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Contents of L-Asparaginase-Related Amino Acids in the Plasma of Children with ALL

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Abstract: Objective To detect the contents of L-asparaginase (L-Asp)-related amino acids in ALL children receiving L-Asp treatment; and to study the relationship between changes in L-Asp contents and clinical efficacy on ALL. **Methods** Plasma concentrations of asparagine (Asn), aspartic acid (Aspa), glutamine (Gln) and glutamic acid (Glu) in different stages of L-Asp treatment were measured by the HPLC-FLD method in 20 children with ALL (17 cases of B-ALL and 3 of T-ALL). **Results** The plasma Asn level after the 1st administration of L-Asp decreased significantly. With the administration of L-Asp according to the induction remission formula of All-XH99, the Asn level remained low, even to nil level. This status remained for 7 days after cessation of L-Asp, and even 10 days in 15 cases of B-ALL, but the Asn level in all the cases of T-ALL and only 2 cases of B-ALL increased and even returned to nomal 7 days after L-Asp treatment ended. The concentration of Glu after the second and the last administrations of L-Asp increased significantly and it returned to normal on the 7th day after cessation of L-Asp, while the concentration of Aspa increased and failed to return to normal on day 10 after cessation of of L-Asp. The concentration of Gln slightly decreased during the course of treatment with L-Asp, but the difference was not significant compared with that before treatment. **Conclusions** The Asn level of children with T-ALL after cessation of L-Asp recovers earier than that of children with B-ALL, indicating that it may be helpful for individualization of L-Asp administration in the treatment of ALL on the basis of the immunophenotype of children. The glutaminase activity of L-Asp does not exert effects on the treatment.

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Key words: L-Asparaginase; Asparagine; Asparagine synthetase; Amino acid

ALL患儿L-Asp治疗相关性血浆氨基酸水平变化及其意义

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[摘 要] 目的 检测ALL患儿血浆左旋门冬酰胺酶(L-Asp)治疗过程中相关氨基酸水平变化,探讨这种变化与临床疗效的相关性,为L-Asp的个体化用药提供依据。**方法** 通过HPLC-FLD技术检测20例初治ALL患儿(17例为B-ALL,3例为T-ALL)在L-Asp使用不同时段血浆中门冬酰胺(Asn)、门冬氨酸(Aspa)、谷氨酰胺(Gln)、谷氨酸(Glu)等水平。**结果** 在L-Asp第一次使用后,患儿血浆内Asn显著下降,随着L-Asp的按序使用,患者血浆中Asn始终保持在低水平甚或测不出,有15例B-ALL患儿可持续到L-Asp停用后约7天左右,并在第10天时仍未恢复正常,但全部3例T-ALL患儿却在L-Asp停用后约7天时Asn浓度明显回升甚至达到正常水平,而仅2例B-ALL患儿出现类似情况。与第一次使用L-Asp前1~2 h血浆浓度相比,第二次、最后一次血浆中Glu浓度均显著升高($P<0.05$),直至L-Asp停用后第7天血浆浓度才恢复正常;而Aspa浓度则持续升高($P<0.05$),到停药后第10天仍未恢复正常;在整个治疗过程中,Gln水平虽略有下降,与治疗前相比并无显著差异。**结论** L-Asp停用后T-ALL患儿血浆Asn水平较B-ALL患儿恢复快,提示在儿童ALL的治疗中,对于L-Asp的使用,应结合患者免疫学分型,这可能为临床L-Asp个体化用药提供理论依据。而L-Asp的部分谷氨酰胺酶活性在L-Asp治疗中作用并不显著。

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L-asparaginase (L-Asp) is one of the effective medications in the treatment of acute lymphoblastic leukemia (ALL). It can help asparagine (Asn) catalyse to aspartic acid (Aspa) and ammonia, causing a depletion of Asn in the blood. In normal cells, the asparagine synthetase is active enough for Aspa to synthetase Asn, and thus the synthesis of protein in cells will not be affected. But in some malignancies such as lymphoblastic leukemia and non-Hodgkin lymphoma, the activity of the asparagine synthetase is lower than that in normal cells and Asn for protein synthesis would be insufficient. So, when there is alack of extracellular Asn, the synthesis of protein, DNA and RNA is inhibited and death of cells would ensue.

In addition, L-Asp has some glutaminase effects that can catalyse glutamine extracellularly and create a glutamine gradient across the cell membrane, thus facilitating glutamine transfers across cells rapidly and its catalysis. This results in intracellular depletion of Gln and limitation of synthesis of Asn and protein[1]. And the effect of glutaminase of L-Asp is thought to be related to such side effects as Asn related encephalopathy and coagulation disorder[2-3].

There are differences in dosages, methods, and attendant side effects of administrating L-Asp in childhood ALL among our country and other countries. To investigate the factors for therapeutic efficacy clinically and the causes of side effects of L-Asp, it is important to study the concentrations of related amino acids in the course of chemotherapy of ALL.

