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Стаття надійшла 29.05.2020 р.

**DOI 10.26724/2079-8334-2021-2-76-238-242**
**UDC 612.0612.561:603.323.4**

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# THE REACTION OF IMMUNOCOMPETENT LIVER CELLS DURING CHEMICAL CASTRATION OF MALE RATS CAUSED BY THE INTRODUCTION OF TRIPTORELIN ACETATE

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The purpose of our study was to elucidate the qualitative and quantitative changes in immunocompetent liver cells during chemical castration of male rats of central origin caused by the introduction of triptorelin acetate solution at different time intervals, to determine the sources of nitric oxide production as well as the effects of quercetin on morphological and quantitative changes of antigenpresenting liver cells. The morphological changes in the experimental groups with central testosterone deprivation and with its subsequent correction with quercetin on the 30th day of the experiment are identical and include signs of stromal – vascular changes in the form of an increase in the thickness of the beam system and minor venous stasis with macrophage infiltration. Central 30 – day deprivation of testosterone synthesis leads to a shift in the functioning of enzymes of the nitric oxide cycle in rat liver towards a predominance of the activity of inducible NO-synthase.

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

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# РЕАКЦІЯ ІМУНОКОМПЕТЕНТНИХ КЛІТИН ПЕЧІНКИ ПРИ ХІМІЧНІЙ КАСТРАЦІЇ САМЦІВ ЩУРІВ, ВИКЛИКАНІЙ ВВЕДЕННЯМ ТРИПТОРЕЛІНУ АЦЕТАТУ

Метою дослідження було з’ясувати якісні та кількісні зміни імунокомпетентних клітин печінки під час хімічної кастрації самців щурів центрального походження, спричинених введенням розчину трипторелiну ацетату через різні проміжки часу, визначити джерела вироблення оксиду азоту, а також вплив кверцетину на морфологічні та кількісні зміни антигенпрезентуючих клітин печінки. Морфологічні зміни в експериментальних групах з центральною депривацією тестостерону та з подальшою його корекцією кверцетином на 30 день експерименту ідентичні і включають ознаки стромально–судинних змін у вигляді збільшення товщини балкової системи та незначний венозний застій з макрофагальною інфільтрацією. Центральна 30–денна депривація синтезу тестостерону призводить до зсуву функціонування ферментів циклу оксиду азоту в печінці щурів у бік переважання активності індуцибельної NO-синтази.

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

*The study is a fragment of the research project “Experimental morphological study of cryopreserved placenta transplants action of diphereline, ethanol and 1 % methacrylic acid on the morphofunctional status in a number of internal organs”, state registration No. 0119U102925.*

Testosterone is an important biological regulator of metabolic processes in the male body. However, with age, the intensity of testosterone production decreases, which leads to its deficiency in the body with further development of anxiety and depressive behaviour, which is associated with apoptosis of hippocampal neurons as a result of the oxidative stress development [10]. In our previous studies, it was shown that central deprivation of testosterone synthesis by triptorelin leads to overproduction of nitric oxide and the development of oxidative stress in the testes of rats [11].

Testosterone deficiency can affect not only the testes and neurons, but also other organs and tissues of the body, in particular the liver. So, with insufficient testosterone production and protein deficiency, fat accumulates in hepatocytes, which is eliminated by an increase in testosterone levels in the body [12]. Exogenous long – term administration of testosterone improves the course of non–alcoholic fatty liver disease by reducing the intensity of fatty liver infiltration [14].

The liver, due to its structure, anatomical location and performance of specific functions plays an important role in the processes of interaction of the organism with the environments. Because the liver is a major barrier to the entry of nutrients, xenobiotics, and microorganisms from the gut into the bloodstream,

the relationship between the hepatobiliary and immune systems is quite complex. To antigen–presenting liver cells belong Kupffer cells that are part of the reticuloendothelial system, sinusoidal endothelial liver cells and dendritic cells [7]. There are very large populations of hepatic macrophages, both resident and recruited, which are involved in the pathogenesis of diseases and actively respond to therapeutic manipulations [3].

Oxidative stress caused by testosterone deficiency is an important pathogenic link in organ and tissue damage [5]. The use of drugs with a powerful antioxidant effect can be a promising method of pathogenetic therapy and prevention of changes in the body that are caused by testosterone deficiency.

