ORIGINAL ARTICLE

CHARACTER AND SPECIFICS OF THE STRUCTURAL ALTERATION OF THE PARENCHYMA AND BLOODSTREAM OF THE TESTES OF WHITE RATS WITH PROLONGED ADMINISTRATION OF HIGH DOSES OF PREDNISOLONE

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ABSTRACT

The aim: To investigate the nature of morphofunctional changes in the parenchyma and bloodstream of the testes of white rats with prolonged administration of high doses of prednisolone.

Material and methods: The experiments were performed on rats fed daily for 1, 3, 7, 14 and 28 days. prednisolone was administered intramuscularly at a rate of 0.4 mg / kg. Massometric, organometric, histological and morphometric studies were performed.

Results: Administration of high doses of prednisolone resulted in a significant increase in body weight and testicular weight and volume. As the drug was administered, spermatogenesis was activated. The number of immature forms of germ cells increased significantly. At the same time, the specific number of mature forms of sperm decreased. This led to a significant increase in the diameter of the convoluted tubules, the thickness of the spermatogenic epithelium, as well as the index of spermatogenesis. The increase in indicators was especially intense until the 7th day of observation, after which its rate decreased, although the dynamics remained unchanged. The thickness of the protein shell tended to decrease. The detected changes occurred against the background of dilation and plethora of arteries, especially up to 7-14 days of the experiment, after which their intensity decreased slightly. This was accompanied by a simultaneous reversible narrowing of the lumen of small arteries and arterioles.

Conclusion: Thus, long-term administration of high doses of prednisolone promotes the activation of spermatogenesis with an increase in immature forms of germ cells and a simultaneous decrease in the proportion of mature sperm. Increased vascular blood supply, especially in the early period. In the long run, the capacity of small arteries and arterioles is reduced, as well as the degree of activation of spermatogenesis.

KEY WORDS: testicles, prednisolone, spermatogenesis, arteries

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INTRODUCTION

Glucocorticoids (GC) today are the most important and frequently used class of anti-inflammatory drugs. Corticosteroids are a class of steroid hormones released by the adrenal cortex, which includes glucocorticoids and mineralocorticoids1. However, the term "corticosteroids" is generally used to refer to glucocorticoids. Named for their effect in carbohydrate metabolism, glucocorticoids regulate diverse cellular functions including development, homeostasis, metabolism, cognition and inflammation. Due to their profound immune-modulatory actions, glucocorticoids are one of the most widely prescribed drugs in the world and the worldwide market for glucocorticoids is estimated to be worth more than USD 10 billion per year. Glucocorticoids have become a clinical mainstay for the treatment of numerous inflammatory and autoimmune diseases, such as asthma, allergy, septic shock rheumatoid arthritis, inflammatory bowel disease, and multiple sclerosis. Unfortunately, the therapeutic benefits of glucocorticoids are limited by the adverse side effects that are associated with high dose (used in the treatment of systemic vasculitis and SLE) and long-term use. These side effects include osteoporosis, skin atrophy, diabetes, abdominal obesity, glaucoma, cataracts, avascular necrosis and infection, growth retardation, and hypertension [1 - 4].

Although the therapeutic effects of HA have been known and used for over 50 years, significant progress in discovering the basic molecular mechanisms of their action has been made only in the last 10-15 years. However, despite this, the causes of GC-mediated side effects remain only partially clear Many previous and current studies have shown that long-term treatment with corticosteroids is associated with a variety of adverse effects, such as infections, gastrointestinal bleeding or ulcers, cardiovascular disease, Cushing's syndrome, diabetes and metabolic syndrome, cataracts, glaucoma. osteoporosis [5 - 7].

Recent data also suggest that adrenal crisis remains a serious problem in clinical practice after glucocorticoid administration. Prolonged use of glucocorticoids on the physiological principle of «negative feedback» inhibits the production of its own hormones by the adrenal cortex. In many patients, there are dysfunctions of the genital area. In

particular, it is a violation of the menstrual cycle in women, the development of impotence in men [8].

However, given that the problem of male infertility is relevant today for all countries, including due to the special difficulties due to the fact that in 30-40% of cases it is not possible to establish the causes of spermatogenesis further awareness of side effects of glucocorticoids and further research in this direction is urgently needed [8, 9].

