5. O. S. Frankfurt, Vopr. Onkol., No. 10, 98 (1967).
6. F. D. Bertalanffy, Nature, 198, 256 (1963).
7. S. M. E. Llanos and A. F. Badran, J. Roy. Micr. Soc., 82, 75 (1963).
8. N. Rosa and S. M. E. Llanos, J. Natl. Cancer Inst., 47, 1007 (1971).

EFFECT OF ONE AND TWO INJECTIONS OF CHALONE ISOLATED FROM MOUSE EHRLICH'S ASCITES TUMOR ON ITS MITOTIC ACTIVITY

Yu. A. Romanov and M. V. Semenova

UDC 616-006-018.15-092.9

Chalone from Ehrlich's ascites tumor has a short and reversible inhibitory action on proliferation of cells of this tumor when administered as both one and two injections. A considerable increase in mitotic activity in the tumor cells compared with the control is observed 10 h after one or two injections (the second injection being given at 6 p.m.) of the chalone, indicating that it acts on the G_2-cell population in the mitotic cycle and that it synchronizes cell division. If the second of two injections of chalone is given at 9 p.m. it leads to more prolonged inhibition of the cells and to a more marked synchronization wave of the G_2-cell population. The duration of inhibition of cells in the G_2-phase of the mitotic cycle after two injections of the chalone thus depends on the state of the cell population on which the chalone acts.

KEY WORDS: chalone; Ehrlich's ascites tumor; mitotic cycle, mitotic index.

During the last two decades, in the problem of regulation of cell proliferation tissue-specific inhibitors of cell division (chalones) have received intensive study, for some workers ascribe to them the role of physiological regulators of cell reproduction [7, 9, 10]. In some investigations chalones have been found to synchronize the cell population in the mitotic cycle, but the data so far obtained are few and further investigation is required. The use of chalones as natural synchronizers, with no toxic action on living organisms, can play an important role in improving the efficacy of tumor chemotherapy. It was accordingly decided to study the synchronizing action of chalone on a tumor cell population by testing its administration by different schemes.

The object of this investigation was to compare the mitotic activity of Ehrlich's ascites tumor (EAT) cells after one or two injections of chalone from that tumor into animals.

EXPERIMENTAL METHOD

Experiments were carried out on 150 noninbred male albino mice (from the Central Nursery, Academy of Medical Sciences of the USSR) aged 1.5 months (weight 18-20 g). The animals were kept under standard conditions: temperature 18°C, food ad libitum, 12 h of daylight and 12 h of darkness (daylight from 6 a.m. to 6 p.m.)

A diploid strain of EAT (Institute of Experimental and Clinical Oncology, Academy of Medical Sciences of the USSR) was transplanted by intraperitoneal injection of 0.2 ml ascites fluid, containing 10^7 cells (with this dose of tumor cells a 100% take was observed with this strain). The EAT chalone was obtained by the method of Hondius and Laurence [8] and of Okulov and Chekulaev [2], modified by S. G. Mamontov and V. B. Zakharov. The stages of obtaining the chalone-containing preparation were as follows. The ascites fluid was isolated from a 13-day tumor on an icebath, after which the cell mass was separated by centrifugation of the ascites fluid at 3000 rpm for 10 min at 4°C. Later the residue was resuspended 3 times in 20 volumes of acetone at 4°C, for 30 min each time, and centrifuged after each treatment for 10 min at 3000 rpm. The resulting acetone powder was dried and homogenized in an agate mortar. An aqueous extract was obtained by adding 20 volumes

Department of Biology and Genetics, Medico-Biological Faculty, N. I. Pirogov Second Moscow Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR V. V. Kupriyanov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 89, No. 2, pp. 189-191, February, 1980. Original article submitted March 1, 1979.

