Ultrastructural and Stereological Study of the Liver in Chronic Mixed HCV+HBV Infection

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The stereological values of the structural density of hepatocyte cytoplasmic organelles were similar in chronic mixed HCV+HBV infection irrespective of the form of HBV infection (seropositive or latent). High incidence of HBsAg- and HBeAg-negative forms of HBV infection determines the leading role of PCR diagnosis and studies of liver biopsy specimens with detection of HBcAg structural markers and HBV DNA in native liver tissue in the diagnosis of mixed HCV+HBV infection.

Key Words: *chronic mixed HCV+HBV infection; liver biopsy; electron microscopy; stereology*

Study of mixed HCV+HBV infection is a serious problem because of a variable and more severe clinical picture in comparison with HCV monoinfection and the presence of seronegative variants of HBV infection [1,3-6,11]. One of important problems of hepatology is cirrhosis of the liver, and mixed HCV+HBV infection is regarded as one of the most significant factors triggering the development of fibrosis and cirrhosis of the liver [12,14].

Latent HBV infection received little attention, but it is significant for the pathogenesis of mixed HCV+HBV infection and other mixed infections and for proper testing of donor blood [9,15]. Numerous data indicate that hepatitis B virus DNA can persist in liver tissue, being absent in the serum, after acute hepatitis C and in chronic hepatitis C with negative HBsAg and HBV DNA tests [8,10,13]. Patients with chronic hepatitis C can have in 16% cases a latent HBV infection, characterized by the absence of HBsAg and HBV DNA in the serum [2]. This indicates serious difficulties in the clinical diagnosis of seronegative or latent HBV infection.

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We studied the ultrastructural and stereological parameters of liver specimens depending on the spectrum of serological markers in chronic mixed HCV+HBV infection, including that under conditions of latent HBV infection.

MATERIALS AND METHODS

A complex pathomorphological, electron microscopic, and stereological study of 112 patients (aged 16-69 years) with markers of chronic hepatitides C and B was carried out. Because of high incidence (45%) of HBsAg-negative cases of mixed HCV+HBV infection, studies of liver biopsy specimens and testing of HBV DNA in native liver tissue played the leading role in the diagnosis of HBV infection. The patients were distributed into 2 groups. Group 1 consisted of 49 patients with serological markers of HCV and HBV infection, group 2 consisted of 63 patients with serological markers of hepatitis C with HBsAg- and HBeAg-negative PCR tests for HBV infection.

Serum markers of viral hepatitides A (HAVAb), B (HBsAg, HBeAg, HBsAb, HBeAb, HBcIgM, HBcIgG), C (HCVAb, HCVcIgM, HCVcIgG, NSAb), and D (HDVAb) were tested by EIA. The HCV RNA and HBV DNA in blood and native liver samples were tested by PCR.

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Transcutaneous punction biopsy of the liver was carried out in all patients. A total of 136 biopsy specimens were studied. Analysis of structural changes in liver biopsy specimens included evaluation of the infectious process activity and fibrosis stage (by 4-point scale). Biopsy specimens were fixed in 4% paraformaldehyde solution. Paraffin sections (stained with hematoxylin and eosin with Pearls reaction, after van Gieson with poststaining of elastic fibrils with Weigert's resorcin-fuchsin, and PAS reaction), semithin sections (stained with Schiff's reagent and Azur II), and ultrathin sections (contrasted with uranyl acetate and lead citrate) were studied. Light microscopy was carried out using a Leica DM 4000B universal microscope with a Leica DFC 320 digital photocamera. Ultrastructural studies were carried out using a JEM 1010 electron microscope at accelerating voltage of 80 kV. Structural density of hepatic lobe cell populations and of hepatocyte cytoplasmic organelles was carried out using multipurpose short fragment test system [7].

RESULTS

High incidence (45%) of HBsAg-negative cases was detected in patients with chronic mixed HCV+HBV infection. Special attention should be paid to the latent form of HBV infection because of the absence of apparent differences in the majority of clinical characteristics of HBsAg-positive and HBsAg-negative (latent) forms of HBV infection. Morphological studies of liver biopsy specimens with detection of hepatocyte ring-shaped nuclei (structural marker of HBcAg) and PCR studies of native liver tissue were the leading methods for the diagnosis of HBV infection in these cases. All clinical cases were therefore divided into two groups: chronic mixed HCV+HBV infection and chronic HCV infection combined with latent HBV infection.

The spectrum of serological markers of chronic mixed HCV+HBV infection included in all cases summary HCVAb, in the majority of cases antibodies to HCV NS antigens and a set of HBV antibodies and antigens, with more rare HBsAg and more incident HBeAg. According to PCR diagnosis, HCV replication predominated (68% cases *vs.* 52% HBV). Importantly that HCV RNA and HBV DNA were significantly more rarely ($p<0.05$) detected in the presence of high biochemical parameters of the cholestatic syndrome, this was paralleled by the absence of simultaneous HCV and HBV viremia.

