ENHANCEMENT OF TUMOR GROWTH AFTER INOCULATION WITH RAT SARCOMA 45 TREATED IN VITRO WITH LONIN-3

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A study of the phenomenon of enhancement of tumor growth began several decades ago. This phenomenon was observed when animals were injected with a sarcomatous extract [1] and frozen, heated [8, 9], or dry [6, 7, 11, 12] malignant cells before inoculation of the tumor. Extensive reviews are available in the literature on this problem [2, 5].

The purpose of this work was to study the phenomenon of enhanced tumor growth, which, instead of the expected increase of antitumor resistance, was obtained upon inoculating rats with tissue of sarcoma 45 treated preliminarily in vitro with the preparation lonin-3.

METHOD

Four series of experiments were carried out. The experimental rats of the first three series were inoculated with sarcoma 45 tissue treated in vitro with lonin-3. The cells of sarcoma 45 after being treated in vitro with this preparation in a dose of 250 mg/kg of tumor tissue for 60 min, losing transplantability, become avirulent [4]. The strain of rat sarcoma 45 we used was characterizer by good resorption. The cells of sarcoma 45 after treatment were not washed free from lonin-3; therefore the experimental rats of the IV series were inoculated with a pure preparation of lonin-3.

In the I, II, and IV series the rats were inoculated three times at one week intervals. In the III series, for investigation of the significance of the inoculation period for tumor growth, the intervals between the 1st and 2nd and between the 3rd and 4th injections were a week and between the 2nd and 3rd, two weeks. Inoculation continued twice as long as in the other series.

The quantity of tissue in the experiments of the I and II series in the 1st and 2nd injections was 50-400 mg and in the 3rd injection 100-800 mg of fresh tumor tissue. The weight of the tissue in the experiments of the III series in the 1st and 2nd injections was 50-300 mg, in the 3rd injection 75-450 mg, and in the 4th, 100-600 mg. The dose of the preparation lonin-3 in the experiments of the IV series in the 1st and 2nd injections was 0.063 mg for each rat and in the 3rd inoculation, 0.125 mg. In conformity with the indicated doses of the preparation we prepared its water-salt suspensions and injected 1 ml into each rat. These doses of the preparation corresponded to the injection of 250-500 mg of sarcomatous tissue treated with 250 mg lonin-3 per 1 kg of tumor tissue.

One (in the I, II, and IV series) or two (in the III series) weeks after the final inoculation, 250 mg of sarcoma 45 in 1 ml of physiological salt solution (25% suspension) were transplanted subcutaneously into the experimental and control rats.

We determined the size of the subcutaneous tumors that developed by the usual method (three measurements) and calculated the average diameter of the tumor in millimeters for a total of 3-4 times every seven days. The tumors were weighed at the end of the experiments.

We used 194 rats in the work. There were 50 rats in the I and II series, 70 in the III series, and 24 in the IV

TABLE 1. Enhancement of the Growth of Sarcoma 45 Upon Intramuscular Inoculation of Rats with Tissue of This Tumor Treated in Vitro with 250 mg Lonin-3 per 1 kg of Tumor for 30 min (average of the data of the four series of experiments)

Experimental variant and index of analysis	Average diameter of tumor (in mm)				Average weight of tumor (in g)
	on day 7	on day 14	on day 21	on day 28	
Experiment	8	24	37	39	60
Control	8	17	21	18	19
Difference	0	7	16	21	41
Significant difference of means (P%)	100	<0.1	<0.1	<0.4	<0.5

TABLE 2. Enhancement of the Growth of Sarcoma 45 Upon Intramuscular Inoculation of Rats with Tissue of This Tumor Treated in Vitro with 250 mg of Lonin-3 per 1 kg of Tumor for 60 min (Average of the data of the four series of experiments)

Experimental variant and index of analysis	Average diameter of tumor (in mm)				Average weight of tumor (in g)
	on day 7	on day 14	on day 21	on day 28	
Experiment	8	21	36	44	59
Control	7	14	16	13	15
Difference	1	7	20	21	44
Significant difference of means (P%)	37	<0.1	< 0.1	0.3	0.3

series (there were 10 control rats in each series). The initial weight of the rats was 110-120 g, age two months, sex—males and females (in some series the rats were of the same sex).

RESULTS

Tables 1 and 2 give the data obtained upon intramuscular injection of rats with tissue of sarcoma 45 treated in vitro by lonin-3 in a dose of 250 mg/kg for 30 min (I series of experiments) and 60 min (II series of experiments).

