ASSESSMENT OF POTENTIAL CLASTOGENIC EFFECT OF THE INSECTICIDE MOSPILAN® 20SP IN HUMAN PERIPHERAL BLOOD LYMPHOCYTES AFTER *IN VITRO* EXPOSURE

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ABSTRACT

Acetamiprid, that is known as the commercial formulation Mospilan® 20SP is the part of the neonicotinoid insecticide group and is widely used against various pests. In our study we assessed the potential clastogenic effects of Mospilan® in human peripheral blood lymphocytes *in vitro* using a chromosome aberration test. The lymphocytes were treated with acetamiprid in the concentration range of 5, 10, 25 and 50 µg.ml⁻¹ for 24 and 48 h. After 24 h exposure, the insecticide induced statistically significant higher levels of chromosome aberrations from the concentration of 10 µg.ml⁻¹ ($P < 0.05$ and $P < 0.001$) and a significant decrease in mitotic index (MI) at the concentrations of 25 and 50 µg.ml⁻¹ ($P < 0.05$ and $P < 0.01$), respectively. After a 48 h exposure, we found a dose dependent increase in the percentage of chromosome aberrations at all concentrations ($P < 0.05$; $P < 0.01$ and $P < 0.001$) and a decrease in MI at concentrations of 25 and 50 µg.ml⁻¹ ($P < 0.05$ and $P < 0.01$). Our results indicated that neonicotinoid insecticide formulations containing acetamiprid may have potential cytotoxic and genotoxic effects.

Key words: acetamiprid; chromosome aberrations; mitotic index; neonicotinoids

INTRODUCTION

Commercial formulations of insecticides are used against various pests. Neonicotinoids' activity is based on their agonistic effect at nicotinic acetylcholine receptors (nAChRs). Due to their selective action on insects, nAChRs located in the central nervous system [10], neonicotinoids are widely used to protect crops and animals from insect attacks. However, neonicotinoids have a relatively low toxicity for humans and other mammals compared to traditional insecticides, but some studies have detected a possible risk in these organisms as well [14]. Acetamiprid also acts as a selective agonist for insect acetylcholine receptors and due to this fact it is considered to be safe for humans. However, the results of several *in vitro* studies detected cytotoxic and genotoxic effects of acetamiprid in mammalian cells. The formation of reactive oxygen species (ROS) has been detected resulting in the oxidative damage of cells [15].

The aim of our study was to evaluate the effects of the selected commercially available insecticide containing acetamiprid on human lymphocytes *in vitro*. After the insecticide exposure, chromosome aberrations (CA) and mitotic index (MI) (genotoxicity and cytotoxicity indicators respectively) were evaluated. Structural aberrations result from damage at the DNA level and high levels of CA generally predict an increased risk of cancer. Therefore, chromosomal analysis using human lymphocytes is a sufficiently sensitive test for genotoxic action [1, 3, 13].

MATERIALS AND METHODS

MOSPILAN® 20SP containing acetamiprid (20.2 %; CAS135410-20-7) was dissolved in pure water and the following concentrations were prepared: 5, 10, 25 and 50 $\mu\text{g}.\text{ml}^{-1}$. The insecticide was added to the cell cultivations for the last 24 and 48 h of their incubation. Whole peripheral blood was taken from the vena cubiti of a young woman (26-year-old) using a sterile syringe containing heparin solution (2000 IU per 10 cm^3). For blood cultivation, a cell cultivation medium containing RPMI 1640, bovine foetal serum (20 %), antibiotic and antimycotic solution (100 $\text{U}.\text{ml}^{-1}$ penicillin, 0.1 $\text{mg}.\text{ml}^{-1}$ streptomycin and 0.25 $\text{mg}.\text{ml}^{-1}$ amphotericin), and phytohaemagglutinin (180 $\mu\text{g}.\text{ml}^{-1}$) was used. Ethyl methanesulfonate (250 $\mu\text{g}.\text{ml}^{-1}$) was used as a positive control. Colchicine, mitotic

venom, was added 90 min before the end of cultivation at the concentration 5 $\mu\text{g}.\text{ml}^{-1}$. The incubation in tubes lasted 72 h at a constant temperature of $37 \pm 1^\circ\text{C}$. After processing the cultures in a standard manner [7], microscopic preparations were prepared, stained with a 2 % Giemsa dye solution and subsequently observed under a light microscope. A hundred of metaphases and a thousand of cells for MI were evaluated for each concentration.

