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Ultrastructural Reactions of Liver Cell Populations Induced by RNA-Containing Hepatitis C and DNA-Containing Hepatitis B Viruses

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Combined hepatitis C and B virus infection is associated with phenotypic heterogeneity of hepatocyte populations. Patchy distribution of hepatocyte ultrastructural changes is due to the diversity of complex cytopathic effects of these viruses. RNA-containing hepatitis C virus impairs mainly the cytoplasmic compartment of liver parenchymal cells and does not affect the nucleus. DNA-containing hepatitis B virus induces degradation of the nuclear compartment.

Key Words: *viral hepatitis C+B; hepatocytes; endotheliocytes; alteration; electron microscopy*

The problem of HCV+HBV coinfection is important for studying viral hepatitides. Viral replicative activity determines the intensity of structural reactions in hepatocytes, which is much higher in HBV than in HCV. The most considerable pathological changes accompany combined replication of both viruses [9].

An extremely early appearance of virus-specific antigen in the hepatocyte cytoplasm is typical of hepatitis C [15]. Of particular interest is the localization of HCV structural proteins in hepatocytes. Immunohistochemical assay of the liver of transgenic mice expressing various levels of structural proteins demonstrated that core- and E2-proteins are primarily localized in the cytoplasm of hepatocytes [12].

Liver biopsy in hepatitis C [4] showed high resistance of the nuclear compartment to HCV; its structure is preserved even after considerable degeneration and loosening of the cytoplasm [3,5]. By contrast, the degradation of hepatocyte nuclei with the formation of ring-like structures is typical of DNA-containing HBV [1].

Here we performed a structural analysis of the nuclear and cytoplasmic compartments of hepatocytes and endotheliocytes in liver samples from patients with HCV+HBV coinfection verified by clinical, biochemical, immunoserological, and pathomorphological markers and confirmed by PCR, and compared these results with the data on HCV infection reported previously [4].

MATERIALS AND METHODS

Forty liver samples taken from 28 patients by transcutaneous biopsy were analyzed by light (paraffin and semithin sections) and electron microscopy. For light microscopy, the samples were fixed in 10% neutral formalin. Paraffin sections were stained by the van

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Gieson's method (with hematoxylin and eosin) combined with the Perls reaction. Elastic fibers were stained with Weigert's resorcin-fuchsin. PAS reaction was then performed.

Samples for electron microscopy were fixed in 4% paraformaldehyde and treated by the method described previously [2]. Semithin sections were stained with azure II and Schiff reagent. Ultrathin sections were contrasted with uranyl acetate and lead citrate and examined under a JEM-1010 electron microscope.

RESULTS

Acidophilic degeneration of hepatocytes observed in all examined samples plays an important role in the cytogenesis of hepatocyte damage caused by coinfection of RNA-positive HCV and DNA-positive HBV. Lipid inclusions (primarily, small lipid droplets) were also found in all samples, but the content of lipid-containing cells was much lower than during HCV monoinfection. Hepatocyte degeneration characterized by reduction of cytoplasmic organelles and loosening of the cytoplasmic matrix was observed. However, its incidence and the volume of hepatocellular compartments involved in this process were lower than in HCV infection. The intensity and distribution of hepatocyte degeneration in HCV+HBV coinfection were lower than in HCV monoinfection.

Signs of moderate or mild intracellular cholestasis were found in more than half of liver samples. Bile pigment granules were diffusely distributed in the cytoplasm of parenchymal cells. Such inclusions were often seen in Kupffer cells.

Alteration of the liver parenchyma was also presented by monocellular and larger (involving 2-4 to 6-10 hepatocytes) necrobiotic foci with concurrent cell infiltration.

Specific changes of periportal hepatocyte nuclei in the form of central lightenings of the nucleoplasm (so-called ring-like nuclei, Fig. 1, *a*) were found in the majority of samples. These changes are morphological markers of HBV replication. The number of binuclear cells indirectly reflecting the intensity of regenerative processes in the liver varied in various samples and only in few samples increased considerably.

Morphological signs of increased activity of sinusoidal cells were less common during HCV+HBV coinfection than during HCV monoinfection. Moderate hyperplasia of Ito cells was revealed in $^1/_3$ of samples. Lymphoid follicles and lymphocyte chains in the stroma and sinusoid lumens (respectively) were less typical of samples taken from patients with the mixed infection.

