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a long-term antihypertensive effect and is safe in this category of patients.

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STRUCTURAL ORGANIZATION OF RAT HEPATIC CELLS AND THEIR CORRECTION WITH CRYOPRESERVED PLACENTA IN EXPERIMENTAL PERITONITIS

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СТРУКТУРНА ОРГАНІЗАЦІЯ КЛІТИН ПЕЧІНКИ ЩУРІВ ТА ЇХ КОРЕКЦІЯ КРІОКОНСЕРВОВАНОЮ ПЛАЦЕНТОЮ НА ТЛІ ЕКСПЕРИМЕНТАЛЬНОГО ПЕРИТОНІТУ

Summary. Currently, cellular and tissue therapy is widely used in treatment of various diseases. The specific state of hepatocytes has been studied histologically and electron-microscopically in simulated aseptic inflammation of the rats' abdominal wall followed by correction with transplantation of cryopreserved placenta within the period from day1 to day 30. Histologically, mainly dystrophic and destructive alterations of the liver cells have been detected during inflammation from day 1 to day 14, which were the same as before treatment. A recovery of the shape and structure of hepatocytes, blood supply and restoration of their functions started on day 14 of the experiment.

However, the findings of the electron microscopic study of the liver specimens in the first three days showed positive changes, manifested by the reactive state of the ultrastructure of hepatocytes and hemocapillaries, which improved in the mid-term time (day 5, 7) of the experiment and the structure of the sinusoid hemocapillaries and hepatocytes came to normal at the late terms (day 21, 30).

Анотація. В даний час для лікування різних захворювань організму все частіше застосовується клітинна і тканинна терапія. На експериментальній моделі асептичного запалення тканин черевної стінки щурів і корекції запалення трансплантацією кріоконсервованої плаценти були вивчені гістологічно і електронно-мікроскопічно особливості стану гепатоцитів в період з 1 до 30 діб. На тлі запалення з 1 до 14

діб були гістологічно виявлені, в основному, дистрофічні і деструктивні зміни клітин печінки, такі ж, як до лікування. Починаючи з 14 доби експерименту, відбувається повернення до норми форми і структури гепатоцитів, кровопостачання і відновлення їх функції.

Однак при електронно-мікроскопічному вивченні препаратів вже в перші три доби можна було побачити позитивні зміни, які полягають в реактивному стані ультраструктури гепатоцитів і гемокапіллярів, які покращились в середні терміни (5, 7 добу) експерименту. У пізні терміни (21, 30 добу) виявленанормалізація структури синусоїдних гемокапіллярів і гепатоцитів.
Keywords: inflammation, hepatocytes, correction, transplantation, cryopreserved placenta.

Ключові слова: запалення, гепатоцити, корекція, трансплантація, кріоконсервована плацента.

The current industrial development, ecological disturbances and availability of huge amount of drugs on the open market lead to accumulation of great deal of foreign toxic substances in the human body that can affect the liver.

The liver is a large gland of the body that is responsible for various vital functions such as metabolic, depositing, barrier (detoxifying) and others [2,5].

More than 80% of liver cells that perform most of these functions are hepatocytes. These cells have a polygonal shape, 1-2 nuclei, granular cytoplasm, containing numerous mitochondria, lysosomes, lipid droplets, particles of glycogen, agranular endoplasmic reticulum (AER) and granular endoplasmic reticulum (GER), elements of the Golgi complex (GC).

Functional activity of hepatocytes is manifested by their participation in the capture, synthesis, accumulation and conversion of various substances, subsequently released into the blood or bile.

Hepatocytes are involved in the metabolism of carbohydrates, lipids, proteins, pigments, and provide depositional and barrier functions.

