MORPHOLOGY AND PATHOMORPHOLOGY

Histopathology and Ultrastructure of the Liver under Combined Action of Narcotics and Hepatitis C and B Viruses

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Liver autopsy material from opioid addicts with viral hepatitis C and C+B showed hepatocyte dystrophy associated with lipid infiltration, hyperplasia of lymphoid tissue, and perivascular fibrosis. Alteration of hepatocytes were most pronounced in patients with hepatitis C+B. Ultrastructure of hepatocytes is characterized by a reduction of protein synthesizing compartment, an increase in volume density of smooth cytoplasmic reticulum, appearance of megamitochondria, and pronounced collagenization of the Disse space.

Key Words: *viral hepatitis; opium addiction; liver autopsy; hepatocytes; light and electron microscopy*

Infection with hepatitis C and B can now be transferred via medical instruments, thus adding to natural ways of infection. Spreading of the infections in intravenous drug addicts plays a special role, since it significantly promotes virus passage and aggravates infectious process by inducing structural rearrangements in the liver. Numerous experimental and clinical studies analyze the effects of narcotics alone [1,9,11,12] or in combination with hepatitis [13-15] on animal and human liver. However, there are only few reports on combined action of opium and hepatitis viruses [10].

Previously, we reported structural reactions of the liver induced by chronic hepatitis C and C+B [4,5]. Here we present the results of examination of liver autopsy material from intravenous opium addicts suffering from chronic hepatitis C and C+B.

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MATERIALS AND METHODS

The diagnosis was based on clinical, biochemical, and immunoserologic data. The following antigens and antibodies were revealed: HBsAg, HBeAg, anti-HBcIgM, anti-HBs, anti-HBe, total anti-HCV, antibodies to core- and NS-antigens of hepatitis C virus. All serum samples were analyzed by polymerase chain reaction for the presence of HVC RNA. Hepatitis C and C+B were verified in 16 and 13 patients, respectively.

Liver samples (n=36) were obtained from 29 patients by transcutaneous puncture biopsy. Paraffin, semithin, and ultrathin sections were examined. For light microscopy the samples were fixed in 10% neutral formaldehyde and stained routinely [7].

For electron microscopy the samples were fixed in 4% paraformaldehyde and treated as described previously [3]. Semithin sections were stained with azure II and Schiff reagent. Ultrathin sections were con-

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trasted with uranyl acetate and lead citrate and examined under a JEM-1010 electron microscope. Structural density of cytoplasmic organells in hepatocytes was analyzed with a universal test system [6].

To evaluate cell immunity, subpopulations of peripheral blood lymphocytes were analyzed by indirect immunofluorescent reaction with monoclonal antibodies. No significant changes in cell immunity were revealed, though the level of T-supressors tended to increase during replicative phase of hepatitis C.

RESULTS

Light microscopy of hepatic tissue showed that the most frequent degenerative alteration of parenchymal cells revealed in all samples was microvesicular lipid infiltration of hepatocytes (Fig. 1, *a*). Monoinfection with HCV caused diffuse lipid degeneration, while in combined HCV and HBV infection degeneration involved less then half of parenchymal cells.

Acidophilic degeneration of hepatocytes localized preferentially in the periportal and pericentral zones played an important role in the morphogenesis of the lesion induced by hepatitis C+B. Faint signs of involutional hepatocyte degeneration were frequently observed. These cells had partially or completely empty cytoplasm and low glycogen content. In 10 samples (mainly hepatitis C+B), necrobiosis of hepatocytes associated with periportal cell infiltration was observed. Postnecrotic granulomas and small lymphocyte-macrophage infiltration were observed in the parenchyma.

