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The effect of chronic intoxication by organophosphate insecticides on the parameters of innate and adaptive immunity and realization of the cholinergic anti-inflammatory pathway

Abstract

Experiments on noninbred albino rats showed that chronic intoxication with organophosphorus compounds (organophosphorus insecticides) malathion and parathion methyl (0.01 LD_{50} daily, for 30 days) significantly reduces the phagocytic-metabolic activity of neutrophils, the activity of natural killer, antibody-dependent cellular cytotoxicity and the production of proinflammatory cytokines TNFα, IL-1β and IL-6. The chronic organophosphorus insecticides intoxication leads to the realization of the cholinergic anti-inflammatory pathway.

Keywords: organophosphate insecticides, neutrophil, immunity, cholinergic anti-inflammatory pathway, cytokines

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Introduction

A large number of anticholinesterase substances are used in agriculture, in various branches of industry, in medicine and in everyday life. Basically, these are substances related to organophosphate insecticides (organophosphorus compounds-OPC). The widespread use of these toxicants as insecticides, as well as the destruction of combat OPC, can lead to environmental pollution, causing intoxication of people and animals.[1–3] There is no doubt that research into the mechanisms of the formation of a post intoxication immunodeficiency state in the poisoning of OPC, in particular, the function of phagocytic-monocyte system (PMS) for the purpose of prevention and treatment, resulting in various infectious complications and diseases.[2,3] With the chronic effect of the OPC, the implementation of the cholinergic anti-inflammatory pathway (mechanism),[4–13] which includes: activation of m-cholinergic receptors (mAChRs) of the brain , which modulate the immunoregulatory function of the vagus nerve; excitation of efferent fibers n. vagus; the action of acetylcholine on receptors; the activation of n-cholinergic receptors (in particular, α7nAChRs) of PMS cells. In PMS cells, the onset of an anti-inflammatory effect is provided by kinase JAK2; transcription factor STAT3; transcription factor NF-κB. Under the influence of cholinergic stimulation, the "inclusion" of these biochemical mechanisms of the cell inhibits the production of TNF-α, B1-HMGB1 protein, macrophage-inflammatory protein-2-MIP-2, interleukins IL-1β, IL-6.[10,11,13,14] Thus, cholinergic stimulation causes acetylcholine α7nAChRs activation of the PMS system cells (macrophages, monocytes and neutrophils), which leads to a decrease in the production of these cells (as well as lymphoid dendritic cells) of proinflammatory cytokines.[2,3,7–10,15,16] The effect of chronic organophosphate insecticides intoxication (malathion and parathion methyl) on neutrophil function and the implementation of the cholinergic anti-inflammatory pathway has not been adequately investigated.

Aim of the study

The aim of the study was to determine the effect of chronic intoxication of organophosphate insecticides on the phagocytic-metabolic activity of neutrophils, function cells of the immune system, the blood levels of the pro-inflammatory cytokines TNFα, IL-1β and IL-6 produced by them, and realization of the cholinergic anti-inflammatory pathway.

Materials and methods

The experiments were carried out on random-bred albino rats of both sexes weighing 18-22g. The control group of rats received isotonic sodium chloride solution (saline) within 30days corresponding to the volume of aqueous solution of an OPC emulsion in groups of rats treated with OPC (0.5-1.0ml). The OPC (malathion–MT–[O,O-Dimethyl-S-(1,2-dicarbethoxyethyl) dithiophosphate] and parathion methyl–PM–[O,O-diethyl O-(4-nitrophenyl) phosphorothioate]) were injected subcutaneously daily for 30 days at a dose of 0.01 LD_{50} (LD_{50} of these insecticides were injected subcutaneously with 815±28 (MT) and 25.3±2.6mg/kg (PM). Used the aqueous solutions of 50% MT emulsion ("AgroWista", Russia) and PM ("AgroVista", Russia) The phagocytic-metabolic activity of neutrophils was assessed by 30 days after the first dose of OPC was administered by determining the phagocytic index, phagocytic number, neutrophil activity index (NAI) in spontaneous and induced Nitro Blue-Tetrazolium Test (NBT, NBT spontaneous, NBT induced) generally accepted methods. *S. aureus* 209P was used to assess the phagocytic index and the phagocytic number.[2,3,17,18]

