DIURNAL RHYTHM OF CELL DIVISION OF HEPATOMA 22a UNDER THE INFLUENCE OF HEPATIC CHALONE

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An important role in the regulation of cell division is played by tissue-specific inhibitors of cell proliferation, or chalones [4, 9]. Chalones have now been isolated from many normal and tumor tissues of man and animals [8].

The study of the regulatory role of chalones in tumor cell division occupies a special place. Investigations [1, 5] have shown a decrease in the number of dividing and DNA-synthesizing cells in Ehrlich's ascites carcinoma after injection of an aqueous chalone-containing extract from the cells of this tumor.

The object of the present investigation was to study the effect of an alcoholic extract of rat liver, possessing chalone activity, on the character of cell division of mouse hepatoma 22a during the 24-hour period.

EXPERIMENTAL METHOD

Experiments were carried out on 95 male C3HA mice weighing 20-23 g, of which 50 served as the control. The experimental group consisted of 45 animals. The mice were inoculated with a solid strain of hepatoma 22a subcutaneously in the thigh by the method adopted in the Laboratory of Tumor Strains (Head, Candidate of Medical Sciences E. S. Revazova), Institute of Clinical and Experimental Oncology. On the 9th day of the experiment at 10 a.m. animals of the experimental group were given an intraperitoneal injection of hepatic chalone in a dose of 15 mg per mouse in 0.2 ml physiological saline. The control animals received 0.2 ml physiological saline alone. The animals were killed by cervical dislocation every 3 h for 27 h after injection of the preparation and also at the time of its injection.

The chalone-containing extract was obtained by the method described in [3, 10] with the author's modification. The livers of adult noninbred mice were washed in cold physiological saline and homogenized in a "Biomix" apparatus. The homogenate was treated three times with 20 volumes of acetone and dried in the cold. The resulting acetone powder was dissolved in 30 volumes of distilled water and kept at room temperature for 1 h, after which it was centrifuged at 3000 rpm for 10 min at 4°C; the residue was discarded and the supernatant (aqueous tissue extract) was treated with cold 96° ethanol to 55% concentration of the solution and kept in a refrigerator for 2-3 h. Centrifugation was then repeated under the same conditions, the residue was discarded, and the supernatant was treated with cold 96° ethanol, up to 81% concentration of the solution, which was allowed to stand overnight in the refrigerator and then centrifuged. The supernatant was poured off, the residue dissolved in distilled water, and the solution clarified by centrifugation and lyophilized. The resulting 81% ethanol fraction of the aqueous extract consisted of a cream or pale brown powder, completely soluble in water and possessing chalone activity.

To estimate the intensity of cell proliferation colchamine (demecolcine) was used, for it has advantages over the method of determining the mitotic index [2]. Colchamine was injected into mice of both groups 3 h before sacrifice in a dose of 5 mg/kg body weight. Pieces of tumor from five experimental and control animals at each time of the investigation were taken for histological examination. To investigate the tissue-specificity of the preparation, a segment of jejunum was taken 6 h after injection of the chalone. The material was fixed in

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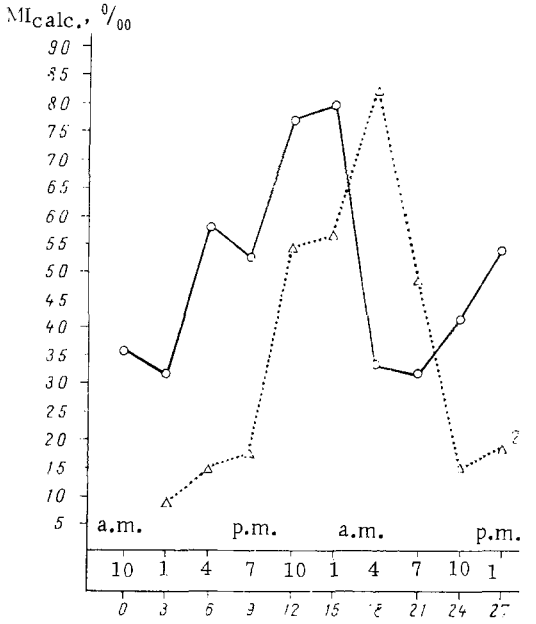

Fig. 1. Changes in number of hepatoma 22a cells undergoing division under normal conditions and after treatment with hepatic chalone. Abscissa: top line — time of day, bottom line — time in h after single injection of hepatic chalone; ordinate, index of mitoses blocked by colchamine (mitotic index, in °/oo).

