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Plasma glutamate—a prognostic marker of cancer and of other immunodeficiency syndromes?

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Elevated plasma levels of glutamate (GLU) have been reported to occur in patients with malignancies and other immunodeficiency syndromes (IDS). To evaluate, whether GLU is useful as prognostic indicator, the plasma concentrations were determined in patients with colorectal carcinoma (CRC), with breast cancer (BRC), and with HIV-infection (HIV). The results were correlated with the disease-stages, and compared with data obtained from patients with benign diseases of the same organ, as well as from sex-matched healthy volunteers. GLU concentrations (volunteers: 27.4±17.6 μmol/l) were elevated in all BRC patients (range of mean values: 53.5–83.2 μmol/l), in CRC patients with T2–T4-tumours (means: 46.8–85.9), and in HIV+ patients of stage WR 5, 6 (means: 53.9–69.7 μmol/l). All CRC- and BRC-patients with metastases showed highly significant elevations of GLU concentrations ($p<0.001$), but there were no direct correlations between disease stages and GLU levels. Pre-operative patients with benign diseases (diverticulitis, adenoma=GID; and mastopathy=MTP) showed increased GLU levels, which were comparable to those of the tumour patients. The glutamine/GLU ratios (volunteers: 19.3±15.0) were decreased only in HIV-WR 6 (7.6±2.1), and BRC-stage 4 (8.0±1.7). From these results we deduce that the plasma GLU concentrations do not allow a discrimination either between patients with malignancies and without, and between persons of different disease stages.

Key words: amino acids, blood; amino acids, metabolism; breast cancer; colorectal cancer; glutamine; HIV-infection; tumour linkage

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Altered concentrations of plasma amino acids have been observed in patients with impaired immune responses during various malignant or infectious diseases (for reviews [1–4]). Elevated glutamate levels, which often coincide with a parallel decrease of plasma glutamine, are regarded as characteristic for progressive malignancies [1, 5, 6]. Recently, Dröge and coworkers [2, 3] were able to demonstrate a direct correlation between elevated plasma glutamate levels and impaired immunological reactivity in patients with colorectal cancer, as

well as with lung cancer and HIV-infection. A similar link was observed in a group of apparently healthy blood donors.

It would be useful to determine whether the plasma concentrations of glutamate parallel the clinical progression of diseases with immunodeficiencies, and thus could be useful indicators of the patients' prognosis.

We analysed the plasma amino acid pattern of patients with colorectal carcinoma, with breast cancer, and with HIV infection, and compared the results with those of patients with benign diseases of the same organs (diverticulitis, adenoma of the colon, mastopathy) and of healthy volunteers.

SUBJECTS AND METHODS

Subjects, disease staging

Surgical patients. All patients with colorectal or mammary diseases were consecutively operated in the First Surgical Department, University of Vienna, on the presumption that they were suffering from a malignant tumour.

In seven of the 52 gastrointestinal patients (age: 65.3±11.5 years, mean±SD, 22 females, 30 males), and in 22 of the mammary group (n=86; age: 59.7±15.0), a malignancy was excluded post-operatively by histological examination. These patients served as controls, as did seven healthy female volunteers for the mammary group, and 16 females and males for the gastrointestinal group (mean age: 37±13 years). The age-distributions of the non-tumour and the tumour subgroups were identical.

The colorectal carcinoma group was staged according to the TNM system [7]. For patients with breast cancer we used the UICC-ACC clinical staging classification [8]: this is based on the TNM system. Stage I: MO and T1a, b NO-1a; stage II: MO and TO-1b N1b or T2a, b NO-1b; stage III: MO and T1a-2b N2 or T3a-b NO-2; stage IV: T4, or N3 or M1.

HIV patients. Sixty-two consecutive male patients (homosexuals, i.v. drug users; mean age: 31.4±8.2 years), who consulted the AIDS ambulance of the University of Cologne hospital because of risk of HIV infection, were examined. Fifty-six were positive with respect to antibodies against HIV, as tested by ELISA (Abbott, Wiesbaden, FRG), and confirmed by Western blot analysis. All patients were classified according to the Walter-Reed staging classification [9], which allows a stricter discrimination during the early disease-stages than the CDC classification:

Stage WR 0=member of the high risk group; WR 1=serological diagnosis of HIV infection; WR 2=additional chronic lymphadenopathy; WR 3=T-helper cells <400/µl; WR 4=additional cutaneous hypergy; WR 5=as WR 3, additional cutaneous anergy, and/or oral candidosis; WR 6=opportunistic infection, additional to HIV infection.

