Influence of quercetin on morphological changes in rats testes after 180 days during central deprivation of luteinizing hormone

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A relevant and popular area of research is the protective effect of the bioflavonoid quercetin, which makes it possible to use it to correct testicular dysfunction of various origins. The aim of the study was to determine the effect of quercetin on the microscopic organization of rat testicles, nitric oxide production and the intensity of oxidative stress in rat testicles on the 180th day of the experiment, during experimental central deprivation of luteinizing hormone synthesis caused by triptorelin solution. The experiment was performed on 20 adult male white rats. Rats were divided into 2 groups of 10 animals in each group: control group (I), group with central deprivation of LH synthesis + quercetin (II). Animals from the group with central deprivation of LH synthesis were injected subcutaneously with triptorelin acetate at a dose of 0.3 mg of active substance per kg and quercetin 100 mg per kg of body weight 3 times a week, while the control group was injected with saline. Ultrathin sections made by ultramicrotome were contrasted with 1 % aqueous uranyl acetate and lead citrate according to the Reynolds method and examined by electron microscopy. According to standard methods, the material was poured into paraffin blocks, from which sections with a thickness of 4 ?m were made and stained with hematoxylin and eosin. Histological specimens were examined using a Вiorex 3 light microscope with a digital microfilter with software adapted for these studies. All biochemical studies were performed in 10 % of testicular tissue homogenate using a Ulab 101 spectrophotometer. Statistical processing of the study results was performed using Microsoft Office Excel software and Real Statistics 2019 extension. The nonparametric Mann-Whitney test was used to determine the statistical significance of differences between groups. Our study of the interstitial space in the testes of white rats showed the heterogeneity of populations of endocrinocytes and macrophages and the variability of structural and functional parameters. In the tissues of the testes in conditions of prolonged central deprivation of testosterone synthesis develops oxidative stress, which on the 180th day of the experiment leads to the development of edema of the interstitial space, followed by tissue fibrosis. Changes in the polarization of macrophages in our opinion may cause oxidative stress in the testes, as evidenced by increased iNOS activity and decreased arginase activity, but use of quercetin protects rat testicular tissue from oxidative damage caused by triptorelin by increasing direct antioxidant system and effects on the lipoxygenase pathway of arachidonic acid metabolism.

Keywords: testis, interstitial endocrinocytes, macrophages, quercetin, NO synthase, rats.

Introduction

The reproductive health of the population is an integral part of the demographic processes taking place in society, therefore, the problems associated with its violation today go beyond the medical, and acquire a social character [1]. In recent decades, men all over the world have seen a sharp deterioration in spermogram indicators, according to reproductive centers, the percentage of fertilization is decreasing, and cases of embryonic mortality are increasing. Over the past decades, a clear tendency towards a decrease in the activity of spermatogenesis in men has been observed throughout the world [13]. For the period from 2015 to 2020 the increase in the absolute number of patients with prostate diseases in our country alone was 36.2 % [14]. There is an increase in infertile
The experiments were carried out on 20 sexually mature male white rats. Rats were divided into 2 groups with 10 animals in each group: the control group (I), the group with central deprivation of testosterone (II). Animals from the group with central deprivation of testosterone synthesis were injected subcutaneously with triporelin acetate at a dose of 0.3 mg of the active substance per kg [9, 16] and quercetin 100 mg per kg body weight 3 times a week [7], while the control group was administered saline. Experiment conducted for 180 days. Animals were kept in standard vivarium conditions of the Poltava State Medical University. Committee on Bioethics of Poltava State Medical University (protocol № 195 From 24.06.2021) found that the studies do not contradict the basic bioethical standards 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 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 [3].

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).

We carried out all biochemical studies in 10 % homogenate of testis tissue using Ulab 101 spectrophotometer. General activity of NO-synthase (gNOS), activity of constitutive isoforms (cNOS), activity of inducible isoenzyme (iNOS) was determined by increase of nitrite concentration after incubation in buffer solution (pH=7.4) containing 0.3 ml of 320 mM L-arginine solution and 0.1 ml of 1 mM NADPH+H solution [5]. Nitrite concentration was measured with help of Griess reagent [5]. Arginase activity was evaluated by increase of L-ornithine content after incubation in buffer solution (pH=7.0) containing 0.2 ml of 24 mM L-arginine solution [5].