Given that hepatocytes are the major cells of the liver and perform its main functions, they are, first of all, altered by the effect of various factors in the body. Alterations of hepatocytes depend on the type of the factor and its duration. It can be trauma, chemicals, inflammatory processes in the body [2, 7, 8]. Hepatoprotectors are traditionally prescribed for patients due to its mechanism of action. Other researchers prescribe drugs of anti-exudative activity, namely, dry licorice extract [1], biologically active compounds with endothelial-protective effect of angioline [7], etc.

Significant structural and functional changes in liver cells in this pathology require long-term treatment and a comprehensive therapeutic approach.

Currently, cellular and tissue transplantation is being often used both in the experiment and in practice, [4, 10-14], which induces tissues and the whole liver to improve regenerative, metabolic and protective properties [3, 4, 6, 9].

The attention has been given to the most common inflammatory diseases and their effect on the structural and functional state of the liver, primarily, hepatocytes.

Therefore, the paper was aimed at the study of the effect of cryopreserved placenta (CPP) for correction of the liver cells damage.

Methods and Material

95 Wistar male rats of 180-200 g body weight were involved into study.

Experimental studies have been made in compliance with the "General Ethic Rules for Conducting Experiments on Animals", adopted by the V National Congress on Bioethics (Kyiv, 2013) that are in concordance with the requirements of international principles of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (Strasbourg, 1986, 2000) and corresponding Law of Ukraine "For the Protection of Pet Animals" (No.3446-IV, as of 21.02.2006, Kyiv) and Declaration of Helsinki on ethical principles for medical research involving human subjects.

Experimental animals were assigned into three groups. Group I (n=5) involved intact animals.

Group II (n=45) involved animals exposed to simulated acute peritonitis (AP). The disease was induced by intraperitoneal administration of λ–carrageenan(5 mg in 1ml saline per one animal).

Group III (n=45) involved rats with simulated AP, induced similarly as for animals from Group II, and subsequently administered with CPP.

CPP was transplanted by the method of subcutaneous administration of placenta fragment, elaborated by the Institute of Cryobiology and Cryomedicine Problems of NAS of Ukraine (Kharkiv) [3].

The experimental study was carried out on day 1, 2, 3, 5, 7, 10, 14, 21 and day 30.

After euthanasia under thiopental anesthesia overdose, sampling of material in the form of a piece of liver, sized 0,5×0,5×0,5 cm, fixed in 10% formalin solution, was made.

Subsequently, the obtained liver fragments of animals from three groups were subjected to morphological studies using conventional histological methods.

For electron microscopic analysis, small pieces of the organ were fixed in 2.5% glutaraldehyde solution, postfixed in 1% solution of osmium tetroxide in phosphate buffer pH 7.2-7.4, dehydrated in alcohols and propylene oxide, and poured into epoxy resin mixture with araldite. Ultrathin sections were made on the LKB-3 ultramicrotome (Sweden), opacified with uranyl acetate and lead citrate by the Reynolds method and studied in a PEM-125K electron microscope.

Results and Discussion

Treatment of the liver disorder, induced by simulated peritonitis, was performed on *day 2*.

The findings of the experimental studies on *day 1* showed alteration similar to the inflammatory process: the structure of the liver was preserved; on *day 1-3* the

veins and capillaries, were plethoric, the vessels were dilated, containing numerous erythrocytes; blood clots were noted.

Following *day 5-7* hepatocytes , different in structure, were noted; some of them changed its shape, had different color intensity, the nuclei were preserved, but had signs of karyopicosis. Partial destruction of hepatocytes around the central veins and triads were detected (Fig. 1).