The physiological effect of testosterone is an increase in the production of nitric oxide (NO) from the endothelial isoform of NO-synthase; however, oxidative stress under conditions of testosterone deficiency can lead to the activation of the inducible isoform of NO-synthase, which can lead to the formation of peroxynitrite [6, 9]

The flavonoid quercetin has capillary–stabilizing properties associated with antioxidant, membrane-stabilizing effects, as well as anti-inflammatory effect by blocking the lipoxygenase pathway of arachidonic acid metabolism, reducing synthesis of leukotrienes, serotonin and other mediators [1]. Quercetin belongs to the group of drugs recommended in the complex treatment of coronavirus disease (COVID-19).

**The purpose** of the study was to elucidate the qualitative and quantitative changes in immunocompetent liver cells during chemical castration of male rats of central origin caused by the introduction of triptorelin acetate solution at different time intervals, to determine the sources of nitric oxide production as well as the effects of quercetin on morphological and quantitative changes of antigen-presenting liver cells.

**Materials and methods.** The experiments were performed on 30 adult male white rats. Rats were divided into 3 groups: group I – control (10), animals from group II (10) were injected subcutaneously with triptorelin acetate at a dose of 0.3 mg of active substance per kg of body weight. Group III animals (10) who received triptorelin acetate in the same dosage and quercetin 100 mg per kg body weight 3 times a week [2], while the control group was administered saline [4].

The animals were kept in standard conditions at 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 of Bioethics (Kyiv, 2001). Animals were sacrificed on day 30 (n=30) by overdose of thiopental anesthesia.

Animals were decapitated, the prepared small pieces of the liver were fixed in a 2.5 % glutaraldehyde solution (pH=7.27.4). Postfixation of the material was carried out with 1 % solution of osmium (IV) oxide, followed by dehydration in propylene oxide and a sample was embedded into the epoxy resins mixture. Ultrathin sections made with an ultramicrotome were contrasted with a 1 % aqueous solution of uranyl acetate and lead citrate according to the Reynolds' method and studied with an electron microscope. Ultrathin sections were made on an ultramicrotome UMTP-4 and mounted on blends. Contrasting of the sections was performed in a solution of uranyl acetate and lead citrate. Sections were studied with an electron microscope MBR-100L at an accelerating voltage of 75 kW.

Using standard methods, the material was imbedded in paraffin blocks, of which sections 4 μm thick were made and stained with hematoxylin and eosin [1]. Histology specimens were examined using Biorex 3 light microscope with digital microfilter with software adapted for these studies (Serial No.5604).

All biochemical studies were carried out in 10 % homogenate of liver tissue using Ulab 101 spectrophotometer. General activity of NO-synthase (gNOS), activity of constitutive isoforms (cNOS), activity of inducible isoform (iNOS), activity of arginases and nitrite concentration was determined by methods described by Yelins'ka A.M. [15].

Statistical processing of the survey 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 $p<0.05$.

**Results of the study and their discussion.** When studying serial semithin liver sections of the control group of rats, the liver corresponded to the general structural organization of the parenchymal organ, which was represented by parenchymal and stromal components, with a predominance of the parenchyma over the stroma. So the liver capsule was represented by a thin connective tissue plate with a small number of vessels in the microvasculature. In the structure of the control group of animals,

the lobular structure of the liver was clearly detected. So the hepatic lobule from the outside was covered by a thin plate of connective tissue, where the bile ducts and components of the hemomicrocirculatory bed of the liver were clearly visualized. On a series of semithin sections they have been branched and passed into interlobular arteries, interlobular veins, and were accompanied by an interlobular bile duct, also in separate lobules were visualized lymphatic vessels. Microvessels had normal blood supply (fig. 1.a.1.b.).

