EFFECT OF FRACTIONS OBTAINED BY GEL-FILTRATION OF CHALONE-CONTAINING PREPARATION FROM EHRLICH'S ASCITES CARCINOMA ON MITOTIC ACTIVITY AND DNA SYNTHESIS IN THE TUMOR OF ORIGIN

N. Ya. Matsak, A. I. Antokhin, and Yu. A. Romanov*

UDC 616-006.3.04-092.9-092:[576.353]-07

KEY WORDS: Ehrlich's ascites carcinoma; chalone; mitotic activity; DNA synthesis.

A chalone-containing preparation (CCP) isolated by alcoholic fractionation from Ehrlich's ascites carcinoma (EAC) cells inhibits entry of the cells into mitosis and the S-phase of the mitotic cycle, and also inhibits DNA synthesis in EAC cells when in the S-phase of the mitotic cycle. The action of CCP is tissue-specific [2, 5]. There is some evidence, although contradictory [6, 7], on the distribution of G_4- and G_2-chalones among different tissues. In relation to EAC [8, 9] an attempt has been made to study the action of the G_1- and G_2-components of CCP. However, it has proved impossible to draw a final conclusion because the effect of these components was assessed by means of an integral parameter, namely the rate of growth of EAC cells in culture. Other workers [11] have studied the action of the G_1-chalone of EAC obtained by chromatography on sepharose. On the basis of differences between kinetic parameters of the effect on EAC cells in the G_1- and G_2-phases of the mitotic cycle, it has been suggested [1] that both G_1- and G_2-chalones exist in CCP for EAC. There is also evidence of the existence of a specific substance, influencing DNA synthesis in the S-phase of the mitotic cycle (S-chalone) [3] in CCP from EAC.

The aim of the present investigation was to separate active substances contained in CCP from EAC and acting on different phases of the mitotic cycle of the cell with this tumor.

EXPERIMENTAL METHOD

The investigation was conducted on noninbred male albino mice of the same age (1.5-2 months) and weighing 20-25 g. The animals were kept under conditions of 12 h for daylight and 12 h of darkness, with food ad libitum for two weeks before the experiment began. The test object was a diploid strain of EAC. The CCP was obtained by alcoholic fractionation [4]. To separate the active component of the CCP and to purify it further, the method of gel-filtration on Ultragel Ac-A-44 (LKB, Sweden) was used. Gel-filtration was carried out on a 2.2 × 80 cm column at the rate of 30 ml/h in 50 mM Tris-HCl buffer, (pH 7.5), containing 0.2 M NaCl. Protein (20 mg) dissolved in 5 ml of buffer was applied to the column. Fractions obtained during gel-filtration were pooled in accordance with the elution profile and material from the peaks was tested for biological activity with respect to its action on 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 (ILN) with ^{3}H-thymidine (^{3}H-T) in the cells of an EAC on the 5th-6th day of its development. MI and IR were expressed in promille. ILN was determined by counting the average number of grains of silver above 50 nuclei. To determine the tissue specificity of action, the effect of the fraction on the above parameters was studied in cells of Novikoff's ascites hepatoma. The total protein conferences between values of the parameters were calculated by the Student-Fisher test. Differences were considered significant for which $p \leq 0.05$.

EXPERIMENTAL RESULTS

The profile of the fractionation curve of CCP from EAC on Ultragel Ac-A-44 is shown in Fig. 1. The animals of group 1 were given an injection of 0.5 ml of fractions 16 + 17, i.e.,

*Corresponding Member of the Academy of Medical Sciences of the USSR.

Department of Biology, Medico-Biological Faculty, N. I. Pirogov Second Moscow Medical Institute. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 106, No. 8, pp. 202-204, August, 1988. Original article submitted October 26, 1987.