Combined HCV and HBV infection induced more pronounced changes of the hepatocyte ultrastructure with HBV-specific degeneration of the nucleus. Some hepatocyte nuclei had deep invaginations of the nucleolemma responsible for the appearance of cytoplasmic regions with cytoplasmic organelle fragments and lipid incorporations in these nuclei (Fig. 1, *b*).

The dynamics of hepatocyte nucleus damage induced by DNA-positive HBV was studied by electron microscopy. Various stages of formation of ring-like structures were observed in euchromatic nuclei of periportal parenchymal liver cells: marginal associations of heterochromatin lumps, progressive lysis of the nucleolemma accompanied by heterochromatin condensation and dislocation, and formation of a torus-like construction of condensed chromatin with moderate electron density and irregular shape (Fig. 1, *c*).

The hepatocyte cytoplasmic compartment was insensitive to such nucleus transformations. This may be due to the fact that DNA-containing animal viruses practically never induce cell lysis [7]; this lysis can result from immunological reaction or chromosomal aberrations.

During ring-like transformations of the nuclei not accompanied by disturbances in their organization in coinfection, the perinuclear region of hepatocyte/cytoplasm retains its intracellular repair potential in the form of parallel profiles of the rough cytoplasmic reticulum and small mitochondria. Sometimes, contacts between mitochondria and lipid inclusions were observed. Considerable heterogeneity of peripheral cytoplasmic regions was due to variations in glycogen content, alteration and reduction of membrane organelles contributing to loosening of the cytoplasm, and polymorphism of lipid drops and residual bodies. The severity of destruction of cell organelles corresponded to the severity of chronic infectious processes.

Hepatocyte mitochondria decreased considerably in number (as during HCV monoinfection), contained lightening matrix, small number of cristae, and one or several lumps close by their structure and electron density to nuclear heterochromatin. Vesicles and channels of the smooth endoplasmic reticulum found during active C+B hepatitis were arranged close to biliary and vascular hepatocyte poles and characterized by heterogeneous content and size.

Tubular (closed channels with osmiophilic content) and reticulotubular (clusters of regular small electron-transparent vesicles) structures considered as pathognomonic markers of HCV infection were found in some hepatocytes [14]. These intracellular structures were shown to be induced by interferon [13].

The Disse spaces were filled with numerous collagen fibers. During HCV+HBV coinfection, the ultrastructure of sinusoidal endothelial cells greatly varied. Endotheliocyte nuclei had irregular shapes and contained moderate amounts of marginally localized heterochromatin. In the cytoplasm, the elements of the

