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# IMMUNOCOMPETENT LIVER CELLS REACTION TO INHIBITION OF LUTEINIZING HORMONE SYNTHESIS ON THE 180$^{TH}$ DAY

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Hepatic macrophages play a central role in maintaining liver homeostasis and in the pathogenesis of liver injury. Liver macrophages are composed of functionally distinct cellular subpopulations. Currently, there is interest in the effect of androgens and their receptors on different liver cells and the development of liver pathology as well as the potential impact of quercetin on androgen synthesis inhibition. The blockade of luteinising hormone synthesis by administration of triptorelin acetate on day 180 of the experiment caused morphological changes in the structure of the rat liver, in particular in the cellular, connective tissue and vascular components. Total activity of NO synthases in the liver rose up as well as nitrite concentration. Arginase activity was decreased. Administration of quercetin leads to a decrease in the total activity of NO synthases. The shift in macrophage polarization toward the predominance of M1 may be a consequence of endothelial dysfunction as a result of luteinizing hormone synthesis inhibition.

**Key words**: liver, macrophage, luteinizing hormone, triptorelin, quercetin, rats.

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# РЕАКЦІЯ ІМУНОКОМПЕТЕНТНИХ КЛІТИН ПЕЧІНКИ НА ПРИГНІЧЕННЯ СИНТЕЗУ ЛЮТЕЇНІЗУЮЧОГО ГОРМОНУ НА 180-Й ДЕНЬ

Макрофаги печінки відіграють центральну роль у підтримці внутрішньоорганного гомеостазу та в патогенезі її пошкодження. Вони складаються з функціонально відмінних клітинних субпопуляцій. В теперішній час існує інтерес до впливу андрогенів та їх рецепторів на різні клітини печінки та розвиток печінкової патології, а також потенційний вплив кверцетину на фоні пригнічення синтезу андрогенів. Блокада синтезу лютеїнізуючого гормону шляхом введення трипторелін ацетату на 180 добу експерименту спричиняла морфологічні зміни в структурі печінки щурів, зокрема в клітинному, сполучнотканинному та судинному компонентах. Сумарна активність NO-синтаз у печінці зростала, як і концентрація нітритів. Активність аргінази знижувалась. Введення кверцетину призводило до зниження сумарної активності NO-синтаз. Зсув поляризації макрофагів у бік переважання М1 може бути наслідком дисфункції ендотелію внаслідок пригнічення синтезу лютеїнізуючого гормону.

**Ключові слова:** печінка, макрофаги, лютеїнізуючий гормон, трипторелін, кверцетин, щури.

*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 No. 0119U102925.*

Macrophages are myeloid immune cells that are abundant in all tissues of the body. They recognize, absorb and neutralize cellular debris, foreign material or pathogenic microorganisms and perform a central function in the organization of inflammatory processes [12]. Hepatic macrophages play a central role in maintaining liver homeostasis, as well as in the pathogenesis of acute or chronic liver damage [11]. Regarding this, they are an attractive target for the development and research of new methods of liver disease treatment. Since macrophages perform a wide range of different functions in the liver and consist

of functionally diverse cell subpopulations, the elaboration of interventional strategies based on the study of this group of antigen-presenting liver cells is a relatively challenging goal today.

Intensive research in animal models of hepatic injury and in samples from patients with liver disease has revealed the complex heterogenity of hepatic macrophages. Monocytes (circulating precursors) and macrophages in the liver can be distinguished based on their origin, migratory behaviour, differentiation, expression of certain markers, and, most importantly, their functions in homeostasis and disease.

However, these characteristics cannot be considered static, as macrophages are extremely versatile cells. For example, macrophages respond to environmental signals from tissue in a very diversified manner, which leads to a wide range of different "polarization states" in vitro and in vivo [8, 13].

Currently, great interest arises in the effect of androgens and their receptors on liver cells and the development of liver pathology, especially in the context of hepatocarcinogenesis. The frequency of hepatocellular carcinoma in men is significantly higher than in women. The hepatocarcinogenic potential of oral anabolic androgens is also known [4].

The flavonoid quercetin has capillary stabilizing properties due to its antioxidant and membrane stabilizing effects. Its protective effect in liver damage has been previously shown [2]. However, the changes in immunocompetent liver cells and macrophage polarization in the liver after inhibition of luteinizing hormone synthesis by administration of tryptorelin and concomitant use of quercetin remain unclear.

**The purpose** of the study was to determine the both qualitative and quantitative changes in immunocompetent liver cells caused by luteinizing hormone synthesis inhibition in male rats, as well as the potential effect of quercetin.

