

ORIGINAL ARTICLE

Experimental study of imiprotin allergic potency in case of inhalation

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ABSTRACT

Probable changes were studied in rats' immune status under experimental conditions with inhalation route of type I pyrethroid-imiprotin administration, which is the main component of a number of household insecticidal agents. The drug at a concentration of 45.0 mg/m³ interrupts immunological homeostasis in experimental animals. Nonspecific cellular component parameters of immune system have changed significantly. Imiprotin is capable of inducing delayed hypersensitivity. Imiprotin induces sensibilization under experimental conditions in more than half of the experimental animals, but the magnitude of the reactions to the intradermal administration of the drug has no probable differences, which allows imiprotin to be attributed to substances with moderate sensibilization potential

KEY WORDS: imiprotin; immunological homeostasis; laboratory rats

Introduction

A steadily increasing tendency is observed within the last decade in the active use of household insecticides including use inside the houses (Sarwar, 2016; Titiek *et al.*, 2011). Pyrethroids are the active ingredients in a large list of these agents. It was reported that the route for these compounds in confined spaces through respiratory tract is a significant negative factor in human living environment (Bradberry *et al.*, 2005), and their long-term effect leads to the development of humoral and cellular reactions, skin hypersensitivity, and respiratory disturbances (Macan *et al.*, 2006). The studies in volunteers showed that after pyrethroids use under the recommended conditions and doses, the modulation of immune components within the physiological range towards the low values during the first days was observed. Although these changes were interpreted as manifestations of immunoregulation compensatory mechanisms (Hadnagy *et al.*, 2003), possible immune system changes caused by pyrethroids should not be neglected. The experiment in rats showed immunotoxic effects induced by aerosol which contained

pyrethroids mixture of imiprotin and deltamethrin (Emara & Draz, 2007).

The purpose of these studies was to detect, under experimental conditions, the probable changes in the immune status of the experimental animals after inhalation of type I pyrethroid-imiprotin, which is the main component in a number of household insecticides.

Methods

Experimental animals and their maintenance

Experimental study was carried out in 20 white, outbred male rats aged 3–3.5 months with a body weight of 180–200 g. Experimental animals received standard granular feed with unlimited access to drinking water. The animal studies were conducted in compliance with the bioethics principles, legislation, and requirements in accordance with the provisions of the “European Convention for the Protection of Vertebrate Animals used for Research and Scientific Purposes” (European Communities, 1986).

The animals were randomly divided into two groups of 10 animals in each: experiment and control.

Chemicals and treatment

Imiprotin is a synthetic insecticide of the pyrethroid group, a derivative of cyclopropanecarboxylic acid. CAS Number 72963-72-5. Molecular weight – 318.37 mg/mol.

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Sensibilization in white rats was carried out by single intranasal administration of imiprotin at a dose corresponding to a concentration of 45.0 mg/m³. Concentration was chosen based on the previous experimental results, where this concentration was established as a threshold value (Hrushka & Turkina, 2017). The conversion of the administered dose into concentration was carried out by the formula:

$$D = C \times V, \text{ where } C - \text{drug concentration, mg/m}^3$$

D – drug dosage (mg) administered to animals

V – air volume inhaled by rats within 4 hours

Control animals were injected intranasally with a solvent (tween oil).

Clinical and immunological tests

Changes in peripheral blood parameters (WBC absolute count and WBC differential) were determined. Based on WBC differential the ratio of individual WBC populations was calculated. Hematological parameters were calculated: lymphocyte to monocyte ratio (LMR), neutrophil to monocyte ratio (NMR), neutrophil to eosinophile ratio (NER).

Humoral immunity parameters

Immunoglobulin (Ig) E in blood serum was determined by ELISA test according to the instructions provided with the enzyme-immunoassay analyzer “STAT FAX PLUS-303”.

Circulating immune complexes (CIC) were determined by precipitation of large globular blood immune complexes with macromolecular polyethylene glycol, spectrofluorometric method.