THE AIM

The aim of the study was to investigate the nature of morphofunctional changes in the parenchyma and bloodstream of the testes of white rats with prolonged administration of high doses of prednisolone.

MATERIALS AND METHODS

The experiments were performed on 42 rats, which were administered intramuscularly daily prednisolone (synthetic glucocorticoid) at a rate of 0,4 mg / kg, which is the maximum single daily dose. Material for morphological examination was collected 1, 3, 7, 14 and 28 days after drug administration, as well as in animals of two control groups: baseline group - control I and group of animals, who were injected with saline in volume, similar to prednisolone on 28-day observation - control II). Testicular weight was determined using a weight of T11 / 500, the volume was determined by immersion in water in a measuring cup. Histological specimens were stained with hematoxylin and eosin, according to Weigert and van Gizon. Animals were removed from the experiment by intraperitoneal administration of large doses of concentrated sodium thiopental. All experimental studies were conducted in accordance with the principles of bioethics set out in the Helsinki Declaration and the Law of Ukraine «On the Protection of Animals from Cruelty» (№ 1759-VI of 15.12.2009).

For morphometric study of intraorgan branches, testicular arteries by caliber and topographic location were divided into three groups: arteries of the protein shell (AB) with an outer diameter of 136-180 μm, intramural arteries of medium diameter (SD) with an outer diameter of 51-135 μm and small (DIA) with an outer diameter of 26-50 microns. According to their morphometric characteristics, these arteries fit into the already known schemes of gradation of vascular channels [10, 11].

Morphometric evaluation of intraorgan vessels was performed using an eyepiece micrometer MOV-1-15Ch. Assessment of the functional state of blood vessels was performed by subtracting the Vaughanworth index (IV) as the ratio of artery wall area to the area of its lumen [12, 13]. Spermatogenesis index (IC) was also caculated as the ratio of the number of layers isolated in each spermatic tube to the number of calculated tubus.

The digital material obtained during morphological and functional studies was subjected to statistical processing using Microsoft Exel for Windows 98 to determine the average values and their standard errors.

RESULTS

The introduction of high doses of prednisolone significantly affected both the general condition of the experimental animals and the condition of their testicles. The appetite of animals significantly increased, which forced to increase their diet. As a result, their body weight in general and the weight and volume of their testicles in particular increased markedly progressively (Table I).

Thus, the average weight of the testicle significantly increased by 16,0% on the 7th day of the experiment, and before its completion significantly exceeded the control figures by 23,1%. Accordingly, there was an increase in the volume of the organ: significant by 32,3% also on the 7th day of the experiment and by 48,1% until its completion. Approximately the same was the difference from the values of the indicators of animals of Control II, which did not differ in principle from Control I, only partially inaccurately exceeded them.

There was also a change in other morphometric parameters (Table II).

According to the results of the study, in particular, it was found that as the drug was introduced, spermatogenesis was activated. The number of germ cells in the lumen of the convoluted tubules increased significantly, especially due to their immature forms (spermatogonia and spermatocytes). The number of mature forms of sperm decreased slightly (Fig. 1).

This led to an increase in the diameter of the seminiferous tubes, as a result of which their number in one field of view gradually decreased (significantly by 10,5 % on the 3rd day of the experiment and by 30,2 % at its completion). The dynamics of the thickness of the spermatogenic epithelium was corresponding (significant thickening by 16,0 % on the 7th day of the experiment and by 20,9 % at its completion), as well as the index of spermatogenesis (significant increase by 8,6 % on the 7th day) experiment and 14,6 % at its completion). As can be seen from the table, the particularly intensive increase in the last two indicators was up to 7 days of observation, after which its rate decreased slightly, although the dynamics remained unchanged. The thickness of the protein shell tended to gradually decrease.

The detected changes occurred against the background of increased blood supply to the testicles, which was confirmed by moderate plethora of arteries, the lumen of which looked dilated, and the inner elastic membranes - smoothed.