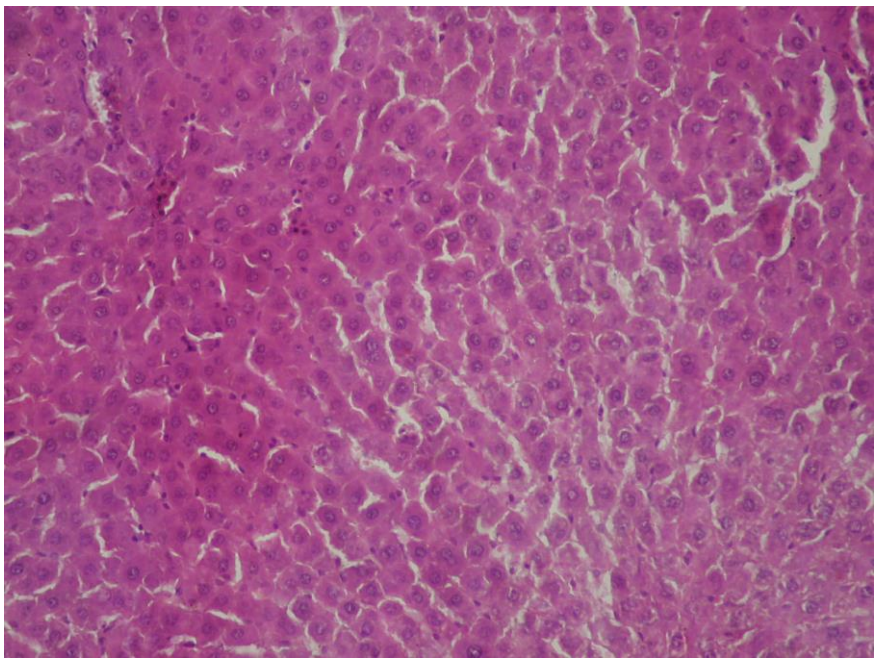

Fig. 1. Aseptic inflammation of the liver, karyopicosis of the nuclei of hepatocytes; day 5. H&E stain. 8×20 magnification.

In the following *day 10 to 14* granular dystrophy of the cytoplasm of hepatocytes was observed, in some hepatocytes its coagulation was detected, lumpy destruction was observed in most hepatocytes, focal necrosis was observed in some hepatocytes (Fig. 2). The Golgi complex underwent reduction and hypertrophy. The bile ducts were of the regular shape, containing a histiocytic infiltrate.

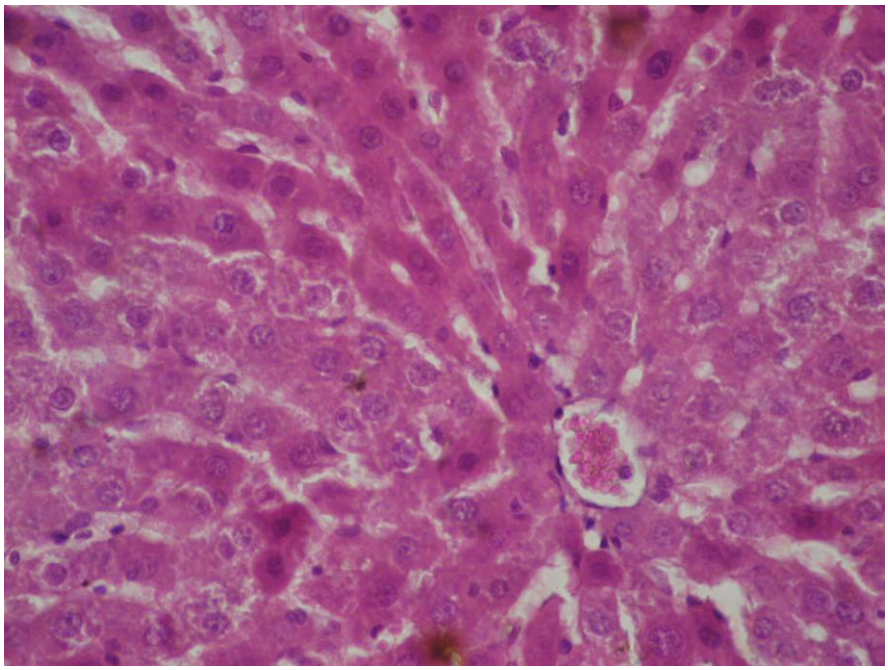

Fig. 2. Aseptic inflammation of the liver; day 14. Granular dystrophy of the cytoplasm of hepatocytes. H&E stain. 9×40 magnification.