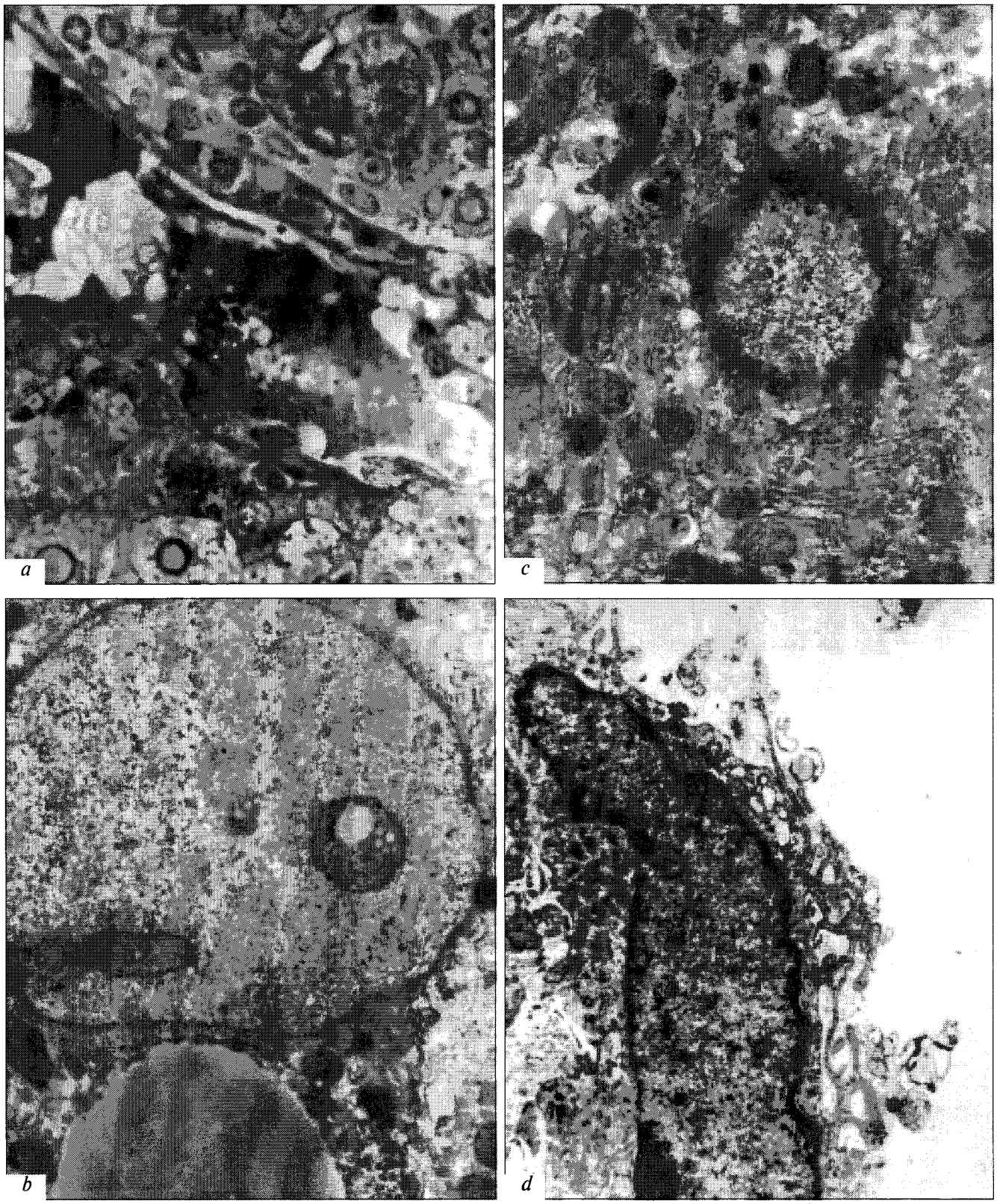

Fig. 1. Ultrastructural changes in liver samples during hepatitis C+B: *a*) two ring-like hepatocyte nuclei in the periportal zone (semithin section stained with Schiff reagent and azure II; ×600); *b*) invagination of the nucleolemma with a nuclear localization of cytoplasmic regions in hepatocyte (large lipid drop in the perinuclear zone; ×4000; *c*) hepatocyte ring-like nucleus (×6000); and *d*) numerous microvilli on the luminal surface of sinusoid endotheliocyte, ×10,000.

protein-synthesizing compartment were reduced, mitochondria were vacuolated, and the cytoplasmic matrix was characterized by high electron density; no signs of pinocytosis were found. In endotheliocytes, a considerable number of polymorphic lysosomes in the cytoplasm and numerous microvilli of the luminal surface were revealed during high-intensity chronic C+B hepatitis (Fig. 1, *d*).

When analyzing hepatocyte alteration and repair during chronic C+B hepatitis, it is necessary to emphasize that hepatocyte cytoplasm is structured, despite the presence of many residual bodies or ring-like nucleus transformation. By contrast, loosened hepatocytes contain nearly unchanged nuclei and well-defined perinuclear focuses of regeneration; no or only solitary degenerative structures are seen in these cells.

Thus, the loosening of the cytoplasm and preservation of the nucleus and perinuclear compartment may be considered as a preventive cell inhibition [11], a universal protective phenomenon, which protects cells from pathogenic factors (for example, viruses and toxins). On the other hand, this loosening can reflect removal of damaged organelles followed by recovery of hepatocyte ultrastructure.

Thus, combined infection with RNA-containing HCV and DNA-containing HBV leads to phenotypic heterogeneity of hepatocyte populations. RNA-containing HCV damages cytoplasmic organelles and does not affect the nucleus. DNA-containing HBV induces degradation of the nuclear compartment and does not affect the ultrastructure of the cytoplasmic compartment.

Survival of damaged cells and the development of adaptive reactions probably depend on many factors including viral replication, intensity of intracellular regeneration [8], buffer properties of polyploid genome of hepatocytes [10], microenvironment formed by parenchymal, sinusoidal, and lymphoid cell populations, and the state of the extracellular matrix involved in hepatocyte differentiation [6].

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