THE EFFECT OF THE CHLORETHYLAMINE PREPARATIONS LONIN-4 AND LONIN-3 ON THE ANTIGENIC AND VIRULENT PROPERTIES OF MALIGNANT CELLS

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After specific antigens had been found in tumor tissue [1-3, 11-14], the question of altering them with x-rays was studied [4, 6, 12, 15], chlorethylamine preparations [9], etc. In addition the literature gave indications that x-rays [5, 7] and chlorethylamine preparations [8, 9] altered the biological characteristics of malignant cells. Such investigations are being carried out in connection with a search for methods to increase the anti-tumor resistance of the organism.

The aim of this paper is the study of changes in the antigenic and virulent properties of malignant cells acted upon by lonin-4 and lonin-3, chlorethylamine preparations synthesized in our institute.

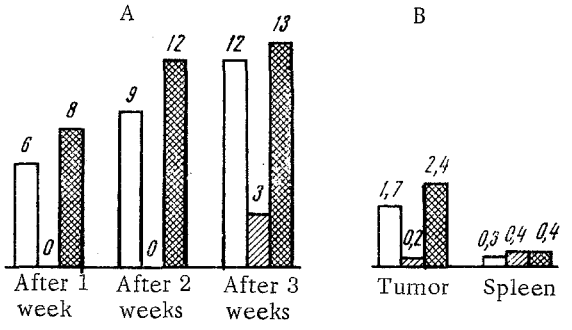

Fig. 1. Growth of Ehrlich adenocarcinoma injected subcutaneously with cells of the same tumor, treated in vivo with toxic doses of lonin-4 and lonin-3. A) Tumor size (in mm); B) weight (in g). Nonhatched bars—treated with lonin-4 in dose of 4000 mg/kg; hatched bars—treated with lonin-3 in dose of 250 mg/kg; doubly hatched bars—control.

METHODS

Malignant cells were obtained after triple centrifugation (for 10 min at 2500 rpm) of ascitic fluid from mice which had been inoculated intraperitoneally with Ehrlich's carcinoma. The sedimented cells were washed with physiological solution. After centrifugation the cells were resuspended and immediately exposed to the doses described below of lonin-4 and lonin-3. After this, 15-30 mg of cells were suspended in 0.4 ml of physiological solution and injected subcutaneously into guinea pigs for sensitization or the cells were diluted with physiological solution to the initial volume of ascitic fluid and 0.1 ml of this was injected subcutaneously into mice. The control mice received 0.1 ml of malignant ascitic fluid.

In studying the antigenic and virulent properties of the Ehrlich adenocarcinoma exposed to the action of these preparations in vivo, we took centrifuged ascitic fluid at the end of a set period after feeding the mice per os lonin-4 or lonin-3. Guinea pig sensitization and inoculation of the mice with tumor was done exactly as in the in vitro experiments.

After 25 days the guinea pigs were desensitized with cells of the Ehrlich adenocarcinoma in control animals, which received the material intravenously every 2 h. To permit intravenous use, the cells were prepared by the same method as for sensitization. The intensity of the anaphylactic reaction was expressed by the signs plus (+) or minus (-) in the conventional manner.

Reactions of Anaphylaxis and Desensitization with Antigens of Ehrlich Adenocarcinoma After in vivo Action of Lonin-4

No. of experiment	No. of guinea pig	Sensitization by cells treated with lonin-4		Desensitization by tumor cells						Permissible introduction of cells treated with lonin-4	
				I		II		III			
		Dose of preparation (in mg per kg of tumor tissue)	Dose of cells (in mg)	Dose (in mg)	Reaction	Dose (in mg)	Reaction	Dose (in mg)	Reaction	Dose (in mg)	Reaction
1st	1	2 000	15	1,25	+	10	+	15,0	—	15,0	—
	2	2 000	15	1,25	+	10	+	22,5	—	22,5	—
	3	2 000	15	1,35	++	10	+	22,5	—	22,5	—
	4	2 000	30	2,50	+++	15	++	45,0	—	45,0	—
	5	2 000	30	3,10	+	15	—	45,0	—	45,0	—
2nd	6	4 000	15	1,25	+++	10	+	15,0	—	15,0	+++
	7	4 000	15	1,25	+++	10	—	22,5	—	30,0	++
	8	4 000	15	1,25	+++	10	—	22,5	—	30,0	+
	9	4 000	30	2,50	+	15	—	30,0	—	30,0	—
	10	4 000	30	2,50	++	15	—	45,0	—	45,0	±
	11	4 000	30	2,50	+	15	—	45,0	—	45,0	—
3rd	12		15	1,25	++++						
	13		15	1,25	+++	10	+++	15,0	—	15,0	—
	14		15	1,25	+	10	+	15,0	—	15,0	—
	15	control	30	3,00	++++						
	16		30	2,50	+++	15	—	30,0	—		—
	17		30	2,50	+++	15	+	45,0	—	5,0	—
	18		×	×		×		×		5,0	—
	19		×	×		×		×		4 5,0	—

