Biochemical effects of acute phoxim administration on antioxidant system and acetylcholinesterase in *Oxya chinensis* (Thunberg) (Orthoptera: Acrididae)

Haihua Wu a, Jingyu Liu a, Rui Zhang b, Jianzheng Zhang a, Yaping Guo a, Enbo Ma a,∗

a Institute of Applied Biology, Shanxi University, Taiyuan, 030006 Shanxi, PR China
b Agricultural and Life Science College, Shanxi Datong University, Datong, 037009 Shanxi, PR China

**A R T I C L E   I N F O**

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**A B S T R A C T**

The study was undertaken to evaluate the effects of different concentrations of phoxim on acetylcholinesterase (AChE) and esterase (EST) activities, and antioxidant system after topical application to *Oxya chinensis*. The results showed that phoxim inhibited AChE activity, and did not cause significant changes in the EST activity and the levels of malondialdehyde (MDA) and reduced glutathione (GSH). After phoxim administration, superoxide (SOD) and catalase (CAT) activities showed a biphasic response with an initial increase followed by a decline in their activities. Glutathione reductase (GR) and glutathione peroxidase (GPx) activities were inhibited in comparison with the control. Glutathione S-transferase (GST) activity showed irregular changes. Its activity increased significantly at the concentrations of 0.06 and 0.12 μg/mL and decreased at the concentrations of 0.09 and 0.24 μg/mL compared with the control. Changes in SOD, CAT, GST, GPx, and GR activities indicated that phoxim caused oxidative damage in *O. chinensis*. However, no significant changes in MDA content suggested that these enzymes played important roles in scavenging the oxidative free radicals induced by phoxim in *O. chinensis*. The formation of oxygen free radicals might be a factor in the toxicity of phoxim.

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1. Introduction

Phoxim (O,O-diethyl phosphorothioate) is an effective organophosphate (OP) pesticide used widely throughout the world for agricultural and domestic purposes. Like other organophosphates, phoxim is known to inhibit acetylcholinesterase (AChE) activity, an effect that is thought to underlie the neurotoxicity elicited by these compounds [1,2]. However, some studies indicate that other biochemical targets may be affected by OP insecticides [3]. Free radicals play an important role in toxicity of pesticides and environmental chemicals. Pesticide chemicals may induce oxidative stress leading to generation of free radicals and alteration in antioxidants or oxygen free radical scavenging enzyme system [4,5]. Lipid peroxidation has been suggested as one of the molecular mechanisms involved in pesticide-induced toxicity [6]. Oxygen free radical enzymatic scavengers like superoxide dismutase (SOD), catalase (CAT), glutathione S-transferase (GST), glutathione reductase (GR), glutathione peroxidase (GPx), etc., may protect the system from deleterious effect of oxygen free radicals [6].

*Oxya chinensis* (Thunberg) (Orthoptera: Acrididae) is a major insect pest in rice. In the previous study, we found that phoxim could control effectively the damage of *O. chinensis* [7]. Now, we were concerned whether phoxim could induce oxidative stress in *O. chinensis* like other organophosphate pesticides. Thus, the objective of the current study was to assess the influence of acute administration of phoxim on the acetylcholinesterase, lipid peroxidation and antioxidant defense system in *O. chinensis*.

2. Materials and methods

2.1. Insects

Fifth-instar nymphs of *O. chinensis* were collected from Jinyuan District, Shanxi Province, China. These nymphs were collected using a sweep net. After they were fed on bulrushes for 2–3 d in the laboratory, the vigorous and uniform fifth instars were selected for phoxim bioassay immediately.

2.2. Topical application treatment

The topical application method was used to treat the grasshoppers. Technical-grade phoxim was dissolved in acetone, and four serially diluted concentrations (0.06, 0.09, 0.12 and 0.24 μg/mL) were prepared. The concentrations of phoxim employed could cause about 10–50% mortality according to the previously determined values of LD50. For each concentration, 4 μL of phoxim was applied to the fifth-instar nymphs’ thorax with a microsyringe to prevent body damage.
solutions were dipped into the membranous lateral region of the second abdominal segment of each of 25–30 grasshoppers using a pipette. Each phoxim concentration was repeated three times, and every replicate included acetone-treated controls. The test individuals were held for 24 h at room temperature with adequate food. After 24 h treatment, the live insects were collected and immediately stored at −80 °C for biochemical analysis.

