The modulatory effect of deltamethrin on antioxidants in mice

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Abstract

Background: Deltamethrin is a \(\alpha\)-cyano pyrethroid insecticide used extensively in pest control. Although initially thought to be least toxic, a number of recent reports showed its toxicity in mammalian and non-mammalian laboratory and wildlife animal species. In fish, it is a potent oxidative stress-inducing agent. We studied the oxidative stress-inducing effect of deltamethrin in mice.

Methods: Male Swiss albino mice were orally administered 2 doses of deltamethrin viz., 5.6 and 18 mg/kg body weight (bw), for 15 days.

Results: Both the doses of deltamethrin significantly induced lipid peroxidation (LPO) in liver and kidney. Along with the induction of LPO, activities of vital antioxidant enzymes such as glutathione peroxidase (GPx), glutathione S-transferase (GST) and catalase (CAT) were also suppressed in both the tissues. Glutathione (GSH) level was also decreased. GSH decrease was more pronounced in kidney than the liver.

Conclusion: Toxicity of many chlorinated and organophosphate insecticides is mediated by the reactive oxygen species (ROS). Findings of the present investigation also suggest a role for ROS in deltamethrin toxicity. An increased LPO indicates that these ROS might have caused degradation of biomembrane in deltamethrin-exposed animals.

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Keywords: Deltamethrin; Oxidative stress; Antioxidant enzymes; Lipid peroxidation; DNA damage

1. Introduction

The use of pyrethroids as insecticidal and anti-parasitic formulations has markedly increased in last 2 decades [1,2]. The main advantages of their use are their photostability, high efficacy at low concentrations, easy disintegration and low toxicity to birds and mammals [3,4]. The selective neurotoxicity of deltamethrin is attributed to their effect on voltage sensitive sodium channels (VSSCs) [5]. Deltamethrin is globally used in crop protection and control of malaria and other vector-borne diseases [1,6]. It has a potent insecticidal activity with an appreciable safety margin [2]. However, a number of studies have demonstrated genotoxic, immunotoxic and tumorigenic effects of deltamethrin in mammalian and non-mammalian species [7–10]. Since intended use of deltamethrin involves spraying in the crop fields to control insect pests and impregnation of bednets to ward off the mosquitoes, concern has been expressed about aquatic ecotoxicological implications of its use. Recently, we have reported on oxidative stress-inducing effect of deltamethrin in a freshwater fish Channa punctata Bloch [11].

For many pesticides, induction of oxidative stress is one of the main mechanisms of their action [12,13]. The damage to membrane lipids, protein and DNA is the endpoint biomarker of oxidative stress-inducing effects of pesticides [12–14]. In our previous study on deltamethrin in fish, we observed that various adaptive and compensatory responses are also induced as a result of exposure to deltamethrin [11]. In the present investigation we report effect of 2 sub-lethal doses of deltamethrin on redox cycle enzymes and glutathione (GSH) in order to understand mammalian response to deltamethrin exposure.
2. Materials and methods

2.1. Chemicals

Butylated hydroxytoluene (BHT), 1-chloro-2,4-dinitrobenzene (CDNB), EDTA disodium salt, and sulfosalicylic acid were procured from Ameresco (Solon, OH). Dithio-bis-2-nitrobenzoic acid (DTNB), Folin reagent, glutathione reduced (GSH), glutathione reductase (GR), and NADP reduced (NADPH) were from Sigma Chemical Co. (St. Louis, MO). o-Phosphoric acid (OPA) was from CDH Chemicals (Mumbai, India). 2-Thiobarbituric acid (TBA) was from Hi-Media Lab (Mumbai) and deltamethrin was from Hoechst Schering Agro Evid Limited (Ankleshwar, India).

2.2. Animals and dose schedule

Swiss albino male mice (30±2g) were provided by the Central Animal House Facility of Jamia Hamdard. The animals were bred and maintained under standard laboratory conditions. Commercial pellet diet and water were given ad libitum. The study was approved by the Institutional Animal Ethical Committee (IAEC) of the university. Animals were divided into three groups. Each group comprised of at least 6 animals. Control animals (group I) were administered vehicle (0.2ml corn oil) orally. Treatment group animals (group II and III) were orally administered 2 doses of deltamethrin: 5.6 and 18mg/kg bw, suspended in corn oil. All the doses were given daily once for 15 days. The dose schedule is based on the preliminary investigation involving a range of doses and previous reports of deltamethrin in mice [6,8,10,15].

