ORIGINAL ARTICLE

Influence of chlorpyrifos on the profile of subpopulations of immunoactive cells and their phagocytic activity in an experimental *in vivo* model

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Abstract

Many environmental factors, including pesticides, cause immunological system disorders by inducing changes in humoral and cellular response. They may stimulate or trigger immunological autoaggression, hypersensitivity and allergy, as well as lead to immunosuppression, thus increasing the incidence of infectious diseases and cancers. Such activity is also attributed to organophosphorus compounds used in agriculture as insecticides, and commonly in households as biocides. The aim of the study was to define possible mechanisms of the immunotoxic activity of the chlorpyrifos (an organophosphorus compound) on experimental animals following their exposure to the compound *via* the oral route. The present study attempts to define the influence of chlorpyrifos on the profile of subpopulations of immunoactive cells: B, T, CD4+, CD8+, and NK, and on their phagocytic activity in an experimental *in vivo* model. For this purpose, the Wistar rats, were exposed orally to increasing doses of chlorpyrifos: $0.1\ LD_{50}$, $0.15\ LD50$, $0.2\ LD_{50}$, $0.3\ LD_{50}$ and $0.4\ LD_{50}$ for 28 days. In the study animals, we failed to demonstrate a statistically significant decrease in the phagocytic activity of the granulocyte.

Key words

immunomodulation, T lymphocytes, B lymphocytes, phagocytic activity

INTRODUCTION

Organophosphorus insecticides are used in plant protection and sanitary hygiene. The mechanism of their action on humans and on laboratory animals consists in inhibiting the activity of enzymes regulating the functioning of the nervous system, mainly the acetyl cholinesterase. Independently of their well known neurotoxic activity [1], organophosphorus insecticides have other biologic activity and influence the production of antibodies [2, 3], interleukins 2 [4], and proliferation of T cells induced by interleukin 2 [5]. Moreover, *in vivo* and *in vitro* studies of organophosphorus compounds [6] indicated histopathological changes in tissues of the immunological system, disorders immunocompetent cells growth, and function and changes in the percentage share of B and T lymphocytes sub-populations [7].

Experiments intended to explain the influence of organophosphorus compounds on the immunological system have been conducted on animal models for many years. The experiments performed by Casale et al. [2] proved that it was possible to initiate a humoral immunological response dependant on the T lymphocytes activity in rodents exposed orally to parathion, malathion and dichlorfos, i.e. compounds which trigger a cholinergic effect, manifested through the stimulation of the parasympathetic nervous system.

In experiments on Wistar rats which received hypodermic injections of DDVP (an organophosphorus insecticide) the authors noted a clear decrease in the activity of NK cells, which have a significant role in eliminating spontaneously-occurring neoplastic cells and antibody-dependent cytotoxicity [8]. It was demonstrated that the organophosphorus compounds trichlorfon and dichlorvos used in the treatment of ectoparasites in carp have the potential to affect the health of consumers who ate treated fish. This suggests a suppressive activity of these compounds on both humoral and cellular response of the immunological system. This suppressive activity is manifested through the decreased phagocytic activity of neutrophils, decreased level of lysozyme in serum, decreased percentage of polymorphonuclear (PMN) cells, and increased activity of ceruloplasmin in plasma [9, 10, 11].

Chlorpyrifos, an active substance in insecticides, is an organophosphorus compound with significant acute toxicity. Chlorpyrifos shows a strong and non-selective activity in humans and animals [12]. Despite these features, the compound was positively verified in a review of active substances in plant protection in the European Union, and was included in Annex 1 to the Directive 91/414/EEC.

An *in vivo* study of chlorpyrifos on human T lymphocytes and bronchial epithelium cells in the presence or in absence of an oxidative stress factor [13] demonstrated a cytotoxicity of this insecticide already at the concentration level $\geq 250\mu M$.

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Thus, immunomodulating activity of this compound may be similar to the majority of the examined cytokine promoters, especially in the presence of an oxidative stress factor strengthened the effects of chlorpyrifos activity.

The use of organophosphates in agriculture constitutes a threat mainly to people performing agro-technical activities and handling these products. Products containing chlorpyrifos are also commonly used in sanitary hygiene to fight flying insects, ants, and other pests in the household. However, there are no studies that would unequivocally demonstrate that exposure to organophosphorus insecticides has no impact on the immunological system. Therefore, the immunomodulating potential of organophosphorus insecticides needs to be elucidated in the situation of sub-threshold exposure.