2.3. Activity assay

For enzyme extraction, the method included in the kit of the Nanjing Jiancheng Bioengineering Institute was followed: grasshoppers were homogenized in buffer (pH 7.4) containing 0.01 mol/L Tris–HCl, 0.0001 mol/L EDTA-2Na, 0.01 mol/L sucrose, and 0.8% sodium chloride. The homogenate (1:10 w/v) was centrifuged at 15,000g (4 °C) for 20 min, and the supernatant was stored on ice for determination of enzyme activity.

The activities of SOD, CAT, GPx, and GR were determined spectrophotometrically according to the method of the Nanjing Jiancheng Bioengineering Institute with a SpectraMAX190 microplate reader and SOFTmax software (Molecular Devices, Sunnyvale, CA). SOD activity was assayed spectrophotometrically at 550 nm by using xanthine and xanthine oxidase system. One unit of SOD activity was defined as the amount of SOD required for 50% inhibition of the xanthine and xanthine oxidase system reaction in 1 mL enzyme extract per milligram of protein. CAT activity was determined spectrophotometrically by monitoring the amount of complex compound at 405 nm due to H2O2 decomposition. The homogenate (1:10 w/v) was centrifuged at 15,000g (4 °C) for 20 min, and the supernatant was stored on ice for determination of enzyme activity.

2.4. MDA and GSH contents assay

Malondialdehyde (MDA) and reduced glutathione (GSH) contents were determined spectrophotometrically according to the method of the Nanjing Jiancheng Bioengineering Institute with a SpectraMAX190 microplate reader and SOFTmax software. MDA level (as an index of in vivo lipid peroxidation) was determined with Thiobarbituric acid reagent (TBA). The MDA–TBA adduct formation was measured spectrophotometrically at 532 nm and content of MDA was expressed as nmol/mg protein. The GSH content was determined spectrophotometrically by monitoring the chromophoric product resulting from reaction of the 5,5-dithiobis-(2-nitrobenzoic acid) with GSH in the presence of NADPH and glutathione reductase at 412 nm. The GSH content was expressed as µg/mg protein.

2.5. Protein assay

Protein contents of enzyme preparation were determined according to Smith et al. [12] by using BSA as a standard. Measurements were performed with the microplate reader at 560 nm.

2.6. Data analysis

All assays were performed in triplicate. Mean and standard deviation values were determined for all the biochemical parameters and the results were expressed as means ± SD (n = 3) from triplicate assay. The data were analyzed employing analysis of one-way variance (ANOVA). Fisher’s least significant difference (LSD) multiple comparisons were then used to separate the means among the treatments for each biochemical parameter. All the statistical analyses were performed using Statistical Product and Service Solutions (SPSS Inc.) software. P-values below 0.05 were regarded as significant.

3. Results

3.1. AChE and EST activities

Fig. 1 shows the changes of AChE and EST activities after phoxim treatment. Phoxim inhibited AChE activity at all the studied concentrations. At the concentration of 0.09 µg/µL phoxim, AChE activity was the lowest and was significantly inhibited about 44% compared with the control. Under phoxim stress, EST activity was lower than that of the control. However, no significant differences were observed.

3.2. MDA content, SOD and CAT activities

Phoxim did not cause significant changes in MDA content (Fig. 2). SOD and CAT activities in phoxim treated O. chinensis increased compared with the control except at the concentration of 0.09 µg/µL phoxim, where SOD and CAT activities decreased to the level of the control (Fig. 2). At the concentration of 0.06 µg/µL...


3.3. GSH content and related enzymes

Significant decreases in GSH content were observed in the phoxim treatments with different concentrations of phoxim in *O. chinensis*. SOD units = U/mg protein, CAT units = mmol/min/mg protein, MDA units = nmol/mg protein. Vertical bars indicate standard deviations of the mean (n = 3). Different letters on the bars indicate that the means are significant different among the treatments with different concentrations of phoxim for the same enzyme/MDA in Fisher’s LSD multiple comparison tests (P < 0.05, ANOVA).

µL phoxim, SOD and CAT activities were the highest and increased significantly about 46% and 30% of the control, respectively.

3.3. GSH content and related enzymes

Fig. 3 depicts the changes of GSH content and its related enzyme activities in *O. chinensis* after phoxim administration. No significant changes in the GSH content were observed in the phoxim treated *O. chinensis*. After phoxim treatment, GR and GPx activities were inhibited compared with the control. At the concentration of 0.24 µL phoxim, GR and GPx activities were the lowest and decreased significantly 40% and 41% of the control, respectively. GST activity showed irregular changes under phoxim stress. At the concentration of 0.12 µg/µL phoxim, GST activity was the highest and increased significantly 77% of the control.