Particularly intense blood supply to the arteries with simultaneous expansion of their lumen was observed up to 7-14 days of the experiment (Fig. 2), after which the growth rate slowed down somewhat, which occurred against the background of simultaneous narrowing of the lumen of small arteries and arterioles (Table 3).

The expansion of mouths of lateral branches as a result of decrease in a tone of the muscular-elastic sphincters, located there drew attention (fig. 3).

The detected morphofunctional changes had their morphometric confirmation. Thus, IP in large arteries (arteries of the protein shell with an outer diameter of

Table I. Dynamics of mass and organometric changes of the testicles of male rats at different times after administration of prednisolone (M ± m)

Parameter	Observation group						
	Control I	Control II	1st day	3rd day	7th day	14th day	28th day
Weight, g	1,69 ±0,06	1,75 ±0,05	1,87 ±0,05	1,94 ±0,04	1,96 ±0,04*	1,95 ±0,04*	2,05 ±0,05*
Volume, ml^3	987,95 ±43,17	1014,79 ±44,54	997,27 ±50,19	1130,46 ±45,09	1287,71 ±49,50*	1378,63 ±43,50*	1431,19 ±56,91*

Note: * - P<0,05 in comparison with groups Control I and Control II

Table II. Dynamics of changes in morphometric parameters of the parenchyma of the testes of male rats at different times after administration of prednisolone (M ± m)

Parameter	Observation group						
	Control I	Control II	1st day	3rd day	7th day	14th day	28th day
The number of tortuous seminal tubules in one field of view	36,59 ±0,74	38,00 ±0,66	34,76 ±0,67	32,84 ±0,66*	29,42 ±0,72*	26,47 ±0,75*	25,40 ±0,82*
The thickness of the protein shell, mcm	38,27 ±2,12	41,02 ±2,09	37,10 ±2,23	35,40 ±2,17	32,91 ±2,44	33,02 ±2,57	31,04 ±2,46
The thickness of the spermatogenic epithelium, mcm	39,09 ±1,45	42,25 ±1,26	39,58 ±1,59	41,23 ±1,41	45,26 ±1,42*	46,44 ±1,37*	47,45 ±1,51*
Spermatogenesis index	3,18 ±0,04	3,20 ±0,05	3,18 ±0,03	3,29 ±0,04	3,37* ±0,03	3,26* 0,04	3,46* ±0,05

Note: * - P<0,05 in comparison with groups Control I and Control II

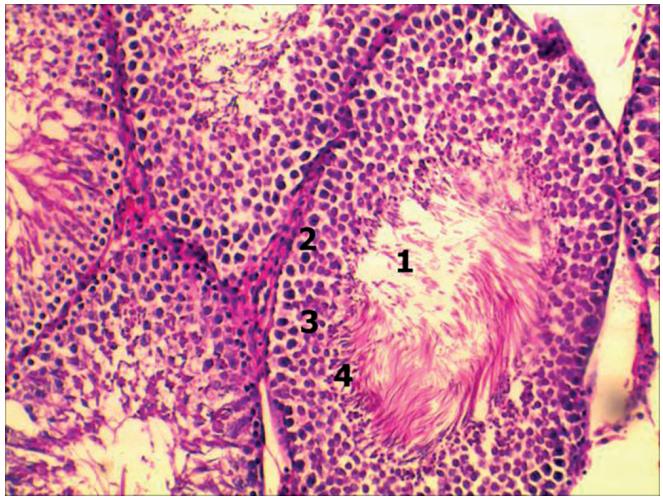

Fig. 1. Histological section of the testicle of a white rat 28 days after administration of high doses of prednisolone. Staining with hematoxylin and eosin. 'x 140.
1 – lumen of the seminiferous tubules;
2 – spermatogonia;
3 – spermatocytes;
4 – sperm cells.

