Intracellular Cholestasis in HCV and HBV Infection

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Structural analysis of hepatocytes in liver biopsy specimens from patients with hepatitis C and C+B with intracellular cholestasis was carried out. Large foci of bile components in hepatocytes led to cell damage, eventuating in cell destruction and death. The cholestatic variant of mixed infection was characterized by destructive necrotic changes in hepatocytes and progressive fibrosis of the liver. Destruction of the hepatocyte cytoplasmic organelles was associated with high activity of the infectious process and pronounced cytolytic syndrome.

Key Words: *hepatites C and B; hepatocyte cholestasis; pathomorphology*

Cholestasis is a condition when bile discharge is poor or completely arrested; depending on its severity, the substances secreted with the bile "return" into the liver or blood [1-3]. The transporting process as the main prerequisite of bile secretion includes the following stages: release of osmotically active substances from the portal venous blood into liver cells, intracellular transport of osmotically active substances to bile tubules, and release of substances from hepatic cells into the tubular lumen. Nonobstructive cholestasis can be caused by exogenous substances, severe inflammatory diseases, hormone therapy, liver tissue injuries of various origin, and other factors. Three cholestasis types are distinguished [10,11]. The first one is characterized by the presence of intracellular pigment granules in hepatocytes, the second by the presence of intracellular granules and extracellular clots, and the third by the presence of intracellular granules, extracellular clots, and bile pigments in Kupffer's cells. The pathological process in intrahepatic cholestasis is located in the liver at a site from hepatocyte microsomes to large bile ducts. Leakage of biliary acid salts through tubular defects, disorders of cholesterol hydroxylation in the course of bile acid synthesis and in the cytoplasmic reticulum lead to reduction of secretion of the biliary acid-dependent fraction of the bile [9].

We studied the structural characteristics of intracellular cholestasis in hepatites C and C+B.

MATERIALS AND METHODS

Comprehensive pathomorphological study of 85 liver biopsy specimens from 62 patients was carried out. Of these, 40 suffered from chronic hepatitis C and 22 from mixed C+B infection at various stages of fibrosis. Slight activity of the process and slight fibrosis stage predominated (33 of 40 cases) in chronic hepatitis C. Stages II-IV were observed in almost half (10 of 22) of patients with chronic mixed infection. Liver fibrosis stages were evaluated by a 4-point system, for which main characteristic was the etiological factor [10,12]. Liver biopsy specimens were fixed in cold paraformaldehyde prepared in Millonig's phosphate buffer (pH 7.2-7.4). Paraffin sections were stained with hematoxylin and eosin with Pearls reaction, after van Gieson with poststaining of elastic fibrils with Weigert resorcin-fuchsine, and PAS reaction was carried out. Semithin sections were stained with Schiff's reagent and azur II, ultrathin sections were contrasted with uranyl acetate and lead citrate. The preparations were examined under a Leica DM 4000b universal microscope; ultrastructural studies were carried out in a JEM 1010 electron microscope.

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RESULTS

The morphogenesis of chronic hepatitis C was analyzed by its three mutually related constituents: hepatocyte involvement, reaction of nonparenchymatous cell populations of the liver, and connective tissue remodeling including fibrosis of the liver [8]. Degeneration was the leading and universal reaction of hepatic parenchymatous cells to destructive exposure. Accumulation of bile components in the hepatocyte cytoplasm could be referred to its "aggressive" forms. This accumulation could be rather significant, leading to disorders of bile secretion, excretion, and passage, which were paralleled by cell death (Fig. 1, *a*, *b*) with subsequent development of liver fibrosis and cirrhosis. Hepatocyte necroses in biopsy specimens from patients with HCV infection were extremely rare; postnecrotic granulomas were sometimes found.

The second constituent of hepatitis C morphogenesis was cell infiltration (reaction of immunocompetent cells to viral infection), associated with migration and proliferation of T-lymphocytes, forming aggregates in the portal tracts and subsequently involving the parenchyma. An important aspect of cell infiltration of the liver was its diffusion from the portal tracts to the parenchyma, where it formed lymphoid aggregates and lymphoid follicles, which was pathognomonic for hepatitis C and was interpreted as response to genetic heterogeneity and changeability of HCV.

