ANTIMETASTATIC EFFECT OF L-LYSINE-α-OXIDASE

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The screening of new preparations with antimetastatic activity is an urgent task. An important place in the antimetastatic resistance of the body in recent years has been ascribed to macrophages [4, 6]. It has been shown that an important role in their normal functioning is played by enzymes of purine metabolism, namely adenosine deaminase (AD) and 5′-nucleotidase (5-N), which regulate the intracellular adenosine content [12, 14]. On the other hand, a polyamine test (determination of the polyamine level in erythrocytes, blood serum, and urine) is widely used in clinical oncology to determine both activity of the tumor process and the effectiveness of treatment of cancer patients [2, 13]. Previously the writers found that the enzyme L-lysine-α-oxidase (LO), isolated from *Trichoderma harzianum* Rifai, possesses an antitumor action [7, 8].

The aim of this investigation was to study the effect of LO on metastasization in mice with Lewis lung carcinoma, and on AD and 5-N activity in alveolar macrophages and on the polyamine concentration in erythrocytes.

EXPERIMENTAL METHOD

Experiments were carried out on C57BL/6 mice weighing 20-25 g. Tumor cells were injected in 0.05 ml of physiological saline in a dose of $2 \cdot 10^5$ into the footpad. The limb with the tumor was amputated at the knee under hexobarbital anesthesia with sterile precautions. The volume and number of lung metastases in the mice were determined by the usual method [10]. The preparation of LO used, with specific activity of 29 IU/kg, was obtained in the Department of Biochemistry, Patrice Lumumba Peoples' Friendship University. The enzyme was injected intravenously in 0.2 ml of physiological saline twice a week, 6 times, starting with the 7th day after transplantation of the tumor cells in doses of 10, 20, and 50 IU/kg. Alveolar macrophages were isolated by the method in [9]. The cells obtained were lysed in a solution of 20 mM Tris-HCl, 5 mM $MgSO_4$, pH 7.4, and activity of AD and 5-N was determined in the lysate with the aid of ^{14}C-adenosine, ^{14}C-AMP, and ascending chromatography on paper [5]. AD activity was expressed in nanomoles of inosine and hypoxanthine per minute per 10^8 cells, and activity of 5-N in nanomoles of adenosine, inosine, and hypoxanthine per minute per 10^8 cells. Polyamines in the erythrocytes (spermine, spermidine, putresceine) were determined after dansylation by our modification of thin-layer chromatography [3], using high-performance liquid chromatography. Polyamine concentrations were expressed in nanomoles/10^9 cells.

EXPERIMENTAL RESULTS

The antimetastatic effect of LO, injected in different concentrations (Table 1), was studied. Reduction of the volume and number of metastases of Lewis carcinoma in mice was observed after injection of the preparation in a dose of 10 IU/kg, whereas the maximal antimetastatic action was observed when the enzyme was used in a dose of 50 IU/kg. As Table 2 shows, surgical removal of the primary tumor (on the 15th day after transplantation) led to a sharp increase in the volume and number of metastases in the lungs compared with intact animals. The possible cause of this phenomenon, according to several investigators,

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TABLE 1. Effect of LO in Different Doses on Metastasization of Lewis Carcinoma in Mice ($M \pm m$, $n = 20$)

Concentration of LO, IU/kg	Number of metastases	Volume of metastases, mm^3
Control	28,2±1,9	198,8±20,3
10	14,6±1,4*	76,1±6,3*
20	12,3±1,3*	50,8±5,2*
50	10,0±0,8*	38,9±4,0*

Legend. Here and in Tables 2 and 3, $*p < 0.05$ compared with values in mice not receiving LO.

TABLE 2. Effect of LO on Metastasization after Operative Removal of Primary Tumor of Lewis Carcinoma in Mice ($M \pm m$, $n = 20$)

Group of experiments	Number of metastases	Volume of metastases, mm^3
Without removal of tumor	26,4±3,2	146,8±15,9
Operative removal of tumor	33,1±1,1**	367,2±19,9**
Operation + LO (50 IU/mg)	9,2±0,7*	36,9±4,2*

Legend. $**p < 0.05$ compared with values for mice not undergoing operation and not receiving LO.

TABLE 3. Effect of LO on AD and 5-N Activity in Alveolar Macrophages of Mice and also on Polyamine Concentrations in Erythrocytes of Mice with Lewis Carcinoma ($M \pm m$, $n = 8$)

Time after transplantation, days	Injection of LO	AD	5-N	Spermine	Spermidine	Putrescine	Putrescine/spermidine
15	—	9,2±1,3	38,2±3,6	3,3±0,4	40,0±12,4	5,7±1,5	0,14
	+	18,2±1,6*	25,9±2,7*	3,0±0,6	38,5±8,7	1,0±0,2*	0,03*
30	—	13,7±1,2	43,8±3,9	1,2±0,2	15,8±2,1	1,3±0,2	0,08
	+	29,1±2,6*	19,7±2,3*	0,7±0,1	16,3±6,5	0,7±0,1*	0,04*

Legend. Concentration of preparation was 50 IU/kg. Parameters of activity given in "Experimental Method."

may be stressor reactions arising in the host after resection of the tumor [1]. When the enzyme was injected into mice in a concentration of 50 IU/kg the number of metastases in the lungs was reduced by 3.6 times and their volume was reduced by 10 times compared with values obtained in animals not receiving the preparation.

In a study of activity of the enzymes of adenosine metabolism in alveolar macrophages, in direct contact with metastatic cells in mouse lungs, as a result of administration of LO, AD activity rose sharply whereas 5-N activity fell at different times of observation (on the 15th and 30th days after transplantation) compared with these parameters in untreated animals (Table 3). This created conditions for a decrease in the intracellular concentration of adenosine — an inhibitor of Macrophagal function [11]. However, it is not yet clear whether the preparation acts directly on the macrophagal membrane, for which there is evidence in the form of weakening of 5-N activity, located on the surface of macrophages [12], or whether trigger mechanisms are involved in this case, their role being played by various modulators of cellular activity. Moreover, on the 15th day after injection of the tumor cells a decrease in the putrescine concentration and an increase in the molar ratio of putrescine/spermidine was observed in the erythrocytes of mice receiving the preparation compared with the group of untreated mice (Table 3). Since the putrescine concentration and the putrescine/spermidine ratio in the erythrocytes (or in blood serum) reflects the rate of proliferation of tumor cells of any kind [13], our results showing a decrease in these parameters can most probably be taken as evidence of the cytostatic action of LO. On the 30th day the polyamine concentrations in the erythrocytes were lower than on

the 15th day, possibly due to the absence of the primary tumor. The spermine and spermidine levels in the two groups of mice studied on the 30th day did not differ significantly. However, the putrescine concentration and the putrescine/spermidine ratio were considerably less in the erythrocytes of the mice receiving LO than in animals not receiving the preparation, and this was probably due to reduction of activity of the metastasization process as a result of injection of the enzyme preparation.

The results thus demonstrate that the enzyme L-lysine-α-oxidase possesses antimetastatic activity. In all probability one of the molecular mechanisms of the observed biological effect may be the restoration of disturbed adenosine metabolism in alveolar macrophages.

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