

ACTION OF A SERUM AGAINST IRRADIATED TUMOR TISSUE ON THE MITOTIC ACTIVITY OF EHRLICH'S ADENOCARCINOMA

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It has been shown that anticarcinoma sera retard the growth and development of tumors, inhibit their metastasization [10, 12, 15, 16], and depress the intensity of cell division [3, 13, 14]. It has also been demonstrated that the antigenic composition, not only of normal [1, 4, 11], but also of tumor [6, 7, 17] tissues, may be modified by the action of ionizing radiation, and that sera against tumor tissue irradiated with roentgen rays inhibit the growth of experimental transplantable tumors in animals irradiated with roentgen rays [8].

In the present paper we describe the results of a study of the mitotic activity of tumor cells in a subcutaneous Ehrlich's adenocarcinoma of mice, irradiated with roentgen rays, when receiving injections of sera against the same tumor tissue irradiated in vitro.

EXPERIMENTAL METHODS

The sera used in the experiments were obtained in our laboratory by I. N. Maiskii, P. P. Filatov, and G. V. Suvarova [9] by immunizing rabbits with extracts of irradiated tumor cells of an Ehrlich's adenocarcinoma and with extracts of untreated, unirradiated cells of this tumor, and in addition sera of healthy unimmunized rabbits also were used. In the present investigation 139 male albino mice were used, and were subdivided into 10 groups. The animals of 8 groups were inoculated subcutaneously in the dorsal region with Ehrlich's adenocarcinoma, and the mice of the 9th and 10th groups were not inoculated with tumor material (Table 1).

The animals of the 9th and 10th groups were used to study the intensity of cell divisions in the epithelium of the cornea and the crypts of the small intestine in animals unaffected by tumors, after irradiation (9th group) and kept in ordinary conditions without any form of treatment (10th group), by comparison with the intensity of cell division in these tissues in mice with Ehrlich's adenocarcinoma (1st-8th groups).

On the day after inoculation of the animals of the 1st-8th groups with tumor material the mice of groups 1, 2, 3, 7, and 9 were placed in plexiglass boxes and irradiated with roentgen rays in a dose of 100 R under the following conditions: current 10 mA, voltage 160 kV, filter 3 mm Al, focus distance 48 cm, duration of exposure 5 min 15 sec, and dose rate 19 R/min. Twenty four hours after irradiation injections of serum began, in doses of 0.3 ml subcutaneously in the dorsal region, repeated 3 times altogether at intervals of 2 days between injections. All the animals were sacrificed at the same time, during the morning, on the 10th day after inoculation of the tumor.

Material taken for investigation of the mitotic activity included the tumor, an area of the small intestine, and the cornea (the latter for comparison of the action of the test sera on the tumor and on tissue not involved in the tumor process).

The tumor and intestine were fixed in Carnoy's fluid and the cornea in 5% acetic acid solution in 70° alcohol. Sections were cut from the tumor and intestine to a thickness of 8 μ , and total preparations of the cornea were made. In all cases the sections were stained with Carazzi's hematoxylin.

After the mitoses had been counted, the mitotic coefficient (MC) was calculated per mill for each object investigated. The coefficient K was also calculated (the ratio between the sum of the early phases of division to the sum of the late phases).

TABLE 1. Distribution of Animals by Groups and by Character of Treatment Given

Group of animals	Number of mice	Experimental conditions
1st	23	Irradiation. Injection of serum against irradiated tumor
2nd	14	Irradiation. Injection of serum against unirradiated tumor
3rd	19	Irradiation. Injection of serum against normal rabbit
4th	17	No irradiation. Injection of serum against irradiated tumor
5th	19	No irradiation. Injection of serum against unirradiated tumor
6th	17	No irradiation. Injection of serum against normal rabbit
7th	5	Irradiation. No serum injected
8th	9	No irradiation. No serum injected
9th	7	Irradiation. No serum injected
10th	9	No form of treatment given

EXPERIMENTAL RESULTS

Counts of the mitoses in the tumors from mice of the 1st-8th groups revealed that the mean percentage of the various phases of division varied within narrow limits for each group (prophase $\approx 11-15\%$, metaphase $\approx 61-74\%$, anaphase $\approx 3-4\%$, telophase $\approx 11-23\%$); the same applied to the values of K (from 3 to 5).

