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CHARACTERISTIC FEATURES OF MORPHOLOGICAL, STRUCTURAL AND HISTOCHEMICAL LIVER DISORDERS AT DIFFERENT STAGES OF EXPERIMENTAL ABDOMINAL SEPSIS

Abdominal surgical sepsis is characterized by a significant number of episodes in case of recorded steady growth and a high mortality rate. The morphological, structural and histochemical changes in the liver tissues at different stages of abdominal sepsis were assessed in 52 experimental animals. The following stage related inflammatory infiltrative changes (hyperemia and edema of interstitial tissue and Disse spaces, hydropic and fatty degeneration of hepatocytes, lymphoid-neutrophil infiltration of portal tracts and intra-lobular stroma) and degenerative-dystrophic necrobiotic disorders (cariopicosis and cariolysis of the cell nuclei, foci of hepatocyte necrosis of the colliquation type, cytoplasmic inclusions of hyaline-like masses, fragmentation of reticular fibers, PNK and glicogen investigation data) underlying the experimental hepatoprive syndrome were identified.

Key words: *abdominal surgical sepsis; stages; morphological, structural, histological and chemical disorders; liver.*

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ОСОБЕННОСТИ МОРФОСТРУКТУРНЫХ И ГИСТОХИМИЧЕСКИХ НАРУШЕНИЙ ПЕЧЕНИ ПРИ РАЗЛИЧНЫХ СТАДИЯХ ЭКСПЕРИМЕНТАЛЬНОГО АБДОМИНАЛЬНОГО СЕПСИСА

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Абдоминальный хирургический сепсис характеризуется существенным числом в случае зарегистрированного роста и стабильно высокой летальностью. Проведена оценка морфоструктурных и гистохимических изменений в тканях печени при различных стадиях абдоминального сепсиса у 52 экспериментальных животных. Установлены стадийные ведущие воспалительно - инфильтративные (полнокровие и отек межуточной ткани и пространств Дассе, гидрогическая и жировая дистрофия гепатоцитов, лимфоидно-нейтрофильная инфинотрация портальных трактов и внутридольковой стромы) и дегенеративно-дистрофические некробиотические (кариопикноз и кариолизис клеточных ядер, очаги некроза гепситоцитов по колликвационному типу, цитоплазматические включения гиалиноподобных масс, фрагментация ретикулярных волокон, исследование РАК и иликосена) нарушения, лежащие в основе экспериментального гепатопривного синдрома.

Ключевые слова: *абдоминальный хирургический сепсис, стадии, морфоструктурные и гистохимические изменения, печень.*

The topicality of the problem connected with generalized surgical infections is due to a steady growth of cases, the number of which has increased more than 8–10 times over the last 50 years. 350,000–500,000 new cases of sepsis with 50,000–180,000 fatal outcomes are diagnosed each year only in the US. According to randomized studies conducted in Western Europe, Canada and Israel the number of cases with surgical sepsis in the intensive care and resuscitation units is more than 15–17% of the total number of patients treated at the above departments.

The mortality rate from sepsis remains high, reaching 30–50% and in case of concomitant multiple organ failure (MOF) and septic shock it is over 70–80%.

A significant part of unfavorable statistics is attributed to abdominal sepsis, which is currently considered as a generalized systemic response, occurring on the background of constant or periodic penetration of microbes and their toxins into the systemic bloodstream from the intra-abdominal foci and initiating the development of MOF caused by the failure of the body's immune system to suppress and localize them in the primary focus.

Bacterial modulins activate the specific receptors of monocytes/macrophages, neutrophils and NK-cells found in the inflammatory focus, which, in turn, trigger the cellular and humoral reactions of natural resistance through the production of intracellular enzymes, matrix and cationic proteins, regulatory peptides and hormones, pro- and anti-inflammatory cytokines, eicosanoids, systemic complement products, free-radical metabolites, acute inflammation and heat shock proteins, cytomedines and neurotransmitters.

With the activation of the inflammatory factors the systemic generalized reaction acquires an uncontrollable nature which causes a number of systemic pathological reactions including increased permeability of the vascular wall, development of perivasal edema, thrombohemorrhagic stasis in the microcirculatory bed, thus forming the prerequisites for multiple organ dysfunctions.

At the same time, despite a significant number of scientific publications on this subject, the dynamics of morphological, structural and histochemical disorders of internal organs in the development of different stages of abdominal sepsis is still elusive and requires further investigation.

