THE EFFECT OF ANTITUMOR SERUM ON THE MITOTIC ACTIVITY OF NEOPLASTIC AND NORMAL TISSUES IN THE MOUSE

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Research by V. N. Dobrokhotov [1] and our own previous investigations [2-4] have shown that 24 hours after administration of an antitumor serum, the intensity of cells division in an Ehrlich's adenocarcinoma is lowered. The antitumor serum does not produce (24 hours after administration) at the same time any essential changes in the mitotic activity of the epithelium of the cornea [4] or of the intestinal crypts [1]. It has also been shown [1] that antitumor serum, when injected in early periods of development of a tumor (24 hours or 4 days after implantation of the tumor material), had after 4-6 days a depressing effect on the mitotic activity of intraperitoneal nodes of an Ehrlich's adenocarcinoma. The trend of the changes in the mitotic activity of the tumor cells and also the mitotic activity of normal meristems were not studied by this author in these experimental conditions.

The aim of the present work was to ascertain the effect of antitumor serum on the changes in mitotic activity in a subcutaneous Ehrlich's adenocarcinoma in the period of its intensive development, and also in normal meristems (the epithelium of the cornea and of the crypts of the small intestine) at different times after the administration of antitumor serum. Since our previous findings [2-4] showed that other specific sera, such as the serum of a normal, nonimmune rabbit, have no depressing effect on the mitotic activity of tumor cells, these sera were not investigated in this particular experiment.

EXPERIMENTAL METHOD

We investigated 3 sera, obtained by the intravenous immunization of rabbits of the chinchilla breed with a saline extract of ascites cells of an Ehrlich's adenocarcinoma. The results of immunological testing of the sera by the complement fixation reaction at 37° were as follows: all 3 sera reacted with tumor antigen in a dilution of 1:400 +++; with antigen from the spleen of a healthy mouse the first serum reacted in a dilution of 1:100 +++, the second, in a dilution of 1:100 ++, the third, in a dilution of 1:200 ++; with antigen from the liver of a healthy mouse the first serum reacted in a dilution of 1:100 ++++ and further dilutions caused hemolysis; the second serum in dilutions of 1:100 and above gave hemolysis; the third reacted in a dilution of 1:100 ++++ and higher dilutions gave hemolysis.

The experiments were performed on 84 male white mice which were divided into 2 groups, each of 42 animals, on the 7th day after implantation of an Ehrlich's adenocarcinoma under the skin of the dorsal region. On the 7th, 8th and 10th days after implantation of the tumor, the mice of the experimental group were given a subcutaneous injection of 0.5 ml of antitumor serum in the dorsal region at some distance from the tumor. No serum was injected into the control group of mice. The mice of the experimental and control groups were then further subdivided into groups according to the time after the last injection of serum that the animals were sacrficed:

Time of sacrifice	Experiment	Control
After 1 day	I group ... 10 mice	II group... 10 mice
" 2 days	III " ... 10 "	IV " ... 10 "
" 4 "	V " ... 10 "	VI " ... 10 "
" 5 "	VII " ... 12 "	VIII " ... 12 "

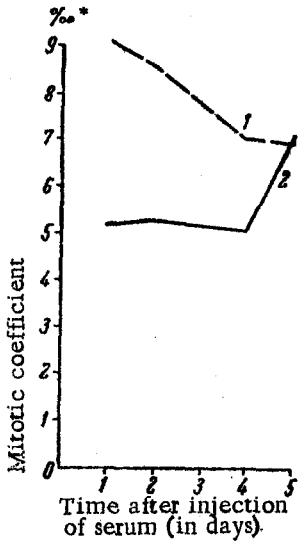

Fig. 1. Mitotic coefficient in an Ehrlich's adencarcinoma after injection of antitumor serum. 1) control; 2) experiment.

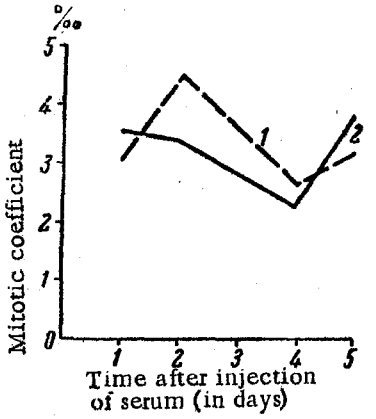

Fig. 2. Mitotic coefficient in the corneas of mice after injection of antitumor serum. 1) control; 2) experiment.

