



MORPHOFUNCTIONAL CHARACTERISTICS OF CELLULAR ELEMENTS OF RAT TESTES UNDER THE INFLUENCE OF INSECTICIDE ROVIKURT

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Abstract: The purpose of the study was to study the characteristics of the response of germ and somatic cells to the action of rovicurt, as well as to study some aspects of the mechanism of this action.

The research materials were the new insecticide Rovicurt, produced by the company “Hinoi” (Hungary) in the form of a 20% emulsion solution, proposed as a systemic acaricide. It mixes well with most organic solvents and consists of 23% Ambush (permethrin) and 2% tetramethrin ($C_{21}H_{20}Cl_2O_3 + C_{17}H_{25}O_4$).

The experiment used white male and female rats weighing 150-200 g. To study this process, male rats (132 pcs.) were taken, subjected to a single action of rovicurt in doses of 380, 150, 75 mg/kg, as well as control animals. The studies were carried out on days 1, 3, 7, 14, 21 and 70 after exposure. And with repeated administration - doses of 38, 19 mg/kg for 2.5 months, as well as control animals.

Research methods. Samples of testicular tissue were fixed in Carnoy's fluid and 2.5% glutaraldehyde solution, followed by additional fixation with 1% osmium tetroxide solution, dehydrated in alcohols of increasing strength, embedded in paraffin and in a mixture of epons. Paraffin sections were stained with hematoxylin-eosin, semi-thin sections prepared on an LKB-4800 ultratome were stained with methylene blue.

Results. When exposed to rovicurt at a dose of 380 mg/kg, the proliferation of testicular stem cells (spermatogonia) is disrupted. This process is based on degenerative changes. Different populations of germ cells react differently to a single action of rovicurt (at doses of 75 and 159 mg/kg):

<p>CC License CC-BY-NC-SA 4.0</p>	<p>differentiation of early and middle spermatids is suppressed, while late spermatids are activated. When exposed to pesticides in doses of 75, 150 and 380 mg/kg, damage to the genome of spermatogenic epithelial cells is observed.</p> <p>Conclusion. With acute and chronic action of pyrethroids, a change in the fine structure of the synaptonemal complex occurs, a delay in the differentiation of spermatocytes and spermatids, the appearance of multinucleated cells, as well as structural changes in sustentocytes and specialized connections between neighboring sustentocytes and germ cells.</p> <p>Key words: rovicurt, synaptonemal complex, spermatid, sustentocyte</p>
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One of the pressing problems facing humanity in connection with scientific and technological progress is environmental pollution. This has brought forward urgent challenges to control pollution and to develop scientific directions that will allow assessing the impact of these processes on humans. Currently, over one million chemicals and pesticides are used annually in the world, and there are more than 900 varieties of them. Their use allows obtaining additional agricultural products.

However, with the sharp increase in industry and the identification of agriculture, a huge number of chemical substances that humans and animals did not encounter in their evolutionary development turned this chemical pressure on the environment into a new environmental factor (Dubinin N.P.).

As the scale of use of chemicals, in particular pesticides, increases, the population that has direct contact with them in production and field conditions also grows. As the work of many researchers has shown, pesticides can contribute to the development of various pathological processes, in particular lead to acute and chronic poisoning, and have mutagenic, teratogenic, allergenic, carcinogenic and other effects. (Skulachev V.I., Kogan Yu.S., Mirakhmedov A.K.)

For the human population, an increase in mutagenic factors in the environment is extremely undesirable, since they create a real basis for an increase in genetic load, primarily a change in the rate of the mutation process (Bochkov N.P.).

Testing newly created chemical compounds on experimental models is aimed at finding ways to prevent or reduce human contact with those that cause negative consequences. At the same time, it is of no small importance to clarify the mechanisms of their effect on the human and animal body of this group of chemicals. This is a problem concerning the general biological patterns of interaction of the body with chemicals - one of the most promising in the development of preventive medicine in general.

Literary data indicate that studying the state of the reproductive system under the influence of pesticides is advisable, since this system is most sensitive to the action of chemicals and primarily suffers from their effects. (Zhuravleva R. A., Gvazova E. A., Gromysz Kalkowska, etc.).

The results of studies on the effect of the pesticide Rovicurt on reproductive systems are contradictory and far from complete; the issue of its effect on spermatogenesis has not been studied. Meanwhile, only knowledge of subtle disorders of proliferation, differentiation and metabolism in cells can fully explain the mechanism of negative reactions of the body to the effects of chemicals, as well as determine the chain of compensatory and adaptive processes.

