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Characterization of deltamethrin metabolism by rat plasma and liver microsomes

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Abstract

Deltamethrin, a widely used type II pyrethroid insecticide, is a relatively potent neurotoxicant. While the toxicity has been extensively examined, toxicokinetic studies of deltamethrin and most other pyrethroids are very limited. The aims of this study were to identify, characterize, and assess the relative contributions of esterases and cytochrome P450s (CYP450s) responsible for deltamethrin metabolism by measuring deltamethrin disappearance following incubation of various concentrations (2 to 400 μM) in plasma (esterases) and liver microsomes (esterases and CYP450s) prepared from adult male rats. While the carboxylesterase metabolism in plasma and liver was characterized using an inhibitor, tetra isopropyl pyrophosphoramide (isoOMPA), CYP450 metabolism was characterized using the cofactor, NADPH. Michaelis–Menten rate constants were calculated using linear and nonlinear regression as applicable. The metabolic efficiency of these pathways was estimated by calculating intrinsic clearance (Vmax/Km). In plasma, isoOMPA completely inhibited deltamethrin biotransformation at concentrations (2 and 20 μM of deltamethrin) that are 2- to 10-fold higher than previously reported peak blood levels in deltamethrin-poisoned rats. For carboxylesterase-mediated deltamethrin metabolism in plasma, Vmax = 325.3 ± 53.4 nmol/h/ml and Km = 165.4 ± 41.9 μM. Calcium chelation by EGTA did not inhibit deltamethrin metabolism in plasma or liver microsomes, indicating that A-esterases do not metabolize deltamethrin. In liver microsomes, esterase-mediated deltamethrin metabolism was completely inhibited by isoOMPA, confirming the role of carboxylesterases. The rate constants for liver carboxylesterases were Vmax = 1981.8 ± 132.3 nmol/h/g liver and Km = 172.5 ± 22.5 μM. Liver microsomal CYP450-mediated biotransformation of deltamethrin was a higher capacity (Vmax = 2611.3 ± 134.1 nmol/h/g liver) and higher affinity (Km = 74.9 ± 5.9 μM) process than carboxylesterase (plasma or liver) detoxification. Genetically engineered individual rat CYP450s (Supersomes) were used to identify specific CYP450 isozyme(s) involved in the deltamethrin metabolism. CYP1A2, CYP1A1, and CYP2C11 in decreasing order of importance quantitatively, metabolized deltamethrin. Intrinsic clearance by liver CYP450s (35.5) was more efficient than that by liver (12.0) or plasma carboxylesterases (2.4).

Keywords: Carboxylesterases; CYP450s; Deltamethrin; Pyrethroid metabolism; Liver microsomes; Plasma; Vmax and Km; Rat

Introduction

Pyrethroid insecticides are used extensively in agriculture and public health (Casida and Quistad, 1998; Soderlund et al., 2000). As a class, pyrethroid insecticides show high insecticidal potency, tend to exhibit slow development of insect resistance, have relatively low acute toxicity in mammals, and are not persistent in the environment (reviewed in Soderlund et al., 2002). Traditionally, pyrethroid insecticides are divided into two classes (type I and II) based on structure and toxicological actions. Type I compounds do not contain a cyano group, but type II compounds do. Tremor is the major sign of

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poisoning by type I pyrethroid insecticides, while choreoathetosis and salivation are the major signs of type II poisoning (Glickman and Casida, 1982; Lawrence and Casida, 1982). Deltamethrin ((*S*)-alpha-cyano-3-phenoxybenzyl-(1*R*,*cis*)-2,2-dimethyl-3-(2,2-dibromvinyl)-cyclopropanecarboxylate) is a type II pyrethroid and is considered the most potent neurotoxic pyrethroid (Pham et al., 1984; Ruzo and Casida, 1977). Unlike most pyrethroid insecticides that exist as at least two isomers, deltamethrin is marketed as a single isomer (*cis*) (Elliott et al., 1974) and is widely used in veterinary products, as well as in agricultural formulations (ATSDR, 2003; CALEPA, 2001; Soderlund et al., 2000).

