Possible Participation of Endotoxin of Gram-Negative Bacteria in Pathogenesis of Liver Damage during Viral Hepatitis

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Possible role of gram-negative bacteria in the genesis of hepatic damage during chronic viral hepatitis B and C was studied. Histological activity, hepatocyte proliferative activity, and expression of α-smooth muscle actin were compared with the severity of endotoxemia in patients with chronic hepatitis B and C. The study revealed a correlation between the degree of intralobular necroses, hepatocyte proliferation, and myofibroblast transdifferentiation of Ito cells with endotoxin concentration in the blood.

Key Words: *endotoxin; chronic viral hepatitis B and C; histological activity index; pathogenesis*

Various mechanisms of liver damage can be subdivided into two major types — biochemical and immune [6]. The latter plays the leading role in viral hepatitis B and C. Our study is focused on lipopolysaccharides (LPS) of gram-negative bacteria or endotoxin (ET), a triggering factor of immune cytopathogenicity during hepatitis abundantly produced in humans by symbiotic microflora, in particular, by the enteric flora.

Different biological effects of ET are mediated by its interaction with various acceptor cells: macrophages (predominantly Kupffer cells), neutrophils, platelets, eosinophils, and endotheliocytes [1]. This interaction stimulates production of the most important cytokines including tumor necrosis factor, interleukin-1, -6, -8, *etc.*, and γ-interferon, stimulates NO-synthase, and activates T cells, the complement system, apoptosis, and stimulates production of acute phase proteins [3,8,9]. Thus, ET can provoke changes characteristic of viral hepatitis.

The liver is the major organ of anti-endotoxin protection. Under physiological conditions, low concentrations of ET entering the liver via the portal blood flow is almost completely scavenged by Kupffer cells. ET can directly affect hepatocytes [4,7]. Since ET activates perisinusoidal Ito cells, the major producers of hepatic collagen [5], in can be hypothesized that ET can stimulate fibrosis.

We studied the correlation between morphologic characteristics of the liver and ET content in patients with chronic viral hepatitis B and C (CVHB and CVHC, respectively).

MATERIALS AND METHODS

We examined 13 patients with CVHB and 33 patients with CVHC. The diagnosis was verified using routine clinical, epidemiological, biochemical, serological, and instrumental tests. The polymerase chain reactions (PCR) for hepatitis B virus DNA and hepatitis C virus RNA were made in PCR Diagnostic Laboratory of Central Research Institute of Epidemiology (Russian Ministry of Health).

All patients were subjected to puncture biopsy with Mengini needles under ultrasonographic control. The specimens were embedded in paraffin. Liver histology was assessed by Knodell index (HI). Proliferating cell nucleus antigen (PCNA) and cells expressing α-smooth muscle actin (α-SMA) were detected immunohistochemically with monoclonal antibodies (Dako).

The number of PCNA-positive parenchymatous cells reflects proliferative activity of hepatocytes (only solitary PCNA-positive parenchymatous cells can be

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detected in healthy subjects). In healthy humans, cells with cytoplasmic α-SMA can be found only in vessel walls. Under pathological conditions, perisinusoidal Ito cells undergo myofibroblast transdifferentiation: they produce procollagens and express α-SMA.

ET was determined by limulus-test with E-Toxate kit (Sigma) [2]. The results were analyzed statistically using Student's *t* test and Pearson's correlation coefficient.

RESULTS

HI provides integral assessment of hepatic changes, including intralobular degeneration and focal necroses, periportal and bridging necroses, and inflammatory reaction. This index revealed no significant difference in morphological parameters between CVHB and CVHC patients (Table 1).

We made an attempt to differentiate the histological data of CVHB and CVHC patients according to the following diagnostic features: intralobular necrosis, periportal necrosis, and inflammatory alterations.

Individual comparison showed that CVHB patients had more pronounced necrotic damages in the central zone of the hepatic lobule (Table 1).

In addition, we assessed the state of intrahepatic bile ducts and expression of PCNA and α-SMA (Table 2).

Analysis showed that damage to the bile ducts were more often seen in CVHB patients compared to CVHC patients. However, ductopenia was observed only in CVHC patients, which results from chronic cholestasis (disappearance of bile ducts) [2].

Proliferative activity of hepatocytes was significantly higher in CVHC patients (Table 2). Immunohistochemical analysis of α-SMA revealed myofibroblast transformation of Ito cells in 100% CVHB patients; in 61.5% patients this process involved the periportal regions. In CVHC patients these changes were observed less frequently.

For evaluation of the role of ET in stimulation of hepatocyte proliferation and transdifferentiation of Ito cell into myofibroblasts, we measured ET concentration in the blood and expression PCNA and α-SMA in hepatic bioptates of CVHB and CVHC patients (Table 3).