0007-4888/88/0008-1147$12.50

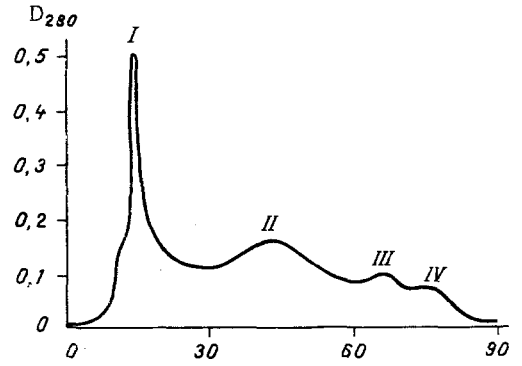

Fig. 1. Fractionation of chalone-containing preparation (alcoholic precipitate) on column with Ultragel Ac-A-44. Rate of elution 30 ml/h; volume of fractions 5 ml. Abscissa, Nos. of fractions.

material from peak I in the elution profile. The protein concentration in these samples was 1360 µg/ml. The animals of group 2 were given an injection of material from peak II (fractions 39 + 40), with a protein concentration of 410 µg/ml, and those of group 3 received material from fraction 65 (the region of peak III), in which the protein concentration was 150 µg/ml. Material of fraction 76 (the region of peak IV), in which protein could not be found to be present, was injected into the animals of group 4. Animals of the control group received an injection of 0.5 ml of buffer. To assess the degree of purification of the preparation, CCP applied to the column was injected into one group of animals in a dose of 15 mg/mouse. The experiment began at 10 a.m. All the animals were given an injection of ^{3}H-T (0.75 µCi/g body weight) 1 h before sacrifice. The results of biological testing are given in Table 1.

The results show that material applied to the Ultragel column (alcoholic precipitate), if injected into animals with EAC, depress MI, RI, and ILN in the cells of this tumor by 58, 25, and 32%, respectively (compared with the corresponding control values). This preparation had no effect on proliferation in Novikoff's ascites hepatoma. Thus the alcoholic precipitate applied to a fractionation column, contained G_1-, G_2-, and S-components of chalone, with tissue-specific action.

The material of peak I in the elution profile possessed inhibitory activity against MI, RI, and ILN in EAC cells but did not affect these parameters in cells of Novikoff's ascites hepatoma. The degree of depression of RI and ILN in EAC cells was greater than in the case of the alcoholic precipitate. Thus this particular fraction of CCP contains G_1- and G_2-chalones, as well as a substance acting on the S-period of the cycle (S-chalone). The effect of all the above-mentioned fractions is tissue-specific.

Material from peak II inhibited mitotic activity in EAC cells by a much greater degree than the fractions of peak I. Meanwhile, it has no effect on the entry of the cells into the phase of DNA synthesis or on the actual process of DNA synthesis in EAC. Material from peak II caused no change in MI, RI, and ILN in Novikoff's ascites hepatoma cells. Thus, the action of the inhibitor of mitotic activity contained in material from peak II is tissue-specific.

Material from peak II inhibited mitotic activity in EAC cells by a much greater degree than the fractions of peak I. Meanwhile it has no effect on the entry of the cells into the phase of DNA synthesis or on the actual process of DNA synthesis in EAC. Material from peak II caused no change in MI, RI, and ILN in Novikoff's ascites hepatoma cells. Thus the action of the inhibitor of mitotic activity contained in material from peak II is tissue-specific.

Material from peaks III and IV contained no active components acting on proliferation in EAC or Novikoff's hepatoma.

Thus, the G_1- and S-components of CCP from EAC, which inhibit the entry of cells into the S phase and inhibit DNA synthesis during it, are completely euluted from the column with material of peak I, i.e., they have a high molecular weight. The G_2-component of CCP is eluted in a sufficiently broad front, being present in material from both peaks I and II. However, its content in the material of peak II is evidently much greater. The presence of G_2-activity of CCP in the material from peak I may perhaps be explained on the grounds that the low-molecular-weight G_2-component of CCP forms a complex with the high-molecular-weight compounds of peak I. The data described above confirm that partial separation of the G_2-

TABLE 1. MI, RI, and ILN in EAC and Novikoff's Ascites Hepatoma Cells 4 h after Injection of Various Fractions of CCP from EAC, Obtained by Gel-Filtration on Ultragel Ac-A-44

