

THE EFFECT OF IONIZING RADIATION ON THE ANTIGENIC AND BIOLOGICAL PROPERTIES OF THE M-1 RAT TUMOR

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In our previous reports [1 - 4] we showed changes in the antigenic and biological properties of Ehrlich's mouse tumor and Brown-Pearce's rabbit carcinoma associated with the action of roentgen rays on these cells in vitro.

The purpose of the present work was to clarify the effect of penetrating radiation, in particular roentgen rays, on the antigenic and biological properties of the M-1 rat tumor.

EXPERIMENTAL

The effect of roentgen rays on the antigenic properties of the M-1 rat tumor was studied in guinea pigs, using the reaction of anaphylaxis and desensitization. Experiments for clarifying the action of roentgen rays on the biological properties of the tissues of this same tumor were carried out on rats by means of transplantation of the irradiated tumor cells to them and subsequent observations for the clinical picture of tumor development.

In the experiments designed to study the effect of ionizing radiation on the antigenic properties we used a 20% suspension of M-1 rat sarcoma cells, irradiated with a dose of 5000 r in vitro.

The cell suspension was prepared in the following manner: tumor tissue, taken from the rat on the 13th day after transplantation, was weighed, placed in a sterile Petri dish, and cut into tiny pieces with scissors until a homogeneous mass was obtained. Physiological saline was gradually added into the Petri dish to make a 1:5 suspension.

Volumes of 2.8 ml of the cell suspensions were irradiated in glass vessels with a diameter of 3.5 cm, using the RUT-60-20 (RUM-7) apparatus with a current of 5 ma at 60 kv, a 0.1 mm aluminum filter, a focal distance of 75 mm, and an irradiation time of 37 seconds.

In order to test their viability, a portion of the cells were transplanted to rats subcutaneously. The rest were used for developing the anaphylactic reaction. The guinea pigs were sensitized subcutaneously. Desensitization and the final injection were performed intracardially, 22 days after sensitization. The intervals between the repeated intracardial injections associated with desensitization were equal to 2 hours.

The guinea pigs of the experimental group were sensitized with tissue from the M-1 tumor irradiated with 5000 r, and desensitized with untreated tissue from this tumor. The final injection was administered after verifying that the desensitization was complete, using tumor tissue irradiated with 5000 r. Guinea pigs sensitized and desensitized with nonirradiated M-1 tumor tissue served as the control. An additional control was provided by pigs on whom we tested the toxicity of the dose used for the final injection.

In the experiments designed to study the effect of roentgen rays on the biological properties of the M-1 tumor we again used a 20% cell suspension, irradiated with 2000, 10,000, and 20,000 r. Irradiation was performed according to the method described above, the only changes being in the corresponding current intensities and irradiation times.

Four groups of sexually mature male rats were used in the experiment, all of the same age and weight, 10 animals in each group. All the rats were simultaneously injected with 0.5 ml of the 20% cell suspension prepared

from the sarcoma M-1, deposition being made under the skin of the back. The animals of the first group received the cell suspension irradiated with 2000 r at a current of 5 ma and an irradiation time of 15 seconds; the animals of the second group were inoculated with cells subjected to the action of 10,000 r at a current of 10 ma and an irradiation time of 37 seconds; the animals of the third group received cells irradiated with 20,000 r at a current of 20 ma and an irradiation time of 36 seconds. The animals of the fourth group served as the control, receiving the same volume of a suspension of untreated tumor cells.

TABLE 1. Results of Setting Up the Anaphylactic Reaction Plus Desensitization

Guinea pig No.	Sensitization	Desensitization with the antigen from untreated sarcoma M-1 tissue			Final injection of 0.5 ml of antigen from the sarcoma M-1 tissue irradiated with 5000 r
		1st injection of 0.2 ml	2nd injection of 0.35 ml	3rd injection of 0.5 ml	
1	0.5 ml of antigen from the sarcoma M-1 tissue irradiated with a dose of 5000 r	+++	+	-	-
2	the same	++++			
3	" "	++++			
4	" "	+	-		-
5	" "	++	-		+
6	" "	+++	-		+
7	" "	+	-		-
8	" "	+++	-		-
9	" "	++	-		-

Symbols: - no reaction; + repeated rubbing of the nose and sneezing; ++ the same, plus coughing and unsteadiness; +++ the same, plus convulsions and involuntary loss of urine and feces; ++++ the same plus death.

