токсичного гепатиту та використання комплексного гепатопротектора. Було проведено експериментальне дослідження на 60 білих щурах-самцях лінії Вістар масою 240-270 г. В процесі роботи щурі були поділені на 3 групи (по 20 щурів у кожній групі): контрольна – включала здорових щурів з травмою нижньої щелепи; дослідна 1 – щурів з травмою щелепи та модельованим токсичним гепатитом; дослідна 2 – щурів з травмою нижньої щелепи + токсичний гепатит+комплексний гепатопротектор. Дослідження гістологічних змін кісткової тканини нижньої щелепи в ділянці травми, при токсичному гепатиті обгрунтувало позитивний вплив гепатопротектора на зміни морфометричних показників. В результаті експерименту виявили, що токсичний гепатит погіршує морфометричні показники загоєння перфораційного дефекту нижньої щелепи. Особливо важливим є те, що комплексний гепатопротектор при токсичному гепатиті збільшує питомий об'єм кісткових балок на 30 та 60 добу та кісткового мозку на 60 добу, що свідчить про його позитивний вплив на процеси регенерації кістки в місці перфорації.

Ключеві слова: експеримент, щурі, травма нижньої щелепи, модельований токсичний гепатит, комплексний гепатопротектор.

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токсического гепатита и использования комплексного гепатопротектора. Было проведено экспериментальное исследование на 60 белых крысах-самцах линии Вистар массой 240-270 г. В процессе работы крысы были разделены на 3 группы (по 20 крыс в каждой группе): контрольная - включала здоровых крыс с травмой нижней челюсти; экспериментальная № 1 - крыс с травмой челюсти и моделированным токсическим гепатитом; экспериментальная № 2 - крыс с травмой нижней челюсти + токсический гепатит + комплексный гепатопротектор. Исследование гистологических изменений костной ткани нижней челюсти в области травмы при токсическом гепатите обосновало положительное влияние гепатопротектора на изменения морфометрических показателей. В результате эксперимента обнаружили, что токсический гепатит ухудшает морфометрические показатели заживления перфорационного дефекта нижней челюсти. Особенно важно то, что комплексный гепатопротектор при токсическом гепатите увеличивает удельный объем костных балок на 30 и 60 сутки и костного мозга на 60 сутки, что свидетельствует о его положительном влиянии на процессы регенерации кости в месте перфорации.

Ключевые слова: эксперимент, крысы, травма нижней челюсти, моделированный токсический гепатит, комплексной гепатопротектор.

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STRUCTURAL ORGANIZATION OF STROMAL AND PARENCHYMAL COMPONENTS OF RAT TESTES DURING CENTRAL DEPRIVATION OF TESTOSTERONE SYNTHESIS ON THE 180 DAY OF THE EXPERIMENT

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Prolonged central deprivation of testosterone synthesis may lead to biochemical and morphological changes in testes. Influence of prolonged testosterone deficiency on reactive nitrogen and oxygen species production, morphological changes in interstitial endocrinocytes and sustentocytes is not yet fully understood. The number of interstitial endocrinocytes is reduced in comparison with the control group, there are interstitial spaces between the convoluted tubules with a complete absence of interstitial endocrinocytes. Interstitial endocrinocytes are reduced in size, their nuclei are heterochromic. When we studied the structural organization of rat sustentocytes from the experimental group in comparison with the control group, we found that hyperplasia of the elements of the smooth endoplasmic reticulum was observed in the cytoplasm of the sustentocytes. The number of mitochondria in the cytoplasm of the sustentocytes decreased, the electron density of the mitochondrial matrix was lowered, protein structures were present either inside the vacuoles or independently located in the cell cytoplasm. Biochemical studies revealed increased NO production from inducible NO-synthase and development of oxidative stress. Experimental central deprivation of testosterone synthesis with diphereline on the 180th day of the experiment leads to shift of NO synthesis from constitutive NO-synthases to inducible NO-synthase and intensification of oxidative stress due to increase of superoxide anion-radical production and decrease in antioxidant protection.

Key words: testes, interstitial endocrinocytes, sustentocytes, NO-synthase, iNOS, cNOS, L-arginine, superoxide dismutase, rats.

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.

