Effect of Baikal Skullcap Extract Administered Alone or in Combination with Cyclophosphamide on Natural Cytotoxicity System in Mice with Lewis Lung Carcinoma

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 137, No. 5, pp. 538-541, May, 2004
Original article submitted December 23, 2003

Baikal skullcap extract was introduced into cytostatic therapy of mice with Lewis lung carcinoma. This extract potentiated the antimetastatic effect of cyclophosphamide in mice with grafted tumors. Combined treatment with cyclophosphamide and extract of Baikal skullcap modulated cytotoxic activity of natural killer cells and peritoneal macrophages during tumor growth.

Key Words: *Baikal skullcap extract; cyclophosphamide; Lewis lung carcinoma; natural killer cells; peritoneal macrophages*

Much attention was given to functional activity of the natural cytotoxicity system during tumor growth [3,5, 8,11,12]. A modern approach to increasing the efficiency of therapy of malignant neoplasms consists in combined treatment with biological modulators and standard therapeutics [9]. Plant preparations hold much promise in this respect. They increase the efficiency and reduce toxicity of cytostatic therapy. Moreover, plant preparations can modulate functional activity of the natural cytotoxicity system during tumor growth [4,9]. One of these preparations is liquid extract of Baikal skullcap (BSE) used to increase the efficiency of antitumor therapy [1,4]. However, the mechanisms of BSE-produced changes in the natural cytotoxicity system are poorly understood.

Here we studied the effects of BSE administered alone or in combination with cyclophosphamide (CP) on the system of natural cytotoxicity in mice with Lewis lung carcinoma.

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MATERIALS AND METHODS

Experiments were performed on 140 male (CBA×C57Bl/6)F_1 mice aging 2-2.5 months and weighing 18-20 g. The animals were obtained from the Laboratory for Experimental Biological Modeling (Institute of Pharmacology, Tomsk Research Center). Lewis lung carcinoma served as an experimental model of malignant neoplasms. The suspension of tumor cells (5-6×10^6 cells) was implanted into the hindlimb muscle.

CP (Saransk Factory Biokhimik) was injected intraperitoneally in a single dose of 125 mg/kg on day 12 after tumor implantation. BSE (State Research Center for Medicinal Plants) was used for correction of immune dysfunction. The preparation (1 ml/kg) was dissolved in 0.2 ml distilled water and administered through a gastric tube for 12 days starting from the 6th day after tumor implantation.

Control animals with implanted tumor cells received an equivalent volume of distilled water. Intact mice of the same age and sex served as the control.

The efficiency of treatment was determined by tumor weight, percent of tumor growth suppression,

0007-4888/04/1375-0471

incidence of tumor cell dissemination [10], mean number of metastases, and index of the inhibition of tumor cell dissemination [2]. Cytotoxic activity of natural killer cells (NKC) was estimated in the membranetoxic test with target cells. The effector/target cell ratio was 100:1 and 50:1 [6]. Cytotoxic activity of peritoneal macrophages was determined as described elsewhere [7].

The results were analyzed by methods of variational statistics.

RESULTS

Administration of CP on day 12 slightly decreased the weight of primary tumor on days 13, 15, and 17 after implantation. The cytostatic had a potent antitumor effect in the terminal stage of tumor growth (days 19 and 22). Pretreatment with CP reduced the incidence of lung metastases on days 13 and 15. Course treatment with BSE did not modulate tumor growth in various periods after implantation. BSE decreased tumor weight only on day 22. CP produced an antimetastatic effect and tended to suppress dissemination of tumor cells. Combined administration of BSE and CP inhibited tumor cell dissemination compared to control animals (days 17, 19, and 22) and CP-treated mice (days 19 and 22). It should be emphasized that BSE potentiated the antitumor effect of CP in mice with Lewis lung carcinoma on day 22 of combination therapy (Table 1).

We studied changes in cytotoxic activity of NKC in the spleen of mice with Lewis lung carcinoma. Single administration of CP and course of treatment with BSE had no effect on this index on days 13 and 15 after implantation of tumor cells.

However, cytotoxic activity of NKC in the spleen of mice markedly increased after combined administration of preparations (day 13, effector/target cell ratio 100:1, Fig. 1, *a*).

On day 17 of tumor growth, cytotoxic activity of NKC in the spleen of mice receiving CP alone (effector/target cell ratios 100:1 and 50:1) or in combination with BSE (effector/target cell ratio 50:1) surpassed that observed at the earlier stage of observations. The course of treatment with BSE decreased the cytotoxicity index of NKC compared to that in untreated animals (effector/target cell ratio 50:1, Fig. 1, *b*).