Despite low number of measurements we revealed a correlation between ET concentration in the blood and PCNA expression in hepatocytes (Table 3). Appearance of extravascular myofibroblasts (α-SMA-positive cells) was accompanied by a significant increase in ET concentration in the peripheral blood. The highest ET concentration was observed in cases with diffuse spreading of α-SMA in the parenchyma, while the presence of α-SMA only in necrotic foci was associated with the lowest ET concentration (Table 3).

TABLE 1. Distribution of CVHB and CVHC Patients According to HI

HI, points		CVHB (*n*=13)		CVHC (*n*=33)	
		abs.	%	abs.	%
Total:	0-3	1	7.6	2	6.1
	4-8	6	46.2	14	42.4
	9-12	5	38.5	12	36.4
	13-17	1	7.6	5	15.1
Division					
intralobular necrosis					
	0-1	1	7.6	14	42.4*
	2-3	10	69.2	8	15.1*
	4	3	23.1	11	33.3*
periportal necrosis					
	0-2	4	30.8	10	30.3
	3-5	9	69.2	19	57.5
	6-9	0	0	4	12.2
inflammatory alterations					
	0	1	7.6	2	6.1
	1-2	6	46.2	12	36.4
	3-4	6	46.2	19	57.5

Note. Here and Table 2: *$p<0.05$ compared to the group with CVHB.

TABLE 2. Distribution of CVHB and CVHC Patients According to Some Morphological and Immunohistochemical Parameters of Hepatic Bioptates

Index	CVHB (*n*=13)		CVHC (*n*=33)	
	abs.	%	abs.	%
Bile duct alterations				
proliferation	4	30.8	2	6.1*
ductopenia	0	0	3	9.1*
Percentage of PCNA-positive hepatocytes 0-1	8	61.5	13	39.4*
1-10	4	30.8	7	21.2*
>10	1	7.6	13	39.4*
Localization of α-SMA				
vessels	0	0	7	21.2
focal necroses	1	7.6	4	12.2
sinusoidal cells	1	7.6	5	15.2
periportal cells	8	61.5	14	42.4
parenchyma (diffuse localization)	3	23.1	3	9.1

Generally, the severity of endotoxemia did not correlate with HI, but the development of necrotic processes in the hepatic lobule was accompanied by a significant increase in blood ET concentration to 0.019±0.006, 0.060±0.014, 0.094±0.016, and 0.333±0.106 EU/μl during necrosis assessed by 2, 3, and 4 points, correspondingly.

There were no cases of pronounced hepatic fibrosis among our patients, we observed only early stages of fibrosis. In patients with 1 (*n*=8) and 0 point (*n*=17) fibrosis, ET concentrations were 0.068±0.041 and 0.379±0.122 EU/μl, correspondingly.

TABLE 3. Serum Concentration of ET (EU/μl) in CVHB and CVHC Patients and Immunohistochemical Parameters of Hepatic Bioptates (*M*±*m*)

Index	ET level
Percentage of PCNA-positive hepatocytes 0-1	0.013±0.007 (6)
1-10	0.142±0.077 (4)
>10	0.119±0.019* (6)
Localization of α-SMA	
vessels	0 (3)
focal necroses	0.044±0.019^{+} (4)
sinusoidal cells	0.093±0.017^{+} (4)
periportal cells	0.101±0.04^{+o} (10)
parenchyma (diffuse localization)	0.173±0.035^{o} (4)

Note. The number of patients is given in parentheses. *p<0.01 compared to the group, in which the percentage of PCNA-positive hepatocytes is 0-1%; p<0.05: $^{+}$compared to the group with vascular localization of α-SMA; ^{o}compared to the group with α-SMA in necrosis foci.

Our study demonstrated a high informative value of morphologic methods in examination of activity and stages of hepatic pathology.

Additional methods of histological analysis, in particular, immunohistochemical tests, provide valuable information on the character and peculiarities of hepatites. Specifically, individual assessment of HI in various hepatic subdivisions helped to reveal the differences between CVHB and CVHC patients in localization of major necrotic foci.

In CVHC patients, immunohistochemical methods revealed activation of reparative regeneration, while in CVHB patients they detected activation of Ito cells fraught with fibrosis.

Our data indicate that ET participates in the pathogenesis of hepatic damage during viral hepatitis, and the degree of this damage determines the level of endotoxemia. It is attested by the direct correlation of ET concentration with the degree of hepatic necroses.

Our data on correlation of the degree of endotoxemia with expression of PCNA and α-SMA in the liver of CVHB and CVHC patients indicate on possible involvement of ET in activation of hepatocyte proliferation. Consequently, they also indicate on stimulation of the regenerator processes on the one side, and on triggering of myofibroblast transdifferentiation of Ito cell leading to the development of fibrosis, on the other.

Further studies with immunohistochemical detection of ET in hepatic tissues are needed to reveal the intimate mechanisms of the action of ET on the antagonistic processes of hepatic damage and reparation in CVHB and CVHC patients.

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