Effect of quercetin administration on electron microscopic changes in testicular interstitial endocrinocytes during long-term central blockade of luteinising hormone in rats


Poltava State Medical University, Poltava, Ukraine

Quercetin is a flavonoid with potential health benefits and it may help prevent cardiovascular diseases, reduce the risk of degenerative brain processes and cancer, and has antioxidant properties that neutralise free radicals. Substances in this group also have antioxidant properties, which help the body protect itself from the harmful effects of free radicals by neutralising these unstable molecules. Research indicates that the impact of free radicals on cell structure is associated with the development of chronic diseases such as diabetes, cancer, and cardiovascular pathologies. The aim of the study was to investigate the effect of quercetin administration on electron microscopic changes in the interstitial endocrinocytes of the testes under long-term central blockade of the synthesis of luteinising hormone by tryptorelin. The experiment involved 35 sexually mature male white rats. They were divided into two groups: the control group (I) received saline, while group II received subcutaneous injections of tryptorelin at a dose of 0.3 mg of active ingredient per kg of rat body weight to induce experimental central deprivation of luteinising hormone synthesis and additionally, quercetin was administered three times a day by gastric tube in terms of body weight.

The study has demonstrated that the administration of tryptorelin results in structural and functional changes in the connective tissue components of rat testes. Specifically, there are quantitative and qualitative disorders in the population of interstitial endocrine cells, as well as electron microscopic changes at the subcellular level. Various pathological changes and abnormalities in the functional activity of the internal components of the cell were detected on days 270 and 365 of the experiment. The frequency, number, and size of Reinke crystals in relation to the cell volume correlated with changes in cells and increased at later stages of the study. A similar correlation with testosterone levels has not been found in the literature, which leads us to classify Reinke crystals as the result of degenerative processes in the cell. Thus, additional administration of quercetin reduces the adverse effect of tryptorelin and delays the onset of changes in the structure of interstitial endocrinocytes from day 180 to later observation periods.

Keywords: testes, electron microscopy, rats, interstitial endocrinocytes, luteinising hormone, quercetin, tryptorelin.

ARTICLE INFO

Received: 26 December 2023
Accepted: 12 February 2024

UDC: 616.682-018.1-02:615.357-076.4:615.356-092.9

CORRESPONDING AUTHOR
e-mail: stetsuk78@gmail.com
Stetsuk Ye. V.

CONFLICT OF INTEREST
The authors have no conflicts of interest to declare.

FUNDING
Not applicable.

DATA SHARING
Data are available upon reasonable request to corresponding author.

Introduction
Quercetin is a flavonoid commonly found in fruits, vegetables, and seeds [25]. Flavonoids can help prevent cardiovascular diseases, reduce the risk of degenerative brain processes and cancer [4, 38]. These substances have antioxidant properties that help the body protect itself from the damaging effects of free radicals by binding and neutralising these unstable molecules [20]. Research has shown that the destructive effects of free radicals are linked to the development of several chronic diseases, such as diabetes, cancer and cardiovascular disease [13].

The distribution of quercetin was investigated in rats and pigs, revealing that it is primarily concentrated in the lungs, colon, kidneys, and liver. Metabolites of this substance were also detected in lower levels in the brain.
If the flavonoid is only obtained from the diet, it is found in the nanomolar range in the plasma, but the concentration can be increased to the low micromolar range by supplementation with aglycones or glycosides [25].

Tryptorelin is a synthetic analogue of gonadotropin-releasing hormone (GnRH) [3, 19, 22, 26-28]. It suppresses receptor expression in the pituitary gland without affecting the functioning of the pituitary-testicular complex as a whole. Deprivation of luteinising hormone synthesis in rats leads to the development of oxidative stress in their internal organs [5, 7, 31, 32]. Nitric oxide production during central inhibition of luteinising hormone synthesis undergoes complex changes. Initially, there is a decrease in NO synthase-dependent nitric oxide production [1, 34] on days 30 and 90, but later there is hyperproduction of nitric oxide from NO synthases in the heart [13, 17].

