
A.A. Zakharov

CHANGES OF ORGANOMETRIC PARAMETERS OF EPIDIDYMIS OF EXPERIMENTAL ANIMALS AFTER ADMINISTRATION OF CYCLOPHOSPHAMIDE

ABSTRACT

The aim of this study was to establish the organometric features of epididymis of immature experimental animals after immunosuppression, simulating the negative impact of exogenous factors on the reproductive system.

Material and methods. The experiment was carried out on 60 immature white rats derived from vivarium of SE "LGMU" with an initial mass 40-50 g. For modeling immunosuppression rats received immunosuppressive drug cyclophosphamide in a dosage of 1.5 mg/kg body weight for 10 days. When working with experimental animals used recommendations ARRIVE manual prepared by National Centre for the Replacement, Refinement & Reduction of Animals In Research. Epididymides were weighed on a torsion balance, calculated relative to body mass, linear dimensions measured using calipers. The data were processed using the statistical program, the significance of differences between the indices of the experimental and control groups was determined by Student-Fisher's test ($p < 0.05$).

Results and discussion. The data may indicate that the epididymis of immature animals undergo some morphologic changes, after "soft" immunosuppression, which is accompanied by inhibition of morphogenesis, manifested by decrease of organometric parameters in comparison with controls. The development of these structural changes is also observed in a number of cases that have been observed by some researchers, under certain conditions of external and internal environment with the action on male sex organs systems of animals and humans. **Conclusions.** Epididymides of immature animals exhibit an active response to the "soft" immunosuppression. The most active change of organ morphometry indicators are set to 1, 7 and 15 days after administration of cyclophosphamide, which is probably due to the imperfection of the structure and functioning of the immature epididymis, as well as changes in the immune organs as a constituent of one of the regulatory systems of the body. Leveling morphometric changes in the later stages of observation (30 and 60 days) may be associated with the normalization of the immune system and the development of adaptation mechanisms in the male reproductive system organs.

Keywords: epididymis, cyclophosphamide, rats, immunosuppression, morphometry.

INTRODUCTION

The deterioration of the reproductive health of the male population is a recognized and widespread problem in the world in recent decades. Through the efforts of the World Health Organization has developed and implemented programs aimed at the formation of long-term policy of the WHO Regional Office, which is part of the European regional strategy in the field of sexual and reproductive health. The results of the latest clinical and laboratory studies support the concern of experts in this field regarding violations of the male reproductive function, increasing the number of which in different countries makes the research of various branches of medical science to draw attention to this problem very urgent [3]. The reasons for the changes in the structure and functioning of the male reproductive system is called a number of exogenous adverse factors, including those that cause systemic immunosuppression, including extensive use of drugs for the relief of immunopathological, cancer and autoimmune conditions [1]. The special position occupied by factors affecting the organism of children and

adolescents due to the imperfection of the morphological and functional elements of the organs of the regulatory systems of the body, including the immune system. The existence of such health and socio-economic components of the problems of modern society stimulates the study of morphological changes in the foundations of the male reproductive function, especially before puberty. In this context, the aim of this study was to establish the organometric features of epididymis of immature experimental animals after immunosuppression, simulating the negative impact of exogenous factors on the reproductive system.

MATERIAL AND METHODS

The study was carried out within the framework of the research program of the Department of Histology, Cytology and Embryology SE "Lugansk State Medical University" "Structural features of organs of the immune and endocrine systems in immunostimulation and immunosuppression" (state registration number 0112U000096). The experiment was carried out on 60 immature white rats obtained from the vivarium SE "LGMU" with an initial body weight of

40-50 g. During the study the animals were kept under standard conditions with natural light and diet recommended for this type of animal. For the simulation of immunosuppression rats were administered an immunosuppressive drug cyclophosphamide in a dosage of 1.5 mg/kg body weight for 10 days. This scheme is widely used in clinical practice to achieve immunosuppression in various autoimmune diseases, transplantation. As a control, animals were administered with 0.9% sodium chloride in equivalent volumes by the same scheme. Rats were taken from the experiment under ether anesthesia at 1, 7, 15, 30 and 60 days of observation. When working with experimental animals used recommendations ARRIVE manual prepared by National Centre for the Replacement, Refinement & Reduction of Animals In Research [5]. Epididymides were weighed on torsion balance WT 1000, calculated relative organ mass, linear dimensions measured using calipers ShTs-I. The data were processed using the licensed program «StatSoft Statistica v6.0», the reliability of the difference between the indices of the experimental and control groups was determined by

Student-Fisher's test ($p < 0.05$).

RESULTS AND DISCUSSION

Epididymis of immature animals of the control group adjacent to the dorsal edge of the testis, and has a head, body and tail. Absolute and relative organ weight increased during the observation period (Table 1). The linear dimensions of the body also underwent similar changes with increasing age of rats: so appendage length was 23.7 ± 0.74 mm, 22.7 ± 0.89 mm, 27.3 ± 0.24 mm, 28.4 ± 1.4 mm, and 43.8 ± 1.3 mm, 1, 7, 15, 30 and 60 days of observation, respectively. Body width also increased in the same period, amounting to 2.67 ± 0.07 mm, 2.79 ± 0.12 mm, 3.94 ± 0.15 mm, 3.9 ± 0.12 mm and 6.94 ± 0.25 mm, respectively. These changes of organometric parameters of epididymis related indicators of ontogenetic morphogenesis.

After administration of cyclophosphamide epididymis remain common morphological features of the structure. However, the immunosuppressive effect of causing significant changes in the morphometric parameters of the body. So, the early stages of observation (1, 7 and 15 days) a decrease of the absolute weight of epididymis indicators at 9.26%, 13.44% and 17.12%, respectively, was discovered (Fig. 1). After 30 and 60 days after treatment significant changes of organometric parameters have been not identified.