Basic production of superoxide anion radical (SAR), 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 SAR with nitro blue tetrazolium [8]. Superoxide dismutase (SOD) activity was determined by inhibition of adrenaline autooxidation, while catalase activity was determined by the amount of hydrogen peroxide, remained after its catalase-dependent reduction [8]. The concentration of free malondialdehyde (MDA) was determined by reaction with 1-methyl-2-phenylindole resulting in formation of specific colored substance [8].

Statistical processing of the study 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.

**Results**

The 180th day of the experiment was characterized by structural changes in both the parenchymal and stromal components of the testes. So in most of the convoluted...
seminiferous tubules, the basement membrane is convoluted, in the rows of the spermatogenic epithelium, an increase in the height of the spermatogenic epithelium was determined due to the disorientation of the cells. The edematous cells are detached from each other. In some convoluted seminiferous tubules, partial desquamation of spermatids was determined, with the appearance of spermatogenic balls. In the nuclei of spermatocytes and spermatids there is a thickening and a decrease in volume in comparison with the control group. Hypochromia of nuclei, with edematous cytoplasm increased in volume. Some convoluted seminiferous tubules were in the stage of complete desquamation, but their number was minimal, and amounted to 2-3% of the total, due to which the height of the spermatogenic epithelium progressively decreased in them and was inferior to the data of the control group.

Studying the interstitial space of the testes of the experimental group, we found that the interstitial tissue is enlarged, edematous due to the microcirculatory and connective tissue components. A significant increase in the number of arterioles and venules was observed in the interstitium, and numerous arterio-arterial anastomoses were identified. The walls of the vessels are edematous, the diameter is increased, the sludge of blood cells inside the vessels was determined (see Fig. 1).

Studying the structure of interstitial endocrinocytes, which were clearly defined against the background of connective tissue by oxyphilic color of the cytoplasm and light-basophilic nuclei of a round or oval shape. Cells are irregular, polygonal, round or oval nuclei. We divided the entire population of endocrinocytes, depending on the density of the nucleus, into light and dark cells, and according to the size of nuclei and the volume of cytoplasm, large and small (Fig. 2).

The total number of interstitial endocrinocytes was significantly reduced in comparison with the control group. The number of active cells is reduced and amounted to 12 % of the total number of cells.

When we studied the macrophage system of the interstitium of the testes of the experimental group, as in our previous studies, we clearly traced two populations of cells by the type of localization in the connective tissue [17]. Parietal macrophages were detected near the basement membrane of the convoluted tubule, their number increased in comparison with both the control group and the previous study periods. Interstitial macrophages are single, inactive (Fig. 3).

Interstitial macrophages were large, nuclei oval or round, electron dense with a predominance of heterochromatin. Karyolemma is dense, two-layer, one nucleus. The cell cytoplasm is small in size with all

![Fig. 1. Seminiferous tubules of experimental rat on the 180th day. Microimage. Stain: hematoxiline and eosine. Lens: 10: Ocular lens: 10. Interstitial space with stasis.](image1)

![Fig. 2. Interstitial space of experimental rat on the 180th day. Microimage. Stain: hematoxiline and eosine. Lens: 40: Ocular lens: 15. 1 - Interstitial space. 2 - Interstitial endocrinocytes - large. 3 - Interstitial endocrinocytes - small.](image2)

![Fig. 3. Interstitial space of experimental rat on the 180th day. Microimage. Stain: hematoxiline and eosine. Lens: 40: Ocular lens: 15. 1 - Interstitial space. 2 - Interstitial endocrinocytes. 3 - Interstitial macrophages. 4 - Parietal macrophages.](image3)
subcellular elements (Fig. 4). The parietal ones had an elongated flattened oval nucleus, which had several nucleoli. The karyolemma is distinct, the karyoplasm is electron-dense, and the chromatin is heterogeneous. The cytoplasm is light, with a small number of lysosomes; mitochondria are small and round. All of the above indicates that the ultrastructural organization of macrophages did not differ from the cells of the control group.

When substantiating the biochemical parameters of testis tissues, we obtained such results as the total NOS activity on the 180th day of central deprivation of luteinizing hormone synthesis was 0.463±0.044 μmol/min, which did not differ much in comparison with the parameters of the control group (Table 1), however, the activity of cNOS decreased by 7.23 times, while the activity of iNOS increased by 3.15 times. The concentration of nitrite in the testes of rats increases 2.45 times. The arginase activity is decreased 4.13 times.