Activity of NKC underwent other changes at the late stage of tumor growth (days 19-22). Single administration of CP increased cytotoxicity of NKC in the terminal stage of tumor growth (effector/target cell ratio 100:1). On days 19-22, cytotoxicity of NKC in

TABLE 1. Effects of Individual or Combination Treatment with BSE and CP on the Growth of Lewis Lung Carcinoma (*M*±*m*)

Days		Tumor weight, g	Tumor growth inhibition, %	Incidence of dissemination, %	Index of inhibition of dissemination, %
13	control	0.83±0.12	—	33	—
	CP	0.63±0.15	24	0***	100
	BSE	0.82±0.1	1	50	151
	CP+BSE	0.77±0.2	7	17	82
15	control	1.32±0.19	—	67	—
	CP	1.13±0.15	14	14***	96
	BSE	1.25±0.25	5	60	25
	CP+BSE	1.19±0.15	10	17***	77
17	control	2.15±0.23	—	86	—
	CP	1.69±0.28	21	57	74
	BSE	2.28±0.28	0	71	46
	CP+BSE	1.75±0.16	19	43***	97
19	control	1.86±0.26	—	100	—
	CP	0.48±0.17*	74	83	43
	BSE	1.84±0.30	1	83	71
	CP+BSE	0.85±0.22*+	54	16*++	98
22	control	4.56±0.15	—	86	—
	CP	2.67±0.30**	41	57	84
	BSE	2.68±0.37**	41	86	48
	CP+BSE	2.21±0.45**	51	0**++	100

Note. *$p<0.001$, **$p<0.01$, and ***$p<0.05$ compared to the control; +$p<0.001$ and ++$p<0.01$ compared to CP.

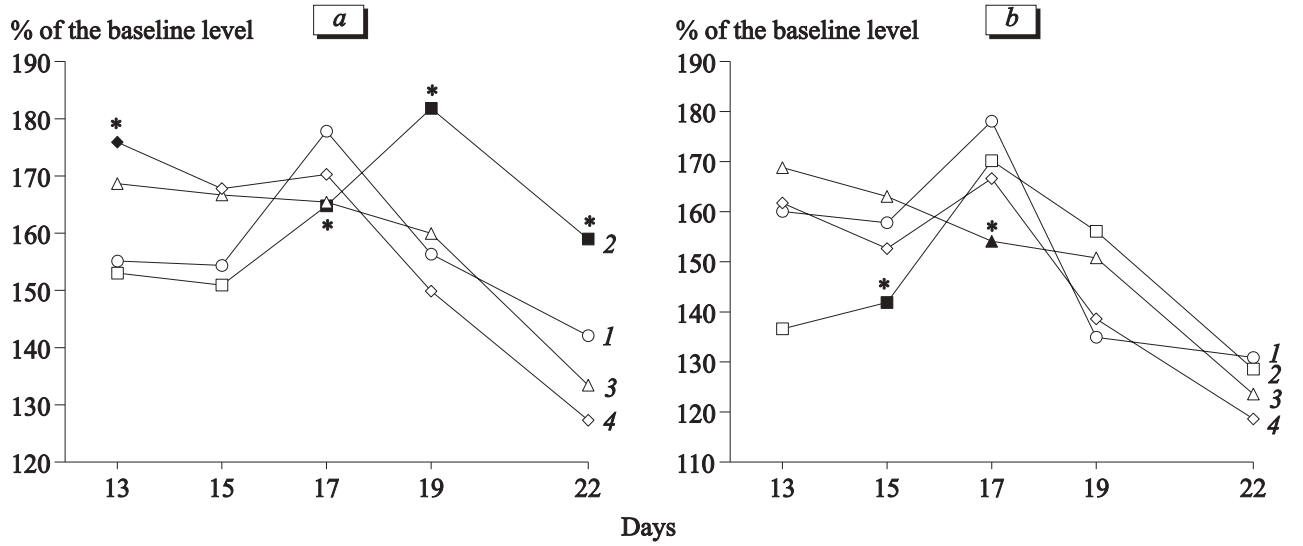

Fig. 1. Cytotoxic activity of natural killer cells in the spleen from mice with Lewis lung carcinoma (*1*) receiving cyclophosphamide (*2*), extract of Baikal skullcap (*3*), and combination therapy with test preparations (*4*) at the effector/target cell ratios of 100:1 (*a*) and 50:1 (*b*). Here and in Fig. 2: **p*<0.05 compared to the control.