In developed European countries, there is a trend towards high sexual activity in older men and the late creation of a family with children, which has certain difficulties in connection with a decrease in testosterone production in old age [2, 10]. At the same time uncontrolled usage of testosterone leads to increase of prostate cancer incidence since androgens play key role in its development [4].

Diphereline is a potent treatment method for prostate cancer treatment, however we showed in our previous works that its usage leads to development of oxidative stress and changes in sustentocytes and interstitial endocrynocites [3, 7, 14]. Minimal duration of diphereline intake in prostate cancer treatment

lasts half a year [6, 8, 9]. It is still unclear if the changes observed on 30th and 90th day of diphereline intake are not part of first adoptive reaction and not disappear later [11, 12].

The purpose of the study was to establish the microscopic organization of rat interstitial endocrinocytes and sustentocytes, to determine the sources of nitric oxide production and the intensity of oxidative stress in the testes in experimental central deprivation of testosterone synthesis with diphereline on the 180th day of the experiment.

Materials and methods. The experiments were carried out on 10 sexually mature male white rats of the Wistar line. Rats were divided into 2 groups: the control group (5) and the experimental group (5). Animals from experimental group were injected subcutaneously with diphereline (Triptorelin embonate) at a dose of 0.3 mg of the active substance/kg [5, 11, 12]. of body weight for 180 days, while control group received injection of saline [13].

Animals were kept in standard vivarium conditions of the Ukrainian Medical Stomatological Academy. 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), as well as with the "General Ethical Principles of Animal Experiments" adopted by the First National Congress on Bioethics (Kyiv, 2001).

After an overdose of ketamine, the animals were decapitated, the prepared small pieces of the testes were fixed in a 2.5% glutaraldehyde solution (pH=7.2-7.4). Postfixation of the material was carried out with 1% solution of osmium (IV) oxide, followed by dehydration in propylene oxide and 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 [1].

Using standard methods, the material was imbedded in paraffin blocks, of which sections 4 μm thick were made and stained with hematoxylin and eosin. Histological preparations 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 testis tissue using Ulab 101 spectrophotometer [11, 12, 15].

General activity of NO-synthase (gNOS), activity of constitutive isoforms (cNOS), activity of inducible isoform (iNOS), activity of arginases and nitrite concentration [11, 12]. Basic production of superoxide anion radical ($O_2^{\bullet-}$), its production by the mitochondrial electron transport chain (ETC) and microsomal ETC was determined by the growth of diformazan concentration, formed in the reaction of $O_2^{\bullet-}$ with nitro blue tetrazolium [15]. Superoxide dismutase (SOD) and catalases activity was determined according to guidelines [15]. The concentration of free malondialdehyde (MDA) was determined by reaction with 1-methyl-2-phenylindole [15].

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

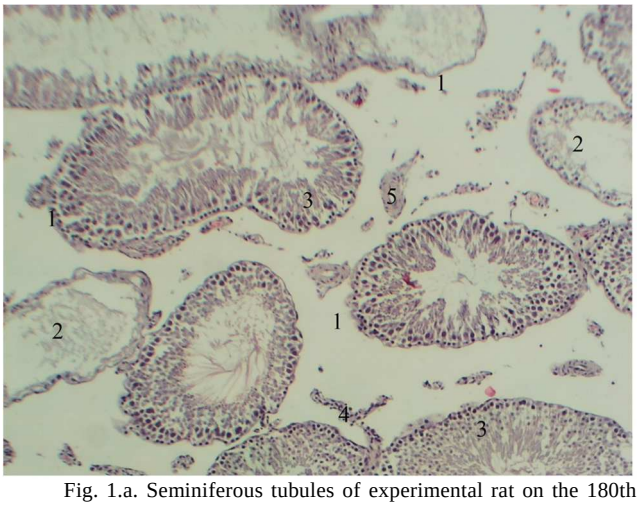

Fig. 1.a. Seminiferous tubules of experimental rat on the 180th day. Microimage. Stain: hematoxiline and eosine. Lens: 10: Ocular lens: 10. 1. Interstitial space - fibrosis. 2. Seminiferous tubule with single sustentocytes. 3. Spermatogenic epithelium of the unhealed tubule. 4. Interstitial cells. 5. The capillary in the Interstitial space with fibroblasts.

