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MORPHOLOGICAL CHANGES IN RAT LIVER STRUCTURE DURING CENTRAL DEPRIVATION OF LUTHEINIZING HORMONE SYNTHESIS AT 365TH DAY OF EXPERIMENT

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Macrophages play a central role in tissue homeostasis and inflammation. Origin, development and involvement of tissue-resident macrophages in defensive processes are fundamental to the future strategies to modulate macrophage functions. Resident liver macrophages include Kupffer cells, which form a significant population of antigen-presenting cells. The androgens plays an important role in the development of the liver in the embryonic stage, the maximum dimorphism of the effects occurs after puberty. Quercetin effect on the inflammatory process is explained by the blockade of the lipoxygenase pathway of arachidonic acid metabolism. The aim of our study was to determine the changes in Kupffer cells, which was caused by the introduction of triptorelin acetate solution. Together with the results of the quercetin effect on antigen-presenting liver cells. The experiments were performed on 30 adult male white rats. Rats were divided into 3 groups: control (10), experimental 1 (10) and experimental 2 (10). Changes in the liver of animals from group 1 occur on cellular and subcellular levels, causing minor venous stasis, erythrocyte sludge and macrophage infiltration. Concomitant oral administration of quercetin minimizes structural and morphological changes in rat liver tissue by increasing the antioxidant protection of liver tissue.

Key words: *resident macrophages, liver, triptorelin acetate, quercetin, rats.*

Relationship between the publication and planned research work. The study is a fragment of the research project «Experimental morphological study of the effect of cryopreserved preparations of cord blood and embryofetoplacental complex, diferelin, ethanol and 1% methacrylic acid on the morphofunctional state in a number of internal organs », state registration № 0119U102925.

Introduction. Macrophages play a central role in both tissue homeostasis and inflammation, performing important tissue-specific functions as well as protecting the body from infection. At the same time, they are also involved in the pathophysiology of many diseases, including cancer and various inflammatory processes. Understanding the origin, development and involvement of tissue-resident macrophages in the regulation of homeostatic processes is fundamental to the development of future intervention strategies to modulate macrophage functions in specific areas [1]. Resident macrophages (MFs) are found in all organs of the body, where they are adapted to perform specific functions necessary for tissue homeostasis. Unlike most immune cells derived from hematopoietic stem cells, tissue-resident MFs develop prenatally from embryonic progenitor cells, including those derived from the yolk sac and fetal liver monocytes. Most importantly, MFs of embryonic origin, regardless of the type of progenitor cells, have the ability to self-repair, and therefore, in most tissues, the niche MF remains inhabited by cells of embryonic origin in adulthood, without the influence of circulating monocytes [2].

Resident liver MFs include Kupffer cells, which together with sinusoidal cells and Ito cells form a significant population of nonparenchymal, antigen-presenting cells. Contrary to popular belief, the location of Kupffer cells is not limited to the lumen of blood vessels, but always a significant part of the cell body is located in the perisinusoidal space of Disse, where they interact closely with dendritic cells and hepatocytes [3].

At the present stage, researchers suggest that the polarization of macrophages may play a critical role in the development of liver diseases such as hepatitis, fibrosis and hepatocellular carcinoma, as well as in the regeneration of hepatocytes.

The androgen / androgen receptor (AR) interaction plays a role in the development of the liver in the embryonic stage, the maximum dimorphism of the effects of androgen / AR occurs after puberty [4] due to the activity of the hypothalamic-pituitary-gonadal axis. Currently, there is considerable interest in the effects of androgens on liver cells and the development of its pathology. In studies of non-cancerous liver disease, models of liver disease in androgen receptor knockout mice have shown that androgen / AR signaling inhibits the development of steatosis, viral hepatitis and cirrhosis [5].

Oxidative stress caused by testosterone deficiency plays a significant role in the development of organ and tissue damage. One of the promising methods of pathogenetic therapy and prevention of changes in the body caused by a decrease in testosterone levels may be the use of drugs with strong antioxidant effects.

