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Effects of thiacloprid, deltamethrin and their combination on oxidative stress in lymphoid organs, polymorphonuclear leukocytes and plasma of rats

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ABSTRACT

Deltamethrin and thiachloprid are an α-cyano class pyrethroid and neonicotinoid insecticide, respectively. Recently, a pesticide combining deltamethrin and thiacloprid has also been released. In the present study, the acute and subacute toxic effects of deltamethrin, thiachlopride, and a combination of these insecticides, on the lymphoid organs (spleen, thymus and bone marrow), polymorphonuclear leukocytes (PMNs) and plasma of rats, were determined to better understand mammalian antioxidant–oxidant and inflammatory system responses. For this purpose, rats were treated orally with different doses of thiacloprid (single acute dose of 112.5 mg/kg); subacute dose of 22.5 mg/kg/day for 30 days; deltamethrin (single acute dose of 15 mg/kg); subacute dose (3 mg/kg/day for 30 days), or a combination of these pesticides. Results were compared with those from a comparable dosing regimen with the known immunosuppressive drug cyclophosphamide. Pesticide treatments caused significant changes in the levels of liver and kidney injury markers. Antioxidant enzyme (catalase and glutathione peroxidase), glutathione and plasma antioxidant levels decreased but lipid peroxidation increased in all lymphoid organs and the plasma. Glutathione-*S*-transferase and especially DT-diaphorase activity, decreased after thiacloprid treatment. Myeloperoxidase activity, carbonyl content, lipid peroxidation and total nitrite levels increased in PMNs and plasma. When evaluated as a whole, the oxidative and inflammatory stresses seen in the pesticide combination groups were not much more pronounced than in the groups treated with a single pesticide. In terms of the evaluated biochemical parameters, the pesticides showed similar effects to cyclophosphamide.

1. Introduction

Deltamethrin is a type II synthetic pyrethroid with strong, neurotoxic insecticidal activity. It inhibited humoral and cellular immune responses [28] and caused thymic atrophy in mice [15]. Thiacloprid acts on the insect nervous system as an agonist of the nicotinic acetylcholine receptor(nAChR). It is neurotoxic and belongs to the new family of the neonicotinoids [40]. In 2007, it was registered in more than 50 countries [6]. Cyclophosphamide (CP) is a cytotoxic drug and is a well known immunosuppressant in mammals [41]. Therefore it was used a positive control in this study.

The primary lymphoid organs are the thymus and bone marrow in which T- and B-lymphocytes differentiate and develop their specialised functions. The secondary lymphoid organ is the spleen which serves as a biological sieve where macrophages mature and interact with T and B cells. Testing for pesticide induced effects on the spleen indicated effects on macrophage development, antibody presentation, and cell mediated immunity [36].

Cells of the immune system are particularly sensitive to changes in antioxidant status because they carry out important functions through the generation of a high number of oxygen free radicals. Before all else circulating PMNs, derived from multipotential stem cells in the bone marrow; the major response cells of acute inflammation, during phagocytosis they generate a variety of microbicidal agents, such as reactive oxygen species (ROS) and reactive nitrogen species (RNS), including hypochlorous acid (HOCl), a highly toxic product of the myeloperoxidase–hydrogen peroxide–halide system [16].

Many xenobiotics, including pesticides, are known to produce oxidative stress in organisms e.g. Koner et al. [25] reported that *in vivo* generation of ROS by injecting xanthine and xanthine oxidase system in animal model led to suppression of humoral and cell-mediated immune responses. Therefore, the widespread use of pesticides in public health and agricultural programs has caused development of various types of cancers, organ toxicity and immunotoxicity [25,29,36,42].

In general, cells have different mechanisms to alleviate oxidative stress and repair damaged macromolecules. The primary defence is by enzymatic and non-enzymatic antioxidants, which have been shown to scavenge free radicals and ROS. The antioxi-

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dant enzymes catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione-*S*-transferese (GST), and the non-enzymatic antioxidants, including glutathione (GSH), have been shown to be significantly affected by pesticide exposure [3]. GST and DT-diaphorase (DTD) are biotransformation enzymes that exert a key role in the metabolism of many pollutants and xenobiotics [37]. For this reason, the simultaneous analysis of some of these enzymes during toxicological experiments may generate important clues about the observed effects. Nitric oxide (NO) has emerged as one of the important neurotransmitters and as a second messenger molecule in the central nervous system. An increase in NOS activity might lead to more production of peroxynitrite and hydroxyl radicals, oxidative stress, DNA damage or apoptosis [5].

There have not been any studies on the effects of either deltamethrin or tiacloprid on oxidative stress in the mammalian immune system. Therefore, the aim of the present study was to examine whether deltamethrin, thiachloprid or their combination induced oxidative damage in rat lymphoid tissues (spleen, thymus, bone marrow), plasma and PMNs.

2. Materials and methods

2.1. Chemicals

Deltamethrin (DECIS 2,5 EC insecticide, BAYER), Thiacloprid (CALYPSO OD 240, BAYER) and Cyclophosphamide ASTA Drug Co., Turkey) were purchased from commercial outlets. All other chemicals were of analytical grade and commercially available.

