EFFECT OF VARIOUS DOSES OF CHALONE-CONTAINING ALCOHOL PRECIPITATE FROM EHRLICH'S ASCITES TUMOR ON MITOTIC ACTIVITY AND DNA SYNTHESIS IN THAT TUMOR

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When the biological action of chalone-containing preparations (CCP) is studied, the method which is widely used to obtain them is by alcoholic fractionation of aqueous extracts, in order to rid them of many nonspecific impurities [5]. The adequate effectiveness, availability, and simplicity of the method have led it to be widely used to investigate the biological characteristics of chalones, and also as the initial stage during isolation of highly purified chalone preparations [2, 4, 6]. CCP isolated by alcoholic fractionation also has been used in investigations on Ehrlich's ascites tumor (EAT) [1, 3, 4]. However, in these investigations the action of the preparation was studied only on mitotic activity (MA).

In the present investigation of effects of different doses of CCP obtained from EAT by alcohol precipitation on MA and on DNA synthesis in that tumor was studied.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male albino mice of the same age (1.5-2 months) and weighing 18-20 g. The animals were kept with alternation of light and darkness every 12 h, and were given food ad libitum for 2 weeks before the beginning of the experiment. A diploid strain of EAT was used as the test object.

CCP was obtained by alcohol fractionation [3], the isolation starting at 10 a.m.

Histological preparations of the tumor and small intestine were made by the standard methods. To prepare autoradiographs of EAT, hydrolysis in 1 N HCl was carried out before application of the nuclear photographic emulsion. As the radioactive marker of replicating DNA, mice with EAT were given an injection of ^{3}H-thymidine, with specific activity of 4.8 Ci/mmole and in a dose of 0.75 of CCP Isolated by Alcohol Fractionation Method at 10 a.m. (M ± m) 6th day of growth of the tumor, 1 h before sacrifice.

TABLE 1. MI in EAT 1, 2, 4, and 6 h after Injection of Different Doses of CCP Isolated by Alcohol Fractionation Method at 10 a.m. (M ± m)

Dose, mg	Time of investigation, h			
	1	2	4	6
Control (physiological saline)	19,0±0,9	18,4±1,4	31,1±3,1	23,5±2,2
15	17,2±1,2	8,8±0,5	12,0±1,5	26,7±2,4
P_1	0,3	0,001	0,001	0,1
10	14,2±0,8	9,0±0,7	15,1±1,1	24,1±3,9
P_1	0,007	0,001	0,001	0,3
5	23,5±1,4	21,1±1,3	19,8±0,8	22,9±1,6
P_1	0,03	0,2	0,007	0,6
P_2	0,013	<0,001	0,001	0,3
P_3	0,001	<0,001	0,005	0,2

Legend. Here and in Tables 2 and 3: P_1) significance of differences from control, P_2) from dose of 15 mg; P_3) from dose of 10 mg.

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TABLE 2. RI in EAT 1, 2, 4, and 6 h after Injection of Different Doses of CCP, Isolated by Alcohol Fractionation Method at 10 a.m. (M ± m)

Dose, mg	Time of investigation, h			
	1	2	4	6
Control (physiological saline)	323,8±6,8	426,5±18,9	388,5±14,5	508,3±28,2
15	370,9±15,8	299,5±13,6	295,8±34,9	380,1±30,4
P_1	0,04	0,001	0,05	0,03
10	485,9±19,0	409,7±19,1	383,7±24,5	552,3±6,0
P_1	<0,001	>0,05	>0,05	>0,05
P_2	0,004	0,002	>0,05	0,001
5	458,4±25,0	467,5±17,0	459,9±24,0	488,0±16,0
P_1	<0,001	—	—	—
P_2	0,02	<0,001	0,005	0,02

TABLE 3. ILN in EAT 1, 2, 4, and 6 h after Injection of Different Doses of CCP, Isolated by Alcohol Fractionation Method at 10 a.m. (M ± m)

Dose, mg	Time of investigation, h			
	1	2	4	6
Control (physiological saline)	51,2±1,0	65,4±7,0	80,5±8,2	130,4±8,2
15	50,5±1,8	44,4±5,8	41,5±4,4	63,7±9,3
P_1	—	0,03	0,003	0,001
10	50,8±5,2	50,9±2,5	53,7±3,9	87,9±3,2
P_1	—	0,03	0,03	0,003
P_2	—	—	—	0,047
5	64,6±2,6	74,3±10,3	69,8±4,9	97,0±6,7
P_2	0,003	0,05	0,001	0,017
P_3	0,048	0,048	0,027	0,013

TABLE 4. Fraction of CCP on MA in EAT and Epithelium of Small Intestine 2 and 4 h after Injection (M ± m)