2.3. Biochemical investigations

After the termination of treatment, animals were sacrificed by cervical dislocation under mild anesthesia and their kidney and liver were removed and used for the measurement of GSH, lipid peroxidation (LPO) and activities of catalase (CAT), glutathione peroxidase (GPx), and glutathione S-transferase (GST). Tissues were homogenized in chilled phosphate buffer (0.1mol/l, pH 7.4) containing KCl (1.17%), using a Potter-Elvehjem homogenizer and the supernatant was centrifuged at 10,500×g for 30min at 4°C to obtain post-mitochondrial supernatant (PMS).

2.3.1. Estimation of GSH

GSH was measured by the method of Haque et al. [16]. Briefly, PMS (1ml) was precipitated with 1ml of sulfosalicylic acid (4.0%). The samples were incubated at 4°C for 1h and then centrifuged at 1200×g for 15min at 4°C. The assay mixture contained 0.2ml of filtered aliquot, 2.6ml of sodium phosphate buffer (0.1mol/l sodium phosphate buffer, pH 7.4) and 0.2ml DTNB (stock 100mmol/l in 0.1mol/l sodium phosphate buffer, pH 7.4) in a total volume of 3ml. The optical density (OD) of reaction product was measured immediately at 412nm using spectrophotometer. The GSH content is expressed as nmol GSH/g tissue.

2.3.2. Antioxidant enzyme measurements

Activities of antioxidant enzymes viz., GST, GPx, and CAT were measured by the method Haque et al. [16]. For GST activity measurement, the reaction mixture consisted of 1.675ml sodium phosphate buffer (0.1mol/l, pH 7.4), 0.2ml GSH (1mmol/l), 0.025ml of CDNB (1mmol/l) and 0.1ml PMS (10%) in a total assay volume of 2ml. The change in absorbance was recorded at 340nm and the enzyme activity was calculated as nmol CDNB conjugate formed/min/mg protein using a molar extinction coefficient of 9.6×10³/l/mol cm. For GPx activity measurement, the assay mixture consisted of 1.44ml sodium phosphate buffer, 0.1ml EDTA (1mmol/l), 0.1ml sodium azide (1mmol/l), 0.05ml of GR (1IU/ml), 0.1ml GSH (1mmol/l), 0.1ml NADPH (0.02mmol/l), 0.01ml H₂O₂ (0.25mmol/l) and 0.1ml PMS (10%) in a total volume of 2ml. Oxidation of NADPH was recorded spectrophotometrically at 340nm at room temperature. The enzyme activity was calculated as nmol NADPH oxidized/min/mg of protein, using a molar extinction coefficient of 6.223×10³/l/mol cm. For CAT activity measurement, the assay mixture consisted of 1.95ml phosphate buffer (0.1M, pH 7.4), 1ml H₂O₂ (0.09mol/l) and 0.05ml 10% PMS in final volume of 3ml. Change in absorbance was recorded at 240nm. Catalase activity was calculated in terms of nmol H₂O₂ consumed/min/mg protein.

2.3.3. LPO measurement

LPO was measured using the procedure of Uchiyama and Mihara [17]. The tissues were homogenized in chilled 0.1mol/l potassium chloride solution. The mixture consisted of 10mmol/l BHT, 0.67% TBA, 1% chilled OPA and tissue homogenate (10%). The mixture was incubated at 90°C for 45min. The absorbance of supernatant was measured at 535nm. The rate of LPO was determined as nmol of TBA-reactive substances (TBARS) formed/hg of tissue using a molar extinction coefficient of 1.56×10⁵/l/mol cm.

2.3.4. Protein estimation

Protein content in various samples was measured by the method of Lowry et al. [18].