The aim of the study is to assess experimentally the dynamics of morphological, structural and histochemical disorders in the hepatic tissue at different stages of abdominal sepsis.

Materials and methods

We analyzed 124 histological sections of the liver obtained from 52 experimental animals (Wistar rats) following all the accepted rules and requirements concerning the work with experimental animals (Strasbourg, France, 1986, EU Directive 86/609 of 24.11.1986).

The operations were performed after preoperative premedication with the mixture of droperidol (0.5 mg/kg), demidrol (1.5 mg/kg) and analgin (60 mg/kg). For general anesthesia we applied intra-flow administration of a 1.0% solution.

Abdominal sepsis combined with multiple organ failure was simulated by modeling widespread peritonitis (by Usikov) via placing a remote vermiform appendage (with the feces content transferred into it from the lumen) into the mesogastric region.

Additionally, we narrowed the terminal section of the small intestine (2–3 cm proximally to the ileocecal angle) by 40–60% applying silk ligature. After 24 hours, we injected the animals with a 24-hour culture of Staphylococcus aureus (strain No. 4293) in the amount of 5 billion units and suspension of epidermal staphylococcus culture (strain No. 3347) in the amount of 10 billion units. Abdominal sepsis was detected in all experimental animals 3 days after the start of the experiment. Lymphostructural and histochemical disorders of internal organs were studied in the dynamics: up to the first signs of sepsis which appeared on the 1st–2nd day and after its full manifestation on the 3rd–4th day.

The studied biopsy material, taken from the liver tissue after its fixation in 10% solution of neutral formalin and Bien solution, was dehydrated with the increasing concentration of alcohol (60°, 70° 80°, 90°, 100°).

At the next stage the biopsy tissues were first treated with paraffin; then we made 5–7 μm thick sections from the prepared blocks, which were later redewaxed. The obtained material was followed by a selective histochemical reaction to determine:

A. Glucosaminoglycans in the intercellular substance (by the PAS reaction).

B. Elements of connective tissue of the liver (by staining with Mallori).

C. Reticular stroma of the liver (by impregnation applying the Foot's technique).

D. Neutral fats (by staining with Sudan IV).

E. Microcirculation (by staining with thionine).

F. Metachromasia (by staining with toluidine blue).

Histological and histochemical reactions were performed many times from different (at least 5–6) sites. The study of micropreparations was carried out by means of "Amplival" Carl Zeiss optical light microscope (Germany, mag.280 times).

Results and discussion

A. Compensated stage of sepsis

Macroscopically it was characterized by an increase in the size and weight of the liver with expressed blood filling. During the incision we determined: the flattened lobe structure; multiple foci of yellow color; thrombosis of the portal vein branches and heterogeneity.

Microscopic examination revealed hydrophilicity, granularity and edema in the hepatocyte cytoplasm. Dystrophy of the liver was detected either as protein-parenchymal disorders or in the form of hyaline-granular and hydropic changes. The cytoplasm of cells with protein dystrophy was characterized by granularity, scarcity of glycogen and RNA, and moderate polymorphism of the nuclei. In a number of cases the damage of the hepatic tissue cellular boundaries was observed.

Mallory's bodies were found in the cytoplasm of the cells subjected to hyaline-granular dystrophy in addition to hyaline grains. Usually they are localized only in the tissue perinuclear region. The main characteristic feature of Mallory's bodies is the wedge-shaped formations with acidophilic inclusions.

At this stage of sepsis, hepatocytes with hydropic destruction were detected. They had cytoplasmic vacuoles with eroded boundaries and almost complete absence of glycogen. Protein-parenchymal and hyaline-granular cell degeneration was found mainly in peripheral and central lobular areas of the hepatic tissue, while hydropically altered hepatocytes were mainly detected in periportal areas. In some cases, balloon-modified hepatocytes were defined, which later turned into a single large vacuolized structure. More often they were localized as cell groups with destroyed nuclei due to pycnosis and lysis.

This stage was also characterized by necrotic foci in the hepatic tissue with hepatocytes fusion with each other. In necrotic foci tinctorial signs of hepatocyte and liver lobe disorders with the destruction of radial structures were revealed. In necrotic cells autolysis prevailed, while in the nuclei we observed marginal or complete lysis.

The triads of the vessels were plethoric and expanded; endotheliocytes had a "cigar-like" form. In the outer layer of the triad we noted the fragility of connective tissue fibers. In some areas around the periphery of the hepatic lobules a weak regeneration of hepatocytes was revealed in the form of areas located as islets characterized by polydisploidy, cell hypertrophy and stromal angiosis. Hepatocytes in necrotic foci were similar to trabeculae located in 2–3 rows; the Disse spaces were extended.

