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Article *in* European Journal of Clinical Investigation · November 1988

DOI: 10.1111/j.1365-2362.1988.tb01049.x · Source: PubMed

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Asparaginase-induced derangements of glutamine metabolism: the pathogenetic basis for some drug-related side-effects

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Received 20 January 1988 and in revised form 19 May 1988

Abstract. Several side-effects of asparaginase therapy have been said to be a consequence of the glutaminase activity of *Escherichia coli* asparaginase, especially the deleterious influence on the liver function. We report here the drug-induced impairments of asparagine and glutamine metabolism in correlation to concentrations changes of plasma proteins, synthesized in the liver, in patients with acute lymphatic leukaemia. One hour after asparaginase application, plasma glutamine decreased to 5% (0–39%: median, range) of the initial values, with a subsequent rise to concentrations slightly lower than those prior to therapy. During the 14 days of drug application the fasting plasma concentrations of glutamine fell to a median of 63% of the pre-therapeutic levels, indicating a depletion of the glutamine pools. Two days after the end of asparaginase application, in one patient the glutamine concentrations increased to the pre-therapeutic range. Plasma concentrations of fibrinogen and antithrombin III decreased to 46% and 56%, respectively, of the initial values, with a slight increase 2 days after the end of therapy. The changes of plasma protein concentrations followed the course of plasma glutamine and asparagine. From that we deduce that the hepatic synthesis of the plasma proteins might be influenced by asparagine and glutamine depletion as a consequence of the therapy with *E. coli* asparaginase.

Keywords. Asparaginase, liver, amino acids, glutamine, glutamate, leukaemia, body weight, antithrombin, fibrinogen.

Introduction

The discovery that asparaginase from guinea-pig serum inhibited the growth of certain tumours, being unable to synthesize L-asparagine, suggested that this enzyme might be used as a specific and selective anti-neoplastic agent [1]. Subsequently, a microbial asparaginase from *Escherichia coli* was shown to act as a potent anti-tumours agent, especially in acute leukaemias [2–4]. Normal tissues possess sufficient asparagine synthetase activity to synthesize and maintain the levels of asparagine that are required for normal metabolic functions [5,6]. Although an absolute need of normal cells for L-asparagine could not be demonstrated so far, a wide spectrum of toxicity involving the liver, pancreas, brain, clotting mechanisms, and the immune system has been described after application of *E. coli* asparaginase [6–19]. Several *in-vivo* and *in-vitro* studies suggest that *E. coli* asparaginase, being able to deaminate not only L-asparagine but also L-glutamine, may be responsible for the drug-related toxicity and immunosuppression [6,7,16,20].

This is why we studied the influence of *E. coli* asparaginase [L-asparagine amidohydrolase (EC.3.5.1.1.)] on the metabolism of glutamine and asparagine. We also correlated changes of the plasma amino acid concentrations with those of plasma proteins synthesized by the liver and with changes of body weight.

Patients and methods

Subjects

We studied four patients with acute lymphocytic leukaemia (one female, three males), who were treated with L-asparaginase from *Escherichia coli* (Crasnitin®, Bayer AG, Leverkusen, FRG) as a part of the cytostatic regimen [4]. Clinical data and the therapy regimen are given in Tables 1 and 2. The laboratory investigations were done during the induction therapy (from day 0 to day 13 of the investigation period), and for 2 days after the end of asparaginase application.

Samples for biochemical monitoring were obtained every morning between 07.00 h and 08.00 h, after a 12-h fasting period and before the application of cytostatic drugs. For post-therapeutical analysis of amino acid courses, blood was drawn at days 4 and 12 over a period of 6 h. During that period of time patients were allowed to take meals and drinks of their choice.

During the investigation period, both antibiotics and blood components were supplied if necessary.

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Table 1. Clinical data of patients with acute lymphocytic leukaemia

Patient	Age (years)	Diagnosis (FAB)	Data before treatment: Leucocytes (10^9 l^{-1})	Platelets (10^9 l^{-1})	Erythrocytes (10^{12} l^{-1})	Lactate dehydrogenase (μkat l^{-1})	Body mass (% IBM)
1	74	L1/C-ALL	124·3	102	3·7	7·24	92
2	33	L1/T-ALL	28·8	22	2·8	14·05	105
3	17	L2/T-ALL	10·4	19	2·2	4·78	87
4	39	L1/C-ALL	2·1	12	3·4	2·53	109

Abbreviations: FAB, FAB classification [36]; ALL, acute lymphocytic leukaemia (C-ALL, common ALL; T-ALL, T-cell ALL); IBM, ideal body mass [37]; L1, uniform lymphoblasts, L2, polymorphic lymphoblasts.