The purpose of our study was to study the characteristics of the response of germ and somatic cells to the action of rovicurt, as well as to study some aspects of the mechanism of this action. In accordance with the purpose of the study, the following tasks were set:

1. To study the features of proliferation, differentiation and functional structure of rat testicular cells under the influence of various doses of rovicurt.
2. To study the mutagenic activity of different doses of rovicurt in the germ and somatic cells of rats.

In implementing the assigned tasks, the nature of the peculiarities of the action of rovicurt on the state of differentiation of cellular elements and spermatogenic cells was studied for the first time, which made it possible to identify the mechanism of action of this drug on the reproductive system. The delay in the development of early and middle spermatids under the influence of rovicurt at doses of 75 and 150 mg/kg and the reduction in the period of differentiation of late spermatids under the influence of all studied doses indicate that these types of cells are sensitive to the effects, and this pathology leads to disruption of the reproductive system and causes an increase in antifertility effect.

A decrease in the number of gonias was established, which indicates the specificity of the response of rapidly renewing stem cells to the impact. The new data obtained indicate that these cells, which have the highest proliferative activity, turned out to be the most vulnerable among the elements of the spermatogenic epithelium under the action of rovicurt.

There was damage to differentiated cells and a violation of the integrity of the elements that make up the structure of the hemotesticular barrier, which serves for genetic and immunological isolation of developing germ cells from the changing internal environment of the body.

For the first time, the effect of rovicurt on oxidative phosphorylation in mitochondria of spermatogenic cells was studied, and a pronounced change in the functional state of the testes and the development of low-energy changes in them were noted, especially at a dose of 380 mg/kg. Which underlie the disruption of a number of functions of the cells of this organ.

The conducted studies provide the possibility of early diagnosis, targeted treatment measures for poisoning with synthetic pyrethroids, in particular Rovicurt, as well as the development of preventive measures for environmental protection, in addition, the research makes it possible to clarify the role of this drug in the formation of certain diseases that are common in the republic and changes in genetic load in the population.

The results of individual effects of rovicurt are interesting not only for practical purposes, but also for theoretical medicine. These data are extremely necessary in embryology, since the obtained materials contribute to revealing the role of germ cells with a damaged genome in the formation of developmental defects. The established features of the development of the pathological process in the elements of the spermatogenic epithelium can serve as a morphological basis for understanding the functional state of the studied organs during pesticide poisoning.

Materials and methods of research. The research materials were the new insecticide Rovicurt, produced by the company "Hinoi" (Hungary) in the form of a 20% emulsion solution, proposed as a systemic acaricide. It mixes well with most organic solvents and consists of 23% Ambush (permethrin) and 2% tetramethrin ($C_{21}H_{20}Cl_2O_3 + C_{17}H_{25}O_4$).

White male rats weighing 150-200g were used in the experiment. To solve the problems, 2 series of experiments were carried out.

Series 1 – comprehensive assessment of the state of spermatogenesis. To study this process, male rats (132 pcs.) were taken, subjected to a single action of rovicurt in doses of 380, 150, 75 mg/kg, as well as control animals. The studies were carried out on days 1, 3, 7, 14, 21 and 70 after exposure. And with repeated administration - doses of 38, 19 mg/kg for 2.5 months, as well as control animals.

Series 2 - we studied the mutation process, which is divided into two main groups: mutagenesis in germ cells and in somatic cells. Mutagenesis in male germ cells was studied after a single (dose 380, 150, 75 mg/kg) and after 2.5 months (dose 38, 19 mg/kg) administration of rovicurt, fixation periods 7-10 days, 14-20 days, 28-40 days and 70 days. To determine mutagenic activity in somatic cells, rovicurt was administered in doses of 380, 150, 75 mg/kg. 48 males were used.

Rovicurt was administered intragastrically using a tube. The animals were decapitated, testes and sperm were taken. During autopsy of females, the number of corpora lutea in the ovaries, live and dead embryos was determined. The choice of doses was made on the basis of methodological recommendations for testing the mutagenic properties of new drugs (Babayan E. et al.).

Samples of testicular tissue were fixed in Carnoy's fluid and 2.5% glutaraldehyde solution, followed by additional fixation with 1% osmium tetroxide solution, dehydrated in alcohols of increasing strength, embedded in paraffin and in a mixture of epons. Paraffin sections were stained with hematoxylin-eosin, semi-thin sections prepared on an LKB-4800 ultratome were stained with methylene blue. Ultrathin sections were also prepared on an LKB-4800 microtome and stained

according to the generally accepted method (Z. A. Butenko et al.). The ultrastructure was studied using an electron microscope at an accelerating voltage of 100 kV. cellular association was determined based on the classification proposed by C.P. Leblond.

In males, the mass of one (right) of the testes was determined, as well as the ratio of this mass to body weight. The thickness of the layer of spermatogenic epithelium, the diameter of the seminiferous tubules, the average number of spermatogonia and sustentocytes, as well as the spermatocytogram were determined using the formulas proposed by Yu. I. Ukhov et al.