Parameters of cellular immune reactions were assessed by generally accepted methods in experimental immunotoxicology and immunology[2,3,19] after chronic intoxication of OPC 30 days after the first injection of organophosphorous substances. The activity of natural killer (NK) was determined by the index of natural cytotoxicity spectrophotometrically. Antibody-dependent cellular cytotoxicity (ADCC) was determined on 4 days after immunization of rats of sheep red blood cells (10^8), using their splenocytes spectrophotometrically.[2,3] Immunization was performed on 26 days after the first injection of OPC. When evaluating phagocytic-metabolic activity of neutrophils

and immune responses, animals received a total dose of OPC (MT and PM), which is 0.3 LD_{50}. The concentration of TNF-α, IL1β and IL-6 was determined in blood plasma of rats after chronic intoxication OPC by enzyme immunosorbent assay (ELISA) using kits (ELISA Kits MyBioSoure) in accordance with the manufacturer's instructions. Monoclonal antibodies MyBioSoure (TNF-α, IL1β, IL-6-#MBS494184, #MBS494492, #MBS335516) were used to determine the concentration of pro-inflammatory cytokines. Blood for research was taken from the retroorbital venous sinus. The data obtained were processed statistically using the Student's t-test. Differences between the parameters were considered reliable at $p<0.05$.

Results

Under the influence of chronic intoxication of OPC a significant reduction of phagocytic-metabolic activity of neutrophils was noted (Table 1). 30 days after the action of MT the phagocytic index, phagocytic number, neutrophil activity index in NBT spontaneous, NBT induced decreased, respectively, to 1.77; 1.49; 1.94 and 1.24 times ($p<0.05$), and after intoxication with PM-1.48; 1.66; 1.55 and 1.33 times ($p<0.05$), respectively. The activity of NK and ADCC under the influence of MT decreased, respectively, 1.30 and 1.46 times ($p <0.05$), and after chronic intoxication by PM-by 1.55 and 1.32 times ($p<0.05$). According to the degree of reduction of phagocytic-metabolic activity of neutrophils, activity of NK and ADCC the effects of OPC in equiletal doses did not differ significantly. The immunosuppressive effect of OPC was accompanied by decrease in the blood concentration of pro-inflammatory cytokines (Table 2). So, after chronic intoxication of MT concentration of TNFα, IL-1β and IL-6 in the blood, respectively, decreased by 1.45; 1.91 and 1.55 times ($p<0.05$), and after PM intoxication - 1.67; 1.63 and 1.40 times ($p<0.05$), respectively.

Table 1 The changes in the phagocytic-metabolic activity neutrophils and the indices of cellular immune responses of rat under the influence of chronic intoxication of organophosphorus compounds (total dose 0.3 LD_{50}, 30 days) (M±m, n=8-10)

Parameters	Control group	Malathion	Parathion methyl
Phagocytic index, %	33,4±2,9	18,9±2,3*	22,5±1,9*
Phagocytic number, c. u.	2,54±0,22	1,70±0,15*	1,53±0,17*
NBT spontaneous, NAI	0,31±0,03	0,16±0,02*	0,20±0,02*
NBT induced, NAI	0,52±0,05	0,42±0,04*	0,39±0,04*
NK activity, %	34,1±3,0	26,3±2,3*	22,0±2,5*
ADCC, %	13,3±1,4	9,2±1,0*	10,1±1,1*

Note: NAI–neutrophil activity index; c.u.–conventional units (average number of microbial cells absorbed by one neutrophil); *-p<0,05 as compared to control.

Table 2 The effect of chronic intoxication of organophosphorus compounds (total dose of 0.3 LD_{50}, 30 days) on the concentration of pro-inflammatory cytokines in the blood of rats, pg/ml ((M±m, n=7)

Series of experiments	ФНОα	ИЛ1β	ИЛ-6
Control group	87±9*	65±8*	115±10
Malathion	60±7*	34±4*	74±9*
Parathion methyl	52±6*	40±5*	82±8*

* -p < 0,05 as compared to control.