Twenty-two males served as controls. All were of the same age as the study group (mean: 35±11 years), and were tested and found to be free of infections.

Amino acid analysis

Amino acids were determined by ion-exchange chromatography with automatic analyser (Biotronic LC 5000, Biotronik, Munchen FRG; Liquimat III, Kontron, Basel, Switzerland). Blood for amino acid analysis was drawn (preoperatively from the surgical patients) after a fast of at least 12 h between 0800 hours and 1000 hours. Methods for deproteinization, internal standards, and analytical procedures are described in detail elsewhere [4, 10, 11]. All specimens were cooled on ice until deproteinization, which was performed within 2 h of venipuncture, and the plasma was stored at −70 °C and −80 °C respectively until analysis. The analytical reproducibility was 5% and comparable to published results [12].

Statistical analysis

For statistical analysis we used the U-test (Wilcoxon, Mann-Whitney). Values of less than $p=0.05$ were considered to be significant.

All values are expressed as means±1 SD.

RESULTS

The results are listed in Tables I–III. Compared with the volunteers (GLU: 28.4±13.8), GLU levels (expressed as µmol/l and as % of total amino acids) are elevated ($p<0.05$) in all breast carcinoma patients, in colorectal carcinoma patients of stages T2–T4, and in

TABLE I. Pre-operative plasmatic amino acid levels of patients with colo-rectal diseases (means±SD)

Subjects	n	Total AA (μmol/l)	GLU (μmol/l)	GLU (% total AA)	GLN/GLU
Controls	16	2708	28.4	1.1	19.3
(volunteers)		(409.2)	(13.8)	(0.5)	(15)
Controls	7	3182.1*	48.3**	1.7**	19.3
(Benign GI-disease)		(525.6)	(21.7)	(0.5)	(13.0)
Colorectal Cancer					
Tumour-stages					
T1 N0 M0	5	3313.4	32.3	1.0	22.2
		(336.2)	(18.3)	(0.6)	(9.5)
T2 N0 M0	11	3560.3**	85.9*	2.2*	12.5
		(799.1)	(83.3)	(1.6)	(6.4)
T3 N0 M0	6	3157.8*	77.9*	2.4*	12.2
		(503)	(60.4)	(1.7)	(7.3)
T2 N1 M0	2	3363***	38.4	1.1	21.4
		(154.1)	(20.1)	(0.5)	(8.4)
T3N1, 2M0	5	3448.4**	46.8***	1.4	15.8
		(606.5)	(11.1)	(0.4)	(4.8)
T4N1, 2M0	4	3072.6	35.3	1.2	19.8
		(610)	(13.4)	(0.4)	(8.8)
T2 N1 M1	1	3202	45.1	1.4	14.5
T3N1, 2M1	3	2940.3	72.5***	2.4**	9.3
		(549.4)	(36.2)	(0.7)	(3.0)
T4N1, 2M1	4	3324.7*	49.4***	1.5**	14.4
		(651.4)	(0.3)	(0.3)	(4.8)
Relapse	4	2984	53.6**	1.8*	14.5
		(395.5)	(21.9)	(0.8)	(6.5)

*p<0.05, **p<0.01, ***p<0.001 (*vs* volunteers). AA, Amino acids; GLU, Glutamate; GLN, Glutamine; n=number of subjects.

TABLE II. Preoperative plasmatic amino acid levels of patients with mammary diseases (means±SD)

Subjects	n	Total AA (μmol/l)	GLU (μmol/l)	GLU (% total AA)	GLN/GLU
Controls	7	2783.2	27.4	1.0	19.7
(volunteers)		(446)	(17.6)	(0.5)	(9.3)
Controls	22	3540†††	58.0††	1.9†	14.9
(mastopathy)		(488.9)	(29.0)	(1.3)	(8.3)
Breast Cancer					
tumour-stages					
S 1	10	3553†††	61.3†	1.7†	12.3
		(647.4)	(28.6)	(0.5)	(4.3)
S 2	27	3405†††	55.5†	1.6†	16.9
		(493.4)	(40.1)	(1.0)	(9.6)
S 3	24	3511.4†††	53.5††	1.5†	15.1
		(548.3)	(26.3)	(0.5)	(5.5)
S 4	3	3192	83.2*†††	2.6†††	8.0**
		(654.7)	(27.0)	(0.3)	(1.7)

*p<0.05; **p<0.01 (*vs* mastopathy). †p<0.05; ††p<0.01; †††p<0.001 (*vs* volunteers).

