Identification of Rat and Human Cytochrome P450 Isoforms and a Rat Serum Esterase That Metabolize the Pyrethroid Insecticides Deltamethrin and Esfenvalerate


ABSTRACT:

The metabolism of (αS)-cyano-3-phenoxybenzyl (1R, 3R)-cis-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropane carboxylate (deltamethrin) and (αS)-cyano-3-phenoxybenzyl 2-(4-chlorophenyl)-3-methylbutyrate (esfenvalerate) by rat and human liver microsomes differs with respect to the biotransformation pathway (oxidation versus hydrolysis) responsible for their clearance. This study aims to further explore the species differences in the metabolism of these chemicals. Using a parent depletion approach, rat and human cytochromes P450 (P450s) were screened for their ability to eliminate deltamethrin or esfenvalerate during in vitro incubations. Rat P450 isoforms CYP1A1, CYP2C6, CYP2C11, and CYP3A2 and human P450 isoforms CYP2C8, CYP2C19, and CYP3A5 were capable of metabolizing either pyrethroid. Human CYP2C9 metabolized esfenvalerate but not deltamethrin. Rat and human P450s that metabolize esfenvalerate and deltamethrin do so with similar kinetics. In addition to the liver, a potential site of metabolic elimination of pyrethroids is the blood via serum carboxylesterase (CE) hydrolysis. The serum of rats, but not humans, contains significant quantities of CE. Deltamethrin and esfenvalerate were metabolized effectively by rat serum and a purified rat serum CE. In contrast, neither pyrethroid was metabolized by human serum or purified human serum esterases (acetylcholinesterase and butyrylcholinesterase). These studies suggest that the difference in rates of oxidative metabolism of pyrethroids by rat and human hepatic microsomes is dependent on the expression levels of individual P450 isoforms rather than their specific activity. Furthermore, these studies show that the metabolic elimination of deltamethrin and esfenvalerate in blood may be important to their disposition in rats but not in humans.

Pyrethroid pesticides are synthetic analogs of pyrethrins, the natural insecticidal products of Chrysanthemum cinerariaefolium. Compared with the pyrethrins, the pyrethroids display enhanced insecticidal activity, greater environmental stability, greater resistance to metabolism, and increased mammalian toxicity (Elliot, 1989; Soderlund, 1992). There is no evidence that metabolites of the pyrethrins or pyrethroids induce neurobehavioral changes or other toxic effects. Thus, the neurotoxicity of pyrethrins and pyrethroids is produced by the parent chemical (Narahashi, 1985; Smith et al., 1997). Furthermore, the toxic potency of pyrethroids in mammals is inversely related to their rates of metabolic elimination (Abernathy and Casida, 1973; White et al., 1976).

Pyrethroids are biotransformed by two pathways: cytochrome P450 (P450)-dependent oxidation and esterase-mediated hydrolysis (Soderlund and Casida, 1977). The type II pyrethroids deltamethrin and esfenvalerate are metabolized primarily by P450-dependent oxidation in mouse and rat liver microsomes (Soderlund and Casida, 1977; Godin et al., 2006). The type II pyrethroids are distinguished from type I pyrethroids by the presence of a cyano group at the α-carbon of the esterified alcohol. In human liver microsomes, esfenvalerate is metabolized primarily by P450 enzymes, whereas deltamethrin is metabolized mainly by esterase-mediated hydrolysis (Godin et al., 2006). Consistent with this finding, recombinant human carboxylesterases...
Pyrethroids (CEs) display greater enzymatic activity toward deltamethrin than esfenvalerate. The CEs appear to be the major human enzyme responsible for hepatic metabolism of deltamethrin (Godin et al., 2006; Nishi et al., 2006). The role of specific P450s in the species differences observed in human and rodent microsomal metabolism of these two chemicals is not clear. Oxidative metabolism of deltamethrin in human liver microsomes is minimal, and although oxidative metabolism of esfenvalerate is relatively efficient compared with deltamethrin, it is still considerably slower than in rat liver microsomes (Godin et al., 2006). Pyrethroids that are metabolized rapidly by esterases are typically less toxic than pyrethroids metabolized by slower oxidative pathways (Abernathy and Casida, 1973; Soderlund and Casida, 1977; Soderlund, 1992; Soderlund et al., 2002). Interindividual variability in the expression or activity of xenobiotic-metabolizing enzymes, which can be caused by genetic polymorphisms, disease state, life stage, and environmental exposures (i.e., induction or suppression of P450s), can lead to altered susceptibility in populations, particularly when a specific enzyme is responsible for the vast majority of a chemical’s clearance. Therefore, it is important to not only characterize the specific enzymes responsible for the metabolism of pyrethroids but also to understand the relative flux through each pathway to determine which is responsible for metabolic elimination of the pyrethroids. In addition to the liver, blood is a site of metabolism for pyrethroids in laboratory animals (Anand et al., 2006; Mirfazaelian et al., 2006). Rat serum contains CEs that are capable of metabolizing pyrethroids (Anand et al., 2006). The activity of serum CEs in the rat may be important in the overall pharmacokinetic disposition of pyrethroids (Anand et al., 2006; Mirfazaelian et al., 2006), particularly because pyrethroids will encounter serum CEs immediately on absorption from the gut. In contrast to laboratory animals, human serum does not contain CE activity (Li et al., 2005). Therefore, whereas blood may be an important tissue for the metabolic elimination of pyrethroids in rats, it may not be in humans. This is supported by the fact that there are currently no literature reports on the metabolism of pyrethroids in human blood or by human serum esterases.

