Effect of Potentiated Antibodies to Cyclophosphamide on the Development of Tumors and Effectiveness of Cytostatic Therapy under Experimental Conditions

E. N. Amosova, E. P. Zueva, T. G. Razina, S. G. Krylova, N. V. Shilova, and O. I. Epstein*

Antibodies to cyclophosphamide obtained by homeopathic potentiation and administered in ultralow doses exhibit no antiblastic activity and did not modulate the effectiveness of cyclophosphamide during antitumor therapy of animals with transplanted tumors (Lewis lung carcinoma and Ehrlich adenocarcinoma).

Key Words: *transplanted tumors; cyclophosphamide; antibodies to cyclophosphamide; ultralow doses*

Combination treatment with antitumor preparations is widely used for the therapy of patients with tumors [3,5]. The search for new means increasing the effectiveness of cytostatic drugs is still in progress.

Previous studies on mice with Lewis lung carcinoma (LLC), melanoma B-16, and lung cancer RL-67 showed that cyclophosphamide obtained by homeopathic potentiation and used in ultralow concentrations increases antiblastic activity of the preparation administered in therapeutic doses [1]. Of particular interest are the data on the possibility of increasing antimetastatic activity of cytostatics with their potentiated forms, since life expectancy in patients with tumors strictly depends on the degree of metastatic dissemination.

It was found that antibodies to various endogenous regulators (interferon, erythropoietin, and histamine) exhibit pharmacological activity in ultralow doses [7-9]. Here we studied the effects of ultralow doses of antibodies to cyclophosphamide on the development of tumors in animals and effectiveness of treatment with this cytostatic.

MATERIALS AND METHODS

The effects of potentiated antibodies to cyclophosphamide (PAB-CP, mixture of homeopathic dilutions C12+C30+C200) on tumor growth and efficiency of cytostatic treatment were studied on 134 mice with transplanted Ehrlich adenocarcinoma (EAC) and LLC. The animals were obtained from the Laboratory of Experimental Biological Modeling (Institute of Pharmacology).

EAC was inoculated intraperitoneally in a dose of $6\text{-}7\times10^6$ cells in 0.2 ml physiological saline to female outbred mice. LLC cells were transplanted into the thigh in a dose of $4\text{-}6\times10^6$ cells in 0.1 ml physiological saline to male C57Bl/6 mice [6].

The anti-cyclophosphamide antiserum (titer 1:800) was routinely obtained from rabbits immunized with a cyclophosphamide-protein conjugate. The antiserum was diluted to ultralow concentrations (equivalent to 10^{-24} wt %) using homeopathic potentiation technique.

PAB-CP or distilled water (control) were administered through a gastric tube in a dose of 0.3 ml per mouse. The therapy was started 24 h after tumor transplantation. Some mice with LLC received this preparation after administration of the cytostatic. The mice with EAC received cyclophosphamide intramuscularly in a single dose of 150 mg/kg on day 3 after transplantation. The animals with LLC received this cytostatic intraperitoneally in a single dose of 125 mg/kg on day 11 after transplantation of tumor cells.

In mice with EAC the volume of ascitic fluid and tumor cells in mice with was estimated on day 8 after transplantation (ascitic fluid was centrifuged at 3000 rpm for 5 min). The index of suppression of tumor growth (IS) was expressed in percents of the control.

The efficiency of therapy in mice with LLC was evaluated on day 20 or 21. These mice received 16 or 17 (treatment from day 2 after transplantation) and 6

Institute of Pharmacology, Tomsk Research Center, Siberian Division of the Russian Academy of Medical Sciences; *"Materia Medica Holding" Research-and-Production Company, Moscow

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TABLE 1. Effect of PAB-CP on the Development of EAC and Effectiveness of Chemotherapy in Female Outbred Mice ($\bar{X}\pm m$)

Parameter	Distilled water (n=9)	PAB-CP (n=10)	Cyclophosphamide	
			without correction (n=9)	+PAB-CP (n=9)
Volume of ascitic fluid, mm^3	6.30±0.45	5.59±0.47	4.70±0.52	4.88±0.64
Volume of tumor cells, mm^3	2.40±0.22	2.74±0.25	1.40±0.18*	1.70±0.22*
IS, %	—	-14	42	29