Noteworthy, the above morphological alterations of hepatocytes were detected from day 1 to 14, regardless transplantation of cryopreserved placenta, performed concurrently for correction of the affected liver cells in the aseptic inflammation.

Marchenko L.N, et al. [6], Rassokhina A.V. [9] has proved experimentally that during transplantation of native and cryopreserved placenta from *day 1 to 14*, the aseptic inflammatory process developed in the surgical site in which the placenta gradually decayed and then resorbed. However, in the native placenta, these processes progressed faster than in cryopreserved one. Only following 2 weeks of the experiment the placental lysis products were absorbed (resorbed) into the surrounding tissues and vessels and the placenta started showing its properties.

On day 14, during the correction, along with morphologically altered hepatocytes, observed on *day 5-7-10*, hepatocytes with restored structure also appeared. We hypothesize that this indicated about processes of restoration as a result of the effect of the administered placenta. Notably, in this period of observation the presence of segmented leukocytes, indicating the antibacterial, anti-inflammatory and neutralizing effect of cryopreserved placenta, which led to a decrease in the manifestations of inflammation.

On *day 21*, the morphological state of the liver cells was normal, regeneration of most hepatocytes, containing 2-3 nucleoli, was noted; vessels were plethoric, and no blood clots were detected. Most hepatocytes were "dark" and only some of them were "clear".

The presence of hepatocytes with nuclei, containing 2-3 nucleoli, as well as "dark" hepatocytes, indicated regeneration of liver cells.

On day 30, regeneration of almost all hepatocytes was noted; their nuclei contained 2-3 nucleoli, no blood clots in veins and capillaries were detected, blood flow was restored.

However, electron microscopic studies of the rat liver specimens in experimental aseptic inflammation and administration of placenta, conducted in the early terms (*day 1, 3*) of the experiment revealed reactive changes in the ultrastructure of hepatocytes and hemocapillaries. Submicroscopic changes in these terms were similar to untreated animals with aseptic inflammation.

Submicroscopic signs of positive effect of cryopreserved placenta on the structure of hepatocytes and hematocapillaries in the liver specimens were established in the middle terms (*day 5, 7*) of the experiment.

The ultrastructural components of most sinusoidal blood capillaries in these terms of the experiment had a more preserved structure than in the liver of animals with aseptic inflammation. The lumens of the vessels were moderately dilated including mainly red blood cells, single leukocytes.

The nuclei of the endothelial cells had orbicular or elongated shape. In their karyoplasm, euchromatin prevailed. Karyolemma was clearly contoured, with a thickened perinuclear space between the nuclear membranes. The nuclear site of the endothelial cell cytoplasm included few small organelles that were less altered than in inflammation without correction. The GER canaliculi were slightly dilated and the matrix of individual mitochondria was enlightened.

Narrow cytoplasmic sections of endothelial cells had well-defined perforations. Well-contoured microvilli were observed in space of Disse (Fig. 3).

