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CYTOSTATIC IMPACT ON SPERMATOGENIC CELLS ULTRASTRUCTURE AND ITS CORRECTION BY CHONDROITIN SULPHATE

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The article presents data on the specific changes in the animal spermatogenic cells ultrastructure under the impact of doxorubicin hydrochloride. It is shown that the toxic effect of cytostatics is accompanied by the development of dystrophic and destructive changes in the rat testes cells. Under the influence of chondroitin sulfate, the gradual restoration of reparative processes in the rat spermatogenic cells exposed to doxorubicin hydrochloride was carried out. Chondroitin sulfate facilitated the turnover of the structure of the filaments plasmatic membrane areas after the toxic effect of the cytostatic, which ensures normal sperm motility.

Key words: testes ultrastructure, spermatogenesis, chondroitin sulfate, cytostatics, doxorubicin hydrochloride.

The study is a fragment of the research project "Mechanisms of development, diagnostics and therapy of infertility in individuals with dysfunction of the gonads and comorbid pathology" (state registration number 0116U007259).

Pharmacotherapy with cytotoxic drugs is relevant for the treatment of inflammatory processes in men. However, the effect of these drugs leads to a cytotoxic effect on spermatogenesis. Therefore, a search is being carried out for drugs neutralizing the negative and toxic effects of antibiotics with cytostatic effect and restarting the process of spermatogenesis. In the arsenal of andrologists today there is a fairly large list of tools, mainly synthetic, for the sexual disorders treatment and improvement of the reproductive system.

However, this therapy is inefficient, so today it is important to search for new therapeutic approaches to the reproductive disorders treatment [5, 6]. Chondroitin sulfate (CS), as a glycosaminoglycan, has a wide range of action and a high safety profile [3].

It has been proved that cholesterol can participate in various regulatory processes of spermatogenesis, positively affects the qualitative status of rat testicular tissue, restores disturbed trophism of germ cells, reduces dystrophic processes in rat testes, which leads to activation of the spermatogenesis process and increases the level of the body androgenic saturation. Thus, cholesterol can be considered as a potential corrector in the pathologies of spermatogenesis [1, 2].

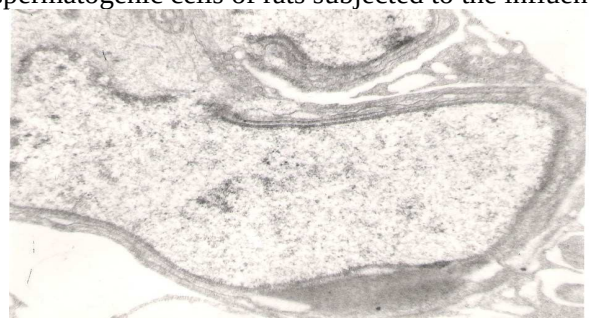
The purpose of the study was to study the specific changes in the submicroscopic architectonics of spermatogenic cells subjected to the destructive impact of doxorubicin hydrochloride (DH) and the correction of this condition with CS.

Materials and methods. The experiment was carried out on sexually mature white male Wistar rats weighing 340–380 g. The animals were divided into 3 groups: group 1 - negative control (intact rats), group 2 - positive control (control pathology), group 3 - animals that, against the background of the cytostatics action, received CS. To simulate cytostatic lesions of the testes, animals were intraperitoneally injected with DH in the dose of 2 mg/kg once a week (3 administrations). CS started to be administered three days before the administration of cytostatics, then three weeks against its background and 9 days after (the total of 33 days). The administration was performed intragastrically once a day with the dose of 60 mg/kg. The introduction of DH cytostatic was carried out with the extension of the exposure time [6]. Animals were sacrificed by decapitation in accordance with the national "General Ethical Principle of Animal Experiments" (Ukraine, 2001), pursuant to the provisions of the "European Convention for the Protection of Vertebrate Animals used for experimental and other scientific purposes" (Strasbourg, 1985). Electron microscopic examination of testicular tissue pieces have been carried out according to well-known method [4]. The testes of intact animals served as the quality control of the tissue histological processing.