The flavonoid quercetin is an aglycone of many plant flavonoid glycosides, including rutin. Due to its capillary-stabilizing properties associated with antioxidant, membrane-stabilizing effects, the drug reduces capillary permeability [6]. Its effect on the inflammatory process is explained by the blockade of the lipoxygenase pathway of arachidonic acid metabolism, reduced synthesis of leukotrienes, serotonin and other mediators of inflammation. Quercetin belongs to a group of drugs recommended in the complex treatment of coronavirus disease (COVID-19).

The aim of our study was to determine the qualitative and quantitative changes in Kupffer cells, as representatives of the population of immunocompetent liver cells, in the chemical castration of male rats of central origin, which was caused by the introduction of triptorelin acetate solution. As well as the results of the effect of quercetin on the morphological and quantitative changes of antigen-presenting liver cells on the background of previous administration of a solution of triptorelin acetate.

Object and methods of research. The experiments were performed on 30 adult male white rats. Rats were divided into 3 groups: control (10), experimental 1 (10) and experimental 2 (10). Animals from experimental group 1 were injected subcutaneously with triptorelin acetate at a dose of 0.3 mg of active substance per kg of body weight. Animals from experimental group 2 received triptorelin acetate in the same dosage and quercetin 100 mg per kg body weight 3 times a week, while the control group was administered 0.9% sodium chloride solution [6]. The animals were kept in standard conditions in the vivarium of Poltava State Medical University. Experimental animals were euthanized in strict compliance with the provisions of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes; (Strasbourg, 1986), as well as in accordance with the "General Ethical Principles of Animal Experiments", adopted by the First National Congress on Bioethics (Kyiv, 2001). Animals from the experimental groups were removed from the experiment on day 365 (n=20).

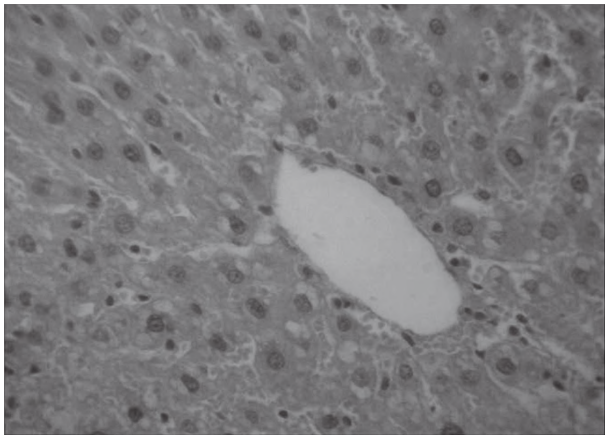

Figure 1 – Control group. Rat liver. Central vein. Haematoxylin and eosin staining. Magnification: lens 40, eyepiece 15.

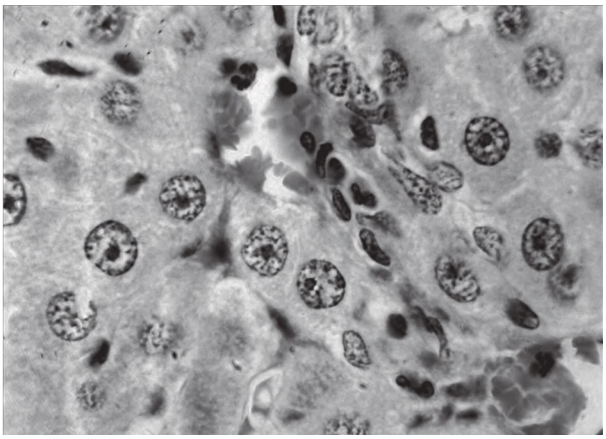

Figure 2 – Stasis in the blood vessels of the rat liver hemomicrocirculatory bed on the 365th day of the experiment. Hematoxylin and eosin stain. Magnification: lens x 100, eyepiece x 10.

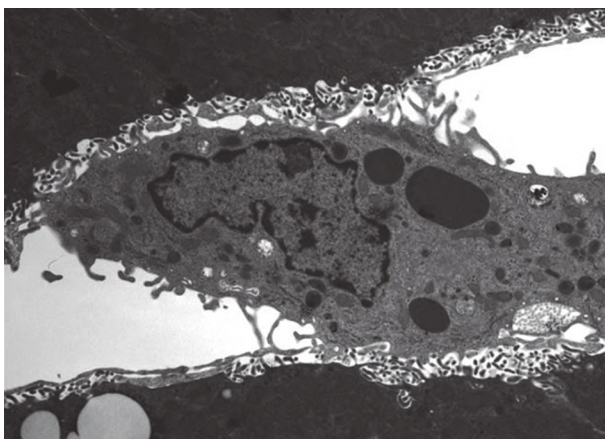

Figure 3 – Group I. Electron micrograph of Kupffer cell of experimental rats on 365th day. Magnification: x7000.