2.2. Animals and experimental design

Fifty-four male Wistar albino rats, each weighing 250–300 g were used in this study. They were procured, maintained and used in accordance with the Animal Welfare Act and the Guide for the Care and Use of Laboratory Animals prepared by the Ondokuz Mayis University's Animal Ethics Committee. The ethics committee approval number is 2010/41 (HADYEK/94). The animals were held in laboratory cages, with a day/night regime of 12 h, temperature range of 21 ± 1 °C, and with standard pellet food and tap water freely available during the experiment. Six rats were randomly assigned to each of six different experimental groups and received any one of two different doses of deltamethrin, thiacloprid or a combination of these, in either a single acute or subacute doses. Dose selection for deltamethrin was based on European Agency for the Evaluation of Medicinal Products (EMEA) report (2001) [19] which showed that the LD50 for deltamethrin was approximately 40 mg/kg b.w. for rats when given orally and using oil as a solvent. The doses of deltamethrin in this experiment were based on approximately 4/10 and 1/10 of this LD50 value for the acute and subacute doses, respectively. A commerical product (Proteus, BAYER) containing thiacloprid and deltamethrin at the ratio of 7.5/1 was used for the combined dose. The doses of thicloprid were therefore standardized at 7.5 times the dose of deltamethrin. A single acute thiacloprid dose of 112.5 mg/kg (aT) a subacute thiacloprid dose of 22.5 mg/kg (sT), a single acute deltamethrin dose of 15 mg/kg (aD), a subacute deltamethrin dose of 3 mg/kg (sD), or combined dose of these pesticides at the same rates [(aTD) and (sTD)] were administered by gavage to rats.

Acute cyclophosphamide (50 mg/kg b.w., i.p.) was used as a positive control (CP). The acute exposure control (aC) and subacute exposure control (sC) rats were administered the equivalent volumes of corn oil (max 1 ml per animal) instead of the single pesticide or combination. The rats were euthanized by cervical dislocation and blood samples were collected at the 24th hour and on the 30th day in the acute and subacute dose groups, respectively.

Some part of blood was used for separation of serum. Another part of blood was drawn from heart of rat with a heparinized disposable syringe and was centrifuged at 2500 rpm for 5 min for separation of plasma, mononuclear cells and erythrocytes. For isolation of PMNs the leukocyte-rich "buffy coat" was removed and then subjected to gelatin sedimentation by mixing with equal volume of 2% gelatin in 0.9% NaCl and incubation at 37 °C for 40 min [21] After centrifugation at 1000 rpm for 10 min, the cell pellet that contained the PMNs was then resuspanded in cold erythrocyte lysing solution (155 mM NH_4Cl, 2 mM $NaHCO_3$, 0.1 mM EDTA). The cell suspansion was centrifuged at 275*g* for 5 min, the supernatant was discarded, the pellet was washed tree times with Hank's balanced solution (containing sodium chloride 138 mmol/L, potassium chloride 2.7 mmol/L, disodium hydrogen phosphate 8.1 mmol/L, potassium dihydrogen phosphate 1.5 mmol/L magnesium chloride 0.6 mmol/L, calcium chloride, 1 mmol/L and glucose 10 mmol/L, pH 7.4). The PMNs suspension then homogenized using liquid nitrogen and supernatant obtained from PMNs by mild centrifugation at 250*g* for 20 min was used for biochemical analyses.

Bone marrows were ejected from femur after cutting the epiphyseal cartilage using 1 ml of ice cold Tris–HCI buffer (0.1 M, pH 7.4). After centrifuged at 1000 rpm for 3 min. pelleted marrow cells kept −80 °C until used. Packeded marrow cells homogenized using liquid nitrogen and 1000 g supernatants were used biochemical analyses.

The spleen and thymus were placed in ice cold 0.15 M NaCI solution, perfused with the same solution to remove red blood cells, blotted on filter paper, quickly weighed and stored at −80 °C until used. Tissues were centrifuged 10,000*g* for 30 min and supernatants were used for enzyme activity and other biochemical parameters.

2.3. Measurement of liver and renal injury markers

Serum aspartate transaminase (AST), alanine transaminase (ALT), urea and creatinine levels were measured with a clinical autoanalyzer (COBAS Integra 800, Roche Diagnostics, Basel, Switzerland).

2.4. Antioxidant and phase II enzymes and reduced glutathione assay in lymphoid organs

The CAT activity in lymphoid organs was measured spectrophotometrically at 240 nm by calculating the rate of degradation of H_2O_2 [1]. Enzyme activity was expressed as μmol of H_2O_2 oxidized per min per mg of protein (U/mg protein). Superoxide dismutase (EC 1.15.1.1) activity was measured by the method described by Winterbourn et al. [43], with one unit of SOD activity being defined as the amount of enzyme activity that causes 50% inhibition of nitroblue tetrazolium (NBT) reduction. The rate of inhibition of NBT reduction by superoxide generated by the photoreduction of riboflavin was determined by measuring the absorbance at 560 nm. SOD activity in lymphoid tissues was expressed as units of SOD enzyme activity per mg of protein. Glutathione peroxidase (EC 1.11.1.9) activity was measured by the coupled assay method, as described by Paglia and Valentine [33]. One unit of enzyme activity was defined as nmole of NADPH consumed per minute per milligram of protein, based on an extinction coefficient of 6.22 mM^{-1} cm^{-1}.

Glutathione-*S*-transferase (EC 2.5.1.18) activity was determined spectrophotometrically at 37 °C, according to the methodology of Habig et al. [22]. The specific activity of GST was expressed as nmole of GSH–CDNB conjugate formed per minute per mg protein, using an extinction coefficient of 9.6 mM^{-1} cm^{-1}. DT-diaphorase (quinone reductase, EC 1.6.99.2) activity was determined by the