**Materials and methods**. The experiments were performed on 30 sexually mature male white rats. The rats were divided into 3 groups: control (10), experimental group I (EG I, 10) and experimental group II (EG II, 10). Animals from experimental group I were subcutaneously injected with tryptorelin acetate at a dose of 0.3 mg of active compound per kg of body mass. In experimental group II, animals received tryptorelin acetate in the same dosage and quercetin 100 mg per kg of body weight 3 times a week, while the control group was administered saline.

The animals were kept in standard conditions of the vivarium of Poltava State Medical University. Permission to conduct biomedical and experimental research was granted at the meeting of the Biomedical Ethics Committee of Poltava State Medical University No. 201 of January 27, 2022. 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 for Animal Experiments adopted by the First National Congress on Bioethics (Kyiv, 2001). Animals from the experimental groups were withdrawn from the experiment on day 180 (n=20).

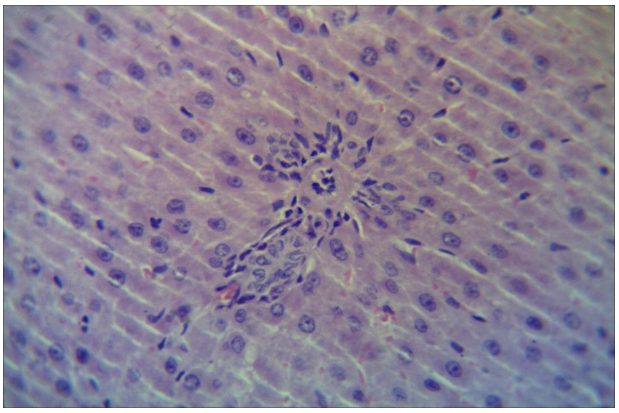

Fig.1. Control group. Fragment of liver parenchyma. Hepatic beams. Haematoxylin and eosin staining. Magnification: approx.:10; vol. 20.

According to the established methodology, the material was embedded in paraffin blocks, from which 4 μm thick sections were made and stained with hematoxylin and eosin [1]. The histological specimens were investigated using a light microscope Biorex 3 with a digital microfilter and software suitable for such studies (serial number 5604).

All biochemical studies were conducted in 10 % liver tissue homogenate using an Ulab 101 spectrophotometer. Total NO synthase activity (gNOS), constitutive isoform activity (cNOS), inducible isoform activity (iNOS), arginase activity and nitrite concentration were determined according to the technique described by Yelinskaya A.M. [14].

Statistical analysis of the data was provided using Microsoft Office Excel and the Real Statistics 2019 add-in. The non-parametric Mann-Whitney test was used to determine the statistical significance of differences between the groups. The difference was considered statistically significant at $p<0.05$.

**Results of the study and their discussion.** In serial semi-thin sections in the control group of animals, the structure of the liver corresponded to the generally accepted principle of the structure of a parenchymal organ, with a certain predominance of parenchymal components over the stroma. The capsule was represented by a thin connective tissue lamina with a small number of microcirculatory vessels. The lobular structure of the liver was clearly observed. Thus, the liver lobule was externally demarcated by a thin plate of connective tissue, with clear visualisation of the bile ducts and components of the haemomicrocirculatory network. In a series of semi-thin sections, hemocapillaries branched and transitioned

into interhepatic arteries, interhepatic veins associated with the interhepatic bile duct, and lymphatic vessels were also identified in some lobes. The blood filling of microvessels was satisfactory. Vessels entered the liver lobules and merged with capillaries originating from portal veins on its periphery. Crossing through the terminal plate of hepatocytes, the portal vein and hepatic artery connected with sinusoids, which passed into the central vein, from which hepatocytes were arranged radially. Each hepatocyte was adjacent to intra-lobular sinusoidal capillaries. In the control group of animals, small oval-shaped Kupffer cells with a hyperchromatic sickle-shaped nucleus and light cytoplasm were detected (Fig. 1).

When we investigated semi-thin sections of the liver on day 180 of the study, in experimental group I the liver structure was preserved, the thickness of connective tissue elements was slightly increased, but statistical significance was not confirmed in comparison with the control group (Fig. 2).

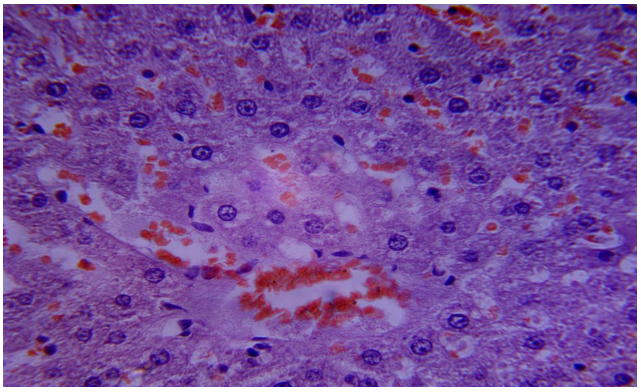

Fig.2. Experimental group I. Rat liver. Central vein. Haematoxylin and eosin staining. Magnification: approx.:10; vol. 40.

