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THE ELECTRON-MICROSCOPIC FINDINGS ON THE LIVER IN CHRONIC ABUSE OF HEROIN

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Summary. *The study included an analysis of 60 autopsies, 50 of which were from the group of intravenous heroin addicts, and 10 served as controls (corpses of young and healthy people who died of mechanical traumas that did not affect the liver). The ultrastructural research has a decisive role in gathering new facts about the liver's response to different drugs. Various changes occur on organelles, plasma membrane of hepatocytes and biliar channels, as well as on the nucleus. The most important ultrastructural findings include: hyperplasia and hypertrophy of the smooth endoplasmic reticulum (SER), which is histologically a vesicular degeneration of hepatocyte developed as a result of the increased synthesis of enzymes of SER due to chronic i.v. heroin intake, and the presence of continuous basal membrane followed by conversion of the sinusoids into capillaries (in cases of chronic active hepatitis and cirrhosis) which leads to a disorder of microcirculation and further progress of cirrhosis.*

Key words: *Intravenous heroin intake, ultrastructural liver lesions*

Introduction

The liver has a key role in removing lipophile substances from the plasma, including both morphine and its derivative heroin. Hepatocyte is the main place of bio-transformational systems which enable their enzymes in that the metabolite of these substances is extracted from the body. Morphological changes in the liver tissue are accompanied by its changed function which results in a different metabolism of heroin and other toxins which are used simultaneously with heroin (alcohol, medicines). Thus, the effects of their abuse are changed and often surprising (1,2).

The liver shows a characteristic of adaptation: its function increases under the influence of various medicines. The adaptation occurs due to the increased production of enzymes that take part in metabolic destruction. This phenomenon is known as enzyme induction. Such changes can be quantitative and manifested in subcellular damage, causing hypertrophy or hyperplasia of hepatocytes known as hepatomegaly (3). The electron microscopic research has a decisive role in gathering the knowledge on the liver's response to the influence of some medicines. It is known that most medicines cause biochemical process such as conjugation, hydrolysis and reduction, which are associated with the oxidative enzyme system of a mixed function (4,5). These changes occur in the smooth endoplasmic reticulum (SER) (6). The discovery of the change of SER under the influence of most medicines is expected. The most common response is proliferation of SER, which is proved in patients treated with phenobarbital, difenilhidantion, diazepam and oral contraceptives, but also in chronic heroin addicts (7). In the case of induction of SER proliferation, SER type 2 component is increased, although views are controversial. The second common change is dilatation and vesicular transformation of SER cistern, which was noticed during the treatment with imurran and tetracycline (8). There are some results which suggest that damage of SER by medicines is insignificant in the human liver when compared to the animal liver. When human hepatocytes are concerned (contrary to rodents' hepatocytes), SER is more abundant than the rough endoplasmatic reticulum (RER). RER is less studied, and its dilatation and degranulation during treatment with oral contraceptives and fenil-butazon derivates are established. Dilatation, vesiculation and RER extension are found during treatment with fenacetine and hypervitaminosis A (9).

Triguero *et al*. (10) performed an analysis of liver biopsies of 5 i.v. heroin addicts. At the ultrastructural level, they analyzed 150 centrolobular sinusoids and compared these results with 90 sinusoids from 3 liver biopsies of patients belonging to controls. EM observation shows the thickening of the sinusoidal wall related to endothelial cell hypertrophy, the increase of the area of the extension of Ito cell and fibrosis of Disse space. Cell hypertrophy can represent hyper-activation of the sinusoid functional capacity of the cells, which initiates fibrogenesis of Disse space. This newly formed mechanical barrier has the power to prevent free exchange of materials through Disse space and can protect the liver from heroin's toxic influences.

Aim

The aim of the study was to perform an ultrastructural analysis of the liver in chronic intravenous heroin addicts.

