ORIGINAL ARTICLE

EFFECT OF IMUNOFAN INFLUENCE ON THE STRUCTURE OF THE TESTES, HORMONAL AND CYTOKINE PROFILE OF IMMATURE EXPERIMENTAL ANIMALS

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Alexey A. Zakharov, Svetlana A. Kashchenko, Inessa V. Bobrysheva, Sergey N. Semenchuk
SAINT LUKA LUGANSK STATE MEDICAL UNIVERSITY, LUGANSK, UKRAINE

ABSTRACT

The aim: It was the establishing the features of changes in the structure of the testes of experimental animals, as well as immunological, hormonal and cytokine parameters of blood plasma during stimulation**.**

Materials and methods: The study was carried out on 60 white male immature rats. Imunofan was used at a dosage of 50 μg. The organs were weighed, the relative mass was calculated, and the linear dimensions were determined. The morphometriv parameters of the epitheliospermatogenic layer were measured. The number of supporting cells and interstitial endocrinocytes was counted, as well as the volume of cell nuclei. The level of reproductive hormones in the plasma and the concentration of cytokines were determined.

Results: The results obtained indicate the development of readaptation processes in the testes after the use of the Imunofan against the background of environmental immunosuppression. The ability of the drug to stimulate the production of cytokines and hormones normalizes the function of immunocompetent cells, which is manifested in the stabilization of the immune homeostasis of the testes.

Conclusions: In response to the immunostimulating effect of Imunofan, a pronounced reaction is observed on the part of the testes of immature animals, which is due to the sensitivity of morphogenetic processes in the organ to external influences and the formation of mechanisms of their regulation, characteristic of this period of ontogenesis.

KEY WORDS: testis, immunostimulation, imunofan, rats, reproductive hormones, cytokines

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INTRODUCTION

Based on comprehensive studies from around the world, WHO has concluded that children's health is significantly affected by environmental phenomena caused by climate change, the presence of persistent organic pollutants, ozone degradation and declining natural flora and plankton diversity. Among the significant consequences for the health of the child is a decrease in immunity [1]. In recent years, there has been an increase in the number of diseases associated with impaired functioning of the immune system, caused by a sharp deterioration in the ecological state of the environment [2, 3]. At the same time, a special position among the causes of this problem is occupied by immunodeficiency states, the occurrence of which is associated with the increasing influence of unfavorable anthropogenic factors, a significant increase in immune-dependent pathological conditions and allergies [4, 5]. It is known that any effect on the body changes its homeostasis, which leads to a complex reaction, primarily from the regulatory systems - nervous, endocrine and immune, which naturally causes morphofunctional rearrangements in all organs that are not directly involved in the processes of modulating the response to environmental changes, including testes [6]. It is known that the critical periods of ontogenesis include long periods of childhood and puberty, which are characterized by increased sensitivity and vulnerability of the organs of the reproductive system to exo- and endogenous influences. However, today, against the background of the abundance of scientific information regarding the clinical and pathogenetic features of the course of certain diseases of the male reproductive system, there are practically no results of studying the problem of the structure of the gonads in the state of immunostimulation, the need for which is justified by the above problems, especially in childhood and puberty. In this regard, the aim of this study was to establish the features of changes in the structure of the testes of experimental animals, as well as immunological and hormonal parameters of blood plasma during stimulation.

THE AIM

It was the establishing the features of changes in the structure of the testes of experimental animals, as well as immunological, hormonal and cytokine parameters of blood plasma during stimulation**.**

