

EFFECT OF PERIODIC ADMINISTRATION OF ANTITUMOR SERA ON MITOTIC ACTIVITY OF TUMOR CELLS

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It has previously been shown [1-6] that an antitumor serum, if injected into experimental animals with developing tumors, depresses mitotic activity of the tumor cells. It has also been found that this effect stops on the 5th day after the last injection of serum [5].

However, the problem of whether the depression of mitotic division of tumor cells may be prolonged by giving repeated injections of antitumor serum has not yet been investigated.

In this investigation the mitotic activity of tumor cells was studied in a subcutaneous Ehrlich's adenocarcinoma in mice receiving antitumor serum injections periodically for a period of 2 weeks.

EXPERIMENTAL METHOD

Serum from chinchilla rabbits immunized intravenously with extract of ascites cells of an Ehrlich's adenocarcinoma was used in the experiments. In the complement fixation reaction at 37° this serum reacted in a dilution of 1:400 with antigen of the same tumor (+++) and in lower dilutions with antigens of spleen and liver. In addition, serum of healthy unimmunized rabbits was used as control.

Three series of experiments were carried out on 124 line A male mice, inoculated subcutaneously in the dorsal region with Ehrlich's adenocarcinoma. Seven days after inoculation of the tumor the mice were divided into 14 groups, and injections of antitumor serum (0.5 ml subcutaneously in the dorsal region) and serum of the healthy unimmunized rabbit began. Serum was injected at intervals of 1-3 days into the various groups of experimental animals. The scheme of injection of the serum, the times of sacrifice, and so on are given in the table.

When the mice were sacrificed the tumors were removed for counting mitoses. The material was fixed in Carnoy's fluid and stained with Carazzi's hematoxylin. After the mitoses had been counted the mitotic coefficient (MC) was calculated in promille for each test object. The results obtained were subjected to statistical analysis by the Fisher-Student method.

EXPERIMENTAL RESULTS

Counts of mitoses in the tumors of mice of the experimental and control groups showed that the mean percentage of the various phases of division was approximately identical in each group ($P \approx 2-4\%$; $N \approx 74-78\%$; $A \approx 1.5-2.5\%$; $T \approx 17-20\%$), and this was also true of the ratio between the early and late phases of division (from 3 to 4.5).

As the figure shows, on the 12th day of development of the tumor, the MC for the mice of group 1 (after 2 injections of antitumor serum) was much lower than in the control (group 3) and after injection of serum of a normal rabbit (group 2).

The difference between the MC of the animals of groups 1 and 2 was close to significant ($P = 0.037$), while that between the animals of groups 1 and 3 was significant ($P = 0.014$).

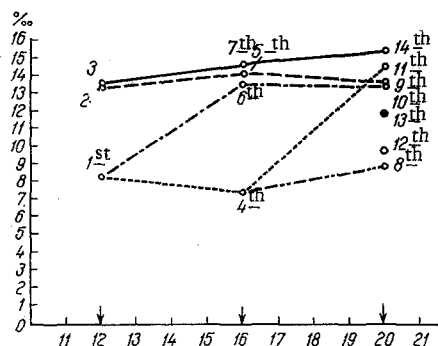
On the 16th day of tumor development, the MC was lowest in the tumors of the mice of group 4, which were sacrificed 24 h after the 3rd injection of antitumor serum. In the mice of group 5 (3 injections

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Distribution of Animals by Groups and Character by Treatment*

Group	No. of animals	Serum	No. of injections	Day of injection of serum after inoculation of tumor	Time of sacrifice (in days)	
					after last injection of serum	after inoculation of tumor
Series I						
1st	8	Antitumor	2	7-th 11-th	1	12
2nd	8	Normal	2	7-th 11-th	1	12
3rd	8	Control (without injection of serum)				12
Series II						
4th	10	Antitumor	3	7, 11, 15-th	1	16
5th	10	Normal	3	7, 11, 15-th	1	16
6th	10	Antitumor	2	7-th 11-th	5	16
7th	5	Control (without injection of serum)				16
Series III						
8th	10	Antitumor	4	7, 11, 15, 19-th	1	20
9th	10	Normal	4	7, 11, 15, 19-th	1	20
10th	10	Antitumor	2	7-th 11-th	9	20
11th	10	"	3	7, 11, 15-th	5	20
12th	10	"	6	7, 9, 11, 13, 16, 19-th	1	20
13th	10	Normal	6	7, 9, 11, 13, 16, 19-th	1	20
14th	5	Control (without injection of serum)				20

* In each of the 9th and 11th groups, 2 mice died during the experiment. In one mouse of group 5 and one mouse of group 13 the mitotic coefficient could not be calculated because of extensive necrosis of the tumor.



Mitotic activity of cells of Ehrlich's adenocarcinoma in mice after repeated injection of antitumor sera. 1st-14th groups of animals (see table). Abscissa) days after inoculation of tumor; ordinate) mitotic coefficient in %.

between the MC of mice of groups 13 and 14. However, the difference between these values in the animals of groups 12 and 14 was significant ($P = 0.006$).

The results of counts of mitoses in the tumors of the experimental animals thus showed that 24 h after 2, 3, 4, and 6 injections of antitumor serum the mitotic activity of the tumor cells was much lower than in the control (injection of normal rabbit serum by the same schemes or no injection of serum). The differences were statistically significant.

of normal rabbit serum), group 6 (5th day after injection of antitumor serum) and group 7 (without injection of serum), the MC was much higher (differences statistically significant; $P < 0.001$). The significance of the difference between the MC for animals of groups 1 and 6 indicates that the inhibitory effect of the antitumor serum stopped on the 5th day after the last injection.

On the 20th day of tumor development the MC was smallest in the mice of group 8—after the 4th injection of antitumor serum. It is clear from the figure that the MC was much higher in the mice of groups 9, 10, 11, and 14.

The difference between the MC of the animals of groups 8 and 9 was significant ($P = 0.002$), as it also was between the mice of groups 8 and 14 ($P < 0.001$). The difference between the MC for the animals of groups 11 and 4 was significant ($P < 0.001$), indicating an increase in MC up to its level in the control on the 5th day after injection of antitumor serum. The MC for the mice of group 12 calculated 24 h after the 6th injection of antitumor serum was slightly less (10.062%) than in the animals of group 13 (12.03%) after the 6th injection of normal rabbit serum, although the difference between these values was not significant, just as

Mitotic activity was also low in the tumors of the mice after the 6th injection of antitumor serum (on the 20th day after inoculation of the tumor), but the inhibitory action of the serum was less obvious in this case.

Mitotic activity in the tumors of the mice on the 5th and 9th days after injection of antitumor serum was practically equal to the control value.

Analysis of the results of these experiments thus shows that repeated injections of antitumor serum into line A mice with a developing subcutaneous Ehrlich's adenocarcinoma caused depression of mitotic activity of the tumor cells throughout the period of injection of the sera. Injection of normal rabbit serum by the same scheme into the experimental animals has no such effect on division of the tumor cells.

The intervals between injections of sera must not exceed 3-4 days, for according to the results of this investigation and to data published previously by the author [5] on the 5th day after the last injection of serum no inhibitory effect of the antitumor serum on mitotic activity of tumor cells of Ehrlich's adenocarcinoma is observed in mice.

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