Human Pharmacology and Toxicology of Succinylated Acinetobacter Glutaminase-Asparaginase

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ABSTRACT

Succinylated preparations of Acinetobacter glutaminase-asparaginase (AGA) were administered i.v. to seventeen children with leukemia and three with solid tumors. The enzyme activity in plasma decreased with a half-life of 27.4 ± 7.4 hr. Doses of 200 IU/sq m or greater reduced the plasma glutamine and glutamate in plasma increased with dose. Urinary excretion of glutamate increased three thousand times, indicating that the high filtered loads exceed the renal capacity for reabsorption. Cerebrospinal fluid levels of glutamine increased despite the depletion of this amino acid in plasma. Pleural fluid levels of glutaminase reached about 15% of plasma level after three daily i.v. doses. Toxocity was very similar to that described with asparaginase enzymes. Nausea, vomiting, weight loss, transient fever, and hyperglycemia without ketosis or acidosis appear to be more frequent with the succinylated AGA preparations than with that reported with Escherichia coli asparaginase. Hypoproteinemia, hypocalcemia, and hypocholesterolemia were seen. No patients developed pancreatitis or changes in renal function. Five leukemic patients became comatose during treatment. All of these patients had central nervous system leukemia or cranial radiation and intrathecal methotrexate. One patient developed an allergic reaction to the succinylated AGA, characterized by a rash, accelerated plasma clearance, and circulating antibodies to AGA.

Antileukemic activity was demonstrated by a reduction in circulating blasts by 1 log, or greater in 50% of the patients and a partial remission in two patients. Two patients with solid lymphoid tumors showed a 50% or greater decrease in the size of the masses.

INTRODUCTION

Enzymes with glutaminase and asparaginase activity have antitumor activity towards transplantable mouse tumors that are resistant to asparaginase (for review, see Refs. 10 and 11). These enzymes are also cytotoxic to human leukemic cells in vitro (23). Initial clinical trials showed that an Achromobacter glutaminase-asparaginase preparation decreased blast counts in patients with acute myelocytic and lymphocytic leukemia (27). This enzyme had a plasma half-life of about 1 hr, necessitating constant infusions of 10,000 to 20,000 IU/sq m/day to deplete plasma glutamine. Preliminary studies showed that AGA also had a very short half-life in humans (8). Chemical modification of free amino groups can increase the plasma half-life of some asparaginase and glutaminase-asparaginase enzymes in animals (12, 20). In previous studies, we have shown that treatment of AGA with succinic anhydride produces a uniform preparation with the same catalytic activity and physical properties, but with an increased plasma half-life in mice, rats, and rabbits. Succinylated enzyme preparations have now been tested in 20 children with leukemia and other tumors refractory to conventional chemotherapy. This report describes the human pharmacology of the succinylated AGA and its effect on amino acid levels in plasma, cerebrospinal fluid, and urine. A preliminary report has appeared describing the antitumor activity and toxicity in the first 14 patients (9).