After overdose with ether solution, the animals were decapitated; pre-prepared small pieces of liver were fixed in 2.5% glutaraldehyde solution (pH=7.2-7.4). Postfixation of the material was performed with a 1% solution of osmium (IV) oxide, followed by dehydration in propylene oxide and introduction of the sample into a mixture of epoxy resins.

According to the standard method, the material was placed in paraffin blocks, from which sections with a thickness of 4 μm were made and stained with hematoxylin and eosin. Histological specimens were examined using a Biorex 3 light microscope with a digital microfilter and software adapted for similar studies (serial number 5604).

Statistical processing of the reserch results was performed using Microsoft Office Excel software and the extension of Real Statistics 2019 to it. The nonparametric Mann-Whitney test was used to determine the statistical significance of differences between groups. The difference was considered statistically significant at $p<0.05$.

The results of the research. In our study of serial semi-thin sections in the control group of animals, the liver corresponded to the general principle of the structure of the parenchymal organ, and was represented by components of the parenchyma and stroma, with a relative predominance of parenchyma over stroma. The structural subunits of the liver, the lobules, were clearly defined. A thin plate of connective tissue was present outside the hepatic lobules, with clear visualization of the bile ducts. The components of the hemomicrocirculatory tract, which branched in a series of semi-thin sections and passed into the interparticle arteries, interparticle veins, accompanied by the interparticle bile duct, were determined. The visualized microvessels were with sufficient blood supply. Passing through the terminal plate of hepatocytes, the portal vein and the hepatic artery connected to the sinusoids, which passed into the central vein **(fig. 1)**.

Hepatocytes were located radially in relation to the vein, each of them was adjacent to the intraparticle sinusoidal capillaries, forming the microcirculatory tract of the circulatory system of the liver. In the control group of animals, small Kupffer cells with oval shape, hyperchromic crescent-shaped nucleus and light cytoplasm were determined.

In the study of semi-thin sections of the liver of animals of experimental group I we found that the liver structure is preserved, the thickness of connective tissue bridges is increased due to the vascular component, but statistically insignificant, compared with the control group. We also did not find, compared with the control group of animals statistically significant changes in the vessels of the hepatic triads, except for the venous com-

ponent, the diameter of the veins was increased by 10-11% **(fig. 2)**.

The bile ducts are slightly dilated. The central veins are full-blooded, erythrocyte sludge is found in some places. Sinusoidal capillaries are dilated by 18% compared to the control group of animals. It was found that number of immunocompetent liver cells, namely Kupffer cells, compared with the control group, increased by 2.3 times, visual inspection revealed minor changes in the ultrastructure of these cells. Thus, on electron microscopic examination of Kupffer cells in animals from experimental group 1 on the 365th day of the experiment, it was determined that the cells were adjacent to the sinusoidal endothelium, some of them partly located by their cytoplasm in the perisinusoidal space and in contact with hepatocytes **(fig. 3)**.

The plasmolemma was uneven, in the form of pseudopodia with numerous microvilli protruding into the lumen of the sinusoids. The nuclei of most cells were irregularly shaped and mostly located in the center of the cell. The karyolema is dense, clear, contoured, the erychromatin predominated in the karyoplasm, and a small nucleolus adjacent to the karyolema was determined. Cytoplasm light, with a small number of primary and secondary lysosomes. Synthetic and metabolic apparatus of the cell was without visual changes.

Histological examination of semi-thin sections of the liver of animals from experimental group 2 on the 365th day of the experiment, the structure of the liver lobule was preserved. The sinusoids had a clear outline, with minimal manifestations of venous stasis, erythrocytes and leukocytes were detected in the lumen, and the walls were not thickened. When conducting electron microscopic examination of Kupffer cells of this group of animals, we did not detect pathological changes.