Note: The results obtained in the guinea pigs used as the control for toxicity of the preparation are not included in Table 1, since the dose of antigen used for the final injection was shown to be nontoxic. The results obtained from the animals in the control group, sensitized and desensitized with untreated tumor tissue, are also not included in Table 1, inasmuch as these animals did not react to the final injection following their complete desensitization.

TABLE 2. Difference in the Clinical Course of the Tumor Process in Rats, Associated with Transplantation to Them of Sarcoma M-1 Tissue Irradiated in Vitro with Various Roentgen Ray Doses

Group of animals	Transplanted tumor	Number of animals	Average latent period (in days)	Average weight of the tumor on the 23rd day after transplantation (in grams)
First	Irradiated with 20,000 r	10	32.0	-
Second	Irradiated with 10,000 r	10	20.5	-
Third	Irradiated with 2000 r	10	9.1	12.66
Fourth	Nonirradiated (control)	10	6.0	25.11

In Table 1 we present the results obtained from studying the effect of roentgen rays on the antigenic properties of the rat tumor M-1.

As can be seen from the data shown in Table 1, in only 2 guinea pigs out of the seven (No. 5 and 6), which were sensitized with irradiated, and desensitized with the untreated tumor tissue, was there even a weak reaction (+) to the final injection of tissue subjected to roentgen irradiation.

The results of the experiments set up to study the effects of penetrating radiation on the biological properties of the cells of rat sarcoma M-1 are presented in Table 2; summarized data is given on the times of appearance of the tumor and its weight (in the rats of the third and fourth groups).

From the data presented in Table 2 it is apparent that the shortest latent period (9 days), approximating in length the latent period of the control animals, was in the rats inoculated with sarcoma tissue irradiated with 2000 r in vitro. The latent period for the development of the tumor in the animals of the other experimental groups was considerably prolonged, and approached an average of up to 20.5 days with inoculation of cells subjected to a dose of 10,000 r, and up to 32.1 days with preliminary radiation of the cells with 20,000 r.

Statistical analysis showed that the difference in the average figures for the duration of the latent period was significant in the case of the first and second groups ($P = 0.008$), second and third groups ($P = 0.004$), and even the third and fourth groups ($P = 0.001$). The significance of the difference between the first and fourth groups is beyond question.

In view of the rapid growth of the tumors in the rats of the third and fourth groups, and the possible death of the animals, they were sacrificed on the 23rd day after transplantation. The weight of the tumor served as the criterion for the effect of roentgen rays on the biological properties of the cells in the animals of these groups. Weighing of the tumors showed that the average weight of those in the rats of the third group was less than half the weight of the tumors in the control animals.

The animals in the first and second groups were left alive. It was established that the average duration of survival of the rats in the first group was equal to 59 days, and in the second group — 61 days, which markedly exceeds the usual duration of survival for animals stricken with the sarcoma M-1 (30-35 days).

It must be stressed that in this series of experiments we failed to note the atypical tumor development which we observed when we studied the effect of penetrating radiation on the biological properties of Ehrlich's adenocarcinoma and the Brown-Pearce carcinoma [2, 4]. In only one case was there noted a relatively insignificant, transient regression of the tumor.

From the data presented it would follow that alteration of the biological properties of rat sarcoma M-1 cells, under the influence of roentgen rays, is manifested by an increase in the latent period of tumor development, in a decrease in the weight of the tumors, and in a prolongation of the duration of survival of the experimental animals. The length of the latent period, in this series of experiments, was directly proportional to the intensity of the cell irradiation prior to their transplantation, in contradistinction to the data obtained by us in studying the effect of roentgen irradiation on the biological properties of Brown-Pearce carcinoma cells [4].

SUMMARY

An inquiry was made into the effect of x-rays on the antigenic and biological properties of the rat M-1 sarcoma. Anaphylactic reaction with desensitization brought evidence that irradiation of a tumor tissue with 5,000 r caused significant changes in its antigenic structure. The doses of 2,000, 10,000 and 20,000 r prolonged the latent period of tumour development following inoculation of irradiated cells.

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