The study of the induction of dominant lethal mutations (DLM) in the germ cells of males was carried out by taking into account dominant lethal mutations that cause hereditary changes in the reproductive cells of males after exposure, leading to the death of the first generation offspring during embryonic development (A. M. Mazheshenko). A single administration was carried out to determine the sensitivity of sequentially all stages of spermatogenesis; after 2.5 months of exposure, the integral effect of small doses of the drug on all spermatogenesis was determined. The change in embryonic mortality reflects sensitivity sequentially: 1 week - mature sperm; 2 – late spermatids; 3 - medium spermatids; 4 – early spermatids and spermatocytes; 8 – spermatogonia.

To detect mutagenic activity in mature germ cells of males, methods for recording abnormal sperm heads (ASH) were used (R. K. Lekyavichyuz). For each dose, 200 sperm were used, a total of 12,800 were examined.

The functional state of spermatozoa obtained from the caudal part of the epididymis of control and experimental animals was assessed according to the method of V.K. Milovanov and G.I. Egorova. The number of spermatozoa was determined by taking their suspension into a melangeur, followed by counting in the Goryaev chamber. Saline solution was used as a liquid to dilute the suspension.

Cytogenetic studies were carried out on males. Preparation of drugs was carried out according to the Ford method (C.K. Ford). For each dose, 600 metaphases obtained from 6 animals were studied.

Analysis of the data obtained after a single intragastric administration of Rovicurt in doses of 380 and 150 mg/kg showed that on the first day of the study (24 hours) changes in the microcirculatory system were noted, which were expressed in the form of several phenomenal phenomena. The first phenomenon is diapedesis of erythrocytes from microvessels, leading to microhemorrhages into the interstitial space. Another phenomenon that occurred 24 hours after administration of these doses (380, 150 mg/kg) was swelling of the capillary walls. It was expressed in thickening and blurring of the contours of the walls. This phenomenon indicates an increase in the permeability of microvascular walls and is reversible. The swelling of the nuclei of individual endothelial cells that make up the walls of microvessels indirectly indicates the swelling of the entire endothelial cell, which leads to an increase in vascular permeability. These changes in microcirculation make it possible to activate

diapedesis of elements of the lymphohistiocytic series, which promotes activation of their function in the extravascular space, which is important for accelerating reparative processes in the testes. The penetration of these elements into the inside of the seminiferous tubules and their location between the germ cells leads to a change in the microenvironment that exists among the evolutionarily established association of cells.

The biological significance of enhancing the isolation of germ cells from the internal environment of the body through the compartmentation of the testes is that the isolation of meiocytes is important by creating a specific microenvironment for the implementation of meiosis (S. S. Raitsina). The blood-testis barrier creates a special physiological environment in the peri-cavitary compartment, different from that existing outside it. The formation of the blood-testis barrier in the ontogenesis of mammals coincides in time with the appearance of seminiferous tubules of germ cells entering meiosis. The blood-testis barrier is characterized by a high strength of the morphological substrate and controls spermatogenesis. Our studies revealed a change in integrity in the elements of the blood-testis barrier; these changes include, firstly, a violation of the microcirculatory system, the lack of parallelism of the layers of the basement membrane, its loosening, disruption of the structure of myoid cells and their tight connection with each other (at a dose of 380 mg/ kg 14, 21 days); secondly, a violation of the structure of specialized connections between neighboring sustentocytes, which are generally considered to be the most effective components of the blood-testis barrier, which led to the lack of genetic protection of germ cells entering meiosis, resulting in the formation of mutations that we discovered by taking into account dominant lethal mutations. Dominant lethal mutations were caused by doses of 150 and 75 mg/kg in spermatocytes and spermatids of various degrees of maturity (2-4 weeks). The absence of a mutagenic effect in early spermatids and spermatocytes (week 4) at a dose of 380 mg/kg is a consequence of massive cell death at these stages, which led to such a seemingly paradoxical effect of a high dose compared to a lower dose (150 mg/kg). Consequently, the most sensitive cells to the effects of rovicurt were the cells that begin and carry out differentiation, and therefore are the most vulnerable. Stem cells are the least active and turned out to be resistant to the effects of the studied doses of rovicurt.

The decrease in spermatogonia we observed when exposed to rovicurt (380-150 mg/kg after 7 days) was due to degeneration, as well as as a result of impaired mitotic divisions (Table 1).

Table 1. Quantitative analysis of spermatogenic epithelium in the testes of rats exposed to a single dose of different doses of Rovicurt.