Results of the study and their discussion. During morphological study of the experimental group of animals on the 180th day of the experiment, we found an increase in the interstitial space of the testes in comparison with the control group of animals (Fig.1a.) due to the proliferation of connective tissue (fibrosis). We determined an increased concentration of fibroblasts in the interstitial tissue, in some places it amounted to 14 cells in the field of view. The number of vessels is increased, they are full-blooded, the walls thickness increased, in some places with the marginal standing of leukocytes.

The number of interstitial endocrinocytes is reduced in comparison with the control group, there are interstitial spaces between the convoluted tubules with a complete absence of interstitial endocrinocytes (fig. 1a).

Interstitial endocrinocytes are reduced in size, their nuclei are heterochromic. Macrophages were also determined in the interstitial space. The number of parietal macrophages prevailed several times over interstitial. In the interstitium, macrophages were determined, which were located 1-2-3 (interstitial) closer to the vessel. Near the wall of convoluted seminiferous tubules, their number was up to 10-12 in the field of view. In the macrophage cytoplasm, secretory granules of various sizes and electron densities were detected. Also, rather large phagosomes containing fragments of dead cells were visualized in the cytoplasm of macrophages. (fig. 1.b). All parietal macrophages were in the phase of phagocytosis, which was not typical of interstitial macrophages.

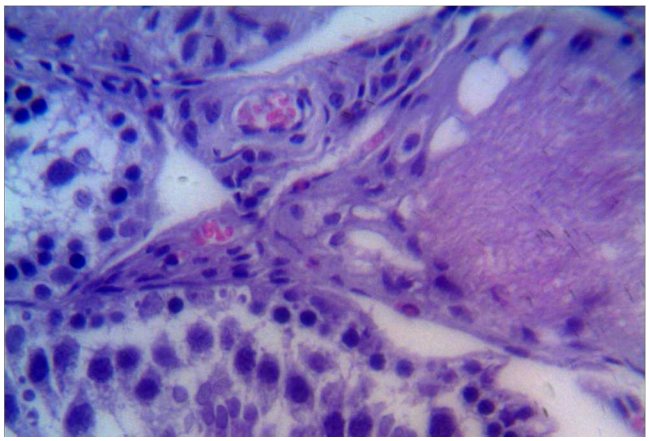

Fig. 1.b. Interstitial space of experimental rat on the 180th day. Microimage. Stain: hematoxiline and eosine. Lens: 40: Ocular lens:15.

The wall of the convoluted seminiferous tubules is compacted, convoluted due to interstitial fibrosis. In the structure of some convoluted seminiferous tubules from the spermatogenic epithelium of the basal layer, we detected following changes: a decrease in the population of both type A spermatogonia and type B spermatogonia. Discompletion and disorientation occurred in the adluminal layer, followed by desquamation of spermatids. Hypochromia and pycnosis were noted in the nuclei of spermatids.

Disorientation, discompletion of secondary and primary spermatocytes was determined in the ranks of spermatogenic epithelium. In most convoluted tubules, their desquamation was traced. Due to the complete or partial desquamation of spermatogenous epithelial cells from the basal membrane in the lumen of the tubules, “seed balls” were formed from undifferentiated cells. There were convoluted seminiferous tubules with a complete absence of spermatogenous epithelium and with single supporting cells (Sertoli cell-only syndrome; fig. 1.a.).

When we studied the structural organization of rat sustentocytes from the experimental group in comparison with the control group, we found that hyperplasia of the elements of the smooth endoplasmic reticulum was observed in the cytoplasm of the sustentocytes. Quite large phagosomes containing cell fragments were detected in the cytoplasm. In the cytoplasm of some spermatocytes and spermatids, deformation of the inner membranes and vacuolization of mitochondria appeared. The number of mitochondria in the cytoplasm of the sustentocytes decreased, the electron density of the mitochondrial matrix was lowered, protein structures were present either inside the vacuoles or independently located in the cell cytoplasm.

The total activity of NOS on the 180th day of central deprivation of testosterone synthesis increased by 51.9% when compared with the control group (tab. 1). At the same time, cNOS activity decreased 5.13 times, and iNOS activity increased 5.69 times. The concentration of nitrite in the testes of rats increases 3.44 times. The activity of arginases is reduced by 4.13 times. Thus, the increased production of nitric oxide under conditions of central deprivation of testosterone synthesis is ensured by the activity of the inducible NOS isoform. At the same time, an increase in iNOS activity with a decrease in the activity of the arginase pathway of L-arginine cleavage may indicate a change in the polarization of the macrophages of the testes with a predominance of the pro-inflammatory phenotype (M_1).

