ONCOLOGY

Biochemical Parameters in the Diagnosis and Monitoring of Neurotoxicity of Antitumor Cytostatics

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The significance of neurospecific proteins in the diagnosis of neurotoxicity in patients with breast, lung, testicular, and ovarian cancer treated by taxane and cisplatin drugs was evaluated. The most pronounced increase in the content of these proteins and titers of autoantibodies to these proteins was observed in patients with clinical manifestations of neurotoxicity induced by cytostatics. A strong correlation was found between the concentration of myelin basic protein and cumulative dose of the drug (R=0.922; p<0.0001). These data suggest that myelin basic protein and gliofibrillar acid protein can be used as markers in the diagnosis and monitoring of antitumor drug neurotoxicity.

Key Words: *neurotoxicity; myelin basic protein; gliofibrillar acid protein; chemotherapy*

The use of antitumor drugs is often limited by their neurotoxicity. Damage to axons and their myelin sheath leads to axonal degeneration and demyelination and underlies the development of neurological symptoms during cytostatic therapy [7]. Drug-induced pathological changes in glial cells impair the blood-brain barrier, which, in turn determines increased cell permeability and the release of some neurospecific proteins, *e.g.,* myelin basic protein (MBP), S-100b protein, gliofibrillar acid protein (GFAP), *etc.* into the liquor and then into the blood. Autoantibodies (a-AB) to neurospecific proteins are transported by the axonal current through damaged blood-brain barrier to various structures and cause damage to the nervous tissue [1,3]. Therefore, MBP and specific a-AB are sensitive markers of demyelination process and can be used in the diagnosis of some nervous and mental disorders [2-4]. GFAP is used in the diagnosis of blood-brain barrier impairment [5,6] and in neurooncology [7,8]. However, little is known on neurospecific proteins as markers of nervous tissue damage caused by toxic effects of antitumor drugs.

We studied the dynamics of serum content of neurospecific proteins in cancer patients treated with cytostatics and evaluated the possibility of using MBP, GFAP, and specific a-AB in the diagnosis and neurotoxicity monitoring during drug therapy of malignant tumors.

MATERIALS AND METHODS

Clinical neurological and biochemical studies were carried out in 46 cancer patients (mean age 53±12 years). Group 1 consisted of 28 patients with breast cancer treated with paclitaxel in combination with doxorubicin, group 2 included 9 patients with breast cancer and lung cancer treated with taxoter (docetaxel) monotherapy (6-9 courses), group 3 consisted of 9 patients with ovarian and testicular cancer treated according to the BEP protocol based on cisplatin (4-6

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courses). Control group included 15 age- and sex-matched healthy individuals. Neurotoxicity was evaluated in accordance with the WHO criteria. The concentrations of MBP, GFAP, the spectrum of a-AB, and the dynamics of their production in the serum were evaluated by enzyme-linked immunosorbent assay with specific monoclonal antibodies before, after every 2-3 course, and after the end of drug therapy. The sample was considered positive (presence of a-AB to neurospecific proteins), if the test parameter 2 or more times surpassed the control.

The differences between the means were considered significant at $p<0.05$ (Student's t test). Analysis of correlations was carried out using Spearman's nonparametrical test (R). The data were processed using Statistica for Windows 5.5 software.

In accordance with standard requirements to statistical analysis, the threshold values of biochemical parameters were estimated on the basis of data in the control group with consideration for their mean values and 95% confidence interval. These values for MBP and GFAP were 6.9 and 5.6 ng/ml, respectively.

RESULTS

The initial levels of MBP and GFAP in cancer patients were higher than in healthy men and women, but this difference was statistically insignificant (Table 1).

After the second course of chemotherapy, serum concentrations of MBP in patients increased significantly in comparison with both the control (1.9 times) and initial values (1.5 times). After the 4th and 6th courses, the content of MBP remained elevated and variability of this parameter considerably increased (Table 1), but this can be attributed to the fact that only 12 of 46 patients received a complete course of drug therapy. The correlation analysis revealed a relationship between MBP concentrations and cumulative dose of the drug (R=0.922; $p<0.0001$). Frequency analysis of serum concentrations of MBP in the course of chemotherapy showed that MBP content most often surpassed the threshold value after the 2nd, 4th, and 6th courses (Fig. 1). After the end of drug therapy the concentration of MBP decreased significantly in 33% patients ($p<0.05$), but the mean value did not return to the initial level.

The content of GFAP underwent similar, but more pronounced changes: after the 2nd, 4th, and 6th courses this parameter increased by 2.6, 4.4, and 6.7 times compared to the initial value, respectively ($p<0.05$). The maximum increase in GFAP concentration in comparison with the initial value (10.4 times, Table 1) was observed after the 8th course. The level of GFAP increased during antitumor therapy in all patients irrespective of the total drug dose. After chemotherapy the content of GFAP decreased in 50% patients in comparison with its level during treatment.

