# ANTITUMOR ACTION OF GLYCOPEPTIDES FROM THE CELL WALL OF Lactobacillus bulgaricus

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The antitumor action of the substance blastolysin, the main components of which are glycopeptide fragments from the cell wall of Lactobacillus bulgaricus, was studied. Blastolysin exhibits a specific antitumor effect against sarcoma S-180, leukemia P-388, plasmacytoma MOPC-315, adenocarcinoma AKATOL, melanosarcoma B-16, carcinoma LLC, and spontaneous tumors of mice. It has low toxicity, does not depress hematopoiesis, and in the character of its action on tumor tissue it differs essentially from known chemotherapeutic preparations.

KEY WORDS: chemotheraphy of tumors; glycopeptides; immunity

The antitumor action of substances contained in lysates of Lactobacillus bulgaricus was discovered in 1959 [1]. The present writers recently showed that this action is due to the presence of glycopeptide fragments of the cell wall in the lysates and determined their structure [3].

It was subsequently found that analogous fragments of the cell wall of other Lactobacilli and of more remote species of Gram-positive bacteria possess a similar antitumor action. A more detailed study was undertaken of the antitumor action of glycopeptides from cell walls of L. bulgaricus.

In the present investigation the antitumor action of the substance blastolysin, obtained from L. bulgaricus, was studied; the main components of this substance are glycopeptide fragments of the cell wall.

## EXPERIMENTAL METHOD

Noninbred albino mice (from the Central nursery, Academy of Medical Sciences of the USSR), mice of strains C57BL and BALB/c, and $BDF_1$ hybrids (from the "Stolbovaya" nursery) were used. The strains of transplantable tumors (the solid type of Crocker's sarcoma S-180, adenocarcinoma of the large intestine AKATOL, adenocarcinoma of the mammary gland KA-755, squamous-cell carcinoma of the lung Lewis-LLC, plasmacytoma MOPC-315, melanosarcoma B-16, lymphatic leukemia P-388, and hemocytoblastosis La) were obtained from the Oncologic Scientific Center, Academy of Medical Sciences of the USSR. The tumors were inoculated into mice of both sexes weighing 18-20 g by the method adopted in the Oncologic Scientific Center, Academy of Medical Sciences of the USSR [2].

Blastolysin was injected intravenously and intraperitoneally, in physiological saline, by single and repeated injections at intervals of 24, 48, and 72 h in a dose of 25-100 mg/kg. The antitumor effect of the substance was assessed in the leukemias by the increase in the period of survival, and in the solid tumors by inhibition of growth of the tumor determined as the mean weight at the end of the therapeutic experiment. In the course of treatment the blood of the experimental animals and histological changes in the normal and tumor tissues were studied.

## EXPERIMENTAL RESULTS

Blastolysin has low toxicity. For intravenous injection into intact mice its $LD_{50}$ was over 2000 mg/kg, and for the animals with sarcoma S-180 it was about 300 mg/kg.

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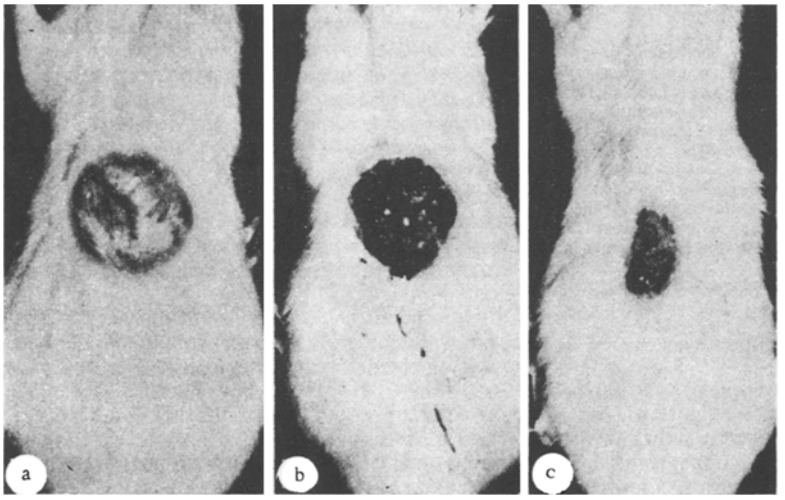


Fig. 1. Action of blastolysin on sarcoma S-180: a) tumor on 9th day after subcutaneous inoculation; b) necrosis of whole surface of tumor on 4th day after single intravenous injection of blastolysin, 50 mg/kg; c) detachment of necrotic scab and epithelization on seventh day.