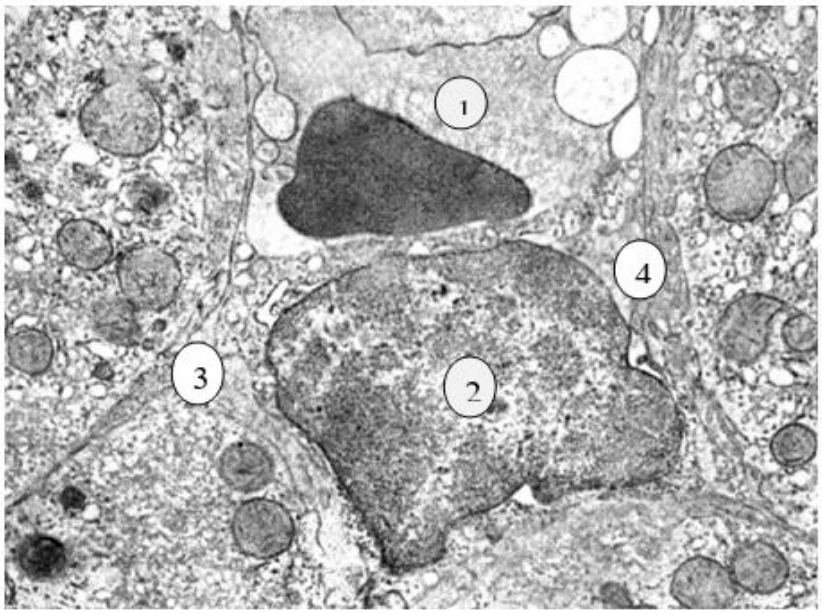

Fig. 3. Ultrastructure of hemocapillary of animal's liver in aseptic inflammation with administration of placenta. Day 5 of the experiment. Red blood cell in the lumen of hemocapillary (1), nucleus (2) and cytoplasm of endothelial cell (3), space of Disse with microvilli (4). ×15 000 magnification.

Macrophages with cytoplasm, containing lysosomes, were observed in some areas of the hemocapillaries.

Electron-microscopically, hepatocytes with different electron density of hyaloplasm, e.i. "dark" and "clear" liver cells were observed. Their ultrastructural components were significantly less altered than in the

liver of a group of animals with aseptic inflammation. In this way, the "clear" hepatocytes contained orbicular nuclei with clear contours of nuclear membranes, small perinuclear spaces and numerous nuclear pores. In the karyoplasm, euchromatin prevailed; ribosomal granules were noted. Large nucleoli were detected in some nuclei. Cytoplasm of hepatocytes included better structured organelles. In "dark" hepatocytes their density was greater. Orbicular or oblong mitochondria were mostly of medium size. Their outer membranes and cristas were clear, mitochondrial matrix was of moderate osmiophylia. In the "clear" cells, small amount of mitochondria was noted; some of them were hypertrophied, though small organelles were present, indicated about hyperplasia.

Electron microscopically, the greatest positive effect on the structural components of the liver was established in the late terms (day 14, 21) of the experiment in administration of placenta after aseptic inflammation. Numerous hepatocytes and hemocapillaries with minor modifications were noted, ultrastructure of which was close to the liver of animals of the intact group.

Hepatocytes contained orbicular nuclei with large nucleoli in the karyoplasm. Karyolemma contained clearly contoured nuclear membranes, the perinuclear space was smooth, and the areas of its extension were not detected.

Mainly orbicular and elongated mitochondria were well structured. Well defined cristas were noted in the moderately osmiophilic matrix. The lumens of the canaliculi of the GER and cisterns of the GC were not thickened; numerous ribosomes and primary lysosomes were detected. Rather narrow lumens of bile capillaries with microvilli, limited by intercellular contacts were noted (Fig. 4).