The aim of the presented study was to define the influence of chlorpyrifos on the profile of sub-populations of immunoactive cells: B, T, CD4+, CD8+, and NK, and on their phagocytic activity in an experimental *in vivo* model using Wistar rats.

MATERIAL AND METHODS

Permission No. 08/2010 was received from the Local Ethical Committee to conduct the experiment.

In the study, Wistar male rats (about 180 at the beginning – 350 g bw at the end of the experiment, 5 animals per group) were subject to alimentary exposure of the compound for 28 days. The test groups were administered a 97% chlorpyrifos concentrate solved in an edible olive oil through a stomach probe. The doses were: $0.4 \, LD_{50}$ (33 mg/kg bw), $0.3 \, LD_{50}$ (25 mg/kg bw), $0.2 \, LD_{50}$ (17 mg/kg bw), $0.15 \, LD_{50}$ (12.5 mg/kg bw), $0.1 \, LD_{50}$ (8 mg/kg bw). The control groups, both positive and negative, received pure olive oil. Additionally, once a day, the positive control group was administered a dose of 0.12 mL of Dexason in order to check the sensitivity of the immunological system responses. After 10 days, as a result of the observed deaths and acute neurotoxic symptoms, the dose of $0.4 \, LD_{50}$ was no longer administered.

On the final day of the experiment, the animals were deeply anaesthetized with a combination of ketamine (Vetaketam) and xylazine (Vetaxyl), and 4-6 mL blood samples were taken from the hearts of the living animals into the test-tubes containing an anticoagulant. A percentage share of B, T, CD4+, CD8+, and NK lymphocytes was identified in the samples of peripheral blood using ready-made monoclonal antibody kits produced by Becton Dickinson, and determined using flow cytometry. Analysis of the obtained data included qualitative tests (non-parametric Dunnett's test – multiple comparison test) and quantitative correlation tests using statistical software SPSS (*Statistical Package for the Social Sciences)* for Windows.

RESULTS AND DISCUSSION

Dunnett's statistical analysis did not show any significant differences in phagocytic activity of granulocytes (phagocyte Orpegon Pharma test adapted to flow cytometry) between the analyzed groups of animals exposed to chlorpyrifos (Fig. 1).
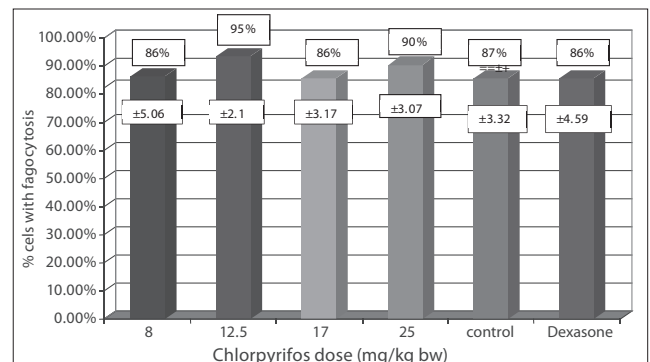

