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THE EFFECT OF QUERCETIN ON MORPHOLOGICAL AND BIOCHEMICAL CHANGES IN RAT LIVER UNDER 270TH DAY CENTRAL DEPRIVATION OF LUTEINIZING HORMONE SYNTHESIS

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Розвиток запального процесу в печінці, в тому числі під впливом вірусів гепатиту В і С, контролюється клітинами імунної системи, а саме синусоїдальними ендотеліальними клітинами, клітинами Ito та клітинами Купфера. Макрофаги відіграють одну з ключових ролей у створенні лінії імунного захисту. Яким саме чином певні популяції макрофагів сприяють захворюванням печінки та її регенерації, є предметом постійних дискусій. Тому виявлення особливостей цих популяцій макрофагів людини має беззаперечну цінність при вивченні їх ролі у розвитку патології печінки. Флавоноїд кверцетин має капіляростабілізуючі властивості завдяки антиоксидантному, мембраностабілізуючому ефекту. Метою нашого дослідження було визначити якісні та кількісні зміни імунокомпетентних клітин печінки, спричинені пригніченням центрального синтезу тестостерону у самців щурів внаслідок введення триптореліну ацетату на 270-ту добу, а також потенційний вплив кверцетину на зміну морфології та кількості антигенпрезентуючих клітин печінки на тлі попереднього введення розчину триптореліну ацетату. Експерименти були проведені на 30 статевозрілих самцях білих щурів. Щурів розподіляли на 3 групи: контрольну (10), експериментальну I (ЕГ I, 10) та експериментальну II (ЕГ II, 10). Тваринам з експериментальної групи 1 підшкірно вводили триптореліну ацетат у дозі 0,3 мг активної речовини на кг маси тіла. У експериментальній групі 2 тварини отримували триптореліну ацетат у такому ж дозуванні та кверцетин 100мг на кг маси тіла 3 рази на тиждень, у той час як контрольній групі вводили фізіологічний розчин. Біохімічні дослідження проводили в 10% гомогенаті тканин печінки. Було визначено основна продукція супероксид-аніонного радикала (SAR), а також активність супероксиддисмутази. Пероксинітрит і супероксид аніон-радикал є потужними окислювачами, які можуть пошкоджувати біологічні полімери (ДНК, білки та біологічні мембрани) і призводити до розвитку окисно-нітрозативного стресу.

Ключові слова: печінка, макрофаг, депривація тестостерону, трипторелін, оксид азоту, NO – синтаза, кверцетин, щури.

The development of the inflammatory process in the liver, including under the influence of hepatitis B and C viruses, is controlled by cells of the immune system, namely, sinusoidal endothelial cells, Ito cells and Kupffer cells. Macrophages play one of the key roles in creating the line of defense. The way in which specific populations of macrophages contribute to liver disease and regeneration is a matter of constant debate. Therefore, identifying the characteristics of these populations of human macrophages is of undeniable value in studying their role in the development of liver pathology. The flavonoid quercetin has capillary-stabilizing properties due to its antioxidant and membrane-stabilizing action. The aim of our study was to determine the changes in immunocompetent liver cells, both qualitative and quantitative, caused by inhibition of central testosterone synthesis in male rats due to the introduction of triptorelin acetate on the 270th day, and the potential effect of quercetin on morphology and liver antigen-presenting cells count against the background of previous administration of triptorelin acetate solution. The experiments were performed on 30 adult male white rats. Rats were divided into 3 groups: control (10), experimental I (10), and experimental II (10). Animals from experimental group I were injected triptorelin acetate subcutaneously at a dose of 0.3 mg of active substance per kg of body weight. In experimental group II, animals received triptorelin acetate in the same dosage and quercetin 100 mg per kg body weight 3 times a week, whereas the control group was administered saline. We conducted biochemical studies in 10% liver tissue homogenate. The main production of superoxide anionic radical (SAR) and superoxide dismutase activity were determined. Peroxynitrite and superoxide anion radical are powerful oxidants that can damage biological polymers (DNA, proteins and biological membranes) and lead to the development of oxidative-nitrosative stress.

Key words: liver, macrophage, deprivation of testosterone, triptorelin, nitric oxide, NO – synthase, quercetin, rats.

The development of the inflammatory process in the liver, including under the influence of hepatitis B and C viruses, is controlled by cells of the immune system, namely, sinusoidal endothelial cells, Ito cells and Kupffer cells [1]. The latter are important part of the mononuclear phagocyte system [3]. Macrophages play one of the key roles in creating the line of defence. The implementation of this function is carried out due to the direct mechanism of influence: recognition, absorption and destruction of a foreign object, as well as due to the indirect mechanism – processing and presentation of antigenic determinants to T lymphocytes. The impact of androgens and their interaction with receptors (AR) can be both temporary and long-term, exerting local or systemic effects on organ functions. The most well-known androgen / AR-dependent cancer is prostate cancer.