The acute toxicity, as well as cellular and molecular mechanisms of toxic action of deltamethrin, has been studied extensively (Narahashi, 1985, 1986). Following acute oral administration of 15 or 50 mg/kg to rats, profuse salivation, coarse body tremors, biting or gnawing, lacrimation, choreoathetosis, and mortality were observed (Soderlund et al., 2000). The marked neurotoxic effects of deltamethrin have been attributed to its binding to sodium channels in the brain (Narahashi, 1982, 1985; Tabarean and Narahashi, 2001) and to interaction with the GABA receptor–ionophore complex (Bloomquist et al., 1986; Crofton and Reiter, 1987; Lawrence and Casida, 1983). The parent compound is considered to be the primary neurotoxicant (Rickard and Brodie, 1985), but the toxicity of the major oxidative metabolite, 4′-OH deltamethrin (Fig. 1), is in question (Anàdon et al., 1996; Dayal et al., 2003; Gray and Rickard, 1982). Other metabolites such as 4′-OH-phenoxybenzoic acid, phenoxybenzoic acid, and thiocyanate elicit toxicity at concentrations that are not achieved from deltamethrin metabolism in vivo (NRCC, 1986).

While the toxicity of deltamethrin has been extensively characterized, toxicokinetic data for deltamethrin and most other pyrethroids are scant and incomplete. Deltamethrin is rapidly absorbed when administered orally or intraperitoneally and enters the central and peripheral nervous systems (Anàdon et al., 1996; Rickard and Brodie, 1985). Deltamethrin is metabolized and its metabolites excreted over a period of days in rats. Anàdon et al. (1996) reported the elimination half-life to be 38.5 h after an oral dosage of 26 mg/kg, while Ruzo et al. (1978) found that deltamethrin and its metabolites were largely excreted within 4 days of oral administration of 0.6–1.6 mg/kg. Ester cleavage and oxidation, primarily at the 4′ position, are the two major means of deltamethrin metabolism (Fig. 1) (Ruzo et al., 1978, 1979; Soderlund and Casida, 1977). Esterases catalyze hydrolysis of the ester bond to form relatively non-toxic acid and alcohol moieties, whereas CYP450s catalyze aromatic hydroxylation of deltamethrin at various positions, notably the 4′ position, followed by conjugation (Ruzo and Casida, 1977; Ruzo et al., 1978, 1979; Shono et al., 1979; Soderlund and Casida, 1977). Ruzo et al. (1978) characterized the metabolites of ester cleavage and CYP450 oxidation, but the metabolic rate constants and relative contribution as assessed by intrinsic clearance of these pathways are unknown.

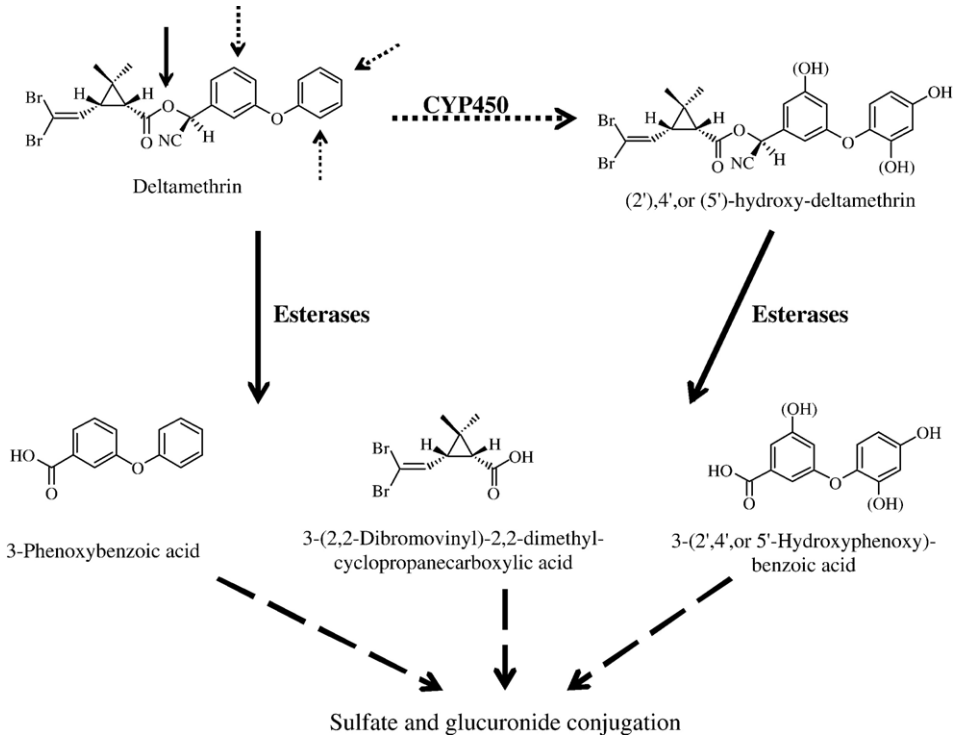

Fig. 1. Metabolism of deltamethrin. It is generally accepted that deltamethrin is detoxified by CYP450-mediated oxidation (dotted arrows) and esterase-mediated hydrolysis (solid arrows) followed by conjugation.