Discussion of the research results. Since balk structure of liver was not changed during term of experiment (365 days) we can state, that changes in the liver of animals from group 1 during this period mostly occur on cellular and subcellular levels as well as in the hemomicrocirculatory vessels, causing dilation of venous beds with minor venous stasis, erythrocyte sludge and macrophage infiltration. Because number of immunocompetent liver cells, namely Kupffer cells, compared with the control group, increased by 2.3 times, and minor changes in the ultrastructure of these cells was revealed further studies needed to distinguish type of macrophages polarization in the rat liver under the influence of central inhibition of testosterone synthesis.

The study and possible influence on the polarization of macrophages in liver disease to block or even reverse pathophysiological changes is considered as a potential strategy for the treatment of pathological processes [7]. However, the origin of liver macrophages and the mechanism of their polarization are complex, and the impact on different types of liver disease and even at different stages of one disease is various [8]. Therefore, the process of macrophage polarization, its role and mechanism in liver disease need further study and elucidation.

In general, macrophage polarization phenotypes can be divided into classically activated M1 and alternatively activated M2 [9]. M1 macrophages are also known as proinflammatory macrophages because they can secrete large amounts of proinflammatory cytokines such as L-1b, inducible nitric oxide synthase (iNOS), tumor necrosis factor-a (TNF-a) [10]. Conversely, M2 macrophages are known as anti-inflammatory macrophages because they mainly produce anti-inflammatory factors such as IL-10, transforming growth factor-b (TGF-b), arginase 1 (Arg1).

The shift in the polarization of macrophages towards the predominance of M1 may be due to the development of endothelial dysfunction, which occurs due to insufficient testosterone production [11]. Endothelial dysfunction can lead to oxidative damage to various organs and tissues due to excessive production of reactive oxygen species [12]. Sources of excess production of reactive oxygen species can be constitutive forms of NO synthase, which with increased activity can produce not only nitric oxide, but also superoxide anion radical [13]. With the simultaneous production of cNOS nitric oxide and superoxide anion radical, the formation of a powerful nitrating agent – peroxynitrite is not excluded.

Peroxynitrite and superoxide anion radical are powerful oxidants that can damage biological polymers (DNA strands, proteins and biological membranes) and lead to the development of oxidative-nitrosative stress. Damage to biological membranes leads to the activation of proinflammatory transcription factors, such as NF-kB, which is able to change the polarization of macrophages to the M1 phenotype [14]. Quercetin is a powerful antioxidant that enhances the protection of cells from oxidative damage both by direct interception of reactive oxygen species and by stimulating the activity of the glutathione system [15]. However, quercetin is also able to inhibit the activation of the transcription factor NF-kB [16]. Quercetin may also prevent the development of endothelial dysfunction [17]. Thus, the use of quercetin affects all pathogenetic links that lead to a shift in the polarization of macrophages to the M1 phenotype and, naturally, leads to the restoration of the predominance of the M2 phenotype in the liver.

Conclusions. Central blockade of luteinizing hormone synthesis by administration of triptorelin acetate on the 365th day of the experiment causes morphological changes in the liver structure of rats, namely in cellular and connective tissue and vascular components.

Concomitant oral administration of quercetin minimizes structural and morphological changes in rat liver tissue from oxidative damage caused by triptorelin injection by increasing the antioxidant protection of liver tissue.

The obtained results provide a theoretical basis for the development of methods of correction for extreme effects on the body. Data on the functional morphology of the liver at the stages of adaptation to changes in endocrine activity of the hypothalamic – pituitary – gonad axis expand the understanding of the causes of metabolic disorders in the structural components of the liver and the possibility of its regulation.

Prospects for further research. Additional studies are necessary to evaluate precise changes in the cooperation between liver immunocompetent cells, the way of the shift in the polarization of macrophages formation towards the predominance of M1 subtype. To compare and estimate the effects of quercetin on morphological and quantitative changes of antigen-presenting liver cells during different terms of experiment. The data can be used in research and teaching at the departments of medical universities and faculties of biology.

