MORPHOLOGY AND PATHOMORPHOLOGY

Activity of Nucleolar Organizers in Hepatocytes of Rats with Cirrhosis of the Liver after Treatment with Bioactive Preparations

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The therapeutic efficiency of bioactive preparations of the liver and spleen in the treatment of experimental toxic cirrhosis of the liver is shown. Evaluation of the nucleolar organizer activity showed the highest efficiency of treatment with human fetal liver extract.

Key Words: *nucleolar organizer activity; liver cirrhosis; CCl_4; liver extract*

Chronic hepatitis and cirrhosis of the liver (CL) rank first in the structure of liver diseases [4]. Inefficiency of treatment of these diseases necessitates the development of new effective therapeutic methods. Therapeutic effects of liver and spleen extracts (LSE) on the course of chronic hepatitis and CL, manifesting by intensification of proliferative processes and reparation of lesions, are studied [5]. The method of evaluation of the nucleolar organizer activity by staining with 50% colloid silver nitrate solution is highly informative for investigation of these phenomena [1]. This method objectively reflects the intensity of ribosomal synthesis and proliferative activity of cells [1,2].

MATERIALS AND METHODS

Experiment was carried out on 48 adult outbred albino rats (180-220 g). The animals were divided into 6 groups, 8 rats per group; one group consisted of intact rats. Experimental animals were subcutaneously injected with 50% oil solution of CCl_4 (0.1 ml) 3 times a week for 2 months in order to induce CL. This was followed by intraperitoneal injections (3 times a week, 2 weeks) of one of the following preparations: donor rat liver extract (1.5 ml/100 g) [6,7]; porcine spleen extract "Splenopide" (1.5 ml/100 g), a gracious gift from Prof. A. B. Tsybin (Institute of Transplantology and Artificial Organs); donor rat liver extract ultrafiltrate (3 ml/100 g); human fetal (8-10 week gestation) liver extract (1.5 ml/100 g).

The nucleolar organizers in hepatocytes were detected in tissue sections [3]. The preparations were examined under a JENAMED-2 microscope at ×1000 under oil immersion and green filter.

The nucleoli, intra- and extranucleolar incorporations, and their sum were counted in hepatocytes. Proliferative processes were evaluated by estimating the percentage of type 1 and type 2 hepatocytes. Argentaffine nucleolar incorporations or diffusely stained nucleoli were detected in type 1 hepatocytes. This distribution of incorporations is observed in nonproliferating cells [1]. Completely stained nucleoli and depositions inside them were

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visualized in type 2 hepatocytes, as well as argentaffine protein granules, dispersed in the karyoplasm. This type of argentaffine granule distribution is characteristic of proliferating cells.

The results were processed by methods of variation statistics. The significance of differences was evaluated by Student's t test and χ^2 test.

RESULTS

Cirrhosis of the liver developed 2 months after CCl_4 injections. It manifested morphologically by the absence of typical hepatic lobules and diffuse growth of the connective tissue. Liver parenchyma consisted of false hepatic lobules; diffuse and zonal fatty degeneration of hepatocytes was observed. Fibrous tissue growth and diffuse lymphocytic and histiocytic infiltration were observed in the portal tracts (Fig. 1).

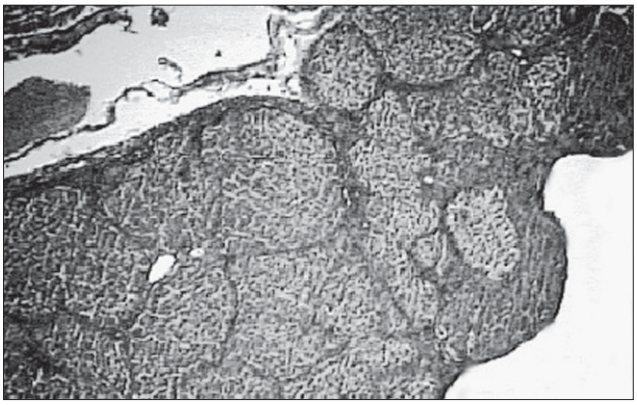

Fig. 1. Toxic cirrhosis of the liver in rats. Hematoxylin and eosin staining; ×1000.

The number of extranuclear incorporations in hepatocytes of rats with CL was significantly higher than in hepatocytes of intact rats (Fig. 2, *a*; Table 1). It is known that emergence of extranuclear granules in the cell karyoplasm is a morphological equivalent of possible activation of ribosomal synthesis in cells. This was paralleled by changes in the sum of intra- and extranucleolar incorporations in rat hepatocytes. This was preceded by changes in the number of proteins regulating the ribosomal synthesis in zones of hepatocyte nucleolar transcription. Presumably, long-term CCl_4 treatment led to hyperplasia of protein granules regulating ribosomal synthesis in the hepatocyte nucleolar organizers, with possible translocation of the argentaffine protein granules from the nucleoli

TABLE 1. Number of Nucleolar Organizers in Rat Hepatocytes before and after Treatment of CL ($M\pm m$)

Experiment conditions	Number of examined hepatocytes	Absolute count of nucleoli per hepatocyte	Absolute count of intranucleolar incorporations per hepatocyte	Absolute count of extranucleolar incorporations per hepatocyte	Absolute sum of incorporations per hepatocyte
Intact rats	140	3.0±0.1	7.7±0.2	0.3±0.1	8.0±0.2
LC	140	6.6±0.1*	17.9±0.3*	4.2±0.2*	22.1±0.4*
LC+rat liver extract	120	2.5±0.1*+	17.2±0.8*	0.3±0.1+	17.5±0.8*+
LC+porcine spleen extract	160	2.5±0.1*+	15.1±0.8*+	0.5±0.1+	15.6±0.1*+
LC+rat liver extract ultrafiltrate	140	1.9±0.1*+	10.8±0.5*+	0.7±0.2+	11.5±0.5*+
LC+human fetal liver extract	160	3.3±0.1+	8.0±0.2+	0.3±0.1+	8.3±0.2+

Note. $p<0.0001$ compared to *intact rats; +CL.

