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CHANGES IN THE STRUCTURE OF SEMINAL VESICLES OF REPRODUCTIVE PERIOD RATS AT THE CONDITIONS OF CYCLOPHOSPHAMIDE-INDUCED IMMUNOSUPPRESSION

ABSTRACT

Objective: to establish the morphometric changes in the seminal vesicles of rats of the reproductive period at the conditions of prolonged systemic immunosuppression.

Material and methods. The study was carried out on 60 white rats of the reproductive period, obtained from the vivarium of laboratory animals of the SI LPR «ST. Luke Lugansk State Medical University». The immunosuppressive state was modeled by administration of cyclophosphamide at a dosage of 1.5 mg/kg body weight intramuscularly tenfold. The rats were taken out from the experiment by decapitation under ether anesthesia on 1, 7, 15, 30 and 60 days after the end of the drugs administration. The organs were weighed, the relative mass was calculated, the length, width and thickness were determined by means of a caliper. The volume of the organ was established by the method of water displacement in a graduated cylinder containing distilled water. At the microscopic level, examined the height and width of epithelial cells, their volume, and also the larger and smaller diameters and the volume of the cell nuclei. Volumetric values were calculated from the formula for the volume of an elongated ellipsoid of revolution.

Results of the study. Statistically significant decrease in all studied organometric parameters occurred both at the early (7, 15 days) and late observation periods (30 days). Micromorphometric parameters after cyclophosphamide administration also decreased with respect to the control group at similar times. The size of the nuclei and their volume decreased on 7, 15 and 30 days: the larger and smaller diameters significantly decreased by 3.99%, 4.98%, 8.38% and 3.22%, 6.56%, 8, 06% respectively. The parameters of the volume of the nuclei of the epithelial cells of the seminal vesicles of the animals of the control groups significantly exceeded the data of the experimental rats at the same time by 7.07%, 11.02% and 15.77%. At 1 and 60 days, statistically significant differences between the parameters studied were not established.

Conclusions. Statistically significant changes in organometric parameters of the organ are noted both at the early (7, 15 days) and late observation periods, which indicates the direct influence of cyclophosphamide on the biosynthetic processes in the organ. Micromorphometric changes, as well as the results of cyto- and karyometry, confirm the organometric data and indicate the development of local disturbances in the morphogenesis of seminal vesicles caused by disregulation of the endocrine-immune homeostasis of the organ.

Keywords: seminal vesicles, cyclophosphamide, immunosuppression, rats.

Introduction

In recent years, the efforts of scientists are aimed at revealing the structural and functional foundations of human interaction and the environment, based on a comprehensive analysis of the influence of external factors on the organism, the structure of anthropological connections, the characteristics of individual manifestations [1]. The first to change the environment are the regulatory systems of the body - immune, endocrine, nervous. It has been proved that unfavorable exogenous effects, including the use of various pharmacological agents, cause pronounced systemic immunosuppression in the body [5]. Violations in the structure and functioning of regulatory bodies naturally stimulate a complex reaction from other structures, including those performing reproductive function. Being in constant functional tension during puberty, these organs are especially sensitive to changes

in homeostasis, which is reflected in a number of clinical experiments. However, against the backdrop of a variety of laboratory-instrumental research methods, a fundamental morphological study of the organs of the male reproductive system in immunosuppression, according to available literature, has so far not been carried out.

Objective- to establish morphometric changes in the seminal vesicles of rats of the reproductive period at the conditions of prolonged systemic immunosuppression.