Figure 1. Influence of chlorpyrifos on phagocytic activity of granulocytes

Results of the statistical analysis of the percentage share of immunocompetent cells (lymphocytes B, T, NK, CD8, CD4) in particular groups of this study, obtained through a flow cytometry, did not indicate any statistically important differences between the analyzed groups of animals (Fig. 2).
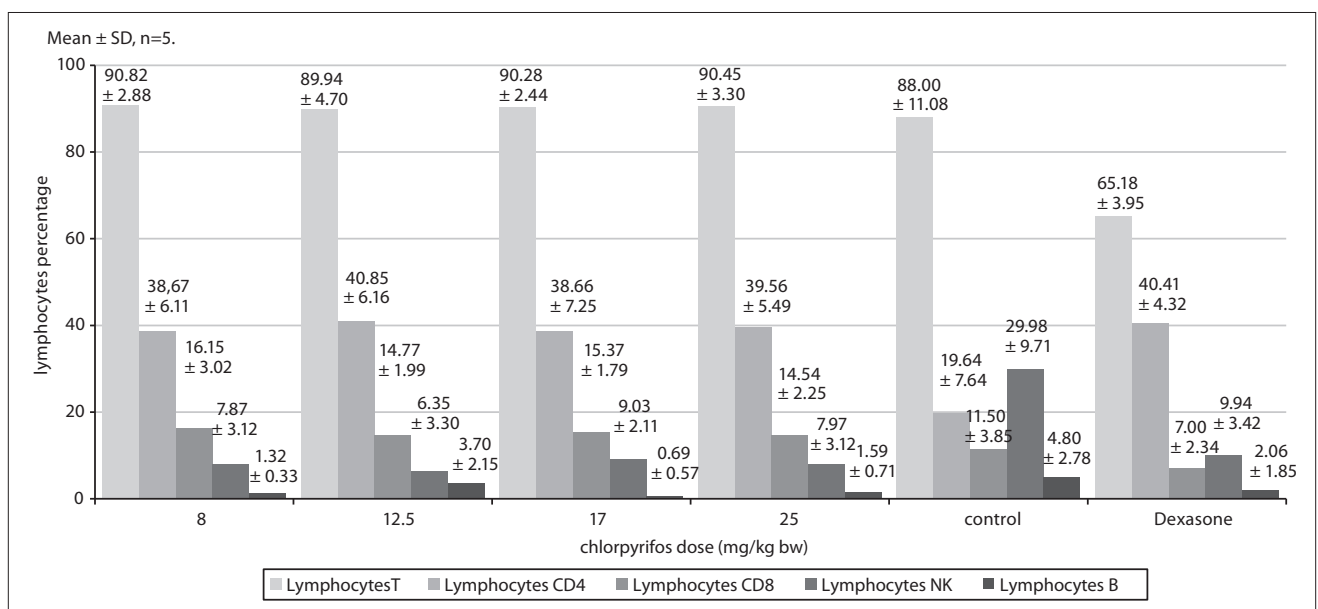

Figure 2. The relationship between the exposure to different doses of chlorpyrifos and the Lymphocytes profiles in the in vivo experiment.

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In a similar study by Blakley et al. [14], Fisher rats were exposed twice a week for 28 days to chlorpyrifos in single doses of 0.5 mg/kg bodyweight; no decrease in bodyweight growth was observed in the exposed animals. The isolated B and T lymphocytes were subsequently cultivated using concanavalin, phytohemaglutinin and lipopolisacharyd dextran stimulation. It was noted that chlorpyrifos weakened blastogenesis of T lymphocytes, induced by concanavalin A and phytohemaglutinin, weakened humoral immunity, expressed in the decreased number of blood erythrocyte antigens, and a relative increase of percentage expression of CD4 and CD8 cells. The results, however, did not show any influence on the macrophage phagocytic activity.

The presence of normal antibodies and a phagocytic response in the situation of a weakened T lymphocytes blastogenesis and increased expression of specific cellular antibodies suggest that chlorpyrifos may induce changes in lymphocytes subpopulations.

Results of studies on 29 human volunteers who were chronically exposed to chlorpyrifos [15] confirmed the above. An increase of CD26 cells expression was noted with simultaneous decrease of percentage share of CD5 lymphocytes, weakening of cellular mytogenesis in response to phytohemaglutinin and concanavalin, and an increase in the frequency of antibodies occurrence.

The results presented by other authors clearly indicate that organophosphorus pesticides used in agriculture may affect the immunological system. The compounds of this group penetrate into the immunological synapses created by T lymphocytes and antigen presenting cells (APC) blocking mAChR receptors, present at the surface of the T lymphocytes [16]. This leads to inhibition of I1-2 synthesis and its receptor, causing disorders of T lymphocytes proliferation and decreased sensitivity to Con A and PHA mitogens [17].

After exposure to organophosphorus compounds, similar effects were observed in NK cells, whose activity clearly decreased as well as the expression of perforin proteins and A granzyme [18]. We can therefore speak about an immunotoxic effect of the organophosphorus compounds on the T and NK cells [17].

These studies show that organophosphorus compounds used in agriculture cause a reaction of the immunological system – a suppression of both humoral and cellular systems. Taking into consideration that immunocompetent cells show different sensitivity to these compounds, the studies on the immunomodulating characteristics are particularly important, especially in the light of a massive increase of various diseases of the immunological system and growing chemical stress. However, the exposure time applied in the presented study seems too short to obtain an expected immunomodulating effect. It would therefore be advisable to continue the study with an extended time of exposure to the examined compound.

CONCLUSION

The lack of immunomodulating effect observed in this study may result from a quick adaptation of the immunological system to the exposure model. However, the lack of significant differences can also be explained by a too short exposure time.

It seems justified to treat the changes in the number and proportion of T and B lymphocytes and NK cells as an indicator of the immunomodulating activity of the organophosphorus compounds.

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