Pathomorphological studies of liver biopsy specimens revealed a sufficiently well-expressed polymorphism. Lipid infiltration was the leading form of hepatocyte degeneration. Diffuse intracellular cholestasis and acidophilic degeneration (Fig. 1, *a*) and the alterative component (necrobiosis foci, Councilman's bodies, and postnecrotic granulomas) were the most pronounced in the presence of high cholestasis markers. Cellular involutive degeneration of hepatocytes, particularly diffuse one, predominated in the presence of manifest extrahepatic symptoms. Ring-shaped transformation of hepatocyte nuclei (structural marker of HBcAg) was found in the majority of biopsy specimens in the presence of extrahepatic symptoms and in half of the cholestatic syndrome cases. Hence, hepatic cholestasis was responsible for more severe involvement of the liver in chronic mixed HCV+HBV infection, which manifested by pronounced symptoms of liver involvement and a trend to disease progress.

In contrast to chronic mixed infection, no serological markers of HBV were detected in chronic hepatitis C in combination with latent HBV infection, and the spectrum of HCV infection serological markers did not differ much. The HCV RNA was detected in 83% blood samples, HBV DNA in 15% native liver tissue specimens.

Pathomorphological studies of liver biopsy specimens showed lipid infiltration of the cytoplasm and acidophilic degeneration of hepatocytes equally often (Fig. 1, *b*); intracellular cholestasis was found in half of cases. Ring-shaped transformation of the nuclei in periportal hepatocytes was found in all biopsy specimens (Fig. 1, *c*). The alterative component (intralobular infiltration, foci of necrobiosis, Councilman's bodies, postnecrotic granulomas) was the most pronounced in high biochemical parameters of cholestasis. The hepatocyte cellular involutive degeneration was a rule diffuse and seen in all the studied biopsy specimens. Hence, similarly as in group 1, the cholestasis syndrome determined a more severe clinical course, significant structural changes in hepatocytes, and a more pronounced trend to fibrosis development.

Electron microscopy of liver biopsy specimens showed pronounced changes in the hepatocyte ultrastructure of different degree. These changes reflected their focal polymorphism together with the complex of alterative and compensatory reactions. Specific ring-shaped nuclei (annular heterochromatin construction without nucleolemma) was found in the periportal hepatocytes in all cases, including latent HBV infection. The biosynthetic potential of the cytoplasmic compartment was retained, this maintaining the reproduction of new viral particles. The hepatocellular regeneratory complex (parallel tubules of granular cytoplasmic reticulum and numerous small mitochondria) was located in the perinuclear zone.

Modified hepatocytes occupied mainly the periportal zones of the lobules; destruction of the cytoplasmic organelles corresponded to the infectious process activity, being the most severe in high cytolysis

and associated with the cholestasis syndrome. This was paralleled by vacuolation and lysis of membrane organelles (Fig. 1, *d*), formation of an appreciable amount of large polymorphic secondary phagosomes and residual bodies located not only near the biliary poles, but also diffused in the cytoplasm. The hepatocyte cellular involutive degeneration was the most pronounced in manifest extrahepatic symptoms of mixed HCV+HBV infection, which was interpreted as defense reaction of the cells.

Stereological analysis of liver biopsy specimens in the two groups revealed no appreciable differences in the surface-volume and volume-volume proportion of the sinusoids and hepatocytes. Ultrastructural comparative stereological analysis of hepatic parenchymatous cells showed negligible differences in the surface and volumic parameters of the main cytoplasmic organelles. The volumic density of smooth cytoplasmic reticulum elements varied slightly: the mean index of its structural components increased negligibly in chronic mixed HCV+HBV infection.

Changes in the hepatocyte stereological parameters were more significant under the effect of opiate toxic charge. A trend to a reduction of the volumic density of granular cytoplasmic reticulum structures in comparison with cases free from opiate dependence was seen (0.46±0.09 and 0.56±0.15 cm^3/cm^3, respectively), as well as a significant increase of the smooth cytoplasmic reticulum volumic density values (0.28±0.04 and 0.07±0.01 cm^3/cm^3, respectively, $p<0.01$).

A trend to an increase in the surface density of granular cytoplasmic reticulum was traced in both groups in cases with high activity of the infectious process in comparison with the parameter in patients with low activity. This resulted in rather high volume-volume proportion of the protein synthesizing com-