Note: Minus (—) indicates absence of a reaction; plus-minus (±) indicates a brief scratching of the snout; + indicates multiple scratching of the snout; ++ the same phenomenon and coughing, sneezing, ruffling of the hair, fall in temperature; +++ the same phenomena (more pronounced), urination and defecation (involuntary); ++++ toxic shock; X indicates that antigen was not injected.

In all, the experiment included 65 guinea pigs, 90 mice of the D strain and 110 white non-pedigreed mice. The average weight of the guinea pigs was 280 g, and age $1^1/_2$ months. The mice weighed 18-20 g and were age 3 months. Field mice were used (in separate groups from each field).

Tumor size was measured by the conventional schema each 7 days during 3-4 weeks. At the end of the experiment the tumors were weighed. The data were treated statistically: the standard error of the arithmetic mean was calculated according to Peters (m), Student's "t", and the probability coefficient P (in percent) [10].

RESULTS

The results of the first experiment show that guinea pigs which have been sensitized with Ehrlich's ascites cells which have been treated for 1 h in vitro with lonin-4 in doses of 1000, 2000, and 4000 mg per kg of tumor tissue, are completely desensitized with native ascites tumor cells. The animals did not react to the introduction of cells that had been treated with lonin-4.

In the 2nd experiment we established that guinea pigs sensitized with tumor cells which had been exposed to the toxic dose of 2000 mg of lonin-4 per kg of tumor tissue in vivo for 24 h, as in the 1st experiment, were desensitized by native tumor cells. However, guinea pigs which had been sensitized with cells treated under the same conditions with a doubly large dose of lonin-4 (4000 mg/kg) were not fully desensitized by native tumor cells: 67% of these guinea pigs reacted to the introduction of tumor cells so treated (see table).

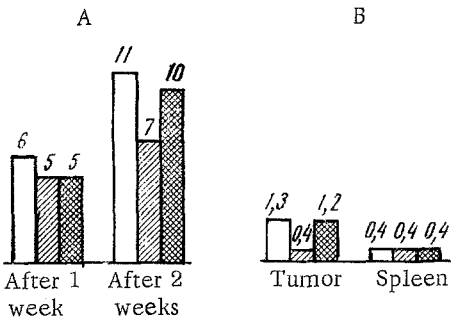

Fig. 2. Growth of Ehrlich adenocarcinoma, injected subcutaneously with cells of the same tumor treated in vivo with therapeutic doses of lonin-4 and lonin-3. Non-hatched bars—treated with lonin-4; hatched bars—treated with lonin-3, 20 mg/kg; doubly hatched bars—control. Remaining designations as in Fig. 1.

In the 3rd experiment the action of lonin-3 on the antigenic properties of adenocarcinoma cells was studied. Guinea pigs were sensitized with cells from the same mouse tumor, after receiving preliminary in vivo treatment for 7 days with therapeutic doses of lonin-3: 20 mg per kg of mouse (total of 140 mg/kg). All guinea pigs in this group were successfully desensitized with native tumor cells.

Study of the sensitizing action of lonin-3 (4th experiment) showed that guinea pigs (there were 6 animals in this group) given 25 days prior to sensitization, a pure preparation in doses triple those administered to the adenocarcinoma cell preparation, did not react to desensitization.

Control guinea pigs gave no reaction to the introduction of cells treated with the stated doses of the preparation. On checking the toxicity of the material likewise no reaction was noted.

