Amino Acid Levels following L-Asparagine Amidohydrolase (EC.3.5.1.1) Therapy

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SUMMARY

The blood levels of the dicarboxylic amino acids and their amides were determined at various periods during the course of L-asparaginase treatment of a number of patients with asparaginase-sensitive and asparagine-insensitive malignancies. Asparagine levels decreased rapidly at the start of treatment, falling essentially to zero with low levels of enzyme. Glutamine levels also dropped rapidly, reaching zero a day or two after the asparagine levels had done so. At this time the glutamate usually reached a peak level; in one case, glutamate was four times the normal level. The results of complete amino acid analyses revealed no remarkable changes in the concentrations of any other amino acid. Glutamine began to reappear as soon as the initial course of therapy (200 IU/kg/day) was interrupted, whereas asparagine levels remained negligible until the plasma asparaginase level was no longer measurable. Study of the bloods of a number of patients being maintained in hematologic remission for a period of months on continuing dosage of enzyme (10 or 50 IU/kg/day) revealed essentially normal glutamine levels in all cases. The body can apparently overcome the effect of at least 50 IU/kg of this Escherichia coli L-asparaginase preparation on the serum glutamine level, possibly because this falls within normal synthetic capacity or because of an increase in glutamine synthetase levels following initial therapy at asparaginase levels high enough to cause large drops in the blood glutamine levels. The observed sensitivity of the tumor to asparaginase could not be correlated with any changes in the amino acid levels during therapy.

INTRODUCTION

In 1953, Kidd (10) observed the inhibition of the growth of transplanted lymphomas in mice and rats by guinea pig serum. In 1956, Neuman and McCoy (13) showed that Walker carcinosarcoma 256, grown in tissue cultures, had an absolute requirement for asparagine. These two apparently unrelated observations were brought together by Broome (4), who in 1963 showed that those lymphomas which were sensitive to guinea pig serum in vivo required asparaginase for growth in vitro and suggested that the asparaginase activity of guinea pig serum was the active antitumor agent. In 1964, Mashburn and Wriston (12) showed that an L-asparaginase isolated from Escherichia coli had the same effect as guinea pig serum asparaginase. By 1966-1967 at least three groups of investigators (3, 16, 17) had reported on the isolation and the purification of the E. coli enzyme. The appearance of a reasonably stable, high specific-activity enzyme preparation from a readily available source such as E. coli made it feasible to test the effect of this enzyme clinically.

This paper presents some biochemical observations on a number of patients being treated and maintained with E. coli L-asparaginase [L-asparagine amidohydrolase, (EC.3.5.1.1)].

MATERIALS AND METHODS

The L-asparagine amidohydrolase (EC.3.5.1.1) used therapeutically was obtained from Worthington Biochemical Corp. as a sterile frozen solution in 0.05% glucose at pH 7.0. This enzyme was made up to 5% with respect to glucose and administered by slow intravenous injection (14).

Blood samples were drawn into heparinized tubes and chilled instantly. The chilled samples were centrifuged in the cold and the resulting plasmas stored in the frozen state. The samples were deproteinized by the method of Stein and Moore (18) for determination of asparaginase levels. Recoveries of asparagine under conditions of asparaginase therapy were checked in a number of cases. Exactly 1.0 ml of a solution of L-asparagine-4-14C (0.25 µg, 2.5 mCi/ml) was added to a known volume of the freshly drawn blood. Asparagine was determined as indicated. Even in the presence of relatively high blood levels of asparaginase, 80% or more of this label could be recovered as asparagine.