The results given in Table 2 show that the MC in groups 1 and 2, 4 and 5, in which injections of serum against irradiated and unirradiated tumor tissue were given to mice irradiated and not irradiated with roentgen rays respectively, was least (2.49 and 2.04, 3.47 and 2.44 respectively). In the remaining groups the value of the MC was significantly higher (3rd group - 9.17, 6th group - 6.43, 7th group - 9.94, 8th group - 8.43). Hence sera against the irradiated and unirradiated tumor had a statistically significant depressant action on the intensity of division of malignant cells (in irradiated and unirradiated mice). Irradiation of mice with roentgen rays (without additional injection of sera) and injections of serum of a normal rabbit (into irradiated and unirradiated animals) did not depress the mitotic activity of Ehrlich's adenocarcinoma.

The value of the MC in the epithelium of the cornea and the crypts of the small intestine was unchanged after injection of the test sera into both the irradiated and unirradiated mice with Ehrlich's adenocarcinoma. Irradiation likewise did not change the intensity of cell division in these tissues (in both inoculated and healthy animals). The latter was evidently in consequence of the late period of the investigation, and also of the comparatively small dose of radiation (100 R) used in these experiments [2, 5].

It should be noted that after injection of serum against the irradiated tumor into mice irradiated with roentgen rays (1st group), in some cases no tumor developed or growth was very weak, or the tumor underwent necrosis (in 74% of the animals of this group). Fewer such cases (57%) were observed after injection of serum against ordinary untreated tumor into the irradiated animals of the 2nd group, and fewer still (20-40%) in the remaining experimental groups (in all these cases it was impossible to count mitoses, which accounts for the smaller number of preparations counted, as indicated in Table 2, by comparison with the number of animals taken in the experiments).

Our observations agree with the findings of other workers [8], and show that a serum obtained against an irradiated carcinoma antigen causes a more definite inhibition of the growth and development of Ehrlich's adenocarcinoma in irradiated animals than the ordinary antitumor and normal sera. It must be assumed that this action of the serum against irradiated tumor tissue is associated with the greater specificity of this serum in respect of the antigens of the tumor and of normal tissues modified by the action of roentgen rays. The resultant effect is evidently to cause stimulation of the protective powers of the organism.

TABLE 2. MC (per mill) in Subcutaneous Ehrlich's Adenocarcinoma after Injection of Sera and Irradiation

Irradiated mice			Unirradiated mice			Mice	
sera						irradiated	untreated
against tumor		of normal rabbit	against tumor		of normal rabbit		
irradiated	untreated		irradiated	untreated			
group							
1st	2nd	3rd	4th	5th	6th	7th	8th
1,25	0,94	3,77	0,47	0,26	2,59	4,24	3,38
1,64	1,86	3,81	1,00	0,85	2,86	7,99	4,58
2,28	2,05	5,25	2,06	1,77	4,97	9,31	6,98
2,32	2,08	5,92	2,51	1,84	5,66	18,22	8,35
3,69	2,32	6,67	2,78	2,14	5,90	—	10,20
3,77	2,96	7,10	3,90	2,30	6,07	—	17,08
—	—	8,18	4,63	2,64	7,64	—	—
—	—	8,67	5,11	2,79	8,01	—	—
—	—	10,50	8,81	2,79	9,76	—	—
—	—	10,76	—	3,70	10,79	—	—
—	—	18,41	—	3,71	—	—	—
—	—	21,02	—	4,50	—	—	—
Mean 2,49	2,04	9,17	3,47	2,44	6,43	9,94	8,43

Probability of chance difference between means (P) in groups

1-й и 3-й	=0,000	4-й и 6-й	=0,023
1-й и 7-й	=0,015	4-й и 7-й	=0,014
1-й и 8-й	=0,016	4-й и 8-й	=0,025
2-й и 3-й	=0,007	5-й и 6-й	=0,000
2-й и 7-й	=0,009	5-й и 7-й	=0,000
2-й и 8-й	=0,009	5-й и 8-й	=0,001

However, in cases in which, after injection of serum against the irradiated tumor into mice irradiated with roentgen rays, tumors nevertheless developed, no sharp difference was observed between the mitotic activity of the tumors in the animals of this group and of the other groups of mice receiving antitumor serum. Such a difference might possibly have been found had the experiments been carried out in other conditions (increase in the titer of the sera, different dosage, etc.).

SUMMARY

Experiments proved that, compared with normal rabbit serum, sera against irradiated and nonirradiated tumors had a statistically significant depressive effect on the intensity of malignant cell division in subcutaneous Ehrlich's adenocarcinoma of mice (both in irradiated and nonirradiated animals).

All the sera mentioned had no significant effect on the mitotic activity of the corneal epithelium and crypts of the small intestine in experimental animals (both healthy and irradiated). The greatest percentages of undeveloped or weakly developed and necrotic tumors (74%) were observed in the group of irradiated mice and in those that were given the irradiated tumor suppressing serum.

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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.