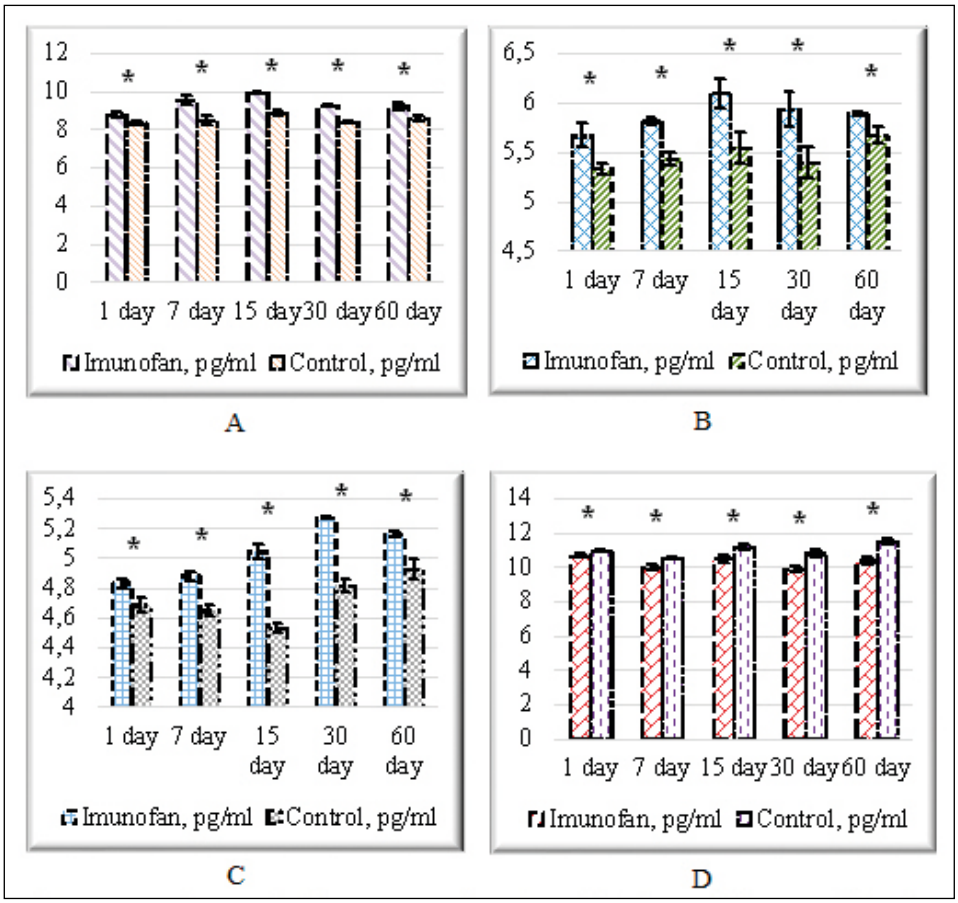

Fig. 1. Changes in the concentration of IL-1β (A), IL-2 (B), IL-6 (C) and TNF-α (D) in the blood plasma of immature rats after application of Imunofan and in the control. * - significant difference from the control data ($p < 0.05$).

MATERIALS AND METHODS

The study was carried out on 60 white outbred male immature rats. When working with animals, we were guided by the current ethical standards [7]. The study protocol was approved by the ethical committee of the Saint Luka Lugansk State Medical University (Protocol No. 1 dated 04/10/2019). To create a model of an immunostimulated state, we used Imunofan, a representative of the IV generation of thymic hormone derivatives. The drug was administered on days 1, 3, 5, 7, 9 of the experiment at a dosage of 50 μg. Rats receiving 0.9% sodium chloride solution in equivalent volumes according to the same scheme served as control. The animals were taken out from the experiment 1, 7, 15, 30 and 60 days after the cessation of drug administration. The organs were weighed, the relative mass was calculated, and the linear dimensions were determined: length, width, and thickness. The volumetric index was calculated using the formula for the volume of an ellipsoid of revolution:

$$V = \frac{\pi ABC}{6},$$

where A is length, B is width and C is thickness.

After standard histological examination, sections of the testes were stained with hematoxylin-eosin and photographed using an automated morphometric complex. At the light-optical level, the larger and smaller diameters, the area of the convoluted seminiferous tubule, the height and area of the epitheliospermatogenic layer were measured. The number of supporting cells and interstitial endocrinocytes was counted per unit area (1725 μm^2), as well as the volume of cell nuclei as an indicator of their functional activity. The spermatogenesis index (I) was calculated using the formula:

$$I = \frac{\Sigma A}{n},$$

where A is the number of rows of spermatogenic cells at different stages of development found in each tubule; n is the number of studied tubules.

The level of hormones in the plasma of peripheral blood, as well as the concentration of mediators of intercellular interaction IL-1β, IL-2, IL-6 and TNFα were determined by enzyme immunoassay. An enzymatic conjugate "Rat Elisa Kit", Elabscience (USA), with antibodies to follitropin, luteinizing hormone and testosterone was added to the studied blood sample, followed by calibration of the solution.

The data obtained were processed using a licensed statistical program StatSoft Statistica 12. The Mann-Whitney U-test was used to assess the statistical significance of the differences. Differences were recognized as statistically significant when the level of reliability (p) was more than 95.0% ($p < 0.05$), in other cases the differences were recognized as statistically insignificant ($p > 0.05$).