In the present study we examined the ability of specific rat and human P450s to metabolize deltamethrin and esfenvalerate in vitro. Deltamethrin and esfenvalerate were chosen for this study because they are two of the most potent and commonly used pyrethroids. In addition, a clear difference exists in the rates of P450-mediated metabolism of deltamethrin and esfenvalerate in human liver microsomes but not in rat liver microsomes (Godin et al., 2006). Therefore, a comparison of the species-specific P450 isoforms that can biotransform these pyrethroids is an ideal approach for examining possible determinants of their rates of clearance. A potential species difference in the metabolism of deltamethrin and esfenvalerate in serum was also explored using rat and human sera and purified serum esterases. The results obtained yield a clearer understanding of the differences in the metabolism of deltamethrin and esfenvalerate between rats and humans. Furthermore, improved characterization of the important pathways that metabolize pyrethroids in rats and humans was obtained. The resulting information will be useful for human health risk assessments by decreasing the uncertainty in extrapolating laboratory animal pharmacokinetic data to humans.

Materials and Methods

Chemicals. Deltamethrin (98.9% purity) was a gift from Bayer Crop Sciences (Research Triangle Park, NC). Esfenvalerate (98.6% purity) was a gift from DuPont (Johnston, IA). Bifenthrin, used as an internal standard, was obtained from Chem Service, Inc (West Chester, PA). These chemicals were used in all the P450 assays and serum elimination studies. Deltamethrin (>98%) and esfenvalerate (>98%) used in the CE and serum metabolite formation assays were obtained from Chem Service, Inc. Liquid chromatography/mass spectrometry (LC/MS) analysis of pyrethroids from the various sources did not reveal any differences in their chemical composition. Chrysosavon acetone and methanol for LC/MS applications were purchased from Riedel-de Haen (Seelze, Germany). Ammonium formate, Trizma base, NADPH, 3-phe- noxybenzyl alcohol (3PBAiC), cis/trans-3-(2’-2’’-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid (a 1:1 mixture of cis and trans isomers; also called cis/trans-dichlororochrysanthenic acid), and 3-phenoxynbenzaldehyde (3PCHO) were purchased from Sigma-Aldrich (St. Louis, MO). Unless otherwise specified all the chemicals were of the highest grade commercially available.

Rat and Human P450s. Rat CYP1A1, CYP1A2, CYP2A1, CYP2B1, CYP2C6, CYP2C11, CYP2C12, CYP2C13, and CYP3A2 and human CYP1A2, CYP2A6, CYP2B6, CYP2E1, CYP2C8, CYP2C9*1, CYP2C9*2, CYP2C19, CYP2D6*1, CYP3A4, and CYP3A5 were purchased from BD Biosciences (Woburn, MA). The concentration of these enzymes ranged from 1000 to 2000 pmol of P450/ml.

P450-Catalyzed Elimination of Pyrethroids: Screening Assays. Each rat and human P450 was screened for its ability to oxidize and eliminate deltamethrin and esfenvalerate from the assay mixture. Assay conditions were 0.5 ml of 0.1 M Tris-HCl, pH 7.4, 10 pmol of P450/ml, and 1 mg/ml NADPH. The assay mixture containing enzyme and NADPH was preincubated for 10 min at 37°C. To initiate the reaction, 5 µl of 100 µM stock solutions of deltamethrin or esfenvalerate dissolved in 50% acetone was added for a final concentration of 1 µM pyrethroid (final concentration of acetone in each reaction was 0.5% v/v). The 1 µM concentration was previously used in microsomal clearance assays and determined to be below the Km for microsomal elimination of these pyrethroids (Godin et al., 2006). Each assay was conducted in duplicate. Reactions were carried out at 37°C over 20 min. At selected time points, 100-µl aliquots were removed, and the reaction was terminated by adding 1 ml of ice-cold 75% acetone containing 1 µg/ml bifenthrin as an internal surrogate of recovery. Samples were vortexed for 10 min and placed into autosampler vials for LC/MS analysis. LC/MS identification and quantification of pyrethroids was accomplished as described previously (Godin et al., 2006). Recovery of internal standard was greater than 95%. The duplicate samples were averaged, and values are reported as the percentage of pyrethroid eliminated over the first 10 min of incubation, a time interval for which the elimination rate was found to be linear. Control reactions were run in the absence of NADPH to verify that metabolism was enzymatic.

