Key words: thyroid gland, macro – and microanatomy, rat.

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MORPHOGENESIS OF PERITUBULAR MACROPHAGES IN THE INTERSTITIAL SPACE OF THE RAT TESTES UNDER CENTRAL BLOCKING OF LUTEINISING HORMONE SYNTHESIS BY TRYPTORELIN ON DAYS 180 AND 270 OF OBSERVATION

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Luteinising hormone (LH) is a glycoprotein hormone, which is formed in the adenohypophysis. Its biological role is to regulate the functions of the human sexual sphere. Together with follicle-stimulating hormone, they are classified as gonadotropic hormones, the synthesis and secretion of which is stimulated by hypothalamic gonadoliberin. Testosterone, the production of which is stimulated by the release of LH, through the activation of testicular interstitial endocrinocytes leads to the activation of macrophage polarisation under the M2 phenotype (anti-inflammatory). Testosterone has a significant impact on the functional state of macrophages in the body. Under the influence of testosterone, macrophage polarisation towards the pro-inflammatory phenotype (M1) is inhibited, resulting in a reduction in the production of cytokines such as interleukin 1β (IL-1β) and interleukin 6 (IL-6). Cytokines suppress steroidogenesis at the level of the adrenal glands, testes and ovaries. In the adrenal glands, macrophages and lymphocytes physiologically infiltrate the adrenal cortex extensively, whereas adrenocortical and chromaffin cells also produce cytokines such as IL-1, IL-6, TNFalpha, leukaemia inhibitory factor (LIF) and IL-18, playing a key role in immune-adrenocortical communication. Thus, testosterone has a significant anti-inflammatory and immunomodulatory effect, and in conditions of its deficiency, tissue damage may develop due to excessive polarisation of macrophages by the M1 phenotype.

The experiment was performed on 20 white male rats randomly selected into 2 groups: control (10 animals) and experimental (10 animals). Animals in the experimental group were injected with a solution of tryptorelin acetate at the dose of 0.3 mg of active ingredient per kg of animal weight to simulate central blocking of luteinising hormone synthesis. The studies were performed using an electron microscope.

It has been established that under the influence of central blockade of LH synthesis, the antigen-presenting cells of the interstitial space of the testis, in particular macrophages, are rearranged. These modifications indicate an inversion of their polarisation, which in turn leads to microscopic reorganisation of cells, specifically their activation (M1) to perform certain functional activity in the organ.

Key words: *testis, hypothalamic-pituitary system, pituitary gland, LH, testosterone, tryptorelin, macrophage, parietal macrophage, electron micrograph, rats.*

The connection of the publication with planned research works.

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

Introduction.

Luteinising hormone (LH) is a hormone of a protein origin, a glycoprotein that is produced in the adenohypophysis. The hormone consists of two subunits, α and β. The biological role of LH is to regulate the functions of the human reproductive system. Together with follicle-stimulating hormone, they are known under the general name "gonadotropic hormones", the synthesis and secretion of which is stimulated by hypothalamic gonadoliberin. LH production is under negative feedback control by the concentrations of sex hormones: increased levels of estrogens and androgens in the blood inhibit LH secretion; decreased functional activity of the gonads (menopause,

surgical or radiation castration) stimulates LH secretion. The hormone binds to specific receptors on the plasma membranes of the Leydig cells of the testes, stimulating the synthesis of testosterone [1] which is necessary for the normal completion of spermatogenesis.

Testosterone has a significant impact on the functional state of macrophages in the body. Under the influence of testosterone, macrophage polarisation towards the pro-inflammatory phenotype (M1) is inhibited, resulting in a reduction in the production of cytokines such as interleukin 1β (IL-1β) and interleukin 6 (IL-6) [2, 3]. Cytokines suppress steroidogenesis at the level of the adrenal glands, testes and ovaries. In the adrenal glands, macrophages and lymphocytes physiologically infiltrate the adrenal cortex extensively, whereas adrenocortical and chromaffin cells also produce cytokines such as IL-1, IL-6, TNFalpha, leukaemia inhibitory factor (LIF) and IL-18, playing a key role in immune-adrenocortical communication [4].

In the testes of adult rats, macrophages belong to one of two subgroups differentiated by the presence or absence of the resident macrophage surface antigen expression, which is recognised by the monoclonal antibody ED2. The local regulation of testicular macrophages was studied in normal and experimental adult rats (4 weeks of observation) with cryptorchidism after subcutaneous injection of testosterone implants (T-implants). Macrophage subsets ED2(+) (resident type) and ED2(-) (monocyte-like) were identified by immunohistochemical analysis and counted in perfusion-fixed frozen sections of testes [5, 6].