Tissue studied	Time of investigation, h			
	2		4	
	control	experiment	control	experiment
EAT	18,4±1,4	8,8±0,5	31,1±3,1	12,0±1,5
P		<0,001		<0,001
Epithelium of small intestine	39,0±2,1	36,0±1,8	38,0±2,2	39,0±2,7

The mitotic index (MI — the relative number of dividing cells), the radioactive index (RI — the relative number of DNA-synthesizing cells), and the intensity of labeling of the nuclei with ^{3}H-thymidine (ILN) were determined on the autoradiographs. MI and RI were expressed in promille. ILN was determined by counting the mean number of grains above 50 nuclei. A cell was considered to be labeled if 5 grains of silver of more could be counted above it. At least 5000 cells were counted in each preparation of the tumor, and at least 2000-2500 cells of the small intestine. The significance of differences between the values of the parameters was calculated by the Student—Fisher test. Differences were considered to be significant if $P \leqslant 0.05$.

EXPERIMENTAL RESULTS

Data on the effect of an 81% ethanol fraction of an extract of EAT cells on MA in this tumor are given in Table 1.

CCP was shown to inhibit mitosis and its activity in this direction was dose-dependent. When 15 mg of CCP was given to an animal maximal inhibition of entry of the cells into mitosis was observed after 4 h, and it amounted to 61%. By 6 h after injection of CCP values of

TABLE 5. Fraction of CCP on Number of DNA-Synthesizing Cells in EAT and in Epithelium of Small Intestine 2 and 4 h after Injection (M ± m)

Tissue studied	Time of investigation, h			
	2		4	
	control	experiment	control	experiment
EAT	427,0±19,0	300,0±14,0 0,001	388,0±15,0	295,0±42,0 0,048
Epithelium of small intestine	276,8±30,9	311,0±21,0	294,0±22,2	321,0±10,3

TABLE 6. Action of CCP on ILN of Cells in EAT and Epithelium of Small Intestine 2 and 4 h after Injection (M ± m)

Tissue studied	Time of investigation, h			
	2		4	
	control	experiment	control	experiment
EAT	65,4±4,8	44,6±6,0 0,03	80,5±4,5	41,5±4,5 0,003
Epithelium of small intestine	9,5±1,4	8,6±1,1	16,2±2,3	15,7±1,3

MI were back to the control level. The strongest inhibitory action of the other two doses of CCP (10 and 5 mg) also was obaerved after 4 h of the experiment; this effect, moreover, depended significantly on the injected dose of the preparation and amounted to 50 and 36%, respectively.

Data on the effect of CCP on RI are given in Table 2. It was found that only the highest of the doses used (15 mg) inhibited entry of the cells into the S phase of the mitotic cycle. RI was reduced after 2, 4, and 6 h by 30, 24, and 25%, respectively. Incidentally, 1 h after injection of all doses of CCP, RI was significantly higher than in the control, by 15, 50, and 42% when the CCP was given in doses of 15, 10, and 5 mg, respectively.

The action of CCP on ILN, i.e., directly on cells in the S phase of the mitotic cyele, was studied in the same experiment (Table 3).

The results showed that the effect of CCP on the intensity of DNA synthesis in EAT cells was more marked than its action on entry of the cells into the S phase (on RI). All three doses of CCP used lowered ILN; inhibition reached maximum, moreover, after 6 h of the experiment. Meanwhile the effect was clearly dependent on the dose of CCP: in a dose of 15 mg it depressed ILN by 61%, in a dose of 10 mg by 32%, and in a dose of 5 mg by 25%. The smallest dose of CCP used (5 mg) caused a significant increase (by 26%) of ILN 1 h after injection of the preparation.

The tissue specificity of action of CCP on EAT was studied. The results showed that the ethanol fraction of the extract of EAT cells in a dose of 15 mg considerably reduced MI in the cells of this tumor 2 h (by 52%) and 4 h (by 61%) after injection of CCP into the animals. MI in the epithelium of the small intestine was unchanged in the action of CCP (Table 4).

RI in EAT 2 and 4 h after injection of the alcohol precipitate were reduced by 30 and 24% respectively, but in epithelial cells of the small intestine under these circumstances they were the same as in the control (Table 5). The same principle was observed when the action of CCP was studied on ILN with ^{3}H-thymidine. In response to its injection ILN in EAT was depressed by 32 and 49% after 2 and 4 h of the experiment, respectively, whereas in the epithelium of the small intestine CCP caused no change (Table 6).

CCP isolated by alcohol fractionation from EAT cells thus depresses proliferative processes in that tumor. It acts both on entry of the cells into mitosis and into the S phase of the mitotic cycle, and also on the process of DNA synthesis in cells in the S phase of the mitotic cycle. The action of CCP on DNA synthesis in cells in the S phase is stronger than its effect on entry of the cells into the phase of DNA synthesis. Changes in all three parameters of cell kinetics that were investigated depended on the dose of CCP. The action of CCP is tissue-specific.

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