Table 2. Induction therapy of patients with acute lymphocytic leukaemia

Drug	Dosage	Days after onset of therapy: 0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Prednison	60 mg m^{-2} BSF	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Daunorubicin	25 mg m^{-2} BSF	X	X						X						X		
Vincristin	1·5 mg m^{-2} BSF								X						X		
Asparaginase	5000 E m^{-2} BSF	X	X	X	X	X	X	X	X	X	X	X	X	X	X		
Biochemical investigations		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

Abbreviations: BSF, body surface.

Amino acid analysis

Heparinized blood used for amino acid determination was centrifuged within 1 h of venepuncture, and 2000 μl of plasma were deproteinized with 200 μl of a 300 g l^{-1} aqueous solution of sulphosalicylic acid containing beta-thienylalanine, 1 mmol l^{-1}, as internal standard. After centrifugation, the supernatant was adjusted to pH 2·2 with 0·2 mol l^{-1} lithium hydroxide and stored at −80°C.

Free amino acids were analysed with an automatic amino acid analyser (Liquimat III; Kontron, Basel, Switzerland) equipped with an automatic computing integrator (Autolab SP 4100; Spectra Physic Inc., Mountain View, CA, U.S.A.). For the separation of the amino acids a Durrum ion exchange resin DC-6A

Table 3. Courses of post-absorptive amino acids during induction therapy

Amino acid	Reference values [21] (μmol l^{-1})	Days after onset of treatment: 0 (μmol l^{-1})*	1 (%)*†	6 (%)*†	13 (%)*†	15 (%)*†
Glutamine	526 425–627	560 282–863	122‡ 99–160	58‡ 49–92	63‡ 61–150	74‡ 68–120
Glutamate	28 13–42	73‡ 62–233	481‡ 319–1027	161‡ 70–646	152‡ 77–210	48‡ 25–100
Asparagine	54 35–73	49 44–61	0‡ 0	0‡ 0	0‡ 0	0‡ 0–41
Aspartate	6 4–9	7 5–26	233‡ 153–732	161‡ 84–362	148‡ 46–241	245‡ 49–385
Total amino acids	2708 2103–3420	3830 2007–4386	124 123–318	107 77–158	97 73–109	118 64–131

* Medians and ranges from four patients.
† Percentage of the initial values (day 0).
‡ $P < 0{\cdot}05$ vs. reference range, and with respect to day 0.

and a lithium buffer system with five buffers were used. The reference values for the plasma amino acids are taken from 16 healthy volunteers [21].

Other laboratory data

Plasma electrolytes, glucose, urea, uric acid, creatinine, LDH, GOT, GPT, GGT and alkaline phosphatase were analysed by means of enzymatic methods with an automatic analyser (Parallel, American Monitor Inc., New York, U.S.A.); blood cells by means of a microcell counter (Sysmex CC-110, Toa, Medical Electronics, Tokyo, Japan). Antithrombin III activity was determined with the Berichrom AT III test (Behring Inc., Marburg, FRG), and fibrinogen levels by means of the method according to Clauss [22]. Reference ranges were taken from the literature.

Statistics

For statistical evaluation we used the median test because of the small study group. All values are expressed as medians and range, respectively, 99% with a confidence range. Probability (P) values of less than 0·05% were considered to be significant.

Results

On day 0, the preprandial concentrations of the total amino acids (Table 3) ranged from 2007 to 4386 μmol l^{-1} with a median of 3830 (normal values: 2708/2103–3420). With the exception of day 1; there was an increase to 124% (123–318) of the initial values ($P<0·05$), the total amino acid concentrations remained within the reference ranges (the absolute concentrations of all amino acids are available on request). The rise on the first day depended primarily on the increases of glutamate (eight-fold of the initial values), as well as arginine, ornithine, leucine, isoleucine, alanine, lysine and proline (1·5–2-fold).