2.3.5. Statistical analysis

Data (means±SE) were compared using one-way analysis of variance (ANOVA). Data significant at p<0.05 in ANOVA were further analyzed by post hoc Bonferroni’s multiple-comparison test to determine statistical differences between the groups. P value<0.05 was considered significant. GraphPad Prism 3 software (GraphPad Software, Inc. San Diego, CA) was used for statistical analysis.
3. Results

3.1. Effect of deltamethrin on antioxidant enzymes in liver of mice

A significant decrease in the activities of GPx, GST and CAT was observed in the liver of deltamethrin-treated animals when compared with vehicle-treated (control) animals (Table 1). Doses of 5.6 and 18 mg/kg bw of deltamethrin resulted in 30.27% (p < 0.001) and 44% (p < 0.001) decrease in GPx activity, respectively, when compared with control animals. The GST activity of 5.6 and 18 mg/kg body weight treatment groups decreased by 4.7% (p < 0.05) and 18.5% (p < 0.001), respectively, whereas CAT activity recorded a decrease of 54.3% (p < 0.001) and 64.86% (p < 0.001). At a higher dose of deltamethrin (18 mg/kg body weight), significant decrease in the activities of GST (p < 0.01) and CAT (p < 0.001) was noticed when compared with lower dose group (5.6 mg/kg body weight). There was no significant difference in GPx activities between both the deltamethrin-treated groups. Also, there was no noteworthy difference between data of vehicle-treated and normal saline animals (data not reported).

3.2. Effect of deltamethrin on antioxidant enzymes in kidney of mice

GPx, GST and CAT activities in kidney of deltamethrin-treated animals were significantly decreased when compared with vehicle-treated animals (Table 2). GPx activity recorded a decrease of 8.06% (p < 0.05) and 20.6% (p < 0.001) in animals treated with 5.6 and 18 mg/kg deltamethrin, respectively. Doses of 5.6 and 18 mg/kg bw of deltamethrin resulted in 55.40% (p < 0.001) and 64% (p < 0.001) decrease in GST activity, respectively, when compared with controls. The CAT activity also decreased by 63.27% (p < 0.001) and 69.19% (p < 0.001) in treatment groups. At higher dose (18 mg/kg), significant (p < 0.05–0.001) inhibition was observed in activities of all the enzymes when compared with lower dose (5.6 mg/kg) group data.

Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>Antioxidant enzyme activity</th>
<th>GPx</th>
<th>GST</th>
<th>CAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (vehicle)</td>
<td></td>
<td>87.19±3.32</td>
<td>143.0±5.03</td>
<td>135.2±0.82</td>
</tr>
<tr>
<td>II (5.6 mg/kg)</td>
<td></td>
<td>60.79±2.25**</td>
<td>136.33±1.56*</td>
<td>61.74±1.12**</td>
</tr>
<tr>
<td>III (18 mg/kg)</td>
<td></td>
<td>48.82±4.23**</td>
<td>116.42±1.11**</td>
<td>47.47±1.92**</td>
</tr>
</tbody>
</table>

Values are means±SE (n=6). GPx is expressed as nmol NADPH oxidized/min/mg protein, GST as nmol CDNB conjugate formed/min/mg protein, catalase as nmol H2O2 consumed/min/mg protein. Significant differences are indicated by *p < 0.05 and **p < 0.001 when compared with vehicle-treated group animals and *p < 0.01 and **p < 0.001 when compared with 5.6 mg/kg body weight of deltamethrin.

Table 2

<table>
<thead>
<tr>
<th>Group</th>
<th>Antioxidant enzyme activity</th>
<th>GPx</th>
<th>GST</th>
<th>CAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (vehicle)</td>
<td></td>
<td>141.5±4.71</td>
<td>248.80±3.02</td>
<td>168.3±2.01</td>
</tr>
<tr>
<td>II (5.6 mg/kg)</td>
<td></td>
<td>130.1±2.55*</td>
<td>110.94±0.88**</td>
<td>61.81±1.63**</td>
</tr>
<tr>
<td>III (18 mg/kg)</td>
<td></td>
<td>112.3±2.52**</td>
<td>89.72±1.85**</td>
<td>51.85±1.40**</td>
</tr>
</tbody>
</table>

Values are means±SE (n=6). GPx is expressed as nmol NADPH oxidized/min/mg protein, GST as nmol CDNB conjugate formed/min/mg protein, catalase as nmol H2O2 consumed/min/mg protein. Significant differences are indicated by *p < 0.05 and **p < 0.001 when compared with vehicle-treated group animals and *p < 0.05, bp < 0.01 and *p < 0.001 when compared with 5.6 mg/kg body weight of deltamethrin.

