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ADAPTIVE AND COMPENSATORY RESPONSES IN RATS AT THE EARLY STAGES OF AN ACUTE INTOXICATION WITH DELTAMETHRIN

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Abstract

The world market of synthetic pyrethroids is estimated more than at 2.5 bn. dollars and will grow in the next years. Numerous works of domestic and foreign authors are devoted to toxicology of pyrethroids, however the issues related to pathogenesis of an acute poisoning of animals with pesticides of that group as well as the principles of laboratory diagnostics at an early stage of intoxication and pathogenetic therapy at poisoning warrant further investigations. For many years deltamethrin has been successfully used in plant growing as well as in animal husbandry creating a poisoning hazard in case of violation of treatment regulations. Morbidity and clinical outcome depend largely on severity of functional endocrine and immune systems disorders. The purpose of the study was to determine dynamics of adaptive and compensatory reactions in animals at an early stage of an acute intoxication with deltamethrin. In experiment white laboratory rats as an established mammal model in biomedical research were used. The experiment was performed on male rats (weight of 180-200 gr) arranged in 6 groups (of 10-12 rats each). Animals from groups II, IV and VI have been subjected to an acute peroral intoxication with deltamethrin (Butox 50, Intervet, Netherlands) in a dose of 43.5 mg/kg of body weight. Rats of groups I, III and V served as control. Rats from different groups were put out of the experiment sequentially: I and II — in a day; III and IV — in three days; V and VI — in a week after the beginning of the experiment. Glucose content was estimated in whole blood of rats, and concentration of insulin and corticosterone was assayed in blood serum. Pieces of animal timus were fixed in 4 % neutral formaldehyde, dehydrated in alcohols with the increasing concentration and embedded into paraffin. Histologic sections at 3-5 microns in thickness were made with the rotational microtome and stained with haematoxylin and eosin, and also according to Van-Giezon. For identification of mast cells the histologic sections were stained with Bismarck brown to Shubich. Increase in blood corticosterone, insulin and glucose levels by 46.3 ($p = 0.0001$), 31.9 ($p = 0.0139$) and 25.6 % ($p = 0.0052$), respectively, was found in a day after poisoning with deltamethrin. On day 3 after poisoning the blood concentration of corticosterone and glucose in rats remained high along with a decrease in insulin content. Hypocorticosteronemia and hypoglycemia were observed on day 7 after poisoning, with the insulin level close to control values. Corticosterone and glucose content has been reduced by 17.3 ($p = 0.0407$) and 19.8 % ($p = 0.0267$), respectively, compared to control. Photomicrographs showed a reduction in the number of thymocytes, activation of apoptosis, an increase in quantity of mastocytes along with an intensification of degranulation as well as development of haemodynamic disorders in thymus when poisoning rats. In a week after intoxication the thymocytes level was partially restored. No animal died during experiment which evidences the efficiency of adaptation and compensatory mechanisms in experimental rats, including hypothalamic-pituitary thymic system. Results of our researches make a contribution to understanding compensatory and adaptive mechanisms under experimental intoxication, and allow estimating functional capabilities of body systems when developing pharmacological correction at poisonings.

Keywords: pesticides, deltamethrin, corticosterone, insulin, glucose, thymus, rats, intoxication

The global market for synthetic pyrethroids is estimated at more than

$ 2.5 billion. In the coming years it will grow and will exceed $ 3 billion by 2019 [1]. The products of this group have high biological activity allowing to effectively combat the insects at various stages of their development. CN-containing substances are more toxic for warm-blooded animals [2, 3].

Deltamethrin — (S)-α-cyano-3-phenoxybenzyl(1R,3R)-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropanecarboxylate — has been successfully used in plant growing and animal husbandry for many years. This synthetic pyrethroid monoizomer of significant toxicity poses a real threat of intoxication when application standards violated. Despite numerous studies on pyrethroid toxicology [4-6], the pathogenesis of acute toxicity due to these products, the principles of laboratory diagnosis and therapy in the early stages of intoxication require in-depth study.

The development of the pathological process in exogenous intoxication is followed by the changes in the interrelation of adaptation and compensatory mechanisms. The severity and outcome of intoxication are largely determined by the severity of functional endocrine and immune system disorders [7, 8]. The early identification helps to improve the diagnosis and prevent complications.

