ONCOLOGY

β-Carotene Correction of Antitumor Immunity in Experimental Chemotherapy of Malignant Tumors

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Changes in cellular immunity parameters and the rate of tumor tissue growth during chemotherapy and immunocorrection with β-carotene are studied in two models of *in vivo* tumor growth (EL-4 lympholeukemia cells injected intraperitoneally and CaOv ovarian adenocarcinoma implanted under the renal capsule). β-Carotene is found to enhance the proliferative response of lymphocytes to concanavalin A (conA) and to increase the activity of specific antitumor T killers, this correlating with prolongation of the mean life span of tumor-bearing animals and inhibition of tumor growth.

Key Words: *β-carotene; antitumor immunity*

A drastic decrease of the body's resistance due to impaired functional activity of immunocompetent cells is one of the complications of antitumor chemotherapy. Recently, a clear-cut immunomodulating effect of β-carotene (BC) has been reported [4,7,11]. It is known that, among other things, activation of the antitumor defense of the organism by BC, mediated by subpopulations of natural killers, T killers and macrophages, is one of the possible causes of inhibition of malignant tumor growth in animals [2,12,14,15].

Our aim was to investigate the immunomodulating properties of BC during chemotherapy of malignant neoplasms in experimental animals. The therapeutic effect (inhibition of tumor growth, intensity of toxic reactions) for combined administration of cytostatics (cyclophosphamide, methotrexate, adriamycin, aranose) and synthetic BC was assessed and the most effective combination of these agents selected, leading to attenuation of the nonspecific toxic effect of antitumor drugs on the immune cells, with due consideration for the fact that the addition of BC to the standard diet does not lead to the build up of an excessive level of retinol in the organism and does not cause the toxic reaction of hypervitaminosis. Two experimental models of *in vivo* tumor growth were used in the study, one of which (EL-4 lympholeukemia) allowed us to assess the efficacy of modified chemotherapy in prolonging life, and the other (ovarian adenocarcinoma CaOv implanted under the renal capsule) the efficacy of inhibition of tumor tissue growth.

MATERIALS AND METHODS

Experiments were carried out with C57Bl/6 (H-2b) and (CBA×C57Bl/6)F_1 (H-2k×b) mice aged 2 months weighing 20 g. The BC preparation, a solution of the active substance in olive oil, was added daily

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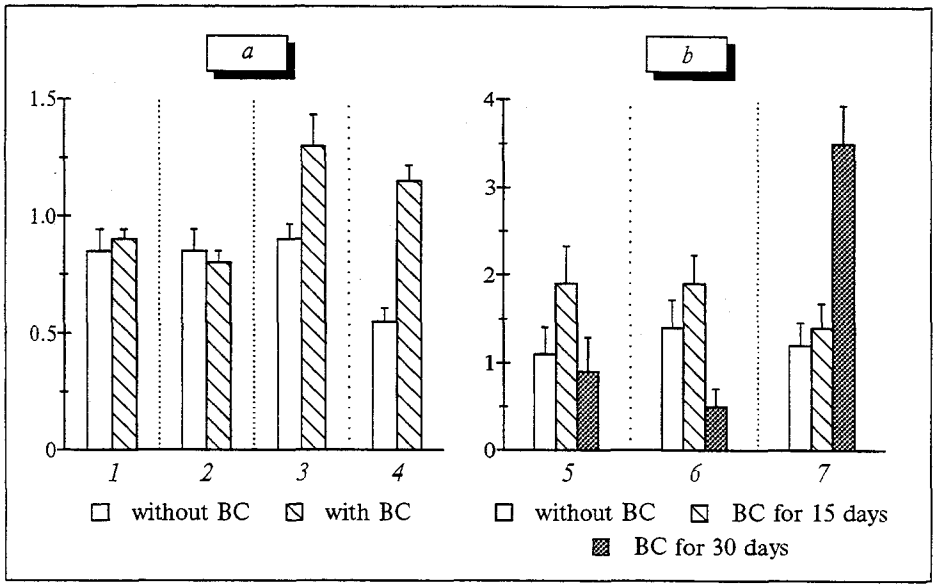

Fig. 1. Proliferative activity of T lymphocytes in mice with EL−4 and CaOv under the influence of BC. Ordinate: splenocyte stimulation index in response to ConA. *a*) in C57Bl/6 mice treated with cyclophosphamide (*2*), methotrexate (*3*), and adriamycin (*4*); *b*) in F_1 hybrid mice treated with cyclophosphamide (*6*) and aranose (*7*); *1*, *5*) splenocytes of untreated mice.

to the basic standard diet in a dose of 0.07 g of active substance per kg body weight.