Table 1.

Nitric oxide cycle function during 180-day central testosterone synthesis deprivation (M±m)

Groups	Parameters				
	gNOS activity, µmol/min per g of protein	iNOS activity, µmol/min per g of protein	cNOS activity, µmol/min per g of protein	Arginase activity, µmol/min per g of protein	NO_2^- concentration, nmol/L
Control	0.54±0.04	0.13±0.02	0.41±0.03	2.48±0.05	3.83±0.25
Experimental	0.82±0.08*	0.74±0.08*	0.08±0.01*	0.60±0.02*	13.19±0.55*

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

The basic production of $O_2^{\bullet-}$ on the 180th day of the experiment increased 8.11 times when compared with the control group (tab. 2). Production of O_2 by mitochondrial ETC increased by 3.7 times, and that of microsomal ETC – by 2.4 times. SOD activity decreased by 2.7 times, meanwhile catalase activity did not change statistically significantly. MDA concentration in rat testes elevated by 3.15 times.

Table 2

Oxidative stress markers in rat testes during 180-day central testosterone synthesis deprivation (M±m)

Groups	Parameters					
	SOD activity, c.u.	Catalase activity, nkat/g of tissue	Basic $O_2^{\bullet-}$ production, nmol/s per g of tissue	Production of $O_2^{\bullet-}$ from mithochondrial ETC, nmol/s per g of tissue	Production of $O_2^{\bullet-}$ from microsonal ETC, nmol/s per g of tissue	Free MDA, µmol/g of tissue
Control	1.87±0.11	182.0±17.0	0.26±0.01	7.84±0.13	9.55±0.19	6.64±1.44
Experimental	0.70±0.17*	181.0±17.0	2.11±0.03*	28.75±1.62*	23.20±0.42*	20.91±0.25*

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

Thus, in the tissues of the testes under conditions of prolonged central deprivation of testosterone synthesis, oxidative stress develops, which on the 180th day of the experiment leads to the development of fibrosis in the testes. Change in the polarization of macrophages may be the reason for the development of oxidative stress in the testes, as evidenced by an increase in iNOS activity and a decrease in arginase activity.

Analyzing the changes described by us in dynamics, it is noteworthy that an increase in iNOS activity and a decrease in arginase activity is observed already from the first month of central deprivation of testosterone synthesis, does not change on the 3rd month of the experiment and continues on the 6th month [5, 6]. Therefore, changes in the polarization of macrophages (transition to the M_1 phenotype) may be the cause of the observed changes. Testosterone produced by interstitial endocrinocytes tends to inhibit the polarization of macrophages by the M_1 phenotype [11, 12].

However, in the tissues of the testes there are 2 populations of macrophages: resident (interstitial) and coming from the bone marrow (parietal). Based on the activities of iNOS and arginases, the number of parietal and interstitial macrophages in the control group, it can be concluded that under physiological conditions, the macrophages of the testes have an anti-inflammatory (M_2) phenotype. The contribution of interstitial and parietal macrophages to the development of morphological and biochemical changes in the testes during prolonged central deprivation of testosterone synthesis requires further study.

Controlling the polarization of testicular macrophages under conditions of prolonged central deprivation of testosterone synthesis may be a promising method for the pathogenetic correction of morphological and biochemical changes observed on 180th day of the experiment.

Conclusion

Experimental central deprivation of testosterone synthesis with diphereline on the 180th day of the experiment leads to shift of NO synthesis from constitutive NO-synthases to inducible NO-synthase and intensification of oxidative stress due to increase of superoxide anion-radical production and decrease in antioxidant protection. At the same time rat interstitial endocrinocytes decrease in number and volume. Their nuclei are heterochromic. Sustentocytes have hyperplasia of the elements of the smooth endoplasmic reticulum, much lesser amount of mithochondria with lower electronic density.