In this study, we performed biochemical and histological estimation of the effects of acute experimental deltamethrin intoxication on the endocrine and immune systems in rats. These findings contribute to the understanding of adaptive mechanisms and functional reserves of the organism in mammals with pesticide toxicosis, which is necessary to develop the methods of appropriate diagnosis and pharmacological correction.

Our purpose was to study the dynamics of adaptive and compensatory reactions in the early stage of acute deltamethrin intoxication.

Technique. White laboratory Wistar rats that are considered the reference objects in numerous biomedical studies on mammals were used in the experiments. Male rats (weight of 180-200 g) were divided into 6 groups. Deltamethrin at a dose of 43.5 mg/kg body weight ($^1/_2$ LD_{50}) was administered in the stomach of group II (n = 12), group IV (n = 12), and group VI (n = 12) animals by gavage. In group I (n = 10), group III (n = 10), and group V (n = 10) (control) rats Deltamethrin was replaced by equivalent amounts of saline. Rats of different groups were withdrawn from the experiment sequentially: I and II — in 1 day, III and IV — in 3 days, V and VI — 1 week after the start of the experiments. Blood was sampled in the same periods.

The Butox 50 deltamethrin formulation (Intervet, Netherlands) was used. The experiments were conducted in compliance with the European Convention for the Protection of Vertebrate Animals Used for Experimentation and other Scientific Purposes № 123 (Strasbourg, March 18, 1986).

Glucose levels were assayed in rat whole blood by standardized glucose oxidase method, insulin and corticosterone concentrations were measured in the serum. Insulin was measured in ELISA test by "sandwich" method, corticosterone content — by direct competitive ELISA with solid-phase immobilized antibodies. Commercial reagent kits manufactured by Cusabio Biotech Co., Ltd (China) were used for enzyme immunoassay.

Fragments of thymus were fixed in 4 % neutral formaldehyde solution, dehydrated through increasing concentrations of alcohol and embedded into paraffin. Histological sections 3.5 μm thick were prepared using a rotary microtome (CUT 4055, SLEE Medical GmbH, Germany) and stained with hematoxylin and eosin, and Van Gieson's stain. To detect mast cells, sections were stained by basic brown according to Shubich [10]. Photomicrography was performed using a digital microscope Altami BIO 1 (Altami, Russia).

The data were processed using Statistica 6.0 software (StatSoft, Inc., USA). Results are presented as the median (Me) and interquartile range (Q_1-Q_3). Inter-

group comparisons were performed using the nonparametric Mann-Whitney U-test. The differences were considered statistically significant at $p < 0.05$.

Results. After single deltamethrin doses of 43.5 mg/kg body weight, excitement and hypersalivation were observed in the animals from the experimental groups. On day 1 of the experiment the animals lost appetite, and water use reduced water sharply. Subsequently, the severity of clinical manifestations of toxicity decreased.

Biochemical studies showed hypercorticosteroidemia in the early stages of intoxication. A day after deltamethrin administration, blood corticosterone concentration in rats was 46.3 % higher than in the control group (Table). Gradually, the hormone level decreased, and in 3 days, differences to control were 30.9 %. Increased corticosterone secretion within 3 days after intoxication could be due to the neurotoxic effects of the drug. The hypothalamus responds to chemical stress by corticotropin releasing hormone which contributes to enhanced synthesis and secretion of adrenocorticotropic hormone by the anterior pituitary [11]. This increases adrenal corticosterone level due to 3-β-hydroxysteroid dehydrogenase activation [12] which results in the development of hypercorticosteroidemia and carbohydrate metabolism disturbance.