MATERIALS AND METHODS

Enzyme Preparations. AGA was purified to homogeneity and succinylated in our laboratory by a slight modification of our method (12) suggested by D. J. Solley and J. Roberts, Sloan Kettering Institute for Cancer Research, Rye, N. Y. The enzyme was prepared from the same lot of frozen Acinetobacter glutaminisidans cells (ATCC 27197). The succinylated enzyme was stored as a lyophilized powder with no loss of activity during 5 months of storage. Eleven preparations of AGA were succinylated in lots of 15,000 to 34,000 IU. After succinylation, they had a specific glutaminase activity of 109 to 150 IU/mg protein, a pI of 5.0 to 5.7, and a plasma half-life of 3.9 to 11.2 hr after i.p. injection in normal male ICRI mice (Sprague-Dawley, Madison, Wis.). The native enzyme had the same specific activity, a pI of 8.2, and plasma half-life of 1 hr. The physical properties of the enzyme are not altered by succinylation (12). Antibodies produced in rabbits to succinylated and native enzyme cross-react completely.4 Endotoxin was monitored by a Limulus amebocyte lysate kit (Worthington Biochemical Corp., Freehold, N. J.). The preparations contained 76 to 690 ng Food and Drug Administration endotoxin equivalents per 1000 IU glutaminase activity. Glutaminase-asparaginase activity was assayed as described previously by ammonia formation from asparagine, except that the ammonia was distilled or assayed by conversion of [14C]asparagine to [14C]aspartate (12). The amino acid concentration of sulfosalicylic acid extracts of plasma, urine, cerebrospinal fluid, and pleural fluid were determined on a Joel 5AH analyzer with lithium citrate buffers, Joel Resin AR-50, or Durran Resin 1A at 39 and 63° (13). Ammonia concentration was measured by distillation of these extracts. The concentration of glutamate was much higher than that of asparagine and

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3 The abbreviations used are: AGA, Acinetobacter glutaminase-asparaginase; ALL, acute lymphocytic leukemia.
4 G. Schmer and J. Holcenberg, unpublished data.

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glutamine in some sulfosalicylic extracts. A 2-ml portion of these extracts was neutralized with lithium hydroxide and chromatographed on 0.4- x 6-cm Pasteur pipet columns of AG 1-X8 (200 to 400 mesh) previously washed and neutralized in 10 mm imidazole buffer, pH 7. The columns were washed with 4 ml of the same buffer and chromatographed on the analyzer. This treatment removed most of the glutamate and aspartate but did not alter the levels of the other amino acids.

Portions of kidney tissue obtained during postmortem examination were homogenized in a sucrose:salts solution and centrifuged at 100,000 x g for 60 min. Glutamyl transferase activity was assayed on the supernatant fluid by the conversion of glutamine to glutamyl hydroxamate. Under the conditions of the assay, it measures glutamine synthetase activity (23).

**Patient and Treatment Protocol.** A protocol was approved by the Human Research Review Committees of the Medical College of Wisconsin and Milwaukee Children’s Hospital. All patients had leukemia or solid tumors refractory to conventional chemotherapy. The patients with “common” and T-cell acute lymphocytic leukemia had relapsed on combination drugs including L-asparaginase.

The enzyme was dissolved in sterile 0.9% NaCl solution at 90 to 550 IU/ml and filtered through a disposable 0.22-μm Millex 25-mm filter (Millipore Corp., Bedford, Mass.). The enzyme solution was kept at 5° and used within 3 days or refiltered. It was stable under those conditions. All doses were administered i.v. over 5 to 10 min. On the first day, all patients received a test dose of 25 IU/sq m. A larger dose was administered in 30 to 60 min, since no immediate reactions were seen with the test dose. Subsequently, patients were treated with a single daily dose of the enzyme. The first five patients began treatment at doses of 200 to 300 IU/sq m. The dose was escalated every 2 to 4 days. Since most patients tolerated 1000 IU/sq m, six patients were started at this dose. Two patients received single doses of native AGA. All nonessential medication was stopped. Nausea was treated with i.v. administration of bolus doses of perphenazine or benzquinamide or by continuous infusions of perphenazine.

All patients were monitored by daily physical examinations, complete blood counts, and reticulocyte and platelet counts. Ammonia, serum electrolytes, pH, glucose, creatinine, liver function tests, amylase, uric acid, blood urea nitrogen, and clotting studies were obtained at least 2 times/week. Weekly calcium and phosphorous levels were followed in 12 patients. Timed urine collections were obtained to monitor amino acid and ammonia excretion. RBC and platelets were administered as needed. Insulin levels were kindly determined by Dr. R. K. Kalkoff, Medical College of Wisconsin.

