DURATION OF THE INHIBITING INFLUENCE OF ANTIRONIDASE SERUM AND ITS γ-GLOBULIN FRACTION ON THE MITOTIC ACTIVITY OF TUMOR CELLS

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In our previous work, we demonstrated a reliable decrease in the number of mitoses of tumor cells in Ehrlich's subcutaneous adenocarcinoma after the administration of antironidase serum and its γ-globulin fraction [8]. However, the mitotic activity of the tumor was investigated only in a definite period—24 h after the injection of the serum and γ-globulin indicated above.

Considering the data of [4, 7] on the more prolonged period of inhibition of cell division of this tumor after injections of antitumor serum, in this work we studied the duration of the inhibiting influence of antironidase serum and its γ-globulin fraction on the mitotic activity of Ehrlich's adenocarcinoma.

PROCEDURE

We used the same sera of horses immunized with ronidase and the γ-globulin fraction obtained from a mixture of antironidase sera, the characteristics of which are cited in the studies of other authors [6], as in our previous investigation [8]. As the control we used normal horse serum. The experiment was conducted on 120 male mice, which were divided into four groups of 30 animals each on the 7th day after grafting of Ehrlich's adenocarcinoma under the skin of the back. The mice of the first group received three subcutaneous injections (on the 7th, 8th, and 10th days after grafting of the tumor) of antironidase serum in doses of 0.5 ml according to the same scheme and the same dose; the animals of the 2nd group received γ-globulin, the mice of the 3rd group normal horse serum, and the animals of the fourth group (control) received no injections.

The dynamics of the changes in the mitotic activity of the tumor cells were investigated for three days after the last injection of the preparation.

All the mice were additionally separated into groups corresponding to the periods of sacrifice after the last injection (see table).

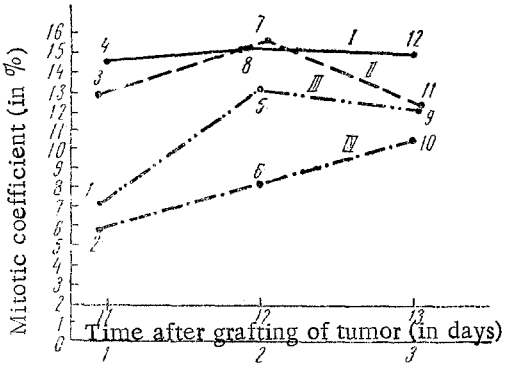
Consequently, the animals of the 1st, 2nd, 3rd, and 4th groups were sacrificed on the 11th day of tumor growth, the mice of the 5th, 6th, 7th, and 8th groups on the 12th day, and the animals of the 9th, 10th, 11th, and 12th groups on the 13th day.

In each period of investigation, the animals of the four corresponding groups were killed simultaneously during the morning hours. The tumors were fixed in Carnoy's fluid; karachchi's hematoxylin was used for staining. In the preparations we determined both the total number of mitotically dividing cells and the number and percent ratio of the phases of mitosis. On the basis of a determination of the phases of division we calculated the ratio of early and late phases of mitosis (coefficient K). The criterion of mitotic activity was the mitotic coefficient (MK) in per mill; statistical treatment was performed according to the Fisher-Student method, as in our previous studies [7, 8].

Separation of Mice into Groups

Period of sacrifice after last injection (in days)	Preparation			
	antironidase serum	γ-globulin fraction	normal serum	condition
1	1 st group (10)	2 nd group (10)	3rd group (10)	4 th group (10)
2	5 th „ (10)	6 th „ (10)	7 th „ (10)	8 th „ (10)
3	9 th „ (10)	10 th „ (10)	11 th „ (10)	12 th „ (10)

Note. The number of animals is indicated in parenthesis.



Mitotic activity in subcutaneous Ehrlich's adenocarcinoma after injection of antironidase serum and its γ-globulin fraction. I) Control; II) normal serum; III) antironidase serum; IV) γ-globulin fraction of antironidase serum. The arabic numerals denote the groups of animals.

RESULTS

The average percentage of various phases of division varied within a small range (P—3.6%; M—64-77%; A—2-4%; T—18-28%), just like the coefficient K (2.5-4.5). In view of this, the intensity of proliferation was judged chiefly according to the value of MK.

The changes in the average value of MK (per group) during the three days after the last injection of antironidase serum and its γ-globulin fraction are shown in the figure.