Asparagine levels became undetectable in all patients until the end of the study, except for one patient who showed a preprandial asparagine concentration of 8 μmol l^{-1} on day 15, i.e. 2 days after the end of asparaginase application. The concentrations of aspartate reached their maxima on day 1, with 233% (153–732) of the initial values. There was another rise of aspartate in three of four patients on day 15. On day 6, the glutamine concentrations were only 60% of the initial values, these ranges were kept until day 13. The glutamate concentrations of all patients were higher than those of the reference group at day 0 ($P<0·05$), and increased to 480% (320–1027: median/ranges) on day 1. The elevated concentrations remained until day 13 in two of the patients. Of all the other amino acids, only phenylalanine and tyrosine increased during the period of asparaginase therapy in those patients who had had almost normal concentrations prior to chemotherapy (patient 2: 50; patient 3: 80 μmol l^{-1}).

Table 4. Courses of GLN, GLU 24 h after asparaginase

	Hours after asparaginase application					
Amino acid	0 (μmol l^{-1})*	1·0 (%)*†	1·5 (%)*†	5 (%)*†	7 (%)*†	25 (%)*†
Glutamine	591	4·5‡	15‡	27‡	39‡	92‡
	413–740	0–39	0–39	0–57	0–121	13–124
Glutamate	121	334‡	325‡	336‡	170‡	107
	54–328	237–557	290–1549	210–1540	78–760	38–488

* Medians and ranges of five samples taken from three patients (patient 1: day 12; patient 3: days 4 and 12; patient 4: days 4 and 12).
† Percentage of the initial values (hour 0).
‡ $P<0·05$ vs. hour 0.

Table 5. Courses of plasma proteins during induction therapy

		Days after onset of treatment				
Plasma proteins	Reference ranges	0 (plasma concentration)	1 (%)*†	6 (%)*†	13 (%)*†	15 (%)*†
Fibrinogen (g l^{-1})		3·6	99	39‡	46‡	51‡
	2·0–4·5	3·5–3·8	95–100	26–51	21–100	30–89
Antithrombin III		100	101	72‡	56‡	58‡
(% of standard)	80–120	100	100–104	32–83	46–58	41–81

* Medians and ranges from four patients.
† Percentage of the initial values (day 0).
‡ $P<0·05$ vs. reference ranges, and with respect to day 0.

The courses of glutamine and glutamate within 24 h of asparaginase infusion are listed in Table 4. There was no homogeneous reaction of any other free amino acid to the application of asparaginase.

The courses of the fibrinogen and antithrombin III are listed in Table 5. The application of an antithrombin III concentrate (Kybernin®, Behringwerke, Marburg, FRG) became necessary in patient 2, with a total of 2000 IU applied on days 13 and 14. Patient 3 got a total of eight units of fresh frozen plasma during days 8–17. Fibrinogen concentrates (Human-Fibrinogen, Kabi-Vitrum, Vienna, Austria) were given to patient 1 (a total of 29 g during days 5–20), patient 2 (3 g on days 6 and 9), and patient 4 (28 g during days 4–16). No patient experienced periods of bleeding or thrombosis.

In spite of the low number of patients, the plasma levels of glutamine and asparagine seemed to parallel the course of the plasma proteins.

Only patient 2 kept a constant glutamine level during the whole study (92–120% of the initial values). This patient was also the only one with a detectable asparagine concentration on day 15 (41% of the initial values) and, furthermore, the only one with a significant rise of fibrinogen (89% of the initial values on day 15, vs. 31% on day 9) without relevant fibrinogen substitution during the therapy. This patients also showed the highest antithrombin III concentrations (81–85% vs. 41–58% in the other patients).

Three of four patients lost weight as a consequence of chemotherapy. In patient 1 body weight fell from 92·3% ideal body weight (IBW) to 84·4, patient 2: 104·7 to 101·1%, and patient 3: 87·1 to 77·0% IBW, i.e. a median weight loss of 9% (range 3·5–11·6) of the initial body weight during 14 days of treatment. We could not find any influence of the other chemotherapeutic agents regarding systematic amino acid or plasma protein concentration changes.

During the whole investigation period the plasma concentrations of all liver enzymes, electrolytes and creatinine were within the normal ranges. Urea concentrations (reference interval: 1·5–9 mmol l^{-1}) increased from a median of 6·68 (6·01–7·35) to 13·69 (8·68–16·03) mmol l^{-1} on day 1, and decreased to 6·01 (4·34–11·02) on day 15. One patient experienced an acute raise of uric acid (reference interval: 0·15–0·42 mmol l^{-1}) at the very beginning of chemotherapy to 0·61 mmol l^{-1}. After that he was treated with allopurinol, as all other patients, and kept his uric acid levels within the normal range.

