EFFECT OF ANTIRONIDASE SERUM AND ITS γ-GLOBULIN FRACTION ON MITOTIC ACTIVITY OF NORMAL TISSUES OF MOUSE WITH DEVELOPING TUMORS

S. V. Sukhorukikh and N. A. Troitskaya UDC 616-006-092.9-085.355:577.154.365.014

Antironidase serum and its γ-globulin fraction, which inhibited mitotic activity of an Erlich's tumor in mice, had no such action on cell proliferation in normal tissues (epithelium of the cornea and intestinal crypts) of the same experimental animals.

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Interest in the study of properties of the enzyme hyaluronidase and its role in the pathogenesis of malignant neoplasms has increased considerably during recent years [1-7, 10-13].

Investigation of the changes in mitotic activity of tumor cells produced by a serum obtained from horses immunized with ronidase* showed that antironidase serum and its γ-globulin fraction significantly reduced the number of cell divisions in a subcutaneous Ehrlich's tumor in mice [8, 9]. It was also found that mitotic activity in normal tissues (epithelium of the cornea and crypts of the small intestine) of these same experimental animals was unchanged 24 h after injection of these preparations [8].

Cell division was investigated in the epithelium of the cornea and crypts of the small intestine in mice with a developing Ehrlich's tumor during the three days after injection of antironidases serum and its γ-globulin fraction, i.e., during the same period of investigation as was used previously when studying mitotic activity of the tumor under analogous experimental conditions [9].

EXPERIMENTAL METHOD

Experiments were carried out on 120 male mice which, 7 days after subcutaneous implantation of an Ehrlich's tumor in the dorsal region, were divided into four groups, with 30 animals in each group. The mice of group 1 received three subcutaneous injections (on the 7th, 8th, and 10th days after inoculation of the tumor) each of 0.5 ml of serum from a horse immunized with ronidase. The serum used was tested for its ability to prevent the formation of a mucin clot, inhibited the activity of hyaluronidase obtained from ronidase in a dilution of 1:8192, and hyaluronidase from the rabbit and mouse testis in a dilution of 1:64, but did not react with rat testicular hyaluronidase in a dilution of 1:16.

The mice of group 2 were injected under the same conditions with γ-globulin extracted from the mixed serum of two horses immunized with ronidase. This preparation, tested in vitro, neutralized ronidase activity in a dilution of 1:2048, rabbit testicular hyaluronidase activity in a dilution of 1:128, mouse testicular hyaluronidase activity in a dilution of 1:64, but had no effect on the activity of rat testicular hyaluronidase in a dilution of 1:16.

The animals of group 3 received injections of normal horse serum under the same conditions. In a dilution of 1:16 this serum did not inhibit the activity of any of the enzyme preparations mentioned above.

The animals of group 4 (control) received no injections.

Each of the four groups was further subdivided into three subgroups (10 mice in each subgroup) depending on the time of sacrifice after the last injection, so that group 1 consisted of subgroups 1, 5, and 9,

*A protein preparation from the bovine testis possessing hyaluronidase activity and manufactured by the Moscow Mikoyan Meat Combine.

Laboratory of Immunology of Growth and Development of Tissues, Institute of Experimental Biology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR N. N. Zukov-Verezhnikov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 68, No. 9, pp. 92-95, September, 1969. Original article submitted February 14, 1969.

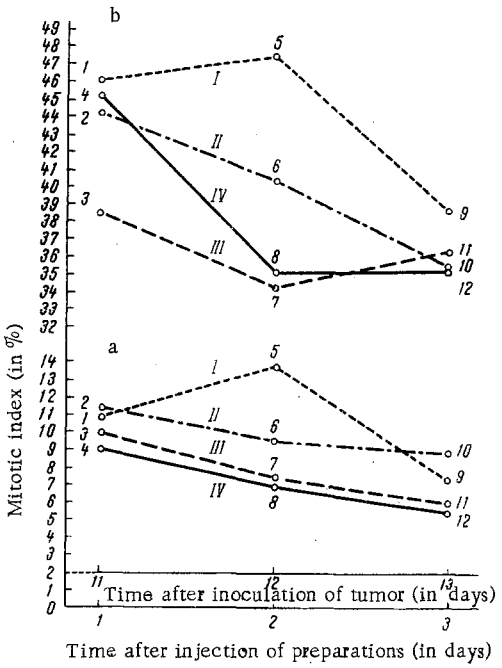

Fig. 1. Mitotic activity in corneal epithelium (a) and in intestinal crypts (b) of mice inoculated with Ehrlich's tumor after injection of antironidase serum and its γ-globulin fraction. I) Antironidase serum; II) γ-globulin fraction of antironidase serum; III) normal horse serum; IV) control. Arabic numbers denote subgroups of animals.

group 2 of subgroups 2, 6, and 10, group 3 of subgroup 3, 7, and 11, and group 4 of subgroups 4, 8, and 12.

