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Oxidative damage induced by chlorpyrifos in the hepatic and renal tissue of Kunming mice and the antioxidant role of vitamin E

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ABSTRACT

Chlorpyrifos is a broad-spectrum, chlorinated organophosphate pesticide employed for pest control in various agricultural and animal husbandries. Acute and chronic exposure to CPF can elicit several adverse effects, including oxidative stress. We investigated neurotoxicity of CPF-treated mice, and evaluated the antioxidant effect of vitamin E against oxidative stress and histological changes in the livers and kidneys of CPF-treated mice. Kunming mice were divided randomly into five exposure groups of six: (A) peanut oil; (B) 3 mg/kg CPF; (C) 6 mg/kg CPF; (D) 12 mg/kg CPF; (E) vitamin E (100 mg/kg), 3 h after administration of CPF (12 mg/kg) and used as a post-treatment group. Oral administration of high-dose groups (12 mg/kg) CPF led to a significant increase in levels of reactive oxygen species, DNA–protein crosslinks, 8-hydroxy-2-deoxyguanosine and malondialdehyde, decreases in acetylcholinesterase activity and glutathione level, as well as causing hepatic and renal histopathological change. Except for AChE activity levels, administration of vitamin E to CPF-treated mice restored these biochemical parameters to within normal levels, and resulted in overall improvement in damage to livers and kidneys. These data suggest that oxidative stress is involved in CPF-induced toxicity and that vitamin E can protect against the tissue damage induced by CPF.

1. Introduction

Pesticides are used widely in the production of grains, fruits and vegetables even though they can have adverse health effects on consumers (Kaushik et al., 2009; Keikotlhaile et al., 2010). Poisoning from organophosphate insecticides from occupational and accidental exposure is an important cause of morbidity and mortality in developing countries (Eddleston et al., 2002).

[O, O-diethyl-O-(3,5,6-trichloro-2-pyridyl) phosphorothioate] is known as chlorpyrifos (CPF). It was first introduced commercially in 1965 as a conventional broad-spectrum, chlorinated organophosphate insecticide to control agriculture and domestic insect pests (Cox, 1994). In outdoor environments, residual low levels of CPF can last for long periods of time. In indoor environments, CPF can persist for several months because of the relative lack of sunlight, water and soil microorganisms that contribute to its rapid degradation in outdoor environments (Eaton et al., 2008; Wauchope et al., 2002). Hence, CPF has become an integral part of the ecosystem and is very stable. Therefore, unintentional releases to the environment and indoor use by unlicensed or untrained applicators of CPF have occasionally resulted in excessive exposure to humans (ATSDR, 1997; Cochran et al., 1995; Eskenazi et al., 1999).

In humans, organophosphate insecticides disturb biochemical and physiological functions. Like other organophosphate insecticides such as malathion and parathion, CPF is a potent nerve agent. It interferes with acetylcholinesterase (AChE), which is necessary for normal transmission in nerves. The resulting accumulation of acetylcholine in the synaptic cleft induces hyperactivity in cholinergic pathways, which leads to neurotoxicity and eventual death (Qiao et al., 2002; Meyer et al., 2004). Because of this cumulative effect, residual levels in other organisms are increased. Hence, the toxicity of CPF is not limited to the acute phase, and its chronic effects have been noted for some time. CPF elicits several chronic adverse effects, including hepatic dysfunction (Goel et al., 2005; El-Shenawy and El-Esia, 2010; Zama et al., 2007), teratogenicity (Akhtar et al., 2006) and immunological abnormalities (Albers et al., 2007; Navarro et al., 2001).

Recent studies have suggested that the acute and chronic toxicity induced by CPF may be associated with the enhanced production of reactive oxygen species (ROS), which has been proposed as a mechanism to produce oxidative stress (Bagchi et al., 1995;

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Gultekin et al., 2001). Oxidative stress was first defined as "a disruption of the pro-oxidant–antioxidant balance in favor of the former" leading to potential damage (Sies et al., 1985), which includes alterations of cellular macromolecules such as lipids, membranes, proteins and nucleic acids (Chen et al., 2013). These alterations probably transform cell function through changes in intracellular calcium or intracellular pH, and eventually lead to cell death and tissue lesions (Majhi et al., 2011).