Material and methods. The study was carried out on 60 white rats of the reproductive period, obtained from the vivarium of laboratory animals of the SI LPR «St. Luke Lugansk State Medical University». The immunosuppressive state was modeled by administration of cyclophosphamide at a dosage of 1.5 mg/kg body weight intramuscularly tenfold. Animals belonging to the control

group received similar volumes of 0.9% NaCl solution in the same way. The rats were taken out from the experiment by decapitation under ether anesthesia on days 1, 7, 15, 30 and 60 days after the end of the drugs administration. When working with animals, they were guided by the Directive 2010/63/ EU of the European Parliament and the Council of the European Union for the protection of animals used for scientific purposes [4]. The organs were weighed on a torsion balance, the relative mass was calculated, the length, width and thickness were determined by means of a caliper. The volume of the organ was established by the method of displacement of water in a graduated cylinder containing distilled water. The organ underwent classical histological wiring, the resulting sections 4-6 μ m thick were stained with hematoxylin-eosin and photographed using the «Olympus CX-41» morphometric complex.

Micromorphometric measurements of objects were carried out by uploading the obtained digital images to the computer program ASCON «Compass-3D 17.0» with calibration by means of photos of the object-micrometer in similar modes of shooting. At the microscopic level, the organs examined the height and width of epithelial cells, their volume, and the larger and smaller diameters and the volume of the cell nuclei. The volume indices were calculated from the formula for the volume of an elongated ellipsoid of revolution:

$$V = \frac{4}{3} \pi A B^2$$

where V is the nucleus volume, A is the larger diameter, and B is the smaller diameter [2].

The obtained data was processed using the specialized program «StatSoft Statistica v6.0». The methods of parametric statistics were used, since the use of the Shapiro-Wilk criterion allowed the establishment of a normal distribution of indicators in the sample. The reliability of the differences between the values of the parameters of the experimental and control groups was determined with the help of the Student-Fisher criterion with an error probability $p < 0.05$ permissible for biomedical research.

Results of the study. Seminal vesicles are paired organs, which necessitates their two-sided study. However, the obtained data indicate that in the control and experimental groups there were no significant differences between the analogous parameters of the right and left seminal vesicle, so the results of the right organ research will be presented in the following.

Seminal vesicles have the appearance of sacciform formations located above the prostate gland, and are covered with a connective tissue capsule. The clearance of each vesicle is irregular in shape; the mucosa is characterized by a specific architecture, giving it the appearance of honeycomb cells (Fig. 1). The epithelium consists of columnar secretory and rounded basal cells.

The parameters of the seminal vesicles of the animals of the control groups underwent regular changes in connection with the peculiarities of their morphogenesis (Table 1).

Macromorphometric parameters of seminal vesicles after administration of cyclophosphamide were also subjected to significant dynamics. A statistically significant decrease in all the studied parameters occurred both at the early (7, 15 days) and late observation periods (30 days). The deviation of the length of the organ from the control group

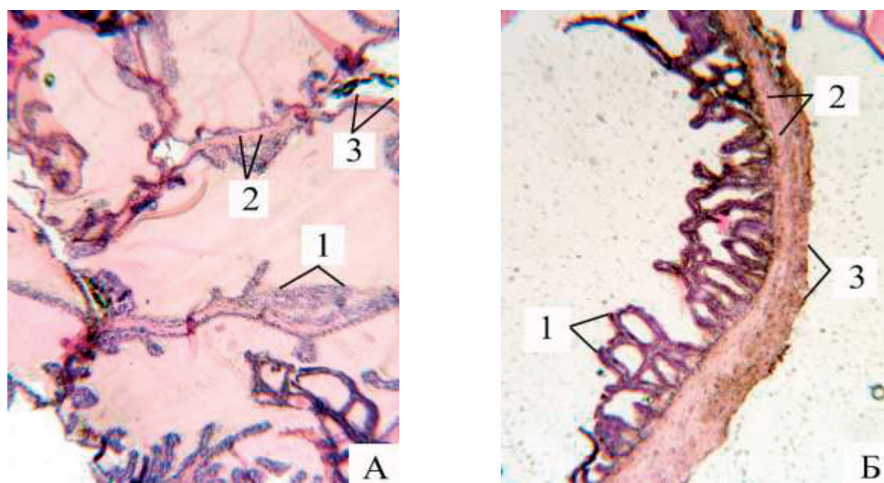


Fig. 1. Seminal vesicles of animals of the reproductive period: A - 7 days after application of cyclophosphamide; B - 7 days after application of 0.9% solution of NaCl. 1 - mucous, 2 - muscle layer, 3 - adventitia. Coloring with hematoxylin-eosin. Zoom: approximation: Zoom 18,5. Objective: Plan C N 10x/0.25 ∞/-FN22.

