ONCOLOGY

Effect of Chalone System on Proliferation of Ehrlich Ascitic Tumor Cells under Conditions of Modified Photoregimen

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We studied the influence of different photoregimens (light/darkness alternation and constant darkness) on the effect of saline and chalone regulation of cell proliferation in Ehrlich ascitic tumor was studied. Saline slightly inhibited proliferation of tumor cells. Chalone-containing preparations from animals exposed to light/darkness alternation and to constant darkness were characterized by different effects on cell proliferation in this tumor. Cell kinetics attested to possible mechanisms of the effect of the chalone system on the status of Ehrlich ascitic tumor; these mechanisms were independent on each other.

Key Words: *chalones; photoregimen; constant darkness; Ehrlich's ascitic tumor*

Study of the time regularities of cell proliferation is an important trend in modern chronobiology [5-7]. The hierarchical structure of levels of regulation of the time organization of proliferative system includes the external and internal factors. Photoperiodicity is particularly important for the regulation of cell reproduction rhythms, including the cells in Ehrlich's ascitic tumor (EAT) [2,3,9,13,14]. Chalones (tissue-specific inhibitors of cell proliferation) play the leading role among factors realizing local (tissue) regulation of the proliferative system [1,4, 8,10-12].

MATERIALS AND METHODS

The study was carried out on 290 male outbred albino mice. Five to seven animals were examined per time point. EAT diploid strain was studied. The results were evaluated by the mitotic index, by graphic parametrical study of biological rhythms, analysis of correlations, and standard methods for statistical data processing [9].

Group 1 animals were kept for 21 days under conditions of standard photoregimen (light:darkness 12:12 h, light from 6.00 till 18.00), after which the animals were transplanted EAT (L/D animals). Group 2 animals were kept under conditions of constant darkness during the same period, after which they were transplanted EAT (D/D animals). Chalone-containing preparations (CCP) were obtained beforehand from mice with 10-11-day EAT (plateau phase). $CCP^{L/D}$ was obtained at 11.00 from L/D animals, $CCP^{D/D}$ at 11.00 from D/D animals. Mice injected with saline served as the control for mice treated with CCP. Intact L/D and D/D animals served as controls for mice injected with saline. The material was collected at 15.00, 17.00, 20.00, 23.00, 5.00, and 9.00 (2, 4, 7, 10, 16, and 20 h postinjection). EAT preparations were made. Total mito-

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tic index (TMI) and mitosis phase indexes were analyzed. Analysis of correlations was carried out.

RESULTS

TMI in EAT decreased during the first hours after injection of saline in both groups, presumably because of development of the G_2-M block in the mitotic cycle (Tables 1, 2). Inhibition of cell multiplication after injection of saline lasted longer in D/D animals than in L/D ones (Table 2). Cell transition from the mitosis prophase into metaphase was disordered in both groups. This was seen from higher prophase index *vs.* the metaphase index during the first hours of the experiment (4 h postinjection). More cells accumulated in the prophase in D/D animals and this process lasted longer (up to 7 h postinjection). Cell process in mitosis was more regular after prophase.

Treatment of L/D mice with $CCP^{L/D}$ led to inhibition, synchronization, normalization, and second phases of TMI inhibition and synchronization in EAT (Fig. 1, *a*). After injection of $CCP^{D/D}$ to these animals the phase of mitotic activity normalization was absent, but the next phase (synchronization) did take place (Fig. 1, *b*).

The effect of $CCP^{L/D}$ on the EAT of D/D animals differed from that in L/D mice: there were no TMI of second waves of mitosis inhibition and synchronization (Fig. 2, *a*). The kinetics of EAT cells in group 2 after injection of $CCP^{D/D}$ was in general similar to that after treatment with $CCP^{L/D}$, but the

TABLE 1. Changes in TMI and Indexes of Mitosis Phases in EAT Cells of L/D Animals Injected with Saline (% of Corresponding Values in Intact L/D Animals)

Time of experiment, day/h	TMI	Prophase mitotic index	Metaphase mitotic index	Anaphase mitotic index	Telophase mitotic index
2/15	40.4	56.0	27.6	40.0	63.0
4/17	62.7	194.1	40.0	0	0
		$p_{15}<0.05$			
7/20	52.1	115.4	56.3	61.5	38.2
				$p_{15}<0.05$	
10/23	84.5	55.5	91.3	300.0	70.4
	$p_{17}<0.05$	$p_{17}<0.05$	$p_{15}<0.05$	$p_{17}<0.01$	$p_{20}<0.05$
16/05	88.8	281.8	71.2	287.5	74.0
		$p_{23}<0.05$			
20/09	82.5	130.0	45.7	280.0	83.9
	$p_{23}<0.05$	$p_{05}<0.05$	$p_{23}<0.05$	$p_{23}<0.05$	$p_{23}<0.05$

Note. Here and in Table 2: figures with *p* show the time of the day with which the data were compared.