The way in which specific populations of macrophages contribute to liver disease and regeneration is a

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matter of constant debate. Bilzer, M. (2006) [5] showed that certain subpopulations of macrophages predominate in liver disease (including liver transplant rejection and liver cancer). Therefore, identification of the characteristics of these populations of human macrophages is of undeniable value in studying their role in the development of liver pathology.

As a result of the activation of Kupffer and Ito cells, mainly due to their collagen production, the process of liver fibrogenesis is initiated; severe forms of chronic hepatitis are developed and transformed into cirrhosis [10]. Liver fibrosis is the most common liver reaction to toxic, infectious or metabolic agents. Induction of this process increases the number of components of plasma membranes, which leads to constant growth of membrane-like structures in the Disse's space and reduces the number of endothelial sinusoidal pores. This leads to a complex process called "sinusoidal capillarization".

Liver damage can lead to the accumulation of matrix proteins, scarring, and changes in tissue structure and function. In the case of fibrosis, chronic compensatory scarring processes begin in the liver. With the occurrence of irreversible distortions of the hepatic architecture and structure of blood vessels, the functional units of the liver begin to undergo cirrhotic changes.

The impact of androgens and their interaction with receptors (AR) can be both temporary and long-term, exerting local or systemic effects on organ functions [8]. The most well-known androgen / AR-dependent cancer is prostate cancer. Androgen / AR ablation is currently the gold standard for treating patients with prostate cancer; however, not all patients respond to such treatment.

The flavonoid quercetin has capillary-stabilizing properties due to its antioxidant, membrane-stabilizing action. There is also a certain anti-inflammatory effect by blocking the lipoxygenase pathway of arachidonic acid metabolism, reducing the synthesis of leukotrienes, serotonin and other proinflammatory mediators [6,7].

The aim of our study was to determine the changes in immunocompetent liver cells, both qualitative and quantitative, caused by inhibition of central testosterone synthesis in male rats due to the introduction of triptorelin acetate on the 270th day, and the potential effect of quercetin on morphology and liver antigen-presenting cells count against the background of previous administration of triptorelin acetate solution.

Materials and methods

The experiments were performed on 30 adult male white rats. Rats were divided into 3 groups: control (10), experimental I (10), and experimental II (10). Animals from experimental group I were injected triptorelin acetate subcutaneously at a dose of 0.3 mg of active substance per kg of body weight [12]. In experimental group II, animals received triptorelin acetate in the same dosage and quercetin 100 mg per kg body weight 3 times a week [2], whereas the control group was administered saline. The animals were kept in standard vivarium conditions at 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 by ether overdose on day 270 (n = 20).

Using standard methods, the material was embedded in paraffin blocks, from which 4 μm thick sections were made and stained with hematoxylin and eosin [1]. Histological specimens were examined using a Biorex 3 light microscope with a digital microfilter and software adapted for similar studies (serial number 5604). For electron microscopic examination, previously prepared small pieces of liver were fixed in 2.5% glutaraldehyde solution (pH = 7.2–7.4). Post-fixation of the material was performed with a 1% solution of osmium (IV) oxide, followed by dehydration in propylene oxide, after which the sample was added to a mixture of epoxy resins.

Using the standard plot method, images were taken at 400 and 1000 magnifications using a Micromed microscope with TSView software adapted for monitoring.

Biochemical studies were performed in 10% liver tissue homogenate using Ulab 101 spectrophotometer. The main production of superoxide anionic radical (SAR) was determined by increasing the concentration of diformazan formed in the SAR reaction with nitro blue tetrazolium. Superoxide dismutase (SOD) activity was determined by inhibition of adrenaline autooxidation, whereas catalase activity was determined by the amount of hydrogen peroxide remaining after its catalase-dependent reduction [12]. The concentration of free malonic dialdehyde (MDA) was determined by reaction with 1-methyl-2-phenylindole.

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

Results and discution

In our study of serial semi-thin sections of the liver in the control group of animals, the liver corresponded to the general principle of the parenchymal organ structure, which was represented by components of the stroma and parenchyma, with a certain predominance of parenchyma over stroma. The liver capsule was represented by a thin connective tissue plate with a small number of microcirculatory vessels. The structural subunits of the liver – lobes – were clearly defined (Fig.1).