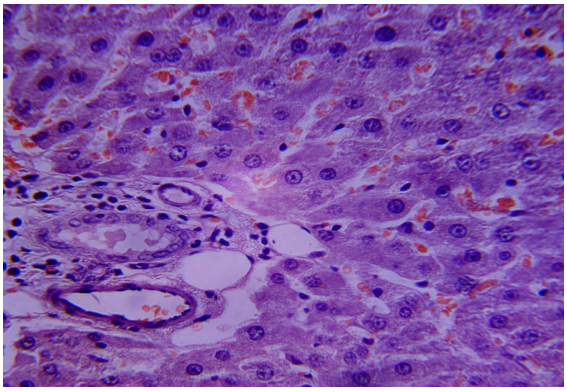

Fig. 3. Experimental group II. Rat liver. Fragment of liver parenchyma, hepatic triad. Haematoxylin and eosin staining. Magnification: approx.: 10; vol. 40

The diameter of the veins of the hepatic triads is increased by 10 % compared to the control group of animals. The lumen of the bile ducts is ellipsoidal, and signs of bile stasis are identified. The central veins were slightly dilated, full-blooded, red blood cells and a significant number of leukocytes were detected in the lumen. Sinusoidal capillaries were expanded. Kupffer's cells were observed in the field of view, their number was increased both in the lumen of sinusoidal capillaries and in Dissé's space compared to the control group.

Histological examination of sections of the liver of animals from experimental group II on day 180 of the experiment revealed that the structure of the liver lobule was preserved, and trabecular extension was noted (Fig.3).

The diameter of the central veins was slightly increased compared to the control group of animals, but the parameters were not statistically significant. There were no red blood cells in the lumen of the central vein. The sinusoids had a clear contour, erythrocytes and macrophages were identified in the lumen. Accumulation of fluid in the connective tissue was detected.

In animals of experimental group I, as a result of 180-day inhibition of luteinising hormone synthesis, the total activity of NO synthases in the liver increased by 103.1 % compared to the control group (Table 1).

Table 1

**Activity of nitric oxide cycle enzymes in rat liver (M±m)**

| Parameters studied | Groups | | |
|---|---|---|---|
| | Control, n=10 | Experimental group I, n=10 | Experimental group II, n=10 |
| NOS activity, μmol/min per g of protein | | | |
| Total activity | 1.27±0.093 | 2.59±0.066* | 0.79±0.036* |
| Inducible isoform | 1.22±0.09 | 2.53±0.072* | 0.71±0.035* |
| Constitutive isoforms | 0.047±0.0004 | 0.058±0.010 | 0.0865±0.002* |
| Arginase activity, μmol/min per g of protein | 1.88±0.04 | 0.8970±0.011* | 1.4398±0.041* |
| Nitrite concentration, nmol/g | 4.99±0.28 | 7.29±0.16* | 10.03±0.21* |

Notes: * – difference is statistically significant when compared to the control group ($p<0.05$)

The activity of the inducible isoform of NO synthase under these conditions rose by 106.5 %, and the activity of the constitutive isoforms did not change statistically notably. The activity of arginase decreased by 47 %. Nitrite concentration increased by 46 %.

Administration of quercetin against the background of inhibition of luteinising hormone synthesis for 180 days leads to a decrease in the total activity of NO synthases by 70.4 %. The activity of the inducible

isoform of NO synthase reduced by 71.8 %, and the activity of constitutive isoforms increased by 48.27 %. Quercetin administration raised the activity of arginase in rat liver by 61.8 % and led to an increase in nitrite concentration by 37.6 % compared to experimental group I.

In general, macrophage polarisation phenotypes can be divided into classically activated M1 and alternatively activated M2 [15]. M1 macrophages are also known as proinflammatory macrophages because they can produce large amounts of proinflammatory cytokines such as L-1b, inducible nitric oxide synthase (iNOS), and tumour necrosis factor-a (TNF-a). And on the contrary, 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.