Material and Method

The study included 60 autopsies, of which 50 were from the group of intravenous heroin addicts, and 10 autopsies served as controls (corpses of young and healthy people who died of mechanical traumas that did not affect the liver).

During autopsy, liver specimens were taken (from 3 to 5). The liver extracts were fixed in glutar-aldehyde and the tissue was emseeded in epon for ultrastructural (EM) research.

Results and Discussion

Various changes occurred on organelles, the plasma membrane of hepatocytes and biliar channels, as well as on the nucleus.

The changes on organelles

The ultrastructural changes were the most prominent on the smooth and the rough ER, and less prominent on the mitochondrias. Namely, proliferation, dilatation, and vesicular transformation of cisterns dominated on SER, especially of type 2 (Fig. 1).

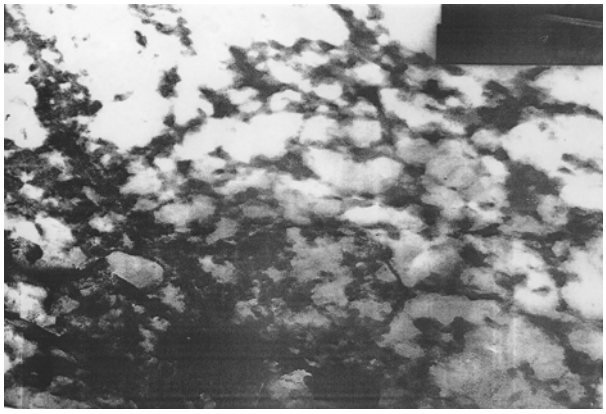

Fig. 1. Hyperplasia and dilatation of SER cisterns. EM × 10 000

Degranulation and fragmentation were also present (Figs. 2 and 3), that is, separation of the ribosomes and their dilatation (Fig. 4). The mitochondrias changed their size and shape, i.e., they became polymorphous with the thickening of their matrix (Fig. 3). The swelling of mitochondrias and even the forming of giant mega-mitochondrias were not rare. The criste in them are shortened (Fig. 4) or fragmented followed by a simultaneous occurrence of crystal inclusion and myelinic figures.

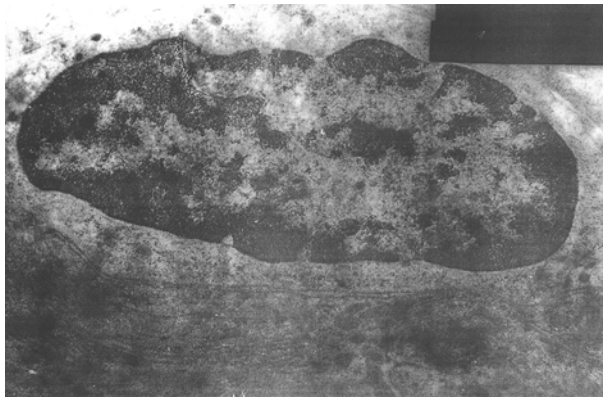

Fig. 2. Chromatin condensation on the nuclear periphery, rare glycogen granules, dense mitochondrial matrix, reduced and partially degenerated RER. EM × 10 000

Fig. 3. Narrowing of the intercellular space, villi protrusion and membrane "capilarisation". Mitochondrial polymorphism with matrix condensation. Focal degeneration (lack of ribosomes) and fragmentation of RER. Glycogen granules are dense on the hepatocyte periphery. Some of them contain crystalloid corpuscles. Matrix condensation is present in some of them. EM × 10 000