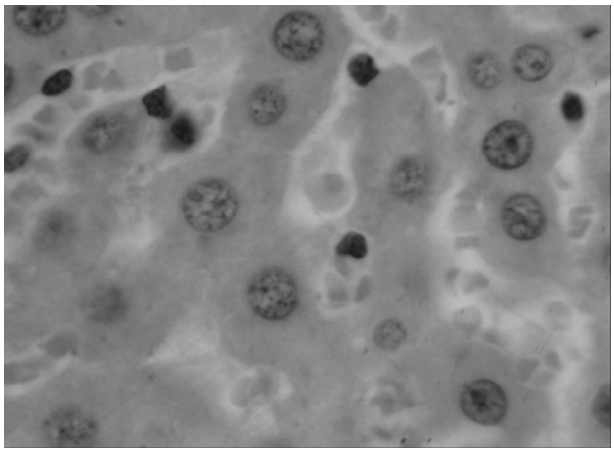

Fig. 1. Stasis in the blood vessels of the rat liver hemomicrocirculatory bed on the 270th day of the experiment. Hematoxylin and eosin stain. Magnification: Lens x 100, Eyepiece x 10.

Thus, the hepatic lobe was externally represented by a thin plate of connective tissue, with clear visualization of the bile ducts. In a series of semi-thin sections, components of the hemomicrocirculatory tract branched and passed into the interparticle arteries, interparticle veins, accompanied by the interparticle bile duct. Lymphatic vessels were also visualized in some lobes.

The microvessels were of sufficient blood supply. The vessels entered the lobes of the liver and at its periphery merged with the capillaries that originated in the portal veins. Passing through the terminal plate of hepatocytes, the portal vein and the hepatic artery connected with the sinusoids, which passed into the central vein, radially from which the hepatocytes were located. Intraparticle sinusoidal capillaries that formed the microcirculatory tract of the hepatic circulatory system were adjacent to each hepatocyte. In the control group of animals, we identified small Kupffer cells of oval shape, which had a hyperchromic crescent-shaped nucleus and light cytoplasm.

Our electron microscopic study of the liver of animals in the control group demonstrated that Kupffer cells acted in the lumen of sinuses. Their plasmolemma was uneven, in the form of pseudopodia or microvilli, which were immersed in Disse's space and approached the endothelial cells of hemocapillaries. Irregularly shaped nuclei were mainly located in the center of the cell. The karyolemma is clear, contoured, euchromatin prevailed in karyoplasm, and a small nucleolus was defined. Primary and secondary lysosomes were found in the cytoplasm of such cells. There were also small mitochondria of round, oval or oblong shape, in moderate quantities, located diffusely throughout the cytoplasm. Separated tanks of the granular endoplasmic reticulum and the Golgi complex were identified.

In our study of semi-thin sections of the liver of animals in 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 as compared to the control group. We did not find statistically significant changes in the vessels of hepatic triads as compared to the control group of animals, except for the venous bed, whose diameter was increased by 9%. The bile ducts are slightly dilated. The central veins are plethoric, sometimes erythrocyte sludge is detected in comparison with the control group of animals. The number of immunocompetent liver cells, namely Kupffer cells, increased by 2.1 times as compared to the control group, their visual inspection reveals minor changes in the ultrastructure. Thus, electron microscopic examination of Kupffer cells with central deprivation of testosterone synthesis on day 270th of the experiment determined that the cells were adjacent to the sinusoidal endothelium, some of which were in the perisinusoidal space and in contact with hepatocytes. Plasmolema was uneven, in the form of pseudopodia with numerous microvilli that protruded into the lumen of the sinusoids. The nuclei of most cells are irregularly shaped, mostly located in the center of the cell. The karyolema is dense, clear, contoured, euchromatin prevailed in the karyoplasm, and a small nucleolus adjacent to the karyolema was determined. Light cytoplasm with a small number of primary and secondary lysosomes. Synthetic and metabolic apparatus of the cell was without visual changes (Fig.2.)

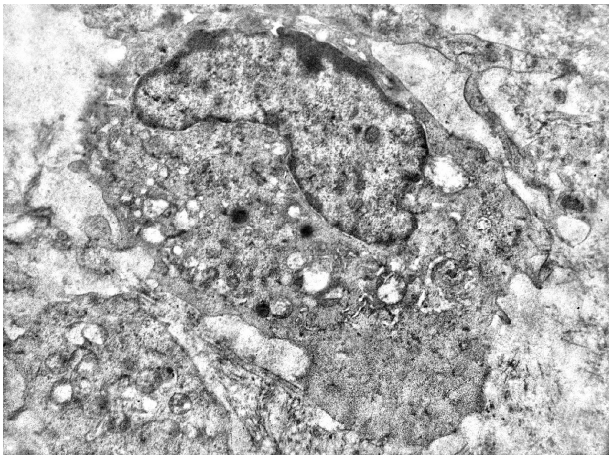

Fig. 2. Macrophage of rat liver in animals of group II. Magnification: x12000. Labeling: 1 – nucleus, 2 – chromatin, 3 – mitochondria, 4 – endoplasmic reticulum, 5 – cytoplasm.