Blood parameters in white laboratory Wistar rats with acute deltamethrin intoxication

Period	Group	Corticosterone, nmol/l	Insulin, nMU/ml	Glucose, mmol/l
Day 1	I (control)	417 (378-500)	457 (394-565)	6.73 (6.02-7.62)
	II	610 (577-711)	603 (486-700)	8.45 (7.57-10.8)
		$p_{[k1]} = 0.0001$	$p_{[k1]} = 0.0139$	$p_{[k1]} = 0.0052$
Day 3	III (control)	395 (366-470)	484 (390-563)	6.35 (5.94-7.81)
		$p_{[k1]} = 0.6683$	$p_{[k1]} = 0.8429$	$p_{[k1]} = 0.8695$
	IV	517 (455-621)	329 (257-412)	8.56 (7.22-10.3)
		$p_{[k3]} = 0.0130$	$p_{[k3]} = 0.0048$	$p_{[k3]}=0.0412$
		$p_{[d1]} = 0.0612$	$p_{[d1]} = 0.0001$	$p_{[d1]}=0.6031$
Day 7	V (control)	428 (368-502)	471 (375-571)	6.40 (5.96-7.81)
		$p_{[k1]} = 0.9741$	$p_{[k1]} = 0.9604$	$p_{[k1]}=0.9548$
		$p_{[k3]} = 0.7053$	$p_{[k3]} = 0.6792$	$p_{[k3]}=0.8201$
	VI	354 (288-410)	389 (294-468)	5.13 (4.29-6.30)
		$p_{[k7]} = 0.0407$	$p_{[k7]} = 0.1209$	$p_{[k7]} = 0.0267$
		$p_{[d1]} = 0.0002$	$p_{[d1]} = 0.0001$	$p_{[d1]} = 0.0001$
		$p_{[d3]} = 0.0001$	$p_{[d3]} = 0.1574$	$p_{[d3]} = 0.0001$

N o t e. Description of the groups is given in the *Technique* section. Me(Q_1-Q_3) values are provided, where Me is median, Q_1-Q_3 is interquartile range; $p_{[k1]}$ — significance level of the differences versus control after 24 hours, $p_{[k3]}$ — versus control after 3 days, $p_{[k7]}$ — versus control after 7 days, $p_{[d1]}$ — compared to the II experimental group rats, $p_{[d3]}$ — compared to the IV experimental group rats.

Blood glucose concentration in rats a day after deltamethrin administration was 25.6 % higher versus control. Insulin level increased by 31.9 %, indicating transient insulin resistance, which could be due to high lipid availability for oxidation.

Glucose is not among the main substrates used by mitochondria for the oxidation, unlike ketone bodies, short- and long-chain fatty acids [13]. Hyperglycemia persisted 3 days after intoxication (see Table) due to gluconeogenesis stimulation by glucocorticoids and splitting of glycogen pool in the liver, as well as reducing glycolysis intensity [14]. However, insulin content decreased by 32.0 % versus control. pancreatic Langerhans islet β cells could be the reason for a decrease in blood insulin concentration over the background of persistent hyperglycemia. A β-cell dysfunction was probably the result of several factors. The first factor was hyperlipidemia caused by an increase in blood corticosterone level in rats after deltamethrin administration. The high content of fatty acids in blood contributes to their accumulation by β-cells, which results in increased free radical processes, primarily through NO synthase activation, which result in cell death by apoptosis [15]. Hyperglycemia was the second factor. The mechanisms of its adverse effects are well understood. These are overproduction of free radicals, glycation processes and changes in regulatory proteins [16). Furthermore,

hyperglycemia may reduce the secretory function of β-cells [17].

A week after single deltamethrin administration, hypocorticosteroidemia developed (see Table). This indicated a reduction in corticotropin releasing hormone and adrenocorticotropic hormone secretion by central endocrine glands, and also could be due to a deficiency of reduced nicotinamide adenine dinucleotide phosphate and ascorbate registered with the activation of free radical processes and lipid peroxidation in the adrenal glands.

A decrease in the activity of 3-β-hydroxysteroid dehydrogenase in the adrenal cortex is of great importance [18]. This enzyme is sensitive to free radical processes activation and an increase in the lipid peroxidation products [19]. Previously, we and other researchers have noted the development of oxidative stress in rats with acute deltamethrin intoxication [20-24].

With the development of hypocorticosteroidemia, a 19.8 % decrease in blood glucose level as compared to control was observed, which was associated with deceleration of gluconeogenesis reactions at glycolysis activation with possible depletion of glycogen reserves in the liver [25]. Hyperglycemia transiency contributed to the normalization of insulin secretion (see Table).

The interrelations of the immune and endocrine systems results not only in the direct involvement of the immune system in stress reactions, but also determines the effects of stress on the immune reactivity. In this regard, we performed a histological study of the central organ of the immune system — the thymus — in the control and experimental rats.