Tumor S-180 was found to be most sensitive to blastolysin. The antitumor effect of blastolysin on this tumor was manifested as early as during the first hours after a single intravenous injection. An extensive area of central necrosis appeared after 24 h in the indurating and shrinking tumors. On the second to third day, in the overwhelming majority of animals receiving blastolysin in doses of 50 and 100 mg/kg, necrosis extended over the whole surface of the tumor (Fig. 1). On the seventh to ninth day total necrosis with detachment of the necrotic scab had taken place in 30% of these animals. Observation of the treated animals for 1 year revealed no recurrence of the tumor. In the remaining 70% of animals a focus of growth was found at the periphery of the necrotic area on the fifth to seventh day after a single injection of blastolysin and the tumor continued to grow, although much more slowly than in the control animals (Fig. 2).

It is interesting to note that this effect of blastolysin was observed constantly only in cases when treatment began when the tumors were already well developed (seventh to ninth day after transplantation). If given earlier (1st-3rd day), no inhibition of tumor growth was observed (Fig. 2), as was also the case when tumor tissue previously treated with blastolysin for 24 h was implanted in the animals.

Reinoculation of sarcoma S-180 was carried out in 10 of the cured animals 1 month after injection of blastolysin, but no tumor developed in any of these animals.

Histological examination of sections and squash preparations of the tumor tissue showed that clear destructive changes in the S-180 cells began to take place 4-6 h after injection of blastolysin. Meanwhile normal tissues, including those with high proliferative activity (epithelium of the small intestine, lymph nodes, spleen, bone marrow), showed no visible pathological changes throughout the period of observation (14 days).

The specific antitumor action of blastolysin was exhibited not only against Crocker's sarcoma, but also against several other transplantable and spontaneous mouse tumors. As Table 1 shows, blastolysin caused significant lengthening of the survival period of mice with leukemia P-388 and sharply inhibited growth of plasmacytoma MOPC-315 and adenocarcinoma AKATOL. Significant inhibition of growth of melanosarcoma B-16 and carcinoma LLC also was observed. Of all the tumors studied, blastolysin had no inhibitory action only on adenocarcinoma AK-755 and leukemia La, but it may be that the conditions in these cases were not optimal for manifestation of antitumor action. The greatest antitumor effect against strains MOPC-315 and AKATOL, incidentally, just as in the case of S-180, was obtained when treatment began in the later stages after implantation of the tumor (Fig. 2).

Spontaneous mouse tumors also were sensitive to blastolysin. Of the 10 experimental mice receiving the substance over a period of 2 weeks in a dose of 50 mg/kg with intervals of 48 h between injections, the tumor was greatly reduced in size in 8, and in 3 of these mice complete necrosis and rejection of the tumor took place after 12 injections.

TABLE 1. Action of Blastolysins on Transplantable Mouse Tumors

| Strain of tumor | Recipient mice | Dose, mg/kg (interval, in h, shown in parentheses) | Number of injections | Day of 1st injection | Method of injection* | Inhibition of tumor growth | | Percent of animals cured |
|---|---|---|---|---|---|---|---|---|
| | | | | | | % | P | |
| S-180 | Noninbred | 100 | 1 | 7 | i.v. | 58 | 0,001 | 30 |
| | | 50 (72) | 3 | 7 | " | 64 | 0,001 | 30 |
| AKATOL | BALB/c | 50 (72) | 4 | 5 | " | 70 | 0,01 | — |
| AK-755 | C57BL | 50 | 1 | 6 | " | 16 | 0,05 | — |
| LLC | C57BL | 50 | 1 | 8 | " | 42 | 0,01 | — |
| MOPC-315 | BALB/c | 50 (48) | 5 | 4 | " | 78 | 0,001 | — |
| B-16 | C57BL | 50 (48) | 5 | 6 | i.p. | 37 | 0,03 | — |
| P-388 | $BDF_1$ | 25 (48) | 7 | 1 | " | 40 | 0,01 | — |
| La | | 50 | 1 | 1 | " | 0 | — | — |

*i.v.) Intravenous; i.p.) intraperitoneal.