P450 Isozyme Kinetic Assays. The kinetic parameters Km and Vmax were determined for the P450s identified as being metabolically active toward deltamethrin and/or esfenvalerate in the initial screening assay. The assay mixture consisted of 0.5 ml of 0.1 M Tris-HCl, pH 7.4, 10 pmol of P450/ml, and 1 mg/ml NADPH. The assay buffer containing enzyme and NADPH was preincubated for 10 min at 37°C. The reaction was initiated by addition of 5 µl of substrate solution. The concentration of various substrates (0.05-2.5 mM) was used to yield final pyrethroid concentrations of 0.5 to 25 µM. Reactions were carried out for 10 min. Reactions were terminated by the addition of 1 ml of ice-cold 75% acetone containing 1 µg/ml bifenthrin. Samples were vortexed for 10 min, and LC/MS analysis was carried out as described previously. Each assay was performed in triplicate. Rates of elimination were converted to product formation velocities and plotted versus substrate concentrations. Km and Vmax parameters were determined using GraphPad Prism version 4.0 (GraphPad Software, San Diego, CA) by fitting the experimental data to the Michaelis-Menten equation by nonlinear regression. Vmax and Km were unobtainable for CYP3A5 as it displayed linear kinetics in the range of concentrations used. Therefore, all the data were also analyzed by linear regression of product formation velocities plotted against substrate concentration in the linear range to obtain catalytic efficiencies (Vmax/Km) from the slope of the regression line.

Rat and Human Serum Elimination Assays. Whole blood was collected from 30 adult male Long-Evans rats (approximately 90 days old) (Charles River, Raleigh, NC). The blood was allowed to clot for 1 h before being centrifuged at 20 min at 2000g to enable serum collection. Three pools of rat serum were generated (n = 3 samples), each from 10 different animals. Pooled human serum (10 donors/pool) was purchased from Bioreclamation (Hicksville, NY) (Lot BRH88162, Lot BRH88163) and Innovative Research (Southfield, MI) (Lot IR05-044). Rat and human sera were diluted to 50% with 0.1

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M Tris-HCl, pH 7.4, buffer. One milliliter of the 50% serum was preincubated at 37°C for 10 min before pyrethroid addition. Ten microliters of 100 μM stocks of deltamethrin or esfenvalerate was added for a final concentration of 1 μM. Serial aliquots (100 μl) were removed at 0, 20, 40, and 60 min and placed in 2 ml of ice-cold hexane containing bifenthrin as an internal surrogate of recovery. Samples were vortexed and centrifuged for 10 min. The supernatant was removed, and the extraction was repeated twice more with 2 ml of hexane. The combined extracts were evaporated to dryness under a stream of N2. Samples were reconstituted in 1 ml of 75:25 (v/v) methanol/water and placed in autosampler vials for LC/MS analysis. Assays were conducted in triplicate. LC/MS analysis was carried out as described previously (Godin et al., 2006). The concentration of pyrethroid was determined over the time course of the assay and plotted versus time. The slope of the linear regression represents the rate of elimination of pyrethroid. Values were scaled to per milliliter of serum.

**Human and Rat Serum Hydrolysis Assays.** Blood was collected from five adult male Sprague-Dawley rats (70–110 days old) (Sprague-Dawley rats were obtained from an in-house colony at Mississippi State University). The blood was allowed to stand for 1 h to clot and was subsequently centrifuged at 2000g for 20 min to enable serum collection. The sera were then pooled together to form a single pool of rat serum. Human serum obtained from a pool of adult male donors was purchased from Sigma-Aldrich. Pyrethroid hydrolysis reactions in human or rat sera were conducted as follows. The pyrethroids were preincubated in 200 to 225 μl of 50 mM Tris-HCl buffer, pH 7.4, for 5 min before adding 25 μl of pooled rat serum or 50 μl of pooled human serum to each sample. For specific activity assays the final concentration of pyrethroid in each sample was 50 μM. When variable pyrethroid concentrations were added to serum incubations, pyrethroid concentrations ranged from 5 to 100 μM. The samples were incubated at 37°C for 30 min before quenching with an equal volume of cold acetonitrile. After centrifugation, the hydrolysis products in the supernatant were analyzed by high-performance liquid chromatography (HPLC)/UV on a Surveyor LC system (Thermo Electron, San Jose, CA) using a reversed-phase HPLC column (2.1 × 100 mm, C18, Thermo Electron) as described previously for trans-permethrin (Ross et al., 2006).