It is apparent from Tables 1 and 2 that there is a significant increase in the average diameter and weight of the tumor for the rats of the experimental groups. We did not note a dependence of the tumor growth rate on the time of action of the preparation. The difference in weight of the tumors was only 3 g, which is statistically insignificant (P = 84%).

Upon immunization under the given conditions of the experimental set up, the quantity of tissue had no bearing on the enhancement of tumor growth. In the experiments of the I series where the primary inoculating doses were 300, 300, 200, and 100 mg of fresh sarcomatous tissue treated with lonin-3, a statistically significant increase of tumor growth was not noted for the animals of the various groups (P = 84, 77, 63%). In the II series where the primary inoculating doses were 400, 200, 100, and 50 mg of fresh treated tumor tissue, the enhancement of growth was also independent of the quantity of sarcomatous tissue.

In the experiments of the III series we investigated the significance of the inoculation period for tumor growth. No difference in tumor growth was observed with four inoculations over a period of four weeks of after transplating the sarcomatous tissue two weeks after the last inoculation (Table 3).

In the experiments of the IV series with the intramuscular inoculation of a water-salt suspension of a pure preparation of lonin-3, the size and weight of the tumors did not differ from the control at all periods of the investigation (Table 4).

TABLE 3. Growth of Sarcoma 45 with Four Intramuscular Inoculations of the Tissue of This Tumor Treated in Vitro with 250 mg of Lonin-3 per 1 kg of Tumor for 30 and 60 min (Data of 6 groups of experiments)

Experimental variant and index of analysis	Average diameter of tumor (in mm)			Average weight of tumor (in g)
	on day 7	on day 14	on day 21	
Experiment	19	34	40	57
Control	22	36	38	53
Difference	3	2	2	4
Significant difference of means (P%)	6	48	92	69

TABLE 4. Growth of Sarcoma 45 with Three Intramuscular Inoculations of Rats with Lonin-3

Experimental variant and index of analysis	Average diameter of tumor (in mm)				Average weight of tumor (in g)
	on day 7	on day 14	on day 21	on day 28	
Experiment	9	18	22	27	23
Control	8	16	24	32	27
Difference	1	2	2	5	4
Significant difference of means (P%)	62	56	62	43	77

Certain authors related the enhancement of tumor growth to the quantity of inoculated tumor tissue [10, 15]. However, the dependence of growth on the dose of the material was not always observed [14]. It is necessary to note that according to the data or our experiments, the amount of sarcomatous tissue for inoculation was also not important. The observed enhancement of tumor growth was not caused by the time of action of the preparation. But tumor growth did depend on the period of inoculation. A difference in growth of sarcoma 45 in comparison with the control was not noted upon doubling the inoculation period. We can assume that the essence of this phenomenon, in addition to other causes, is associated with the age of the animals since the tumors of the rats in the control group from the series of those inoculated for a long time grew better than the tumors of rats of the control groups from the series of those inoculated for a shorter period.

As is known, during the development of any tumor there are two aspects of the process: protective and adaptive reactions of the organism and growth of the malignant cells. Proceeding from the hypothesis of Möller [13] that in malignant neoplasms stimulated immunologically the tumor antigens are covered by antibodies and are blocked, i.e., lose the capacity for antigenic stimulation, and in connection with our data on the significance of the age of the rats we can hypothesize that inoculation of a younger organism leads to a predominating blocking effect. This means that in rats inoculated for a sufficiently long time the absence of a change in tumor growth is not an index of areactivity but only a consequence of the adaptive reaction of the organism. Thus, enhancement of tumor growth completes the "unsuccessful" immune reaction of the organism to an external stimulus.

Based on the hypothesis that in the phenomenon of enhancement of tumor growth the tumor cells grow more quickly as a consequence of the antibodies formed on them [12], we can assume that in our experiments the antibodies that formed after immunization with tumor cells damaged in vitro by lonin-3 react with the undamaged transplanted cells of the same tumor, i.e., the tumor cells enter an unusual, altered environment of the organism surrounding them. They change, quickly adapt to the new conditions, become more stable, and begin to multiply at an accelerated rate. We can assume that the opinion concerning the change of reactivity of the cells (the precancer state) and the creation of a population of vigorously multiplying cells as a consequence of damage [3] must be under stood in a wider aspect.

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All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.