RESULTS

After the use of Imunofan, the dynamics of the levels of intercellular interaction mediators in the blood plasma of immature rats was found, indicating the development

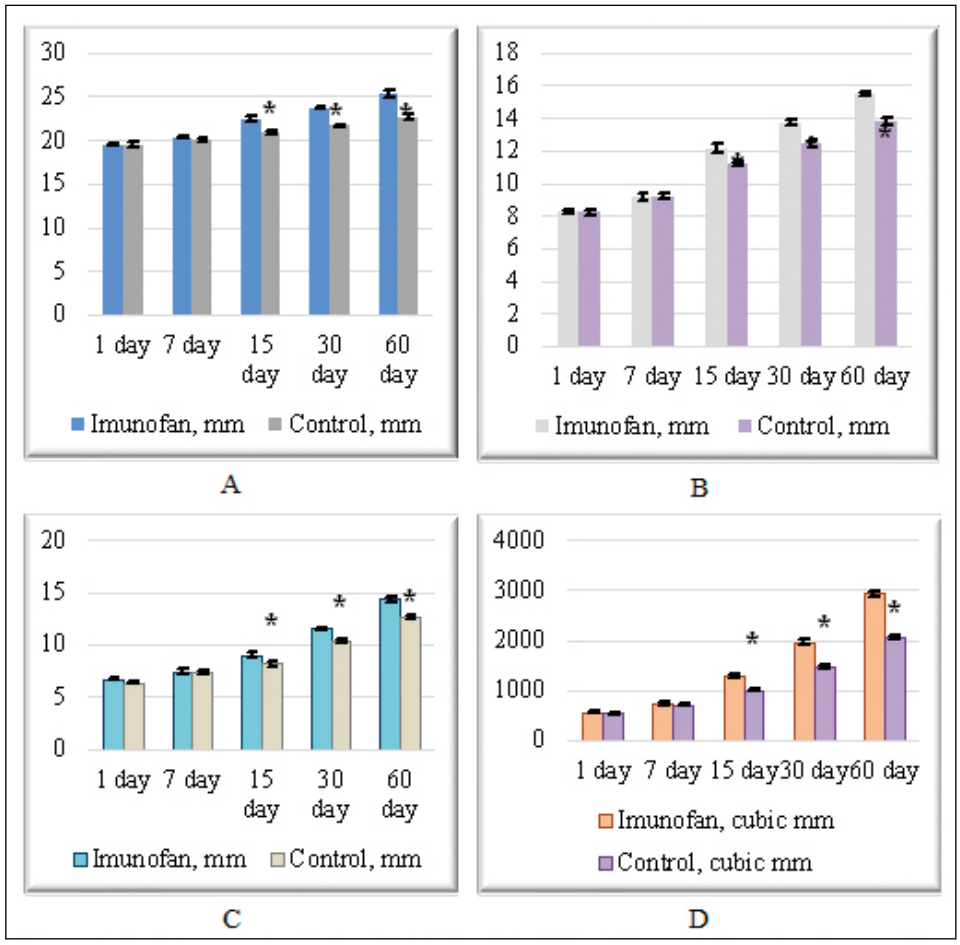

Fig. 2. Changes in the length (A), width (B), thickness (C) and volume (D) of the testes of immature rats after the use of Imunofan and in the control. * - significant difference from the control data ($p < 0.05$).