Antioxidants are molecules that can slow down or prevent the oxidation of other molecules by ROS or other chemical events. Antioxidant defenses are extremely important because they represent the direct removal of pro-oxidants, thereby providing maximal protection for biological sites (Owuor and Kong, 2002). Antioxidants include enzymatic systems and non-enzymatic systems. Vitamin E is a non-enzymatic antioxidant. It is considered to be a major membrane-bound antioxidant employed by the cell (Burton and Ingold, 1989).

Several pesticides can produce adverse effects in the biological systems of the liver and kidney. CPF activation to its oxon is mediated by CYP450 primarily in the liver. The kidney also has a relatively high level of enzyme activity, and the role of this organ in converting xenobiotics and endogenous substances into excretable forms is considerable (Lee et al., 2008). Dermal absorption and inhalation exposure pathways are likely to be the main occupational exposures to CPF, but ingestion is likely to be the predominant pathway for exposure to the general public (especially children) (Eaton et al., 2008).

We checked the level of AChE activity in brain tissue to investigate neurotoxicity of CPF. And we evaluated the antioxidant effect of administration of vitamin E against the oxidative stress, lipid peroxidation, DNA damage, and tissue lesions induced by acute oral intoxication of CPF in experimental animals. This was achieved by recording histopathological alterations, and measuring changes in the levels of ROS, glutathione (GSH), malondialdehyde (MDA), DNA–protein crosslinks (DPC) and 8-hydroxy-2-deoxyguanosine (8-OH-dG) in liver and kidney tissue. Such changes may have implications for the care of humans accidentally exposed to CPF.

2. Materials and methods

All experimental procedures were approved by the Office of Scientific Research Management of Huazhong Normal University (Wuhan, China). The certification on Application for the Use of Animals was dated 8 November 2011 (approval identification code: CCNU-SKY-2011-008).

2.1. Chemicals and kits

Chlorpyrifos, 2′7′-dichlordihydrofluorescein (DCFH-DA), 2-thiobarbituric acid (TBA), hematoxylin and eosin (H&E), 3-Carboxy-4-nitrophenyl disulfide (DTNB), Hoechst 33258 and (+)-α-Tocopherol (vitamin E) were purchased from Sigma–Aldrich (St. Louis, MO, USA). A colorimetric kit for AchE was purchased from Jiancheng Bioengineering Institute (Nanjing, China), an enzyme-linked immunosorbent assay (ELISA) kit for 8-OHdG was purchased from R&D Systems (Minneapolis, MN, USA). All other chemicals were of the highest grade available commercially.

2.2. Animals

Male Kunming mice (7–8 weeks, 24 ± 1 g) were purchased from the Hubei Province Experimental Animal Center (Wuhan, China) and housed in standard environmental conditions (12-h light–dark cycle, 50–70% humidity, 20–25 °C). Food and water were provided *ad libitum*. Mice were quarantined for ⩾7 days before study initiation. Six mice in each group were utilized so as to minimize the number of experimental animals needed while ensuring the validity of statistical power.

2.3. Experimental groups

Different concentrations of CPF were dissolved in corn oil and administered orally in a single dose by gavage. Control mice received peanut oil. Animals were divided randomly into five exposure groups of six mice and treated every day for 7 days as follows: (A) peanut oil control; (B) 3 mg/kg CPF; (C) 6 mg/kg CPF; (D) 12 mg/kg CPF; (E) vitamin E (100 mg/kg) 3 h after administration of CPF (12 mg/kg) and used as post-treatment group. The selected low dose of the insecticide was based on previous studies in which the 1/20 median lethal dose (LD_{50}) of CPF induced biochemical alterations in mice without morbidity (Luo et al., 2011). Vitamins E administers daily was selected based on taking the Vitamins E once a day in normal human life. The vitamin E 3 h after administration CPF was selected based on vitamins E was used to improve and repair CPF-induced oxidative damage, not prevent it, in the design of our study. After 7 days, all mice were killed. Brain, livers and kidney were collected and processed according to the requirements of the tests described below.