Allergic tests *in vivo*

Ear swelling challenge test (ESCT) was performed one week after sensibilization to characterize the delayed-type allergic reactions.

ESCT setting: thickness of the middle external ear was pre-measured in mm using micrometer, then 25 µl of the experimental substance was applied on both surfaces of the middle third of the fixed ear in concentration that does not cause non-allergic contact dermatitis. Ear thickness was re-measured in 24 hours with the calculation of EST (swelling challenge test) value based on the thickness difference before and after application. The severity of compound's allergic effect was determined in comparison with the control by swelling intensity. EST is considered positive with a value of 0.03 mm or above. In order to exclude the influence of nonspecific factors on delayed hypersensitivity assessment by ESCT absolute terms in control and experimental animals, as well as to determine the integral evaluation criteria for delayed hypersensitivity (by incidence and severity of skin reactions) ESCT score was used: ESCT absolute value in mm with a gradation of 0.03–0.07; 0.08–0.12; 0.13–0.17; 0.18–0.22; 0.23 and corresponds to 1; 2; 3; 4; and 5 points, respectively

Immunological tests *in vitro*

In order to perform quantitative assessment, a detection method was used ten days after animal sensibilization of

blood cells reaction to allergen *in vitro* – specific leukocyte agglomeration reaction (SLAR), specific leukocyte lysis reaction (SLLR) and neutrophil damage index (NDI). These tests allow detecting the delayed allergic reaction caused by cell sensibilization.

SLAR setting: two test tubes were added with 0.04 ml 5% citrate each. Citrate was added to test tube, which contained imiprotin, the second tube was a control one. Then, 0.2 ml of blood was added to all test tubes and incubated at 37°C for 2 hours. Subsequently, smears were prepared on degreased slides, dried at room temperature within 24 hours and dyed with 0.1% aqueous methylene blue solution for 10 minutes, then rinsed gently with water. 500 leukocytes were counted in each smear, estimating the number of cells forming aggregates of three or more cells. Then the percentage of agglomerated leukocytes was calculated.

SLLR setting: test tubes were added with 0.05 ml of saline solution, where the experimental test tube was added with saline solution and imiprotin. Then, 0.1 ml of experimental animals' blood was added to the test tubes and incubated at 37°C for 2 hours. The resulting solution was then transferred in 0.02 ml into plate wells containing 0.4 ml of Turk solution. The absolute number was counted and calculations made by the formula:

$$SLLR = (Lc - Le) / Lc * 100, \text{ where } L - \text{absolute number of leukocytes in control (Lc) and experiment (Le).}$$

The reaction was considered positive only with an indicator more than or equal to 10%.

For NDI setting as an anti-coagulant 5% aqueous solution of sodium citrate in saline solution was used. The working concentration of the experimental substance is prepared based on the same anticoagulant, which is added to the blood. First silicone centrifuge tube (experimental sample) was added with 0.1–0.2 ml of the experimental substance solution, second (control) was added with the same amount of the experimental animal blood, after which the test tubes are stirred and kept at room temperature for 1 hour. At the end of incubation smears are prepared on slides, which are fixed and stained using the method for leukocyte count. Count 100 neutrophils in immersion, taking into account the amount of chromatinolysis, picnose, fragmentation of nuclei, hyperchromatosis or carriolysis. The reaction rate is calculated by dividing difference in the number of damaged neutrophils by 100 in experimental and control samples.

Statistical analysis

Statistical processing of results was carried out using Microsoft Excel. The verification of obtained data compliance with the normal law of distribution was carried using Shapiro-Wilk's W test. Accuracy of changes received for comparative values was estimated using Student's t-test. Changes were considered accurate, if significance level exceeded 95% ($p < 0.05$) (Glantz & Slinker, 1990).

