ULTRASTRUCTURAL DEMONSTRATION OF CHOLESTEROL IN THE LIVER IN CHRONIC ALCOHOL POISONING

A. S. Loginov and V. V. Ul'yanova

UDC 616.89-008.441.13-036.12-092.9-07:
616.36-008.939.22-076.4

KEY WORDS: hepatocyte ultrastructure; cholesterol; alcohol.

Chronic alcohol poisoning leads to profound disturbances of lipid metabolism in the human and animal liver. This is manifested as accumulation of lipids, including cholesterol, in the liver. Morphological investigations have been mainly concerned with deposition of triglycerides in the hepatocyte cytoplasm [2, 5], and the effect of cholesterol on the state of the intracellular structures remains virtually unstudied. The aim of this investigation was to study ultrastructure of the liver in lipid hepatosis induced by chronic alcohol poisoning, paying particular attention to deposition of cholesterol and its relationship to the development of destructive changes in the liver cells.

EXPERIMENTAL METHOD

Specimens obtained by diagnostic liver biopsy from 19 patients with fatty degeneration of alcoholic etiology were investigated electron-microscopically and cytochemically. The control group consisted of five clinically healthy individuals with no history of episodic alcohol consumption. Liver tissue for routine electron-microscopic study was treated by the usual method. Electron-cytochemical demonstration of free cholesterol was carried out by a reaction based on the formation of a cholesterol-digitonin complex [8, 9]. To determine the precise distribution of free cholesterol in the cell and its binding with organoids, which can take place during perfusion fixation and incubation, experiments were carried out on rats. Daily intragastric injections of alcohol in a dose of 2 g/kg body weight were given to 15 animals for periods of 45, 60, and 90 days. The control group consisted of nine intact rats. Samples of liver tissue from biopsy material and from the experimental rats, after appropriate fixation and dehydration, were embedded in Araldite [1]. Ultrathin sections were stained with uranyl acetate and lead citrate and examined in the JEM-1200EX electron microscope. The instrumental magnification was 5000-50,000.

EXPERIMENTAL RESULTS

In patients with alcohol-induced liver damage sharp changes were observed in the liver cells. The cytoplasm of most of them contained lipid inclusions, which varied considerably in number and size. Lipid drops were closely bound with mitochondria and the endoplasmic reticulum, and various degrees of destruction were observed in them. Sometimes, besides lipids, peroxisomes and lipofuscin were found. The mitochondria varied greatly in size irrespective of their binding with lipids. Besides normal mitochondria, others were observed which were swollen and dense, sometimes with widened intracristal spaces, and containing crystalline inclusions. Some individual mitochondria were 7 μm in diameter (megamitochondria), and crystalline structures and deposition of calcium salts also were observed in them. The rough endoplasmic reticulum was greatly widened and fragmented, and its membranes were degranulated in places. The smooth endoplasmic reticulum consisted of a large number of vesicles of different sizes, which occupied a considerable volume of the cytoplasm. Destructive changes in the hepatocytes were accompanied by an increase in the number of primary and secondary lysosomes, and also of segresomes, most frequently grouped at the biliary pole of the cell. The intercellular spaces were widened around the altered liver cells and sometimes extensive regions of them contained outgrowths of hepatocytes and also bundles of collagen fibrils. More powerful collagen bundles were located in Disse's space.

Central Research Institute of Gastroenterology, Moscow. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 109, No. 6, pp. 619-621, June, 1990. Original article submitted September 14, 1989.

