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ADHESION OF LEUKOCYTES IN CHRONIC DIFFUSE LIVER DISEASES

A. S. Loginov, V. P. Yamskova, N. B. Tumanova,
V. D. Tkachev, and V. I. Reshetnyak

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Histological changes arising in the liver in chronic diseases may be associated with disturbances of intercellular relations between hepatocytes. Electron-microscopic investigations have shown that during cholestasis in patients with primary biliary cirrhosis, partial or total separation of the hepatocytes takes place, and leads to an increase in the interhepatocytic space [1]. In addition, investigation of laboratory animals with experimentally induced cholestasis revealed considerable disturbances in the region of the tight junction, namely a decrease in the area of the contacting surfaces of the hepatocytes in animals with cholestasis by 50% compared with control animals [12]. Existing approach techniques enable some of the characteristics of intercellular interactions to be determined [3]. A special place among them is occupied by methods of estimation of the adhesive characteristics of intercellular junctions (IJ), determining their mechanical strength [4, 5]. In the present investigation one such method was used, based on determination of the number of separating whole cells and nuclei during a standard dispersion procedure [8, 10]. Several workers have shown that the number of single cells (hepatocytes, enterocytes) separated as a result of mechanical action on the tissue reflects the state of IJ: weakening of IJ leads to an increase in the number of cells separating from the tissue [3]. This approach technique was used successfully previously in a study of the macromolecular factors involved in the adhesive process [2, 6, 8-10].

The aim of this investigation was to study the adhesive properties of hepatocytes using biopsy material from patients with chronic diffuse liver diseases.

EXPERIMENTAL METHOD

The test object consisted of liver biopsy material from 72 patients, divided into eight groups on the basis of their final clinical diagnosis: 1) with primary biliary cirrhosis — PBC, including chronic cholestatic hepatitis — CCH, as the initial stage of PBC (11 patients), 2) with chronic hepatitis — CH, with moderate activity (five patients); 3) with chronic active hepatitis — CAH (four patients); 4) with chronic persistent hepatitis — CPH (11 patients); 5) with Gilbert's unconjugated hyperbilirubinemia — UH (11 patients); 6) with fatty degeneration of the liver — FD (11 patients); 7) with cirrhosis of the liver — CL (three patients); 8) with gastroenterologic diseases but without any histological changes in the liver: gastric and (or) duodenal ulcer, chronic gastritis, chronic pancreatitis, chronic cholecystitis, etc. — control (16 patients). Liver biopsy was carried out by Menghini's method with a needle 1.8 mm in diameter [2]. Liver biopsy tissue was cut into fragments measuring about 1 mm^3. Each tissue fragment was dispersed in 0.1 ml of 0.1% trypan blue in Ringer's solution, using a glass tissue disintegrator with 50-μ gap. Each fragment was dispersed under strictly standard conditions [8, 10]. Single hepatocytes (N_{sh}) and free cell nuclei (N_n) separated on dispersion were then counted in a Goryaev chamber. From these results the coefficient of dissociation (K_d) was calculated by the equation:

$$K_d = \frac{N_{sh}}{N_{sh} + N_n}.$$

At least five tissue fragments were counted for each patient and K_d was determined for each fragment. Changes in size of the cells were recorded by means of an ocular micrometer. The area of a hepatocyte from patients of the control group was chosen as the unit. The results were compared by Student's test.