The shift in macrophage polarisation towards M1 predominance may be a consequence of the development of endothelial dysfunction, which occurs as a result of inhibition of luteinising hormone synthesis [8]. Endothelial dysfunction can lead to oxidative damage to various organs and tissues due to excessive production of reactive oxygen species [7]. The sources of excessive production of reactive oxygen species may be constitutive forms of NO synthase, which, with increased activity, can produce not only nitric oxide but also superoxide anion radical [8]. With the simultaneous production of nitric oxide and superoxide anion radical by cNOS, 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 can change the polarisation of macrophages to the M1 phenotype [9]. Quercetin is a powerful antioxidant that enhances cellular protection against oxidative damage both by directly intercepting reactive oxygen species and by stimulating the activity of the glutathione system. Quercetin can reduce the expression of iNOS genes, which explains the results of reduced iNOS and total NOS activity [10]. At the same time, quercetin can also inhibit the activation of the transcription factor NF-kB [5]. Quercetin can also prevent the development of endothelial dysfunction [6]. Thus, the use of quercetin affects all pathogenetic links that lead to a shift in macrophage polarisation to the M1 phenotype and consequently leads to the restoration of the predominance of the M2 phenotype in the liver.

## Conclusion

The blockade of luteinising hormone synthesis by administration of tryptorelin acetate on day 180 of the experiment causes morphological changes in the structure of the rat liver, in particular in the cellular, connective tissue and vascular components.

The simultaneous oral administration of quercetin leads to the minimisation of structural and morphological changes in rat liver tissue from oxidative damage caused by tryptorelin injection by increasing the antioxidant defence of liver tissue.

The obtained results provide a theoretical basis for the development of correction methods for extreme effects on the body. The data on the functional morphology of the liver at the stages of adaptation to changes in the endocrine activity of the hypothalamus-pituitary-gonad axis expand the understanding of the reasons for metabolic disorders in the liver's structural components and the possibilities of its management.

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

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## THE PECULIARITIES OF LOW-DOSE IONIZING RADIATION INFLUENCE ON MUSCLES METABOLISM IN EXPERIMENTAL ANIMALS

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The purpose of this study was to research the peculiarities of glycolytic processes manifestation in cardiac and striated muscles as a result of total irradiation exposure. 120 male rats were divided into 2 groups. Group 1 (n=20) – intact rats, group 2 (n=100) – rats that were exposed to ionizing gamma radiation. The animals were euthanized, blood was collected, the heart and the frontal group of thigh muscles were removed in which the pyruvate kinase and lactate dehydrogenase activities with its isozymes spectrum together with lactate and pyruvate content were measured. The pathophysiological mechanisms of radiation-induced energy supply reformation are aimed at strengthening of short-term processes of the energy supply to vital organs and systems for destroyed biochemical, physiological, functional and regulatory processes restitution and the sanogenetic mechanisms activation.

**Key words:** total irradiation, pyruvate kinase, lactate dehydrogenase, lactate, pyruvate, metabolism, pathophysiological mechanisms.

**Г.Ф. Степанов, Р.С. Вастьянов**

## ОСОБЛИВОСТІ ВПЛИВУ МАЛИХ ДОЗ ІОНІЗУЮЧОГО ВИПРОМІНЮВАННЯ НА МЕТАБОЛІЗМ М'ЯЗІВ ЕКСПЕРИМЕНТАЛЬНИХ ТВАРИН

Метою дослідження було вивчення особливостей перебігу гліколітичних процесів у серцевому м'язі та поперечно-смугастих м'язах внаслідок впливу тотального опромінення. 120 щурів-самців були розділені на 2 групи. 1 група (n=20) – інтактні щури, 2 група (n=100) – щури, яких піддавали впливу іонізуючого гама-опромінення. Тварин виводили із досліду через евтаназії, збирали кров, видаляли серце і передню групу м'язів стегна, в яких вимірювали активність піруваткінази, лактатдегідрогенази з її ізоферментним спектром та вміст лактату і пірувату. Патофізіологічні механізми спричиненої радіацією перебудови енергозабезпечення спрямовані на короткочасні процеси посилення надання енергії для життєво важливих органів та систем задля відновлення зруйнованих біохімічних, фізіологічних, функціональних та регуляторних процесів та активацію саногенетичних механізмів.

**Ключові слова:** тотальне опромінення, піруваткіназа, лактатдегідрогеназа, лактат, піруват, метаболізм, патофізіологічні механізми.

*The study is a fragment of the research project "Mechanisms of epigenetic disorders of the leading links of bioenergetics and nitrogen metabolism in irradiated animals and their descendants", state registration No 0121U114601*

In the current conditions of large-scale artificial radiation pollution of the environment and radiation load on the biosphere, the assessment of the total biological effectiveness of prolonged exposure is extremely relevant. The effect of radiation at a sufficiently high dose on biological objects in some cases is comparable to the effect of radiation at a dose ten times lower [13].

The radiation hazard of low-dose exposure has been shown to be significantly higher than that of maximum dose exposure. It should be emphasized that there is a pronounced alternative effect of acute and chronic low-dose radiation on the cellular genetic apparatus [11, 14].

Most of the results in the range of low doses of ionizing radiation indicate the existence of the effect of radiation hormesis, which is characterized by increased fertility, accelerated cell growth and