Results and discussion

Changes in leukocyte count and leukocytal indices

Imiprotin inhalation route does not affect the total leukocyte count in experimental animals as compared to the control group. Leukocyte count analysis showed the destabilizing effect of the drug on blood functional state, which was manifested by an increase in the absolute and relative number of eosinophils and neutrophils, severe lymphopenia, with no changes in relative and absolute quantitative parameters of other leukocyte subpopulations (Table 1).

Allergic disorders caused eosinophilia by binding IgE antigen complexes to the surface of mast cells. It should be noted that eosinophilic granulocyte plays a significant role in the pathophysiological phase of hypersensitivity. Eosinophil cationic protein (ECP) induces the secretion of histamine from mast cells and basophils, actively involved in inflammatory reactions; inhibits the proliferation of T-lymphocytes, provides regulation of cell-mediated immune responses. In modern literature, it is considered an active actor in developing allergic diseases and an important element in maintaining immunological homeostasis (Hogan *et al.*, 2003).

In addition, substantial effect of imiprotin on the immune status of experimental animals in our experiment is evidenced by an increase in the number and content of neutrophils. Neutrophils play one of the leading roles in developing and maintaining inflammatory reactions that can rapidly increase metabolic rate and can be used as an objective criterion for assessing the effector component of immune system. Due to antigen-presenting and effector functions, neutrophils can be the actors and regulators of immune response. The implementation significance of granulocytes functional potential in developing and overcoming allergic diseases is justified by the experimental data from several authors (Nathan, 2006). Engagement of eosinophils and neutrophils in allergen-specific reactions is confirmed by the presence of Fcε receptors to IgE on their surface (Gounni *et al.*, 2001).

The main role in immune responses is played by lymphocytes that recognize antigens. Our study found relative lymphopenia in experimental animals against the background of neutrophilia. This indicates the defect in cellular immunity, namely the failure of the lymphocyte component. In clinical practice, this pattern is characteristic of acute allergenic pneumonia (Kurup *et al.*, 2006).

Hematological indices analysis shows an increase in NMR indicating an imbalance in the components of microfagal-macrophage system and LMR decrease indicating the suppression of the effector component of the immune response. In general, experimental animals show abnormal immunological reactivity.

The pattern of leukocytes count found in experimental animals is characteristic of immune-dependent inflammatory processes in the body and suggests a shift in immunological homeostasis under imiprotin effect. The analysis of identified changes allows classifying them as allergenic ones. The experiment results obtained are

Table 1. Changes in the immunological status of rats exposed to imiprotin test

Parameters	Control	Experiment	t _{exp}
Leucocytes, G/L*	7.97±0.7	8.63±1.26	1.45
Leukocyte count:			
basophils,%	0.2±0.42	0.3±0.48	0.5
basophils, G/L*	0.0166±0.035	0.027±0.044	1.0
eosinophils,%	2.4±0.7	4.4±1.35	4.2
eosinophils, G/L*	0.19±0.06	0.39±0.17	4.0
neutrophils,%	17.1±2.47	23.4±3.4	4.7
neutrophils, G/L*	1.37±0.28	2.04±0.5	3.7
monocytes,%	2.2±0.42	2.4±0.52	1.0
Monocytes, G/L*	0.176±0.04	0.207±0.054	1.5
lymphocytes,%	78.1±3.14	69.6±3.44	5.8
lymphocytes, G/L*	6.22±0.48	5.98±0.7	0.9
Haematological parameters:			
NER	7.72±2.7	5.81±2.06	1.8
NMR	8.0±1.8	10.2±2.73	2.1
LMR	36.47±5.81	30.1±5.88	2.4
Humoral immunity:			
CIC, cu	91.10±39.53	124.10±30.63	0.8
IgE, IU d/ml	5.74±2.6	7.82±3.8	1.4

The values are expressed as mean ± SDev. (n=10); p<0.05 compared with respective control rats; Student's t-test - t_{th} (theoretical) = 2.1; t_{exp} - experimental t-value; *G/L - Giga cells per liter

Table 2. Results of allergic tests in vivo and in vitro in rats exposed to imiprotin test.