Test system	Fraction injected	MI ± m	RI ± m	ILN ± m
EAC	Control	44,6±1,7	287,1±12,5	30,6±3,9
	16+17	18,4±4,1 (41,3)	171,4±9,5 (59,6)	12,6±2,0 (41,2)
	p_K	<0,001	<0,001	0,001
	39+40	10,8±22,0 (24,2)	269,4±13,4 (94,0)	34,1±3,2 (111,4)
	p_K	<0.001	>0,1	>0,1
		p_{16+17}=0,1	p_{16+17}<0,001	p_{16+17}<0,001
	65	48,0±1,0 (107,6)	280,3±11,0 (97,6)	32,1±3,2 (105,0)
	p_K	>0,1	>0,1	>0,1
		p_{16+17}<0,001	p_{16+17}<0,001	p_{16+17}<0,001
	76	49,1±2,3 (110,0)	275,0±12,2 (95,8)	29,3±3,9 (95,8)
	p_K	>0,1	>0,1	>0,1
	Alcoholic precipitate	18,8±0,7 (42,2)	215,3±14,3 (75,0)	20,7±1,1 (67,7)
	p_K	<0,001	0,01	0,03
			0,03	0,01
Novikoff's ascites hepatoma	Control	24,5±2,0	272,9±12,2	40,3±3,3
	16+17	21,1±0,6 (86,0)	272,2±8,3 (99,7)	40,8±2,6 (101,0)
	p_K	>0,1	>0,1	>0,1
	39+40	21,8±0,6 (89,0)	278.2±10,4 (101,9)	43,2±4,9 (107,0)
	p_K	>0,1	>0,1	>0,1
	65	25,3±1,2 (103,0)	271,9±9,3 (99,6)	39,8±5,6 (99,0)
	p_K	>0,1	>0,1	>0,1
	76	26,9±1,3 (108,0)	262,9±16,9 (96,3)	45,7±5,3 (113,0)
	p_K	>0,1	>0,1	>0,1
	Alcoholic precipitate	24,7±2,1 (101,0)	279,2±12,1 (102,3)	39,1±3,0 (97,0)
	p_K	>0,1	>0,1	>0,1

Legend. Numbers in parentheses are percentages of control. Subscript attained to p indicates fraction with which comparison is made.

component of CCP from components inhibiting the entry of the cells into the phase of DNA synthesis and DNA synthesis itself can be achieved by gel-filtration.

LITERATURE CITED

1. A. I. Antokhin, "Chalone regulation of proliferative processes in Ehrlich's ascites tumor," Author's Abstract of Dissertation for the Degree of Candidate of Biological Sciences, Moscow (1979).
2. N. Ya. Matsak, Yu. A. Romanov, and A. I. Antokhin, Byull. Éksp. Biol. Med., 103, No. 1, 89 (1987).
3. Yu. A. Romanov, S. A. Ketlinskii, A. I. Antokhin, and V. B. Okulov, Chalones and Regulation of Cell Division [in Russian], Moscow (1984).
4. T. V. Savchenko, V. B. Zakharov, S. G. Mamontov, and Yu. A. Romanov, Vestn. Akad. Med. Nauk SSSR, No. 11, 54 (1980).
5. M. V. Semenova, "Cell division in Ehrlich's mouse ascites tumor after administration of a chalone-containing extract of this tumor and bryomycin," Author's Abstract of Dissertation for the Degree of Candidate of Biological Sciences, Moscow (1981).
6. N. M. Barfod, Cell Tissue Res., 110, 225 (1977).
7. J. Bichel, Natl. Cancer Inst. Monogr., 38, 197 (1973).
8. W. Lehnmann, H. Graets, M. Schütt,and P. Langen, Acta. Biol. Med. Germ., 36, 43 (1977).
9. W. Lehnmann, H. Graets, R. Lamtleben, et al., Acta Biol. Med. Germ., 39, 93 (1980).
10. O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall, J. Biol. Chem., 193, 265 (1951).
11. G. S. Nakai and H. Gergely, Cell Tissue Kinet., 13, 65 (1980).