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Short communication

Reduction of B16 melanoma metastases by oral administration of egg-white lysozyme*

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Summary. The oral administration of hen egg-white lysozyme to mice bearing B16 melanoma significantly reduces the formation of spontaneous lung metastases and, when combined with surgical removal of the primary tumor, prolongs the survival of the treated hosts. The antimetastatic effect, comparable with that found in the Lewis lung carcinoma and MCa mammary carcinoma systems, is independent of the direct interaction of lysozyme with tumor cells and tends to indicate the suggested intervention of an indirect action mediated by the induction of host responses.

Introduction

The formation of pulmonary metastases can be significantly reduced by lysozyme treatment in animals bearing Lewis lung carcinoma and MCa mammary carcinoma [8 – 10, 12]. Antimetastatic activity has been reported after either i. v. injection [8] or oral administration [9, 10, 12] of numerous doses of hen egg-white lysozyme. Lysozyme has been shown to be a direct activator of immune cells such as monocytes and lymphocytes [4, 6, 7] and, after oral administration, acts as an inducer of immune responses with a potency comparable with that obtained using the broth of in vitro digested cell walls of *Bifidobacterium longum*, given by the same route [5]. Thus, after oral administration, it could be supposed that lysozyme, by interaction with intestinal bacteria, can liberate peptidoglycans of high and low molecular weight that become the agents responsible for the observed immunostimulation [2, 3] and, hence for the antitumor activity in the treated animals.

The aim of the present study was to examine the interesting antimetastatic effects of the oral administration of lysozyme in another solid, metastasizing tumor, the B16 melanoma model.

Materials and methods

Tumor-cell suspensions from a B16 melanotic melanoma line obtained from the Tumor Repository Bank (NIH, National Cancer Institute, USA) were prepared by filtering through a double layer of sterile gauze 2-week-old tumors obtained from $C_{57}B_1$ mice and finely minced with scissors in 10 vol. phosphate-buffered saline. After washing and centrifugation of the filtered material at 250 *g* for 10 min, tumor-cell viability was checked by trypan blue exclusion; only suspensions having at least 65% viable cells were used. Lysozyme obtained from SPA (Milan, Italy) was given admixed with the powdered food, and the dose was adjusted based on a daily food intake of 3.5 g/animal. Variations of food consumption were responsible for changes in the delivered dose of lysozyme of $<10\%$, which, given the spectrum of active doses found in the Lewis lung carcinoma system [10], must be considered to be noninfluential on activity.

Results

The oral administration of hen egg-white lysozyme at 50 mg/kg per day to BD_2F_1 mice on days 1 – 7 after the i. m. implantation of 10^6 B16 melanoma cells caused a significant reduction in the development of lung metastases as compared with that in untreated mice (Table 1). The reduction in the number of lung metastases is of the same magnitude as that observed in mice bearing Lewis lung carcinoma [8, 10]; the effect on primary tumor growth is less pronounced. The effect of orally given lysozyme on lung metastases is also evident when the compound is used after surgical removal of the primary tumor (Fig. 1). Radical surgical amputation of the whole tumor-bearing leg was done with the animals under Ketalar anesthesia (i. p., 125 mg/kg), using a synthetic, absorbable suture for ligating the femoral and circumflex arteries and a silk suture to close the skin at the stump. The oral administration of a daily dose of 35 mg/kg after surgical removal of the primary tumor in mice bearing i. m. implants of B16 melanoma significantly prolonged the survival of treated animals, whose death occurred due to the growth of lung metastases, as shown by necroscopic examination. In vitro incubation of lysozyme with B16 tumor cells [10^6 tumor cells/ml and 50 μg/ml lysozyme kept at 37° C for 30 min in Minimum Essential Medium (MEM) containing antibiotics] did not reduce tumor-cell viability (measured by trypan blue exclusion at the end of incubation) or

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Table 1. Effects of lysozyme on primary tumor growth and on the formation of pulmonary metastases in mice bearing B16 melanoma

Lysozyme treatment	Primary tumor weight:		Lung metastases (*n*):	
(mg/kg per day)	Mean ± SE	%Var	Mean ± SE	%Var
0	2.92 ± 0.23	–	7.9 ± 1.5*	–
50 on days 1–7	2.46 ± 0.23	− 16	2.7 ± 0.7*	− 66

Groups of 10 BD_2F_1 mice, implanted i.m. with 10^6 B16 melanoma cells on day 0, were given 50 mg/kg lysozyme daily on days 1–7
%Var, Percentage of variation as compared with control values
* Statistically significant difference according to the computerized *t*-test for grouped data [11]; $P = 0.05$

clonogenicity (determined by the capacity to form lung tumors after i.v. injection of 0.1 ml incubated mixture in groups of ten BD_2F_1 mice). The number of metastatic tumors counted on the surface of the lungs, which were freshly removed 15 days after tumor implantation, increased from 62 ± 16 (group of controls) to 80 ± 9 (treated group) (differences not statistically significant). Similar results were obtained using lysozyme at 500 μg/ml.

Discussion

The oral administration of lysozyme inhibits B16 melanoma growth by a mechanism unrelated to a direct cytotoxic effect on tumor cells. The low activity of lysozyme at the primary site is in agreement with previous observations indicating a reduced efficacy of the treatment in large Lewis lung tumors, particularly in tumors growing i.m. [10]. The effects of lysozyme are more evident in the lung tumor, and this action is paralleled by a significant prolongation of the host's life. The lack of inhibition of tumor growth after in vitro incubation of lysozyme with tumor cells seems to support the hypothesis of an indirect action of dietarily administered lysozyme on tumor growth, probably due to the induction of host factors capable of eliciting host responses against tumor metastases, as shown by the effects of samples of whole plasma and peritoneal resident cells in mice bearing MCa mammary carcinoma [9] and by the effects of dietary lysozyme on the spleens of mice carrying implants of Lewis lung carcinoma [1].

These observations stress the need to extend the evaluation of other lysozyme preparations for the management of solid tumor metastases, with particular emphasis on the role of a dietary intake of lysozymes or lysozyme-like substances in combined treatments with conventional cytotoxic drugs.

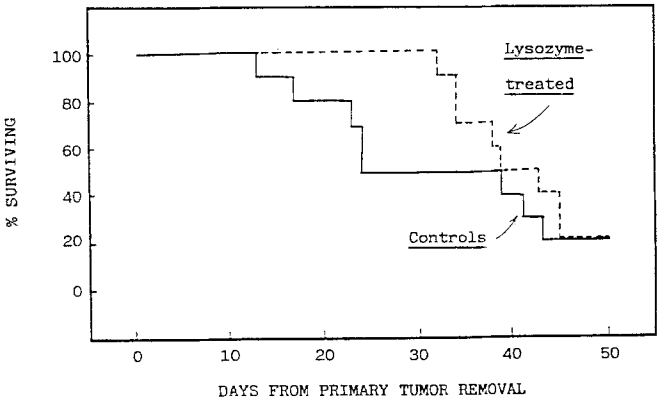

Fig. 1. Effects of the postsurgical treatment with lysozyme on the survival of mice bearing B16 melanoma. Groups of 10 BD_2F_1 mice, implanted i.m. with 10^6 B16 melanoma cells on day 0 and undergoing surgical amputation of the whole tumor-bearing leg on day 12, were treated with 35 mg/kg lysozyme daily from day 13 until their death. The difference between the actuarial curves of the two groups is statistically significant according to the computerized Mann-Whitney U-test [12] for two-tailed comparisons; $P = 0.013$

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