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BIOLOGICAL ACTIVITY OF CHALONES ISOLATED FROM THE NORMAL AND REGENERATING LIVER

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UDC 612.35.014.3.018:612.6.03

KEY WORDS: liver chalones; partial hepatectomy; regulation of mitotic homeostasis of the liver.

It has now been shown that an essential role in the regulation of mitotic homeostasis of the liver is played by tissue-specific inhibitors of proliferation, or chalones [1, 4, 6]. After partial hepatectomy, leading to intensive proliferation of hepatocytes in the residual part of the organ, chalone activity disappears: The chalone-containing extract isolated from regenerating liver 24 h after the operation cannot inhibit growth of hepatoma cells in an *in vitro* system [5]. The mechanism of disappearance of biological activity of the chalones has not yet been explained.

The object of this investigation was to study the effect of chalones, isolated from the regenerating liver at various times after partial hepatectomy, on the entry of hepatocytes into the phase of DNA synthesis and mitotic division in an *in vivo* system.

EXPERIMENTAL METHOD

Aqueous extracts of normal and regenerating liver, fractionated with ethanol, were used as the chalones; the method used to obtain them was described previously [3]. The fraction precipitated between 70 and 81% saturation with ethanol possessed activity. Analytical disc electrophoresis of the resulting extracts was carried out by Maurer's method [2] in 7.5% polyacrylamide gel at pH 8.9. Preparative electrophoresis was carried out using the same system of buffers in a block of polyacrylamide gel measuring 160 × 230 × 4 mm for 14 h with a current of 50 mA. After the end of electrophoresis the block of gel was cut into four regions (Fig. 1A-D), corresponding to groups of proteins with different mobility, and after elution, concentration, and dialysis against physiological saline, the effect of these groups of proteins on the level of hepatocyte proliferation was determined in an *in vivo* system. For this purpose two series of experiments were conducted on noninbred male rats from the Rappolovo Nursery, Academy of Medical Sciences of the USSR, weighing 70-80 g, hepatectomized by the method of Higgins and Andersen. In the experiments of series I, simultaneously with colchicine (0.2 mg/kg body weight), chalones isolated from the normal or regenerating liver 22 h and 28 days after the operation (in a dose of 15 mg/100 g body weight) were injected into the hepatectomized animals 25 h after the operation. In the experiments of series II, chalones isolated from normal or regenerating liver 28 days after the operation (15 mg/100 g body weight per animal) and also various protein components of these chalones (equivalent to the protein content in 15 mg of whole extract per 100 g body weight) were injected into hepatectomized rats 21 h after the operation. One hour before sacrifice, ^{3}H-thymidine was injected (1 μCi/g body weight) intraperitoneally into the animals of this group. Instead of chalones, physiological saline in the same volume was injected into the control hepatectomized rats in both series. All the animals were killed 29 h after hepatectomy. Activity of hepatic chalones was assessed by the decrease in number of C-mitotic activity, and in the fraction of ^{3}H-thymidine-labeled hepatocytes (in 3000 cells from each animal).

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0007-4888/81/9207-0971$07.50

TABLE 1. Effect of Chalones Isolated from Normal and Regenerating Liver on Proliferative Activity of Hepatocytes (M ± m)

Preparation	n	MC_{colch}, ‰	MC, ‰	ILN, ‰
Control	7	147,6±16,4	25,1±5,2	52,5±5,1
Chalones from normal liver	6	78,3±7,6*	11,7±2,3*	16,8±6,6*
Chalones from regenerating liver:				
22 h after operation	5	141,9±16,5		
28 days after operation	6	118,3±17,6	41,4±7,1	22,4±3,3*
Individual fractions of chalones from regenerating liver 28 days after operation				
A	3	—	27,1±3,4	5,1±2,1*
B	3	—	38,0±6,1	94,8±48,4
C	3	—	49,7±8,5*	42,9±26,4
D	3	—	4,3±1,3*	10,8±3,1*

*P < 0.05 compared with control.