TABLE 2. Changes in TMI and Mitosis Phase Indexes in EAT Cells in D/D Animals Injected with Saline (% of Corresponding Values in Intact D/D Animals)

Time of experiment, day/h	TMI	Prophase mitotic index	Metaphase mitotic index	Anaphase mitotic index	Telophase mitotic index
2/15	46.9	253.3	33.9	12.5	33.3
4/17	44.5	200.0	33.7	0	10.9
					$p_{15}>0.05$
7/20	26.5	246.2	18.8	0	13.0
	$p_{15}<0.05$		$p_{17}<0.05$		
10/23	68.4	84.6	77.8	113.3	61.1
		$p_{20}<0.01$	$p_{20}<0.05$	$p_{15}<0.01$	$p_{20}<0.05$
16/05	71.0	62.3	70.2	141.2	46.7
	$p_{20}<0.05$	$p_{23}<0.05$	$p_{23}<0.05$	$p_{23}<0.05$	$p_{23}<0.05$
20/09	57.7	127.3	58.1	63.2	25.0
	$p_{05}<0.05$	$p_{05}<0.05$	$p_{05}>0.05$	$p_{05}<0.05$	

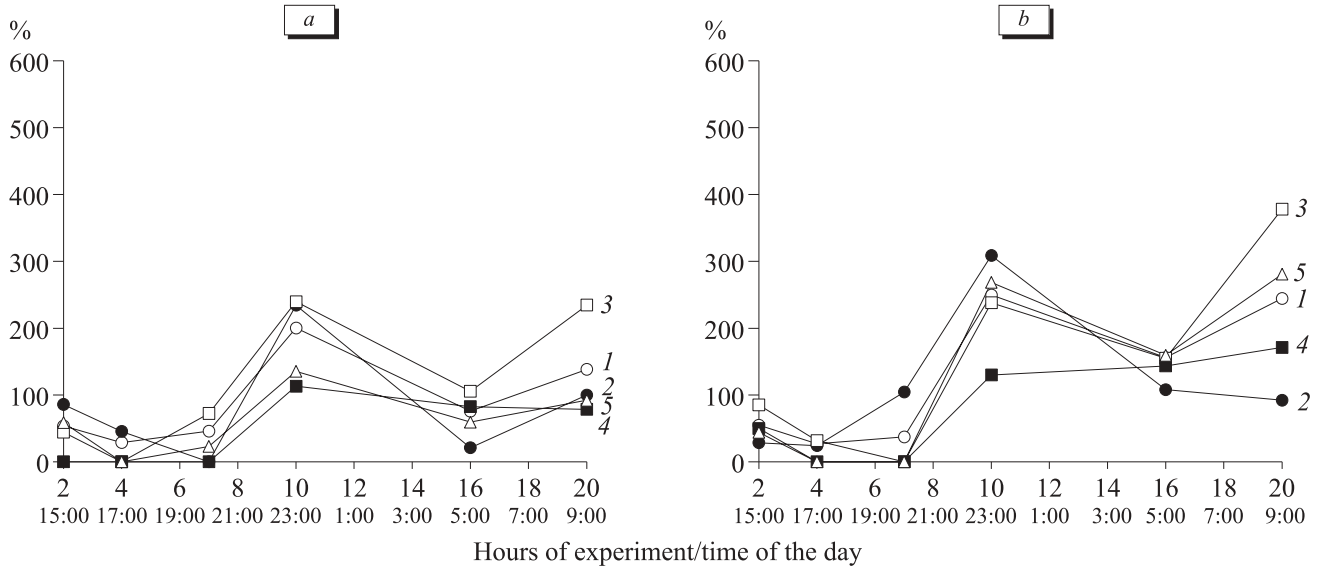

Fig. 1. Changes in the total mitotic index (TMI) and indexes of mitosis phases in Ehrlich ascitic tumor (ATE) cells in animals kept under conditions of light/darkness alternation after injection of $CCP^{L/D}$ (*a*) and $CCP^{D/D}$ (*b*). Percentage of respective values in animals kept under light/darkness conditions, injected with saline, is shown. Here and in Fig. 2: *1*) TMI; *2*) prophase mitotic index; *3*) metaphase mitotic index; *4*) anaphase mitotic index; *5*) telophase mitotic index.