0007-4888/80/8902-0169$07.50

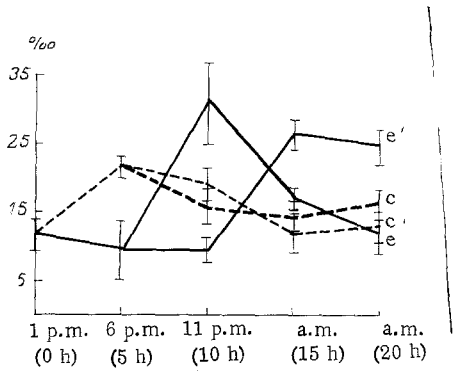

Fig. 1

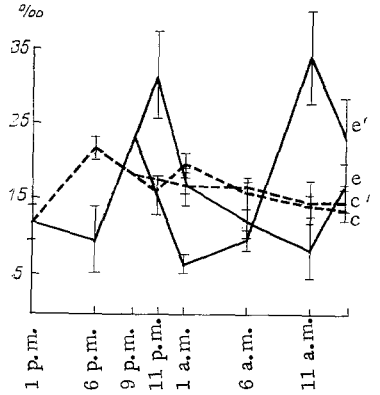

Fig. 2

Fig. 1. Changes in mitotic activity of EAT cells after one (at 1 p.m.) and two (at 1 and 6 p.m.) injections of chalone. Here and in Fig. 2, one injection: c) control, e) experiment; two injections: c') control, e') experiment. Mean values of M ± m given). Abscissa, time of day; time after single injection of chalone given in parentheses (in h); ordinate, MI (in ‰).

Fig. 2. Changes in mitotic activity of EAT cells after one (at 1 p.m.) and two (at 1 and 9 p.m.) injections of chalone.

of distilled water to the powder, and the mixture was incubated for 1 h at 4°C. The extract was then centrifuged at 3000 rpm for 10 min at 4°C. The supernatant obtained by centrifugation was treated with 96°C ethyl alcohol to obtain a 55% alcoholic fraction, which was then incubated for 1 h in the cold. This fraction was then centrifuged at 3000 rpm for 10 min and the supernatant was treated with 96° ethanol up to a concentration of 81%. The resulting alcoholic fraction was incubated for 24 h at 4°C. The residue obtained after centrifugation of the 81% alcoholic fraction at 3000 rpm for 30 min was resuspended 3 times in acetone and again centrifuged at 3000 rpm for 10 min. The residue obtained after the last centrifugation was dried, pulverized, and used as the chalone.

Experiments were carried out on six groups of animals. Mice of experimental groups 1, 3, and 5, 5 days after transplantation of the tumor received an injection of 0.5 ml of chalone solution (dose 10 mg per mouse) at 1 p.m. Animals of groups 2, 4, and 6, which served as controls for the above-mentioned groups, were injected with physiological saline at the same time. Animals of groups 1 and 2 were then killed 5, 10, 12, 15, 17, 20, 22, and 25 h later. Animals of group 3 received a second injection of the same dose of chalone at 6 p.m. The control mice of group 4 received another injection of physiological saline. These animals were then killed 5, 10, and 15 h later. The animals of group 5 received a second injection of chalone at 9 p.m. and they were killed 4, 9, 14, and 16 h later. The mice of group 6 received physiological saline and were killed at the same time as the mice of group 5. At each period of the experiment five animals were taken from each experimental and control group. Films were prepared from the ascites fluid taken from the tumors, fixed twice for 10 min each time with methyl alcohol, and the histological preparations were stained with methylene blue. In each EAT preparation 5000 cells were analyzed and the mitotic index (MI) calculated in pro mille. The results were subjected to statistical analysis by Student's t-test.

EXPERIMENTAL RESULTS

As Figs. 1 and 2 show, the mitotic activity of the EAT cells in the control animals was inconstant during the 24-h period. Its highest values were observed at 6 p.m. and lowest at 1 p.m. ($P < 0.01$). These changes in MI are evidence that there is a diurnal rhythm of the number of mitoses in EAT, as other workers showed previously [1, 4, 5].

MI of the tumor cells 5 h after injection of chalone was 57.1% lower than in the control ($P < 0.01$; Fig. 1). However, 5 h later during the experiment a sharp increase in MI was observed, to 198% of the control value ($P < 0.01$). Inhibition of cell proliferation during the first few hours after injection of chalone can be explained by development of a block to the cells in the G_2-phase of the mitotic cycle, for the duration of this phase in EAT is known to be 4 h [1]. The subsequent rise in MI can evidently be regarded as a result of synchronization by the chalone of the G_2-cell population, expressed as the more or less simultaneous entry of the cells into mitosis after removal of the chalone block. These results agree with those obtained by other workers [2] who studied the action of an aqueous extract of EAT cells on cell division in this tumor after a single injection. Differences

in the values of MI at later stages of the experiments in the experimental and control animals were not statistically significant ($P > 0.1$), evidence of normalization of the level of mitotic activity.