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TABLE 1. Arithmetic Mean Values ($\bar{X}$) of Coefficient of Dissociation (K_d) in Patients with Different Nosologic Forms of Chromic Diffuse Liver Disease

No. of group	Type of chronic diffuse liver disease	Number of patients	K_d ($\bar{X} \pm S$)	Range of values of K_d
1	PBC, CCH	11	0,61±0,03	0,41—0,84
2	CH	5	0,11±0,02	0,06—0,15
3	CAH	4	0,18±0,04	0,08—0,24
4	CPH	11	0,10±0,02	0,00—0,20
5	UH	8	0,11±0.02	0,00—0,16
6	FD	11	0,07±0,02	0,00—0,14
7	CL	3	0,18±0,03	0,14—0,24
8	Control	16	0,09±0.02	0.00—0,18

TABLE 2. Identification of Cholestasis by Adhesiometric and Histologic Methods

Preliminary clinical diagnosis	$K_d(\bar{X} \pm S)$	Result of histologic investigation	Final clinical diagnosis
CAH of viral etiology	0.41 ± 0.03	Picture of chronic aggressive hepatitis resembling PBC in its formal features	CH of virus etiology with signs of cholestasis
5 months after treatment, CH of viral etiology	0.11 ± 0.01	Mild aggressive hepatitis	CH of virus etiology
CAH? CCH?	0.52 ± 0.04	PBC	CCH with transformation into PBC
PBC?	0.71 ± 0.03	PBC	PBC
CCH?	0.09 ± 0.01	Moderate fibrosis	Polyp of the sigmoid colon, dyskinesia of the large intestine

EXPERIMENTAL RESULTS

The results showed a statistically significant difference in the adhesive properties of hepatocytes from patients with PBC and those from all other groups of patients studied, but the latter did not differ from each other; in all cases $p < 0.001$ (Table 1).

In patients with cholestasis, considerable separation of free hepatocytes (K_d = 0.41-0.84) was observed after dispersal of the liver tissue; this value of K_d is 3-8 times greater than that for the control group. In terms of area, the isolated hepatocytes were 1.5-2 times less than cells from patients of the control group.

For the group of patients with PBC the increase in the yield of hepatocyte after dispersion of the tissue was evidently connected with weakening of IJ on account of increased pressure in the biliary capillaries, arising during cholestasis. The same reasons evidently apply to the smaller size of the hepatocytes in the patients of this group.

The results are in agreement with those of electron-microscopic investigations, which showed that disturbances of IJ take place in the liver during cholestasis [1], in particular, in the zone of a tight junction [5]. The tight junction zone between hepatocytes is known to surround the lumen of a biliary capillary, and does not permit bile to penetrate into the intercellular space or into the region of the sinusoids [3], and it is the determinant structure in adhesive interactions between hepatocytes. After mechanical dispersion of liver tissue in which adhesive interaction between the cells has not been weakened (control group), mainly rupture of the plasma membranes of the hepatocytes is observed, and as a result of this, the number of whole cells separated by dispersion is reduced and the number of cell nuclei increased. Weakening of IJ in the tight junction zone in patients with cholestasis, however, leads to an increase in the number of whole cells released by dispersion and to a decrease in the number of released cell nuclei.

In groups 4, 5, and 7 there were three patients (one in each group) for whom K_d differed from the average value of K_d of the corresponding group, and was 0.44-0.56. Disturbances of adhesive properties of the hepatocytes which we observed in these patients were evidently associated with histologic changes found in them, which likewise may lead to weakening of intercellular junctions. However, these cases were solitary, they were not found in all the groups, and they did not affect the significance of the differences ($p < 0.001$) between K_d in the patients of groups 4, 5, and 7 compared with K_d of patients with PBC.

The results of investigation of the adhesive properties of the hepatocytes in patients shown in Table 2 are particularly interesting. Punch biopsy was performed on these patients to clarify the diagnosis. Values of K_d obtained by the adhesiometric method correlated completely with the results of histologic investigation of biopsy material and were confirmed by the final clinical diagnosis.

Thus a disturbance of the adhesive properties of the liver cells, evaluated quantitatively for the first time by the use of a coefficient of dissociation K_d, was found in patients with PBC and with CCH. In other forms of chronic diffuse liver disease no disturbances of the adhesive properties of the liver cells were found. By means of the adhesiometric method it is possible to determine sufficiently accurately and very quickly (in 15-20 min) the presence of a cholestasis syndrome in patients.

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