**Determination of Antibodies to the Enzyme.** Serum antibodies to AGA were tested by a radioimmunoassay. Native and succinylated AGA were labeled with 125I in a lactoperoxidase-catalyzed reaction (21). Preliminary experiments indicated that 33% saturated ammonium sulfate could separate enzyme from enzyme-antibody complexes. This concentration of ammonium sulfate precipitated 10 ± 3% S.D. of 125I-labeled AGA alone or mixed with normal rabbit or human serum, and 80 ± 5% of 125I-labeled enzyme and anti-AGA rabbit serum. The specificity of this reaction was tested by adding increasing concentrations of nonlabeled AGA to the iodinated AGA:antibody mixture. The amount of 125I precipitated by antibody decreased linearly with nonlabeled enzyme concentration. Based on these data, the following radioimmunoassay was developed. 125I-Labeled AGA (0.2 μg) in 50 μl of phosphate-buffered saline (15 mm phosphate in 160 mm NaCl, pH 7.4) was mixed with 50 μl patient’s serum undiluted or serially diluted with phosphate-buffered saline. Controls included 125I-labeled AGA alone, 125I-labeled AGA plus rabbit anti-AGA serum (1:20 and 1:40), and 125I plus normal rabbit serum or human serum. Plasma samples from the patients were tested in duplicate. The reagents were incubated at 37° for 30 min, and at 4° for an additional 30 min. After the addition of 200 μl of fetal calf serum and 1 ml of 43% saturated ammonium sulfate, the reagents were mixed, incubated at room temperature for 30 min, and centrifuged at 1000 x g for 15 min. The radioactivity in the pellet and supernatant was measured in a γ spectrometer.

**RESULTS**

**Plasma Levels.** Chart 1 shows the change in plasma activity of succinylated AGA with time. The values can be described by the sum of 2 exponentials. The half-life of the initial phase was 1.2 to 1.8 hr in 4 patients studied. This phase may be due to distribution of the enzyme in the body or to a small amount of native enzyme in the succinylated preparations.

Values obtained 10 or more hr after a dose show a linear decrease in enzyme activity on this semilog plot. The half-life of the terminal phase is 27.4 ± 7.4 hr (range, 17 to 44) in 14 patients. The values in Chart 1 cannot be used directly to calculate the volume of distribution, since these levels were often obtained after repeated daily doses. An approximate volume of distribution was calculated from the enzyme activity one day after the first dose and the elimination rate constant of each patient. The volume of distribution in 11 patients was
2900 ± 910 ml/sq m (range, 1910 to 4725). This value is greater than twice the average plasma volume in children (1).

Glutaminase activity was routinely measured 17 to 24 hr after a dose. Chart 2 shows that a plot of enzyme activity increases linearly with the dose administered. Glutamine and asparagine levels decreased from pretreatment values of 439 ± 114 and 43 ± 20 μM (N = 17), to undetectable levels (<2 μM) after doses of 200 IU/sq m or greater. The levels of these amino acids remained undetectable until the plasma glutaminase activity decreased to less than 0.06 IU/ml. Glutamate and aspartate levels before treatment were 46 ± 20 and 13 ± 11 μM, respectively (N = 8). Chart 3 shows that the levels of these amino acids increased with plasma glutaminase activity. At a glutaminase activity of 0.56 IU/ml, the average glutamate and aspartate levels were 50 and 6 times their respective pretreatment levels. Threonine levels also increased in 12 of these patients; the average increase was 2.8-fold (range, 0.7- to 7.2-fold). In contrast, urea, serine, glycine, alanine, valine, isoleucine, leucine, methionine, tyrosine, and phenylalanine levels increased less than 2-fold. In 5 patients, urea levels fell to 20 to 70% of pretreatment levels. Ammonia levels also increased in all patients during therapy; maximal values were 210 to 750 μM. The plasma ammonia levels did not correlate with glutaminase or glutamate levels.