4. Discussion

ACHé is the main target of OP pesticides, which functions at cholinergic synapses by terminating the chemical impulse of the neurotransmitter acetylcholine (ACh). OPs irreversibly inhibit ACHé, causing death of the insect by nerve hyperexcitability and extreme exhaustion [13]. The present study had shown that ACHé activity was inhibited after phoxim administration in *O. chinensis* as it did in the other species [1]. The inhibition caused by phoxim was not dose-dependent. The reason might be that the enzyme was extracted from the live grasshoppers treated with phoxim.

Recent studies have implicated oxidative stress as a possible causative mechanism for the non-target toxicity of OPs [14,15]. A change in SOD, CAT, GST, GPx, and GR activities was found in *O. chinensis* after phoxim treatment. This suggested that phoxim caused oxidative damage in *O. chinensis*, possibly by generating reactive-oxygen stress in the bodies.

SOD catalyses the destruction of the superoxide radical and protects oxygen-metabolizing cells against harmful effects of superoxide free radicals. The SOD activity in phoxim treated grasshoppers showed a biphasic response with an initial increase followed by a decline in its activity. The early increase in SOD activity might be considered a contingent response of *O. chinensis* to phoxim stress. However, the superoxide radicals by themselves or after their transformation to H2O2 cause an oxidation of the cysteine in the enzyme and decrease SOD activity [16]. This might be the reason that SOD activity declined at the higher concentrations of phoxim.

CAT is a hematin-containing enzyme located in peroxisomes and facilitates the removal of hydrogen peroxide (H2O2), which is metabolized to molecular oxygen (O2) and water. Therefore, the SOD–CAT system provides the first defense against oxygen toxicity. CAT activity is directly regulated by the concentration of H2O2 [17]. The present results showed that the tendency of CAT was consistent with the changes of SOD under phoxim stress. Furthermore, CAT activity showed a positive relationship with SOD activity (r = 0.9204). This indicated that H2O2 generated by SOD was removed by the induced activity of CAT [18].

GPx, responsible for enzymatic defense against hydrogen peroxide (H2O2), is strictly linked with the concentration of GSH because it catalyses the reaction between glutathione and hydrogen peroxide, resulting in the formation of glutathione disulphide (GSSG) [19]. In this study, phoxim caused a decrease in the GPx activity. The decreased activity of GPx indicated its reduced capacity to scavenge H2O2 and lipid hydroperoxides. The decreased activity of GPx may be the result of O2 production [20] or a direct action of pesticides on the synthesis of the enzyme [21]. It has been reported that trichlorfon and methidathion caused a decrease in the GPx activity [22,23].

In our study, phoxim caused an inhibition in the GR activity. GR plays a role in the antioxidant defense processes, by reducing GSSG to GSH with consumption of NADPH, thus maintaining a high intracellular GSH/GSSG ratio. According to Zhang et al. [24] and Moreno et al. [25], the inhibition of GR activity could be due to the change in the availability of NADPH in the cell.

GSTs are detoxifying enzymes that catalyze the conjugation of variety of electrophilic substrate to the thiol group of GSH, producing less toxic forms [26]. In the present study, GST activity showed an irregular change after phoxim administration. The response of GST to toxicants is controversial because it is highly dependent on the type of xenobiotic, on the concentration and time of exposure and on the species involved [27]. The changes of GST showed that its response was dependent on the concentration of phoxim.
GST is an inducible enzyme and its activity can be induced by certain pesticides [28]. Furthermore, GST activity can also be inhibited by some pesticides [29]. The increase in GST activity indicated its role in detoxification of phoxim.

GST plays a pivotal role in the maintenance of intracellular redox status and antioxidant enzyme functions. Its content is critical for cellular thiol homeostasis and cellular protection against free radicals damage [30]. In the present study, GST content in phoxim treated O. chinensis did not show significant changes in comparison with the control. This phenomenon indicated that GST content was kept at a balance between synthesis and consumption, and there was no net loss of GST after phoxim administration in O. chinensis [31]. GST content in the organisms is closely related with the activities of some enzymes, such as GR, GPx, and GST. The changes of these enzymes activities make GST content keep a dynamic balance in organisms. The reduced GPx and GR activities might be correlated with no altered changes in GST content after phoxim administration. The inhibition of GPx reduced the GST conversion to GSSG while the inhibition of GR reduced the GSSG conversion to GSH.

The most widely used index of lipid peroxidation is MDA formation often assayed with the thiobarbituric acid assay. Lipid peroxidation has been suggested as one of the molecular mechanisms related with no altered changes in GST content after phoxim administration. The inhibition of GPx reduced the GST conversion to GSSG while the inhibition of GR reduced the GSSG conversion to GSH.

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