2.4. Histopathological examinations

The hepatic and renal tissues of mice were isolated for the preparation of histopathological slides. Tissue samples were incubated in fixative (saturated 2,4,6-trinitrophenol: formalin: glacial acetic acid [15:5:1 *v*/*v*/*v*]) for 24 h at room temperature, then cut into pieces. Slides of lung tissues were stained using H&E according to standard protocols (Apgar et al., 1998). Stained pieces were embedded in paraffin, sectioned into 10-μm slices and observed using a BX51 microscope (Olympus, Tokyo, Japan). Tissue sections were examined qualitatively by two experienced pathologists in a blinded fashion.

2.5. Preparation of tissue cell suspensions and homogenates

2.5.1. Cell suspensions

Hepatic and renal issues were placed in 10 ml/g ice-cold 1 × phosphate-buffered saline (PBS; pH 7.5) and fragmented into sections of ≈1 mm^3 using dissection scissors. The mixture was filtered thrice by passing through gauze. The cell suspension was centrifuged at 200*g* for 5 min. Cells were counted using a hemacytometer and adjusted to 10^7/ml in PBS for DPC detection.

2.5.2. Tissue homogenates

Brain: Tissue was place in 10 ml/g ice-cold 1 × PBS (pH 7.5) and homogenized using a glass homogenizer. Homogenate was centrifuged at 500*g* for 10 min at 4 °C, and supernatants were collected for AchE activity detection.

Liver and kidney: Tissue was place in 10 ml/g ice-cold 1 × PBS (pH 7.5) and homogenized using a glass homogenizer. Three-quarters of the homogenate was centrifuged at 9300*g* for 10 min at 4 °C. Supernatants were collected for detection of ROS, GSH and MDA. The other quarter of the homogenate was centrifuged at 2300*g* for 10 min at 4 °C, and the supernatants collected for 8-OH-dG detection.

2.6. AchE activity detection

Levels of AchE activity in brain supernatants were measured using colorimetric kit according to manufacturer protocols, samples were analyzed using a microplate reader at a wavelength of 412 nm. The protein concentration was determined using Lowry assay (Lowry et al., 1951).

2.7. ROS assay

ROS was measured by monitoring the increasing fluorescence of DCFH-DA using a previously described procedure (Crow, 1997) with minor modifications. Supernatants were diluted 200-fold in PBS, and 100 μL of diluted supernatant was mixed with 100 μL DCFH-DA (20 μM; diluted 100-fold from stock solution dissolved in dimethyl sulfoxide (DMSO)) and placed into the well of a microplate. The reaction mixture was allowed to sit for 5 min in darkness and the ROS level detected by a fluorescence reader (FLx 800, BioTek Instruments, Vinooski, VT, USA) with an excitation wavelength go 485 nm and emission wavelength of 520 nm.

2.8. GSH depletion assay

GSH was measured using a previously described procedure (Anderson, 1985) with minor modifications. Thiols such as GSH can react with DTNB in the dark and form yellow compounds. In the case of disturbance of thiols from proteins, 10% trichloroacetic acid (TCA) was used to delimitate these proteins. Afterwards, the pH was adjusted to 7.5 to yield the color-change reaction with DTNB (60 μg ml^{-1}, diluted 50-fold from stock solution dissolved in DMSO). Experimental and standard samples were analyzed using a microplate reader at a wavelength of 412 nm. Based on the standard curve, the calculation was: GSH (nmol/L) = OD412/0.0023, where OD412 is the optical density at 412 nm.

2.9. MDA determination

MDA was measured using a previously described procedure (Janero, 1990) with minor modifications. MDA and TBA can bind to each other at the boiling point of the mixture to form pink pigments. Briefly, a 0.5-ml tissue sample was mixed evenly with 0.6% TBA solution (dissolved in 10% TCA; acidic pH was neutralized by sodium hydroxide). Proteins were precipitated and pink pigments formed in the mixture during a 15-min bath in boiling water. After a rapid cooling process, pink solutions