Urinary Excretion. The extremely high levels of glutamate and aspartate exceed the renal capacity for reabsorption. Table 1 presents the changes in renal clearance and excretion of these amino acids during treatment of 12 patients. The clearance and excretion of aspartate and glutamate increased in each patient studied. Glutamate excretion was most affected; the average increase was 3 thousand-fold. Ammonia excretion increased approximately 10-fold. The clearance and rates of excretion of threonine, serine, and glycine were also calculated. The clearance of these amino acids increased 3- to 5-fold; the excretion rates increased 4- to 14-fold. Urea clearance did not change appreciably.

Table 1 also shows that the patients continued to excrete glutamine despite depletion of plasma levels of glutamine to below 2 μM. This persistent glutamine excretion was surprising in light of reports that the human kidney has very little capacity to synthesize glutamine (16).

The ability of the kidney to synthesize glutamine was investigated in 2 ways. First, glutamyl transferase activity was measured in 100,000 × g supernatant fractions from postmortem samples of kidney cortex of 2 patients who died during therapy, 1 patient who died 1 month after stopping therapy, and 2 patients with unrelated nonrenal diseases. All samples were obtained within 6 hr of death. The enzyme activity was 4.1 to

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**Table 1**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Maximal clearance (ml/min/1.73 sq m)</th>
<th>Maximal urinary excretion (mmol/hr/1.73 sq m)</th>
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<tr>
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</table>

Values before treatment in 9 of these patients

| Av. ± S.D. | 17 ± 17 | 1.7 ± 3.9 | 0.006 ± 0.004 | 0.004 ± 0.008 | 0.025 ± 0.016 | 1.2 ± 0.4 |

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**Chart 2.** Plasma glutaminase activity 17 to 24 hr after a dose of glutaminase. Points, means; bars, S.E. —, linear least-squares fit of the means.

**Chart 3.** Plasma free glutamate and aspartate at various plasma glutaminase levels. Points, mean; bars, S.E. —, linear least-squares fits of the means.
Effect of treatment on cerebrospinal fluid amino acids

<table>
<thead>
<tr>
<th>Time on therapy (days)</th>
<th>Concentrations in acid extracts of spinal fluid (μM)</th>
<th>Plasma glutaminase (IU/ml)</th>
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<tr>
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</table>

Av. ± S.D. of treated (N = 11): 7.5 ± 6.9, 4.0 ± 3.9, 79 ± 67, 1225 ± 556

Av. ± S.D. of control (N = 7): 1.4 ± 0.8, 5.3 ± 2.7, 1.0 ± 1.5, 402 ± 83

p<0.05, NS<0.005, <0.005

a Control patients have leukemia without central nervous system disease.
b From Student’s t test.
c NS, not significant.

6.5 nmol/min/mg protein in the samples from patients off therapy and 11.1 and 18.9 nmol/min/mg protein in the samples from patients on treatment. Second, the degree of glutamine synthesis was assessed by amino acid levels in the kidneys from these patients. Free glutamine levels in the kidneys on treatment were 0.12 and 0.11 μmol/g wet weight and 0.26 and 1.00 μmol/g wet weight in the controls. In contrast, asparagine was undetectable in treated samples and 0.10 and 0.27 μmol/g wet weight in controls. Thus, the rate of glutamine synthesis was sufficient during treatment to maintain a measurable intracellular level and to excrete this amino acid in the urine.

Cerebrospinal Fluid. Lumbar spinal taps were obtained in 8 patients during therapy. Glutaminase activity was 0.001 to 0.004 IU/ml in 5 of these patients; the average activity was 0.5% of the plasma values at the time of the collection. This percentage is similar to the values reported for Escherichia coli asparaginase (6, 24). Table 2 shows the levels of free aspartate, asparagine, glutamate, and glutamine in these patients. Control values were obtained from 7 children with leukemia (4 were subsequently treated with the enzyme). The levels of aspartic and glutamic acid were near the level of detection of the analyzer in the control samples. Aspartic and glutamic acid levels increased an average of 5- and 79-fold during treatment (p < 0.05). Asparagine levels were lower than controls in only some of the samples. Surprisingly, glutamine levels rose an average of 3-fold during treatment (p < 0.005), despite the absence of circulating glutamine in these patients. This elevated glutamine level may explain the observation that 2 of our patients had progressive central nervous system leukemia during treatment.