The third component of morphogenesis was fibrosis of the liver – a result of a complex of destructive proliferative reactions over the course of chronic viral infectious. Evolution of fibrosis in viral hepatitis could be presented as primary fibrosis of the portal tracts with subsequent dissemination towards the central vein and adjacent portal tracts with the formation of porto-portal and porto-central septae [14]. Another variant of fibrosis was metabolic, starting from the central vein region and disseminating towards the portal tracts with the formation of porto-central septae. The third variant was perihepatocellular, prognostically the most significant, determining the functional and metabolic status of the organ.

Fibrosis of the liver was found in all examined liver biopsy specimens. Slight portal fibrosis predominated, central was less incident. Fibrosis stage did not depend on the probable duration of HCV infection. Cases with slight signs of liver fibrosis predominated, even if the duration of infection was 10-20 years and longer.

Studies of the cell biosynthetic reactions detected a new form of degeneration: cell involution, consisting not in destruction of cytoplasmic organelles, but in their involution because of deficient plastic resources of the cell [7]. This type of degeneration predominated in HCV infection [4,5]. One of the main markers of hepatitis C was lipid infiltration of hepatocytes [13]. Hepatitis C replication phase (PCR) corresponded to small vesicular subplasmalemmal lipid infiltration, cell involution degeneration of hepatocytes, and formation of lymphoid aggregates and follicles in the portal tracts [6].

A characteristic feature of chronic HCV infection was pronounced increase of cholestasis markers during the stage of unfolding cirrhosis of the liver. As a rule, HCV cirrhosis of the liver was associated with induction of proliferative reactions. Proliferation of the ductal epithelium with the formation of numerous bile ducts in fibrosis fields (Fig. 1, *c*) was found in biopsy specimens of HCV cirrhosis. The model showed that fetal hepatic stem cells or precursor cells, transported to the portal zone, differentiated into bile duct cells. If these cells were transferred into the parenchyma, they differentiated into hepatocytes [15].

Importantly that the reserve compartment of hepatic stem cells is stimulated to proliferation and differentiation into hepatocytes and bile duct cells and restores the liver volume only under very specific conditions, when the proliferation of mature hepatocytes is limited or blocked.

In chronic mixed HCV+HBV infection in comparison with HCV monoinfection the minimum activity was rarely seen; moderate activity was more incident, characterizing the course of chronic mixed C+B hepatitis as more severe. The distribution of patients with C+B hepatitis by disease stages was similar to that in HCV monoinfection. Cases with slight portal fibrosis predominated, while the terminal stage – cirrhosis of the liver – was more incident.

Acidophilic degeneration of hepatocytes, detected in 90% specimens, played the key role in the morphogenesis of hepatocyte population lesions in chronic mixed infection (RNA genome hepatitis C virus and DNA genome hepatitis B virus). The population of parenchymatous cells was characterized by pronounced polymorphism because of hepatocyte degeneration of different origin (cell involution, acidophilic, lipid) and was heterogeneous by the content of intracellular glycogen. Lipid incorporations (mainly small droplet) were detected in all examined biopsy specimens, but the counts of lipid-containing cells were significantly less than in HCV monoinfection. Cell involution degeneration, characterized by reduction of the cytoplasmic organelles and devastation of the cytoplasm matrix, developed rather often, but was detected in a lesser number of specimens than in HCV monoinfection and with a lesser volume of hepatocellular compartment involved in this process.