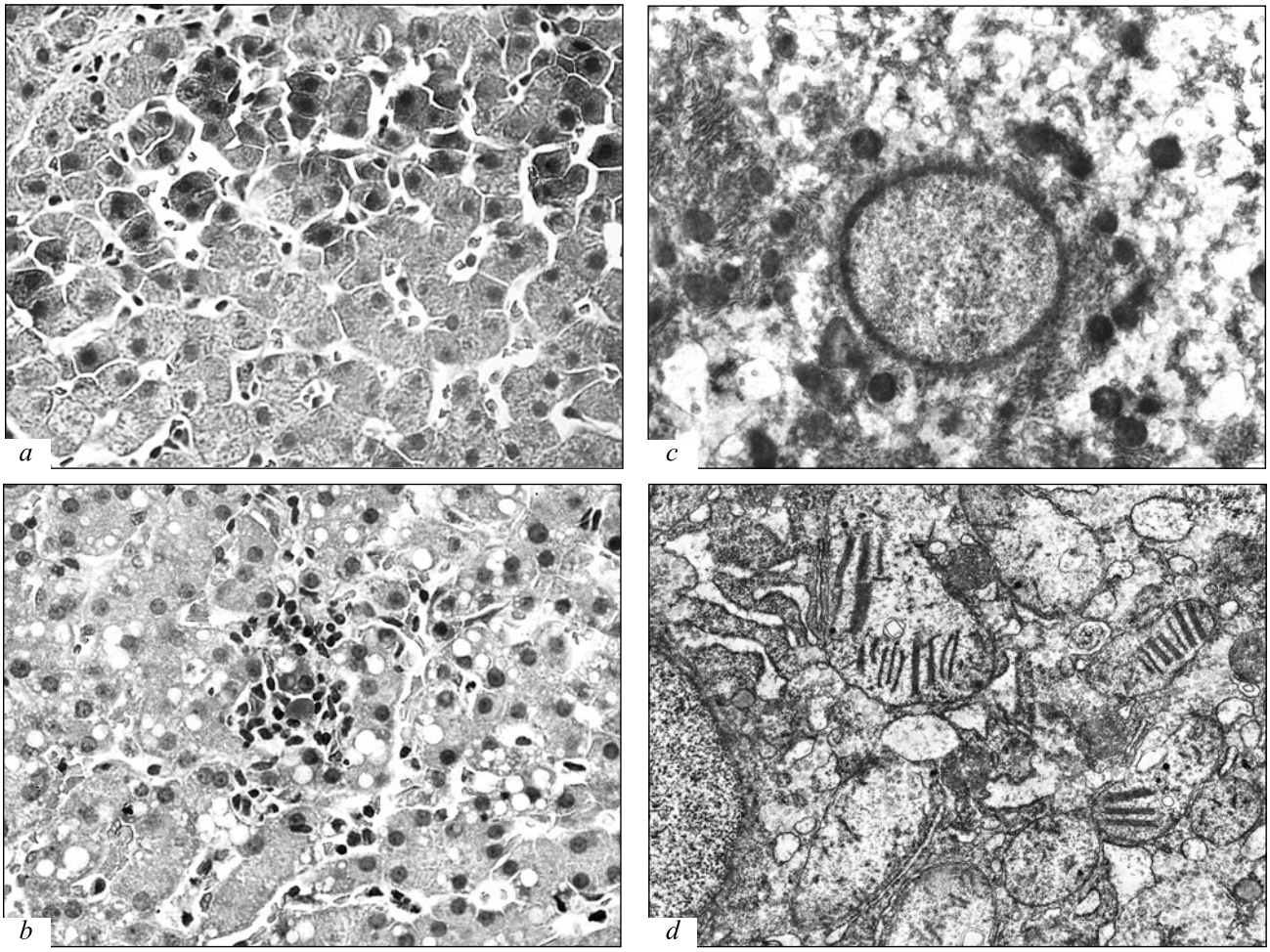

Fig. 1. Pathomorphology of liver biopsy specimens from patients with chronic mixed HCV+HBV infection (*a*, *d*) and chronic HCV infection combined with latent HBV infection (*b*, *c*). *a*) hepatocyte acidophilic degeneration, ×500; *b*) diffuse polymorphic lipid infiltration of hepatocytes, intralobular mononuclear infiltration in the center, ×400; *c*) ring-shaped transformation of hepatocyte nucleus, ×4000; *d*) hepatocyte cytoplasm: mitochondrion degeneration, dilatation of cytoplasmic reticulum tubules, ×15,000. *a*, *b*: hematoxylin and eosin staining; *c*, *d*: electronograms.

partment and mitochondrial organelles *vs.* hepatocyte cytoplasm. In addition, high activity of mixed hepatitis was associated with a somewhat higher structural density of lipid incorporations and a lesser volume density of cytoplasm devastation zones.

On the whole, the majority of the main cytoplasmic organelles in hepatocytes had similar structural density parameters irrespective of the infectious process activity and HBV infection form (seropositive or latent). The main discriminative feature of mixed infection and of mixed hepatitis in combination with opiate effect was an increase in the primary stereological parameters of the smooth cytoplasmic reticulum. The presence of drug addiction was responsible for a lesser volume of granular cytoplasmic reticulum. High activity of the infectious process was characterized by a less pronounced cytoplasm devastation compartment due to a higher structural density of protein synthesis organelles.

For many years recovery from HBV infection was assumed to be associated with the disappearance of HBsAg from the serum and normalization of aminotransferase levels [2]. Introduction of PCR, testing of HBV DNA in the blood and liver tissue revealed a new level of HBV and chronic hepatitis B persistence. Hepatitis B virus DNA can insert in the normal hepatocyte genome and persist throughout many years, causing latent forms of HBV infection, diagnosed only by detection of hepatocyte ring-shaped nuclei in liver biopsy specimens.

Hence, high incidence of HBsAg-negative (latent) HBV infection in the structure of chronic mixed HCV+HBV infection necessitates the use of a wider spectrum of serological markers, obligatory PCR testing, and pathomorphological analysis of liver biopsy specimens.

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