In the subsequent series of experiments the action of lonin-4 and lonin-3 on the virulence of the Ehrlich's carcinoma was studied. In experiments with mice of the 1st group it was found that in comparison with the control, on subcutaneous injection of adenocarcinoma cells treated in vitro for 1 h with 2000 mg of lonin-4 per kg of tumor tissue, there was no statistically evident difference in tumor size after 4 weeks (P = 60-12). Again there was no significant difference when a dose of 4000 mg was used under the same conditions (P = 27-18). Upon subcutaneous injection into mice of adenocarcinoma cells treated in vitro for 1 h with lonin-4 in the dose of 20,000 mg/kg tumor tissue, the tumor cells were not observed to grow in the first 2 weeks ($P < 0.1$). However, in the 3rd week the tumors began to grow; they were 90% smaller in comparison to controls ($P < 0.1$), and in the 4th week, 42% smaller (P = 1.6).

In experiments with mice of the 2nd group, the action of lonin-3 on the virulence of Ehrlich's cells was studied. With subcutaneous injection of cells treated in vitro for 1 h with 2000 mg lonin-3/kg of tumor tissue, the tumors did not grow. Observations were carried out for 4 weeks. No tumor growth was observed also after injection of cells which had been treated in vitro for 30 min with 2000 mg of lonin-3/kg of tumor tissue and for 60 min with 1000 mg/kg of tissue. The significance of these results was extremely high ($P < 0.1$).

In experiments on mice of the 3rd group we studied the action of toxic doses of lonin-4 and lonin-3 on the virulence of the adenocarcinoma cells in vivo. As Fig. 1 shows, the size of the tumors which developed after injection of cells treated in vivo for 24 h with 4000 mg of lonin-4 per kg, was in the first and second weeks 25% less than in controls. During the 3rd week there was no significant difference in tumor size (P = 33). The weight of the tumors was 29% less than in control mice (P = 9). After 24 h treatment in vivo with 250 mg of lonin-3/kg the tumors did not grow in the first 2 weeks ($P < 0.1$), and in the 3rd week were 77% smaller than the controls ($P < 0.1$). The weight of the tumors was 92% that of the controls ($P < 0.1$). At the same time the weight of the spleen in the experimental mice was almost the same as in the controls (P = 100).

In experiments with the 4th group of mice the effect of therapeutic doses of lonin-4 and lonin-3 on Ehrlich's tumor cells was studied in vivo. The data presented in Fig. 2 show that with treatment of the cells in vivo for 8 days with 80 mg of lonin-4 per kg of mouse weight (total of 640 mg/kg for each mouse) the tumor size in experimental animals over 2 weeks did not differ from the controls (P = 49-62). There was also no difference in tumor weight (P = 69). With the use of 20 mg lonin-3/kg of mouse weight for 8 days (total of 160 mg/kg per mouse) in vivo, the tumor size in experimental animals for the 1st week also did not differ from controls (P = 100). However, in the 2nd week they were 36% smaller (P = 2). The tumor weight was 67% less than in controls (P = 0.4). No significant difference was found when the spleens were weighed (P = 49-69).

The results of the investigation show that with in vivo and in vitro treatment using different doses of lonin-4 and lonin-3 the virulence of adenocarcinoma cells is only weakened; for sure, the virulence is diminished only with use of extremely large doses—20,000 mg/kg of tumor tissue. With the use of lonin-3 in vitro the virulence is diminished but in vivo, in all doses used, the cells retained full virulence.

In an earlier paper [8] we established that cells of the Ehrlich tumor, when treated with 2000 mg of lonin-3 per kg of tumor tissue in vitro for 30 min, are able to increase the resistance of mice to growth of the same tumor by 50%

It may be that the cell virulence, obtained with the use of half the dosage would further increase cell immunogenicity.

SUMMARY

A study was made of the antigenic and virulent properties of the Ehrlich adenocarcinoma cells subjected to the action of the preparations lonin-4 and lonin-3. Investigations were carried out on guinea pigs by means of anaphylaxis with reaction desensitization, as well as on mice subcutaneously vaccinated with adenocarcinoma cells, treated in vivo and in vitro with lonin-4 and lonin-3. The results of investigations clarified to a degree the action mechanism of the studied chlorethylamino preparations and some problems concerning the immunobiological cancer cell variability.

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