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Redox status in liver of rats following subchronic exposure to the combination of low dose dichlorvos and deltamethrin

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ABSTRACT

Organophosphates and pyrethroids are widely used pesticides with prominent toxicity to humans. However, their joint toxicity has not been thoroughly investigated. In this study, we investigated the oxidative damages induced by low dose dichlorvos (DDVP) and deltamethrin (DM), the representative organophosphate and pyrethroid, respectively, and their mixtures in the liver of rats for 90 consecutive days. Two oxidative stress markers, malondialdehyde (MDA) and protein carbonyl (PCO) levels, were measured to reflect the extent of lipid peroxidation and protein oxidation, respectively. DDVP, DM, and their mixtures induced levels of MDA and PCO dose-dependently, although no toxic signs and pathological changes of liver were found in the rats following 90-day exposure. DDVP and DM induced greater increase of MDA than PCO, which indicated that lipids were particularly sensitive to the oxidative damage. We found that DDVP, DM and their mixtures could inhibit the activity of two antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT). The effects of DM on SOD activity, lipid peroxidation and protein oxidation were greater than those of DDVP. The combined effect of DDVP and DM was lower than the sum of their individual effects. Thus the interaction between dichlorvos and deltamethrin may be antagonistic on the induction of oxidative stress in rat liver.

1. Introduction

Pesticides have been widely used in agriculture and household. Studies have shown that both short-term and long-term exposure to pesticides could cause serious toxicity [1–5]. The two pesticides (dichlorvos and deltamethrin) used in this study are highly effective pesticides and they belong to the two main pesticide classes: organophosphates (dichlorvos) and pyrethroids (deltamethrin). Although they have prominent neurotoxicity, liver is also the major target organ of these pesticides [6–9]. Induction of oxidative stress was found to be the main mechanism of their chronic and subchronic toxicities [8,10,11].

The toxicity of two chemicals when applied in combination was often found to be different compared to their individual toxicity [11–14]. By comparing the combined toxicity with the individual toxicity, the interactions between different compounds can be categorized as additive, synergistic or antagonistic. When the combined toxicity is greater than, less than or equal to the sum of the individual toxicity, the interaction between the chemicals was characterized as synergistic, antagonistic and additive, respectively [15,16]. Since a variety of pesticides are used on vegetables, fruits and cereals every day, people may be exposed to a mixture of pesticides. Thus the study on their combined toxicity is of importance for the risk assessments of pesticides.

A metabonomic study showed that dichlorvos (DDVP) and deltamethrin (DM) induced serum and urine metabolite changes in rats [17]. However, the joint liver toxicity of DDVP and DM and the underlying mechanisms are still unknown. In the present study, we investigated the oxidative stress in rat liver induced by three different doses of these two pesticides after a 90-day exposure.

2. Materials and methods

2.1. Chemicals

Dichlorvos (purity > 96%) was obtained from Zhangjiagang Aihua Chemical Co., Ltd (Jiangsu, China). Deltamethrin (purity > 98%) was purchased from Nanjing Ronch Chemical Co., Ltd (Nanjing, China).

Abbreviations: CAT, catalase; DDVP, dichlorvos; DM, deltamethrin; DNPH, 2,4-dinitro-phenylhydrazine; LD_{50}, half-lethal dose; MDA, malondialdehyde; PCO, protein carbonyl; SOD, superoxide dismutase; TBA, thiobarbituric acid; WST-1, sodium salt of 4-[3-(4iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio]-1,3-benzene disulfonate.

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Thiobarbituric acid and guanidine hydrochloride were obtained from Beijing Xinjingke Biotechnology Co., Ltd (Beijing, China). SOD and catalase were purchased from Beyotime (Nantong, China). Diethylenetriamine pentaacetic acid, hypoxanthine and xanthine oxidase were purchased from Sigma-Aldrich Chemical Company (St. Louis, MO, USA). WST-1 was obtained from Dojindo Laboratories (Kumamoto, Japan). All other reagents were of analytical grade and obtained from commercial providers.

2.2. Animals and treatment

Six to eight week old male Wistar rats were obtained from Weitong Lihua Laboratory Animal Technology Company (Beijing, China) and were housed individually in standard cages. Animals were acclimatized for at least 1 week before the commencement of the study. During the experiment, the environment (room temperature at 22 ± 2 °C and humidity at 50%–60%) and a light/dark cycle of 12 h were maintained. Animals had free access to water and commercially prepared laboratory animal diet.

Fifty rats were randomly assigned to 10 groups with 5 rats in each group. Previous studies showed that the acute oral half-lethal doses (LD_{50}) of dichlorvos and deltamethrin were 80 mg/kg, 128 mg/kg for male rats, respectively [18,19]. In this study we chose the doses of 1/125 LD_{50}, 1/50 LD_{50}, and 1/20 LD_{50} of each pesticide as low-, mid-, and high-dose for the pesticide treatment groups, respectively. Therefore, rats in the low-, middle-, and high-doses of DDVP groups received 0.64, 1.60, and 4.00 mg/kg/day respectively, and those in the corresponding dosage levels of DM groups received 1.02, 2.56, and 6.40 mg/kg/day respectively. The low dose mixture group of DDVP and DM received 0.64 mg/kg/day DDVP plus 1.02 mg/kg/day DM; the middle dose mixture group of DDVP and DM received 1.60 mg/kg/day DDVP plus 2.56 mg/kg/day DM. The high dose mixture group of DDVP and DM received 4.00 mg/kg/day DDVP plus 6.40 mg/kg/day DM (for details see Table 1).