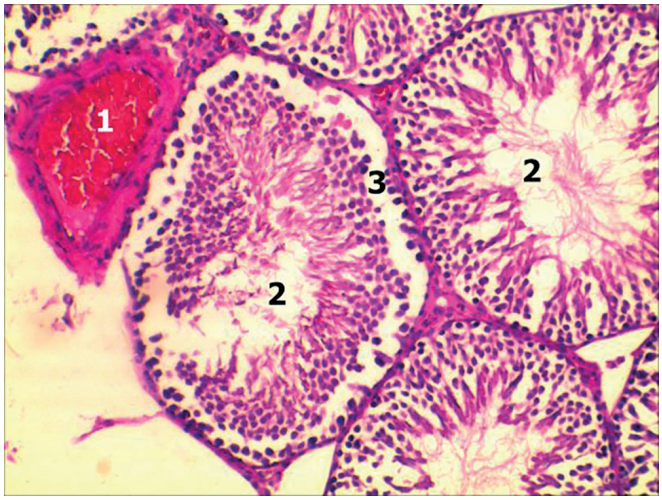

Fig. 2. Histological section of the testicle of a white rat 7 days after administration of high doses of prednisolone. Staining with hematoxylin and eosin. 'x 140.
1 – full-blooded artery;
2 – lumen of seminiferous tubules;
3 – detachment of spermatogenic epithelium from the wall of seminiferous tubules.

136-180 μm) gradually decreased throughout the experiment and became smaller than the control figures: on the 7th day significantly by 9,4 % and on the 28th day - by 12,1 %, in intramural arteries of medium caliber (with an outer diameter of 51-135 μm) such a decrease on the 7th day was 5,9% and 10.4 % - on the 28th day, also significantly different from the control figures. As for the small intramural arteries (with an outer diameter of 25-50 μm), they tended to reduce IP by 4,2 % was observed only during the first day. On the third day, the indicator returned and became close to the control values, and then gradually increased, significantly exceeding the initial level on the 7th day by 10,0 % and in the final stage of the experiment - by 18,1 %.

Table III. Dynamics of changes in morphometric parameters of the testicular arteries of male rats at different times after administration of prednisolone (M ± m).

Duration of observation	The order of branching of vessels and their parameters											
	Protein sheath arteries				Intramural arteries of medium caliber				Small intramural arteries			
	Diameter external (mcm)	Diameter internal (mcm)	Media thickness (mcm)	IV	Diameter external (mcm)	Diameter internal (mcm)	Media thickness (mcm)	IV	Diameter external (mcm)	Diameter internal (mcm)	Media thickness (mcm)	IV
Control I	158,83 ±1,30	98,50 ±0,09	30,17 ±0,49	160,02 ±2,64	91,67 ±1,17	53,33 ±0,80	19,17 ±0,21	195,54 ±2,13	36,00 ±1,13	17,33 ±0,49	9,33 ±0,33	331,40 ±7,85
Control II	161,33 ±1,20	99,33 ±0,11	31,00 ±0,43	163,79 ±2,25	91,00 ±1,15	53,00 ±0,52	19,00 ±0,34	194,77 ±2,67	37,00 ±1,39	17,67 ±0,56	9,67 ±0,42	338,03 ±6,72
1st day	157,67 ± 1,38	97,33 ±0,42	30,17 ±0,49	162,38 ±2,52	89,83 ±1,45	52,50 ±0,85	18,67 ±0,33	192,85 ±2,43	38,83 ±1,19	19,00 ±0,68	9,92 ±0,27	318,70 ±6,99
3rd day	159,17 ±1,40	99,33 ±0,49	29,92 ±0,51	156,76 ±2,69	89,67 ±1,33	53,00 ±1,00	18,33 ±0,25	186,55 ±3,82	38.17 ± 1,08	18,17 ±0,60	10,00 ±0,26	342,31 ±7,94
7th day	162,17 ±1,35	102,67 ±0,49	29,75 ±0,46	149,49 ±2,19*	90,00 ± 0,97	53,50 ±0,72	18,25 ±21	183,18 ±3,16*	38,67 ±1,54	18,00 ±0,68	10,33 ±0,44	361,51 ±7,13*
14th day	161,50 ±1,31	102,50 ±0,50	29,50 ±0,45	148,25 ±2,17*	92,67 ±1,17	55,50 ±0,89	18,58 ±0,24	179,02 ±3,50*	37,83 ± 1,19	17,33 ±0,56	10,25 ±0,34	376,75 ±7,98*
28th day	160,83 ±1,25	103,50 ±0,43	28,67 ±0,44	141,47 ±2,12*	92,17 ±1,30	55,50 ±0,76	18,33 ±0,33	175,86 ±3,14*	39.17 ± 0,83	17,67 ±0,33	10,75 ±0,28*	391,78 ±10,25*

