>
<td>Erwinia L-asparaginase</td>
<td>1965</td>
<td>1.3</td>
<td>5.8</td>
</tr>
<tr>
<td></td>
<td>793F</td>
<td>0.1</td>
<td>6.1</td>
</tr>
<tr>
<td>Acinetobacter glutaminase</td>
<td>914</td>
<td>0.4</td>
<td>5.0</td>
</tr>
<tr>
<td>asparaginase (succinylated)</td>
<td>1965</td>
<td>0.4</td>
<td>5.1</td>
</tr>
</tbody>
</table>

- Injected with E. coli L-asparaginase.
- Mean ± S.D.
- Monkey injected with 113H-labeled E. coli L-asparaginase. Kinetic values presented are averages of data from enzyme activity and radioactivity.
- Significantly different from DTPA by Student t test (p < 0.005).

L-asparaginase achieved and the prolonged disappearance half-time of this enzyme. The plasma asparagine was undetectable when the plasma L-asparaginase concentration was greater than 0.03 IU/ml.

Chart 2 is a plot of plasma L-asparaginase activity and CSF asparagine in simultaneous samples obtained after i.v. L-asparaginase treatment. The data from the monkeys show that CSF asparagine is detectable only when the plasma L-asparaginase activity has decayed to less than 0.1 IU/ml. CSF L-asparaginase activity was <0.001 IU/ml in all the samples plotted in this chart. These data suggest that CSF asparagine is derived from plasma asparagine and that sufficient depletion of the plasma asparagine results in depletion of the CSF asparagine.

In contrast, after i.t. administration of this enzyme, CSF asparagine was still <0.2 μM when plasma L-asparaginase was 0.001 to 0.006 IU/ml and CSF L-asparaginase was <0.001 IU/ml. Thus, following i.t. L-asparaginase, CSF asparagine is depleted even if the plasma L-asparaginase concentration is lower than 0.03 IU/ml and plasma asparagine is detectable. The minimal CSF L-asparaginase activity needed to deplete CSF asparagine was below the limits of our assay sensitivity.

Chart 3. Plasma and ventricular CSF enzyme activity following 3 consecutive daily doses of L-asparaginase 6000 IU/sq m/day in 3 monkeys. Plasma and CSF enzyme activity are shown by open and closed symbols, respectively. O, •, Monkey 765; ▲, △, V, A, △ Monkey 914; □, ■, Monkey 1965.
Although measurable in extracts of monkey brain in vitro, is negligible in vivo (15).

**CSF Asparagine Levels in Humans following L-Asparaginase Therapy.** Five patients treated with i.m. *E. coli* L-asparaginase (6000 IU/sq m) on a 3-times-a-week schedule had no detectable asparagine in their CSF for at least 1 week after the L-asparaginase therapy was stopped. Two patients treated with i.v. L-asparaginase (10,000 IU/sq m) on a weekly schedule had a CSF asparagine concentration of <0.02 μM at 3 days following the dose. Four patients treated with this weekly schedule had detectable CSF asparagine at 5 to 8 days after the dose. One of the 2 patients treated with 10,000 IU/sq m twice weekly had detectable CSF asparagine at 3 days after a dose. The CSF asparagine levels and plasma L-asparaginase activity for 7 of these patients is shown as solid circles in Chart 5. These values were consistent with the monkey data, indicating that CSF asparagine is usually detectable when plasma L-asparaginase is <0.1 IU/ml.

**DISCUSSION**

Although CNS prophylaxis has improved the prognosis of patients with acute lymphoblastic leukemia, CNS leukemia still occurs and is generally associated with a poor prognosis. The need for improved chemotherapy of meningeal leukemia is well appreciated. Since L-asparaginase is an effective systemic antileukemic agent, the current study was undertaken to explore the pharmacological rationale for using this drug to treat CNS leukemia.

A drug chosen to treat meningeal leukemia by the i.t. route optimally should be slowly cleared from the CSF (2, 19). The results of the present study indicate that *E. coli* L-asparaginase does not appear to possess this characteristic. *E. coli* L-asparaginase was cleared from the CSF of monkeys faster than the rate of normal bulk flow when injected i.t. A clearance rate faster than bulk flow might be explained by rapid transcapillary movement, uptake by brain cells, or degradation of the enzyme in the CSF. The experiment with 125I-labeled *E. coli* L-asparaginase showed no breakdown of enzyme in the CSF. Whether active transport into brain capillaries or uptake by brain cells is responsible for the clearance of L-asparaginase from the CSF could not be determined from these studies. However, the rapid clearance found in this monkey model is consistent with previously reported data in a single patient treated with i.t. L-asparaginase (23).