**Purified Rat Serum CE.** Rat serum CE protein was purified to homogeneity as described by Crow et al. (2007). Hydrolysis reactions catalyzed by purified rat serum CE were performed in 100-μl volumes at 37°C. Varying amounts of pyrethroid (5–100 μM) were preincubated for 5 min in 50 mM Tris-HCl buffer, pH 7.4, at 37°C. The hydrolytic reactions were initiated by addition of the pure CE (2.5 μg protein/reaction). After 30 min of incubation, the reactions were quenched by the addition of an equal volume of ice-cold acetonitrile. The samples were centrifuged, and an aliquot of the supernatant was analyzed by HPLC to quantify the hydrolysis products. Rates of hydrolysis reactions catalyzed by pure CE have been shown to be linear up to 60 min (Ross et al., 2006). Nonenzymatic controls were also included and found to have negligible rates. Serum CE reactions at each substrate concentration were performed in duplicate.

**Human Serum Esteras.** Human butyrylcholinesterases (BChEs) and acetylcholinesterases (AChEs) were purchased from Sigma-Aldrich. Incubation of pyrethroid with BChEs or AChEs was done in the same manner as the reactions catalyzed by rat serum CE.

**Immunoblotting of Pooled Human Liver Microsomes: Human CE 1 and Human CE 2 Protein Levels.** The recombinant human CE proteins [human CE 1 (hCE-1) and human CE 2 (hCE-2)] were expressed in baculovirus-infected Spodoptera frugiperda cells and purified (Morton and Potter, 2000). Polyclonal antibodies against hCE-1 and hCE-2 were provided by Dr. M. Hosokawa (Chiba University, Chiba, Japan) and Dr. P. Potter (St. Jude Children’s Research Hospital, Memphis, TN), respectively. Pooled human liver microsomes from four different vendors [CellzDirect, Phoenix, AZ (Lot HMMC-PL020), Cedra, Austin, TX (Lot 821-1), Xenotech, Lenexa, KS (Lot 0310241), and BD Biosciences, San Jose, CA (Lot 26738)] were subjected to SDS-polyacrylamide gel electrophoresis using standard protocols (Ross and Borzajani, 2007). After electrophoresis, the proteins were transferred to polyvinyl difluoride membranes and probed with either anti-h-CE-1 (1:4000, v/v) or anti-h-CE-2 (1:5000, v/v) polyclonal antibody in Tris-buffered saline/5% milk. Immunocomplexes were localized on the membrane with a horseradish peroxidase-conjugated goat anti-rabbit secondary antibody and the SuperSignal West Pico chemiluminescent substrate (Pierce, Rockford, IL). The chemiluminescent signal was captured using a digital camera (gel documentation system, Alpha Innotech, San Leandro, CA). Bands on the digital images were quantified using NIH Image J software (version 1.33a). Known quantities of recombinant hCE-1 and hCE-2 proteins were loaded on the same gels to establish calibration curves.

**Kinetic Analysis and Statistics for CE Studies.** Nonlinear regression of substrate concentration versus reaction velocity plots was analyzed using SigmaPlot version 8.02 software (Systat Software Inc., San Jose, CA) by fitting experimental data to the Michaelis-Menten equation. Each substrate concentration in the kinetic experiments was evaluated in duplicate. The specific activity data obtained using pooled rat serum (n = 5 animals/pool) are reported as the mean (±S.D.) of three replicates.

**Results**

**Rat P450 Screening Assays.** The ability of rat P450 isoforms to metabolize deltamethrin and esfenvalerate was studied by evaluating the elimination of 1 μM pyrethroid. Rat CYP1A1, CYP2C6, CYP2C11, and CYP3A2 metabolized both deltamethrin and esfenvalerate (Fig. 1). However, rat CYP1A2, CYP2B1, CYP2B12, and CYP2C13 did not metabolize either compound (Fig. 1). Rat CYP2C6 eliminated the greatest percentage of deltamethrin followed by CYP1A1 > CYP2C11 > CYP3A2 (Fig. 1). Rat CYP2C6 also eliminated the greatest percentage of esfenvalerate followed by CYP2C11 > CYP3A2 > CYP1A1 (Fig. 1). CYP2C11 eliminated a significantly greater percentage of deltamethrin than esfenvalerate, whereas CYP3A2 eliminated a greater percentage of esfenvalerate than deltamethrin (Fig. 1). These P450 isoforms were chosen for this study based on their relatively high expression in rat liver microsomes (e.g., CYP2C6, CYP2C11, CYP3A2) (Güengerich et al., 1982) and because previous studies indicated they may be involved in pyrethroid metabolism (e.g., CYP1A1, CYP1A2, CYP2B1) (Dayal et al., 2001; Anand et al., 2006). CYP2C12 and CYP2C13 were chosen because of their sex-specific expression, thus allowing the potential influence of gender on the metabolism of deltamethrin and esfenvalerate to be determined.