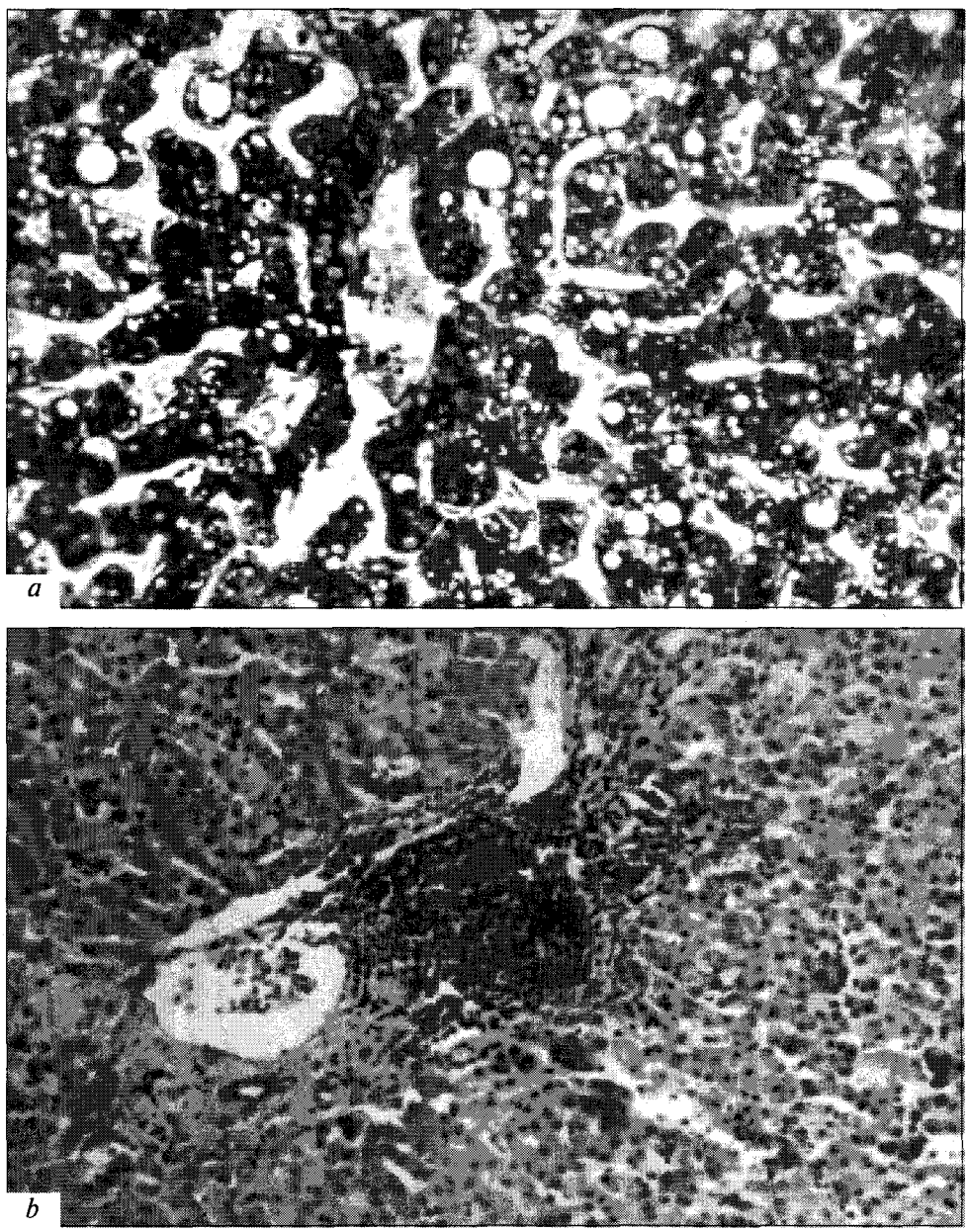

Fig. 1. Light microscopy of liver sample in hepatitis C combined with opium addiction. *a*) diffuse microvesicular lipid infiltration of hepatocytes, enlargement of sinusoids. Semithin section, staining with Schiff reagent and azure II, ×200; *b*) lymphoid follicle in portal tract. Staining with hematoxylin and eosin, ×60.

The most pronounced alterations in hepatocyte nuclei (hyperchomism, pyknosis, and, sometimes, rhexis) were found in cells with acidophilic degeneration. Hepatitis C+B induced the formation of hepatocytes with cricoid nuclei (marker of HBcAg); their number varied from single to numerous in different samples. Sometimes, the number of binucleate hepatocytes significantly increased.

