DEPENDENCE OF MITOSIS-INHIBITING ACTIVITY OF A CHALONE PREPARATION FROM EHRLICH'S ASCITES TUMOR ON TIME OF ITS ISOLATION

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UDC 616-006.6-018.15-02:615.362.014.3.018:612.6

KEY WORDS: Ehrlich's ascites tumor; chalone; mitosis-inhibiting activity.

An urgent problem in the chronobiology of cell reproduction is the study of mechanisms of the circadian rhythm of cell multiplication. In 1969 Bullough postulated that tissue-specific inhibitors of cell division (chalones) participate in the regulation of circadian rhythms of mitotic activity (MA) in the tissues. In his opinion, cell proliferation is regulated at the tissue level by a chalone–adrenalin complex, and rhythmic changes in MA are associated with circadian fluctuations in adrenalin concentration. However, evidence that adrenalin is not essential for the action of chalones has been obtained in recent years [3, 5-7]. At the same time, work showing the presence of fluctuations in cell sensitivity to chalones and also fluctuations in production or activity of chalones has been published [1, 2].

This paper describes an investigation of circadian changes in production or activity of the G_2-chalone of Ehrlich's ascites tumor (EAT) of mice.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male albino mice of the same age (1.2-2 months) weighing 18-20 g. The diploid strain of EAT was used as test object. The tumor was obtained from the Institute of Oncology, Academy of Medical Sciences of the USSR, and maintained by intraperitoneal injection of 0.5 ml ascites fluid. The tumor used in the experiments was at the 5th day of development.

Seven times in 24 h (at 9 a.m., 1, 5, and 9 p.m., and 1, 5, and 9 a.m.) at 4-hourly intervals ascites material was collected from the animals and chalone-containing preparations were obtained by the method in [4], modified by S. G. Mamontova and V. B. Zakharova. The ascites fluid was collected in a glass jar in the cold. The cell mass was separated by centrifugation for 10 min at 3000 rpm (4°C). The supernatant was discarded. The residue was resuspended in 20 volumes acetone in the course of 30 min. The suspension was then allowed to stand for 30 min and the supernatant was poured off. The material was put under a vacuum to evaporate the acetone, and an acetone powder was obtained, which was dissolved in 20 volumes of distilled water. The supernatant was treated with 96% ethanol to a concentration of 55% and then incubated for 1 h at 4°C. After incubation, the mixture was centrifuged for 30 min under the same conditions. The supernatant was treated with 96% ethanol to obtain an 81% concentration and incubated for 12 h at 4°C. After centrifugation under the same conditions 20 volumes of acetone was added to the residue and it was resuspended for 20 min. It was then allowed to stand for 30 min and the supernatant was poured off. The residue was dried under a vacuum. The resulting acetone powder was used in the experiments as an aqueous solution.

Mitosis-inhibiting activity of the chalone-containing preparation was investigated on a temporary suspension culture of EAT cells. Cells from a 5-day EAT were placed in medium containing 20% calf serum and 80% of a mixture of Eagle's medium with RPMI medium and glutamine (from Flow Labs) (in the ratio of 3:7). Cells were added to the culture medium in the proportion of 10^6 to 1 ml. The culture was incubated at 37°C to adapt the cells. The culture was used in the experiments 2 h after the beginning of culture, when the mitotic index

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 N. P. Bochkov. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 91, No. 3, pp. 343-345, March, 1981. Original article submitted April 21, 1980.

 0007-4888/81/9103-0360$07.50

TABLE 1. Effect of Different Doses of Chalone-Containing Preparation from EAT on MA in EAT Culture (M ± m)

Experimental conditions	Time of determination of MA, min					
	15		30		60	
	MI, ‰	inhibition, ‰	MI, ‰	inhibition, ‰	MI, ‰	inhibition, ‰
Control	7,3±0,32	—	9,0±0,63	—	12,0±0,42	—
Chalone						
5 μg/ml	6,0±0,57	19	6,0±0,57	33*	4,0±0,29	67*
12.5 μg/ml	5,0±0,76	32*	5,0±0,57	44*	4,2±0,29	65*
25 μg/ml	2,5±0,65	66*	4,8±0,79	50	10,0±1,15	17

*P ⩽ 0.05 compared with control.

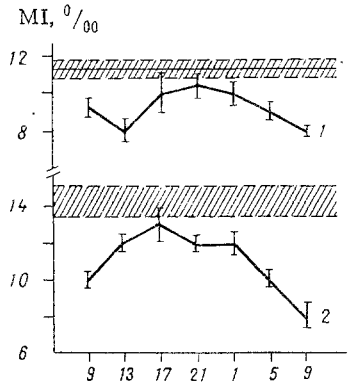

Fig. 1. Effect of chalone-containing preparation from EAT, isolated at different times of 24-h period, on MI of cells in culture of this tumor. Effect recorded 30 min (1) and 60 min (2) after administration of preparation. Abscissa, time.