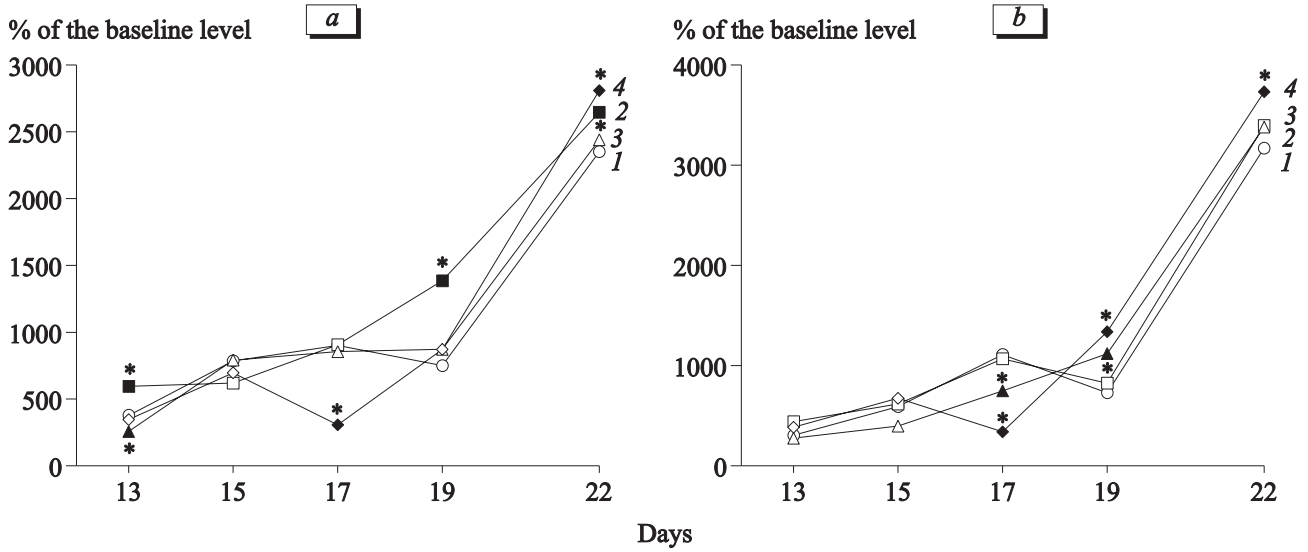

Fig. 2. Cytotoxic activity of peritoneal macrophages from mice with Lewis lung carcinoma (*1*) receiving cyclophosphamide (*2*), extract of Baikal skullcap (*3*), and combination therapy with test preparations (*4*) at the effector/target cell ratios of 10:1 (*a*) and 5:1 (*b*).

mice receiving BSE did not differ from the control. Combined treatment with CP and BSE tended to decrease cytotoxicity of NKC (compared to untreated animals, Fig. 1).

We studied the effect of test preparations on cytotoxic activity of peritoneal macrophages from mice with Lewis lung carcinoma. Single administration of CP significantly increased this index. However, cytotoxicity of macrophages in BSE-treated mice was lower than in control animals on day 13 of observations (effector/target cell ratio 10:1, Fig. 2, *a*). On days 15 and 17 the index of macrophage cytotoxicity surpassed that observed at the earlier stage, but did not differ from the control. The only exception was day 17, when BSE treatment markedly decreased this index compared to the control (effector/target cell ratio 5:1). On day 17 after implantation of tumor cells, the index of cytotoxicity of macrophages in mice receiving CP and BSE was lower than in untreated animals (effector/target cell ratios 10:1 and 5:1, Fig. 2).

Single administration of CP significantly increased the index of cytotoxicity of peritoneal macrophages at the late stage of tumor growth (days 19 and 22, effector/target cell ratio 10:1). Cytotoxic activity of macrophages from mice receiving BSE (effector/target cell ratio 10:1) did not differ from the control. BSE stimulated effector cells in the system of natural cytotoxicity at an effector/target cell ratio of 5:1 (day 19).

Compared to control animals, combined treatment with the test preparations markedly increased the index of cytotoxicity of macrophages on days 19 (effector/target cell ratio 5:1) and 22 after tumor implantation (effector/target cell ratios 10:1 and 5:1, Fig. 2).

Our results suggest that course treatment with BSE decreased tumor weight in mice with Lewis lung carcinoma on day 22 of the neoplastic process. During combination therapy of mice with Lewis lung carcinoma, BSE potentiated the antitumor effect of CP on day 22 after tumor implantation and prevented dissemination of tumor cells in the lungs. BSE had no effect on cytotoxic activity of NKC and peritoneal macrophages (effector/target cell ratios 100:1 and 10:1, respectively). Combined treatment with CP and BSE produced wave-like changes in cytotoxic activity of peritoneal macrophages (effector/target cell ratio 10:1). The index of cytotoxicity decreased on day 17, but significantly increased on day 22 after tumor implantation. Other changes were observed after combined administration of the test preparations. The increase in cytotoxic activity of NKC on days 13 and 15 after tumor implantation (effector/target cell ratio 100:1) was followed by a decrease in this index at the late stage of tumor growth (statistically insignificant compared to the control).

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