

CHANGE IN THE RATE OF TUMOR CELL DIVISION UNDER THE EFFECT OF ANTIRHONIDASE ACTIVITY

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It has been established that human and animal malignant tumors [1, 4, 6, 9, 10, 13], the tissues adjacent to them [6, 13], and metastases of tumors [9, 10] are characterized by high hyaluronidase activity. In the opinion of researchers this causes the filtrating character of the growth of malignant neoplasms.

According to the data of many investigators, sera containing the antibody against the spreading factor, in particular the serum against Brown-Pearce carcinoma which has an antihyaluronidase effect, reduces metastasis of this tumor [3, 7, 8]. An even more evident degree of the suppression of metastasis of Brown-Pearce carcinoma, which is expressed as fewer affected organs and metastases in them and as a prolongation of the life of the experimental animals, was noted with the use of sera from goats and horses immunized with rhonidase* [7, 8].

It has been demonstrated in individual works carried out on mice that the administration of antihyaluronidase serum to experimental animals after removing their spontaneous mammary-gland tumors reduced the percent of recurring tumors [14]. The addition (in vitro) and "antifactor" (antitumor) serum to tumor cells before inoculating mice inhibits the growth of Ehrlich adenocarcinoma by lowering its hyaluronidase activity [2, 4].

Taking into account these data and the results of works that demonstrated that antitumor serum causes a suppression of the rate of tumor cell division in Ehrlich adenocarcinoma [5, 11, 12], we investigated the mitotic activity of this tumor upon injection of antirhonidase serum into experimental animals.

This study also cites data on the effect of this serum on the intensity of division of normal tissues (in corneal epithelium and the crypts of the small intestine).

EXPERIMENTAL METHOD

In the experimental setup we used antirhonidase serum of horses and the γ -globulin fraction of this serum, which were obtained by I. N. Maiskii and N. A. Kozlova. As a control we used normal horse serum.

The experiment was conducted on 40 white male mice which were simultaneously inoculated under the skin of the spine with Ehrlich adenocarcinoma. On the 7th day after inoculation, when the tumors were easily palpable, the mice were divided into 4 groups of 10 mice each, and the administration of the appropriate sera began. The mice of the 1st group received antirhonidase serum; of the 2nd group, the γ -globulin fraction of this serum; those of the 3rd group, normal horse serum; and the mice of the 4th group did not receive serum. The serum was administered three times (on the 7, 8, and 10th day after tumor transplant) in a dose of 0.5 ml under the skin of the spine at some distance from the tumor.

A day after the last injection of the serum (i.e., on the 11th day of development of Ehrlich adenocarcinoma), the mice of all four groups were killed simultaneously in the morning.

* A protein preparation from bull tests having hyaluronidase activity. Produced by the A. I. Mikoyan Meat Combine, Moscow.

Mitotic Coefficient (in %) in Ehrlich Adenocarcinoma
for Various Groups of Mice

No. of mouse	Group of mice			
	1st	2nd	3rd	4th
1	0,52	1,76	8,56	5,45
2	4,68	3,83	9,32	8,69
3	5,24	3,95	9,51	13,25
4	6,82	4,62	10,78	13,87
5	7,81	4,65	13,58	13,92
6	8,66	6,14	14,09	15,37
7	8,97	6,19	14,24	18,40
8	9,52	9,29	15,18	18,50
9	9,93	9,43	23,05	18,78
10	11,52	9,47	(Preparation was ruined during glucolytic treatment)	
Average	7,37	5,93	13,14	14,54

Note. The probability of a random difference of the means (P) was: 1st and 3rd groups, 0.005; 1st and 4th groups, 0.001; 2nd and 3rd groups, 0.000; 2nd and 4th groups, 0.00. The difference between the 1st and 2nd groups and between the 3rd and 4th groups was unreliable.

We took the tumor, a portion of the small intestine, and the cornea to investigate the number of mitoses. The tumor and the intestine were fixed in Carnoy's fluid and the cornea in a 5% solution of acetic acid in 70° alcohol. Caracci's hematoxylin was used for staining in all cases.

In addition to the total number of mitoses, we calculated the number of all division phases and on this basis calculated the ratio of the early division phases to the late phases (coefficient K). The rate of cell division in the investigated objects was judged by the value of the mitogenic coefficient calculated in parts per thousand. The significance of the obtained results were checked by the Fisher-Student method.

EXPERIMENTAL RESULTS

The analysis of the mitotic activity in mouse tumors showed that the average percent of the various division phases in all investigated groups somewhat varied (prophase \approx 3-6, metaphase \approx 64-75, anaphase \approx 2-4, telophase \approx 19-28). The ratio of the early and late phases of division in all four groups fluctuated within narrow limits (2.5-3.9), which permitted us to consider that the rate of cell division was approximately the same in the control and experimental mice. Therefore, the rate of cell division, as in the previous works, was judged by the value of the mitotic coefficient.