Indicators	Control M ±	380 mg/kg M ±	150 mg/kg M ±	75 mg/kg M ±
Average tubule diameter	198±2,3	200±0,7	223±2,9**	206±2,1*
Spermatogonia	16,4±0,1	9±0,9**	11±1,0**	12±0,3**

Sertoli cells	22,3±0,8	24±0,5	23±0,3	24±0,6*
Spermatids and spermatocytes	347±4,5	334±23,7	331±1,9*	329±0,1*
Sertoli cell index	24,8±0,5	19,8±1,6*	21,5±0,4*	20,6±0,5*

Note: * P<0.05, ** P<0.001

In sustentocytes, the following was observed: a decrease in the elements of the granular cytoplasmic reticulum and the number of free polysomes, which leads to pronounced changes in the protein-synthesizing components; expansion and segmentation of the cisterns of the smooth cytoplasmic reticulum, elements that are the specific structure through which androgens reach developing germ cells.

As a result of exposure to rovicurt, we discovered a sharp degeneration of cellular organelles, sustentocytes, expansion of cisterns and tubules, agranular cytoplasmic reticulum, and the appearance of a large number of large vacuoles. The number of lysosomes, phagosomes, and various types of inclusions sharply increased: lipid droplets, residual bodies, remnants of phagocytosed sperm. In the area of contacts between neighboring sustentocytes, compaction of the cytoplasmic membranes, moderate expansion of the submembrane cisterns of the cytoplasmic reticulum, and a decrease in the number of filaments until complete disappearance are detected.

The description of changes in the morphofunctional state of sustentocytes is morphological indicators of a violation of the most important functions of the blood-testis barrier, creating a specific microenvironment of cells and providing their immune protection.

Electron macroscopic studies conducted by us made it possible to detect a disturbance in the structure of the synaptonemal complex (SC) in spermatocytes on the 56th day after exposure, which, apparently, may be the result of the formation of dominant lethal mutations, which subsequently manifest themselves in the offspring of treated males.

In spermatogenesis, during the complex differentiation of germ cells, the formation of sperm and spermatids occurs. Our quantitative analysis of the frequency of occurrence of various stages of the spermatogenic epithelium cycle showed a delay in the development of early and middle spermatids with a single exposure to doses of 75 and 150 mg/kg. All studied doses of Rovicurt act as differentiation activators on late spermatids. Probably, these changes occurred due to a decrease in the number of proliferating precursor cells with a decrease in the number of proliferating cells; the duration of stay of early spermatids in the profiling pool increases, while for late spermatids the transit time is reduced due to more rapid passage of the later stages of proliferation. It is assumed that the factor limiting cell reproduction is the state of the cytoskeleton. The changes we discovered in the cytoskeleton of sustentocytes led to a number of membrane and intracellular changes. Damage to the cytoskeleton also causes degeneration of the entire pool of germ cells and disrupts the normal course of spermatogenesis, which is reflected in a decrease in the number of spermatogonia and the number of all germ cells.

The morphological changes we discovered, such as damage to the nuclei of spermatids with a normal configuration of chromatid bodies, disruption of the structure of the plastic complex from which the acrosome is formed, make it possible to assume the effect of rovicurt on the synthesis of RNA and protein, which leads to a lack of materials necessary for the formation of acrosomes. These changes in the structure of spermatids led to asynchronization of differentiation of the intracellular association of germ cells.

The appearance of multinucleated spermatids 5-6 days after exposure to a dose of 380 mg/kg also indicates a delay in sperm formation.

The results obtained made it possible to establish that all cells of the seminiferous tubules have different sensitivity to the action of rovicurt. The most sensitive are type "B" and intermediate spermatogonia, and the most resistant to the effects of this drug are type A0 spermatogonia.

Based on the studies conducted, a change in the fine structure of the synaptonemal complex, a delay in the differentiation of spermatocytes and spermatids, the appearance of multinucleated cells, as well as structural changes in sustentocytes and specialized connections between neighboring sustentocytes and germ cells were discovered. They revealed the absence of genetic protection of differentiating germ cells, there is a way in which the formation of atypical sperm is carried out, and their number in the sperm is determined by the degree of infertility of males.

Conclusion

1. When exposed to rovicurt at a dose of 380 mg/kg, the proliferation of testicular stem cells (spermatogonia) is disrupted. This process is based on degenerative changes.
2. Different populations of germ cells react differently to a single action of rovicurt (at doses of 75 and 159 mg/kg): differentiation of early and middle spermatids is suppressed, while late spermatids are activated.
3. When exposed to pesticides in doses of 75, 150 and 380 mg/kg, damage to the genome of spermatogenic epithelial cells is observed.
4. Atypical sperm are formed as a result of changes in sustentocytes and specialized connections between neighboring sustentocytes and germ cells, as well as the appearance of multinucleated cells and disruption of the synaptonemal complex.

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