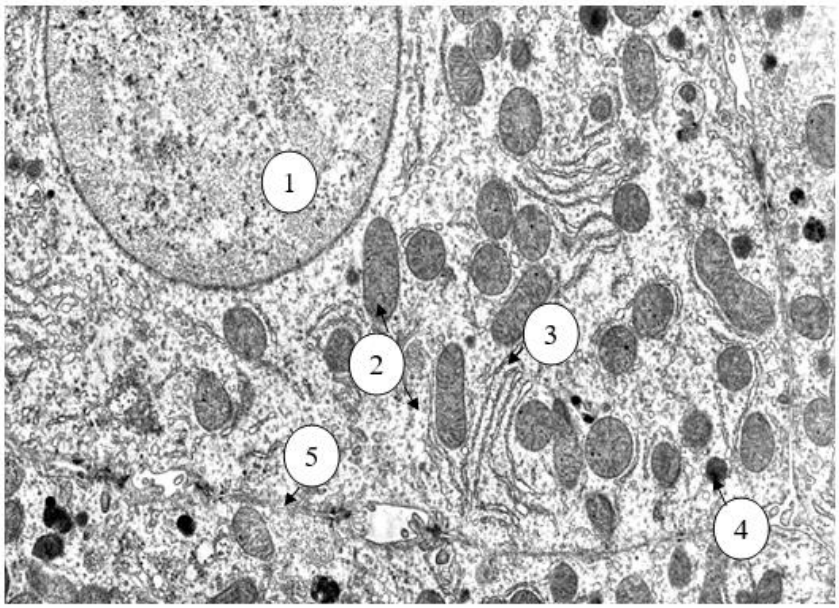

Fig. 4. Ultrastructure of hepatocytes in the liver specimen of the animal in correction of aseptic inflammation. Day 14 of the experiment after administration of placenta. Fragment of the nucleus (1), mitochondrion (2), canaliculi of the granular endoplasmic reticulum (3), lysosomes (4), bile capillary (5). ×17 000 magnification

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Hepatocytes with two large nuclei were observed. They contained well expressed fibrillar and granular components.

Conclusions

Experimental studies of liver cells in rats with cryopreserved placenta transplantation confirm all the properties of the placenta, as evidenced by numerous studies.

Starting from day 14, the histological specimens showed regeneration of hepatocytes, disappearance of blood clots, appearance of such cells as segmental nuclear leukocytes, confirming acceleration of inflammation, activation of Kupfer apparatus, with high phagocytic activity, regeneration of Golgi apparatus, responsible for metabolic processes.

Findings of electron microscopic studies of the liver of animals in correction of experimental aseptic inflammation with cryopreserved placenta have found that right in the early stages less damage to the structural components of the organ occurs.

Gradually, and especially at the later stages of the experiment, regeneration of the structure of the wall of sinusoidal hemocapillaries and liver hepatocytes was noted.

The use of the agent prevents and reduces damage to membrane organelles, nuclear and plasma membranes of endothelial cells and hepatocytes.

Consequently, administration of cryopreserved placenta promotes acceleration of inflammatory process realization and exhibits its multiple properties: immunomodulatory and immunostimulatory, desensitizing, antitoxic, powerful hepatoprotective, activation of blood flow, stimulates regeneration, reveals its antiaggregated properties, prevents formation of blood clots, activates regulation of the major functions of the organism.

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ARTISTRY IN HAIR TRANSPLANT BY MICRO-GRAFTING WITH FUE TECHNIQUES

Summary. Hair transplant in cosmetic surgery is no less than an art in which slitting is done with concentrating on proper depth and angulation of hair follicle. The most important aspect is to maintain proper angulation and direction of hair follicles while performing graft implantation and trying to achieve as much density as possible to give most natural results.

Key words: Hair Loss, Androgenetic Alopecia, Hair Transplant, Follicular Unit Extraction, PRP, Micro-grafting, Artistic Hair Transplant.

Background. Hair loss is a widespread non-malignant pathology with varying severity, age of onset, and scalp location. This disease affects more than 21 million people worldwide and by the age of 70 years, 80 % of the population will probably develop some type of hair loss. Alopecia is more prevalent in men than in women (70 % and 40 %, respectively. The loss of hair can have profound effects on one's self esteem and emotional well-being, as one's appearance plays a role in the work place and interpersonal relationships. Severe psychological disturbance has been reported in both genders including psychiatric symptoms such as depression and anxiety that lead to a reduction in the quality of life.

Hence, providing an appropriate treatment which gives durable and natural results against follicular degeneration is of paramount importance. It is therefore not surprising that Hair transplant surgery (HTS) with micro-grafting with FUE techniques has become increasingly popular, and the results that we are able to create today are quite remarkable, providing a natural appearance when the procedure is performed well.

Introduction. Hair transplantation has become a well-established procedure for the treatment of hair loss