Quercetin, a flavonoid, serves as a substrate for the synthesis of NO. It reduces the activation and adhesion of white blood cells and thrombocytes to the vascular endothelium, inhibits the synthesis of the adhesion proteins VCAM-1 and MCP-1, and prevents the formation and development of atherosclerotic plaque. Quercetin inhibits the synthesis of endothelin-1, a potent vasoconstrictor that stimulates the proliferation and migration of smooth muscle cells in the vascular wall [20]. According to reference [20], the substance exhibits antioxidant, antiplatelet, antiradical, and membrane-stabilising properties. It also prevents an increase in calcium levels in cells, has an angioprotective effect, inhibits protein kinase, and has a pronounced cytoprotective (endothelioprotective) activity. Quercetin can directly affect the pathophysiological processes associated with endothelial dysfunction as an endothelial protective agent. This is evidenced by a decrease in individual markers, leading to improved vasomotor reactions, better control of major blood pressure parameters, and an overall improved prognosis for patients [38].

Quercetin is a modulator of several enzymes involved in phospholipid degradation, such as phospholipases, phosphoglycerases and cyclooxygenase. These enzymes affect free radical processes and are responsible for the biosynthesis of nitric oxide and proteinases in cells. The inhibitory effect of quercetin on membranotropic enzymes, especially on 5-lipoxygenase, affects the inhibition of the synthesis of leukotrienes LTC4 and LTB4 [20]. In addition, quercetin increases the level of nitric oxide in endothelial cells in a dose-dependent manner, which explains its cardioprotective effect in ischemic and reperfusion myocardial damage [13]. Quercetin has antioxidant and immunomodulatory properties. It reduces the production of cytotoxic superoxide anion, normalises the activation of lymphocyte subpopulations, and reduces the level of their activation. By inhibiting the production of anti-inflammatory cytokines IL-1β and IL-8, it helps to reduce the volume of necrotic myocardium and enhance reparative processes [6]. The protective action mechanism is also linked to preventing an increase in platelet intracellular calcium concentration and aggregation activation, while simultaneously inhibiting thrombogenesis. The drug restores regional blood circulation and microcirculation without significantly altering vascular tone, thereby increasing microvessel reactivity. Quercetin normalises cerebral haemodynamics in ischaemic lesions and reduces the cerebral blood flow asymmetry ratio in ischaemic stroke [38]. The mechanisms of action of quercetin can be used experimentally to correct the pathological effects on testicular interstitial endocrinocytes. This has been demonstrated in our study.

The aim of the study was to investigate the effect of quercetin administration on electron microscopic changes in the interstitial endocrinocytes of the testes under long-term central blockade of the synthesis of luteinising hormone by tryptorelin.

Materials and methods

The study was carried out on 35 sexually mature male white rats, which were randomly divided into two groups: control (10 animals) and experimental (25 animals). The control rats were injected with saline. The experiment lasted for 365 days. To model central withdrawal of luteinising hormone synthesis, animals in the experimental group were administered a solution of tryptorelin at a rate of 0.3 mg active ingredient per kg animal weight [12, 21]. In addition, quercetin was administered orally three times a day using a gastric tube, taking into account the body weight of the animals.

The animals were kept under standard conditions at the vivarium of Poltava State Medical University. The investigation is a part of the research project "Experimental and morphological study of the effect of cryopreserved preparations of cord blood and embryofetal placental complex (EFPC), dipherelin, ethanol and 1 % methacrylic acid ester on the morphological and functional state of a number of internal organs" (state registration number 0119U102925). The animals were euthanised in accordance 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 the General Ethical Principles for Animal Experiments adopted by the First National Congress on Bioethics (Kyiv, 2001). The animals were withdrawn from the experiment on days 30, 90, 180, 270 and 365 by an overdose of ether anaesthesia. The study was approved and confirmed by the Bioethics Committee of Poltava State Medical University (protocol No. 195 - 24.06.2021).

For electron microscopic studies [11], the organ fragments were fixed in a 2.5 % glutaraldehyde solution, fixed in a 1 % osmium tetroxide solution in phosphate buffer (pH 7.2-7.4), dehydrated in alcohols and propylene oxide, and poured into a mixture of epoxy resins with araldite. Ultrathin sections were prepared on a ZKB-3 ultramicrotome (Sweden) and grids were made. The sections were contrasted first in a 1 % uranyl acetate...
solution in methanol and then with lead citrate according to Reynolds. The preparations were examined on a PEM-125 K electron microscope (serial number 38-76, TU 25-07-871-70), accelerating voltage 50-75 kW.

Morphometric quantitative analysis was conducted using Microsoft Office Excel and its Real Statistics 2019 extension, following generally accepted statistical methods.