Animals of groups I and II thus were sacrificed on the 11th day of development of the tumor, those of groups III and IV on the 12th day, of groups V and VI on the 14th day and groups VII and VIII on the 15th day.

At each time of the investigation the mice of the experimental and control groups were sacrificed simultaneously, starting at 11 a.m.

The tumors and intestine were fixed in Carnoy's fluid, the cornea in a 5% solution of acetic acid in 70% alcohol. Sections of tumor and intestine were cut to a thickness of of 8μ, and total preparations of the cornea were made. In all cases the films were stained by Carazzi's hematoxylin.

The mitoses in the tumor were counted in 300 fields of vision, and in the cornea — on both sides of two perpendicular lines drawn in ink on the cover glass of the preparation. In each tumor an average of 60 000-70 000 cells was counted, and in the corneal epithelium — 30 000-35 000 cells. The number of mitoses in the intestine was determined in 50 crypts, cut longitudinally, in which roughly 3500 cells were usually counted. As a result of the counting of the mitoses in the phases of division, the coefficient K (ratio between the sum of the early phases of division to the late) and the mitotic coefficient MK (the ratio between the number of dividing cells and the total number of cells counted in the particular specimen, per 1000) could be calculated for each test object. The experimental results were treated statistically by the Fisher-Student method.

EXPERIMENTAL RESULTS

The counts of the mitotic activity in the tumors of all the experimental and control groups of mice showed that the mean percentage of the different phases of division was approximately equal for each group (P $\approx$ 3-6%, M $\approx$ 74-79%; A $\approx$ 2-4%; T $\approx$ 13-17%), as also was the coefficient K (from 4 to 5). The trend of the changes in the mean value of MK in the tumor in the course of the 5 days after the last injection of serum is indicated in Fig. 1.

*$^0/_{00}$ = parts per thousand.

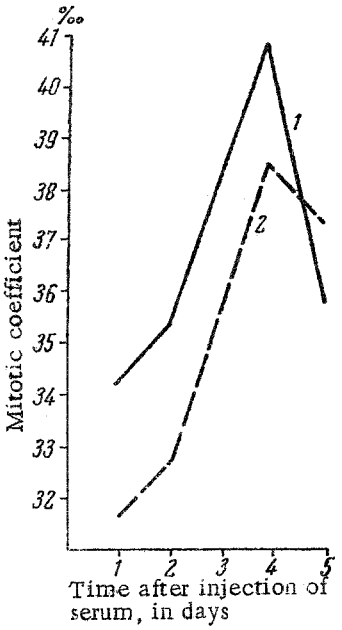

Fig. 3. Mitotic coefficient in the intestinal crypts of mice after injection of antitumor serum. 1) Experiment; 2) control.

It is clear that the value of MK in the subcutaneous Ehrlich's adenocarcinoma of the experimental mice was roughly the same on the 1st, 2nd and 4th day after injection of the serum (5.18; 5.26 and 5.05 per 1000) and only on the 5th day did it increase to 7.05 per 100 (for the interval between the 4th and 5th days p = 0.04).

The value of MK in the tumors of the control mice in the first 3 days of the investigation considerable exceeded the corresponding values inthe experimental group (9.07; 8.59 and 7.05 per 1000 respectively). At a later period of the investigation the value of MK in the tumors of the control animals was 6.95 per 1000, i.e., it was practically equal to the value of MK in the tumors of the experimental mice sacrificed on the same day (on the 5th day after injection of the serum). The difference between the values of MK in the experimental and control animals one day after injection of the serum was significant (p = 0.001), and after 2 days – P=0.022. On the 4th day after injection of the serum the difference between the values of MK in the experimental and control mice was less clearly shown, as the result of a slight fall in the value of MK in the tumors of the control animals (p = 0.041). Consequently, the mitotic activity of the tumor cells of a subcutaneous Ehrlich's adenocarcinoma was at a lower level in the course of the first 4 days (especially so during the first 2 days) after the injection of antitumor serum than in the control experiments.