In hepatitis C, mononuclear infiltration of portal tracts was not intense, though a tendency to the formation of "penetrating" infiltrates in the parenchyma was observed. Monoinfection with HCV induced the formation of numerous lymphoid follicles and aggregates in the portal stroma (Fig. 1, *b*).

Pathomorphological examinations revealed more intense pathological process in hepatic tissue of addicts with hepatitis C+B. Cell infiltration of the portal stroma was more pronounced, it penetrated the parenchyma and caused progressive necrosis of periportal hepatocytes. Lymphoid follicles in the stroma were seldom.

In 50% samples, eosinophils were found in cell infiltrates or in the sinusoid lumen. The presence of eosinophils in infiltrates is typical of drug-induced liver damage [8].

In most liver samples, weak or moderate fibrosis of portal tracts (mostly in hepatitis C+B) was observed. Long-term drug addiction and chronic hepatitis induced pronounced fibrosis associated with the forma-

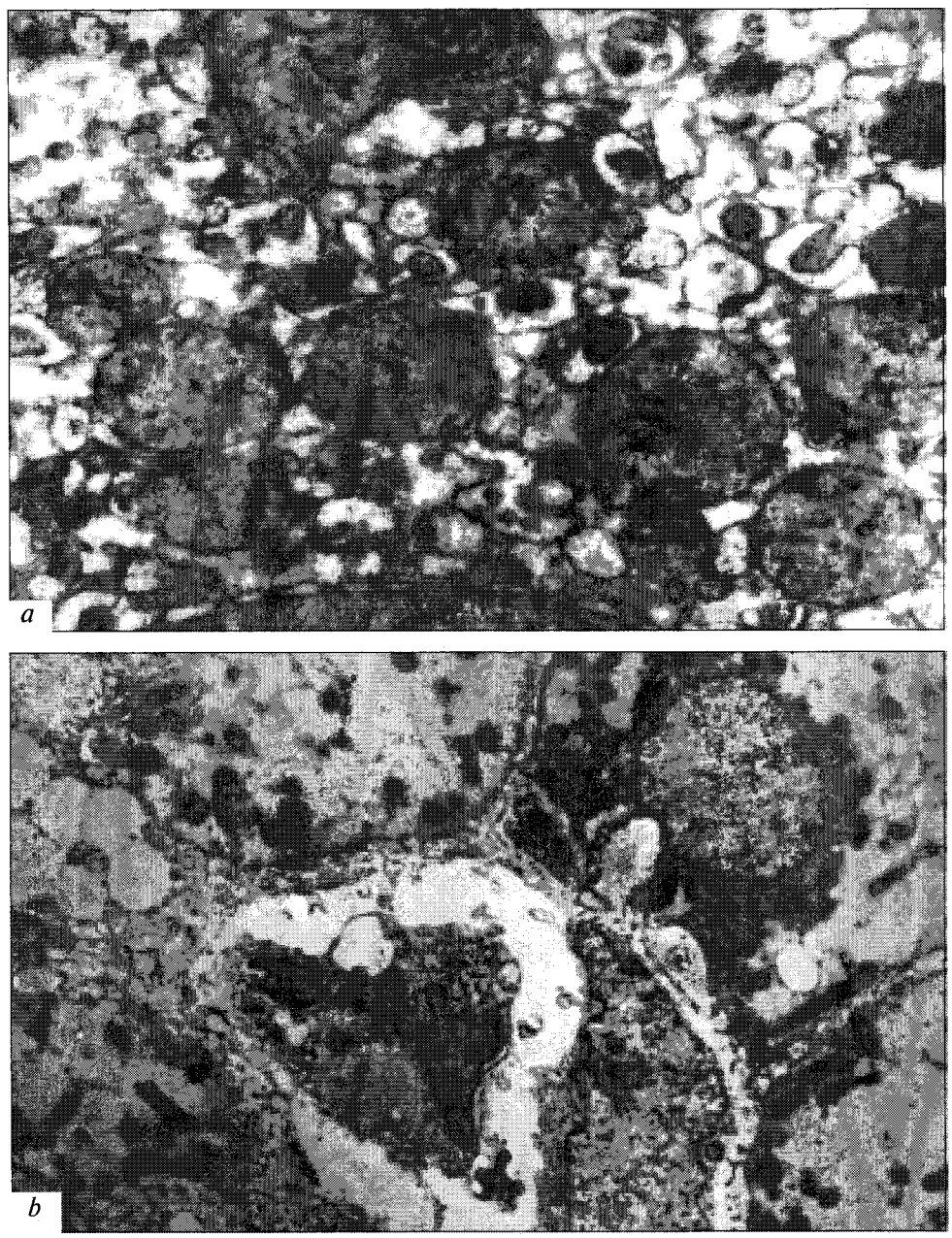

Fig. 2. Ultrastructure of hepatocytes in chronic viral hepatitis combined with opium addiction. *a*) hepatocyte fragment: pronounced hyperplasia of smooth endoplasmic reticulum, ×6000; *b*) sinusoid, perisinusoidal fibrosis, and Ito cell with large lipid inclusions, ×2000.

tion of porto-portal and porto-central septa, which culminated in cirrhosis of the liver. Even weak fibrosis of the portal stroma was accompanied by sclerosis of the central veins, formation of collagen fibres in the perivenular zone along sinusoids, and perihepatocellular sclerosis.

Electron microscopy of liver samples from opium addicts with chronic hepatitis C+B revealed typical ultrastructural alterations in parenchymal cells, which correlated with activity of pathological process and were most pronounced under conditions of coinfection. Moderate polymorphism of hepatocytes was noted. Some cells retained normal ultrastructure, however cells with the signs of degeneration prevailed. The following alterations were observed: microvesicular lipid infiltration, significant destruction of most mitochondria, partial or extensive destruction of hepatocyte cytoplasm with areas of complete electron transparency.

The foci of intracellular hepatocyte regeneration were presented by parallel profiles of granular cytoplasmic reticulum and mitochondria with dense matrix. These foci had no preferential perinuclear localization and were less abundant than in hepatocytes from patients with hepatitis alone. This was confirmed by stereological analysis of the ultrastructure of hepatic parenchymal cells: volume density of granular cytoplasmic reticulum in addicts tended to decrease in comparison with patients without opium dependency.