The data shown in the table indicate that the average values of the mitotic coefficient in the tumors of the mice in the 1st and 2nd groups, i.e., with the injection of antirrhonidase serum and its γ -globulin fraction, were the lowest (7.37 and 5.93% respectively).

The mean values of the mitotic coefficient in the tumors of the mice in the 3rd group, which received normal serum, and the mice in the 4th group, which received nothing, were appreciably higher (about double) than those of the mice in the 1st and 2nd groups (13.14 and 14.54% respectively).

These data indicate a statistically significant suppression of the intensity of tumor-cell division a day after the last injection of antirrhonidase serum or its γ -globulin fraction, which is not noted upon injection of normal nonimmune serum.

Upon counting the mitoses in the corneal epithelium of the experimental mice of all four groups, it was calculated that the average percent of the various phases of division did not have significant differences (prophase $\approx 16-19$, metaphase $\approx 37.5-49$, anaphase $\approx 2-3$, telophase $\approx 29-43$) just as for the coefficient K ($\approx 1.3-2.3$). The mean values of the mitotic coefficient varied negligibly, the difference between them was insignificant (1st 11.29%; 2nd group, 11.45%; 3rd group, 10.13%; 4th group, 9.29%). Consequently, the mitotic activity of the corneal epithelium of the experimental mice did not change upon injection of the studied sera.

The average percent of the various division phases in the intestinal crypts was approximately the same (prophase $\approx 3.4-4.6$, metaphase $\approx 44-50$, anaphase $\approx 2.5-4.5$, telophase $\approx 42-48$) just as the coefficient K (≈ 1.0). The mean values of the mitotic coefficient had certain variations in the different groups (1st group, 46.07%; 2nd group, 44.27%; 3rd group 38.61%; 4th group, 44.18%). However, the difference between them was insignificant, which indicates approximately the same level of the intensity of cell division in the intestinal crypts of the experimental and control mice.

Thus, the threefold injection of antirhoidase serum or its γ -globulin fraction into mice with developing Ehrlich adenocarcinoma had a suppressing effect on mitotic activity of the cells of this tumor and did not affect the mitotic activity of corneal epithelium or the intestinal crypts of the same mice. This is possibly explained by the more specific action of antirhoidase antibodies with respect of Ehrlich adenocarcinoma. Apparently the antibodies by acting on metabolic processes of tumor cells affect also their mitotic activity.

SUMMARY

The object of the work was to study the effect produced by antirhoidase horse serum and of its gamma-globulin fraction on the mitotic activity of subcutaneous Erlich's adenocarcinoma and of normal tissues (corneal epithelium and small intestine crypts). Normal horse serum was used as control in mice.

As compared to the normal horse serum, antirhoidase serum, as well as its globulin fraction, had a depressive effect on the intensity of tumor cell division without affecting however, the mitotic activity of the normal tissues under study.

LITERATURE CITED

1. K. P. Balitskii, *Medichn. zh.*, 2 (1949), p. 31.
2. K. P. Balitskii, *Medichn. zh.*, 2 (1950), p. 53.
3. L. S. Biryukova, *Byull. éksper. biol.*, 11 (1960), p. 90.
4. F. A. Gluzman, *Medichn. zh.*, 2 (1949), p. 50.
5. V. N. Dobrokhoto, *Byull. éksper. biol.*, 3 (1955), p. 59.
6. I. N. Maiskii and N. A. Troitskaya, *Byull. éksper. biol.*, 12 (1951), p. 464.
7. I. N. Maiskii and N. A. Kozlova, *Byull. éksper. biol.*, 10 (1960), p. 101.
8. I. N. Maiskii, N. A. Kozlova, and M. N. Nilovskii, *Byull. éksper.*, 11 (1960), p. 86.
9. M. D. Podil'chak, *Vrach. delo*, 1952, 1, line 33.
10. M. D. Podil'chak, Abstracts of Reports of the First Ukrainian Conference of Pathophysiologists [in Russian], Kiev (1959), p. 170.
11. S. V. Sukhorukikh, *Byull. éksper. biol.*, 7 (1959), p. 83.
12. S. V. Sukhorukikh, *Byull. éksper. biol.*, 11, p. 97.
13. N. A. Troitskaya, In the book: Problems of Immunology of Normal and Malignant Tissues [in Russian], Moscow (1956) p. 105.
14. A. Lacassagne, J. Loiseleur, and G. Rudali, *Compt. rend. Acad. Sci. (Paris)*, Vol. 244 (1957), p. 1587.