Conversely, testosterone stimulates a change in macrophage polarisation towards the predominance of the anti-inflammatory (M2) phenotype, even when macrophages are stimulated with bacterial lipopolysaccharide [7, 8].

Thus, testosterone has a pronounced anti-inflammatory and immunomodulatory effect, and in conditions of its deficiency, tissue damage may develop due to excessive polarisation of macrophages according to the M1 phenotype.

The aim of the study.

To determine the prevalence and morphogenesis of parietal macrophages in the interstitial space of the testes under central blockade of LH synthesis by tryptorelin.

Object and research methods.

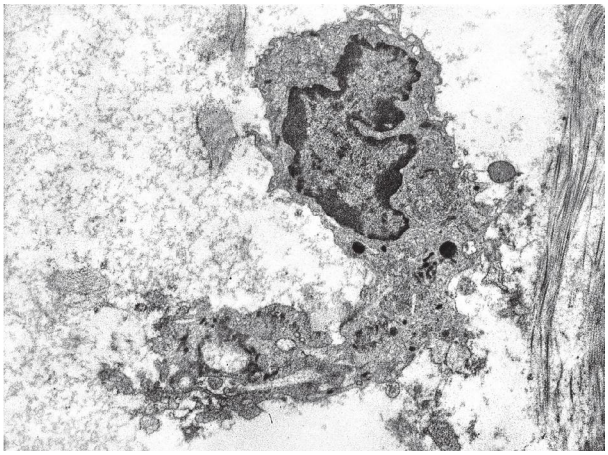

Figure 1 – Electron micrograph of a parietal macrophage on day 180 of observation with central LH blockade by tryptorelin. Magnification: x10000.

The study was performed on 20 sexually mature white male rats. The animals were randomly divided into 2 groups: control (10 animals) and experimental (10 animals). Animals in the experimental group were injected with a solution of tryptorelin acetate at the dose of 0.3 mg of active ingredient per kg of animal weight to simulate central blocking of luteinising hormone synthesis [9]. Animals from the experimental group were withdrawn from the experiment on days 180 and 270 by an overdose of ether narcosis. Rats in the control group were injected with saline. Animals were kept under standard vivarium conditions of the Poltava State Medical University. Experimental animals were sacrificed 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) and the "General Ethical Principles of Animal Experimentation" adopted by the First National Congress on Bioethics (Kyiv, 2001).

The animals were decapitated and the prepared small pieces of testes were fixed in 2.5% glutaraldehyde solution (pH=7.2-7.4). The material was postfixed in a 1% solution of osmium (IV) oxide, dehydrated in propylene oxide and embedded in epoxy resin. Ultrathin sections were prepared on an ultramicrotome, contrasted with a 1% aqueous solution of uranyl acetate and lead citrate according to the Reynolds method, and examined by electron microscopy [10].

Statistical processing of the study results was performed using Microsoft Office Excel and its extension Real Statistics 2019. The non-parametric Mann-Whitney test was used to evaluate the statistical significance of the intergroup differences. The difference was statistically significant at $p<0.05$.

Research results and their discussion.

In the electron micrographs on the 180th day of observation, PMs were situated singly near the convoluted seminiferous tubules. The cells were functionally active, at the phagocytosis stage They had relatively large electron-dense nuclei with a predominance of dispersed euchromatin and clumps of condensed heterochromatin. The shape of the nuclei is mostly oval. In some places, polymorphic nuclei with a small number of invaginations were found. The caryolemma is clear, dense, two-layered, with a large number of pores. One nucleolus was present in the karyoplasm. The cytoplasm of the cells was smaller than that of interstitial macrophages. The endoplasmic reticulum was well developed, with numerous ribosomes on the membranes. Few mitochondria were present, round in shape. Also, a large number of lysosomes of different sizes and electron density were present in the cytoplasm of the cells, along with a significant number of inclusions, and some phagosomes were detected. A small number of pseudopodia and invaginations were observed on the cell surface **(fig. 1).**