On the whole, liver biopsy specimens from patients with mixed HCV+HBV infection were characterized by deeper and more extensive degenerative changes in hepatocytes. The predominant pathogenetic

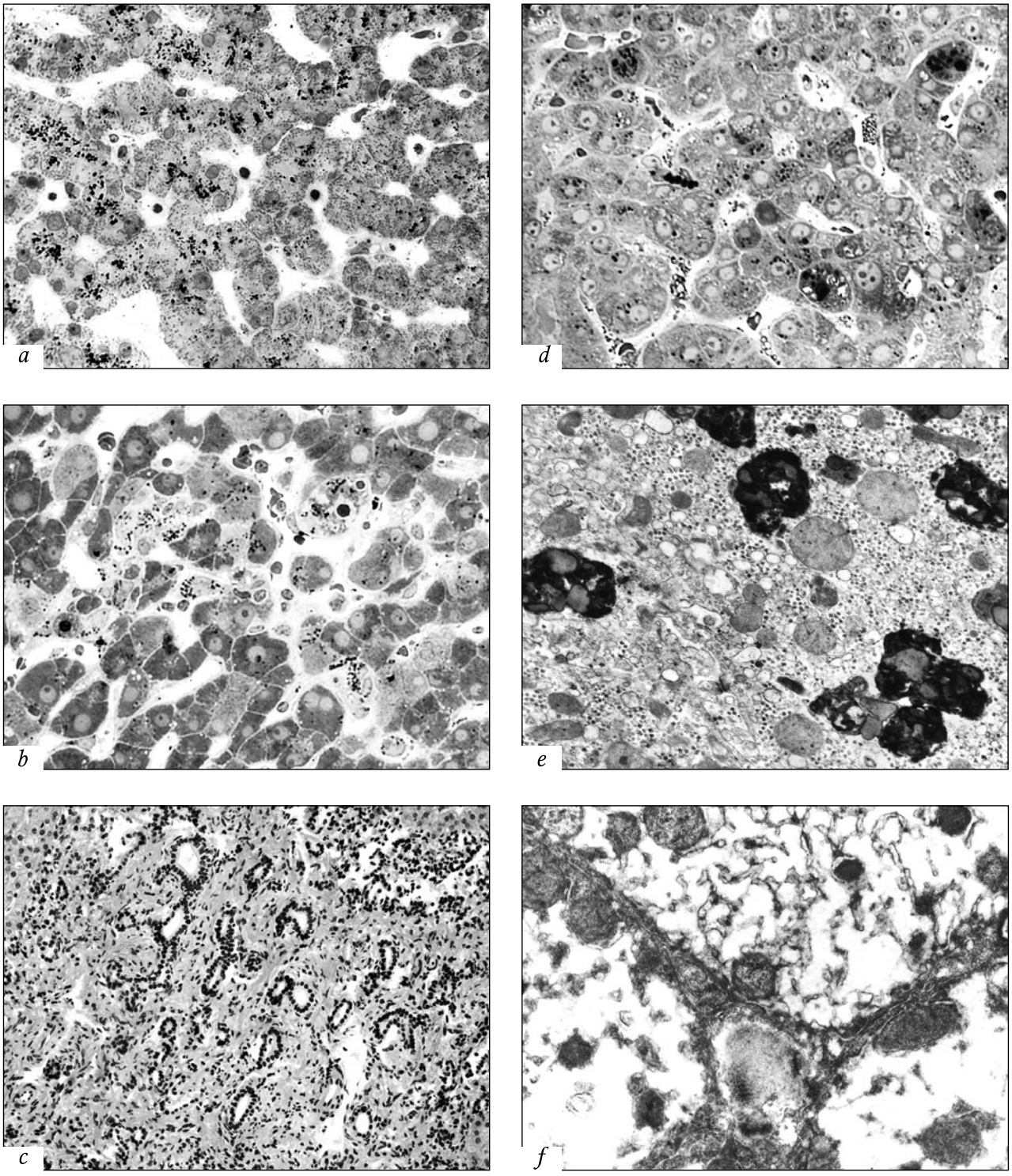

Fig. 1. Pathomorphology of liver biopsy specimens in chronic HCV infection (*a*-*c*) and chronic mixed HCV+HBV infection (*d*-*f*). Semithin sections, stained with azur II (*a*, *b*, *d*), hematoxylin and eosin (*c*); electronograms (*e*, *f*). a) intracellular cholestasis, ×600; *b*) marked intracellular cholestasis, hepatocyte destruction, ×600; *c*) HCV cirrhosis of the liver, ductal epithelium proliferation, formation of numerous bile ducts, ×160; *d*) intracellular cholestasis: large accumulations of bile components in hepatocytes, ×800; *e*) hepatocyte fragment: large autophagosomes containing bile components, ×10,000; *f*) fragments of three hepatocytes; a bile capillary with bile components between them, ×8000.

role of hepatitis C or B could be indirectly identified by the predominant type of degenerative changes in the specimen.