The cerebrospinal fluid levels of other neutral and acidic amino acids were increased an average of 1.7 to 3.8 times the control values. Levels of urea were similar to control values.

Pleural Fluid. Three patients had large pleural effusions. Chart 4 shows the glutaminase activity in plasma and pleural fluid in one of these patients. With 3 daily i.v. doses, the enzyme activity in pleural fluid slowly increased to about 16% of the plasma level. An intrapleural dose produced high levels that decreased with a half-life that appeared to be longer than the plasma half-life calculated from an i.v. dose. In another patient, the intrapleural glutaminase level was 13% of the plasma value 24 hr after 3 daily i.v. doses. The enzyme activity in the pleural fluid was 1.5 times the plasma level in samples obtained 4 days after 7 daily i.v. doses administered to a third patient.

Toxicology. One patient died of intestinal obstruction from lymphoma 2 days after starting enzyme therapy. Table 3 summarizes the toxicity in the other 19 patients. All but 4 patients had nausea, vomiting, and anorexia that appeared to increase with AGA dose. These symptoms were adequately controlled.
with antiemetic drugs in all but 2 patients. Weight loss averaged 0.6 ± 0.3% of body weight per day (N = 16). A transient fever of 1–2°, usually associated with a chill, occurred about 1 hr after the first dose of enzyme in 7 patients and after the first 2 doses in one patient. This fever was less frequent in patients who received enzyme preparations with low endotoxin levels.

Seven patients developed hyperglycemia without ketosis or acidosis after 5 to 30 doses of 500 to 1500 IU/sq m/day. Peak blood sugar levels were 170, 260, 410, 440, 450, 480, and 590 mg/dl. Plasma insulin levels were less than 30 pm units/ml during the hyperglycemia in 4 of these patients. These levels returned to normal within days after stopping the enzyme therapy in 6 of these patients; 4 patients were treated with 3 to 15 units of insulin. The seventh patient died of a gastrointestinal hemorrhage while his blood sugar level was returning to normal.

Albumin, fibrinogen, and cholesterol levels decreased in some patients. Prothrombin times were prolonged by 2 to 4.5 sec in 9 patients; partial thromboplastin time was elevated in only 3 patients. Most patients developed a mild respiratory alkalosis with a venous total CO2 of 14 to 20 mEq/liter. Calcium was decreased in one-half the patients tested. No abnormalities were noted in urinalysis, serum amylase, blood urea nitrogen, creatinine, or bilirubin. The serum glutamic oxaloacetic transaminase and alkaline phosphatase rose slightly in one and 5 patients, respectively.

Myelosuppression is difficult to evaluate in these children because of extensive prior chemotherapy or radiotherapy and bone marrow replacement by tumor. Ten of these patients did not require transfusions of RBC or platelets prior to enzyme therapy. During therapy, the granulocyte, platelet, and reticulocyte counts decreased by more than 50% in 8, 7, and 9 patients, respectively. Hemoglobin decreased more than 20% in 3 patients. Two patients without bone marrow disease had no myelosuppression.