After immunosuppression the length indicator of epididymis undergone similar changes. At 1, 7 and 15 days of observation was significant reduction at 9.77%, 12.48% and 17.95% respectively, compared with those of control animals. Organ width index was significantly decreased in the specified time observations at 10.11%, 13.47% and 16.84% relative to the control data (Table 2). In the long-term period of the experiment (30 and 60 days), significant deviations of the experimental data from the control has not been established.

The data may indicate that the epididymis of immature animals undergo

Table 1

Changes in the absolute and relative mass of epididymis of immature animals in the control group ($M \pm m$, $n=30$)

Group	Days	Absolute mass of epididymis, mg	Relative mass of epididymis, mg
Control	1	27 ± 0.55	0.19 ± 0.001
	7	31 ± 1.5	0.23 ± 0.01
	15	37 ± 1.8	0.23 ± 0.008
	30	38 ± 2.74	0.25 ± 0.012
	60	58 ± 3.1	0.36 ± 0.014

some morphologic changes, after "soft" immunosuppression, which is accompanied by inhibition of morphogenesis, manifested decrease of organometric parameters in comparison with controls. The development of these structural changes is also observed in a number of cases that have been observed by some researchers, under certain conditions of external and internal environment with the action on male sex organs systems of animals and humans. In particular, S.S. Ostrovsky et al. point to the oppression of morphological and functional parameters of the reproductive system of rats after exposure to heavy metals [4]. P.V. Loginov, P.A. Ivanov proved the reduction of the endocrine function of the male reproductive system in the background of nutritional stress [2].

CONCLUSIONS

1. Epididymis of immature animals exhibits an active response to the "soft" immunosuppression.

2. The most active change of organ morphometry are set to 1, 7 and 15 days after administration of cyclophosphamide, which is probably due to the imperfection of the structure and functioning of the immature epididymis, as well as changes in the immune organs as a constituent of one of the regulatory systems of the body.

3. Leveling the morphometric changes in the later stages of observation (30 and 60 days) may be associated with the normalization of the immune system and the development of adaptation mechanisms in the male reproductive system organs.

4. The obtained results are of interest for the study of the structural features of epididymis in an immunostimulation.

References

1. Dudenkova N.A., Shubina O.S. Izmeneniya morfofunktsional'nogo sostoyaniya i produktivnosti semennykh zhelez belykh kryss pri vozdeystvii atsetata svintsa [Changes of morpho-functional status and productivity of the seminal glands in white rats under the influence of lead acetate]. Fundamental'nye issledovaniya [Fundamental research]. 2013. № 10 (chast' 6). S. 1253 – 1259.
2. Loginov P.V., Ivanov P.A. Izmenenie funktsional'nogo sostoyaniya semennikov kryss v usloviyakh pishchevogo stressa po urovnyu polovykh gormonov [Changing

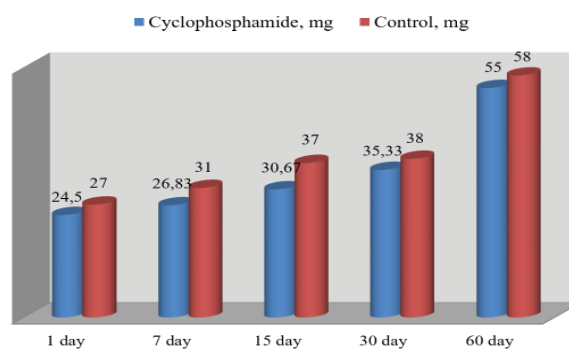


Fig. 1. Changes in the absolute weight of epididymis after use of cyclophosphamide and in control.

Table 2

Linear parameters of epididymis of immature animals after the administration of cyclophosphamide ($M \pm m$, $n=30$)

Group	Days	Epididymis length, mm	Epididymis width, mm
Cyclophosphamide	1	$21.38 \pm 0.42^*$	$2.4 \pm 0.09^*$
	7	$19.87 \pm 0.84^*$	$2.42 \pm 0.03^*$
	15	$22.4 \pm 0.38^*$	$2.38 \pm 0.15^*$
	30	25.25 ± 0.25	3.68 ± 0.1
	60	42.65 ± 1.19	6.72 ± 0.21

* – significant difference from control, $p < 0.05$.

of the functional state of the testes of rats under nutritional stress on the level of sex hormones]. Mezhdunarodnyy zhurnal eksperimental'nogo obrazovaniya [International Journal of Experimental Education]. 2014. № 8-3. S. 86 – 87.

3. Malolina E.A., Kulibin A.Yu., Yu.A. Tyulenev Tyulenev Destruktivnye izmeneniya v semennikah myshi pri retrogradnom puti ih zarazheniya virusom prostogo herpesa [Destructive changes in the testes of mice during retrograde path of the herpes simplex virus]. Urologiya [Urology]. 2013. № 4. S. 55 – 59.

4. Ostrovskaya S.S., Shatornaya V.F., Kolosova I.I. Sochetannoe vozdeystvie svintsa i kadmiya na organizm [The combined effects of lead and cadmium in the body]. Vestnik problem biologii i meditsiny [Bulletin of the problems of biology and medicine]. 2014. Vyp. 4, № 3 (115). S. 25 – 29.

5. Kilkenny C. Improving Bioscience Research Reporting: The ARRIVE Guidelines for Reporting Animal Research / C. Kilkenny, W.J. Browne, I.C. Cuthill [et al.] // PLoS Biol. – 2010. – № 8 (6): e1000412. doi:10.1371/journal.pbio.1000412.

The author: Zakharov Alexei Alexandrovich, PhD, docent of the Lugansk SMU, e-mail: masterhist@mail.ru.