Note. *p<0.05 compared to distilled water.

or 9 (treatment after cyclophosphamide administration) injections of PAB-CP, respectively. Tumor weight was determined and IS was calculated. The intensity of metastatic dissemination was evaluated by the number of lung metastases and their area. The incidence of metastatic dissemination was calculated as the percentage of animals with metastases from total number of animals in the group. The index of suppression of metastasizing (ISM) reflecting the degree of metastatic dissemination was calculated by the formula [2]:

$$ISM = [(A_C \times B_C - A_E \times B_E)/(A_C \times B_C)] \times 100\%,$$

where A_C and A_E are the incidence of lung metastases and B_C and B_E are the mean number of lung metastases in control and experimental mice, respectively.

TABLE 2. Effect of PAB-CP on the Development of LLC and Effectiveness of Chemotherapy in Male C57Bl/6 Mice on Day 20 after Tumor Transplantation ($\bar{X}\pm m$, n=10)

Parameter	Distilled water	PAB-CP	Cyclophosphamide		
			without correction	+PAB-CP	
				16 injections	6 injections
Tumor weight, g	4.86±0.61	6.62±0.33*	4.70±0.33	4.1±0.3	4.37±0.44
IS, %	—	-36	3	16	10
Incidence of metastasizing, %	100	100	100	90	90
Number of metastases	41.70±6.17	33.70±3.54	10.30±1.84*	6.70±2.08	6.80±1.82
Area of metastases, mm^2	66.01±16.86	48.42±10.04	4.43±2.08*	3.44±1.59*	2.03±0.68*
ISM, %	—	19	75	86	85

Note. *p<0.05 compared to distilled water.

TABLE 3. Effect of PAB-CP on the Development of LLC and Effectiveness of Chemotherapy in Male C57Bl/6 Mice on Day 21 after Tumor Transplantation ($\bar{X}\pm m$)

Parameter	Distilled water (n=10)	PAB-CP, 17 injections (n=10)	Cyclophosphamide		
			without correction (n=9)	+PAB-CP17 injections (n=10)	
				17 injections (n=10)	9 injections (n=8)
Tumor weight, g	4.39±0.35	4.50±0.36	3.95±0.43	3.26±0.40	4.14±0.72
IS, %	—	-2	10	26	6
Incidence of metastatic dissemination, %	100	100	78**	70**	88
Number of metastases	34.20±3.95	32.20±1.96	7.33±2.67*	4.20±1.96*	5.88±2.17*
Area of metastases, mm^2	37.93±7.62	49.47±9.65	3.72±1.80*	1.06±0.48*	4.24±2.12*
ISM, %	—	6	83	91	85

Note. *p<0.01 and **p<0.05 compared to distilled water.

The results were analyzed by nonparametric Mann-Whitney *U* test and Fischer's angular transformation [4].

RESULTS

The course of treatment with PAB-CP had no effect on the development of tumors (Table 1). Cyclophosphamide markedly inhibited tumor growth. Administration of PAB-CP had no effect on antiblastic activity of the cytostatic (Table 1).

On day 20 PAB-CP significantly stimulated primary tumor growth in animals with LLC (Table 2). Single treatment with cyclophosphamide did not suppress the growth of primary tumor node, but markedly suppressed metastasizing. The mean number and area of metastases decreased by 4.0 and 14.9 times, respectively, compared to the control (Table 2). During combination therapy PAB-CP did not modulate the antitumor effect of cyclophosphamide (Table 1).

On day 21 we did not observe the stimulatory effect of PAB-CP on tumor growth (Table 3). Cyclophosphamide markedly suppressed metastatic dissemination (similarly to the previous series) and decreased the number and area of lung metastases by 4.7 and 10.2 times, respectively (Table 3). PAB-CP did not modulate the antitumor effect of cyclophosphamide (Table 3).

Our results show that PAB-CP have no antiblastic activity and do not modulate the efficiency of cyclophosphamide in animals with transplanted tumors.

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