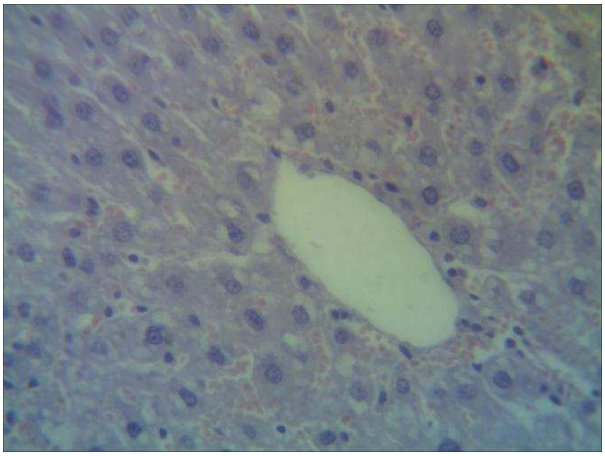

Fig. 1.a. group I. Rat liver. Central vein Haematoxylin and eosin staining. Magnification: lens x 40, eyepiece x 10.

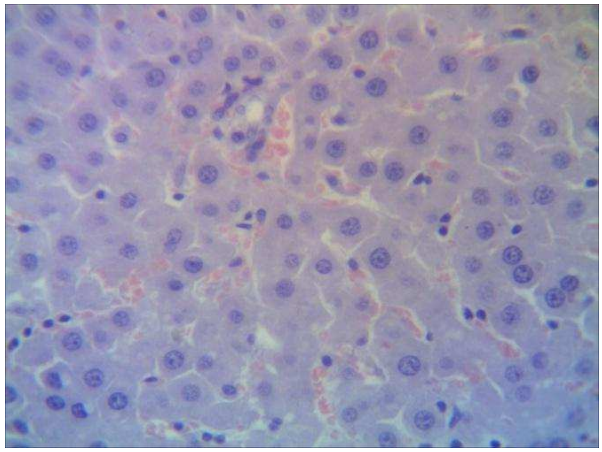

Fig. 1.b group I Liver structure Haematoxylin and eosin staining. Magnification: lens x 40, eyepiece x 10.

The vessels entered the liver lobules and merged with the capillaries on its periphery, which originated from the portal veins. Passing through the terminal plate of hepatocytes, the portal vein and the hepatic artery were connected to sinusoids that passed into the central vein, and hepatocytes were located radially from it. Intralobular sinusoidal capillaries adjoined each hepatocyte, which formed the microvasculature of the liver circulatory system In the first group of animals, small oval Kupffer cells were determined, which had a hyperchromic sickle-shaped nucleus and a light cytoplasm.

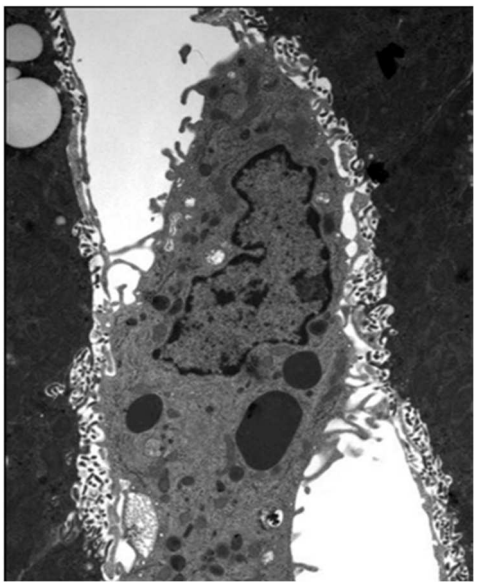

Fig. 2. Group I. Electron micrograph of Kupffer cell of experimental rats on 30th day. Magnification: 7000.

Our electron microscopic study of the liver of animals of the control group showed that Kupffer cells protruded into the lumen of the sinusoids (fig. 2).

Their plasmolemma was uneven, in the form of pseudopodia or microvilli of the plasmolemma, which were in contact with the hemocapillary and had numerous microvilli that were immersed in the Disse space and approached the endothelial cells of the hemocapillaries. Nuclei of irregular shape, predominantly located in the center of the cell, but there were cells with a peripheral location of the nucleus. The nuclear membrane is clear, contoured, euchromatin predominated in the karyoplasm, and a small nucleolus was visualized. Primary and secondary lysosomes appeared in the cytoplasm of such cells. Small mitochondria were scattered throughout the cytoplasm and had a rounded, oval or oblong shape. Individual cisterns of the granular endoplasmic reticulum were identified as well as Golgi complex

In the group II of the studied animals, the structure of the liver was preserved; the thickness of the connective tissue bridges was increased, but not statistically significant in comparison with the control group of animals. (fig 3.a, 3.b.).