Thus, the increased production of nitric oxide under conditions of central disturbance in the synthesis of luteinizing hormone, with a direct effect on the endocrinocytes of the interstitial space of the testes to testosterone production, is provided by the activity of the inducible isofrom of NOS. 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 testes macrophages with a predominance of the pro-inflammatory phenotype (M1), which we have in an increase in active parietal forms.

Basic production of O$_2^-$ on the 180th day of the experiment increased 52.96 times compared with the control group (Table 2). O$_2^-$ production of mitochondrial ETC decreased 6.37 times, and microsomal ETC - 35.23 times. The SOD activity decreased 1.37 times, while the catalase activity did not change statistically significantly. The concentration of MDA in the testes of rats was reduced by 2.36 times.

**Discussion**

Thus, our study of interstitial endocrinocytes and macrophages in the testes of rats showed the heterogeneity of the populations of these cells, the variability of the structural and functional parameters of endocrinocytes and macrophages. The results obtained agree with the literature data, which set out the basic principles and regularities of the organization of the cell population of the interstitial space of the testis [20].

Also, in the tissues of the testicles 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 edema of the interstitial space with subsequent tissue fibrosis. A change in the polarization of macrophages can be the cause of the

![Fig. 4. Interstitial macrophage (IM) of rats of the control group of animals. Magnification x12 000. 1 - nucleus, 2 - chromatin, 3 - mitochondria, 4 - endoplasmic reticulum, 5 - cytoplasm.](image)

**Table 1. Nitric oxide cycle function during 180-day central deprivation LH synthesis + quercetin (M±m).**

<table>
<thead>
<tr>
<th>Groups</th>
<th>gNOS activity, μmol/min per g of protein</th>
<th>iNOS activity, μmol/min per g of protein</th>
<th>cNOS activity, μmol/min per g of protein</th>
<th>Arginase activity, μmol/min per g of protein</th>
<th>NO2-concentration, nmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.548±0.041</td>
<td>0.130±0.022</td>
<td>0.411±0.032</td>
<td>2.480±0.052</td>
<td>3.834±0.252</td>
</tr>
<tr>
<td>Experimental</td>
<td>0.463±0.044*</td>
<td>0.414±0.040*</td>
<td>0.057±0.002*</td>
<td>1.013±0.051*</td>
<td>11.55±0.21*</td>
</tr>
</tbody>
</table>

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

**Table 2. Oxidative stress markers during 180-day central deprivation LH + quercetin synthesis (M±m).**

<table>
<thead>
<tr>
<th>Groups</th>
<th>SOD activity, c.u.</th>
<th>Catalase activity, nkat/g of tissue</th>
<th>Basic O$_2^-$ production, nmol/s per g of tissue</th>
<th>Production of O$_2^-$ from mitochondrial ETC, nmol/s per g of tissue</th>
<th>Production of O$_2^-$ from microsomal ETC, nmol/s per g of tissue</th>
<th>Free MDA, μmol/g of tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.87±0.110</td>
<td>182.0±17.0</td>
<td>0.284±0.012</td>
<td>7.840±0.131</td>
<td>9.553±0.192</td>
<td>6.640±1.442</td>
</tr>
<tr>
<td>Experimental</td>
<td>1.363±0.011*</td>
<td>12.72±0.11*</td>
<td>13.77±0.11*</td>
<td>1.234±0.035</td>
<td>0.271±0.004</td>
<td>15.70±0.18*</td>
</tr>
</tbody>
</table>

**Note:** * indicates that the difference is statistically significant when compared with control group (p<0.05).
development of oxidative stress in the testes, as evidenced by an increase in iNOS activity and a decrease in arginase activity, but our use of quercetin protects rat testicular tissue from oxidative damage caused by the administration of triptorelin by increasing direct antioxidant protection and affecting the lipoygenase pathway of metabolism arachidonic acid [11].

The results obtained are a theoretical basis for the development of methods for correcting violations of the generative and endocrine function of the testes under extreme influences on the body, with damage to endo- and paracrine regulation. Data on the functional morphology of the testes at the stages of adaptation to changes in the endocrine and immune function of the testes expand the existing understanding of the causes of disturbances in spermatogenesis and its regulation. The data can be used in research and teaching at the departments of medical universities and biological faculties of universities.