Parameters	Control	Experimental	t _{exp}
specific leukocyte lysis reaction (SLLR)			
H	1/10	8/10	
Absolute count	7.74±0.44	9.80±0.80	1.59
%	6.29±1.06	12.11±1.20	1.46
Specific leukocyte agglomeration reaction (SLAR)			
H	7/10	10/10	
Absolute count	7.9±4.01	9.9±3.41	1.2
%	0.83±0.57	1.41±0.14	3.2
Neutrophil damage index (NDI)			
H	0/10	2/10	
Absolute count	5.8±0.9	7.9±0.56	1.96
%	0.013±0.002	0.04±0.006	3.66
Ear swelling challenge test (ESCT)			
H	4/10	9/10	
10-2 mm score	0.18±0.06	0.24±0.06	0.4
	1.6±2.27	3.6±0.5	1.1

Note: The values are expressed as mean ± SDev. (n=10); p<0.05 compared with respective control rats; Student's t-test - t_{th} (theoretical) = 2.1; t_{exp} - experimental t value; H - number of positive animals in numerator, denominator - total in experiment.

consistent with changes in cellular component of immune system in the experimental animals under imiprotin effect, which we have discovered earlier (Hrushka & Turkina, 2017).

Humoral component of immune system

Humoral component of immune system showed no probable differences in the circulating immune complexes count in sensitized and control animals (Table 1). This shows the absence of immunocomplex pathology in experimental animals.

IgE is unchanged in relation to control, presumably because the pathological processes pass through IgE-independent pathway. This mechanism of respiratory hypersensitivity is discussed in modern scientific literature for non-atopic allergies (Cochrane *et al.*, 2015). It is important that in clinical practice with a confirmed diagnosis of allergenic pneumonia serum IgE in patients remain within the limits of normal (Kurup *et al.*, 2006).

Results of allergic tests *in vivo* and *in vitro*

The sensitized animals were found with an increase in blood cells response to imiprotin effect, in particular, a significant increase in percentage of SLAR – 1.7-fold, NDI – 2.5-fold vs control (Table 2).

Agglomeration is the first phase of cells allergenic reaction. SLAR results indicate the development of delayed-type allergenic response after inhaled imiprotin effect.

NDI changes from 0.02 to 0.07 were observed in the experimental group (critical value increase of 0.05 was observed in two animals from experimental group), while this index in all control animals did not exceed 0.02. NDI increase observed in experiment with imiprotin action is due to the allergen effect on growth of neutrophil granulocytes, which have amoeboid activity, and indicates a specific sensitivity the body.

SLLR results are not unambiguous. No significant differences were found in leukolysis parameters of the experimental and control groups. At the same time, there was a steady tendency to this parameter increase in animals of the experimental group. 10% increase in its critical value was observed in 80% of experimental animals. It ranged from 0 to 8% in control, and only one animal exceeded the critical value of 10%.

ESCT increase in the experimental animals in the integrated assessment has no statistically significant differences compared to the control group. In absolute terms, after an intranasal administration of the drug, a positive ear swelling challenge test (ESCT) was detected in 9 out of 10 experimental animals. The average absolute value 1.5-fold exceeded the control values.

Thus, *in vivo* and *in vitro* tests confirmed the assumptions on allergic nature of changes in peripheral blood parameters in experimental animals. In general, based on the results obtained, the immune system reaction to imiprotin inhalation effect can be attributed to a neutrophil-dependent type (Gounni *et al.*, 2001).

Consequently, the sensitizing effect analysis indicates that delayed hypersensitivity is the most probable

outcome of body sensitivity with this imiprotin route of administration at a dose of 45 mg/m³. Taking into account the important role of immune system in preserving body balance and the risk of developing pathological conditions in case of its abnormality, it is important to develop regulations for imiprotin in environmental setting (Cochrane *et al.*, 2015).

Conclusions

The inhalation effect of imiprotin at a concentration of 45.0 mg/m³ causes an impairment of immunological homeostasis in experimental animals. Nonspecific cellular component parameters of immune system have changed significantly due to imiprotin effect, which reflects systemic processes occurring in the body as a whole.