The mean percentage of the different phases of division in the corneal epithelium of all the experimental and control groups of mice varied within narrow limits (P ≈ 17-25%; M ≈ 33-41%; A ≈ 5-9%; T ≈ 26-37%). The coefficient K in all the groups was also roughly identical (between 1.4 and 2.0). The changes in the mean values of MK in the corneal epithelium of the mice in the course of the 5 days after injection of the antitumor serum are illustrated in Fig. 2.

As is clear from Fig. 2, the value of MK in the corneal epithelium of the experimental groups of mice was roughly the same at all periods of the investigation (after 24 hours – 3.57 per 1000; after 2 days – 3.43 per 1000; after 5 days – 3.98 per 1000). The lowest value of MK (2.32 per 1000) was observed on the 4th day after injection of the serum (14th day of development of the tumor). The value of MK in the corneal epithelium of the control mice also varied between narrow limits (after 24 hours – 3.16 per 1000, after 2 days – 4.55 per 1000; after 4 days – 2.71 per 1000, after 5 days – 3.3 per 1000), and moreover the lowest value of MK (2.71 per 1000) was observed at the same period of the investigation as that in the experimental animals (14th day of development of the subcutaneous Ehrlich's adenocarcinoma).

The difference between the values of MK in the control epithelium of the experimental and control mice at each period of the investigation was not statistically significant. Consequently, the changes in the mitotic activity in the corneal epithelium of the experimental and control mice in the course of the 11-15 days of development of the adenocarcinoma were identical and did not depend on the effect of the antitumor serum.

The mean percentage of the various phases of division in the epithelium of the intestinal crypts of all the experimental and control groups of mice varied between narrow limits (P≈3-9%; M≈52-60%; A≈3-6%; T≈26-29%), as also did the coefficient K (betwen 1.3 and 2.3).

The curves showing the changes in the value of MK in the intestinal crypts on the 1st, 2nd, 4th and 5th days after injection of the serum are given in Fig. 3.

It will be seen that the value of MK was almost the same one and 2 days after injection of the serum (34.20 and 35.25 per 1000), rose to 40.8 per 1000 on the 4th day and then fell on the 5th day to 35.74 per 1000. However, the difference between the value of MK for these groups was not statistically significant. The changes in the value of MK in the intestinal crypts of the control mice were similar to those observed in the experimental

animals (31.67; 32.67; 38.48 and 37.31 per 1000), and moreover the highest value of MK (38.48 per 1000) was observed at the same period of the investigation as in the experimental mice. The differences between the values of MK of all the control groups were not statistically significant. Nor was the difference between the values of MK for the intestinal crypts of the experimental and control groups of mice at each period of the investigation statistically significant.

The small increase in MK in the intestinal crypts on the 4th day after injection of serum therefore did not depend on the injection itself, for a similar increase was observed in the control groups also at this period of the investigation.

The experimental results thus show that the depressing effect of antitumor serum on the mitotic activity of the tumor cells of a subcutaneous Ehrlich's adenocarcinoma lasts for 4 days after injection of the serum, and furthermore that a more intensive depression of cell division is observed during the first 2 days. The injection of antitumor serum in mice with a subcutaneously developing Ehrlich's adenocarcinoma causes no essential changes, during the 5 days after injection of the serum, in the mitotic activity in the epithelium of the cornea and the crypts of the small intestine of the experimental animals.

SUMMARY

This work deals with the study of the effect of antitumor serum on the dynamics of changes of mitotic activity in Ehrlich's subcutaneous adenocarcinoma, as well as in the epithelium of the cornea and crypts of the small intestine in the same experimental animals. The results of the experiment conducted on 84 male mice demonstrated that the antitumor serum has a depressing effect on the mitotic activity of Ehrlich's adenocarcinoma for a period of 4 days after the administration of the serum; the depression of cell division is more intense during the first 48 hours. There are no significant changes of mitotic activity in the corneal epithelium and crypts of the small intestine in mice with subcutaneously inoculated Ehrlich's adenocarcinoma for 5 days after the administration of the anti-tumor serum.

LITERATURE CITED

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