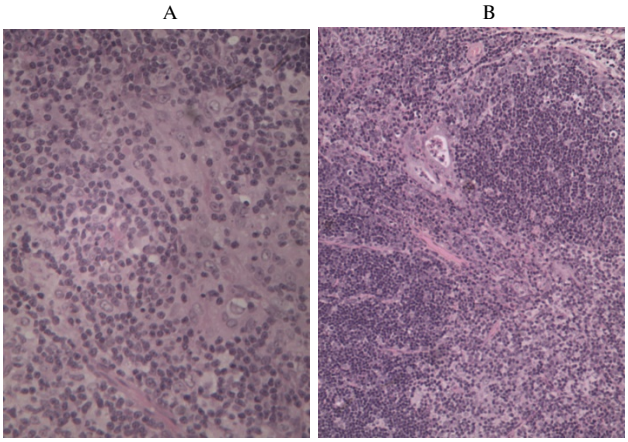
Histological structure of the thymus in the control rats was represented by individual lobes separated by connective tissue septa, capsulated by unformed fibrous connective tissue. The cortex and medulla zones separated by a clearly visible boundary were well differentiated in each lobe. Thymocytes, lymphoblasts, and epithelial cells were clearly visible in the cortex; the density of cellular elements was lower in the medulla, thymus vascular network was moderately filled with blood.

Increased secretion of hypothalamic-pituitary-adrenocortical system hormones at acute intoxication reduced the number of lymphoid cells in the thymus. Three days after deltamethrin administration, a marked decrease in the number of thymocytes in the lobes was observed in histological sections (Fig., A) with cortex and medulla inversion making it difficult to visually define their borders. This was typical of all thymus lobes and registered in all the animals of the experimental groups within 3 days after intoxication, the only difference consisting in the severity of these changes. The reduction in the number of thymocytes was followed by more intensive apoptosis in the organ, fragmentation of cell nuclei and formation of apoptotic bodies. The development of hemodynamic disorders was indicated by the accumulation of blood cells in the blood vessel lumen. Deltamethrin intoxication was followed by an increase in the number of mast cells located in the interlobular connective tissue near the blood vessels predominantly and in the organ parenchyma much rarer. Intensification of mast cell degranulation processes was observed in the animals of the experimental groups/

The reported pathomorphologic changes in the thymus of animals exposed to deltamethrin demonstrate a decrease in their immune reactivity under hypercorticosteroidemia. Glucocorticoids are known to have a pronounced immunosuppressive effect, violate the cooperation of immune system cells, and induce apoptosis of thymocytes [26, 27].

A decrease in corticosterone level in rats 1 week after intoxication was followed by partial thymic cellularity recovery (see Fig., B). During the experiment, animal deaths have not been reported, indicating the effectiveness of adaptive and compensatory mechanisms, including the one of hypothalamic-pituitary-

thymus system [28] involved in the inhibition of the hypothalamic-pituitary-adrenal axis which dominates in all stress types.



Histological structure of the thymus in white laboratory Wistar rats after deltamethrin administration at a dose of 43.5 mg/kg: A — day 3, a decrease in the density of thymocytes is observed in the medulla (×600), B — day 7, thymic cellularity is recovered partially (×300) (microscope Altami BIO 1, Altami, Russia; hematoxylin and eosin staining).

The nature of endocrine and immune disorders at an early stage of acute deltamethrin intoxication evidenced the high risk of immunodeficiency in remote post-intoxication period, which is confirmed by numerous examples of pathologies in organs when exposed to pesticides [29].

Thus, acute deltamethrin intoxication in rats is followed by the development of hypercorticosteroidemia and hyperglycemia at insulin resistance. Within 3 days, the level of blood insulin reduces sharply and returns to normal gradually with the decrease of glucose and corticosterone concentrations, reaching the reference values a week after intoxication. A decrease in the number of lymphoid cells in the thymus and activation of apoptosis evidence the suppression of immune reactivity in animals. The increased mast cell counts and degranulation processes indicate the allergenic effects of the drug. The results of our research make a contribution to the understanding of compensatory and adaptive mechanisms under experimental intoxication, and make it possible to estimate the functional capabilities of body systems when developing the ways of pharmacological correction at intoxication.

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