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The Antitumor Effect of Bleomycin Combined with Bestatin against Ehrlich Ascites Carcinoma in Mice (42667)

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Abstract. The effect of bleomycin against Ehrlich ascites carcinoma transplanted subcutaneously to mice used in combination with bestatin was investigated. Male Balb/c mice weighing approximately 20 g and bred in our laboratories were used in this study. Each mouse was injected in its left lateral abdominal region subcutaneously with 7×10^6 tumor cells in 0.2 ml of ascites fluid. The mice were divided into four groups: control, bestatin alone (5 mg/kg intraperitoneally on Days 9–14), bleomycin alone (10 mg/kg intraperitoneally on Days 7 and 8), and bestatin plus bleomycin. Our results show that bestatin enhances the antitumor effect of bleomycin against Ehrlich ascites carcinoma as measured by the increased survival rates. Being an agent of very low toxicity, bestatin should be considered as a part of the chemoimmunotherapy protocol.

Various drug combinations are currently used in cancer chemotherapy (1). One of the schedules used for this purpose is to combine an antineoplastic agent with an immunomodulator in order to stimulate the components of the immune system that are effective against tumor cells (2–4).

Bestatin is a low-molecular-weight immunomodifier which was isolated from *Streptomyces olivoreticuli* by Hamao Umezawa *et al.* in 1976 (5). It inhibits aminopeptidases (6), binds to cell surfaces (7, 8), enhances immune responses (9–11), retards the induction of skin cancer induced by methylcholanthrene, prevents bacterial infections in mice treated with cyclophosphamide (11), reduces the bacterial persistence in experimental chronic *S. typhimurium* infection (12), and exhibits antitumor effects against mouse tumors (10, 13–15).

In previous studies, bestatin was reported to enhance the antitumor effect of various cytotoxic agents. It was also reported that bestatin administration after treatment with the cytotoxic agents was more effective than that made before the treatment (16). This result suggests that bestatin shows a positive effect on the small number of remaining tumor cells after most of tumor cells are previously killed by action of preadministered antitumor agents.

In this study, we investigated the effect of bestatin combined with bleomycin, a chemotherapeutic agent with which this immunomodulator had not been combined previously, on the survival rates of Ehrlich ascites carcinoma (EAC)-bearing mice.

Materials and Methods. Male Balb/c mice, 7–8 weeks old, weighing approximately 20 g, and inbred at the laboratories of the Center for Experimental Medical Research and Application of İstanbul University, were used. The animals were fed *ad libitum* with standard laboratory diet and water, purchased from İstanbul Yem Sanayi, Topkapı. EAC was obtained from a male mouse into which the ascites tumor had been implanted intraperitoneally 14 days previously. Bestatin (Sigma) was dissolved in phosphate-buffered saline (PBS) at a concentration of 1 mg/ml and administered at a dose of 5 mg/kg intraperitoneally (ip). Bleomycin (Mustafa Nevzat Drug Companies) was dissolved in physiological saline at a concentration of 0.2 mg/ml and administered at a dose of 10 mg/kg ip.

Twenty animals were assigned to the following four groups: 1, control; 2, bestatin alone; 3, bleomycin alone; and 4, bestatin plus bleomycin. There were five animals in each group.

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On the first day of the experiment, 0.2 ml of ascites fluid containing approximately 7 $\times 10^6$ cells was given subcutaneously to each animal in its left lateral region. On the 7th and 8th days, bleomycin was administered to the animals of the third and fourth groups. From the 9th to the 14th days, bestatin was administered to the animals in the second and fourth groups. The life spans of all animals were subsequently followed.

Statistical analyses were carried out by using the nonparametric Mann–Whitney U test.

Results. Survival rates are shown in Fig. 1. The animals of the control group had an average survival of 28 days. The animal with the longest life span survived for 35 days. Animals of the second, third, and fourth groups had average survival rates of 50, 58, and 75 days, respectively.

The third and fourth groups had longer survival periods (statistically significant) than the control group. In both groups, the P values approximate 0.01. Bestatin and bleomycin had also a beneficial effect on the survival rates of mice when compared with bleomycin alone ($P = 0.05$). Bestatin alone failed to delay the growth of EAC.