We observed edema around the bile ducts on the background of neutrophilic leukocyte infiltration and expansion of interlobe biliary tract as well as smooth epithelial cells with the signs of microvillosis in the lumen (figure 1).

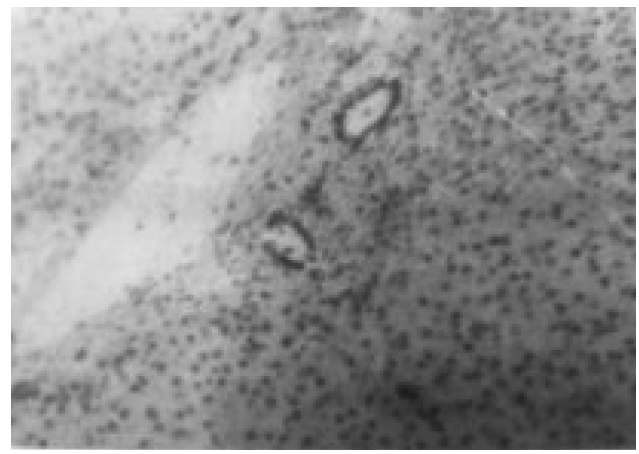

Figure 1. Hepatic tissue with the compensated stage of experimental sepsis. Staining: hematoxylin-eosin. Magnification: ×280

In a number of bile ducts surrounded by a fibrous connective tissue cylindrical epithelium was partially replaced by the cubical one.

B. Subcompensated stage of sepsis

Macroscopically this stage was characterized by a decrease in the liver volume, consolidated tissue and tuberous surface; on the slice it was brown with the inclusion of small gray foci.

Microscopic investigation revealed infiltrative development of fibrous connective tissue caused by its proliferation into the liver lobules. In some places we revealed «islands» of fibrous tissue. The majority of hepatocytes were hypertrophied, less often – atrophied. Hypertrophied cells were located along the periphery of the liver lobes, while atrophied cells were noted in the periportal spaces. Protein-hydrophilic degeneration predominated in hepatocytes, while in the cytoplasm we observed expressed vacuolization and granularity.

Radial columnar structure of hepatocytes has been subjected to decomposition, and reticular fibers – to fragmentation. Multiple lymphoid infiltrates, edema, fragmentation of the collagen stroma were discovered in the interlobular connective tissue. Hepatocyte cell borders were indistinct. We also revealed shrinkage of the atrophied cells cytoplasm with the signs of karyopicosis and karyolysis in the nuclei, small inclusions of chromatin aggregates and necrobiotic changes accompanied by the loss of nucleoli.

In comparison with the previous stage we observed fatty degeneration of the central hepatic lobular zones. In the sites of fatty degeneration complete disappearance of glycogen, predominance of macrophages filled with crystals, fat and protein grains were noted.

One of the characteristics of this stage of sepsis was presence of multiple foci of colliquative necrosis. In hepatocytes, located in these necrotic foci, irreversible changes with the absence of radial columns in the composition were revealed. Along with the above changes, we found hepatocytes with nuclear destruction caused by karyopicosis and karyolysis and large hyaline-like masses observed in the cytoplasm. A marked lympho-plasmocyte infiltration was defined around hepatocytes; in the foci of necrosis multiple hemorrhages and the expansion of the Disse spaces were identified (figure 2).

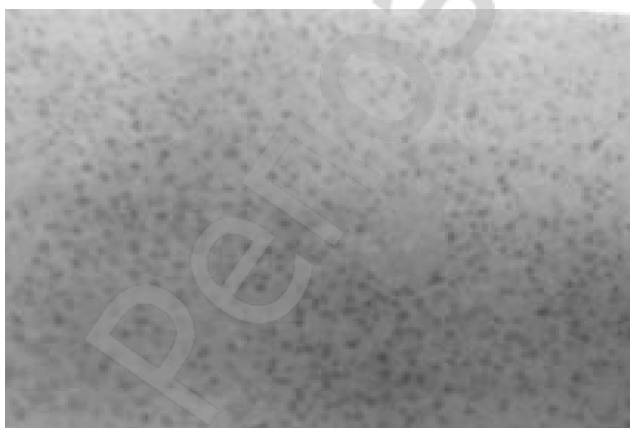

Figure 2. Hepatic tissue micropreparation at the subcompensated stage of experimental sepsis. Fatty and hyaline-droplet dystrophy of hepatocytes, moderate swelling of Disse spaces, narrowing of sinusoids on the background of weak lymphohistocytosis infiltration of portal tracts are detected there. Staining: hematoxylin-eosin. Magnification: ×280

C. Decompensated stage of sepsis

Macroscopically it is characterized by liver consolidation, decrease in the size with granularity detected on the surface; yellow patchy inclusions on the incision surface.