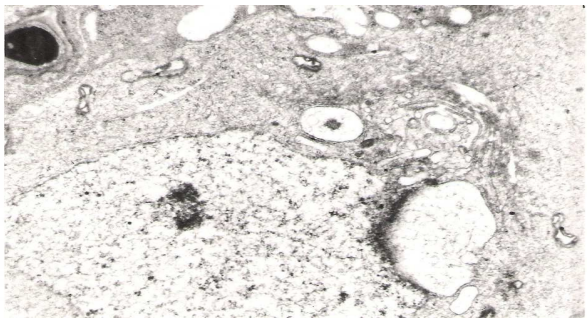
Results of the study and their discussion. The electron microscopic study of the interstitial endotheliocytes ultrastructure in animals exposed to DH showed that the toxic effects of cytostatics are accompanied by the development of dystrophic and destructive changes of organelles and a decrease in the activity of synthetic, reparative processes, as well as piezobiosynthesis. In spermatocytes, a reduction of the condensation degree occurs, the detachment of the acrosome from the nucleus is observed, the forms of the acrosome are changing, which is a sign of the spermatozoa inferiority.

Destruction of the filaments plasma membrane areas, mitochondrial matrix swelling with clearing, cristae destruction, absence of the dynein legs near the microtubule doublets indicate impaired sperm motility. Under the influence of CS, after the destructive effect of DH, the cytoplasmic membrane in contact with the hemato-testicular barrier loses its clearly contoured structure, looks thickened and electron dense. The basement membrane of the blood capillaries endotheliocytes is thickened, has a loose structure. In loose connective tissue myoid cells are located, their cytoplasm having an increased electron density. After the introduction of cholesterol, the myoid cells ultrastructure is not changed, which indicates an increase in the activity of rhythmic contractions of the tubule wall, which causes obstacles to the development of stagnant processes that were caused by the cytotoxic effect of DH. These cells are surrounded by fine-fibrous substance and collagen fibers (fig. 1). Thus, under the influence of CS, the bioenergetic supply recovery of metabolic, reparative processes in the rat testes exposed to DH was carried out. It should be noted that the ultrastructural organization of spermatogenic cells retains the features of dystrophic and destructive changes. The cells nuclei mainly contain decondensed chromatin, which granules are diffusely scattered over the karyoplasm. The nuclear matrix has a low electron density. The nuclear membrane contains small destruction foci (fig. 1 A). Acrosomal granules are osmiophilic and closely adjacent to the nuclear membrane, which forms fairly deep invaginations (fig. 1 B). In the cytoplasm, numerous small mitochondria are revealed, having a rounded and slightly elongated shape.

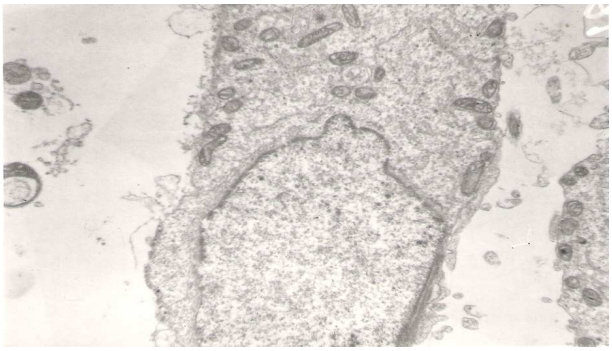
The mitochondrial matrix is moderately cleared, has a middle electron density. Mitochondrial crista are randomly oriented. The outer membranes are moderately loosened, there are no destruction foci. The cytoplasm contains numerous ribosomes and polysomes (fig. 1 C). Along with this, spermatids with nuclei containing dense condensed (mature) chromatin and formed acrosome were present in the preparations (fig. 1 D). Under the influence of CS, gradual restoration of reparative processes in spermatogenic cells of rats subjected to the influence of DH was carried out.



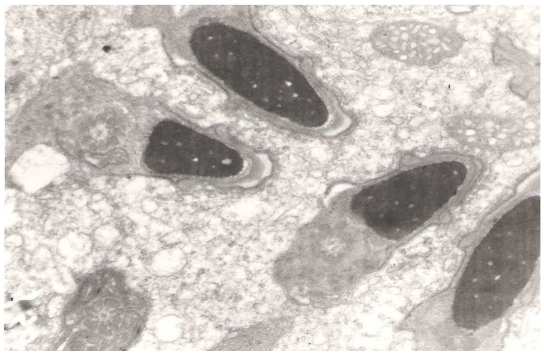
A. Ultrastructure spermatids of rats exposed to cytostatics and chondroitin sulfate. Decondensed chromatin, focal lysis of the nuclear membrane. X35000.



B. Ultrastructure spermatids of rats exposed to cytostatics and chondroitin sulfate. Acrosomal granule, nuclear membrane invagination. X36000



C. Ultrastructure spermatids of rats exposed to cytostatics and chondroitin sulfate. Mitochondria rounded and elongated, disordered orientation of the cristae, many ribosomes and polisomes. X32000.