Materials and methods

Subjects

Twenty newly diagnosed childhood ALL patients (12 males and 8 females, median age of 57 months) admitted to our hospital from October, 2001 to April, 2002 were enrolled in this study. Of these patients, 17 cases were B-ALL and 3 were T-ALL. The treatment schedule (ALL-XH-99) was described in Table 1. (L-Asp was prepared by Kyowa Hakko Kogyo. Co, Ltd, Tokyo, Japan).

Main apparatus and reagents

The standard amino acids, L-Asn, Aspa, Gln and Glu were obtained from Sigma. HPLC-FLD was HP1100 G1321A and its chromatography column was Diamonsil™ C18 (250 mm×4.6 mm, 5-μm). Fluorescence detection was conducted at $\lambda_{EM}=450$ nm and $\lambda_{EX}=334$ nm.

Sample collection

Blood samples (3 ml) (100 U/ml heparin was used as the anticoagulant) were collected 1-2 hours prior to administration of asparaginase and at the 4th, 7 th and 10 th day after cessation of L-Asp. Within 20 minutes after collection, the samples were centrifuged and the plasma was stored in a −70℃ refrigerator.

Detection of amino acids

The standard curves were established using four kinds of standard amino acids according to the reference[2] with minor modifications. Firstly, the plasma (500 μl) was immediately deproteinized by adding 10% 200 μl sulphosalicylic acid (w/w). After centrifugation, 200 μl supernatant was collected and mixed with 20 μl OPA reagent. Two minutes later, 50 μl mixture was monitored by HPLC-FLD. The limit of quantification was 0.2 μmol/L.

Standards of the results

Asn ≤0.2 μmol/L was classified as complete depletion. The Asn concentration of near-complete depletion was between 0.2 and 0.5 μmol/L. The moderate reduction was between 0.5-1.0 μmol/L and the incomplete reduction was between 1-40 μmol/L. Asn >40 μmol/L was classified as no depletion[3].

Statistical analysis

The comparisons of Asn concentrations were performed according to the method of Jonckheere-Terpstra.

Results

Stability of the amino acids in the plasma

Each derivative amino acid level in the plasma was measured using the HPLC technique (See Figure

1). The daily calibration curves showed linearity >0.99 for aspartic acid (Aspa, calibration linear from 1.059 to 52.95 μM), asparagine (Asn, calibration linear from 1.099 to 54.95 μM), glutamic acid (Glu, from 1.054 to 52.7 μM) and glutamine (Gln, from 1.122 to 56.1 μM) (See Figure 2). The variation assay showed that the coefficients of variation of the standard curves were lower than 10%.

Changes of amino acid levels in the plasma during each phase of administration of L-Asp

Table 1 Induction remission formula of ALL-XH-99

Prednisone	60 mg/m²/d	0～28 days
Vincristine	1.5 mg/m²	8th, 15th, 22nd, 29th day
Daunorubicin	30 mg/m²	8th, 9th, 10th day
L-Asp	6000 U/m²	once every other day from the tenth day (six to ten times)

Table 2 Changes of the Asn level in different phases of treatment [Number (%)]

Asn	First administration	Second administration	Last administration	7 days after cessation of L-Asp	10 days after cessation of L-Asp
≤0.2 μmol/L	—	13(65)	13(65)	13(65)	9(45)[a]
0.5 μmol/L<Asn≤1 μmol/L	—	1(5)	—	1(5)	—
1 μmol/K<Asn≤40 μmol/L	—	6(30)	7(35)	1(5)[a]	6(30)
>40 μmol/L	20(100)	—	—	5(25)[a]	5(25)[a]

Note: a vs the second application $P<0.05$

Table 3 Concentrations of Aspa, Glu and Gln in different phases of treatment ($\bar{x} \pm s$, μmol/L)

	Aspa	Glu	Gln
First administration	20.97±7.48	28.35±9.62	661.64±22.34
Second administration	49.92±28.18[a]	48.67±34.10[a]	632.70±238.17
Last administration	43.56±25.38[a]	50.34±30.26[a]	603.39±233.95
7 days after cessation of administration	34.40±23.06[a]	30.49±12.54	539.65±160.96
10 days after cessation of administration	32.13±20.06[a]	29.18±16.00	646.95±160.59