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Реферати

СТРУКТУРНА ОРГАНІЗАЦІЯ СТРОМАЛЬНИХ ТА ПАРЕНХІМАТОЗНИХ КОМПОНЕНТІВ СІМ'ЯНИКІВ ЩУРІВ ПРИ ЦЕНТРАЛЬНІЙ ДЕПРИВАЦІЇ СИНТЕЗУ ТЕСТОСТЕРОНА НА 180 ДЕНЬ ЕКСПЕРИМЕНТУ

Стецук Є.В., Акімов О.Є., Шепітько К.В., Гольцев А.Н.

Тривала центральна депривація синтезу тестостерону може призвести до біохімічним і морфологічних змін в яєчках. Вплив тривалого дефіциту тестостерону на вироблення активних форм азоту і кисню, морфологічні зміни в інтерстиційних ендокріноцитів та сустентоцитів ще повністю не вивчені. Кількість інтерстиційних ендокріноцитів знижена в порівнянні з контрольною групою, між звивистими канальцями є інтерстиційні простори з повною відсутністю інтерстиційних ендокріноцитів. Інтерстиційні ендокриноцити зменшені в розмірах, їх ядра гетерохромні. При вивченні структурної організації сустентоцитів щурів експериментальної групи в порівнянні з контрольною групою, ми виявили, що гіперплазія елементів гладкої ендоплазматичної сітки спостерігалася в цитоплазмі сустентоцітов. Кількість мітохондрій в цитоплазмі сустентоцитів зменшилася, електронна щільність мітохондріального матриксу була знижена, білкові структури були присутні або всередині вакуолей, або незалежно розташовувалися в цитоплазмі клітини. Біохімічні дослідження виявили збільшення продукції NO з індуцібельною NO-синтазою і розвиток окисного стресу. Експериментальна центральна депривація синтезу тестостерону з Диферелином на 180-й день експерименту призводить до зрушення синтезу NO з конститутивних NO-синтази до індуцібельної NO-синтази і посилення окисного стресу за рахунок збільшення продукції супероксид-аніон-радикалів і зниження антиоксидантного захисту.

Ключові слова: сімяники, інтерстиціальні ендокріноціти, сустентоціти, NO-синтаза, iNOS, cNOS, L-аргінін, супероксиддисмутаза, щури.

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СТРУКТУРНАЯ ОРГАНИЗАЦИЯ СТРОМАЛЬНЫХ И ПАРЕНХИМАТОЗНЫХ КОМПОНЕНТОВ СЕМЕННИКОВ КРЫС ПРИ ЦЕНТРАЛЬНОЙ ДЕПРИВАЦИИ СИНТЕЗА ТЕСТОСТЕРОНА НА 180 ДЕНЬ ЭКСПЕРИМЕНТА

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Длительная центральная депривация синтеза тестостерона может привести к биохимическим и морфологическим изменениям в яичках. Влияние длительного дефицита тестостерона на выработку активных форм азота и кислорода, морфологические изменения в интерстициальных эндокриноцитах и сустентоцитах еще полностью не изучены. Установлено, что количество интерстициальных эндокриноцитов снижено по сравнению с контрольной группой, между извитыми семенными канальцами имеются интерстициальные пространства с полным отсутствием интерстициальных эндокриноцитов. Интерстициальные эндокриноциты уменьшены в размерах, их ядра гетерохромны. При изучении структурной организации сустентоцитов крыс из экспериментальной группы по сравнению с контрольной группой, мы обнаружили, что гиперплазия элементов гладкой эндоплазматической сети наблюдалась в цитоплазме сустентоцитов. Количество митохондрий в цитоплазме сустентоцитов уменьшилось, электронная плотность митохондриального матрикса была снижена, белковые структуры присутствовали либо внутри вакуолей, либо независимо располагались в цитоплазме клетки. Биохимические исследования выявили увеличение продукции NO из индуцибельной NO-синтазы и развитие окислительного стресса. Экспериментальная центральная депривация синтеза тестостерона с диферелином на 180-й день эксперимента приводит к сдвигу синтеза NO из конститутивных NO-синтаз в индуцибельную NO-синтазу и усилению окислительного стресса за счет увеличения продукции супероксид-анион-радикалов и снижения антиоксидантной защиты.

Ключевые слова: семенники, интерстициальные эндокриноциты, сустентоциты, NO-синтаза, iNOS, cNOS, L-аргинин, супероксиддисмутаза, крысы.

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