D. Ultrastructure of late spermatids in rats exposed to cytostatics and chondroitin sulfate. Condensed chromatin with the formed acrosome. X30000.

Fig. 1.

In the nuclei of spermatocytes, there was predominantly mature chromatin, which is characteristic of its condensation degree increase. The detachment of the acrosome from the nucleus and the change in the shape of the acrosome was practically not observed, which is a sign of the spermatozoa full-value condition, i.e. initiation of recovery processes after the introduction of CS against the background of the DH toxic effect. The structure of the filament plasma membrane areas was restored, the mitochondrial matrix was electron-dense, the formation of cristae and dyneinic legs was observed around the microtubule doublets, which indicates the normal mobility of spermatozoa.

This research was aimed to show the recovery effect of CS on the sperm ultrastructure after destructive effects of DH. This can be used by men after chemotherapy to preserve fertility. In this way actual task is searching for ways of prevention means and treatment of male reproductive disorders caused by cytotoxic drugs. Such a drug as CS stimulates the metabolic processes of the organism. Numerous studies have confirmed its important role in the male reproductive function [7]. It have been shown under the influence of CS, gradual restoration of reparative processes in spermatogenic cells of rats subjected to the influence of DH was carried out.In the nuclei of spermatocytes, there was predominantly mature chromatin, which is characteristic of its condensation degree increase. The detachment of the acrosome from the nucleus and the change in the shape of the acrosome was practically not observed, which is a sign of the spermatozoa full-value condition, i.e. initiation of recovery processes after the introduction of CS against the background of the DH toxic effect. The structure of the filament plasma membrane areas was restored, the mitochondrial matrix was electron-dense, the formation of cristae and dyneinic legs was observed around the microtubule doublets, which indicates the normal mobility of spermatozoa.

Conclusions

1) Under the influence of CS, gradual restoration of reparative processes in spermatogenic cells of rats subjected to the destructive impact of DH was carried out.

2) In the cytoplasm of spermatogenic cells, after the CS introduction, there are numerous ribosomes and polysomes. Spermatids have nuclei containing dense condensed (mature) chromatin and formed acrosome.

3) CS contributed to the restoration of the filament plasma membrane structure after the toxic effect of cytostatics.

Prospects for further development lie in the fact that the obtained scientific results are necessary for further research on the effect of CS on the male reproductive system after exposure to cytostatics.

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Реферати

ВПЛИВ ЦИТОСТАТИКІВ НА УЛЬТРАСТРУКТУРНУ ОРГАНІЗАЦІЮ СПЕРМАТОГЕННИХ КЛІТИН І КОРЕКЦІЯ ХОНДРОЇТИН СУЛЬФАТОМ

Н. М. Бречка, В. П. Невзоров, Н. Ю. Селюкова, Е. М. Коренєва, О. В. Щербак

В роботі наведені дані про характерні зміни ультраструктури сперматогенних клітин тварин, які зазнали впливу доксорубіцину гідрохлориду. Показано, що токсичний вплив цитостатиків супроводжується розвитком дистрофічних і деструктивних змін в клітинах сім'яників щурів. Під впливом ХС здійснювалося поступове відновлення репаративних процесів в клітинах сперматогенезу щурів, під впливом доксорубіцину гідрохлориду. ХС сприяв відновленню структури ділянок плазматичної мембрани джгутиків, після токсичного впливу цитостатика, що забезпечує нормальну рухливість сперматозоїдів. В ядрах сперматоцитов перебував переважно зрілий хроматин, що характерно для підвищення ступеня його конденсації. Відшарування акросоми від ядра і зміна форми акросоми практично не спостерігалася, що є ознакою повноцінності сперматозоїдів, тобто початку відновлювальних процесів

ВОЗДЕЙСТВИЕ ЦИТОСТАТИКА НА УЛЬТРАСТРУКТУРНУЮ ОРГАНИЗАЦИЮ СПЕРМАТОГЕННЫХ КЛЕТОК И КОРРЕКЦИЯ ХОНДРОИТИНА СУЛЬФАТОМ

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В работе приведены данные о характерных изменениях ультраструктуры сперматогенных клеток животных подверженных воздействию доксорубицина гидрохлорида. Показано, что токсическое воздействие цитостатиков сопровождается развитием дистрофических и деструктивных изменений в клетках семенников крыс. Под влиянием ХС осуществлялось постепенное восстановление репаративных процессов в клетках сперматогенеза крыс, подвергшихся деструктивному влиянию доксорубицина гидрохлорида. ХС способствовал восстановлению структуры участков плазматической мембраны жгутиков, после токсического влияния цитостатика, что обеспечивает нормальную подвижность сперматозоидов. В ядрах сперматоцитов находился преимущественно зрелый хроматин, что характерно для повышения степени его конденсации. Отслойка акросомы от ядра и изменение формы акросомы практически не наблюдалась, что является признаком