Our submicroscopic examination of electron micrographs on the 270th day of observation revealed the following changes in the structure of the PM. The number of cells decreased significantly compared to both the control group and the samples from the previous follow-up period. Single PMs in a condition of functional activity were detected: nuclei were elongated, flat, the karyolemma was osmophilic, and chromatin heterogeneous. Most cells are of high electron density. The nucleus was single, blurred, the karyolemma was not clearly visualised, and the number of pores was rather small. The cytoplasm was

light, occupied a minor part of the cell volume, and large, light phagosomes were detected in the middle. A large quantity of lysosomes was noted, differing in size and electron density. Mitochondria were small, single, with a lightened matrix and a small number of cristae. A large amount of pseudopodia and invaginations were detected on the cell surface **(fig. 2).**

Based on the results of our previous studies [11, 12], we identified two populations of macrophages located in the interstitial space of the testes, considering their location and functional capacity. Since we did not find a sufficient amount of literature data on this issue, we use the terminology defined earlier during the first stages of our investigation. In the total population of seminiferous tubules macrophages, we distinguish between interstitial and parietal or peritubular macrophages. This study is focused on a detailed consideration of the morphogenesis of parietal macrophages (PM) at the long term observation periods, i.e. 180 and 270 days. The results of our studies reveal that under the influence of central blocking of LH synthesis, morphogenesis of antigen-presenting cells of the interstitial space of the testis, namely macrophages, occurs. These modifications indicate an inversion of their polarisation, which in turn leads to microscopic reorganisation of cells, specifically their activation (M1) to perform certain functional activity in the organ.

Conclusions.

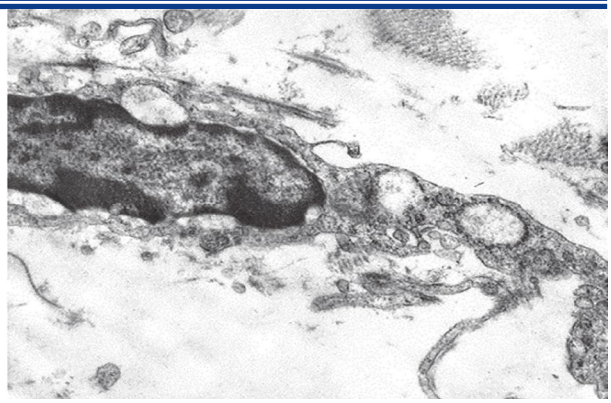

Figure 2 – Electron micrograph of a parietal macrophage on day 270 of observation with central LH blocking by tryptorelin. Magnification: x10000.

The administration of tryptorelin causes quantitative and qualitative changes in the structure of the interstitial space of the rat testes, characterised by morphological changes in the number and nature of macrophages. The number of macrophages increased due to a gradual increase in the pool of peritubular "parietal" macrophages, with a maximum on the 180th day of observation.

Prospects for further research.

It is planned to investigate the morphogenesis of the interstitial space of the testes under blocking of LH synthesis with inversion of macrophage polarisation with the simultaneous use of quercetin.

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МОРФОГЕНЕЗ ПЕРИТУБУЛЯРНИХ МАКРОФАГІВ ІНТЕРСТИЦІАЛЬНОГО ПРОСТОРУ СІМ'ЯНИКІВ ЩУРІВ ЗА ЦЕНТРАЛЬНОГО БЛОКУВАННЯ СИНТЕЗУ ЛЮТЕЇНІЗУЮЧОГО ГОРМОНУ ТРИПТОРЕЛІНОМ НА 180 ТА 270 ДОБИ СПОСТЕРЕЖЕННЯ

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Резюме. *Вступ.* Тестостерон має істотний вплив на функціональний стан макрофагів в організмі. Під впливом тестостерону поляризація макрофагів у бік прозапального фенотипу (М1) пригнічується, що призводить до зниження вироблення цитокінів, таких як інтерлейкін 1β та інтерлейкін 6. Цитокіни пригнічують стероїдогенез на рівні надниркових залоз, яєчок та яєчники. У надниркових залозах макрофаги та лімфоцити фізіологічно широко інфільтрують кору надниркових залоз, тоді як адренокортикальні та хромафінні клітини також виробляють цитокіни, такі як TNFalpha, фактор інгібування лейкемії та IL-18, що відіграє ключову роль у зв'язку між імунною системою та корою надниркових залоз. Таким чином, тестостерон має виражену протизапальну та імуномодулюючу дію, а в умовах його дефіциту можливий розвиток тканинного ураження внаслідок надмірної поляризації макрофагів за фенотипом М1.

Об'єкт і методи. Дослідження проводили на 20 дорослих самцях щурів. Тварин випадковим чином розподіляли на 2 групи: контрольну (10 тварин) та інтактну (10 тварин). Тваринам контрольної групи в стегно вводили

фізіологічний розчин у дозі 0,3 мл. Підготовку матеріалу для електронно-мікроскопічного дослідження структур інтерстиціального простору яєчка проводили за загальноприйнятою методикою.