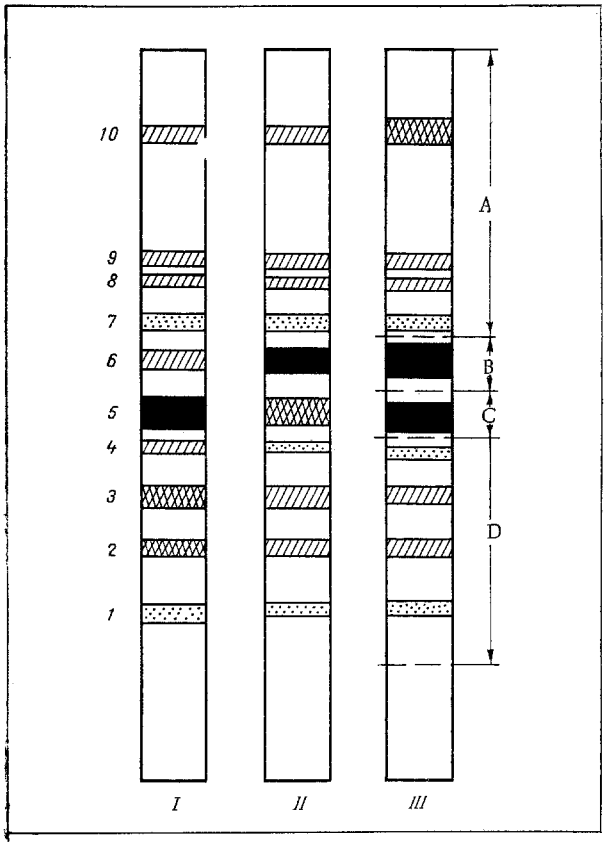

Fig. 1. Protein spectra of chalone-containing extract isolated from normal and regenerating rat liver: 1) normal liver; II) regenerating liver 22 h after hepatectomy; III) regenerating liver 28 days after hepatectomy. 1-10) Protein components of extracts. A-D) Regions of subdivision of gel after preparative electrophoresis.

EXPERIMENTAL RESULTS

The experiments of series I showed that chalones isolated from the regenerating liver 22 h after hepatectomy, unlike chalones from the normal liver, did not affect the level of mitoses among hepatocytes arrested by colchicine. The antimitotic effect of the chalones was not restored even 28 days after the operation (Table 1).

To study the causes of disappearance of chalone activity in the regenerating liver, in the experiments of series II the effect of protein fractions A, B, C, and D, composing the chalone-containing extract, on the regenerating liver 28 days after the operation, and on the level of proliferative activity of the hepatocytes, was studied (Fig. 1). Despite the absence of any effect of this extract on the mitotic coefficient of the hepatocytes, an active

mitotic inhibitor was found among its components in the group of proteins with high electrophoretic mobility (group D). It was also shown that this extract contains protein stimulating mitotic activity of hepatocytes (group C, Table 1).

The chalone-containing extract isolated from the regenerating liver 28 days after the operation inhibited DNA synthesis in the hepatocytes by 55%; inhibitors of DNA synthesis were found among two groups of proteins — those with high (group D) and with low (group A) electrophoretic mobility. No factor significantly stimulating DNA synthesis could be found in the chalone extract of the regenerating liver 28 days after the operation (Table 1). These data are evidence that chalone activity inhibiting DNA synthesis (the G_1 effect) was either more stable or was restored more rapidly in the course of regeneration of the liver than chalone activity inhibiting mitosis (the G_2 effect).

The protein spectrum of the chalone-containing extract of the regenerating liver did not differ qualitatively from the protein spectrum of normal liver at different times after partial hepatectomy; such differences as were found were quantitative in character, namely a decrease in the relative content in the regenerating liver of proteins with high electrophoretic mobility (group D) and changes in the ratio between protein fractions C and B toward predominance of the latter (Fig. 1).

The results suggest that regulation of mitotic homeostasis in the liver, both under normal conditions and during regeneration, is effected through the combined action of tissue-specific inhibitors (chalones) and stimulators of proliferation. The problem of the tissue-specificity of these stimulators requires further study. Disappearance of the biological activity of chalones in the regenerating liver is due not to the loss of the tissue-specific inhibitor by the liver cells, but to changes in competitive relations between inhibitors of proliferation, in favor of stronger activity of the latter.

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