In histological examination of semi-thin sections of the liver in animals from group II on the 270th day of the experiment, the structure of the liver lobe was preserved. The sinusoids had a clear contour, without manifestations of venous stasis, erythrocytes and leukocytes were determined in the lumen, the walls were not enlarged. We did not detect pathological changes when conducting electron microscopic examination of Kupffer cells of animals in this group.

When we analyzed the biochemical parameters of the two experimental groups and the control group, we found changes in the activity of NO synthases and arginases (Table 1). Prolonged administration of triptorelin leads to an increased activity of gNOS by 51.97% and iNOS by 51.64% as compared to the control group. CNOS activity also increases by 76.60%. ARG activity decreases by 37.23%. The concentration of nitrites in the liver of rats does not change statistically significantly.

Table 1.

The activity of nitric oxide cycle enzymes in rat liver under 270th day central deprivation of luteinizing hormone synthesis and quercetin administration (M ± m)

Biochemical parameters	Groups		
	Control	I (9 months)	II (9 months + quercetin)
gNOS, μmol / min per g of protein	1.27±0.09	1.93±0.11*	0.65±0.11**
iNOS, μmol / min per g of protein	1.22±0.09	1.85±0.11*	0.61±0.11**
cNOS, μmol / min per g of protein	0.047±0.0004	0.083±0.00*	0.040±0.0004**
NO2, nmol / g	4.99±0.28	4.34±0.16	4.32±0.18
ARG, μmol / min per g of protein	1.88±0.04	1.18±0.05*	1.54±0.04**

** - data are statistically significantly different from the control group (p <0.05)*

*** - data are statistically significantly different from experimental group I (p <0.05)*

The use of quercetin against the background of long-term administration of triptorelin leads to a decrease in the activity of gNOS by 66.32% and iNOS by 67.03% as compared to the control group. CNOS activity is also reduced by 51.81%. ARG activity increases by 30.51%. The concentration of nitrites in the liver of rats under these conditions does not change statistically significantly.

Results and discussion

INOS activity can be used as a marker of macrophage polarization by the M1 phenotype, whereas ARG activity is a clear marker of the M2 phenotype. This is due to the fact that these enzymes are expressed by macrophages polarized by a specific phenotype, and they cannot be expressed together [4]. It can be stated that with prolonged central deprivation of luteinizing hormone synthesis by triptorelin, the polarization of liver macrophages is shifted towards the predominance of the M1 phenotype, as the iNOS / ARG ratio increases to 1.57 versus 0.65 in the control group.

The shift in the polarization of macrophages towards the predominance of M1 may appear due to the development of endothelial dysfunction, which occurs because of insufficient testosterone production [3]. Endothelial dysfunction can lead to oxidative damage of various organs and tissues due to excessive production of reactive oxygen species [9]. Sources of excessive 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. 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, proteins and biological membranes) and lead to the development of oxidative and nitrosative stress. Damage to biological membranes provides further activation of proinflammatory transcription factors, such as NF-kB, which is able to change the polarization of macrophages for the M1 phenotype [10]

Quercetin is a powerful antioxidant that can enhance the protection of cells from oxidative damage by both direct interception of reactive oxygen species and by stimulating the activity of the glutathione system [14]. However, quercetin is also able to inhibit the activation of the transcription factor NF-kB [6]. Quercetin may also prevent the development of endothelial dysfunction [7]. 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 day 270 of the experiment causes morphological changes in the liver structure of rats, which are characterized by alterations in the cellular and tissue ratio of connective tissue elements and variations in the vascular component of the liver.

Concomitant oral administration of quercetin protects rat liver tissue from oxidative damage caused by triptorelin injection by increasing the antioxidant protection of liver tissue, which is manifested in the increased arginase-dependent cleavage of arginine.

These findings provide a theoretical basis for the development of methods for correction of extreme effects on the body. The data on the functional morphology of the liver at the stages of adaptation to alterations in endocrine status are expanded by understanding the causes of metabolic disorders in the structures of the liver and the possibilities of its regulation.

The obtained data can be used in research and teaching work at the departments of medical universities and faculties of biology.

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