From the figure it is evident that the mitotic activity in subcutaneous Ehrlich's adenocarcinoma of the control mice was almost the same on the 1st, 2nd, and 3rd days (MK in the animals of the 4th group was equal to 14.54%, in the 8th group 15.44%, and in the 12th group 15.01%).

In the tumors of mice that received injections of normal serum, during these periods of the investigation certain fluctuations in the mitotic activity was observed: on the 1st day (3rd group), MK was 13.14%, on the 2nd day (7th group)—15.66%, and on the 3rd day (11th group)—12.65%. The differences between these indices were unreliable, just like the difference between MK in the tumors of mice of these groups and of the control animals, which received no injections, at each period of the investigation (i.e., between the 3rd and 4th, 7th and 8th, and 11th and 12th groups).

Consequently, the mitotic activity was practically identical in the control, and after the injection of normal serum, i.e., the latter did not exert any inhibiting influence on the mitotic division of malignant cells, which corresponds to the data of our previous work [7].

The antironidase serum, as can be seen from the figure, induced a drop in the mitotic activity in the tumors of the experimental animals (MK 7.37%) a day after its last injection (1st group), which was reliable in this period of investigation in comparison with the level of cell division after the injection of normal serum (1st and 3rd groups, $P = 0.005$) and in the control (1st and 4th groups, $P = 0.001$). However, on the 2nd day (just as on the 3rd day), no distinctly pronounced influence of antironidase serum was observed: although the mitotic activity in the tumors of the animals was somewhat lower during these periods of the investigation (MK 13.25 and 12.32%, respectively) than after the injection of normal serum and in the control, this difference was statistically unreliable.

Moreover, the increase in the mitotic activity between the 1st and 2nd days is reliable (1st and 5th groups, $P = 0.001$), just as in the interval between the 1st and 3rd days (1st and 9th groups, $P = 0.009$). This in turn is evidence of reliability of the cessation of inhibition of the proliferation of tumor cells 2 days after the last injection of the antironidase serum.

The mitotic activity in the tumors of animals that received injections of the γ-globulin fraction was lower in all three periods of the investigation, as is shown in the figure, than in the remaining groups of the experimental

animals: on the 1st day the MK was equal to 5.93% (2nd group), on the 2nd day 8.37%, (6th group), and on the 3rd day 10.51% (10th group). Moreover, on the first day the degree of inhibiting influence of γ-globulin on the mitotic activity of the tumor was approximately the same as that of antironidase serum: the difference is unreliable between the indices MK in the 1st and 2nd groups; at the same time, the difference between the action of γ-globulin and normal serum, as well as the control, is reliable (2nd and 3rd groups, $P < 0.001$; 2nd and 4th groups $P < 0.001$). But on the 2nd day a difference was already distinctly manifested between the action of antironidase serum and its γ-globulin fraction: the inhibition of cell division under the influence of the latter is reliable (6th and 5th groups, $P = 0.002$; 6th and 7th groups, $P < 0.001$). A certain increase in the MK (between the 1st and 2nd days) after the injection of γ-globulin is unreliable, i.e., the mitotic activity on the 2nd day was practically at the same level as on the 1st. On the 3rd day after injection of γ-globulin, the decrease in the mitotic activity (10th and 12th groups, $P = 0.003$) was reliable in comparison with the control, but unreliable in comparison with the action of antironidase and normal serum. Between the 2nd and 3rd days, the difference in the values of MK was unreliable, while between the 1st and 3rd days it was reliable (10th and 2nd groups, $P = 0.003$).

Thus, the results obtained indicate that antironidase serum and its γ-globulin fraction induce an inhibition of the mitotic activity in subcutaneous Ehrlich's adenocarcinoma, while normal serum does not exert this action. These data correspond to the observations of the authors, noted in the use of sera that possessed antihyaluronidase activity; inhibition of the growth of the tumor indicated above (in treatment of its cells in vitro before grafting [1, 3], as well as a decrease in the metastasis of Brown-Pierce carcinoma [2, 5, 6], is especially distinct in the case of injections of antironidase sera [5, 6].

In this work it was also shown that the duration of the inhibiting influence of antironidase serum is less (1 day) than that of its γ-globulin fraction (3 days); moreover, when the latter was injected, a sharper inhibition of cell division was noted during the 1st 2 days. The more distinctly pronounced inhibiting influence of the γ-globulin fraction of antironidase serum on the mitotic activity of the tumor is evidently explained by the fact that this fraction contains no nonspecific components, which might exert a cytotoxic effect upon the organisms of the experimental animals.

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