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STRUCTURAL AND FUNCTIONAL STATE OF THE SEMINAL GLANDS IN THE DYNAMICS OF CHRONIC IMMUNE INFLAMMATION

Zalyubovska O. I.[1], Tiupka T. I.[1], Berezniakova M. E.[1], Minaieva A. O.[2], Avidzba Y. N.[1], Karabut L. V.[1]
[1]Kharkiv National Medical University
[2]V. N. Karazin Kharkiv National University
e-mail: kkld1@ukr.net

СТРУКТУРНО-ФУНКЦІОНАЛЬНИЙ СТАН СІМ'ЯНИХ ЗАЛОЗ У ДИНАМІЦІ ХРОНІЧНОГО ІМУННОГО ЗАПАЛЕННЯ

Залюбовська О. І.[1], Тюпка Т. І.[1], Березнякова О. І.[1], Мінаєва А. О.[2], Авідзба Ю. Н.[1], Карабут Л. В.[1]
[1]Харківський національний медичний університет
[2]Харківський національний університет імені В. Н. Каразіна

Summary/Резюме

It is known that chronic inflammation is characterized by the persistence of a pathogenic agent, dysfunction of the immune system and immunological deficiency, which determines the peculiarity of morphological changes in tissues in the focus of inflammation. The result of acute or chronic inflammation, pathology of the immune system is often anomalies in the anatomical structure of the reproductive system, accompanied by damage to the spermatogenic epithelium, which lead to male infertility. Therefore, there is great practical interest in studying the mechanisms of the development of chronic immune inflammation in the seminal glands.

The aim of our work was to determine the morphological criteria for the formation of chronic inflammation in the seminal glands of rats on a model of chronic immune inflammation.

Experimental studies were performed on 40 nonlinear male rats weighing 180-200 g. Microscopic and histochemical studies were performed on the 7th, 14th, and 28th day.

In the study of the seminal glands of experimental animals, depending on the length of stay under the conditions of modeling adjuvant arthritis (chronic immune inflammation), we found distinct morphological and morphometric changes characterizing the function of the gland at 7, 14, and 28 days.

On the 7th day, a histochemical study draws attention to a decrease in protein-synthetic function in spermatogenous epithelial cells, as evidenced by an increase in the intensity of the reaction to DNP in the nuclei and a decrease in the intensity of the reaction to RNP in the cytoplasm, as well as a decrease in the optical density of the PAS-positive material in the cytoplasm of spermatogenic epithelial cells and Leydig cells.

On the 14th day, morphological changes become less pronounced compared with those on the 7th day and approach those described in the control group. Histochemical reactions indicate an increase in the function of spermatogenic epithelial cells, which indicates an improvement in the protein-synthetic function of the cells.

On the 28th day of experimental chronic immune inflammation, morphological changes in the testes are observed, which are characterized by hyperplasia of the seminiferous

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tubules with the restoration of all stages of development of spermatogenic epithelium and the appearance of spermatogenesis, as evidenced by the large number of spermatozoa, both in the lumen of the tubules and between the epithelial cells in the adluminal zone.

Conclusions: 1. When studying the seminal glands of rats under conditions of modeling chronic immune inflammation, more pronounced morphological and morphometric changes characterizing the function of the gland were found on the 7th and 14th day of the experiment. 2. The repair of the seminiferous tubules and the restoration of the clone of spermatogenic epithelial cells with normalization of the endocrine function of the gonads under conditions of modeling of chronic immune inflammation are observed on the 28th day of the experiment. The indicated morphological changes are accompanied by the restoration of the protein-synthetic function of spermatogenic epithelial cells, as evidenced by histochemical reactions to DNP, RNP, and PAS-reaction.

Key words: chronic immune inflammation, seminal glands, microscopic study, histochemical reactions.

Відомо, що хронічне запалення характеризується персистенцією патогенного агента, дисфункцією імунної системи та імунологічною недостатністю, що обумовлює своєрідність морфологічних змін тканин у вогнищі запалення. Результатом гострого або хронічного запалення, патології імунної системи найчастіше є аномалії анатомічної будови статевої системи, що супроводжуються ушкодженням сперматогенного епітелію, які призводять до чоловічої безплідності. Тому виникає великий практичний інтерес до вивчення механізмів розвитку хронічного імунного запалення в сім'яних залозах.

Метою нашої роботи стало визначення морфологічних критеріїв формування хронічного запалення у сім'яних залозах щурів на моделі хронічного імунного запалення.