The ability of imiprotin to induce delayed hypersensitivity has been identified. Imiprotin, under conditions of experimental reproduction and detection of delayed hypersensitivity, causes sensibilization in more than half of experimental animals, but the magnitude of reactions to intradermal administration of the drug has no probable differences, which allows imiprotin to be classified as a substances with moderate sensitizing potential.

Thus, the data obtained indicate the need for further studies of imiprotin's lower concentrations in order to establish safe levels of its effect. It should be noted that at low concentrations, there appears a problem of controlling the factors that may lead to a lowered dose, namely mechanical loss in the esophagus, mechanical loss to other regions in the nose, and mechanical loss anteriorly from nose. Therefore, there is a need to control the concentration of the substance in the blood plasma. An emphasis of the future research should be put on sensitive tests for detection of the allergic alterations in the body at the interleukin or chemokine levels.

REFERENCES

- Bradberry SM, Cage SA, Proudfoot AT, Vale JA. (2005). Poisoning due to pyrethroids. *Toxicol Rev* **24**(2): 93–106.
- Cochrane SA, Arts JH, Ehnes C, Hindle S, Hollnagel HM, Poole A, Suto H, Kimber I. (2015). Thresholds in chemical respiratory sensitisation. *Toxicology* **333**: 179–194.
- Emara AM, Draz EI. (2007). Immunotoxicological study of one of the most common over-the-counter pyrethroid insecticide products in Egypt. *Inhal Toxicol* **19**(12): 997–1009.
- European Communities (EC) [webpage on the internet] (1986). – European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes. Strasbourg, 18.III.1986. European Treaty Series No. 123. [accessed on 11 April 2005] Available from: www.conventions.coe.int/treaty/treaties/html/123.htm
- Glantz SA, Slinker BK. (1990). *Primer of Applied Regression and Analysis of Variance*. New York: McGraw-Hill, Health Professions Division.
- Gounni AS, Lamkhioued B, Koussih L, Ra C, Renzi PM, Hamid Q. (2001). Human neutrophils express the high-affinity receptor for immunoglobulin E (FcRI): role in asthma. *FASEB J* **15**(6):940–949.
- Hadnagy W, Leng G, Sugiri D, Ranft U, Idel H. (2003). Pyrethroids used indoors – Immune status of humans exposed to pyrethroids following a pest control operation – a one year follow-up study. *Int J Hyg Environ Health* **206**(2): 93–102.

- Hogan SP, Rosenberg HF, Moqbel R, Phipps S, Foster PS, Lacy P, Kay AB, Rothenberg ME. (2008). Eosinophils: biological properties and role in health and disease. *Clin Exp Allergy* **38**(5): 709–750.
- Hrushka OI, Turkina VA. (2017). Дослідження впливу іміпротрину на клітинний імунітет лабораторних тварин. *International research and practice conference "Innovative technology in medicine: experience of Poland and Ukraine"* 165–168.
- Kurup VP, Zacharisen MC, Fink JN. (2006). Hypersensitivity pneumonitis. *Indian J Chest Dis Allied Sci* **48**(2): 115–128.
- Macan J, Varnai MV, Turk, R. (2006). Zdravstveni učinci piretrina i piretroida. *Arh Hig Rada Toksikol* **57**: 237–243
- Nathan C. (2006). Neutrophils and immunity: challenges and opportunities. *Nat Rev Immunol* **6**(3): 173–182.
- Sarwar M. (2016). Indoor risks of pesticide uses are significantly linked to hazards of the family members. *Cogent Medicin* **3**(1): 1155373
- Titiek SY, Hermanu T, Kooswardhono M, Nurmala K. P, dan Sjafrida M. (2011). Pesticide Use of Behavior: A Case Study of Household Pest Control in DKI Jakarta Urban Settlement. *Forum Pasca Sarjana* **34**(3): 195–212.