Five leukemic patients became comatose during therapy. Two of these patients had central nervous system leukemia that progressed during enzyme therapy. A third patient had hyperglycemia and severe gastrointestinal hemorrhage. These 3 patients died. Another patient had staphylococcal septicaemia and a rapid rise in peripheral blasts. He awakened 2 days after enzyme therapy was stopped, and antibiotics and hydroxyurea were administered. The fifth patient had prior central nervous system leukemia, cranial irradiation, and extensive intrathecal methotrexate. She became unresponsive, with intermittent screaming and decorticate posturing 7 hr after 2 daily doses totaling 1500 IU/sq m/day. Generalized seizures and left-sided weakness developed. Cerebrospinal fluid pressures were normal. One dose of mannitol was given; anticonvulsants and dexamethasone were started. She awakened in 5 days and appeared normal 2 days later. She was discharged and died of leukemia 1 month later. Autopsy showed perivascular leukemic infiltrates in the cerebral parenchyma and spinal cord and reactive astrocytes in the insular cortex.

All patients were monitored for allergy to AGA by clinical signs and plasma enzyme and antibody levels. Immune response to AGA was seen in only 1 of 19 patients, despite treatment for up to 60 days. That patient developed an erythematous rash, chills, and fever after the 16th daily dose of enzyme. Plasma enzyme activity was at the expected level, so treatment was continued with AGA and diphenhydramine. The systemic symptoms disappeared by the 20th dose. One day after the 21st and 22nd doses, enzyme activity was near zero, and glutamine and asparagine had reappeared in the plasma. This is the only patient with detectable serum antibodies to AGA. Free AGA antibodies were first demonstrated in the plasma sample obtained one day after the 22nd dose. The reaction of this antibody with AGA was inhibited by excess unlabeled AGA, but not by E. coli asparaginase or human serum.

Autopsies were performed on 11 patients; 7 died within 5 weeks after the conclusion of the enzyme therapy. Three of these 7 had moderate to marked fatty changes and infiltrates of leukemia or lymphoma in the liver. One patient showed focal areas of pancreatic necrosis without hemorrhage and slight renal tubular swelling. All other pathological changes were consistent with widespread cancer.

**Antitumor Activity.** Table 4 presents the effect of the succinylated AGA on the patients with various subtypes of leukemia. The treatment decreased the blast count by greater than 1 log10 in one-half the patients with "common" and T-cell leukemia and in the only patient with acute myelogenous leukemia. The patients with T-cell and "common" ALL were all clinically resistant to asparaginase. In 4 of these patients, lymphoblasts were eliminated from their blood. One patient with "common" ALL developed bone marrow aplasia after 15 doses of enzyme, and treatment was stopped. Normal marrow returned in 2 weeks with less than 25% lymphoblasts. One patient with T-cell leukemia had a partial bone marrow remission. A patient with B-cell leukemia and one with lymphoma had greater than 50% decreases in measurable masses during therapy. No response was seen in individual patients with Ewing's sarcoma and ovarian teratocarcinoma.
DISCUSSION

Glutamine depletion therapy by an enzyme requires a preparation with high activity, a low \( K_m \) for glutamine, low endotoxin levels, no product inhibition, and no cofactor requirements. These criteria are met by native AGA and the Achromobacter enzyme studied by Spiers and Wade (27). Unfortunately, these enzymes are rapidly cleared from circulation, necessitating constant infusions of large amounts of enzyme to maintain low glutamine levels. Our data show that single daily doses of succinylated preparations of AGA deplete circulating glutamine levels and produce plasma enzyme levels that decrease with a half-life of about 1 day. This half-life is similar to those of native \( E. \) coli and Erwinia asparaginase preparations. Also, like asparaginase, succinylated AGA appears to be distributed in a volume larger than the plasma space, a distribution that produces appreciable levels in pleural fluid and very low levels in cerebrospinal fluid (3, 4, 6, 24).

Succinylated AGA differs from asparaginase in its effects on amino acid levels in body fluids. Asparaginase produces prolonged depletion of plasma asparagine, transient depletion of plasma glutamine, marked elevation of ammonia, 3- to 4-fold elevation in glutamate, and little effect on other amino acids (15, 17). Succinylated AGA produces prolonged depletion of both asparagine and glutamine, similar elevation of ammonia, and much greater elevation of glutamate, aspartate, and threonine levels. During treatment with succinylated AGA, the very high levels of glutamate and aspartate exceed the renal threshold for reabsorption and lead to large urinary losses of these amino acids.