of the rats was 13.55%, 16.05% and 14.62%, respectively, of the above-mentioned days. Similarly, the width and thickness of seminal vesicles decreased with immunosuppression: the changes were 5.62%, 4.77%, 3.16% and 10.78%, 12.25%, 4.82% at the same time. A statistically significant decrease in body volume was observed on days 7, 15 and 30, and were 27.16%,

29.86% and 21.30%, respectively. The micromorphometric parameters of the epithelial cells of the seminal vesicles of the reproductive period in the control groups underwent a regular age dynamics (Table 2).

After the administration of cyclophosphamide, the decrease in the height and width of the epithelium was 4.61, 5.02, 7.09 and 4.87, 6.64, 7.91%,

Table 1

Organometric indices of the seminal vesicles of rats in the reproductive period of the control groups ($M \pm m$, $n=30$)

Parameters	Dates of observation, days				
	1	7	15	30	60
Absolute mass, mg	395,80±17,45	420,52±6,57	455,00±10,28	498,34±6,25	525,71±16,65
Relative mass, mg/g	1,91±0,12	1,93±0,08	1,91±0,04	2,21±0,09	2,27±0,11
Length, mm	21,53±1,08	24,32±1,12	26,28±0,66	28,87±1,24	30,15±1,53
Width, mm	8,88±0,31	9,20±0,17	9,95±0,12	10,97±0,11	11,43±0,19
Thickness, mm	6,40±0,32	6,65±0,12	6,80±0,15	7,60±0,14	9,30±0,10
Volume, mm ³	640,75±45,64	779,04±29,87	930,99±21,24	1260,24±28,74	1678,04±109,56

Table 2

Micromorphometric indices of the seminal vesicles of rats in the reproductive period of the control groups ($M \pm m$, $n=30$)

Parameters	Dates of observation, days				
	1	7	15	30	60
Height of epitheliocyte, μm	17,14±0,12	17,66±0,11	18,53±0,29	19,66±0,18	20,24±0,34
Width of the epitheliocyte, μm	11,97±0,24	12,65±0,19	13,25±0,17	13,95±0,19	15,62±0,32
Volume of epitheliocyte, μm^3	1285,83±27,35	1479,65±20,31	1703,31±34,56	2003,17±35,63	2585,58±69,21
Larger nucleus diameter, μm	11,20±0,12	11,45±0,05	11,86±0,05	12,69±0,12	12,39±0,28
Smaller nucleus diameter, μm	7,65±0,07	7,98±0,07	8,23±0,06	8,64±0,08	8,79±0,14
Nucleus volume, μm^3	89,68±1,76	95,63±1,15	102,16±0,96	114,76±0,94	113,99±5,17

respectively, on 7, 15 and 30 days of follow-up. The size of the nuclei and their volume decreased at the same time: the larger and smaller core diameters significantly decreased by 3.99%, 4.98%, 8.38% and 3.22%, 6.56%, 8.06%. The parameters of the volume of the nucleus of the epithelial cells of the seminal vesicles of the animals of the control groups significantly exceeded the data of the experimental rats at the same time by 7.07, 11.02 and 15.77%.

At 1 and 60 days statistically significant differences between the parameters studied were not established.

The established changes in the parameters of the seminal vesicles indicate an intensive reaction from the organ in response to the immunosuppressive effect. It is known that the use of cyclophosphamide according to this scheme induces the development of the systemic immunosuppression state [6]. Under these conditions, changes in the organ indicators can be explained by the direct cytotoxic effect of the drug on actively dividing cells, including secretory epitheliocytes. S.S. Ostrovskaya et al. (2014) observed the active reaction of the organs of the male reproductive system in the experiment after exposure to salts of heavy metals [7]. Changes in micromorphometric indicators, indicative of the degree of functional activity of seminal vesicles, may explain the disregulatory effect of cyclophosphamide on local endocrine and immune homeostasis. Thus, Yu.S. Khramtsova et al. proved the fact of spermatogenesis disturbances under conditions of changes in the cooperation of the endocrine apparatus of the testes with the local complex of immunocompetent cells [3].