**Human P450 Screening Assays.** The ability of human P450 isoforms to metabolize deltamethrin and esfenvalerate was also studied by evaluating the elimination of 1 μM pyrethroid. Deltamethrin was metabolized by human CYP2C8, CYP2C19, CYP3A4, and CYP3A5 (Fig. 2). Esfenvalerate was metabolized by human CYP2C8, CYP2C9*1, CYP2C9*2, CYP2C19, CYP3A4, and CYP3A5 (Fig. 2). However, human CYP1A2, CYP2A6, CYP2B6, CYP2E1, and CYP2D6*1 did not metabolize either deltamethrin or esfenvalerate (Fig. 2). CYP2C19 eliminated the greatest percentage of deltamethrin followed by CYP2C8 > CYP3A5 > CYP3A4 (Fig. 2). CYP2C19
also eliminated the greatest percentage of esfenvalerate followed by CYP2C8 > CYP2C9*1 > CYP3A5 = CYP2C9*2 > CYP3A4. CYP2C8, CYP2C19, CYP3A4, and CYP3A5 eliminated a similar percentage of esfenvalerate and deltamethrin (Fig. 2), whereas CYP2C9*1 and CYP2C9*2 metabolized esfenvalerate but not deltamethrin (Fig. 2). These P450 isoymes were chosen for study based on their relatively high expression in human liver microsomes and their known contributions to xenobiotic metabolism (Rodrigues, 1999).

Kinetic Analysis of Deltamethrin Metabolism by Rat and Human P450s. The kinetic parameters of deltamethrin metabolism by rat CYP2C6, CYP2C11, and CYP3A2 and human CYP2C8 and CYP2C19 were examined because they appear to contribute significantly to pyrethroid metabolic clearance. Rat CYP2C6 and CYP2C11 had the highest \( K_m \) and \( V_{max} \) values among rat P450s examined for deltamethrin metabolism (Table 1). Rat CYP3A2 eliminated deltamethrin at a significantly slower rate than CYP2C6 and CYP2C11; however, it also exhibited a lower \( K_m \) value (Table 1).

Human CYP2C8 and CYP2C19 have similar \( K_m \) and \( V_{max} \) values for deltamethrin (Table 1). \( K_m \) and \( V_{max} \) values were unobtainable for CYP3A5 using nonlinear regression as the data appeared to display linear kinetics across the range of pyrethroid concentrations used in these experiments. Therefore, catalytic efficiencies (\( V_{max}/K_m \)) for each enzyme were obtained from the slopes of the linear region of the substrate-velocity plots and used to compare with the catalytic efficiency for CYP3A5 (Table 1). The catalytic efficiency of CYP3A5 was found to be greater than both CYP2C8 and CYP2C19 for deltamethrin.

\[ K_m, V_{max}, \text{ and } V_{max}/K_m \text{ parameters for deltamethrin were similar for rat and human P450s. The large standard error associated with the estimates of } K_m \text{ and } V_{max} \text{ for some enzymes is likely caused by the inability to accurately determine } V_{max} \text{ because of the solubility limits of the pyrethroids under the experimental conditions used.} \]

Kinetic analysis of rat CYP1A1 was not attempted because of its very low constitutive expression in the mammalian liver (Nebert et al., 2004). In addition, because the induction of CYP1A1 is minimal in human livers (Xu et al., 2000; Silkworth et al., 2005) and deltamethrin is predominately metabolized by esterases in humans, CYP1A1 metabolism is less interesting for the purposes of this study. Because of the minimal metabolism of both deltamethrin and esfenvalerate by human CYP3A4, kinetic parameters were not determined for this P450. Therefore, it is unlikely that human CYP3A4 plays any role in the metabolism of these pyrethroids.