**Results**

At the 1-month follow-up, electron microscopic photographs were studied in the experimental group. It was observed that the testicular connective tissue reacted asynchronously and underwent various changes in the structure of interstitial endocrinocytes at the cellular and subcellular levels. The population of interstitial endocrinocytes in the studied animals showed significant quantitative changes, with a variety of cell shapes ranging from round to polygonal. A few spinous and spindle-shaped cells were also observed (Fig. 1).

The cell size was 16.33±2.34 µm. In addition, cells up to 18 µm in diameter were observed due to the number and size of intracellular lipid granules. The cell nuclei were round or ellipsoidal, with euchromatin evenly distributed throughout the entire area. The content of parietal heterochromatin varied. The analysis of the entire population of endocrinocytes on day 30 revealed three distinct cell types based on their degree of development and differentiation, as determined by chromatin distribution and the number and quality of lipophilic granules in the cytoplasm. A functionally active state was observed in the majority of cells (65-70 % of the population). The cells were arranged in groups of 3-5, primarily located near capillaries. Single macrophages and stromal cells (fibroblastic cells) were found in close proximity.

The nuclei reflected the shape of the cell and were either rounded or elliptical, with 1-2 nucleoli. Depending on the cell activity, the amount of hetero- and euchromatin varied, with a predominance of heterochromatin. The nuclear envelope appeared clear and contained a large number of pores. The cytoplasm revealed the endoplasmic reticulum, formed by numerous branching tubes filled with a thin fibrous substance; many ribosomes were present on the membranes, and individual ribosomes were also found in the cytoplasm of the cells.

The mitochondria were clearly visible, numerous and varied in shape. The matrix was dense, with tubular and mixed-type cristae rarely visible. Various cytolemma protrusions were observed on the cell surface, and contacts between cell processes and blood and lymphatic capillaries were frequently detected. The endocrinocytes were found to be connected to each other by desmosomes and gap junctions. Additionally, curvatures of the biological membrane were detected in some areas. The lipophilic granules in the cytoplasm varied in size, shape, and density.

On the 90th day, the experimental group exhibited connective tissue membrane swelling and an increase in macrophage count, with parietal macrophages predominating over paravascular ones. The number of endocrinocytes decreased. Cell nuclei remained normal in size and shape (Fig. 2).

Minor destructive changes in the ultrastructural organization of the lamellar cytoplasmic Golgi complex were detected in the structure of interstitial endocrinocytes. The content of lysosomes in the cytoplasm of endocrinocytes was low, and lipid granules were large in size and present in each cell. Cells with a large number of lipid inclusions and small electron-dense hormonal granules in the cytoplasm are observed in some cases. A characteristic feature of these cells is the presence of numerous mitochondria with cristae immersed in a matrix of moderate electron density. The mitochondria of interstitial cells were of medium size, with an osmiophilic matrix and a small number of cristae. The cytoplasm of the cells was cleared, contained very few free ribosomes and polysomes.
compared to the control group of animals and the previous observation period. The cytoplasmic membrane matched the structure of the elementary membrane.

On the 180th day of observation, there are signs of connective tissue edema, the number of macrophages is increased, and fibroblasts are sporadic. The number of endocrinocytes is reduced. Cell groups are still being determined, but the number of such groups is significantly reduced compared to the previous observation periods. In the interstitial space, there are cells in both the stages of destruction and dystrophy. Some cells appear unchanged. The nuclei of the cells vary in shape and size, with reduced density due to euchromatin. Heterochromatin is adjacent to the inner membrane of the nucleolemma in small depths. Most cells do not have nuclei. The cytoplasm is clear with a small amount of lipid inclusions. The endoplasmic reticulum shows signs of disruption. Lysosomes are single, clear and small. Single, few mitochondria are present in the cytoplasm. They are variable in size and shape, but mostly rounded, the matrix is not seen, cristae are not visualised. The outer membrane is blurred and loose. Intercellular contacts are mostly absent. Cell remnants are found in the interstitial space (Fig. 3).