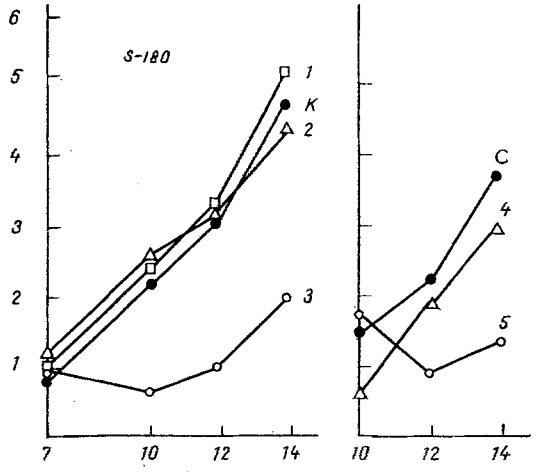


Fig. 2

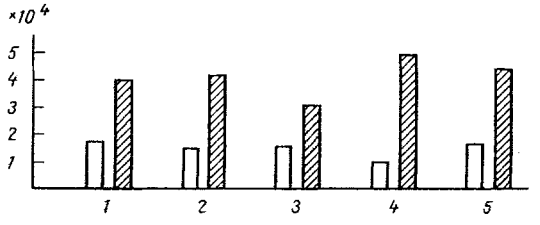


Fig. 3

Fig. 2. Dynamics of growth of S-180 and AKATOL tumors after injection of blastolysin (a single dose of 50 mg/kg) at different times after transplantation. C) Control; 1-5) injection of blastolysin 1, 3, 7, 3, and 9 days respectively. Abscissa, days after transplantation of tumor; ordinate, mean volume of tumor (in $mm^3 \times 10^3$).

Fig. 3. Total leukocyte count in animals with tumors on 4th day after single injection of blastolysin, 50 mg/kg. 1) S-180; 2) AK-755; 3) LLC; 4) P-388; 5) B-16. Unshaded columns represent mean leukocyte count in control animals; shaded columns the same in animals with tumors.

Blastolysin, it should be mentioned, inhibits neither erythropoiesis nor leukopoiesis. Moreover, in the animals with tumors an increase in the total leukocyte count was found 24 h after injection of this substance (Fig. 3). This increase took place mainly on account of monocytes, stab cells, and polymorphs. An increase in the peripheral blood leukocyte count after injection of blastolysin also took place in the intact animals, but by a much lesser degree and after much larger doses.

By the character of its action on tumor tissue and the absence of any inhibitory effect on hematopoiesis, blastolysin thus differs substantially from most of the known chemotherapeutic agents. Meanwhile, in some of the features of its antitumor effect (rapid necrosis of the tumor, maximal effect on tumors already developed) blastolysin resembles the endotoxins of Gram-negative bacteria [5] and the so-called tissue necrosis factor (TNF) [4]. It should be pointed out, however, that blastolysin has low toxicity and does not cause reactions of the recipient to its injection such as are characteristic of the endotoxins.

The fact that blastolysin has no action on tumors in vitro, the leukemoid reaction which it induces, and also the development of lasting immunity in the treated animals all indicate that activation of the immunological mechanisms of the recipient plays an important role in the antitumor action of blastolysin.

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# MIGRATING ABILITY OF TUMOR CELLS TREATED WITH IMMUNE SERA

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The action of antitumor sera (ATS) on the adhesive properties of L cells and of Ehrlich's ascites carcinoma cells was studied. The ability of the tumor cells to adhere to plastic and glass and to form rosettes with sheep's red blood cells was reduced after treatment with immune serum and, when injected into mice, fewer of these cells were held up in the lungs, spleen, and liver. The results show that antibodies may play an essential role in the metastasization of tumor cells.

KEY WORDS: adhesion; migration of cells; tumor cells; metastasization of tumors; antibodies

The action of antibodies on the proliferation and viability of tumor cells is well known [7]. However, the problem of whether antibodies can alter the migration of tumor cells in vivo remains unsolved. However, such an effect is possible, for antibodies are known to be able to affect the distribution of lymphocytes in the body [4]. The importance of the solution to this problem is determined by the fact that metastasization of tumors is an activity which largely depends on the migrating ability of the neoplastic cells [1].

In the investigation described below the distribution of tumor cells treated with antitumor sera (ATS) and changes in their adhesive properties in an in vitro system were studied.

## EXPERIMENTAL METHOD

In the experiments L cells and Ehrlich's ascites carcinoma cells were used. The ATS were obtained by immunizing rabbits with these cells [7]. The cytotoxic titer of the sera for L cells was 1 : 512 and for Ehrlich's carcinoma cells 1 : 256. The cells were incubated for 30 min at 37°C in Hanks' solution without Ca and Mg ions (to reduce aggregation of the cells), and with 20% ATS or with normal rabbit serum, after which they were washed or diluted with medium 199 to a certain concentration. The distribution of $^{51}Cr$-labeled tumor cells in the body was studied by the method described previously [3, 6]. CBA mice and (CBA × C57BL)$F_1$ hybrids were used. The reaction of spontaneous rosette formation of the cells with sheep's red blood cells was carried out by the method described previously [5]. To obtain adhesion, tumor cells in medium 199 with the addition of 20% bovine serum were placed in sterile plastic dishes (obtained from Corning) and in tubes with glass wool and incubated for 90 min at 37°C in an atmosphere of air with 5% $CO_2$ [2].

## EXPERIMENTAL RESULTS

In the experiments of series I the distribution of tumor cells in the mice was studied (Table 1). As a result of treatment of these cells with ATS their distribution among the organs was disturbed, as reflected in an increase in radioactivity in the blood and a decrease in the lungs, spleen, and liver, and with no significant

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