Kinetic Analysis of Esfenvalerate Metabolism by Rat and Human P450s. The kinetic parameters of esfenvalerate metabolism by rat CYP2C6, CYP2C11, and CYP3A2 and human CYP2C8, CYP2C9*1, and CYP2C19 were examined. Of the rat enzymes that metabolized esfenvalerate, CYP2C6 and CYP2C11 had the highest \( K_m \) and \( V_{max} \) values and were similar to the values for deltamethrin (Table 1). Rat CYP3A2 had lower \( K_m \) and \( V_{max} \) values than

<table>
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<th>Enzyme</th>
<th>( K_m ) (( \mu )M)</th>
<th>( V_{max} ) (pmol/min/pmol P450)</th>
<th>( V_{max}/K_m )</th>
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* Calculated value using kinetic parameters obtained from nonlinear regression analysis.

* Catalytic efficiency estimated from the slope of the linear regression analysis of concentration versus velocity plots.
CYP2C6 and CYP2C11 (Table 1). Of the human P450s examined each had similar $V_{\text{max}}$ values (Table 1). However, the $K_m$ for CYP2C19 was $\sim$5-to 6-fold lower than the $K_m$ for CYP2C8 and CYP2C9*1. As with deltamethrin, $K_m$ and $V_{\text{max}}$ values were unobtainable for human CYP3A5 because the data displayed linear kinetics for esfenvalerate oxidation. Therefore, the catalytic efficiency was estimated from the slope of the linear regression, and as with deltamethrin, CYP3A5 had a higher value than the other human P450s (Table 1). As previously noted, kinetic parameters for human CYP3A4 were not determined because of a lack of significant metabolism of esfenvalerate.

**Elimination of Deltamethrin and Esfenvalerate by Pooled Rat and Human Sera.** The ability of rat and human sera to metabolize deltamethrin and esfenvalerate was examined by incubating 1 mM pyrethroid in 50% rat or human sera. In rat serum, deltamethrin and esfenvalerate were eliminated at rates of $15.33 \pm 3.24$ (mean $\pm$ S.D.) and $9.97 \pm 2.94$ pmol/min/ml serum, respectively. Neither deltamethrin nor esfenvalerate was eliminated during incubation in human serum.

**Hydrolysis of Pyrethroids.** When the prototypical type I pyrethroid trans-permethrin is hydrolyzed by purified rat or human CE, the two products formed are 3PBAlc and trans-dichlorochrysanthemic acid. Both metabolites can be analyzed by HPLC (Fig. 3). Hydrolysis of the type II pyrethroid deltamethrin by purified CE liberates cis-dibromochrysanthemic acid, which is a stable metabolite, and a cyanohydrin that spontaneously converts to 3PBCHO at pH $>7$ (see scheme in Fig. 4). cis-Dibromochrysanthemic acid and 3PBCHO are also conveniently quantified by HPLC analysis (Fig. 3), and thus product formation rates can be determined. The hydrolysis of esfenvalerate also liberates the same cyanohydrin that spontaneously yields 3PBCHO. When deltamethrin is incubated with hepatic microsomes, 3PBCHO can undergo redox reactions to produce 3PBAc and 3-phenoxycinnamic acid (Fig. 4), which are likely catalyzed by alcohol and aldehyde dehydrogenases, respectively, present in the heterogeneous protein mixtures (Choi et al., 2002).

**Rates of Deltamethrin and Esfenvalerate Hydrolysis: Pooled Rat Serum and Purified Rat Serum CE.** The hydrolysis of deltamethrin and esfenvalerate by pooled rat serum and a purified rat serum CE was examined. The pooled rat serum sample and the purified rat serum CE hydrolyzed both pyrethroids but at different rates (Fig. 5, A and B). The specific activity of deltamethrin hydrolysis by the purified CE was nearly 2-fold greater than the activity for esfenvalerate (Fig. 5B). This compares well with the greater specific activity of the pooled rat serum with deltamethrin than with esfenvalerate (Fig. 5A). Concentration-velocity plots were analyzed for deltamethrin using both the pooled rat serum and the purified CE. In the
pooled serum sample, deltamethrin displayed linear kinetics (Fig. 5C). In contrast, deltamethrin displayed hyperbolic kinetics with the purified CE (Fig. 5D). The estimated $k_{cat}$ and $K_m$ values for the rat serum CE-catalyzed deltamethrin hydrolysis (Fig. 5D) were 0.48/min and 12.6 μM, respectively, and the calculated $k_{cat}/K_m$ was 38/min/mM. Furthermore, the hydrolysis of deltamethrin and esfenvalerate by two human esterases, BChE and AChE, present in human serum was also examined. No evidence of hydrolysis was detected (data not shown), which is consistent with the lack of pyrethroid elimination in human serum (see above).