When we examined the electron micrographs of the experimental group of animals on day 270, the interstitial space between the convoluted tubules was enlarged and the number of hormone-producing cells was significantly reduced. The cells were mostly solitary. The number of blood vessels is increased, and macrophages are observed in large numbers near them. The endocrinocytes are small, elongated, with acidophilic cytoplasm, vacuolated at the periphery, and no smooth endoplasmic reticulum is seen. The nuclei are elongated and occupy most of the cell. Nuclei are present in some of the cells but not in all of them. The caryolemma is indistinct, and the number of pores is small. Fragmented nuclei with dense chromatin are sometimes observed. Mitochondria are single and rounded. In certain cells, the smooth membranes of the Golgi complex were randomly oriented and surrounded by large, electron-transparent vacuoles, lipid inclusions, and secretory granules. The cytoplasmic membrane was indistinct. A significant number of cells had a fragmented smooth endoplasmic reticulum (Fig. 4).

On Day 360, there was an increase in connective tissue layers due to fibroblastic cells. The number and quality of blood vessels increased several times compared to previous periods of the experiment. In addition, the interstitial endocrinocytes were observed to be small in size and to be solitary. In certain locations, there were regions where these cells were entirely absent. If there were pairs of cells, they were not connected to each other. The cell membrane lacked clear boundaries, and there were no cell junctions. Isolated lipid granules were seen in the cytoplasm, and the endoplasmic reticulum and Golgi complex were poorly developed. The nuclei show light-optical alterations, and may be absent or in the process of

![Fig. 3. Lipid inclusions of the endocrinocyte in the testicular stroma on the 180th day of observation. 1 - endocrinocyte nucleus, 2 - inclusions. Magnification x12 000.](image)

![Fig. 4. Testicular stromal endocrinocyte on the 270th day of observation. 1 - endocrinocyte nucleus, 2 - part of the cytoplasm outside the cell, 3 - lipid inclusions. Magnifications x12 000.](image)

![Fig. 5. Testicular stromal endocrinocyte on day 360 of observation. 1 - intracellular Reinke crystals, 2 - inclusions. Magnification x12 000.](image)
Discussion

The article discusses the structural characteristics of testicular interstitial endocrinocytes under long-term (365 days) blockade of luteinising hormone synthesis by tryptorelin, as a result of the effect on the hypothalamic-pituitary-testicular system. The presented results of the study of the structure, differentiation and regulation of interstitial endocrinocytes in the rat testis prove that in rats, as in humans, we have recognised two different populations of these cells, namely, fetal and functional interstitial endocrinocytes, which were actually detected only at the early stages of the experiment. The ultrastructure, lifespan, ability to synthesise androgens, and regulatory mechanisms of these two populations' cells are quite different [15, 29].

The observed differences in the morphological parameters of endocrine cells under tryptorelin administration at different follow-up periods clearly confirm that tryptorelin is capable of inducing oxidative stress [8, 30, 34], which is manifested by increasing destructive alterations in the ultrastructural organisation of these cells. Various pathological changes and abnormalities were observed in the functional activity of the internal components of the cell. The analysis of the structure of interstitial endocrinocytes revealed that the population of these cells consists of round, oval or polygonal cells of medium size with well-developed cytoplasm and average, sometimes large nuclei. These are mature, specialised cells that have acquired the ability to synthesise androgens through determination and differentiation. The younger, less differentiated cells are small and spindle-shaped, sharing some structural features with fibroblasts. They are likely to maintain the constancy of cell composition in this population. Literature data [9, 29, 36] and our results confirm that no mitotic activity is detected. The stability of the population is therefore ensured by a dynamic equilibrium between the processes taking place in the pericellular space after tryptorelin administration in our experiment is an indication of the cessation of androgen synthesis in these cells and of degenerative changes in the cells.

The population of rat endocrinocytes is a stable type of cell population. This is evidenced by the fact that no mitoses were detected in the interstitial endocrinocytes of the testes in any of the mature rats studied. Resident macrophages are important for the differentiation and function of interstitial endocrinocytes [22, 23]. The apoptosis of these cells in the late stages of the experiment contributes to the regulation of the number and quality of these cells, which can be induced by cytotoxins [2]. The characteristics of the ageing of these cells in rats are likely to be species specific. It is known [32] that 11-beta-hydroxysteroid dehydrogenase protects testosterone synthesis in interstitial endocrinocytes of stressed rats. In the cytoplasm of endocrinocytes of experimental animals on the 365th day of observation, hexagonal prisms with clearly defined edges and corners, formations in the form of a "wasp's nest" - Reinke crystals (layers arising from the disintegration of smooth endoplasmic reticulum membranes) are sometimes detected. The stability of the population is therefore ensured by a dynamic equilibrium between the processes taking place in these structures. In our experiment, we used quercetin as a drug to correct the oxidative stress of the testicular interstitial space caused by tryptorelin, which has both antioxidant and immunomodulatory properties, reducing the production of cytotoxic superoxide anion, which we believe normalises the structure of the endoplasmic...