 0007-4888/90/0006-0832$12.50

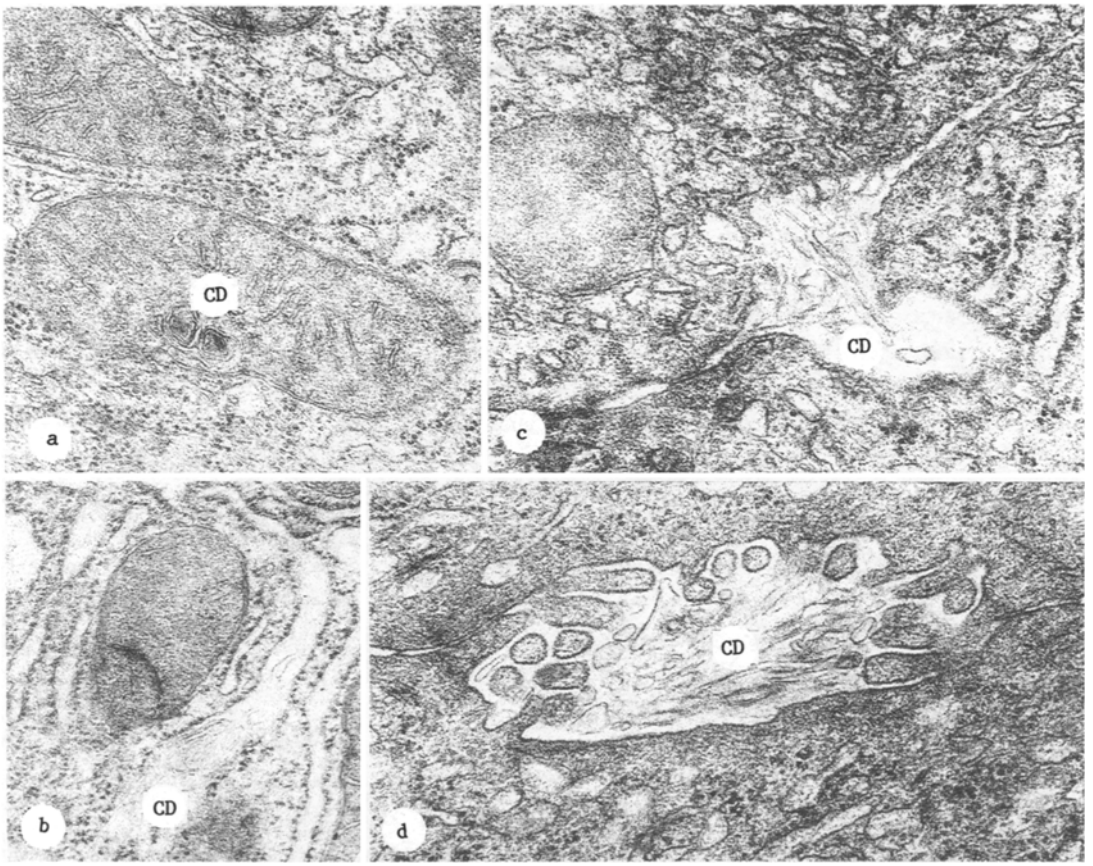

Fig. 1. Deposition of free cholesterol in liver in chronic alcohol poisoning. a) Cholesterol inside a mitochondrion, 32,500×; b) cholesterol deposits in lumen of tubule of rough endoplasmic reticulum; c) concentration of cholesterol in intercellular space with destruction of plasma membrane of cells and penetration into cytoplasm; d) cholesterol in biliary capillary; b, c, d) 50,000×. CD) Cholesterol deposits.

The histochemical reaction for cholesterol revealed an uneven distribution it both in neighboring cells and within the same hepatocyte. It appeared in the form of whorls, cylinders, and lamellar structures with varied packing density of their components, and it was distributed in different parts of the cell: free-lying in the hyaloplasm in the form of small and large concentrations, and in close association with mitochondria and the endoplasmic reticulum, the membranes of which were largely destroyed. Incidentally, the membranes of some organoids were not always clearly revealed even at a distance from the cholesterol deposits. Concentrations of cholesterol were also found at the periphery and within the lipid drops. Cumulation of cholesterol was observed outside the cell — in the widened intercellular spaces, Disse's space, and biliary capillaries. The plasma membrane at points of contact of cholesterol was ruptured.