Discussion. Stimulation of the immune functions involved in tumor defense mechanisms by immunopharmacological agents is a promising field in cancer research. Bestatin, one of the agents that serves this purpose, is being examined as an immunomodulator for cancer treatment. Bestatin is suggested to stimulate the immune system mainly by activating macrophages (8) and also to stimulate T-lymphocyte responses as measured by delayed-type hypersensitivity (DTH) testing (17). In previous studies, bestatin stimulated the differentiation of bone marrow granulocyte stem cells in a medium containing colony-stimulating factor (11) and promoted the production of interleukin-1 and interleukin-2. As reported by Müller and his group (18), intraperitoneal injection of bestatin increased the activity of DNA polymerase in T cells and terminal deoxynucleotidyltransferase in bone marrow cells.

These findings, together with tests showing the extremely low toxicity of the compound, have led to the establishment of clinical trials

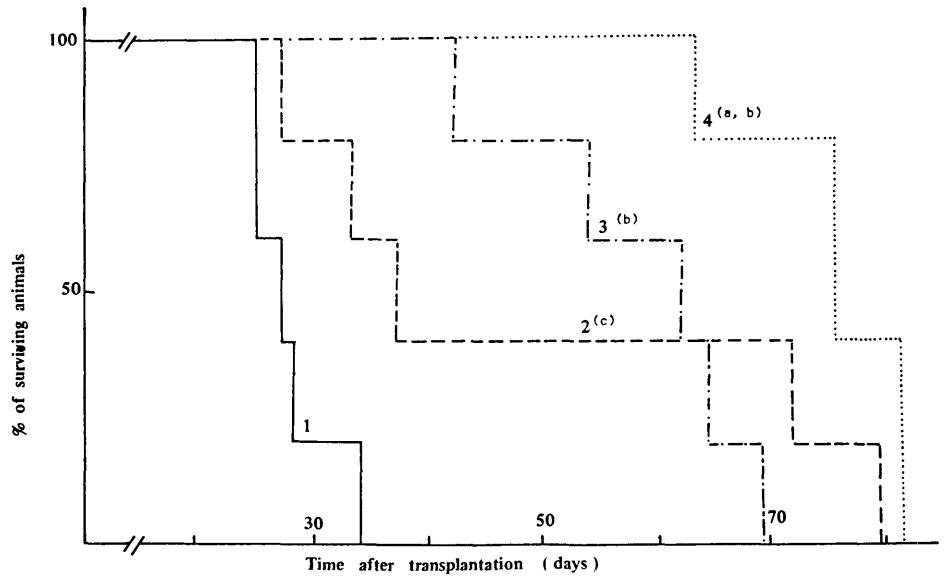

FIG. 1. Survival rates of EAC-transplanted BALB/c mice treated with bestatin and bleomycin. 1, Control; 2, bestatin (5 mg/kg) ip from Days 9 to 14; 3, bleomycin (10 mg/kg) ip on Days 7 and 8; 4, bestatin + bleomycin. (a) $P = 0.05$ when compared with group 3; (b) $P < 0.05$ when compared with control group; (c) $P > 0.05$ when compared with control group.

to evaluate its role as an immunological adjuvant in cancer treatment (19). Clinical studies have shown that bestatin inhibits recurrences and prolongs the survival periods of patients with *e.g.*, melanoma, bladder cancer, esophagus and stomach carcinomas, head and neck tumors, and leukemia. Our study also confirms the above findings, which suggest a beneficial role for bestatin in tumor management protocols.

It was found that bestatin inhibits aminopeptidase-B and leucine aminopeptidase competitively. One explanation for the immunomodulating effect of bestatin is that this effect could be due to binding with the cell membrane-associated aminopeptidases in lymphoid cells, for which it acts as a competitive inhibitor. In a recent study conducted by Aoyagi and his group, bestatin was demonstrated to trigger oscillative movements of enzyme networks in the spleen (20, 21). Since this phenomenon is likely to be accompanied by metabolic changes in spleen cells, it may somehow be related to the immunomodulating actions of this agent (22).

In our study, the enhancing effect of bestatin on the antitumor activity of bleomycin is likely to be due to the immunomodulating action of the former agent. Another role that can be suggested for this action of bestatin originates from the fact that metabolic inactivation of bleomycin is through an aminopeptidase-B-like activity in tumor and normal tissues. Since bestatin has an aminopeptidase-B-inhibiting activity, it might reduce the metabolic inactivation of bleomycin and thus increase its antitumor activity. We think that the estimation of blood levels of bleomycin after bestatin administration will provide valuable information on this undisclosed mechanism of the action of bestatin.

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