Lympholeukemia EL-4 and ovarian adenocarcinoma CaOv cells were used to model tumor growth. C57Bl/6 mice were intraperitoneally injected 5×10^6 cells of tumor culture EL-4, the BC preparation was added to the food, and after 24 h cytostatics were injected. CaOv cells were cultured in medium 199 with 10% fetal calf serum (FCS) and embedded in fibrin clots as described previously [1]. The recipients were F_1 hybrid mice which were fed BC for 15 and 30 days prior to the experiment. One day before tumor transplantation under the renal capsule the animals underwent total-body irradiation in a dose of 4.5 Gy. Implantation of fibrin clots under the renal capsule was carried out as described previously [1]. Cytostatics were injected on day 4 after tumor implantation.

The officinal forms of cytostatics were dissolved in normal saline and injected intraperitoneally one time in the following doses: aranose, 500; cyclophosphamide, 200; methotrexate, 20; and adriamycin, 7.5 mg/kg.

For the study of the effect of BC on immunological parameters in the course of experimental chemotherapy of malignant tumors, C57Bl/6 mice were sacrificed by dislocation of cervical vertebrae on days 13-14, and F_1 mice on days 8-9. After this, the spleens were removed under sterile conditions and splenocyte suspensions were prepared using Potter homogenizers. In all experiments the isolated cells were cultured in medium RPMI-1640 containing 10% FCS, 1% 200 mM L-glutamine, and 40 U/ml gentamicin. In the lymphocyte blast transformation test (LBTT), 3×10^5 cells were incubated with 1-5 μg ConA in 96-well plates for 3 days at 37°C in an environment with 5% CO_2 in triplet variants. At the end of incubation 1.0 μCi of ^{3}H-thymidine was added to each well, the cell cultures were transferred to membrane filters with a harvester, and the radioactivity of the samples was measured. The stimulation index was estimated as the ratio of the isotope incorporation in test cultures to that in control cultures. The cytotoxic activity was assessed in a 16-h test with ^{51}Cr-labeled tumor target cells. In this test 1×10^4 target cells labeled with the isotope were mixed with 5×10^5 effector lymphocytes in triplet variants. The cytotoxic effect was estimated by the formula $100(Ex\text{-}S)/M\text{-}S$, where *Ex* is the supernatant radioactivity in the experiment,

TABLE 1. Effect of BC on Survival of C57Bl/6 Mice with EL−4 Lympholeukemia Treated by Modified Chemotherapy

Group	Survival, days			
	chemotherapy			no chemotherapy
	cyclophosphamide	methotrexate	adriamycin	
Control	21.35±2.78	15.78±0.22	22.99±2.87	13.41±0.26
BCK	26.52±0.81*	20.14±0.43*	25.84±1.41	13.0±0.57

Note. Asterisk shows reliable differences ($p<0.05$) *vs.* the respective control.

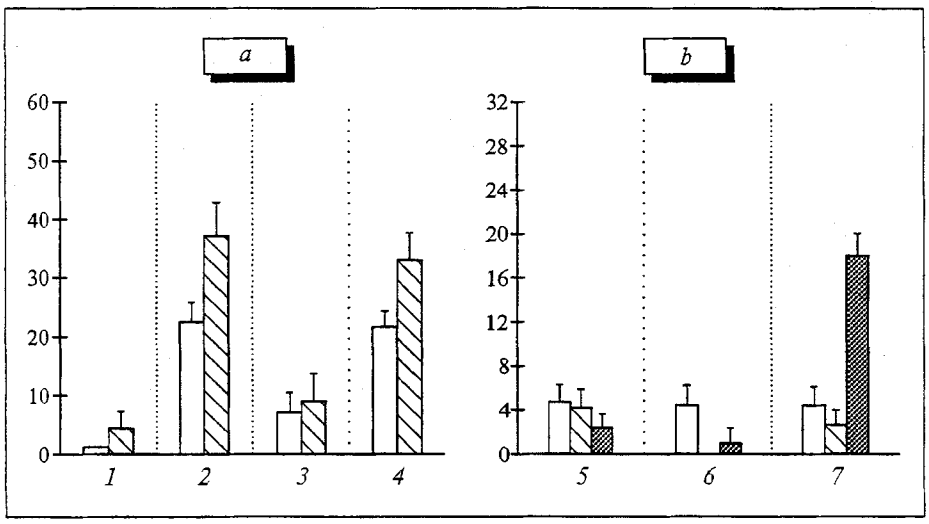

Fig. 2. Cytolytic activity of T lymphocytes of mice with EL-4 and CaOv under the influence of BC. Ordinate: cytotoxic activity of splenocytes, %. *a*) in C57Bl/6 mice against EL-4 target cells on day 13 of growth of syngeneic tumor; *b*) in F_1 hybrid mice against CaOv target cells on day 9 of tumor growth. Effector to target cell ratio 50:1. Other symbols as in Fig. 1.