TABLE 2. Number of Hepatocytes of Different Types in Rats before and after Treatment of CL ($M\pm m$)

Experiment conditions	Total	Number of type 1 hepatocytes		Number of type 2 hepatocytes	
		abs.	%	abs.	%
Intact rats	140	120	85.7	20	14.3
LC	140	11	7.9	129	92.1
LC+rat liver extract	120	106	88.3	14	11.7
LC+porcine spleen extract	160	131	81.9	29	18.1
LC+rat liver extract ultrafiltrate	140	115	82.1	25	17.9
LC+human fetal liver extract	160	133	83.1	27	16.9

Note. Significant differences with intact rats and rats with LC ($p<0.0001$) according to χ^2 test.

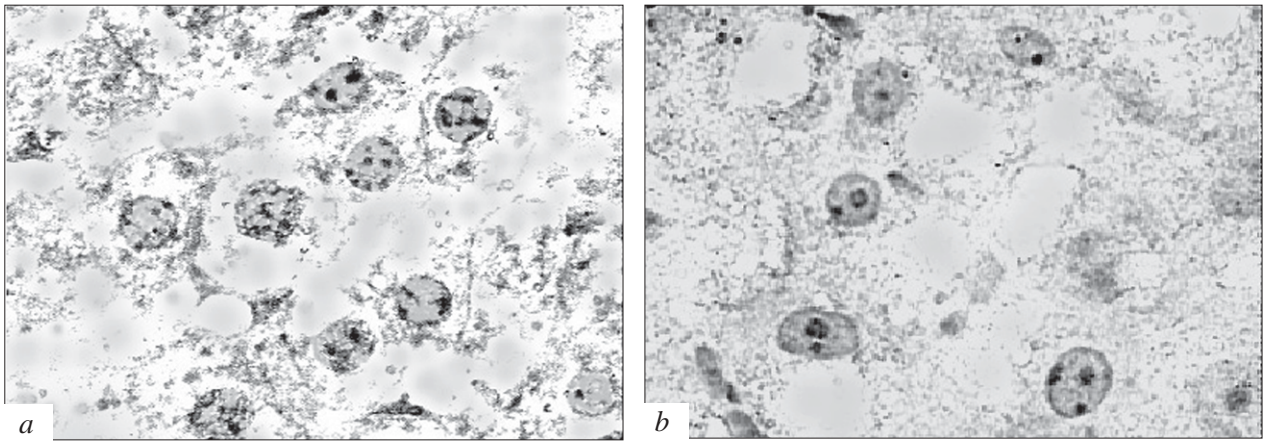

Fig. 2. Hepatocytes of rats with cirrhosis of the liver before (*a*) and after treatment by liver and spleen extracts (*b*). *a*) high; *b*) low activity of nucleolar organizers. Argentaffine reaction with 50% colloid silver nitrate; ×1000.

into the karyoplasm. This leads to an increase in the number of extranucleolar incorporations and, hence, to activation of ribosomal synthesis resultant from the increase in the number of regulator proteins associated with the nucleolar organizer regions. These data indicate that hepatocytes possess a hyperplastic mechanism of adaptation to chronic intoxication, which agrees with the second principle of structural base of material support of homeostasis [8].

Treatment with LSE led to a reduction in the number of nucleoli, intranuclear, extranuclear incorporations, and the sum of argentaffine granules in rat hepatocytes, indicating less pronounced activation of the ribosomal synthesis in them (Fig. 2, *b*). The only exception was the number of intranucleolar incorporations in hepatocytes of rats treated with donor rat liver extract. Presumably, injection of bioactive LSE to rats with CL caused the appearance of proteins blocking biosynthetic and proliferative potential in hepatocytes. The number of incorporations in hepatocytes of rats treated with liver extract decreased at the expense of a lesser number of extranucleolar incorporations. This attests to a sharp decrease in the ribosomal synthesis activity. The number of nucleoli, intra-, extranucleolar incorporations, and sum of argentaffine granules in hepatocytes of rats treated with LSE differed significantly from those in hepatocytes of untreated rats. These data indicate that these therapeutic methods are effective in toxic CL, because the number of nucleoli and argentaffine incorporations decreases, ribosomal synthesis by hepatocytes is inhibited, particularly at the expense of decrease in the content of extranucleolar argentaffine protein granules in the cell karyoplasm. The number of nucleoli and argentaffine granules in hepatocytes of rats treated with human fetal liver extract did not differ from those in intact rat hepatocytes. This indicates that the values were similar to those in intact rats and hence, treatment with human fetal liver extract was the most effective method of treatment of CL.

The absolute count of type 1 hepatocytes was lower and that of type 2 cells higher in rats with CL than in intact rats (Table 2). These facts indicate that activity of the nucleolar organizers in hepatocytes of rats with CL increased as a result of hepatotoxin CCl_4. The absolute count of type 2 hepatocytes in rats treated with LSE was lower and of type 1 hepatocytes was significantly higher than in untreated rats and virtually did not differ from the corresponding values in intact rats. Hence, all treatments for toxic CL were effective, as resulted in a decrease in activity of ribosomal synthesis and proliferative potential (by 75%).

Human fetal liver extract exhibited the most pronounced effect on liver recovery. Typical hepatic lobules appeared, represented by somewhat enlarged hepatocytes with hyperchromatic nuclei and having connective-tissue septae. Orientation of hepatic cords in hepatic lobules was common (radian).

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