Carnoy's fluid and histological sections were stained with Mayer's hematoxylin. The number of cells undergoing division was counted by examining 7000-10,000 cells in the tumor and 2000-2500 cells in 50 crypts of the small intestine. The mitotic index was expressed in promille, by adding together the number of colchamine-blocked mitoses and the number of prophases.

The numerical results were subjected to statistical analysis by the Fisher–Student method.

EXPERIMENTAL RESULTS

The results are given in Table 1 and Fig. 1. It will be clear from Table 1 that the mitotic index for the animals of the control group rose significantly from 10 a.m. to 4-7 p.m. and reached a maximum by 10 p.m.-1 a.m. The mitotic index fell significantly until 4-7 a.m., when it reached a minimum ($P = 0.003$). This was followed by a further increase in the number of cells commencing division, but this was not significant ($P = 0.179$).

In the animals of the experimental group minimal values of the mitotic index were found 3, 6, and 9 h after injection of the chalone. The mitotic index then increased significantly to 10 p.m. and reached a maximum by 4 a.m. (P between 7 p.m. and 4 a.m. was 0.009), after which it fell to reach a minimum between 10 a.m. and 1 p.m. ($P = 0.006$).

During the period of increased mitotic activity (from 4 p.m. to 1 a.m. in the control and from 10 p.m. to 7 a.m. in the experimental group) the mean index of colchamine-blocked mitoses did not differ significantly (66.3 and 60.6 °/oo respectively). However, the fraction of cells undergoing division in this period relative to the total number of mitoses in the 24-h period was significantly higher (75.6%) in animals of the experimental group than of the control (57.6%). Meanwhile the total number of cells dividing in the course of the 24-h period in animals of the experimental group was less, namely 31.9% (45.8% in the control).

After a delay of 9 h of the cells in the G_2-period of the mitotic cycle the fraction of cells starting to divide during the period of increased mitotic activity increased, i.e., an effect of synchronization of the dividing cell population due to the action of the chalone was observed.

TABLE 1. Diurnal Changes in Number of Dividing Cells in Hepatoma 22a at Various Times after Injection of Hepatic Chalone (M ± m)

Time of day	Time of injection, h	Mitotic index		% of control	P
		control group	exptl. group		
10	—	35,1±2,1			
13	3	32,0±1,4	8,6±2,6	26,8	0,0001
16	6	57,9±3,5	15,4±1,4	26,6	0,001
19	9	52,5±6,2	17,9±1,3	34,1	0,001
22	12	75,2±10,4	54,7±10,7	72,7	0,2
1	15	79,7±22,7	55,9±9,3	70,1	0,26
4	18	33,4±6,3	83,5±13,2	250,0	0,007
7	21	32,5±6,1	48,6±9,0	149,5	0,165
10	24	41,8±7,9	15,2±1,3	36,4	0,008
13	27	53,4±8,8	19,9±1,7	37,3	0,04
Mean values for 24-h period		49,4	35,5	71,8	

Between 10 a.m. and 1 p.m. the index of c-mitoses fell significantly (P = 0.008) compared with the control. This fact can evidently be explained by the blocking effect of the chalone in the G_1-phase and by a reduction in the number of cells commencing DNA synthesis. This suggestion is also confirmed by the fact that the duration of the G_2 and S phases in hepatoma 22a is approximately 12 h [6]. The same time interval also lies between the end of the blocking action of the chalone on the G_2-population and the beginning of the next sharp fall in mitotic activity.

Under the influence of the chalone, cells in the rapidly growing hepatoma 22a are probably blocked at the boundary between the G_2-M and G_1-S phases, and some of them probably pass into the phase of proliferative rest (G_0) or die, for otherwise it is difficult to explain the reduction in the total number of cells dividing during the 24-h period by approximately 15% in the tumors of the experimental animals.

No statistically significant differences were found between the mice of the control and experimental groups in the mitotic index of the epithelium of the small intestine: 85.6 ± 4.9 and 83.1 ± 6.9 respectively. Consequently, the preparation used was tissue-specific in its action.

The presence of tissue-specificity of the preparation isolated, together with the absence of species-specificity (the chalone was obtained from rat liver), are the principal biological characteristics of the action of substances with chalone activity. Furthermore, the results are evidence that chalone isolated from normal liver cells actively influences cell division in a tumor of hepatic genesis, namely hepatoma 22a; they also indicate that the hepatoma remains susceptible to the action of regulatory factors at the tissue level.

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