При дослідженні сім'яних залоз експериментальних тварин залежно від термінів перебування в умовах моделювання ад'ювантного артриту (хронічне імунне запалення) відмінні морфологічні та морфометричні зміни, що характеризують функцію залози, нами виявлені у терміні 7, 14 та 28 діб.

На 7 добу при гістохімічному дослідженні звертає увагу зниження білково-синтетичної функції у клітинах сперматогенного епітелію, про що свідчить підвищення інтенсивності реакції на ДНП у ядрах та зниження інтенсивності реакції на РНП у цитоплазмі, а також зменшення оптичної щільності ШІК-позитивного матеріалу у цитоплазмі клітин сперматогенного епітелію та клітин Лейдіга.

На 14-ту добу морфологічні зміни стають менш вираженими порівняно з такими на 7 добу. Гістохімічні реакції свідчать про підвищення функції клітин сперматогенного епітелію, що підтверджується покращенням білково-синтетичної функції клітин.

На 28 добу експериментального хронічного імунного запалення відзначаються морфологічні зміни у сім'яниках, які характеризуються гіперплазією сім'яних канальців з відновленням усіх стадій розвитку сперматогенного епітелію та появою сперматогенезу, про що свідчить велика кількість сперматозоїдів, як у просвіті канальців, так і між клітинами епітелію в адлюмінальній зоні.

Висновки. 1. При дослідженні насіннєвих залоз щурів в умовах моделювання хронічного імунного запалення більш виражені морфологічні та морфометричні зміни, що характеризують функцію залози, виявлено на 7 та 14 добу експерименту. 2. Репарація сім'яних канальців та відновлення клону клітин сперматогенного епітелію з нормалізацією ендокринної функції гонад в умовах моделювання хронічного імунно-

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го запалення спостерігаються на 28 добу експерименту. Зазначені морфологічні зміни супроводжуються відновленням білково-синтетичної функції клітин сперматогенного епітелію, про що свідчать гістохімічні реакції на ДНП, РНП та ШІК-реакція.

Ключові слова: *хронічне імунне запалення, сім'яні залози, мікроскопічне дослідження, гістохімічні реакції.*

Inflammation, as a typical pathological process that has developed in the process of evolution in response to any damage to body tissues, underlies many diseases of an infectious and non-infectious nature [1]. In modern theoretical and clinical medicine, the problem of inflammation remains one of the main. Extensive experimental and clinical material has been obtained on the study of inflammation at various levels of body organization using the latest models and previously inaccessible methods for research. Despite the enormous significance of these data, the problem of inflammation is far from exhausted and has not lost its relevance, and the number of patients suffering from chronic inflammatory diseases continues to increase [1, 2]. Unfortunately, the current stage of the study of inflammation is characterized by insufficient knowledge about the nature of chronic inflammation. Its reasons, mechanisms and dynamics of development remain not quite clear [3, 4]. Knowledge of the main external manifestations of inflammation, the essence of the processes underlying it, the mechanisms of their development and consequences is necessary for the timely correct diagnosis of inflammatory diseases, rational pathogenetic therapy at any stages of the development of the inflammatory process [1].

It is known that chronic inflammation is characterized by the persistence of a pathogenic agent, dysfunction of the immune system and immunological deficiency, which determines the peculiarity of morphological changes in tissues in the focus of inflammation [5]. The result of acute or chronic inflammation, pathology of the immune system is often anomalies in the anatomical structure of the reproductive system, accompanied by damage to the spermatogenic epithelium, which lead to male infertility [6]. Therefore, there is great practical interest in studying the mechanisms of the development of chronic immune inflammation in the seminal glands.

The aim of our work was to determine the morphological criteria for the formation of chronic inflammation in the seminal glands of rats on a model of chronic immune inflammation.

Materials and methods

Experimental studies were performed on 40 nonlinear male rats weighing 180-200 g, bred in the vivarium of the Kharkiv National Medical University following the recommendations for conducting medical and biological research using animals according to the general ethical principles of animal experiments adopted by the First National Congress of Ukraine on Bioethics (Kyiv, 2001), "European Convention for the Protection of Vertebrate Animals used for Research and Other Scientific Purposes" (Strasbourg, 1986), Law of Ukraine №3447-IV dated 21.02.2006 "On the protection of animals from cruel treatment".

Animals were kept on a normal diet in standard vivarium conditions. Slaughter of animals was performed under hexenal anesthesia (50 mg/kg body weight intragastrically) by bloodletting.