Note: a vs first administration $P<0.05$

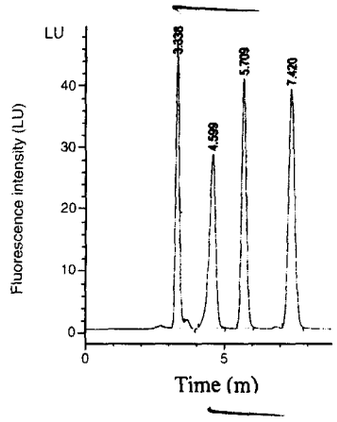

Figure 1 HPLC-FLD picture of 4 kinds of standard amino acids

The 1st peak is Aspa, 2nd is Glu, 3rd is Asn and 4th is Gln

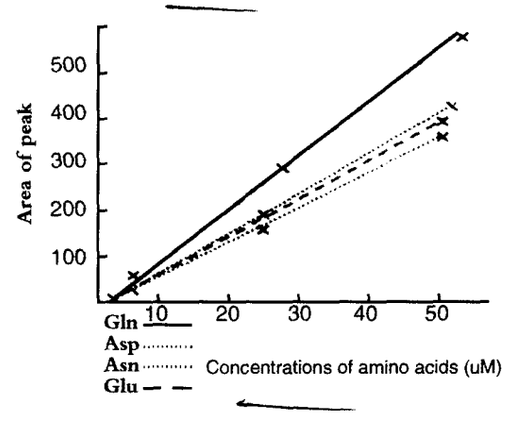

Figure 2 Standard curves of 4 kinds of amino acids

Amino acid concentrations (Asn, Aspa, Glu, Gln) changed after treatment with L-Asp. The Asn level reduced significantly between the first and second administrations of L-Asp according to the schedule. The Asn level of the children was within the lower range or even to nil level. This status remained for 7 days after cessation of treatment with L-Asp, and the Asn level was still below the normal range even on the 10th day. But on the 7th day after termination of treatment with L-Asp, the Asn level of 5 patients (T-ALL 3 cases, Pre-B-All 2 cases) increased significantly or even returned to the normal level (See Table 2). Compared with the Glu level 1 – 2 h before the first administration of L-Asp, the Glu level after the second and the last administrations of L-Asp increased significantly ($P < 0.05$) and it declined to normal on the 7th day after L-Asp treatment, while the Aspa level was higher than normal ($P < 0.05$) even on the 10th day after L-Asp treatment. The Gln level decreased slightly during the course of treatment, showing no significant difference compared with that before administration (See Table 3).

Discussion

Boos[4] reported that on the first and third days after administration of asparaginase (all patients were enrolled into ALL-BFM 90 schedule), the level of aspartic acid and glumatic acid rose significantly, while the level of glutamine decreased markedly, and the level of asparagine almost vanished 2 weeks after the last administration of L-Asp. In our study Asn concentration in most patients did not recover and was even undetectable 10 days after cessation of L-Asp treatment. In 5 cases, Asn concentration almost recovered 7 days after cessation of L-Asp treatment. Three cases of them were T-ALL and 2 were Pre-B-ALL. Aspartic acid was elevated significantly before the 2nd and the last L-Asp administrations, and it continued for 10 days after cessation of L-Asp treatment.

Pieter[4] observed that drug resistance to L-asparaginase in T-ALL patients was stronger than that in common and pre-B-ALL patients. Dübbers[7] found that there was no significant difference in asparaginase synthetase (AS) activity between ALL and AML. But significantly lower AS activity was found in the B-lineage ALL and AML-M5 subgroups, while the activity of AS were rather higher in T-ALL. The therapeutic effect of L-Asp is closely related to the trough activity level, and the expression and activity of AS were inversely related to the sensitivity of asparaginase in human leukemia cells. In addition, L-Asp can cause a depletion of Asn in tumour cells, and consequently the synthesis of protein in tumor cells is blocked with cell death. Therefore we postulated that a certain dose of L-Asp can diminish the Asn level, even to nil level. In some blast cells such as T-ALL blast cells, the AS activity was so high that it can synthesize enough Asn to keep the cells alive and rendering it insensitive or resistant to L-Asp, the Asn concentration of these patients would increase or even return to normal soon after L-Asp administration stopped. In this study we found that the Asn level of 3 cases of T-ALL recovered 7 days after cessation of L-Asp treatment, and the Asn level in one of these cases increased significantly 4 days after cessation of L-Asp treatment. Thus we suggested that the method and dosage of L-Asp in the treatment of children with T-ALL should be modified by increasing the dosage of L-Asp per time or the times of administrating L-Asp.

Sheng[9] reported that altering the coding sequence Cys-1 of As to either Ala or Ser could eliminate its activity of glutaminase and the Asn synthesis activity of As was also inhibited by Gln. This indicated that the mutation of AS gene would result in the change of As activity. In our study, the Asn level in 2 cases of pre-B-ALL returned to normal 7 days after cessation of L-Asp. We postulated that perhaps the genetic polymorphism of As in human beings might cause different levels of sensitivity to L-Asp in different people. In some people, the AS activity increases due to some special genetic polymorphism, so they are insensitive to L-Asp. Therefore to administer appropriate chemotherapy schedule and to elevate the clinic efficacy, it is important to study the genetic polymorphism of AS gene and the relationship among the genetic polymorphism and AS activity and clinic efficacy.

In our study, the Glu level increased and continued for 7 days after cessation of L-Asp administration, while the Gln level decreased a little but there was no significant difference compared with that before treatment. Thus we regarded that the glutaminase activity of L-Asp did not have any significant effect on the therapy. Although some authors reported that the glutaminase activity of L-Asp contributed to the abnormality of blood coagulation, we found few of these side-effects in our study, which is probably related to the lower dosage of L-Asp in our treatment or the mutation of G1691 A and G20210A of factor V.

In our opinion, it is important to determine a reasonable administration method with L-Asp treatment for childhood ALL on the basis of immunophenotype. Further reseach of genetic polymorphism and activity of AS would be greatly beneficial to the improvement of therapeutic efficacy.

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