M the maximal chromium release in the presence of 1% sodium dodecylsulfate solution, and *S* the spontaneous release of the isotope without the addition of effectors. In addition, the mean duration (in days) of mouse survival during *in vivo* growth of EL-4 cells under the influence of chemotherapy combined with BC was assessed in experiments with these tumor cells. In the experiments with CaOv the ratio of the physical size (in mm^2) of tumor tissue on days 8-9 of its growth to the size of the fibrin clot of CaOv cells implanted under the renal capsule was estimated in F_1 hybrids. This value was used to assess the magnitude of tumor growth inhibition under the influence of chemotherapy.

The results were statistically processed using Student's method.

RESULTS

Two systems of *in vivo* modeling of tumor tissue growth were used to assess the advisability of using BC and its efficacy as an immunomodulating agent in protocols for chemotherapy of malignant tumors. In experiments with C57Bl/6 mice, cells of a syngeneic EL-4 tumor were intraperitoneally injected, and after chemotherapy with cyclophosphamide, methotrexate, or adriamycin and immunocorrection the immunological parameters were compared. The data of three variants of LBTT (Fig. 1) showed a trend toward an increase of the lymphocyte proliferative ability under the action of the T cell mitogen ConA after the use of the following combinations: methotrexate+BC and adriamycin+BC. A negligible increase of the stimulation index may be due to the ability of cytostatics to block cell mitosis. In none of the groups was a marked drop of the stimulation index observed after therapy with cytostatics and BC. In the cytotoxicity test BC stimulated the activity of specific T killers (Fig. 2), reliable changes being observed in the groups administered BC alone and in combination with cyclophosphamide. An increase of antitumor cytotoxicity as a result of immunocorrection included in the chemotherapeutic protocol correlated with a prolongation of the mean life span of animals after tumor implantation (Table 1). The most pronounced positive shifts were observed after administration of cyclophosphamide. Note that the toxicity of cytostatics was not boosted when they were used in combination with BC.

In another series of experiments CaOv tumor cells in fibrin clots were implanted under the renal capsule of (CBA×C57Bl/6) F_1 mice. BC in combination with aranose was found to enhance splenic lymphocyte proliferation in response to

TABLE 2. Effect of BC on CaOv Tumor Growth Inhibition in Hybrid Mice F_1 Treated by Modified Chemotherapy

Group	Ratio of tumor size at autopsy to volume of implanted tumor tissue		
	chemotherapy		no chemotherapy
	aranose	cyclophosphamide	
Control	9.95±2.83	20.4±3.51	98.22±12.44
BC	0.48±0.14*	6.15±2.11*	20.73±4.81*

Note. Asterisk shows reliable differences ($p<0.002$) *vs.* the respective control.

ConA (Table 1), this increase being more marked after the addition of the agent to the food during 30 days; this correlated with an increase of antitumor cytotoxicity (Fig. 2). The inclusion of BC to the protocol of antitumor chemotherapy of F_1 mice increaseded the inhibition of tumor growth, which, again, was the most intensive after BC was combined with aranose (Table 2).

Hence, the incorporation of BC in antitumor chemotherapy suppressed tumor tissue growth in mice. The effect was most marked when the combination of cyclophosphamide and BC was used in EL-4 lympholeukemia, whereas in therapy of CaOv ovarian adenocarcinoma the combination of aranose with BC was the most effective. Similar data were previously reported by scientists who used vitamin A, a product of enzymatic transformation of BC, to boost the antitumor effect of cyclophosphamide, 5-fluorouracil, and other agents *in vivo* and *in vitro* [3,6,8,10,13]. Since in our experiments tumor growth inhibition correlated with an increase of antitumor cytotoxicity, it may be assumed that, besides the cytostatic effect of the drugs on tumor cells, the efficacy of combined therapy is also related to BC activation of T-killer generation. The stimulation of antitumor immunity resulting from the use of low cyclophosphamide doses may be attributed to elimination of T suppressors from the population of immunocompetent cells [5,9].

The results encourage the hope that BC preparations will find use in practical oncology for the combined treatment of malignant tumors.

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