Chronic immune inflammation (adjuvant arthritis) was caused by the administration into the subplantar aponeurosis of rats of a complete Freund's adjuvant at a dose of 0.1 ml [7].

Macroscopic and microscopic studies were performed in dynamics on the 7th, 14th and 28th days. Such macroscopic characteristics were evaluated: the size of the seminal glands, the condition of the membranes of the organ, the presence of hemorrhages in the areas of necrosis on the incision.

Tissue samples of seminal glands fixed in 10% neutral formalin were subjected to

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histological study, which were subjected to standard wiring through alcohols of increasing concentration, Nikiforov liquid (96% alcohol and diethyl ether in a 1: 1 ratio), chloroform, and then paraffin was poured. Serial sections with a thickness of 4-5Ч10^{-6} m were made from the blocks prepared in this way. In all cases, traditional histological methods of hematoxylin staining with eosin, as well as picrofuchsin solution by Van Gieson were used [8]. Micropreparations were studied on an "Olympus BX-41" microscope with subsequent processing using the program "Olympus DP-soft version 3.1" and Microsoft Excel, with which morphometric studies were performed.

To determine the level of protein-synthetic activity, the content of the ribonucleoprotein (RNP) in the cytoplasm and the deoxyribonucleoprotein (DNP) in the cell nuclei were determined by measuring the optical density in sections, processed by histochemical methods for the determination of the DNP according to Felgen-Rossenbek (the control is hydrolysis with HCl), the RNP was determined by the Brachet reaction (the control with crystal ribonuclease), and the detection of glycogen using the Periodic Acid Schif (PAS) reaction (the control is amylase) [9]. The control group was the seminal glands of intact rats.

The obtained results were processed statistically using Student's t-factor for independent samples [10].

Table 1

Organometric indicators of the seminal glands of rats of the control and experimental groups, (M±m)

Experiment conditions	Seminal glands			
	Weight, kg×10 $^{-3}$		Sizes, m×10 $^{-3}$	
	right	left	right	left
Control group (intact animals), n=10	6,3±0,22	6,8±0,24	2,2 x 1,2	2,2 x 1,2
Chronic immune inflammation (7th day), n=10	7,7±0,27*	7,2±0,24	3,1 x 1,3	3,8 x 1,3
Chronic immune inflammation (14th day), n=10	6,0±0,34	5,9±0,19*	3,8 x 1,5	4,8 x 1,5
Chronic immune inflammation (28th day), n=10	5,9±0,19*	4,8±0,17*	2,6 x 1,2	2,3 x 1,0

Note. * Differences in comparison with the control group are significant (p <0.05)

Table 2

The intensity of histochemical reactions to DNP, RNP and glycogen in the cells of the spermatogenic epithelium of the seed glands, Leydig cells (n=10, M±m)

Cells of the spermatogenic epithelium of the seminal glands	DNP in cell nuclei, c.u. of optical density	RNP in the cytoplasm of cells, c.u. of optical density	PAS-reaction in the cytoplasm of cells, c.u. of optical density
Control group (intact animals)			
Type A light spermatogonia	0,210±0,007	0,298±0,011	0,285±0,011
Type A dark spermatogonia	0,245±0,019	0,275±0,010	0,244± 0,003
Type B spermatogonia	0,224±0,008	0,198±0,007	0,221±0,005
Leydig cells	0,198±0,007	0,132±0,005	0,297±0,012
Chronic immune inflammation (the 7th day)			
Type A light spermatogonia	0,214±0,008	0,251±0,024	0,270±0,026
Type A dark spermatogonia	0,252±0,009	0,270±0,029	0,238± 0,002
Type B spermatogonia	0,229±0,004	0,190±0,006	0,218±0,003
Leydig cells	0,211±0,005	0,121±0,001	0,281±0,005
Chronic immune inflammation (the 14th day)			
Type A light spermatogonia	0,223±0,009	0,218±0,011	0,287±0,011
Type A dark spermatogonia	0,255±0,009	0,246±0,010	0,224±0,012
Type B spermatogonia	0,242±0,011	0,211±0,007	0,208±0,006
Leydig cells	0,216±0,007	0,112±0,004	0,274±0,009
Chronic immune inflammation (the 28th day)			
Type A light spermatogonia	0,216±0,008	0,318±0,011	0,328±0,012
Type A dark spermatogonia	0,225±0,008	0,296±0,010	0,271±0,010
Type B spermatogonia	0,202±0,009	0,221±0,007	0,243±0,008
Leydig cells	0,179±0,007	0,154±0,006	0,332±0,012

Note. * Differences in comparison with the control group are significant (p <0.05)

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Results and discussion

In the study of the seminal glands of experimental animals, depending on the length of stay under the conditions of modeling adjuvant arthritis (chronic immune inflammation), we found distinct morphological and morphometric changes characterizing the function of the gland at 7, 14, and 28 days.