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Clinical case histories for patients L. K. and M. J., details of enzyme administration, pyrogenicity tests, and clinical criteria for enzyme sensitivity and disease remission have been presented in detail by Oettgen et al. (14).
RESULTS

Chart 1 shows serum levels of asparagine and glutamine and the plasma L-asparaginase level found in blood samples during the initial course of treatment of a patient (L. K.) with an asparaginase-sensitive tumor. Blood samples were taken just prior to enzyme administration. The results from the trinitrophenylation procedure (solid symbols) and those from the amino acid analyzer were in essential agreement. Certain interesting features emerge. First, the asparagine levels decreased rapidly after institution of treatment, falling to a very low level before any residual serum asparaginase level could be measured. The serum glutamate level rose to a peak value about three times the starting value on 4/17 at the same time that the amide concentration reached zero. There was no remarkable change in the level of aspartate during treatment.

Chart 2 gives the data for the initial course of treatment of a patient (M. J.) with an asparaginase-insensitive tumor. The changes seen here, for the most part, duplicate those seen in the first case. Asparagine fell almost to zero with a dose of 0.2 IU/kg/day, which was only one-thousandth of the maximum daily dosage used in this course of therapy. The serum glutamate values showed no consistent pattern. In this case, however, the asparaginase began to rise to normal levels even with continued therapy and a serum asparaginase level of over 3 units/ml and then returned to zero. The higher asparagine values seen in these intermediate days were confirmed with both analytical methods, and acid hydrolysis of these samples resulted in the quantitative conversion of the asparagine to aspartic acid. The asparaginase level remained negligible after asparaginase administration was halted, and serum asparaginase levels fell below detectable limits. Glutamine levels began to rise when therapy was stopped despite the fact that serum levels of asparaginase were high for several additional days.

A number of analyses were performed on sera of patients in various stages of treatment. The first set of figures in Table 1, 2

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Chart 1. Serum asparagine, glutamine, and asparaginase levels during an initial course of L-asparagine amidohydrolase (EC. 3.5.1.1) therapy on a patient (L. K.) with an asparaginase-sensitive tumor. Solid symbols represent values obtained by the trinitrophenylation procedure. All other values are from the Amino Acid Analyzer. Solid lines represent asparaginase levels; dashed lines represent glutamine levels. The histogram blocks represent L-asparaginase levels. Pretreatment levels were as follows: asparagine, 30 μM; glutamine, 276 μM; aspartic acid, 13 μM; glutamic acid, 117 μM.

Chart 2. Serum asparagine, glutamine, and asparaginase levels during an initial course of L-asparagine amidohydrolase (EC. 3.5.1.1) therapy on a patient (M. J.) with an asparaginase-insensitive tumor. Solid symbols represent values obtained by the trinitrophenylation procedure. All other values are from the Amino Acid Analyzer. Solid lines represent asparaginase levels; dashed lines represent glutamine levels. The histogram blocks represent L-asparaginase levels. Pretreatment levels were as follows: asparagine, 18 μM; glutamine, 240 μM; aspartic acid, 12 μM; glutamic acid, 230 μM.

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2 1.0 International Enzyme Unit (IU) of enzyme will hydrolyze 1.0 μmoles of substrate per minute from 0.01 M substrate solution at 37°C.
Amino Acid Levels following L-Asparaginase Therapy

WEB 3/28 and 4/6, are from another patient with an apparently insensitive tumor (14) during his initial course of therapy. Both glutamine and asparagine fell to zero and glutamate showed a four-fold increase. The second patient in this table, L. K., is the patient referred to in Chart 1. This blood was taken about three months after the initial course and during a period when asparaginase was being withheld. The levels of both asparagine and glutamine appear to be normal. The remaining patients in this group were maintained in remission, on asparaginase therapy, over a period of months. Two were maintained on 70 IU/kg/week and four on 350 IU/kg/week, (two-sevenths of the weekly dose on Monday and Wednesday, three-sevenths on Friday). In every case the asparagine level was zero and the glutamine levels close to normal. The patients on the lower dosage maintained low but measurable serum asparaginase levels; those on the larger dosage showed proportionately higher serum enzyme levels.