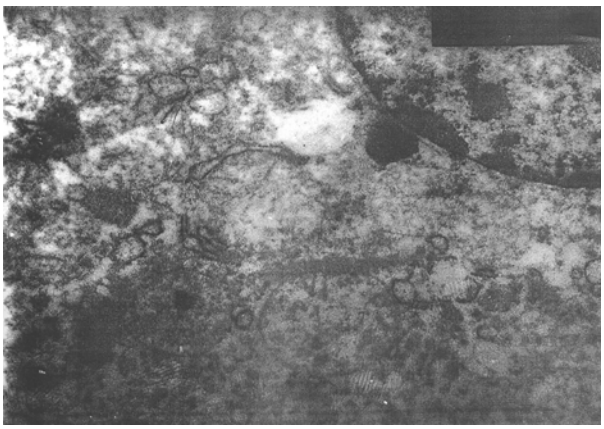

Fig. 4. Lipid vesicles, mega-mitochondrias, fragmented cysts, crystalloid corpuscles, vesicular RER dilatation, mitochondrial membrane thinning and multifocal fragmentation. EM × 10 000

The lysosomes were not directly affected by drugs. The changes in them were of secondary nature and are the result of digestion of products of the damaged membranes of other organelles. In the cytoplasm of hepatocyte and even in the cytoplasm of biliary epitheliums, the autophagal vacuoles, the increased number of lipofuscin pigment granule, paracrystal inclusions-myelin figures were found (Fig. 3).

The changes on the cells membranes

The swelling and thinning of microvilles on the vascular pole, accompanied by the protrusion cytoplasm in Disse space, were found. This phenomenon is known as a shedding figure and represents an unspecific response to shedding (Fig. 5). Much more important is the widening of intercellular space together with the fearing of desmosoms.

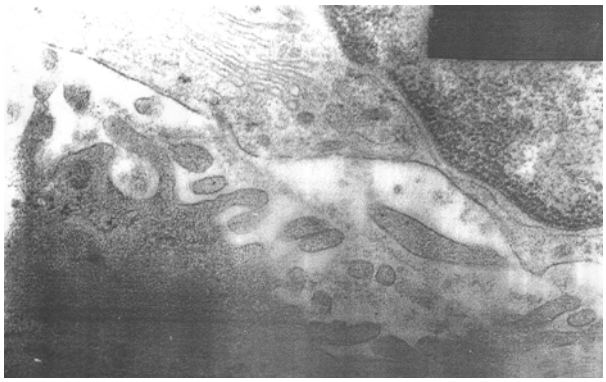

Fig. 5. Kupffer cells' dominant hypertrophy with the narrowing of the sinusoidal lumen. Kupffer cells' hyperactivity i.c. hyperplasia of the Golgi's zone and RER. Thinning of microvilli and peeling of the vascular site of hepatocytes. Marginalization and condensation of the hepatocyte nuclear chromatin is present on some of them. EM × 26 000

The changes on the nucleus

Condensation of the chromatin and destruction of the nucleus were dominant (Fig. 6).

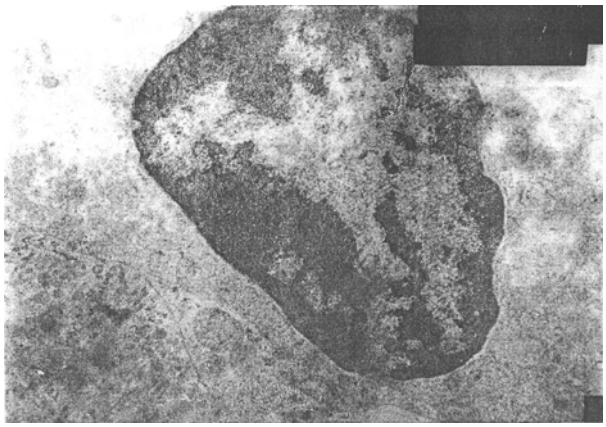

Fig. 6. Condensation and marginalisation of nuclear chromatin. EM × 10 000

Degenerative changes

In the case of serious degeneration type lipid changes, depletia of glycogen in the cytoplasm of hepatocyte was noticeable (Fig. 7).