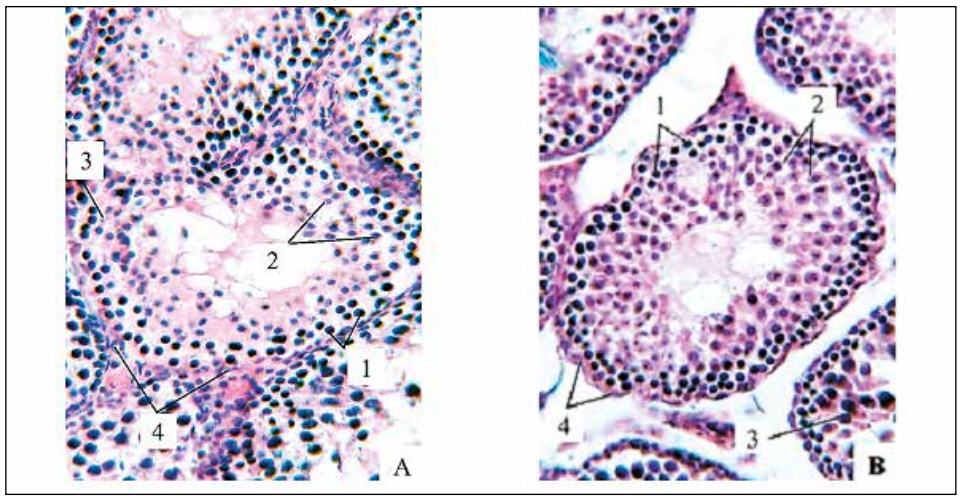

Fig. 3. Fragment of the testis of immature animals on the 30th day of observation: A - in the control; B - after using Imunofan. 1 - spermatogonia, 2 - spermatocytes, 3 - sustentocyte, 4 - tubule wall. Hematoxylin-eosin staining. Magnification: Approximation: Zoom 18.5; lens: PlanCN 40x / 0.65 ∞ / 0.17 / FN22.

of the state of immunostimulation throughout the entire observation period (Figure 1).

The general plan of the structure of the testes of immature animals after the administration of the drug retained typical morphological features: the gonads were covered with a thin connective tissue capsule, the convoluted seminiferous tubules, predominantly of a rounded shape, were densely packed in the lobules of the organ, on the histological section many did not have a lumen and were lined with rows of cells of the epitheliospermatogenic layer without presence of mature sperms.

At the same time, after the administration of Imunofan, changes in the absolute and relative weight of the testes of immature animals were revealed. Thus, a statistically significant increase in these indicators was observed on 15, 30 and 60 days by 9.38%, 10.13%, 11.00% and 5.94%, 6.07%, 6.83%, respectively. The linear-volumetric parameters of the gonads changed in the same way (Figure 2).

Administration of imunofan also caused changes in the micromorphometric parameters of the testes. Thus, the larger and smaller diameters of the convoluted seminiferous tubules increased statistically significantly on days 15,

30 and 60 of observation, the positive dynamics was 7.92%, 13.44%, 14.69% and 8.05%, 10.06%, 12.66% respectively.

The immunostimulating effect was also reflected in the sizes of the tubule and epitheliospermatogenic layer areas. The height of the germinal epithelium was statistically significantly higher than the control values in the same observation period by 10.68%, 11.75% and 16.07%, respectively. The spermatogenesis index, which reflects the functional activity of the testes, increased under conditions of immunostimulation. Thus, the indices of the control groups of immature animals after 7, 15, 30 and 60 days were statistically significantly lower than those of the rats receiving Imunofan, by 4.02%, 7.58%, 10.40% and 15.91%, respectively (Fig. 3).

When determining the number of sustentocytes and endocrinocytes, an increase was noted during immunostimulation in both indicators on the 15th, 30th and 60th days of observation in comparison with the data of the control groups of animals by 7.00%, 13.81%, 14.63% and 8.02%, 11.41%, 14.34% respectively. The volume of the nuclei of Sertoli and Leydig cells changed in a similar way. The positive dynamics was 5.19%, 7.33%, 10.27%, 14.51% and 4.68%, 9.70%, 12.00%, 14.29% at 7, 15, 30 and 60 days of immunostimulation.

The study of the parameters of the gonads of immature animals did not reveal statistically significant differences between the data of the experimental and control groups at the early stages of observation (1 and 7 days).

The immunostimulating effect, simulated by the introduction of Imunofan, led to moderately pronounced shifts in the hormonal parameters of the blood plasma of immature animals. Thus, an increase in testosterone concentration was found on the 15th, 30th and 60th days of observation after the end of the drug administration by 5.42%, 7.92% and 11.22%, respectively.

At the same time, there was a slight decrease in the level of luteinizing hormone by 4.85% (7 days), 7.31% (15 days), 7.63% (30 days) and 9.05% (60 days). On 15, 30 and 60 days after the cessation of the administration of Imunofan, a moderate decrease in the concentration of follitropin in the blood plasma was found by 7.43%, 8.75% and 9.77%, respectively.

