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Effect of DDVP on urinary excretion levels of pyrethroid metabolite 3-phenoxybenzoic acid in rats

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ABSTRACT

Pyrethroid insecticide (PYR) is used worldwide in agriculture and for indoor extermination of harmful insects. Urinary PYR metabolites (e.g. 3-phenoxybenzoic acid, 3PBA) have been used as the most sensitive biomarker for environmental PYR exposure since the late 1990s. In this study, we examined the effect of organophosphorus insecticide (OP) dichlorvos (DDVP) on excretion levels of urinary *cis*-permethrin-derived 3PBA in rats. Concentration of urinary 3PBA and *cis*-permethrin in plasma was monitored using gas chromatography–mass spectrometry and high-performance liquid chromatography after *cis*-permethrin injection (20 mg/kg) via the tail vein of rats pretreated intraperitoneally with DDVP (low dose, 0.3 mg/kg; high dose, 1.5 mg/kg). The amount of urinary 3PBA excretion over 48 h after *cis*-permethrin administration in control was 21.5 ± 5.1 μg (mean ± S.D.). In the low- and high-dose DDVP groups, the amounts of urinary 3PBA excretion were decreased to 81.1% (17.4 ± 2.7 μg) and 70.3% (15.1 ± 2.6 μg) of control, respectively. The plasma concentrations of *cis*-permethrin-derived 3-phenoxybenzyl alcohol (3PBAlc), which is a metabolite derived following hydrolysis of *cis*-permethrin, in high-dose DDVP group (0.18 ± 0.01 μg) were significantly lower than in control (0.23 ± 0.03 μg) 1 h after *cis*-permethrin injection. Both in the control and high-dose DDVP group, no differences were observed in the excretion levels of urinary 3PBA after injection of 3PBAlc (25 mg/kg, i.v.). These results suggested that the effect of DDVP on the amount of urinary 3PBA excretion was caused by the DDVP-induced modification of the *cis*-permethrin metabolic pathway. In conclusion, the possible decrease in urinary excretion level of 3PBA due to co-exposure to OPs should be considered in the biological monitoring of PYR exposure.

1. Introduction

Pyrethroid insecticides (PYRs) have been widely and effectively used for applications in agricultural settings, public health, commerce, and individual households throughout the world to increase efficiency of agricultural crop production and maintain hygienic conditions (Bardin et al., 1994). Combined application of PYRs and organophosphorus insecticides (OPs) has often been observed in agricultural and public health settings due to their quick, persistent impact, and insecticidal effectiveness against drug-resistant insects (Ozaki et al., 1984). Since it is possible that simultaneous exposure to PYRs and OPs can occur in pesticide applicators, some researchers have shown the effect of the co-exposure on the toxicodynamic interaction in experimental animals (Timchalk et al., 2005; Kanbur et al., 2008; Tuzmen et al., 2008; Zhang et al., 2010). However, few data have thus far been available about the toxicokinetic interaction of PYRs and OPs.

Numerous researchers have studied the acute and chronic mammalian toxicity of PYRs. Especially in recent years, cross-sectional studies have accumulated on the low-level and chronic toxicity of PYR exposure in human without acute toxic symptoms (Bian et al., 2004; Xia et al., 2008; Han et al., 2008; Meeker et al., 2008, 2009). The relevant studies have used urinary PYR metabolites as a biomarker of PYR exposure, since urinary metabolite concentrations can reflect individual dose levels absorbed through comprehensive exposure routes, i.e., ingestion, inhalation and dermal absorption. However, one must consider the limitation of the estimation of PYR exposure level from the urinary PYR metabolite concentration, because excretion kinetics of the urinary metabolites is a consequence of some factors including pre-

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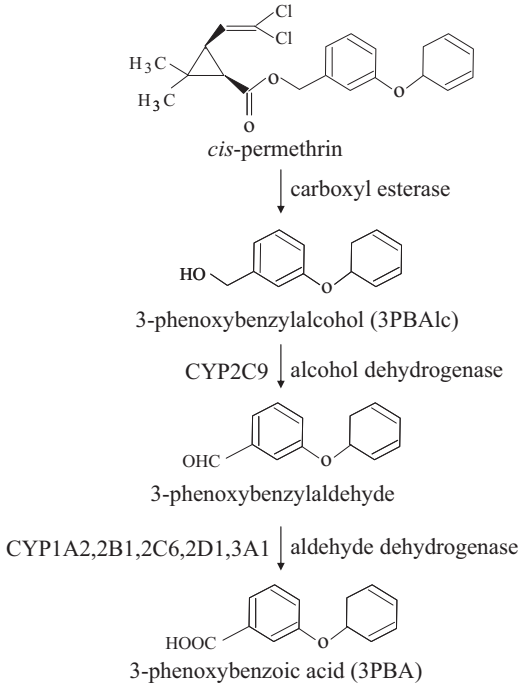