From the side of the vessels of the hepatic triads, in comparison with the control group of animals, we did not reveal statistically significant changes, except for the venous bed. The diameter of the veins of the triads is increased in comparison with the control group of animals by 12 %. The bile ducts are unchanged. The central veins are slightly dilated, full blooded in comparison with the control group of animals, in the lumen of which erythrocytes and an insignificant amount of leukocytes were determined. Sinusoidal capillaries are dilated. Kupffer cells are determined, the number of which is increased in comparison with the control.

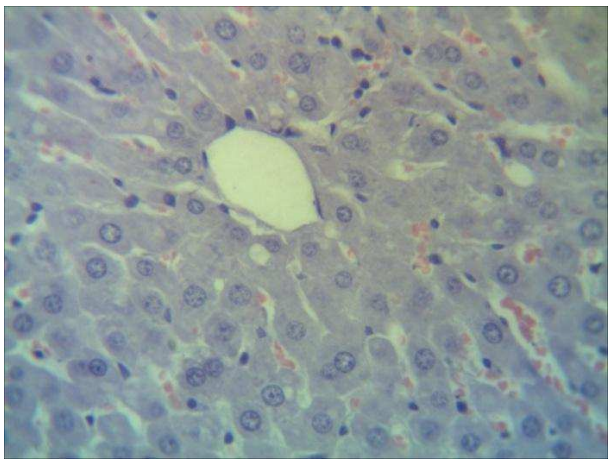

Fig. 3.a. II group on 30th day of experiment. Rat liver. Central vein. Haematoxylin and eosin staining. Magnification: lens x 40, eyepiece x 10.

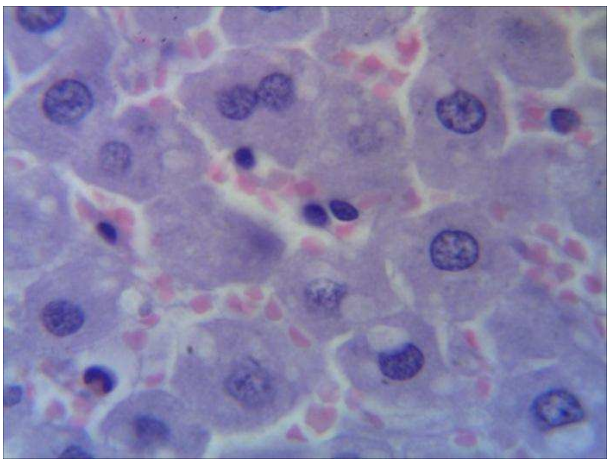

Fig. 3.b. II group on 30th day of experiment. Kupffer cells in rats' liver. Haematoxylin and eosin staining. Magnification: lens x 100, eyepiece x 10.

Electron microscopic examination of liver Kupffer cells of animals from the group II revealed that cells adjoined the endothelium of the sinusoids; some with a smaller part of the cytoplasm were in the perisinusoid space and in the contact with hepatocytes. (fig. 4.)

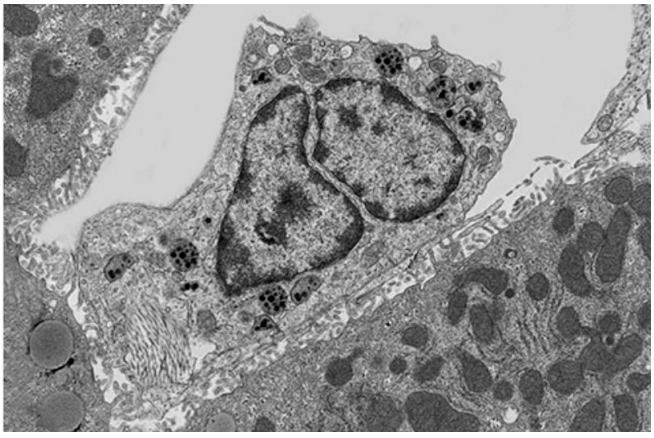

Fig. 4. Group II. Electron micrograph of a Kupffer cell of experimental rats on 30th day. Magnification: 7000.