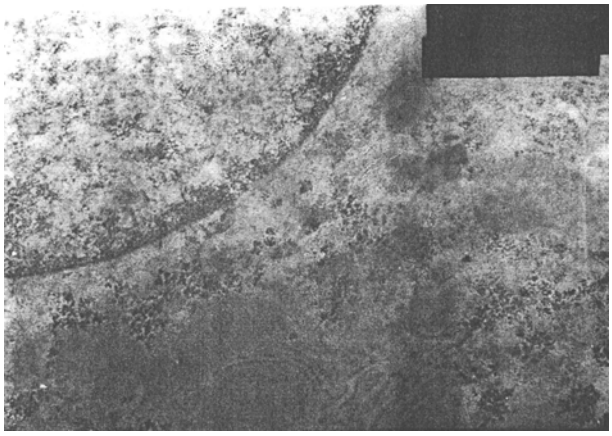

Fig. 7. Evident depletion of glycogen or the glycogen's absence in the hepatocyte cytoplasm. EM × 13 000

Inflammatory changes

A mesenchymal inflammatory reaction was present in the lobules' port space and periportal.

Kupffer's and sinusoidal endothelial cells were intralobulary increased (Fig. 5). In the cytoplasm of Kupffer's cells, there were various inclusions, corpuscles, above all, the granules of the lipofuscin pigment, hemosiderin, ceroids and biliary inclusions.

The expanded Disse space contained the cells of inflammation of the lymphocyte type and polymorphonuclears, which are closely related with hepatocytes.

An important change was capillarisation of the sinusoid (Fig. 3). First, the thin amorphous basal membrane was created along the vascular pole. The basal membrane was followed by the increase in collagen fibers in Disse space, but also in the intercellular space. Those damages caused a disorder of microcirculation and a further progress of cirrhosis.

Chronic active hepatitis

In chronic active hepatitis, the lymphocyte-plasma cell infiltrate extended from the portal space to the nearby parenchyma (11). Lymphocyte-plasma cells showed different stages of maturation and were stuck between hepatocytes. During this process, their cell membranes were in close contact. In the case of a limited lysis of the cytoplasm membrane hepatocytes, the processes of activated lymphocytes were invaginated into hepatocytes, which is known as emperiopolesis (Fig. 8). Plasma cells were in close connection with the hepatocyte.

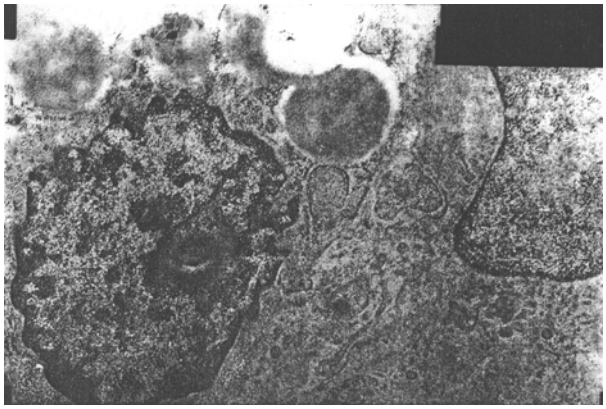

Fig. 8. Activated lymphocyte with protrusion of its extensions in the hepatocyte cytoplasm - the process of emperiopolesis, the presence of paracrystalloid inclusions in the mitochondrial matrix. EM × 10 000

In macrophage, there were phagosomes together and with fibroblasts. They increase the synthesis of proteins enabling the multiplication of collagen fibers. With the multiplication of fibrils, hepatocytes become isolated from the food source, which leads to irregular lobular organization. Collagen was especially prominent in Disse space near the vascular pole of the hepatocyte. The lumen of the sinusoids was reduced because of the hypertrophic sinusoidal macrophages (Fig. 5) and multiplied lymphocytes and plasma cells. Condensation of the collagen fibers causes intralobular septa which connect the portal zones with the lobular center damaging the lobular structure even more. Besides fibrosis and mononuclear thick infiltrate, cholangiole are multiplied in the portal space, but have the shape of solid stripes without lumen.