(MI) was 8.14‰.

In the experiments of series I the chalone containing preparation was added to the culture in doses of 5, 12,5, and 25 μg/ml and MA was investigated after 15, 30, and 60 min. In the control 0.5 ml of physiological saline was added. In the experiments of series II the mitosis-inhibiting activity of chalone preparations isolated at different times of the 24-h period was studied. All preparations were added to the culture in a dose of 5 μg/ml and the results were read after 30 and 60 min. Physiological saline was used as the control. MI was counted in 3000-5000 cells in histological preparations stained with methylene blue. Four samples of culture were tested each time.

EXPERIMENTAL RESULTS

The results of the experiments of series I are given in Table 1. They show that all doses of the preparation had an inhibitory action on MA in the EAT culture. Maximal inhibition for each dose was observed at different times after administration of the preparations. The effect of a dose of 25 μg/ml was maximal after 15 min, and that of doses of 5 and 12.5 μg/ml after 60 min. The mitosis-inhibiting effect of the chalone-containing preparations from EAT thus depended on the dose of the preparation and the duration of its action on cells of the EAT culture.

In view of these results in the experiments of series II a dose of 5 μg/ml was used and the inhibitory effect was analyzed after 30 and 60 min. The results showed that chalone preparations isolated at different times of the 24-h period differ in their mitosis-inhibiting activity. Chalones isolated at 1, 5, and 9 a.m. and 1 p.m. significantly inhibited MA 30 min after administration. The degree of inhibition was greater when chalones isolated at 5 and 9 a.m. and 1 p.m. were used — by 16-29% (P ⩽ 0.03) compared with the action of the 1 a.m. chalone (by 10%, P = 0.07). Extracts obtained at 5 and 9 p.m. did not reduce MI. The mitosis-inhibiting effect of the individual preparations was increased 60 min after the beginning of the experiment. Chalones isolated at 5 and 9 a.m. depressed MI by 29-43% (P ⩽

0.001), those isolated at 1 and 9 p.m. and 1 a.m. by 14% (P = 0.07; P = 0.17); the 5 p.m. extract did not cause significant inhibition of cell division in EAT.

The results of these experiments thus show that the mitosis-inhibiting activity of chalone-containing preparations of EAT depends significantly on the time of the 24-h period when they were isolated. Similar results were obtained in experiments *in vivo* with EAT chalone [1] and with hepatic chalone [2], although in these studies activity of the chalones isolated only at 4 and at 2 different times of the 24-h period was analyzed. Unfortunately, because of the low degree of purity of the chalone preparations it is impossible to give a precise answer to the question whether it is the production of chalones or their activity that changes in the course of the 24-h period.

The results point to the possibility of a role of G_2-chalone of EAT in the formation of the circadian rhythm of MA in this tumor by periodic changes either in the quantity of chalone produced by the tumor cells or in its activity. In addition, they are evidence that the mitosis-inhibition action of this chalone in an EAT culture may be manifested without the participation of adrenalin.

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ACTION OF SPECIFIC PLACENTAL PROTEINS — TROPHOBLASTIC β-GLYCOPROTEIN AND CHORIONIC α_1-MICROGLOBULIN — ON PROLIFERATION OF LYMPHOCYTES AND MALIGNANT FIBROBLASTS *in vitro*

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UDC 616-006-008.939.6-092.9

KEY WORDS: placental proteins; proliferation; lymphocytes; malignant cells.

During the development of malignant tumors renewal of biosynthesis of embryonic and placental proteins is observed [4, 6, 8, 9], although their role in carcinogenesis has not been studied. Possibly in tumor-bearing animals, just as in pregnant women, the presence of embryonic proteins in the blood leads to the appearance of areactivity of the tumor and fetus toward transplantation antigens, for some of these proteins have the property of depressing lymphocyte proliferation in mixed cultures [10-12]. It is interesting to investigate whether embryonic proteins can inhibit the division of other intensively proliferating cells, i.e., tumor and embryonic cells. Although no such action is observed *in vivo*, one or two studies have demonstrated a possible inhibitory effect of fetoplacental factors on embryonic and tumor cells *in vitro* [1, 2, 7]. The mechanism of protection *in vivo* against the inhibitory effect of fetoplacental factors on proliferating tissues of embryo and tumor is unknown.

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(Presented by Academician of the Academy of Medical Sciences of the USSR V. V. Kupriyanov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 91, No. 3, pp. 345-347, March, 1981. Original article submitted May 10, 1980.

0007-4888/81/9103-0362$07.50