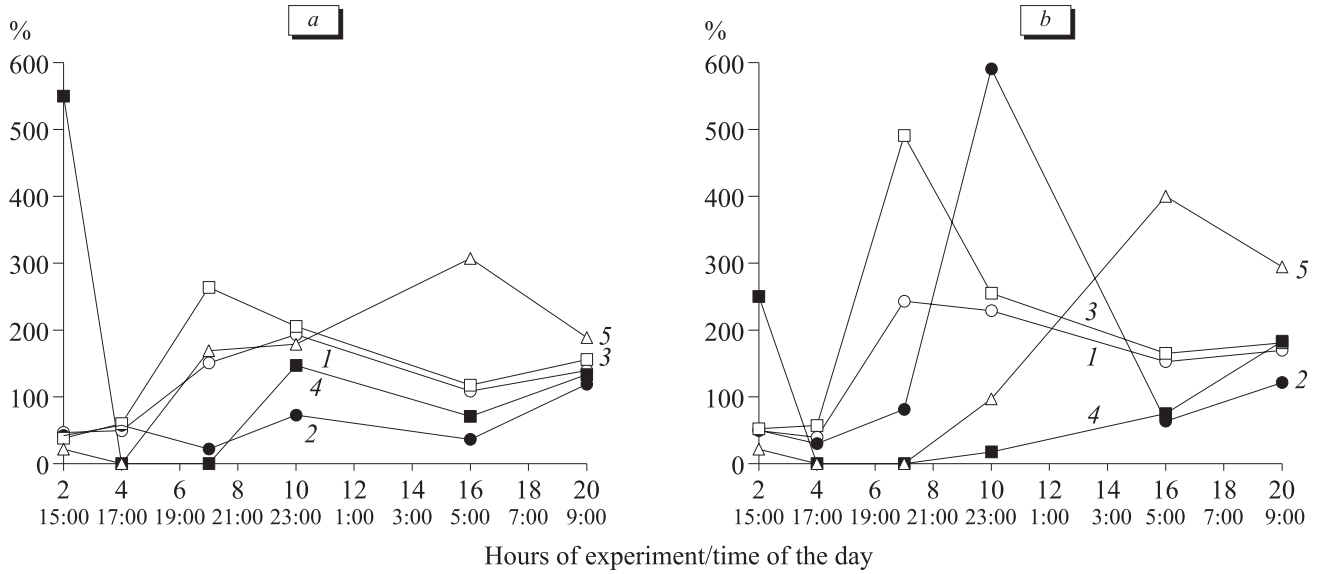

Fig. 2. Changes in TMI and indexes of mitosis phases in ATE cells of animals kept under conditions of darkness after injection of $CCP^{L/D}$ (*a*) and $CCP^{D/D}$ (*b*). Percentage of respective values in animals kept in darkness, injected with saline, is shown.

initial phase of cell division synchronization was more pronounced (Fig. 2, *b*).

The data also demonstrate progress of EAT cells through mitosis in animals of both groups after treatment with CCP of two types. After injection of $CCP^{L/D}$ to L/D mice in addition to the G_2-M block in EAT we observed prophase-metaphase block (Fig. 1, *a*), manifesting by cell accumulation in prophase (by the 4th hour of experiment), while the number of cells in metaphase was very low. These blocks were also observed in L/D animals after injection of $CCP^{D/D}$ (Fig. 2, *a*). The effects of these two mechanisms regulating EAT cell proliferation was independent in both cases.

The effect of $CCP^{L/D}$ on EAT of D/D animals differed from that in L/D mice: long G_2-M block and markedly uneven progress of the cells through mitosis were observed (Fig. 2, *a*). The G_2-M block in D/D animals injected with $CCP^{D/D}$ was shorter than after treatment with $CCP^{L/D}$, but cell progress through mitosis phases was also uneven (Fig. 2, *b*).

Correlation analysis of changes in the mitosis phase indexes for EAT in both cases after injections

of $CCP^{L/D}$ and $CCP^{D/D}$ indicate that cell progress through mitosis, generally even after treatment of L/D animals by two types of CCP, was uneven after treatment of D/D mice by these CCP.

Hence, injection of normal saline to animals with EAT exposed to photoperiodicity conditions and to constant darkness reduced mitotic activity in Ehrlich tumor and impairs transition of tumor cells from mitosis prophase into metaphase. Treatment with "light" and "darkness" CCP differently modified the time course of cell division in the tumor, which attests to different biological activities of CCP originating from animals kept under different conditions of illumination. The effects of light and darkness CCP on EAT cell proliferation in animals exposed to photoperiodicity and constant darkness seems to be characterized by two independent mechanisms: G_2-period mitosis block and prophase-metaphase block.

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