The results were statistically evaluated by χ^2 test in Graph Pad Prism. The level of significance was set to $P < 0.05$.

RESULTS

After 24 h exposure (Fig. 1), we found a dose-dependent increase in the percentage of breaks with statistical significance ($P < 0.05$ and $P < 0.001$); the highest level was detected at the highest concentration (50 $\mu\text{g}.\text{ml}^{-1}$; $P < 0.001$). At the lowest concentration of 5 $\mu\text{g}.\text{ml}^{-1}$, the values were not statistically significant. We also analysed the MI. At the same time, the MI decreased as the insecticide concentration and the percentage of breaks increased. At concentrations of 5 and 10 $\text{mg}.\text{ml}^{-1}$, the decrease in MI was insignificant; other concentrations showed a statistically significant reduction of MI ($P < 0.05$ and $P < 0.01$). After 48 h of exposure (Fig. 2), we found a statistically significant induction of breaks from the lowest concentration (5 $\mu\text{g}.\text{ml}^{-1}$; $P < 0.05$), the maximum was detected at the

Fig. 1. Induction of chromosomal aberrations and changes in MI after 24 h exposure to the neonicotinoid insecticide Mospilan® 20SP

CA—chromosome aberrations; MI—mitotic index
*— $P < 0.05$; **— $P < 0.01$; ***— $P < 0.001$

Fig. 2. Induction of chromosomal aberrations and changes in MI after 48 h exposure to neonicotinoid insecticide Mospilan® 20SP

CA—chromosome aberrations; MI—mitotic index;
*— $P < 0.05$; **— $P < 0.01$; ***— $P < 0.001$

highest concentration (50 µg.ml⁻¹; P<0.001). The MI values decreased in a dose dependence relationship. The MI reduction was initially devoid of statistical significance (5 and 10 µg.ml⁻¹); a statistically significant decrease was detected at the concentrations of 25 µg.ml⁻¹ (P<0.05) and 50 µg.ml⁻¹ (P<0.01).

DISCUSSION

In our study we assessed the potential genotoxic and cytotoxic effects of the acetamiprid-based insecticide formulation Mospilan® 20SP in human peripheral lymphocytes *in vitro*. We found a statistically significant induction of chromosome aberrations after 24 h (P<0.05 and P<0.001) and 48 h (P<0.05; P<0.01 and P<0.001). The reduction of MI was seen at the highest concentration (P<0.05; P<0.01) at both exposure times. Our results indicated the potential cyto- and genotoxic effects of the insecticide.

The genotoxic effects of acetamiprid have been shown in the human colon [4] and lymphocytes cells *in vitro* [11]. Data on the actual level of acetamiprid residue in humans are limited. Kocaman and Topaktas [11] first published an article examining the relationship between a commercially available formulation containing acetamiprid and its genotoxic potential. The results of this study demonstrated that there were significant increases in sister-chromatid exchanges (SCE) and frequencies of chromosome aberrations in human lymphocytes after exposure to 25–40 µg.ml⁻¹ of acetamiprid for 24 h and 48 h. The induction of structural CA, SCE and micronuclei (MN) after exposure to acetamiprid indicated the clastogenic potential of the compound due to DNA damage. These results support the findings of Feng et al. [6]. They tested imidacloprid, a neonicotinoid insecticide that significantly induced MN, SCE, and DNA damage (comet assay) in human lymphocytes.

The molecular mechanisms of the genotoxicity of acetamiprid are not yet known. The DNA damage may be caused by the formation of reactive oxygen species [8]. Neonicotinoids exhibit high toxicity to pollinating insects, especially for honey bees (*Apis mellifera*), in which they cause behavioural disorders, orientation difficulties, and worsening social activities [9]. The symptoms of poisoning similar to those that have already been observed after various neonicotinoid insecticides exposure [2, 12] could affect

the survival of bees in the field. In addition, if the honey bee was able to return to the colony, its memory and communication skills would be so altered that it would not be able to survive [5].

CONCLUSIONS

In conclusions, we have demonstrated that the insecticide Mospilan® 20SP was able to cause genotoxic damage and the reduction of lymphocyte proliferation. Further research should be carried out to confirm its genotoxic and cytotoxic effects using other *in vitro* and *in vivo* test systems.

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