Table 2 shows the analyses of the red blood cells of two patients and one normal subject. The first patient showed no clear clinical response at this time, 2.5 weeks after the initiation of therapy; the second was in complete hematologic remission 3.5 months after initial therapy (14). In both cases the red cells were devoid of both asparagine and glutamine. Glutamine was present in the sera of the second patient before, after, and, presumably, on the day on which the red cells were collected. Serum values for the first patient are not available. In the normal control, the values of both amides were quite high.

DISCUSSION

The initial course of asparaginase therapy results in a rapid and marked depression in the serum level of asparagine. The drop begins promptly even when relatively small doses are given. In the initial trials, dosage was increased rapidly so as to rapidly establish a measurable blood asparaginase level. The drop in asparagine was followed closely by a dramatic drop in the glutamine level to zero, a result not anticipated, but explainable on the basis of the small but definite glutaminase activity of this enzyme (8). We have confirmed the presence of glutaminase activity in this enzyme preparation and have measured the glutaminase activity of our particular sample as 3—4% of the asparaginase activity. These in vitro assays are performed at pH 8.6 with 0.01 M substrate concentrations (8). During the initial course of therapy, the serum enzyme level averaged about 2 units/ml. This amount of enzyme can hydro-
lyze the amount of asparagine normally found in the serum, about 40 μmoles/ml, in 1.2 seconds under assay conditions for this enzyme. Although the enzymatic rate would be considerably lower in the blood due to the lower substrate level, it is still not surprising that the asparagine level does fall to zero rapidly and seems to stay there as long as asparaginase therapy is continued. Since, for this enzyme preparation, the glutaminase activity was only 3% of the asparaginase activity, and the glutamine level is normally about 400 μM, 10 times as great as the asparagine, about 7 minutes would be required for 2 units of this enzyme to completely hydrolyze the serum glutamine under assay conditions. This could account for the drop in serum glutamine as well. The increased glutamate can certainly be accounted for on the basis of glutamine hydrolysis. There is a considerable amount of both glutamine and asparagine present in the red blood cells, and this store of amides is also depleted. Since the enzyme does not enter cells (7), the amides must diffuse out of the red cells.

Another fact that stands out in these studies is that, although asparagine concentrations remain negligible during maintenance therapy (10 or 50 IU/kg/day), glutamine concentrations return to normal rapidly after the initial course of therapy (200 IU/kg/day) and remain normal in the face of continuing measurable blood levels of E. coli asparaginase and, therefore, glutaminase. In Charts 1 and 2, it can be seen that glutamine levels began to rise before the high blood enzyme levels resulting from initial therapy were reduced appreciably. It would appear that either the body can increase its rate of glutamine synthesis enough to replenish the blood level after the initial therapeutic trauma or that normal glutamine synthetase activity can overcome the effect of at least 50 IU/kg per day of this enzyme preparation. It should be noted, however, that the glutamine level within the red blood cells does not appear to return to normal.

The temporary reappearance of asparagine in the sera of M. J. (Chart 2) at the height of the initial therapeutic effect is difficult to explain. It represents the only occurrence of this kind that we have seen and its explanation, at this moment, is a matter for conjecture.

If one assumes that tumors having a requirement for asparaginase in vitro would also require it in vivo, the primary effect of asparaginase therapy can be considered to be the resultant drop in the blood asparagine levels to zero. The secondary effect of the E. coli enzyme on glutamine may actually enhance its effectiveness. It causes an initial drop in the level of serum glutamine to zero, at which time glutamine is apparently leached out of the red cells. It may not be unreasonable to suppose that the glutamine content of leukemic cells may also be decreased at this time. There have been reports that those tumors which are asparaginase-sensitive have very low levels of asparagine synthetase activity (6, 9), while those of cell lines which have become asparaginase-resistant show relatively high asparagine synthetase levels. Since asparagine synthetase requires glutamine as a nitrogen donor (1, 11, 15), the glutaminase activity of E. coli asparaginase may enhance its antitumor effect.

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