Chronic hepatitis C and C+B combined with opium addiction frequently induced hyperplasia of smooth endoplasmic reticulum (Fig. 2, *a*) presented mainly by diffuse polymorphic vesicles and narrow channels seldom associated with biliary poles of hepatocytes. This resulted in a significant increase in the volume density of smooth endoplasmic reticulum (0.26± 0.03 and 0.07±0.02 cm^3/cm^3 in addicts and nonaddicts, respectively), a structural marker of intense metabolism of xenobiotics.

The volume density of mitochondria tended to increase in opium addiction, probably due to appearance of megamitochondria. Giant mitochondria were also observed in alcohol-induced damage to the liver [8] and can be regarded as an adaptive response to opiate toxicity.

Vascular hepatocyte pole was characterized by destruction and reduction of microcilia and enhanced exocytosis. Cytoplasm of sinusoidal endotheliocytes showed marked ultrastructural heterogeneity with predominance of alterations of cytoplasmic organells. Disse (Fig. 2, *b*) and intercellular space contained numerous collagen fibres and residual bodies. Collagenization of the Disse space is regarded as a mechanism protecting hepatocytes against toxic action of narcotics [12].

Thus, combined action of opiates and hepatitis C and B viruses induced structural changes in the hepatic tissue, which by their light microscopic characteristics did not significantly differ from those described previously for hepatitis C and C+B [4,5]. However, some tissue and structural changes were probably associated with metabolism of narcotics. Thus, even minor sclerosis of the portal and periportal tissue was accompanied by specific hepatic fibrosis with predominant sclerosis of the central veins and centrolobular zones. Ultrastructural reorganization of hepatic tissue is manifested as hyperplasia of smooth endoplasmic reticulum, formation of giant mitochondria, and collagenization of the Disse space, which can be regarded as compensatory adaptive reactions [2]. Despite extensive degenerative alterations of hepatocytes, necrotic changes were minor, which probably suggests preserved number and histogenetic potential of parenchymal cells.

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