Effect of quercetin administration on electron microscopic changes in testicular interstitial endocrinocytes during ...

destruction. Nucleoli are not present. Mitochondria are single and rounded, with no detected cristae. Reinke's crystals are present in the cytoplasm. The cell membrane is unclear (see Fig. 5).
Conclusions

1. Tryptorelin administration induces structural and functional abnormalities in the connective tissue components of the rat testis, characterised by quantitative and qualitative changes in the population of interstitial endocrine cells, as evidenced by electron microscopic alterations at the level of subcellular structures.

2. The additional administration of quercetin reduces the negative effect of tryptorelin and delays the appearance of disturbances in the structure of interstitial endocinocytes from day 180 to later observation periods.

References


структури інтерстиційних ендокриноцитів яєчок та функціональної активності внутрішніх компонентів клітин щурів введення триптореліну призводить до структурно-функціональних змін у будові сполучнотканинних компонентів яєчок рази на добу за допомогою гастрального зонду у перерахунку на вагу тіла тварин. Проведені дослідження показують, що інтенсивний вплив вільних радикалів на структуру клітин, відбувається патогенетично зумовлено відносно вільних радикалів. Експеримент проведений на 35 статевозрілих самих білих щурів. Єщо відсутність патогенетичних змін у відповідь на виклики, конкуренти з утворенням відповідної зміни структури клітин, сприяє високому ризику відповідної зміни структури клітин. З моменту проведення експерименту зміни структурно-функціональних змін, включно з генетичними аспектами, відбувається структурно-функціональна зміна, яка приводить до субклітинних структур. На 270 та 365 день експерименту виявлено патологічні зміни в структурі інтерстиційних ендокриноцитів яєчок та функціональної активності внутрішніх компонентів клітин.

ВПЛИВ ВВЕДЕННЯ КВЕРЦЕТИНУ НА ЕЛЕКТРОННО-МІКРОСКОПІЧНІ ЗМІНИ ІНТЕРСТИЦІЙНИХ ЕНДОКРИНОЦИТІВ ЯЄЧОК ПІД ДІЄЮ ТРИПТОРЕЛІНУ ПІД МІКРОСКОПОМ

В підгрупі тварин, які приймали кверцетин, відбувається зниження відповідної зміни структури клітин, що сприяє високому ризику відповідної зміни структури клітин. З моменту проведення експерименту зміни структурно-функціональних змін, відбувається структурно-функціональна зміна, яка приводить до субклітинних структур. На 270 та 365 день експерименту виявлено патологічні зміни в структурі інтерстиційних ендокриноцитів яєчок та функціональної активності внутрішніх компонентів клітин.
Встановлено, що частота знаходження кристалів Рейнке, їх кількість і розмір по відношенню до об'єму клітини корелює зі змінами в клітинах і збільшується на більш пізніх термінах дослідження. Відомостей щодо аналогічної кореляції з рівнем тестостерону в літературі виявлено не було, що дозволяє позначити кристали Рейнке, як продукт дегенеративних процесів у клітині. Таким чином, додаткове введення кверцетину зменшує негативний вплив триптореліну і відстережує виникнення змін у структурі інтерстиційних ендокриноцитів зі 180-ї доби на більш пізні часові проміжки спостереження.

Ключові слова: яєчка, електронна мікроскопія, шури, інтерстиційні ендокриноцити, лютеїнізуючий гормон, кверцетин, трипторелін.

Author's contribution
Stetsuk Ye. V. - work concept and design, data collection and analysis, writing the article.
Shepitko V. I. - work concept and design.
Pronina O. M. - final approval of the article.
Zaporozhets T. M. - critical review, final approval of the article.
Boruta N. V. - responsibility for statistical analysis.
Vilkhova O. V. - data collection and analysis.
Lysachenko O. D. - data collection and analysis.
Pelypenko L. B. - work concept and design, data collection and analysis.
Voloshyna O. V. - writing the article.
Levchenko O. A. - data collection and analysis, responsibility for statistical analysis.