Discussion

It is possible that the reduction of phagocytic-metabolic activity of neutrophils may be associated with inhibition of cytosol esterase by PMS cells (α-naphthyl acetate esterase, α-naphthyl butyrate esterase, α-naphthyl-AS-D-acetasterase, α-naphthyl-AS-D-chloroacetate esterase) and also with initiation of OPC by lipid peroxidation.[2,3] The suppression of the activity of NK and ADCC is probably due to the inactivation of OPC acetylcholinesterase of NK and cells participating in the ADCC.[2,3,20] The effect of OPC on phagocytic-metabolic activity of neutrophils is probably due to the interaction of toxicants and their metabolites with NADP·H and $NADH^+$. The action of OPC can also be associated with inhibition by toxicants and products of their biotransformation FAD^+, FAD·H, reduced and oxidized ubiquinone, cytochrome b_{245} of leukocytes, or other mechanisms for disrupting the functioning of the NADP·H oxidase complex of neutrophils. OPC also affects oxygen-dependent microbicidal systems of phagocytes (neutrophils) besides to oxygen-dependent anti-infective systems of phagocytosis.[2,3,17–19]

As already mentioned, a decrease in the production of pro-inflammatory cytokines by neutrophils, monocytes, macrophages (and, to a lesser extent, other cells of the immune system) is due to the cholinergic anti-inflammatory pathway (activated by acetylcholine α7nAChRs cells) under the influence of cholinergic stimulation PMS, leading to a reduction in the synthesis of pro-inflammatory cytokines).[2,3,7,8,10,11,15,16] Probably a decrease the production of these cytokines during intoxication OPC is not only induced as a result of the mechanisms associated with the cholinergic anti-inflammatory pathway.[3,11,21] The excitation of neutrophils with acetylcholine mAChR leads to an increase in their phagocytic-metabolic activity.[3,21] However, as shown our experiments, with the chronic effect of OPC the opposite effect was observed, due to the action of acetylcholine on α7nAChRs neutrophils.[3,21] It is possible that this effect is significantly higher than the activating effect of acetylcholine on the mAChRs of PMS cells.

There is reason to believe that when OPC is intoxicated, the phagocytic-metabolic activity of neutrophils is determined by numerous effects, a number of which are differently directed (for example, activation of neutrophil mAChRs and α7nAChRs.[3,21] It is interesting to note that a decrease in the synthesis of pro-inflammatory cytokines during cholinergic stimulation, in particular, under the influence of OPC, can lead to a decrease in the mortality of animals in the early stage of sepsis.[2–5,9] With the defeat of toxicants, in particular, OPC, the phagocytic-monocytic system plays a very important role in the manifestation of both inflammatory and anti-inflammatory effects. The reduction of phagocytic-metabolic activity of neutrophils and the synthesis of pro-inflammatory cytokines can be considered as a negative reaction during the chronic action of OPC. In this case,

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possible violations associated with the destruction and removal of tissues affected by a toxicant, and processes associated with their action.[22] That is the realization of inflammation, as a reaction that bears a protective-adaptive nature (and not only pathological), is disturbed during chronic intoxication of OPC.

Conclusion

a. The chronic organophosphorus compounds intoxication (malathion, parathion methyl) for 30 days in a total dose of 0.3 LD_{50} leads to a decrease of phagocytic-metabolic activity of neutrophils, activity of natural killer and antibody-dependent cellular cytotoxicity.

b. The function of the phagocytic-monocytic system after chronic poisoning of organophosphorus compounds decreases, which is manifested by reduction in the blood concentration of pro-inflammatory cytokines TNF-α, IL-1β and IL-6.

c. The chronic intoxication with organophosphate insecticides (organophosphorus compounds) leads to the realization of the cholinergic anti-inflammatory pathway.

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Conflict of interest

Author declares that there is no conflict of interest.

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