Fig. 1. The metabolic pathway of permethrin in mammals. This figure was adapted from Nakamura et al. (2007) with slight modifications.

sampling interval after exposure, the urinary concentration, and drug-metabolizing enzyme activities. Therefore, it is important to clarify the confounding factors affecting PYR metabolite levels for estimation of the PYR exposure level.

The metabolic pathway of *cis*- and *trans*-permethrin, a typical PYR compound, in mammals was shown in Fig. 1. Carboxylesterases (CEs) catalyzes hydrolysis of PYRs in a detoxification pathway. Then 3-phenoxybenzoic acid (3PBA) is derived, and it has been frequently measured in urine samples for the assessment of individual PYR exposure (Ueyama et al., 2009; Han et al., 2008; Meeker et al., 2008, 2009). CE activity is reportedly decreased by OPs (Su et al., 1971; Cohen and Ehrich, 1976), raising the possibility of alteration of PYR metabolism and urinary 3PBA excretion levels by simultaneous OP exposure. Thus, the principal aim of this study is to determine whether or not urinary excretion levels of 3PBA after PYR exposure are modified by concurrent administration of OP in rats.

2. Materials and methods

2.1. Chemicals

Dichlorvos (DDVP) standard (purity >98%), *cis*-permethrin (purity >98%), Florisil (60–100 mesh), 5,5′-dithiobis-2-dinitrobenzoic acid (DTNB) and phenylthioacetate (PTA) were obtained from Wako Pure Chemical Industries Ltd. (Osaka, Japan). 3PBA and 2PBA as an internal standard (I.S.) were purchased from Tokyo Kasei Kogyo (Tokyo, Japan). 3-phenoxybenzyl alcohol (3PBAlc) (purity >97%) and glycerol formal (mixture of 1,3-dioxan-5-ol and 4-hydroxymethyldioxolane) were purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). The solvent was glycerol formal (0.2 ml/kg) for *cis*-permethrin and 3PBAlc, and 0.9% sodium carboxymethylcellulose (0.6 ml/kg) for DDVP. Control animals received an equivalent volume of 0.9% sodium carboxymethylcellulose in place of DDVP. All other chemicals were commercially available and were of analytical reagent grade.

2.2. Animals

Eight-week-old male Wistar rats (approximately 250 g) were purchased from Japan SLC (Hamamatsu, Japan). The rats were housed under controlled environmental conditions (temperature of 23 ± 1 °C and humidity of 55 ± 5%) with a commercial food diet (Clea rodent diet CE-2, CLEA Japan, Tokyo, Japan) and water freely available to animals under a 12 h light-dark cycle (lights on from 800 to 2000 h) for at least 3 days before the experiment. The procedures involving animals and their care conformed to the international guidelines, Principles of Laboratory Animal Care (NIH publication No. 85-23, revised 1985) and Guiding Principles for the Care and Use of Laboratory Animals of Nagoya University.

2.3. Urinary 3PBA excretion levels

Intravenous injection of *cis*-permethrin and 3PBAlc and intraperitoneal injection of DDVP were adopted in this study for the following reasons. First, the effect of DDVP on bioavailability through gastrointestinal absorption of *cis*-permethrin and 3PBAlc after oral administration remains unclear. Second, the plasma concentration curves of DDVP or *cis*-permethrin after the oral route showed large variations due in part to feeding status. Third, intravenous injection of DDVP can cause a sharp increase in the blood DDVP concentration and can result in unbearable stronger cholinergic signs. Three days before the start of the experiments, rats were placed in individual metabolism cages. Each rat received *cis*-permethrin (20 mg/kg) or 3PBAlc (25 mg/kg) via tail vein. High- (1.5 mg/kg) or low-dose (0.3 mg/kg) DDVP was intraperitoneally injected 2 h before the *cis*-permethrin injection.