Their plasmolemma was uneven, in the form of pseudopodia with numerous microvilli that protruded into the lemen of the sinusoids. The nuclei with irregular shape located in the center of the cell, predominantly. The karyolemma is dense, clear, contoured; euchromatin predominated in the karyoplasm, and a small nucleolus, which adjacent to the karyolemma was visualized. The cytoplasm is light with a small number of primary and secondary lysosomes. Synthetic and metabolic apparatus of the cell were without visual changes.

When using quercetin against the background of central deprivation of testosterone on the 30th day of the experiment (group III of animals), on histological examination of the hepatic lobule structure was preserved, the central veins were slightly dilated, compared with the control group of animals, but the indicators were not statistically significant. There were no erythrocytes in the lumen of the central vein. Sinusoids were clearly contoured, erythrocytes and macrophages were determined in the lumen

With a 30 day central deprivation of testosterone synthesis in rat liver, the total activity of NO-synthases increases by 31.5 % when compared with the control group (table 1.).

Table 1

**Activity of nitric oxide cycle enzymes in the liver of rats after 30 days of central deprivation of testosterone synthesis and its correction with quercetin. (M±m)**

| Studied parameters | Groups | | |
|---|---|---|---|
| | Group I n=10 | Group II, n=10 | Group III, n=10 |
| NOS activity, μmol/min per g protein<br>Total<br>Inducible<br>Constitutive | <br>1.27±0.09<br>1.22±0.09<br>0.047±0.0004 | <br>1.67±0.09*<br>1.62±0.09*<br>0.046±0.0005 | <br>1.16±0.09**<br>1.12±0.09**<br>0.043±0.0011 |
| Arginase activity, μmol/min per g protein | 1.88±0.04 | 1.71±0.02* | 1.97±0.07** |
| Concentration of nitrites, nmol/g | 4.99+0.28 | 7.54+0.31* | 4.99+0.28** |

Note: * the difference is statistically significant when compared with the control group ($p<0.05$)

** the difference is statistically significant when compared with the experimental group ($p<0.05$)

The activity of the inducible isoform of NO synthase under these conditions increases by 32.8 %, while the activity of the constitutive isoforms does not change significantly. Arginase activity is reduced by 9 %. The concentration of nitrites increases by 1.51 times.

The introduction of quercetin against the background of a 30day central deprivation of testosterone synthesis leads to a decrease in the total activity of NO-synthases by 30.5 %. The activity of the inducible isoform of NO-synthase decreases by 30.9 %, while the activity of constitutive isoforms does not change significantly. Quercetin increases the activity of arginase in the liver of rats by 15.2 % and decreases the concentration of nitrites by 1.51 times in comparison with the experimental group.

Since bulk structure of liver was not changed during term of experiment (30 days) we can state, that changes in the liver during this period mostly occur on cellular and subcellular levels. Testosterone can positively modulate activities of endothelial and neuronal nitric oxide synthases; therefore testosterone deficiency may decrease their activity [13]. Decreased cNOS activity may contribute to endothelial dysfunction observed in microvascular bed on 30th day of experimental central deprivation of testosterone synthesis [15]. Further endothelial dysfunction can leads to insufficient blood flow in liver and hypoxia. In our previous studies, it was shown that central deprivation of testosterone synthesis by triptorelin leads to overproduction of nitric oxide and the development of oxidative stress in the testes of rats [11]. Further studies are necessary to evaluate precise changes in the cooperation between Kupffer cells, hepatocytes and endotheliocytes as well as the effects of quercetin on morphological and quantitative changes of antigen-presenting liver cells.

## Conclusion

Thus, the morphological changes in the experimental groups with central testosterone deprivation and with its subsequent correction with quercetin on the 30th day of the experiment are identical and include signs of stromal vascular changes in the form of an increase in the thickness of the beam system and minor venous stasis with macrophage infiltration.

Central 30day deprivation of testosterone synthesis leads to a shift in the functioning of enzymes of the nitric oxide cycle in rat liver towards a predominance of the activity of inducible NO synthase.

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Стаття надійшла 28.05.2020 р.