Note: * - P<0,05 in comparison with groups Control I and Control II

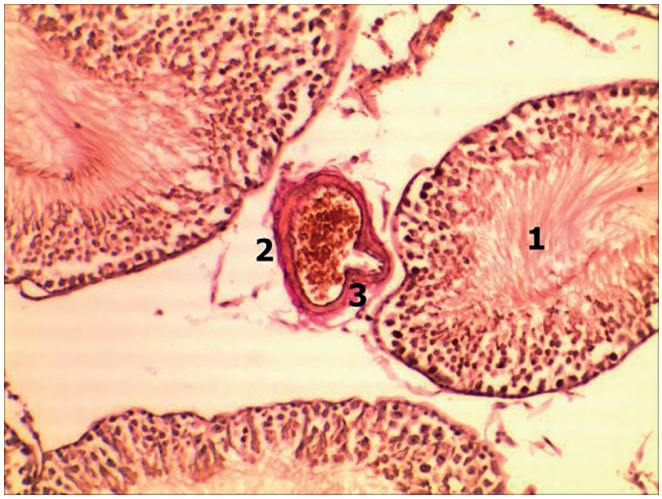

Fig. 3. Histological section of the testicle of a white rat 7 days after administration of high doses of prednisolone. Coloring by Weigert and van Gizon. 'x 140.
1 – lumen of the seminiferous tubule;
2 – intraorgan branch of the testicular artery;
3 – mouth of the lateral branch from the intraorgan branch of the testicular artery.

DISCUSSION

Our data on structural and functional changes in the parenchyma of the testes and their bloodstream are fully consistent with modern ideas about the nature of hormonal effects on the genital area and may be a consequence of the side effects of prednisolone, which are embedded in Cushing's syndrome, diabetes and metabolic syndrome [5, 6, 7]. These include a significant increase in animal body weight as a result of increased appetite and the development of alimentary obesity, as well as an increase in the weight and volume of the testes with increased functional activity on the background of full blood vessels due to dilation of their lumen. It is known that the mineralocorticoid action of prednisolone contributes to the retention of salts and water in the body, which in turn can lead to the development of hypertension. The potential development of arteritis in such cases can play a potentiating role [14]. Therefore, it is quite logical that we have registered an increase in wall tone and narrowing of the lumen of small arteries and arterioles with a decrease in their capacity in the long term of the experiment, which is confirmed by the corresponding dynamics of the Vaughanworth index. In the scientific literature, such reactions to high blood pressure are known as the Chinese reflex. They are initially aimed at preventing congestion of the hemomicrocirculatory tract. However, in the future may be the cause of ischemia of the organ with a violation of its functional capacity [15].

Regarding the morphofunctional rearrangement of the testes of experimental animals in terms of spermatogenesis, when using high doses of prednisolone, this process is markedly activated with its simultaneous qualitative and quantitative deviation from the norm by significantly increasing the number of immature forms and reducing the proportion of mature spermatozoa. be the cause of infertility [16]. Also, the further probable development of infertility is threatened by sudden withdrawal of the drug due to secondary insufficiency of the adrenal cortex (Addison's syndrome), and the development of nodular periarteritis, which requires further research in this direction [8, 14].

CONCLUSIONS

1. Prolonged administration of high doses of prednisolone promotes the activation of spermatogenesis with a progressive increase in immature forms of germ cells and a simultaneous decrease in the proportion of mature sperm in white rats.
2. Activation of spermatogenesis occurs against the background of increased testicular blood flow with dilation

of the lumen and increased blood supply to blood vessels of all calibers, especially in the early period (7-14 days from the start of use).
3. In the long term (14-28 days) there is a decrease in the capacity of small arteries and arterioles, as well as the rate of activation of spermatogenesis, which may be a reaction to congestion of the hemomicrocirculatory tract of the testes and cause further ischemia of the organ with its functional insufficiency.

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Conflict of interest:
The Authors declare no conflict of interest

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