Analysis of distribution of biochemical values with consideration for the severity of neurological symptoms showed a relationship between MBP concentration, moment of detection and severity of toxic distal peripheral polyneuropathy (PNP). An increase in the protein level preceded clinical manifestations of PNP in 24.2% cases, developed simultaneously with it in 62.7% cases, and was delayed in only 13.1% patients. The mean MBP concentration in patients with signs of of PNP (11.2±6.7 ng/ml) was significantly ($p<0.001$) higher than in patients without clinically manifest PNP (6.1±2.6 ng/ml). The increase in serum MBP concentration in patients with PNP caused by cytostatic treatment in comparison with the threshold values was observed in 67.3% cases. Protein concentrations increased in 20 (76.9%) of 26 patients after the 2nd course of chemotherapy, in 24 (72.7%) of 33 after the 4th course, and in 24 (64.9%) of 37 after the 6th course (Fig. 1). After the 8th course MBP content increased in only 4 (36.4%) of 11 examinees. Less frequent elevation of MBP level can be due to a lower drug dose and positive effect of combined therapy for PNP in patients with clinically manifest condition. After chemotherapy the mean MBP concentrations decreased (8.3±4.6 ng/ml) in comparison with the values during treatment, as did the percentage of PNP patients with high (in comparison with the threshold)

TABLE 1. Concentrations of MBP and GFAP (ng/ml) in Cancer Patients Receiving Antitumor Chemotherapy and in Controls (X±s)

Group	MBP	GFAP
Control (n=15)	4.7±1.1	4.4±0.6
	(2.8-6.4)	(3.6-5.4)
Cancer patients		
before therapy	6.9±3.6	4.5±1.9
	(1.9-11.7)	(1.1-6.7)
after 2nd course	9.1±5.1*	8.2±1.2*
	(1.8-25.8)	(6.8-10.1)
after 4th course	11.3±6.6*	14.4±1.7*
	(1.7-26.2)	(12.28-16.80)
after 6th course	10.7±7.7*	24.3±5.7*
	(1.8-33.2)	(15.8-31.8)
after 8th course	9.6±7.2	29.1±6.1*
	(1.4-21.8)	(24.0-41.1)
3 months after chemotherapy	8.26±4.60*	23.8±5.6*
	(1.4-13.9)	(16.7-34.8)

Note. In parentheses: range of fluctuations. *$p<0.05$ compared to the control group and values before therapy.

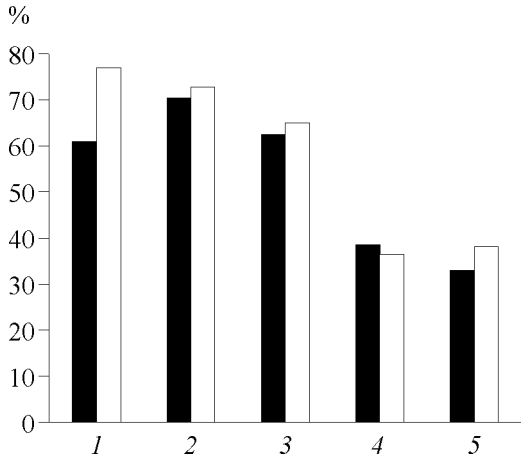

Fig. 1. Percentage of patients with increased concentration of the myelin basic protein during cytostatic therapy. Dark bars: total group; light bars: patients with peripheral polyneuropathy. *1-4*) after 2nd, 4th, 6th, and 8th chemotherapy courses, respectively; *5*) 3 months after chemotherapy.

MBP levels (Fig. 1). It is noteworthy that we revealed a statistically significant relationship between MBP concentrations and the severity of PNP. In first-degree PNP the mean concentration of MBP was 10.3±1.6 ng/ml and in second degree PNP it was 13.7±1.5 ng/ml ($p<0.05$).

The content of GFAP increased in all patients with clinical manifestation of neurotoxicity. Interestingly, that the development of clinical manifestations of central neurotoxicity (encephalopathy) was paralleled by a drastic increase inGFAP concentration (25.8-41.1 ng/ml).

High titers of a-AB to MBP and GFAA were observed during the same period as the increase in the concentrations of these proteins ($p<0.02$). After the 2nd-3rd courses of chemotherapy the titers of a-AB to MBP and GFAP increased in all groups by on average 2.5-2.8 times and reached a maximum after the 4th-6th courses. It is noteworthy, that differences in the titers of a-AB to MBP and GFAP in first- and second-degree toxicity did not reach the level of statistic significance. In general, changes in the titers of a-AB to neurospecific proteins were less pronounced than the time course of these proteins concentrations during chemotherapy. Analysis of the distribution of the studied biochemical parameters with consideration for chemotherapy protocols and tumor location showed no significant differences, which indicates the possibility of using these proteins as independent markers of antitumor drug neurotoxicity.

Hence, serum levels of MBP, GFAP, and a-AB to them increased significantly in cancer patients with clinical manifestations of neurotoxicity during cytostatic therapy. The development of PNP, increase in its incidence and augmentation of its severity were paralleled by increases in MBP and GFAP concentrations. Regression of neurological symptoms was associated with a decrease in protein levels and a-AB titers to them. Measurement of neurospecific proteins is significant evaluation of neurotoxicity, which suggests measurements of MBP and GFAP as the main and a-AB to them as an accessory tests in the diagnosis and monitoring of the cytostatic neurotoxicity.

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