Cholesterin concentrations (reference interval: 3·38–6·24 mmol l^{-1}) were lowered in two patients (patients 1 and 2) from 5·12 and 5·62 to 2·13 and 4·19 mmol l^{-1} on day 13, with a subsequent increase to 2·47 and 5·41 mmol l^{-1} on day 15, respectively.

Triglyceride levels (reference interval: 0·4–2·05 mmol l^{-1}) decreased in all patients from a median of 2·11 (range 1·33–5·86) to 1·00 (0·84–2·08) mmol l^{-1} on day 13, with an increase to 1·27 (0·91–3·51) after chemotherapy.

Discussion

Despite the great number of studies on asparaginase there are few data available on the effects of *E. coli* asparaginase administration on plasma amino acid levels in humans [23,24].

We were able to demonstrate for the first time the deleterious effect of *E. coli* asparaginase on glutamine homeostasis, which had already been postulated by several investigators [11,23,24], by means of the post-therapeutic courses of the plasma amino acids. From the uniformly observed fall of glutamine to nearly or completely undetectable plasma concentrations, we deduce that this is an obligatory side-effect of this drug.

Glutamine is of central importance in the metabolic pathways, comprising about one-half of the whole body resources of all free amino acids [25,26]. Glutamine is the principal carrier of nitrogen from the periphery to visceral organs, and it is also an important respiratory substrate for rapidly dividing cells such as enterocytes, reticulocytes and tumour cells [27]. The concentration of glutamine in normal subjects is closely regulated within a narrow range without significant diurnal variations [28].

Glutamine concentrations in whole blood and skeletal muscle decrease markedly following multiple organ failures, in which the intracellular glutamine stores become depleted more than 50% while plasma levels fall to 50–70% of the reference intervals [29,30].

The glutamine concentrations of our patients exceed these ranges distinctly. With one exception, the leukaemic patients showed a loss of 40–50% of the pre-therapeutic plasma levels, which lasted for nearly 2 weeks. One has to assume that the extravascular glutamine pools and glutamine-dependent organs also become depleted as a consequence of the asparaginase application [31,32].

The drug-induced disturbances of glutamine metabolism are much better reflected by the plasma concentrations during the first hours after asparaginase application than by the fasting amino acid levels. One patient of the study group had undetectable glutamine levels, another one showed values below 175 μmol l^{-1} over a period of about 3–6 h. The courses of glutamate and ammonia [11] are reciprocal to that of glutamine.

The asparaginase-induced courses of fibrinogen, anthrombin III, triglycerides and cholesterin are in accordance with other studies [6,7,12–15,17,18].

The mechanism of asparaginase-induced hepatotoxicity is not completely understood. Investigations on glutaminase-free asparaginase, lacking hepatotoxicity and immunotoxicity, indicate that the glutaminase activity of *E. coli* asparaginase might be the primary pathogenetic factor [6,7,20].

Despite the dramatic effects of *E. coli* asparaginase on glutamine homeostasis we were not able to obtain a direct statistical correlation between the absolute plasma level of the amino acids and the protein

concentrations before and during the therapy. Several factors might influence the plasma concentrations, e.g. the fibrinogen substitution, as mentioned above. It seems to be of interest for this discussion that the only patient who was able to maintain his original glutamine concentrations during the whole therapy was the only person with detectable asparagine values 2 days after the end of asparaginase application. This patient was also the only one to show the highest antithrombin III levels, as well as a significant rise of fibrinogen concentrations (without relevant substitution of this substrate), during the study period. That is why the depletion of glutamine alone, or a synergistic effect together with the asparagine depletion [6], appear to be possible pathogenetic factors of *E. coli* asparaginase side-effects on hepatic synthesis of plasma proteins.

Glutaminase-induced glutamine deficiency is said to impair the hepatic protein synthesis, comparable to the situation in several protein malnutrition [6,33]. There are preliminary data indicating that a high protein diet may partly overcome the negative effects of asparaginase therapy on nutritional status [34,35]. Whether a post-chemotherapeutical application of high dosages of glutamine, which would restore the depleted glutamine pools, will be able to accelerate patients' regeneration after *E. coli* asparaginase is now under investigation.

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