TABLE III. Plasma amino acid levels of patients with HIV infection (means±SD)

Subjects	n	Total AA (μmol/l)	GLU (μmol/l)	GLU (% total AA)	GLN/GLU
Controls (volunteers)	22	2357 (233.4)	43.6 (19.4)	1.85 (0.9)	15.1 (8.2)
Controls (risk group)	6	2508 (302.2)	37.0 (10.8)	1.9 (0.4)	10.8 (3.7)
Disease stages					
WR 1	4	2144 (378.7)	61.3 (39.2)	2.7 (1.7)	14.4 (16.1)
WR 2	20	2352.9 (664.2)	53.0 (30.8)	2.2 (1.2)	13.3 (8.1)
WR 3	10	2342.5 (306.0)	50.3 (31.0)	2.1 (1.3)	10.8 (4.5)
WR 4	8	2392.6 (252.1)	56.0 (42.7)	2.4 (1.9)	10.7 (6.6)
WR 5	9	2230.3 (297.9)	59.3* (18.2)	2.7* (0.9)	11.6 (5.8)
WR 6	5	2253.8 (366.3)	69.7*** (17.8)	3.0*** (0.6)	7.6** (2.1)

*p<0.05; **p<0.01; ***p<0.001 (*vs* controls).

anergic HIV+ patients (WR 5, 6). All tumour patients with metastases have highly significant elevations ($p<0.001$). There are no significant correlations between the disease stages and the glutamate levels. Patients with benign gastrointestinal or mammary diseases show raised glutamate levels similar to those of the tumour patients. The GLN/GLU-ratios are decreased only in HIV-WR 6, and in breast carcinoma patients of stage 4.

DISCUSSION

Our data are in agreement with the results of other groups, showing elevated plasma concentrations of glutamate in patients with malignancies of the colorectum or the breast, and with AIDS [1–3, 13–16], when compared with healthy volunteers. The highest glutamate concentrations were found in the most advanced disease stages, e.g. in HIV+ patients with opportunistic infections (stage WR 6), and in tumour patients with metastases, as other groups could already demonstrate [1, 13].

There are several problems that make the plasma glutamate useless as a reliable disease marker:

(i) Gastrointestinal or mammary patients with malignancies cannot be discriminated from controls.

(ii) Patients with benign diseases have glutamate elevations similar to that of the tumour patients.

(iii) There is no correlation between the absolute or relative (to the total amino acid amount) glutamate concentrations and the disease stages; e.g. colorectal cancer patients of T2 and T3 show higher glutamate concentrations than those of T4 N1 M1.

Thus, valid information with respect to the prognosis of an individual patient cannot be obtained by the determination of plasma glutamate. Furthermore, plasma glutamate concentrations show striking intra-individual variations [17, 18].

The reasons for the increased plasma glutamate concentrations of patients with malignancies and other immunodeficiencies are not completely understood. From the data on tumour patients [1, 13, 19, 20], Holm and coworkers deduced both glutamate release from tumour tissue to the plasma pool and reduced glutamate utilization by peripheral tissues of the tumour-bearing host. The nutrient intake and the nutritional status of the patients have no influence on the glutamate concentrations [1]. On the other hand, the tumour-induced stress metabolism of the host is characterized by catabolism of muscle proteins. The released free amino acids are shifted to the visceral organs, where they are used as precursors of the stimulated visceral protein synthesis

[21]. The amino acids may be used also as energy-yielding substrates. This is why one can speculate, that the glutamate pool is increased as a consequence of increased amino acid degradation (e.g. of arginine, ornithine, proline, histidine), and ammonia detoxification [22].

In addition, further pathological conditions could contribute to elevated glutamate levels: e.g. the incomplete oxidation of glutamine to glutamate as a consequence of hypoxaemia [23]; or changes of glutamate uptake from plasma to other compartments [24, 25].

Because of the different metabolic pathways of glutamate, several factors are responsible for the changes of plasma glutamate. This may explain why we did not find any systematic correlation between disease stage and the plasma levels of glutamate.

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