The levels of glutamine in body fluids and cells probably depend on the rates of synthesis and utilization of this amino acid, the concentration of glutaminase in surrounding fluids, and the rate of transport of glutamine into these fluids. These multiple factors may account for our observations that the levels of glutamine were undetectable in plasma, elevated in cerebrospinal fluid, and detectable in urine and kidney extracts during treatment. Our experience with 2 patients suggests that the cerebrospinal fluid may be a sanctuary for leukemic cells that are sensitive to glutaminase treatment in other sites.

The observed toxicity of succinylated AGA is very similar to that reported with L-asparaginase (5, 14, 15, 19). For instance, neurotoxicity was observed in 5 of 20 patients during therapy with succinylated AGA. All of these patients had central nervous system leukemia, septicemia, or severe hemorrhage and hyperglycemia that may have contributed to the coma. Somnolence, lethargy, disorientation, convulsions, and/or coma have been reported in up to 50% of adults and 28% of children treated with asparaginase (5, 14, 15, 19).

The mechanism for the central nervous system toxicity of asparaginase and succinylated AGA is not known. Blood ammonia levels do not correlate with symptoms (15). Furthermore, no consistent differences were seen between the levels of neutral and acidic amino acids from cerebrospinal fluid or plasma in treated patients, with and without neurotoxicity.

Treatment with succinylated AGA increased the level of glutamine in cerebrospinal fluid in our patients. Glutamine levels are increased in the brain of experimental animals by AGA therapy, hepatic coma, portacaval shunting, and loads of ammonia and glutamate (13). These increased levels are probably caused by high glutamine synthetase activity in brain tissue. High glutamine levels can lead to accumulation of \( \alpha \)-ketoglutaramate, a metabolite that is elevated in cerebrospinal fluid of patients with hepatic coma (28). The spinal fluid level of \( \alpha \)-ketoglutaramate was not elevated in one of our comatose patients (courtesy of Dr. T. E. Duffy, Cornell University Medical College).

Hyperglycemia with low insulin levels has been reported in patients treated with asparaginase, but the frequency may be higher in patients receiving succinylated AGA (15, 29). At the levels of enzyme used in this study, hyperglycemia could be controlled with low doses of insulin and, therefore, need not be a dose-limiting toxicity.

Myelosuppression was difficult to evaluate in our patients because of extensive bone marrow infiltration, prior chemotherapy, and radiotherapy. Most of our patients with bone marrow disease showed a progressive decrease in blood counts during therapy with this enzyme. These observations are consistent with the myelosuppression noted in normal mice (22) and in 5 of 6 patients treated by Spiers and Wade (27). In contrast, 2 of our patients without bone marrow disease had no myelosuppression. Further observations are needed in patients without bone marrow disease to evaluate the myelosuppression produced by this enzyme.

Although none of our 20 patients achieved a complete remission, a marked reduction of circulating blasts was observed in one-half of the patients, and 2 of them achieved a partial bone marrow remission. A similar decrease in blast counts but no remissions was observed in 6 patients treated with an Achromobacter glutaminase-asparaginase (27). Much higher doses and constant infusions were needed in that study because of the rapid clearance of that enzyme.

The toxicity is tolerable at doses up to 2000 IU of succinylated AGA per sq m per day. Further clinical trials should explore the antileukemic effect at higher dosages. The limited effect of AGA and asparaginase on solid tumors and central nervous system leukemia may be due to high levels of glutamine provided by surrounding tissues. We have observed a potentiation of glutaminase effect by a new glutamine analog (NSC 163601) on s.c. Ehrlich ascites carcinoma tumors in mice (7). Hopefully, similar combinations may extend the antitumor spectrum of AGA in humans.

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