All pesticides were dissolved in corn oil (1 ml/kg body weight) and administered via oral gavage. The rats in the control group received an equivalent volume of corn oil. Rats were given pesticides daily for 90 consecutive days. All animal procedures were performed in accordance with current China legislation and approved by the CAS Institute of Zoology Animal and Medical Ethics Committee.

2.3. Sample preparation and histopathology

The body weight, toxicity signs and mortality were recorded during the treatment period. After 90-day treatment, animals were subjected to anesthesia (sodium pentobarbital). Livers were removed from rats immediately after death. Each liver sample was divided into two aliquots. One aliquot was used for histopathological examination and the other to obtain homogenate for biochemical analysis. The liver samples were homogenized in cold 10 volumes of 50 mM phosphate buffer (pH 7.4) using glass homogenizers. The homogenates were centrifuged for 15 min at 3000 *g* and supernatants were collected for the biochemical determination. The liver samples for histopathological examination were fixed in 10% formalin, cut into 4-μm sections and stained with hematoxylin and eosin.

Table 1
Experimental design for joint toxicity of dichlorvos and deltamethrin (unit of dosage: mg/kg/day).

Group	Dichlorvos (DDVP)	Deltamethrin (DM)
CON	–	–
DDVP-L	0.64 (1/125 LD_{50})	–
DDVP-M	1.60 (1/50 LD_{50})	–
DDVP-H	4.00 (1/20 LD_{50})	–
DM-L	–	1.02 (1/125 LD_{50})
DM-M	–	2.56 (1/50 LD_{50})
DM-H	–	6.40 (1/20 LD_{50})
DDVP-L + DM-L	0.64 (1/125 LD_{50})	1.02 (1/125 LD_{50})
DDVP-M + DM-M	1.60 (1/50 LD_{50})	2.56 (1/50 LD_{50})
DDVP-H + DM-H	4.00 (1/20 LD_{50})	6.40 (1/20 LD_{50})

CON: control; DDVP: dichlorvos; DM: deltamethrin; L: low dose; M: middle dose; H: high dose; LD_{50}, half-lethal dose.

2.4. Serum chemistry

Standard spectrophotometric methods were used for the measurement of the following serum parameters: alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and cholinesterase (ChE) using an Autolab-PM4000 automatic analyzer (AMS Company, Rome, Italy). Briefly, 20 μl of the serum sample was used for each measurement. The samples were dispensed into sample pools and reacted with the corresponding substrate for 5 min at 37 °C. Then the amount of the reaction product was determined automatically at the corresponding wavelength (570 nm, 450 nm, 405 nm, or 412 nm, respectively, for ALT, AST, ALP, or ChE). The enzyme activity was then calculated based on the amount of reaction product and the respective standard curve.

2.5. Superoxide dismutase (SOD) activity assay

The activity of SOD in liver was measured according to the method of Peskin and Winterbourn [20] based on the measurement of WST-1 (sodium salt of 4-[3-(4iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio]-1,3-benzene disulfonate) reduction rate. The reaction mixture contained 19.3 ml assay buffer (sodium phosphate, 50 mM, pH 8.0, containing 0.1 mM diethylenetriamine pentaacetic acid and 0.1 mM hypoxanthine), 100 μl WST-1 solution (10 mM), 100 μl catalase solution (2 mg/ml) and 5 μl xanthine oxidase (4.5 mU/ml final concentration). Twenty microliters (20 μl) of diluted liver homogenate with protein concentration around 100 μg/ml was transferred into each well of a 96-well plate. Blank wells contained 20 μl 0.01 M PBS (pH 7.4). Aliquots of 200 μl reaction mixture were transferred to the sample wells and blank wells. Plates were then mixed immediately and read at 450 nm in a microplate reader at 37 °C for 5 min. The SOD activity was calculated by the SOD standard curve, which was made according to the method described above [20].

2.6. Catalase activity assay

The assay was carried out according to the method of Sinha [21]. The reaction mixture contained 0.5 ml of 0.01 M phosphate buffer (pH 7.0) and 0.4 ml of 0.2 M H_2O_2. The 0.1 ml diluted liver homogenate with protein concentration around 300 μg/ml was added to the reaction mixture, and vortexed immediately and accurately incubated for 1 min at 37 °C. Then, 2 ml of dichromate/acetic acid reagents was added to the reaction mixtures, mixed and incubated in a water bath at 100 °C for 10 minutes to terminate the reaction. The reaction solutions were cooled to room temperature and measured at 570 nm. The amount of consumed H_2O_2 was calculated by the standard curve. Catalase activity was expressed as μmoles of H_2O_2 consumed/min/mg protein.

2.7. Measurement of malondialdehyde (MDA) level

The level of malondialdehyde (MDA) in the liver was assayed according to the method of Stocks and Dorman [22]. The level of MDA was determined based on its ability to react with thiobarbituric acid (TBA). In brief, 1 ml of diluted liver homogenate (with protein concentration around 5 mg/ml) was precipitated with 0.5 ml of 20%