The pharmacokinetics of *E. coli* L-asparaginase in plasma was studied by measuring both enzyme activity and the radio-labeled compound. Identical results were obtained with both methods. Following i.v. administration of *E. coli* L-asparaginase, the enzyme activity present in the CSF never exceeded 0.2% of the plasma levels. These results are consistent with the findings in humans (8, 23).

L-Asparaginase exerts its antileukemic activity by hydrolyzing asparagine, an amino acid required by L-asparaginase-sensitive leukemia cells which have little or no asparagine synthetase. Therefore, the CSF asparaginase level present following a dose of L-asparaginase should be a more reliable indicator of the potential antileukemic effect of L-asparaginase therapy.

Our studies demonstrate that although i.t. L-asparaginase is rapidly cleared from the CSF, it is able to deplete CSF asparagine for at least 5 days. It has been reported that a carrier process mediates the transport of asparagine across the blood-brain barrier (17). The rate of asparagine transport by this saturable system is believed to be very low. Asparagine can be synthesized in monkey brain to a limited extent (15). The prolonged depletion of CSF asparagine also suggests that both transport and synthesis of asparagine are slow.

The CSF pharmacokinetics of *Erwinia* L-asparaginase and succinylated *Acinetobacter* glutaminase-asparaginase was also studied since these enzymes have antileukemic activity in humans and differ from *E. coli* L-asparaginase in isoelectric point and activity towards glutamine. Both enzymes were rapidly cleared from the CSF but at a slightly slower rate than *E. coli* L-asparaginase (Table 1). After an i.t. injection of the same asparaginase activity, these enzymes depleted CSF glutamine for longer than *E. coli* L-asparaginase. This longer glutamine depletion is probably due to the greater glutaminase activity of three enzymes (9). One monkey had seizures after i.t. injection on April 15, 2017. © 1981 American Association for Cancer Research. cancerres.aacrjournals.org Downloaded from
of succinylated Acinetobacter glutaminase-asparaginase. Two other monkeys had no toxicity after i.t. injection of that enzyme. Both monkeys vomited during these experiments. However, seizures or other CNS signs were not seen after injection of E. coli or Erwinia L-asparaginase. Therefore, transient depletion of CSF glutamine and more prolonged depletion of CSF asparagine did not lead to consistent CNS signs.

The results of the current study also indicate that a moderate dose of i.v. E. coli L-asparaginase (6000 IU/sq m on 3 consecutive days) depletes CSF asparagine for an equally prolonged period of time. Depletion of CSF asparagine was seen even when the CSF L-asparaginase activity was below the level of detection by our assay (<0.001 IU/ml). We could not determine whether the effect of i.v. L-asparaginase on CSF asparagine was secondary to depletion of circulating levels of this amino acid or to undetectable enzyme activity that entered the CSF. Nevertheless, the plasma L-asparaginase activity appeared to correlate with CSF asparagine.

Analyses of the data obtained from patients undergoing L-asparaginase therapy confirmed the results obtained in the subhuman primate model. The minimal plasma level of L-asparaginase necessary to deplete CSF asparagine is similar in the 2 species. Furthermore, the human data indicate that the CSF asparagine depletion that occurs following systemic L-asparaginase therapy is dose and schedule dependent. Weekly doses of 10,000 IU/sq m of L-asparaginase that are commonly used for treatment of childhood acute lymphoblastic leukemia did not maintain the CSF asparagine level at <0.2 μM for the entire week. Two or 3 doses/week are probably needed for sustained depletion of asparaginase.

Systemic L-asparaginase treatment may be associated with neurotoxicity. The results of the present study are consistent with the hypothesis that L-asparaginase-induced CNS dysfunction may be the result of CNS asparaginase depletion. The present data also offer an explanation for the reversal of L-asparaginase-related neurological symptoms with infusion of L-asparagine, a phenomenon which has been recently reported (12). Whether systemically administered L-asparaginase plays a role in the long-term neurotoxic sequelae being observed in patients treated on current acute lymphoblastic leukemia treatment regimens is yet to be determined.

The results of this study suggest that systemic L-asparaginase therapy may be a feasible method of treating meningeval leukemia in the patient with acute lymphoblastic leukemia. Further studies are needed to better define the optimal dose and schedule required to induce depletion of CSF asparagine in humans and to correlate this depletion with the clinical antileukemic response in patients.

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