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МОРФОЛОГІЧНІ ЗМІНИ СТРУКТУРИ ПЕЧІНКИ ЩУРІВ ПРИ ЦЕНТРАЛЬНІЙ ДЕПРИВАЦІЇ СИНТЕЗУ ЛЮТЕЇНІЗУЮЧОГО ГОРМОНУ НА 365 ДЕНЬ ЕКСПЕРИМЕНТУ

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Резюме. Макрофаги відіграють центральну роль у тканинному гомеостазі та запаленні. Розуміння походження, розвитку та участі макрофагів, що є резидентними для тканин, у регуляції гомеостатичних процесів є основоположним для розробки майбутніх стратегій втручання для модуляції функцій макрофагів у певних ділянках. Резидентні макрофаги печінки включають клітини Купфера, які разом із синусоїдними клітинами та клітинами Іто утворюють значну популяцію непаренхімних, антигенпрезентуючих клітин. Взаємодія андроген/андрогенний рецептор відіграє роль у розвитку печінки в ембріональній стадії, максимальний диморфізм ефектів настає після статевого дозрівання за рахунок діяльності гіпоталамо-гіпофізарно-гонадної осі. Вплив кверцетину на запальний процес пояснюється блокадою ліпоксигеназного шляху метаболізму арахідонової кислоти, зниженням синтезу лейкотрієнів. Метою нашого дослідження було визначення якісних та кількісних змін у клітинах Купфера при хімічній кастрації самців щурів центрального походження, яка була спричинена введенням розчину трипторелін ацетату. А також визначення результатів дії кверцетину на антигенпрезентуючі клітини печінки на тлі ефектів, викликаних використанням розчину трипторелін. Експерименти проводили на 30 дорослих самцях білих щурів. Щурів розділили на 3 групи: контрольну (10), дослідну 1 (10) та дослідну 2 (10). Тваринам 1 дослідної групи вводили підшкірно трипторелін ацетат у дозі 0,3 мг діючої речовини на кг маси тіла. Тварини 2 дослідної групи отримували трипторелін ацетат у такому ж дозуванні та кверцетин 100 мг на кг маси тіла 3 рази на тиждень. Відмічені зміни в печінці тварин 1 групи на клітинному та субклітинному рівнях, а також у гемомікроциркуляторних судинах, що призвели до незначного венозного застою, сладжу еритроцитів та інфільтрації макрофагами. Одночасне пероральне застосування кверцетину мінімізує структурні та морфологічні зміни в тканині печінки щурів від окисного пошкодження, спричиненого ін'єкцією трипторелін, за рахунок підвищення антиоксидантного захисту тканин печінки.

Ключові слова: резидентні макрофаги, печінка, трипторелін ацетат, кверцетин, щури.

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population of non-parenchymal, antigen-presenting cells. The androgen / androgen receptor (AR) interaction plays a role in the development of the liver in the embryonic stage, the maximum dimorphism of the effects of androgen / AR occurs after puberty due to the activity of the hypothalamic-pituitary-gonadal axis. Quercetin effect on the inflammatory process is explained by the blockade of the lipoxygenase pathway of arachidonic acid metabolism, reduced synthesis of leukotrienes. The aim of our study was to determine the qualitative and quantitative changes in Kupffer cells during chemical castration of male rats of central origin, which was caused by the introduction of a solution of triptorelin acetate. And also to elucidate the results of the quercetin action on antigen-presenting liver cells on the background of the effects caused by the use of triptorelin solution. The experiments were performed on 30 adult male white rats. Rats were divided into 3 groups: control (10), experimental 1 (10) and experimental 2 (10). Animals from experimental group 1 were injected subcutaneously with triptorelin acetate at a dose of 0.3 mg of active substance per kg of body weight. Animals from experimental group 2 received triptorelin acetate in the same dosage and quercetin 100 mg per kg body weight 3 times a week. Changes in the liver of animals from group 1 occur on cellular and subcellular levels, causing minor venous stasis, erythrocyte sludge and macrophage infiltration. Concomitant oral administration of quercetin minimizes structural and morphological changes in rat liver tissue by increasing the antioxidant protection of liver tissue.

Key words: resident macrophages, liver, triptorelin acetate, quercetin, rats.

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Conflict of interest statement
The authors declare no competing interests.

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