3.3. Effect of deltamethrin on reduced glutathione in liver and kidney of mice

GSH content in liver was significantly decreased by 43.55% (p < 0.001) to 63.67% (p < 0.001), as a result to exposure of 5.6 and 18 mg/kg deltamethrin, respectively, when compared with vehicle-treated animals (Fig. 1). GSH in kidney also decreased significantly by 93.18% (p < 0.001) and 89.23% (p < 0.001) at 5.6 and 18 mg/kg dose, respectively (Fig. 1). Comparison between data of both the treatment groups showed no significant difference in GSH content in either liver or kidney.

3.4. Effect of deltamethrin on LPO in liver and kidney of mice

LPO in liver was significantly increased by 95.83% (p < 0.001) and 130% (p < 0.001) at 5.6 and 18 mg/kg doses of deltamethrin, respectively, when compared with vehicle-treated animals (Fig. 2). The percent increase in LPO in kidney was 67.63% (p < 0.001) and 83.9% (p < 0.001) at 5.6
and 18mg/kg, respectively, over control values (Fig. 2). The LPO was also found to be significantly greater at 18mg/kg dose as compared to 5.6mg/kg dose for kidney (p < 0.05) as well as liver (p < 0.001).

4. Discussion

The orally administered deltamethrin-induced LPO and decreased various vital antioxidants in liver and kidney of mice. Both the organs showed almost a similar response. In most of the in vivo toxicity studies in mammal oral route of administration of deltamethrin has been employed, as gastrointestinal tract is the main site of its absorption. The main site of metabolism of deltamethrin is liver [1,19]. The disruption of antioxidant balance in liver may indirectly affect its activation balance and disposition of deltamethrin.

Deltamethrin induced discernible oxidative stress response in mice as measured by increased LPO. Oxidative damage has been recognized as one of the primary causes of subcellular toxicity of pesticides [12]. Studies on pyrethroid insecticides have also suggested a putative role for free radicals in LPO and other oxidative stress-mediated injuries [11,20]. In the present study liver and kidney showed almost a similar pattern of induction of LPO with greater degree of LPO at the higher dose. LPO is caused by the action of ROS. ROS also cause damage to DNA and proteins resulting in various harmful consequences [12,13]. El-Gohary et al. [21] reported deltamethrin-induced LPO and nitric oxide (NO) production in plasma of rats and its role in testicular apoptosis. Recently, Imamura et al. [22] have reported deltamethrin-induced expression of activity-dependent gene expression of brain-derived neurotrophic factor (BDNF) in SD rats. Li et al. [23] advocated a role for oxidative stress in deltamethrin-induced neurotoxicity. The findings of present investigation on liver and kidney warrant further investigation on induction of redox cycling by deltamethrin in brain and its correlation with BDNF. LPO induction was found to be dose-dependent in both the tissues. Both the liver and kidney showed an equal level of susceptibility to deltamethrin exposure. However, when the relative responses of LPO and GSH were compared, effect of deltamethrin was more pronounced in case of decrease in GSH than the LPO induction in both the tissue. Manna et al. [24] showed that deltamethrin exposure (15mg/kg for 30 days p.o.) led to 13-fold increase in LPO in rat liver. On the pattern of our observations, they also found that the activities of hepatic CAT and GSH decreased significantly in deltamethrin-treated animals. Yarsan et al. [25] have also reported on LPO inducing effect of deltamethrin in plasma and decrease in activities of Cu–Zn–SOD and GPx in erythrocytes in mice.

In the present study decrease in GSH content was found to be more pronounced in kidney than the liver. This shows that kidney is relatively more susceptible to deltamethrin toxicity than the liver. There is no report in support of this observation. Decrease in the GSH level and disruption of activities of antioxidant enzymes as reported here might contribute to histological changes. Tos-Luty et al. [15] reported on degenerative changes with inflammatory foci in mouse liver exposed to 5mg/kg of deltamethrin with more pronounced at higher dose (25mg/kg). Inflammatory changes are a clear indication of involvement of free radicals in deltamethrin-induced toxicity. Findings of the present study also suggest a role for free radicals in deltamethrin-induced heptotoxicity and nephrotoxicity. Furthermore, role of oxygen free radical-mediated oxidative stress is strengthened by the findings of Jayasree et al. [26] who have shown that administration of vitamin E (an antioxidant) was helpful in reversing the toxic effects of deltamethrin as measured by various oxidative stress biomarkers in broiler chicks. Similar studies in mammals may provide means of abrogation of deltamethrin-induced oxidative stress and its deleterious consequences.

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References