On microscopic examination we identified fibrosis of the connective tissue capsule (Glisson) with deep proliferation into lobes and lobules accompanied by the development of a false lobular structure. We also revealed thrombosis of the vessels located between the connective tissue fibers, obliteration of their lumen with the development of connective tissue (the phlebosclerosis and endophlebitis type). The normal lobular structure of the liver parenchyma was not determined in the absolute majority of cases; we identified only isolated islets, false lobules and signs of lipoidosis.

Most of the tissue cells were atrophied due to hydrophobic (balloon) dystrophy. The existing cells had the phenomena of karyopicosis, karyolysis, karyorexis with the destruction of the nuclear membrane, which confirmed the development of irreversible processes.

The above changes were more expressed around the foci of hemorrhage and hematoma of the tissue. In comparison with the previous stages of sepsis (compensated and subcompensated), the number and size of hemorrhage foci were considerable. Changes of this type were more frequently observed in the periportal areas, where focal sites of necrosis were defined along with total vacuolohydropic and granular dystrophy. Foci of necrosis were characterized by necrobiosis of hepatocytes, accumulation of tissue detritus products and perifocal lymphoid infiltrates (figure 3).

Hepatocytes were more susceptible to protein-hydropic changes in the central lobular and intermediate areas, where nucleus cell pycnosis with condensed chromatin was determined.

Sinusoidal capillaries were wavy and in some regions expanded. They contained only plasma capillaries, while in other regions they were narrowed and included uniform elements of blood and detritus masses. In some atrophied lobules, necrotic foci were found in the intermedial and periportal areas.

Protein-parenchymal dystrophy of hepatocytes and lipid accumulation were discovered in the reactions with Sudan IV staining mainly in the peripheral areas of the liver. In addition we also noted fragments and lysis of reticular fibers. Their destruction was most often determined around the vessels and in the central lobular

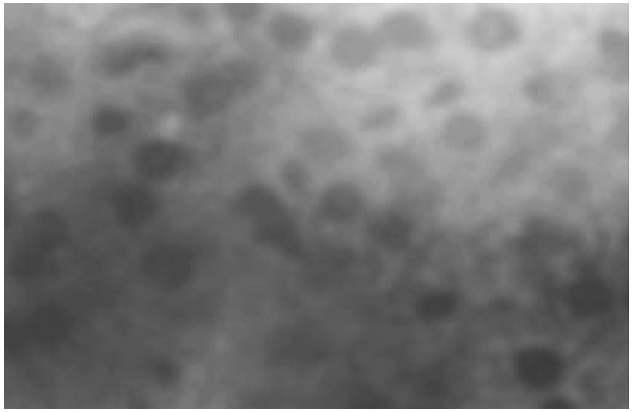

Figure 3. Hepatic tissue at the decompensated stage of sepsis. Visible: stagnation in the blood vessels with expansion of the Disse spaces; hepatocyte dystrophy; plasma cells in the region of the hepatic triad. Staining: hematoxylin-eosin. Magnification: ×280

zones. At the same time multiple thrombi were isolated in the lumen of the blood vessels and signs of necrobiosis were observed in the surrounding tissues. In histochemical studies a decrease or complete disappearance of RNA and glycogen was identified in the lumen of hepatocytes on the background of the Disse spaces expansion.

Conclusions

1. A severe degree of hepatic insufficiency (hepatoprive syndrome) was noted in the absolute number of cases with the onset of sub- and decompensated stages of abdominal experimental sepsis.

2. Morphological, structural and histochemical manifestations of experimental hepatoprive syndrome are a combination of inflammatory infiltrative and degenerative-dystrophic necrobiotic processes including hyperemia (plethora) and edema of interstitial tissue, Disse spaces, hydropic and fatty degeneration of hepatocytes, lymphoid-neutrophil infiltration of portal tracts and intra-lobular stroma.

3. The discovered pathology stipulates the need for targeted hepatotropic correction in combination therapy of abdominal sepsis.

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