Conclusions
1. Central blockade of LH synthesis by the introduction of triptorelin with parallel administration of quercetin to the studied animals on the 180th day of the experiment causes changes in the structure of the interstitial space of the testes of rats and is characterized by high variability in the populations of interstitial endocrinocytes and macrophages, which affects the change in spermatogenesis in the testes of rats.
2. Biochemical parameters on the 180th day of the experiment showed a shift in NO synthesis from constitutive NO synthases to inducible NO synthase and increased oxidative stress due to an increase in the production of superoxide anion radical and a decrease in antioxidant protection.
3. Quercetin protects rat testicular tissue from oxidative damage caused by the administration of triptorelin on the 180th day of the experiment by increasing the antioxidant defense and reducing reactive oxygen species.

References
ВПЛИВ КВЕРЦЕТИНУ НА МОРФОЛОГІЧНІ ЗМІНИ СІМ’ЯНИКІВ ЩУРІВ НА 180 ДЕНЬ ПІД ЧАС ЦЕНТРАЛЬНОЇ ДЕПРИВАЦІЇ ЛЮТЕЇНІЗУЮЧОГО ГОРМОНУ

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Актуальним і затребуванням напрямом досліджень є протективна дія біофлавоноїду кверцетину, яка дає можливість використовувати його для корекції тестикулярної дисфункції різного ґенезу. Метою дослідження було установити вплив кверцетину на мікроскопічну організацію яєчок щурів, продукцію оксиду азоту та інтенсивність окисног о стресу в яєчках щурів на 180-ту добу експерименту, під час експериментальної центральної депривації синтезу лютеїнізуючого гормону, викликаної введенням розчину триптореліну ацетату. Експеримент проводили на 20 статевозрілих самцях білих щурів. Щури були розділені на 2 групи по 10 тварин у кожній групі: контрольна група (І), група з центральною депривацією синтезу ЛГ + кверцетин (ІІ). Тваринам із групи з центральною депривацією синтезу ЛГ підшкірно вводили триптореліну ацетат у дозі 0,3 мг діючої речовини на кг та кверцетин 100 мг на кг маси тіла 3 рази на тиждень, при цьому контрольній групі вводили фізіологічний розчин. Ультратонкі зрізи, виготовлені ультрамікротомом, контрастували з 1 % водним розчином уранілацетату та цитрату свинцю за методом Рейнольдса та досліджували за допомогою електронного мікроскопа. За стандартними методами матеріал заливали в парафінові блоки, з яких виготовляли зрізи товщиною 4 мкм і забарвлювали гематоксиліном та еозином. Гістологічні препарати досліджували за допомогою світлового мікроскопа Вiorex 3 з цифровим мікрофільтром з програмним забезпеченням, адаптованим для цих досліджень. Усі біохімічні дослідження проводили в 10 % гомогенаті тканини яєчка за допомогою спектрофотометра Ulab 101. Статистичну обробку результатів дослідження проводили за допомогою програмного забезпечення Microsoft Office Excel та розширення Real Statistics 2019 до нього. Для визначення статистичної значущості відмінностей між групами використовували непараметричний критерій Манна-Уїтні. Проведене нами дослідження інтерстиційного простору в сім’янихках білих щурів показало неоднорідність популяції ендокриноцитів та макрофагів та мінливість структурно-функціональних параметрів. У тканинах яєчок в умовах тривалої центральної депривації синтезу тестостерона розвивається окисний стрес, який на 180-й день експерименту призводить до розвитку набряку інтерстиціального простору з наступним фіброзом тканини. Зміна поляризації макрофагів, на наш погляд, може бути причиною розвитку окисного стресу в сім’янихках, про що свідчить підвищення активності iNOS та зниження активності аргінази, але використання кверцетину захищує тканину яєчок від окисного пошкодження, викликаного введенням триптореліну за рахунок підвищення прямої антиоксидантної системи та вплив на ліпоксигеназний шлях метаболізування арахідонової кислоти.

Ключові слова: сім’яник, інтерстиційні ендокриноцити, макрофаги, кверцетин, NO-синтаза, щури.