Результати і обговорення. Ми ідентифікували дві популяції макрофагів, розташованих в інтерстиціальному просторі яєчок, враховуючи їх розташування та функціональну здатність. У загальній популяції макрофагів сім'яних канальців ми розрізняємо інтерстиціальні та пристінкові або перитубулярні макрофаги. Дане дослідження зосереджено на детальному розгляді морфогенезу пристінкових макрофагів (ПМ) за віддалені періоди спостереження, тобто 180 та 270 днів. Результати наших досліджень показали, що під впливом центрального блокування синтезу ЛГ відбувається морфогенез антигенпрезентуючих клітин інтерстиціального простору яєчка, а саме макрофагів. Ці модифікації вказують на інверсію їх поляризації, що, у свою чергу, призводить до мікроскопічної реорганізації клітин, зокрема їх активації (М1) для виконання певної функціональної діяльності в органі.

Висновки. Введення трипторелін викликає кількісні та якісні зміни в структурі інтерстиціального простору сім'яників щурів, що характеризується морфологічними змінами кількості та якості макрофагів. Кількість макрофагів зростала за рахунок поступового збільшення пулу перитубулярних «пристінкових» макрофагів з максимумом на 180-ту добу спостереження.

Ключові слова: яєчко, гіпоталамо-гіпофізарна система, гіпофіз, ЛГ, тестостерон, трипторелін, макрофаг, пристінковий макрофаг, електронна мікрофотографія, щури.

MORPHOGENESIS OF PERITUBULAR MACROPHAGES IN THE INTERSTITIAL SPACE OF THE RAT TESTES UNDER CENTRAL BLOCKING OF LUTEINISING HORMONE SYNTHESIS BY TRYPTORELIN ON DAYS 180 AND 270 OF OBSERVATION

Stetsuk Ye. V., Shepytko V. I., Boruta N. V., Rud M. V., Skotarenko T. A., Vilkhova O. V.

Abstract. *Introduction.* Testosterone has a significant impact on the functional state of macrophages in the body. Under the influence of testosterone, macrophage polarisation towards the pro-inflammatory phenotype (M1) is inhibited, resulting in a reduction in the production of cytokines such as interleukin 1β and interleukin 6. Cytokines suppress steroidogenesis at the level of the adrenal glands, testes and ovaries. In the adrenal glands, macrophages and lymphocytes physiologically infiltrate the adrenal cortex extensively, whereas adrenocortical and chromaffin cells also produce cytokines such, TNFalpha, leukaemia inhibitory factor and IL-18, playing a key role in immune-adrenocortical communication. Thus, testosterone has a pronounced anti-inflammatory and immunomodulatory effect, and in conditions of its deficiency, tissue damage may develop due to excessive polarisation of macrophages according to the M1 phenotype.

Object and methods. The study was performed on 20 adult male rats. Animals were randomly divided into 2 groups: control (10 animals) and intact (10 animals). Animals in the control group were injected with saline in dosage 0.3 ml in the thigh. Preparation of material for electron microscopic studies of the interstitial space structures of the testis was performed according to the generally accepted method.

Results and discussion. We identified two populations of macrophages located in the interstitial space of the testes, considering their location and functional capacity. In the total population of seminiferous tubules macrophages, we distinguish between interstitial and parietal or peritubular macrophages. This study is focused on a detailed consideration of the morphogenesis of parietal macrophages (PM) at the long term observation periods, i.e. 180 and 270 days. The results of our studies reveal that under the influence of central blocking of LH synthesis, morphogenesis of antigen-presenting cells of the interstitial space of the testis, namely macrophages, occurs. These modifications indicate an inversion of their polarisation, which in turn leads to microscopic reorganisation of cells, specifically their activation (M1) to perform certain functional activity in the organ.

Conclusions. The administration of tryptorelin causes quantitative and qualitative changes in the structure of the interstitial space of the rat testes, characterised by morphological changes in the number and nature of macrophages. The number of macrophages increased due to a gradual increase in the pool of peritubular «parietal» macrophages, with a maximum on the 180th day of observation.

Key words: testis, hypothalamic-pituitary system, pituitary gland, LH, testosterone, tryptorelin, macrophage, parietal macrophage, electron micrograph, rats.

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Conflict of interest:
The authors of the article confirm the absence of conflict of interests.

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