Conclusions.

1. In conditions of prolonged immunosuppression, an active response is observed on the part of the seminal vesicles of animals of the reproductive period.

2. Statistically significant changes in the organ's organ indicators are observed both at the early (7, 15 days) and late observation periods, which indicates the direct influence of cyclophosphamide on the biosynthetic processes in the organ.

3. Micromorphometric changes, as well as the results of cyto- and karyometry, confirm the organometric data and indicate the development of local disturbances in the morphogenesis of seminal vesicles caused by the dysregulation of the endocrine-immune homeostasis of the organ.

4. The results obtained give rise to interest in the study of the structure of seminal vesicles in acute age-related immunosuppression.

References:

1. Alekseeva N.T. Otechestvennaya jekologicheskaja morfologija: tradicii, opyt, perspektivy, nauchnye shkoly [Domestic ecological morphology: traditions, experience, prospects, scientific schools]. Zhurnal anatomii i gistopatologii [Journal of Anatomy and Histopathology]. 2015. Vol. 4, №3. P. 9-13.
2. Bessalova E.Ju. Biometricheskie pokazateli semennikov krysa pri parenteral'nom vvedenii spinnomozgovoj zhidkosti [Biometric parameters of testis of rats with parenteral administration of cerebrospinal fluid]. Visnik problem biologii ta medicine [Bulletin of Biology and Medicine]. 2011. №4 (90). P. 195-197.
3. Hramcova Ju.S., Artashjan O.S., Jushkov B.G. i soavt. Vlijanie tuchnyh kletok na reparativnuju regeneraciju tkanej s raznoj stepen'ju immunologicheskoy privileirovannosti [Influence of mast cells to reparative tissue regeneration with varying degrees of immunological privilege]. Citologija [Cytology]. 2016. Vol. 58, №5. P. 356-363.
4. Direktiva Evropejskogo Parlamenta i Soveta Evropejskogo Sojuza po ohrane zivotnyh, ispol'zuemyh v nauchnyh celjah ot 2010 g. № 2010/63/EU [Directive 2010/63 / EU of the European Parliament and of the Council of the European Union on the protection of animals used for scientific purposes from 2010 № 2010/63/EU]. NP «Ob#edinenie specialistov po rabote s laboratornymi zivotnymi» [«Association of experts on work with laboratory animals»]. St. Petersburg, 2012. 48 p.
5. Kashchenko S.A., Petizina

O.N., Morozova E.N. Korreljacionnaja zavisimost' mezhdru parametrami limfaticeskikh uzlov krysa posle korrekcii immunodeficitnogo sostojanija [Correlation relationship between parameters of lymph nodes of rats after correction of immunodeficiency state]. Ukraïns'kij morfologichnij al'manah [Ukrainian morphological almanac]. 2014. Volume 12, No. 3. P. 34-37.

6. Koveshnikov V.G., Frolov V.M., Kashchenko S.A. Ul'trastruktura timusa v uslovijah immunostimuljacji i immunosupressii [Ultrastructure of thymus under conditions of immunostimulation and immunosuppression]. Ukraïns'kij medichnij al'manah [Ukrainian medical almanac]. 2005. Vol. 3, No. 2. P. 36-40.

7. Ostrovskaja S.S., Shatornaja V.F., Kolosova I.I. Sochetannoe vozdejstvie svinca i kadmija na organism [The combined effect of lead and cadmium on the body]. Vestnik problem biologii i mediciny [Bulletin of the problems of biology and medicine]. 2014. Issue 4, No. 3 (115). P. 25-29.

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