A second injection of chalone at 6 p.m. led to maintenance of this low level of mitotic activity due to the first injection of chalone for a further 5 h thereafter (Fig. 1). MI at this time remained lower than in the control ($P < 0.01$). Evidently just as after a single injection, in this case also the chalone prevented the cells from entering into mitosis. A marked rise in MI was observed 10 and 15 h after the second injection of the chalone, to values 2.2 and 1.9 times higher respectively than the control ($P < 0.01$). This rise also must evidently be explained by the synchronizing effect of the chalone on the G_2-cell population, just as after a single injection of the preparation. The second injection of chalone at 6 p.m., given before the increase in mitotic activity of the EAT cells after the first injection of chalone, thus prolonged the time of preservation of low mitotic activity and led to a longer wave of synchronization of the dividing cells than after a single injection. Meanwhile, in both cases the beginning of the synchronization wave was found after the same time interval (10 h).

Changes in MI after one and two injections of chalone (the second injection at 9 p.m.) are illustrated in Fig. 2.

A decrease in MI, which was 42.9% of the control value ($P < 0.01$), was found 5 h after a single injection of chalone, evidently because of the delay of the cells in the G_2-phase of the mitotic cycle induced by it. MI rose sharply 10 h after injection of the chalone – by 98% compared with the control ($P < 0.01$). This rise evidently reflects synchronization by the chalone of cells of the G_2-population. At the next period a sharp fall in MI was observed, and it did not differ significantly in value from the control. At the next two periods of sacrifice of the animals the values of MI likewise did not differ from the control. At the end of the experiment MI increased a little, but did not differ significantly from the control ($P > 0.1$).

A marked decrease in MI was observed 4 h after the second injection of the chalone at 9 p.m.: By 62% compared with the control ($P < 0.01$). MI continued to remain below the control level ($P < 0.02$) at the next period of the experiment also (9 h later). Later still, after 14 h, a sharp rise in MI was observed, when it was 238.5% relative to the control ($P < 0.02$). Another 5 h later during the experiment a decrease in MI was found, but the differences from the control were not statistically significant ($P > 0.1$). A second injection of chalone given during the period of increase of MI after the first injection, thus led to prolongation of the inhibitory action of the chalone on EAT cells (the duration of inhibition was about twice as long as after a single injection) and to an increase in the wave of synchronization of the G_2-cell population.

When the curves showing changes in mitotic activity after a second injection of the chalone at 6 p.m. (Fig. 1) and at 9 p.m. (Fig. 2) are compared it will be noted that, whereas in the first case the second injection of chalone led to delay of cell division for 5 h, in the second case the inhibitory effect lasted for 9 h. Meanwhile in the first case a smaller wave of synchronization was observed than in the second. The differences can probably be explained on the grounds that the action of chalone in the two experimental schemes used was realized in different cell populations. Whereas in the first case its action a second time was directed to cells most of which had been delayed in the plane of the mitotic cycle, in the second case the effect of the chalone also extended to cells in which the $G_2 \rightarrow M$ block was absent.

This investigation confirms previous observations showing a temporary and reversible effect of inhibition of proliferation of EAT cells after a single injection of chalone from that tumor. After a second injection of chalone the cell population does not lose its ability to respond to the tissue-specific inhibitor of cell division. Two injections, like a single injection, are accompanied by synchronization of the G_2-population of tumor cells, but this effect of the chalone on EAT cells after a second injection, like its inhibitory action, depends on what cell population is exposed to its action.

These results demonstrate the importance of choice of schemes of administration of chalone in order to obtain the maximal effect of synchronization of the tumor cell population.

LITERATURE CITED

1. S. N. Gor'kova and A. T. Tusuz'yan, in: The Biology of Cell Reproduction [in Russian], Moscow (1972), p. 64.
2. V. B. Okulov and L. I. Chekulaeva, Arkh. Anat., No. 1, 106 (1976).
3. Yu. A. Romanov, V. P. Rybakov, and A. I. Antokhin, Byull. Éksp. Biol. Med., No. 12, 1477 (1976).
4. H. R. Brown, Growth, 38, 413 (1974).
5. H. R. Brown and E. R. Burns, Transplantation, 16, 199 (1973).

6. G. Brugal, Cell Tissue Kinet., 6, 519 (1973).
7. W. S. Bullough, Natl. Cancer Inst. Monogr., 38, 5 (1975).
8. W. B. Hondins and E. B. Laurence, Europ. J. Biochem., 5, 191 (1968).
9. O. H. Iversen, Cancer Res., 30, 1481 (1970).
10. T. Rytomaa, Cell Tissue Kinet., 1, 329 (1968).