Quantitative Immunoblotting: hCE-1 and hCE-2. Quantitative immunoblotting of samples of pooled human liver microsomes using anti-hCE-1 and anti-hCE-2 antibodies showed that the average amount of hCE-1 protein expressed in four separate pools of human liver microsomes was 64.4 ± 16.5 μg of hCE-1/mg of microsomal protein (mean ± S.D.) (Fig. 6). In contrast, the level of hCE-2 protein (1.4 ± 0.2 μg of hCE-2/mg of microsomal protein) in the same samples of liver microsomes was nearly 50-fold lower than the level of hCE-1 protein (Fig. 6). Thus, hCE-1 is clearly the most abundant CE isozyme in human liver microsomes and is found at much higher concentrations compared with hCE-2.

Discussion

The relative rates of oxidation and hydrolysis of the pyrethroids deltamethrin and esfenvalerate differ between human and rat hepatic microsomes (Godin et al., 2006). The current work examined the role of specific P450 isozymes responsible for deltamethrin and esfenvalerate metabolism in rat and human liver microsomes. The difference between rat and human serum hydrolysis rates and the substrate specificities of a purified rat serum CE were also examined for these pyrethroids.

In rat liver microsomes, both deltamethrin and esfenvalerate are cleared at comparable rates by P450-mediated oxidation (Godin et al., 2006). In agreement with this observation, the elimination of both compounds by rat CYP1A1, CYP2C6, CYP2C11, and CYP3A2 in the current study was similar. CYP2C6, CYP2C11, and CYP3A2 are highly expressed in rat liver (Guengerich et al., 1982) and likely contribute the bulk of the oxidative metabolism of deltamethrin and esfenvalerate in this organ. The kinetics of deltamethrin and esfenvalerate metabolism by CYP2C6, CYP2C11, and CYP3A2 were very similar (Table 1). Rat CYP2C6 and CYP2C11 displayed higher $K_m$ and $V_{max}$ values than CYP3A2, suggesting they are responsible for the largest proportion of the metabolism of pyrethroids in rat liver at saturating concentrations.
In contrast to rat liver microsomes, human liver microsomes primarily metabolize deltamethrin (hydrolysis) and esfenvalerate (oxidation) by different pathways (Godin et al., 2006). We have previously shown that hCE-1 is likely the principal enzyme responsible for human hepatic microsomal metabolism of deltamethrin (Godin et al., 2006). In contrast, esfenvalerate is not hydrolyzed efficiently by hCE-1 but is primarily metabolized by oxidative processes in human liver microsomes (Godin et al., 2006). Because of these metabolic pathway differences, it was expected that there would be a considerable variation in the substrate specificity of human P450s that are capable of metabolizing these pyrethroids. However, this was generally not the case. Deltamethrin and esfenvalerate were each metabolized at comparable rates by CYP2C8, CYP2C19, and CYP3A5 (Fig. 2). An important exception was their metabolism by the human CYP2C9 isozymes (Fig. 2; Table 1). Whereas esfenvalerate was metabolized effectively by the CYP2C9 isozymes, deltamethrin was not. Although CYP2C19 eliminated the greatest percentage of both deltamethrin and esfenvalerate (Fig. 2), CYP2C9 has the highest expression in human liver (Rodrigues, 1999). CYP2C9 is expressed at approximately 4- and 2-fold greater levels than CYP2C19 and CYP2C8, respectively, and at nearly 100-fold greater levels than CYP3A5 (Rodrigues, 1999). Thus, the higher expression level of CYP2C9 and its ability to oxidize esfenvalerate, but not deltamethrin, may account for the greater rate of oxidative metabolism of esfenvalerate by human liver microsomes (Godin et al., 2006).

In terms of the P450s that metabolized the pyrethroids investigated in this study, individual rat and human enzymes had comparable \( K_m \) and \( V_{max} \) values for deltamethrin and esfenvalerate. However, we had previously observed that the rates of pyrethroid oxidative metabolism were slower in human hepatic microsomes than in rat hepatic microsomes (Godin et al., 2006). This difference is likely caused by the levels of P450 expression in rat and human hepatic microsomes. According to Guengerich et al. (1982), expression of rat CYP2C6, CYP2C11, and CYP3A2 ranges from 300 to >1000 pmol of P450/mg of microsomal protein. In contrast, estimates of average P450 isozyme expression in human liver microsomes are much lower, ranging from 1 to 100 pmol/mg microsomal protein (Rodrigues, 1999). Thus, the abundance of P450 isozymes in rat liver compared with human liver, and not the individual enzyme’s activity or specificity, likely accounts for the difference in oxidation rates of deltamethrin and esfenvalerate that was previously observed (Godin et al., 2006).