On the 7th day, with a macroscopic examination of the membrane of the gonads, they are slightly strained, the consistency of the seminal glands is cerebral, grayish-whitish in color, there are focal point hemorrhages in the section.

The mass and size of the seminal glands of the control and experimental groups in the dynamics are presented in Table 1.

Microscopic examination of the gonads reveals the devastation of a few seminiferous tubules. In the individual tubules, there is a partial destruction of the basement membrane with the absence of cellular elements of spermatogenic epithelium.

At the same time, in the seminal glands between the tubules at the periphery, proliferation of small young Leydig cells of an oval shape with a small weakly saturated chromatin nucleus is detected. In some gonads, there is proliferation of connective tissue cells with the formation of tender young connective tissue stained red by Van Gieson.

A histochemical study draws attention to a decrease in protein-synthetic function in spermatogenous epithelial cells, as evidenced by an increase in the intensity of the reaction to DNP in the nuclei and a decrease in the intensity of the reaction to RNP in the cytoplasm, as well as a decrease in the optical density of the PAS-positive material in the cytoplasm of spermatogenic epithelial cells and Leydig cells (table. 2).

On the 14th day, macroscopic changes become less pronounced compared with those on the 7th day and approach those described in the control group.

Microscopically revealed moderate hyperplasia of the seminiferous tubules, which are closely adjacent to each other. The oval shape of all tubules is noteworthy. The tubule wall is thickened due to the proliferation of spermatogenic epithelium. Spermatogonia type A and B are located on the basement membrane. Spermatocytes, spermatids and spermatozoa, which are located both in the lumen of individual tubules and between the cells of the spermatogenic epithelium, are located in the adluminal zone of the tubules. As in the control group, a significant number of Sertoli cells are found between the cells of the spermatogenic epithelium in the adluminal zone. Leydig cells (in the amount of 1-2) are found in the intercanalicular stroma. Small arterioles are dilated, full-blooded. Histochemical reactions indicate an increase in the function of spermatogenic epithelial cells, which indicates an improvement in the protein-synthetic function of the cells (Table 2).

On the 28th day of the experiment, with a macroscopic examination, the gonads have an ovoid shape in the section of the cerebral consistency, grayish-whitish in color. Punctate hemorrhages are detected in places.

A microscopic examination reveals tightly fitting tubules with an expanded lumen. Noteworthy is the increase in the number of rows of spermatogenic epithelium. Spermatogonia type A and B are located on the basal membrane, with dark type A spermatogonia predominating. Light spermatogonia type A with a moderately saturated chromatin nucleus and moderate intensity of reaction to DNP, intense reaction to RNP in the cytoplasm, and intense PAS-reaction, as evidenced by optical density indices (Table 2). In spermatogonia, moderate chromatin saturation with corresponding histochemical reactions is also detected (Table 2). Spermatogonia type B is somewhat larger, with a moderate reaction to DNP and with a more intense reaction to RNP (Table 2). There are spermatids of both the last stages of maturation and the early stages of development, as well as dividing spermatocytes of the first order. In the lumen and

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between the epithelial cells there are a few sperm.

A few mature Leydig cells, a small number of small immature cells, lymphohistiocytic infiltration are found in the intercanalous stroma around full-blooded vessels with an expanded lumen.

Thus, on the 28th day of experimental chronic immune inflammation, morphological changes in the testes are observed, which are characterized by hyperplasia of the seminiferous tubules with the restoration of all stages of development of spermatogenic epithelium and the appearance of spermatogenesis, as evidenced by the large number of spermatozoa, both in the lumen of the tubules and between the epithelial cells in the adluminal zone.

Conclusions.

1. When studying the seminal glands of rats under conditions of modeling chronic immune inflammation, more pronounced morphological and morphometric changes characterizing the function of the gland were found on the 7th and 14th day of the experiment.

2. The repair of the seminiferous tubules and the restoration of the clone of spermatogenic epithelial cells with normalization of the endocrine function of the gonads under conditions of modeling of chronic immune inflammation are observed on the 28th day of the experiment. The indicated morphological changes are accompanied by the restoration of the protein-synthetic function of spermatogenic epithelial cells, as evidenced by histochemical reactions to DNP, RNP, and PAS-reaction.

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