A lesser incidence of lymphoid follicle formation in the stroma in comparison with HCV monoinfection presumably reflected the predominant role of hepati-

tis B virus in mixed infections. Much more incident alterative changes in the liver parenchyma – monocellular and larger foci (from 2-4 to 6-10 hepatocytes) of hepatocyte necrobiosis and necrosis with the formation of postnecrotic granulomas – were found.

The cholestatic variant of mixed HCV+HBV infection was characterized by pronounced intrahepatic cholestasis combined with hepatomegalia and jaundice, hyperbilirubinemia, splenomegalia, and pronounced cytolytic syndrome. The pathognomonic sign of the cholestatic variant was intrahepatocellular cholestasis, leading to hepatocyte death. Signs of slight or moderate intracellular cholestasis were detected in all biopsy specimens; bile pigment granules sometimes tended to diffuse in the parenchymatous cell cytoplasm. Incorporations of this kind were often found in Kupffer's cells (Fig. 1, *d*, *e*).

Cell infiltration of the portal stroma was as a rule mononuclear with dissemination into the parenchyma; active lymphodiapedesis was seen. Fibrous changes in the liver were rather significant in the cholestatic variant and were associated with the ductal epithelium alteration and proliferation.

The cholestatic variant of mixed infection was characterized by the most pronounced alterative component (necrobiosis and necrosis foci, Councilman's apoptotic bodies, postnecrotic granulomas) and fibrous changes in the liver. This suggested regarding this variant of mixed infection as the most unfavorable for the development of cirrhosis of the liver. Hence, intracellular cholestasis was responsible for more severe involvement of the liver in chronic HCV+HBV infection. Clinically it manifested in pronounced symptoms of liver involvement and a trend to disease progress.

Electron microscopy of liver biopsy specimens from patients with hepatites C and C+B showed significant heterogeneity of hepatocytes, manifesting by varying electron density of the cytoplasm matrix, reduction of membrane organelles, and polymorphic lipid droplets and residual bodies. The quantities, location, and size of bile components accumulations varied greatly. Numerous components of the bile and secondary phagosomes with accumulation of lipofuscin osmiophilic granules and residual bodies were found near the biliary poles and diffused in the cytoplasm; in solitary cases, they were found in the sinusoidal and biliary capillary lumens (Fig. 1, *f*). Elements of granular cytoplasmic reticulum and mitochondria with reduced numbers of cristae and clarified matrix were found in some hepatocytes in the perinuclear zone of devastated cytoplasm. Destruction of the hepatocyte cytoplasmic organelles corresponded to the activity of the infectious process, being the most manifest in high cytolysis and associated with the cholestasis syndrome.

The intracellular pigment granules in cholestasis may accumulate before elevation of serum bilirubin concentration [12]; hence, serum bilirubin "lags behind" the initial accumulation of biliary pigment in liver tissue during the early period of cholestasis. This observation can be highly significant in the pathogenesis of cholestasis. Normally the mechanism of irreversible excretion of bilirubin works adequately. When this mechanism is disordered, total cleansing capacity of the liver is low and bilirubin starts to precipitate in the liver cell cytoplasm. This precipitated pigment is presumably an inert form of bilirubin, not involved in rapid exchange between liver cells, plasma basin, and excretion routes. This accumulation of bilirubin and other bile components eventually leads to hepatocyte damage and death. When loss of hepatocyte population as a result of their death reaches the level below which the regeneration of mature parenchymatous cells is impossible, the stromal reaction, activation of matrix-producing cells starts to predominate – fibrosis and subsequent cirrhosis of the liver are unfolding, that is, the strategy of disease progress is changing – alteration is replaced by proliferation.

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