DISCUSSION

It has been proven that immunosuppressive effects change the morphogenesis of the testes, which is accompanied by their dysfunction in the form of impaired spermatogenesis up to azoospermia [9, 10]. The results obtained indicate the development of readaptation processes in the testes after the use of the immunomodulator against the background of environmental immunosuppression, which is reflected in the prevalence of the morphometric parameters of the studied organ over the data of the control groups of animals. So, J. Gold and V. Vardhani in studies on mice proved an increase in the level of testicular DNA after the use of an immunostimulant [11]. This can be explained by the properties of imunofan to increase the resistance of the genetic material of cells to unfavorable exogenous influences. The ability of the drug to stimulate the production of cytokines normalizes the function of immunocompetent cells, which is manifested in the stabilization of the immune homeostasis of the testes. At the same time, the long-acting phase of Imunofan is accompanied by an intense antioxidant and detoxifying effect, which prevents damage to actively dividing gonadal cells. The development of such changes, apparently, is associated with the normalization of metabolic processes in the body of animals, including restoration of the balance of redox reactions, optimization of anabolic and catabolic processes, and adaptation of regulatory systems to disorganizing exogenous influences [12]. Optimization of the parameters of the antioxidant system and cell lipid peroxidation, as well as the membrane stabilizing effect resulting from prolonged use of Imunofan, stimulated the activation of proliferative and synthetic processes, which was manifested by an increase in the index of spermatogenesis, as well as linear-volumetric and micromorphometric parameters of the testes. Research conducted by L.M. Yaremenko et al. proved that the systemic use of Imunofan led to the normalization of the expression of neuronal cytoskeleton proteins and an increase in the amount of mRNA encoding them [13]. A similar effect can take place among germ cells of the reproductive system, mediating an increase in the stability of mitotic and intracellular transport processes. At the same time, the dynamics of the hormonal parameters of blood plasma indicates the active involvement of systemic endocrine mechanisms in the regulation of testes morphogenesis during immunostimulation at a later follow-up period. Changes in the concentration of the above regulators were reflected both in the intensity of the processes of differentiation of germ cells and in the activity of sustentocytes and interstitial endocrinocytes. The effect of the drug on the levels of cytokines in blood plasma is associated with its effect on the morphogenesis of the organs of the reproductive system, which is determined by the property of imunofan to change the formation and concentration in the cytoplasm of immunocompetent cells of secondary messengers: cAMP, inositol-1,4,5-triphosphate, diacylglycerol, calcium ions and nitric oxide (II) with subsequent signal transmission to various inductors. Imunofan is able to stimulate the synthesis of group E prostaglandins, the action of which is probably realized through cGMP. Also, cytokines acting on sustentocytes regulate the formation of integral proteins in their plasmolemma that control endocytosis processes using clathrin-mediated mechanisms, which optimizes the activity of the blood-testis barrier.

CONCLUSIONS

1. In response to the immunostimulating effect of Imunofan, there is a pronounced reaction of the testes of immature animals, which is due to the sensitivity of morphogenetic processes in the organ to external influences and the formation of mechanisms of their regulation, characteristic of this period of ontogenesis.

2. The most intense immunostimulating, membrane stabilizing and detoxifying effect of Imunofan was observed on the 15th, 30th and 60th days of observation, which was confirmed by the statistically significant dynamics of morphometric parameters of the organ.
3. Micromorphometric data of the testes supplement the results obtained at the macroscopic level at the later stages of observation after the use of Imunofan and indicate the full participation of all structural components of the organ in its response to immunostimulation.
4. The established dynamics of the hormonal parameters of the blood plasma of animals suggests the development of a systemic endocrine reaction in response to the use of imunofan, which indicates a close integration of the immune and humoral mechanisms of regulation of the morphogenesis of testes at immature age.
5. The data obtained allow us to conclude about the positive effect of immunostimulation on morphogenetic processes in the testes in childhood, which must be considered when carrying out the immunotropic therapeutic and prophylactic measures in practical pediatric health care.

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ORCID and contributionship:

Alexey A. Zakharov: 0000-0002-7377-2891 [A-C,]
Svetlana A. Kashchenko: 0000-0001-6793-1975 [E,F]
Inessa V. Bobrysheva: 0000-0003-3087-1600 [C,F]
Sergey N. Semenchuk: 0000-0002-4308-3382 [B]

Conflict of interest:

The Authors declare no conflict of interest.

CORRESPONDING AUTHOR

Alexey A. Zakharov
Saint Luka Lugansk State Medical University
50 years of Lugansk Defense bl., 291045 Lugansk, Ukraine
tel: +380509678705
e-mail: masterhist@mail.ru

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