In the control animals cholesterol was distributed in the hyaloplasm in the form of separate elements. After exposure to alcohol for 45 days the liver cells differed from one another in their lipid content. Cholesterol was located in different parts of the cell in the form of whorls, cylinders, and lamellar bodies, with loose or dense packing of their elements. Laminar structures were found in the hyaloplasm, within the mitochondria (Fig. 1a), and in the lumen of the tubules of the endoplasmic reticulum (Fig. 1b); as a rule, the membranes of these organoids were largely destroyed. Cholesterol also was observed within the lipid drops, filling them partly or almost completely. Membranes of cellular organoids not in contact with cholesterol remained intact. The noncellular deposition of cholesterol in the form of lamellar formations or, less frequently, of walls and cylinders, was demonstrated in the widened intercellular spaces (Fig. 1c) and was accompanied by destruction of the plasma membranes of neighboring cells over a considerable area. After exposure to alcohol for 60 and 90 days, the quantity of lipids, including cholesterol, in the liver increased and the destructive changes in the liver cells were more marked.

Cholesterol also was found in the biliary capillaries. They were either diluted, with a small number of villi, and filled with cholesterol masses (Fig. 1d), sometimes narrow, and containing single elements of cholesterol deposits, in which case the plasma membrane at points of contact with cholesterol was destroyed. Cholesterol was particularly abundant in Disse's space. Powerful bundles of collagen fibrils were seen in these same areas.

The results of the investigation showed that chronic alcohol poisoning leads to deposition of cholesterol in liver cells and to structural damage of the hepatocytes. Excess formation of acetyl-CoA, an intermediate product of ethanol metabolism, leads to increased synthesis of cholesterol, whose catabolism is slowed under these circumstances [3, 4]. This was expressed morphologically by the appearance of cholesterol deposits in different parts of the hepatocyte. It is important to note that during contact between cholesterol and the cellular organoids, the membranes of the latter were destroyed. This applies in particular to mitochondria, in which destruction was observed not only of the outer, but also of the inner membrane. We also found deposition of cholesterol in dilated tubules of the rough endoplasmic reticulum, accompanied by degranulation, and often also by destruction of its membranes. This is evidence of disturbance of protein synthesis in the hepatocytes, in agreement with the results of biochemical investigations [6, 7]. Changes in protein metabolism also were observed in the mitochondria, as shown by the appearance of paracrystalline structures in them. Comparison of different methods of demonstration of free cholesterol (immersion and perfusion methods of fixation and incubation) revealed identical localization of cholesterol in the liver, although with perfusion the membranes of the organoids not in contact with cholesterol were somewhat better preserved.

Thus chronic alcohol poisoning leads to cholesterol accumulation inside and outside the liver cells, and this is accompanied by destruction of cellular organoids and also of certain parts of the plasma membrane of the hepatocytes. Bundles of collagen fibrils are observed in the neighborhood of cholesterol deposits in the intercellular spaces and, in particular, in Disse's space.

LITERATURE CITED

1. A. S. Loginov, S. M. Chebanov, and S. P. Arbuzov, Lab. Delo, No. 4, 216 (1981).
2. A. S. Loginov, K. D. Dzhalalov, and Yu. E. Blok, Pathogenesis, Diagnosis, and Treatment of Alcohol-Induced Liver Damage. A Scientific Review [in Russian], Moscow (1985).
3. L. A. Maksimova, B. R. Gedulina, and Ya. I. Filler, Changes in Lipid Metabolism in Pathology of the Internal Organs [in Russian], Riga (1987), p. 73.
4. Ya. I. Filler, "Epidemiological, clinical, biochemical, and morphological characteristics of liver damage in patients with chronic alcoholism," Author's Abstract of Candidate's Dissertation, Medical Sciences, Riga (1983).
5. E. Baraona and C. S. Lieber, J. Lipid Res., **20**, 289 (1979).
6. C. S. Lieber, Gastroenterology, **79**, 373 (1980).
7. M. C. Mitchell and E. Mezey, Hepatology, **7**, 390 (1987).
8. T. J. Scallen and S. E. Dietert, J. Cell Biol., **40**, 802 (1969).
9. J. R. Williamson, J. Ultrastruct. Res., **27**, 118 (1969).