The doses of DDVP were set at a level which does not cause any acute symptoms such as salivation, seizures and paralysis except for plasma BChE inhibition. Urine samples were collected for 1 week after *cis*-permethrin or 3PBAlc injection at certain intervals (0–6, 6–12, 12–18, 18–24, 24–48 h and 48 h to 1 week). Collected urine samples were immediately measured for their volume and stored at −80 °C until analyses.

2.4. Enzyme assay

Plasma samples obtained from the toxicokinetic study of *cis*-permethrin were also used for monitoring butyrylcholinesterase (BChE) and CE activity. The rats were sacrificed before or 2 h after DDVP injection by exsanguinations from the abdominal aorta under light anesthesia, and the liver was taken. The preparation of microsomes was described elsewhere (Omura and Sato, 1964). Briefly, the liver (approximately 0.5 g) was homogenized at 4 °C with a Teflon homogenizer using 1.15% KCl. The homogenate was centrifuged at 12,000 × *g* for 25 min at 4 °C. The supernatant was further centrifuged at 100,000 × *g* for 90 min at 4 °C to obtain the microsomal fraction. The pellet obtained was suspended in 1.15% KCl. After the quantitative analysis, the microsome concentration was adjusted at 1 mg/ml using 1.15% KCl (microsome solution).

Activities of BChE in plasma were measured with the acetylthiocholine–DTNB procedure (Voss and Sachsse, 1970). Activities of CEs in plasma and microsome were measured according to the previous report (Basack et al., 1998). One hundred-fold dilution plasma and microsome solution were added into a glass test tube containing 2 ml of DTNB buffer. The DTNB buffer consisted of a Sörensen phosphate buffer (pH 8.0) containing 0.01% of DTNB and 0.45% of NaCl. After the test tubes were incubated at 30 °C for 5 min, 1 ml of substrate solution (1.5 mg/ml of ethanol) was added to the test tubes. Colorimetric monitoring for 405 nm was 3 and 10 min after PTA solution addition. The plasma CEs activity was represented by the extent of hydrolyzed substrate (μmol/min/ml for plasma and μmol/min/mg of protein for hepatic microsome).

2.5. Analysis of 3PBA in urine

Concentrations of 3PBA in urine were measured by a slightly modified version of GC/MS method reported previously (Leng and Gries, 2005). The apparatus used for GC/MS was a GC/MS-EI (Agilent 5975 inert MSD system) equipped with an auto sampler (Santa Clara, CA, USA). Briefly, a 50 μl urine sample was placed in a 10-ml screw-capped glass centrifuge tube to which 2 ml of water, 20 μl of I.S. solution (10 mg of 2PBA/l of acetonitrile) and 0.5 ml of 6 M HCl was added. After incubation at 100 °C for 2 h in heat block, 3 ml of *tert*-butyl methyl ether was added. After shaking vigorously, the test tube was centrifuged at 2000 × *g* for 5 min. The organic phase (upper layer) was transferred to a screw-capped glass tube. Residues were re-extracted with 2 ml of *tert*-butyl methyl ether and centrifuged. Combined extract was evaporated under a stream of nitrogen at 45 °C. The residue was dissolved in 250 μl of acetonitrile. For derivatization, 30 μl of 1,1,1,3,3,3-hexafluoroisoproanol (HFIP) was added with gentle mixing followed by addition of 20 μl of diisopropylcarbodiimide (DIC). After incubation for 10 min at room temperature with slight shaking, 1 ml of 1 M sodium hydrogen carbonate solution and 250 μl isooctane were added. The mixture was shaken vigorously and centrifuged at 3000 × *g* for 5 min. One microliter aliquot of the isooctane phase was injected into GC/MS. The GC operating conditions were as follows: GC column, HP-5MS (30 m × 0.25 mm I.D. × 0.25 μm film thickness, Agilent, USA); column temperature was modified from 70 °C (hold for 1 min) to 300 °C at the rate of 15 °C/min and hold there for 6 min; injector temperature, 250 °C; carrier gas, helium (99.99% purity); flow rate, 1 ml/min. Splitless was changed to split 15:1 at 2 min after the sample injection. The MS operating conditions were as follows: ionization source temperature, 230 °C; electron ionization, 70 eV; interface temperature, 300 °C. Selected ions for confirmation and quantification of 3PBA were *m/z* 197 and *m/z* 364. The within-run imprecision was less than 15% in the assay of the pooled urine spiked with 3PBA concentrations of 0.4, 4 and 40 μg/L ($n = 6$). The recovery rate was 96–102% 3PBA concentration at 0.4, 4 and 40 μg/L ($n = 5$).