Chronic persistent hepatitis

In the portal space, there were crowded lymphocytes, histiocytes, plasma cells and fibroblasts, accompanied by proliferation of the bille channels. The border plates were intact and the lobular structure was preserved. Hepatocytes were very often without changes, although they sometimes contained HbsAg. On the periphery, the stasis of the bilirubin pigment was found and it was the result of periportal inflammation and fibrosis. The multiplied billiary channels were surrounded by the collagen sheaf, but they had a lumen and basal membrane, as well as a microvillus on the apical poles.

Mitochondria of the hepatocytes changed the shape and size due to the paracrystaloid inclusions (Fig. 8). Sometimes, a nucleoid corpuscle can be seen.

Cirrhosis

In cirrhosis, the nodules in Disse space show a different level of dilatation, as well as increased EM thickness (12). The presence of the continuous basal membrane with conversion of the sinus into capillaries was described (Fig. 2). A bundle of collagen fibers around the basal membranes were polarized in parallel formations, thus creating thick or thin sheaths.

The forming of basal membranes and sinusoidal capillarization (Fig. 3) played an important role in the fibrogenesis of Disse spaces. Basal membranes surrounded the portal space and proliferating sinuses, while the capillarisied sinusoids represented the focus for the occurrence of cirrhotic fibrogenesis. Collagen fibers were formed even when the basal membrane was not present (13).

The increase of the reticular and collagen fibers was found in both extended intercellular spaces and cirrhotic nodules, which is known as pericellular fibrosis.

It has been suggested that a changed Disse space represents a barrier for diffusion of substrates from the sinusoids. That is why the pathological microvillous borders on some occur, as an adaptive answer.

In the synthesis of collagen fibers, Ito cells of the perisinusoidal spaces have the main role. Mitochondrias have greater dimensions compared to the megamitochondrias. In hepatocytes, there were Mallory corpuscles (14).

Cholostasis was present in the hepatocytes and small canals and was accompanied by the hypertrophy of the Golgi zone and promination of SER (15).

Conclusion

The most important ultrastructural findings on the liver of intravenous heroin addicts include:

- hyperplasia and hypertrophy of the smooth endoplasmic reticulum, which is histologically a vesicular degeneration of hepatocyte occurring as a result of the increased synthesis of enzymes of SER, due to chronic i.v. heroin intake;
- the presence of a continuous basal membrane accompanied by conversion the sinusoids into capillaries (in cases of chronic active hepatitis and cirrhosis), which causes disorders of microcirculation and a further progress of cirrhosis.

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ELEKTROMIKROSKOPSKI NALAZI JETRE KOD HRONIČNE ZLOUPOTREBE HEROINA

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Kratak sadržaj: *Studija obuhvata analizu 60 autopsija i to 50 iz grupe intravenskih heroinomana, a 10 obdukcija je predstavljalo kontrolnu grupu (leševi mladih i zdravih osoba smrtno stradalih zbog mehaničkih trauma koje nisu zahvatile jetru). Ultrastrukturna istraživanja imaju odlučujuću ulogu u dobijanju novih podataka o odgovoru jetre na različite droge. Različite promene se odvijaju na organelama, na citoplazmatskoj membrane hepatocita i žučnih kanalića, kao i na jedru. Najznačajniji ultrastrukturni nalazi su: hiperplazija i hipertrofija glatkog endoplazmatskog retikuluma, koja se histološki sagledava kao vezikulatna degeneracija hepatocita, nastala kao posledica povećane sinteze enzima endoplazmatskog retikuluma zbog hroničnog intravenskog unošenja heroina i postojanje kontinualne bazalne membrane uz konverziju sinusoida u kapilare (u slučajevima sa hroničnim aktivnim hepatitisom i cirozom), što uzrokuje poremećaj mikrocirkulacije i dalju progresiju ciroze.*

Ključne reči: *Intravensko unošenje heroina, ultrastrukturna oštećenja jetre*