At each time of investigation the animals of the four corresponding groups were sacrificed simultaneously during the morning. The piece of small intestine taken for investigation was fixed in Carnoy's fluid, while the cornea was fixed in 5% acetic acid solution in 70% alcohol. In both cases the material was stained with Carazzi's hematoxylin.

During counting of the total number of mitoses in the sections the number and relative percentages of all phases of division were determined and the ratio between the early and late phases of division (the coefficient K) was calculated. The mitotic index (MI) was calculated in promille for each test object. Statistical analysis was carried out by the Fisher — Student method.

EXPERIMENTAL RESULTS

The mean percentage of the various phases of division and the coefficient K in the corneal epithelium of all the experimental animals were as follows: P (prophase) ≈12-18%, M (metaphase) ≈37-54%, A (anaphase) ≈2-6%, T (telophase) ≈ 26-24%, K≈1.3-2.5.

The mitotic activity in the corneal epithelium of the control mice (Fig. 1) gradually fell during development of the tumor (MI in subgroup 4 was 9.3%, in subgroup 8 it was 7.15%, and in subgroup 12 it was 5.71%), the decrease in intensity of proliferation by the 13th day of tumor development being significant in subgroups 4 and 12 (P = 0.002).

Similar changes were observed with respect to the mitotic activity of the corneal epithelium of the mice receiving normal horse serum (MI for subgroup 3 was 10.08%, subgroup 7 was 7.58%, and subgroup 11 was 6.08%; difference between subgroups 3 and 11 statistically significant, P = 0.015). A tendency for MI to diminish was also observed in the corneas of mice receiving the γ-globulin fraction, although this decrease was not significant (MI in subgroup 2 was 11.49%, in subgroup 6 was 9.55%, and in subgroup 10 was 9.06%). The differences between the mean values of MI in the corneas of mice of the groups listed above and the control group were not statistically significant at any time of the investigation. Conseqeuntly, mitotic activity in the corneal epithelium was virtually identical in the control animals and in those receiving injections of normal horse serum and the γ-globulin fraction.

The mitotic activity on the 1st and 3rd days after injection of antironidase serum was not significantly different from that observed in the corneal epithelium of the experimental animals of the other subgroups (MI for subgroup 1 was 10.96% and subgroup 9 was 7.31%). On the 2nd day antironidase serum caused a temporary increase in mitotic activity (MI in the subgroup 5 was 13.79%), significantly different from the level of cell division in the corneas of mice receiving injections of γ-globulin (for subgroups 5 and 6, P = 0.024) or normal horse serum (for subgroups 5 and 7, P = 0) and in the controls (for subgroups 5 and 8, P = 0).

On the whole, after injection of antironidase serum, just as in the other groups, a significant decrease in the intensity of proliferation was observed in the cornea during development of the tumor (in subgroups 1 and 9, P = 0.006), in agreement with findings reported by Utkin [11], who observed a decrease in mitotic activity in the corneas of mice inoculated with Ehrlich's tumor.

The mean percentage of the various phases of division in the crypts of the small intestine varied within narrow limits at all periods of investigation (P≈2-5%, N≈44-53%, A≈2-7%, T≈36-48%), and this was also true of the ratio between early and late phases of division (K≈1-1.5). During the three days of

observation (Fig. 1) MI fell both in the control group (for subgroups 4 and 12, $P = 0.004$) and after injection of γ-globulin (for subgroups 2 and 10, $P = 0.02$). A tendency for MI to diminish was also found after injection of normal and antironidase sera, although this was less clearly defined (difference between MI on the 1st and 3rd days not statistically significant).

On the 2nd day, in mice receiving antironidase serum, a slight increase in mitotic activity was observed, whereas in the other groups at the same period of investigation MI was much lower (for subgroups 5 and 6, $P = 0.034$; for subgroups 5 and 7, $P = 0.001$; for subgroups 5 and 8, $P = 0.01$). In the intestinal crypts the same tendency for the intensity of proliferation to diminish as the tumor developed was observed as in the corneal epithelium.

The results thus show that antironidase serum and its γ-globulin fraction, when injected into mice with a developing Ehrlich's tumor, in contrast to their action on the mitotic activity of malignant cells [9], have no inhibitory effect on cell proliferation of normal tissues (epithelium of the cornea and intestinal crypts).

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