The current study also quantified the expression of the two major CEs in human liver microsomes, hCE-1 and hCE-2 (Fig. 6). hCE-1 is robustly expressed in human liver at >60 \( \mu \)g (1000 pmol) per milligram of microsomal protein. hCE-2 is expressed at much lower levels, 1.4 \( \mu \)g (23 pmol) per milligram of microsomal protein. If one assumes the average molecular mass of a P450 is 52 kDa (Lewis, 2001), then the expression of the major individual human P450s in the liver ranges from approximately 0.05 to 5 \( \mu \)g (~1–100 pmol) of P450 enzyme per milligram microsomal protein (Rodrigues, 1999). Thus, the expression of hCE-1 is approximately 12- to 1200-fold greater than the levels of individual P450s in human liver microsomes. Therefore, the results of these studies suggest that the relative levels of expression of both hCE-1 and CYP2C9 are important determinants of the rate and pathway of metabolism of pyrethroids in human liver microsomes.

The blood is a potential site of pyrethroid metabolism. Rat serum possesses significant CE activity that can hydrolyze pyrethroids (Anand et al., 2006; Crow et al., 2007), whereas human serum lacks CE activity (Li et al., 2005). Consistent with these previous findings, deltamethrin and esfenvalerate were hydrolyzed in rat serum (see Fig. 5A). Neither pyrethroid was eliminated or hydrolyzed after incubation in human serum or with purified preparations of human AChE and BChE esterases, consistent with previous results for the pyrethroid trans-permethrin (Ross et al., 2006). The rate of the hydrolysis catalyzed by purified rat serum CE of deltamethrin was 2-fold greater than the rate of esfenvalerate (Fig. 5B). Similar results were observed in rat serum (Fig. 5A). Concentration-velocity plots for deltamethrin in rat serum revealed nonhyperbolic kinetics (Fig. 5C), which is similar to the kinetic plot observed by Anand et al. (2006) up to 100 \( \mu \)M in rat serum. However, when deltamethrin hydrolysis was studied using the purified rat serum CE, we found that it exhibited hyperbolic kinetics characteristic of a classical Michaelis-Menten enzymatic mechanism (Fig. 5D). One possible explanation to account for the discrepancy in kinetics between whole serum and purified serum CE is that deltamethrin may bind noncovalently to serum albumin, thus reducing its effective concentration available for hydrolysis by the serum CE enzyme. This could account for the much higher apparent \( K_m \) for deltamethrin when investigated in whole serum.

Hydrolase A is the most abundant rat hepatic CE (Morgan et al., 1994; Sanghani et al., 2002). The \( K_m \) value obtained for deltamethrin hydrolysis by pure rat serum CE (this study) was ~2-fold greater than the \( k_{cat} \) for hydrolase A (Godin et al., 2006). However, because of a slightly higher \( k_{cat} \) value for the serum CE, the calculated catalytic efficiencies (\( k_{cat}/K_m \)) are similar. Therefore, the contribution of rat serum CE to deltamethrin elimination is likely to be important, particularly at low serum concentrations. These results highlight a significant species difference between rats and humans with respect to pyrethroid metabolism in the blood because no hydrolysis of deltamethrin or esfenvalerate occurs in human serum.

In vitro metabolism studies using rodent and human tissues have been used to estimate in vivo pharmacokinetic parameters such as half-life and clearance (Iwatsubo et al., 1997). In vitro metabolism parameters do not always directly scale to the in vivo situation, and often a correction factor is used (Obach et al., 1997; Naritomi et al., 2001). The species differences noted in this and previous work (Godin et al., 2006) indicate that the rat may not be a good model for understanding human metabolism of all the pyrethroids. As such, any correction factor used for scaling the rodent in vitro data to in vivo may not apply to scaling the human data. However, understanding these species differences provides information on data gaps and uncertainties inherent in these extrapolations.

The results obtained in this study qualitatively and quantitatively provide information on the relative importance of the liver and blood to the metabolic clearance of pyrethroides in rats and humans, which can be used to estimate metabolism parameters in a physiologically based pharmacokinetic model. These results also address potential human variability in pyrethroid metabolism. Identifying and quantifying the role of oxidative and hydrolytic enzymes in the metabolism of pyrethroids in humans can provide insight into how variability in the expression of these enzymes will affect exposure-dose relationships. For example, the expression of both P450s and esterases can vary greatly in human populations because of genetic polymorphisms, disease states, life stage, and environmental exposures (i.e., induction or suppression of metabolizing enzymes). If a single enzyme is primarily responsible for a chemical’s metabolic elimination, variability in the expression or activity of that enzyme can lead to altered susceptibility within a subpopulation. Significant human variability exists in enzymes such as CYP2C9 (Stubbs et al., 1996) and hCE-1 (Hosokawa et al., 1995). Populations with decreased CYP2C9 expression may have slower elimination of esfenvalerate and potentially greater risk associated with those exposures. Similarly, populations with decreased hCE-1 activity could have significantly reduced deltamethrin clearance rates.