після введення ХС на тлі токсичної дії ДГ. Відновлювалася структура ділянок плазматичної мембрани джгутиків, матрикс мітохондрій був електронно-щільний, спостерігалося утворення крист і дінеїнових ніжок близько дуплетів микротрубочек, що свідчить про забезпечення нормальної рухливості сперматозоїдів.Під впливом ХС здійснювалося поступове відновлення репаративних процесів в клітинах сперматогенезу щурів, які зазнали впливу ДГ.Ключові слова: ультраструктура сім'яників, хондроїтину сульфат, доксорубіцину гідрохлорид.

Ключові слова: ультраструктура сім'яників, хондроїтину сульфат, доксорубіцину гідрохлорид.

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полноценности сперматозоидов, то есть начала восстановительных процессов после введения ХС на фоне токсического действия ДГ. Восстанавливалась структура участков плазматической мембраны жгутиков, матрикс митохондрий был электронно-плотным, наблюдалось образование крист и динеиновых ножек около дуплетов микротрубочек, что свидетельствует об обеспечении нормальной подвижности сперматозоидив.Под влиянием ХС осуществлялось постепенное восстановление репаративных процессов в клетках сперматогенеза крыс, подвергшихся воздействию ДГ.

Ключевые слова: ультраструктура семенников, хондроитина сульфат, доксорубицина гидрохлорид.

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MORPHOLOGICAL AND MORPHOMETRIC REARRANGEMENTS OF THE RAT ADENOHYPOPHYSIAL-THYROID SYSTEM UNDER THE EXPERIMENTAL EXTRACELLULAR DEHYDRATION

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Disruption of homeostasis cause significant changes in all the body systems, including the endocrine one. The purpose of the work is to study the morphological and morphometric rearrangements of the adenohypophyseal-thyroid axis in rats under conditions of experimental moderate degree extracellular dehydration. The experiment was performed on 12 rats of control and experimental group. The experimental group's rats for 60 days modeled the moderate degree of extracellular dehydration. Histological, morphometric and statistical methods of investigation were used. Disturbance of the water-salt balance of the body causes a slight increase in secretory activity on the part of adenohypophysis and the development of hypofunction of the thyroid gland, which negatively affects the development of compensatory and adaptive processes in the body.

Key words: adenohypophysis, extracellular dehydration, thyroid gland, stress

The work is a fragment of the eresearch projects "Morphofunctional aspects of the body homeostasis" (state registration No. 0118U006611)

Homeostasis provides the necessary conditions for the life of both individual cells and the body as a whole. Constancy of water-electrolyte composition is part of homeostasis [7, 12]. In forming the regulation of the compensation mechanism for various extreme factors affecting the body, the endocrine system plays one of the key roles [4, 14]. Adequacy, nature of the body's adaptive changes depends on changes in the hormonal secretion that provide the body homeostasis [5]. Steady and multiple stress can be the basis of stress-induced pathology development [5, 15]. The pituitary - thyroid axis of the endocrine system, together with the pituitary gonadal, sympathoadrenal and hypothalamic - pituitary adrenocorticular systems, play a key role in providing the adaptive response of the body to stress effects. The thyroid gland's hormones are known to take an active part in the development of adaptive responses and have the ability to increase the sensitivity of adrenoreceptors to adrenaline and noradrenaline [5]. Conditions associated with changes in the activity of regulatory stress systems (in acute or chronic stress, post-traumatic stress disorders, changes in physical activity), also affect the functioning of the hypothalamic-pituitary-thyroid axis of the endocrine regulation [5]. To date, the thyroid gland's status has been studied under the influence of heavy metals salts [6], moderate chronic hyperthermia [11], geochemical factors [10], tiotriazolini [5], and antipsychotic therapy depending upon the age [1]. According to the results of the literature sources search, there is no works on studying morphological and morphometric indices of adenohypophysis blood vessels in rats under the conditions of experimental moderate degree extracellular dehydration.

The purpose of the study was to study morphological and morphometric rearrangements of the adenohypophysial-thyroid axis structural components in sexually mature male rats under the conditions of experimental extracellular dehydration.