Stereoselective Degradation of alpha-Cypermethrin and Its Enantiomers in Rat Liver Microsomes

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ABSTRACT Alpha-cypermethrin (α-CP), [(RS)-a-cyano-3-phenoxy benzyl (1RS)-cis-3-(2, 2-dichlorovinyl)-2, 2-dimethylcyclopropanecarboxylate], comprises a diastereoisomer pair of cypermethrin, which are (+)-(1R-cis-αS)–CP (insecticidal) and (−)-(1S-cis-αR)–CP (inactive). In this experiment, the stereoselective degradation of α-CP was investigated in rat liver microsomes by high-performance liquid chromatography (HPLC) with a cellulose-tris- (3, 5-dimethylphenylcarbamate)-based chiral stationary phase. The results revealed that the degradation of (−)-(1S-cis-αR)-CP was much faster than (+)-(1R-cis-αS)-CP both in enantiomer monomers and *rac*-α-CP. As for the enzyme kinetic parameters, there were some variances between *rac*-α-CP and the enantiomer monomers. In *rac*-α-CP, the V_{max} and CL_{int} of (+)-(1R-cis-αS)–CP (5105.22 ± 326.26 nM/min/mg protein and 189.64 mL/min/mg protein) were about one-half of those of (−)-(1S-cis-αR)–CP (9308.57 ± 772.24 nM/min/mg protein and 352.19 mL/min/mg protein), while the K_m of the two α-CP enantiomers were similar. However, in the enantiomer monomers of α-CP, the V_{max} and K_m of (+)-(1R-cis-αS) -CP were 2-fold and 5-fold of (−)-(1S-cis-αR)-CP, respectively, which showed a significant difference with *rac*-α-CP. The CL_{int} of (+)-(1R-cis-αS)–CP (140.97 mL/min/mg protein) was still about one-half of (−)-(1S-cis-αR)–CP (325.72 mL/min/mg protein) in enantiomer monomers. The interaction of enantiomers of α-CP in rat liver microsomes was researched and the results showed that there were different interactions between the IC_{50} of (−)- to (+)-(1R-cis-αS)-CP and (+)- to (−)-(1S-cis-αR)-CP($IC_{50(-)/(+)}$ / $IC_{50(+)/(-)} = 0.61$). *Chirality 28:58–64, 2016.*

KEY WORDS: alpha-cypermethrin; stereoselectivity; liver microsomes; rat

Cypermethrin (CP) [(RS)-a-cyano-3-phenoxybenzyl (1RS)-cis-trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate], a type II synthetic pyrethroid used to control pests in industrial and agricultural situations, possesses high insecticidal activity, enhanced stability, and considerably lower mammalian toxicity compared with other pesticides.[1,2] It exerts insecticidal effects by prolonging the open time of sodium channels, which increases the duration of neuronal excitation.[3] Cypermethrin has three chiral centers at 1C and 3C in the cyclopropane carboxylic acid moiety and αC in the alcohol component, resulting in eight diastereomers showing different biological activity with different configurations. (+)-(1R-cis-αS)-CP and (+)-(1R-trans-αS)-CP showed the strongest insecticidal activity among eight diastereomers.

Alpha-cypermethrin (α-CP) is highly effective against a wide range of chewing and sucking insects (particularly Lepidoptera, Coleoptera, and Hemiptera) in crops,[4,5], made up of (+)-(1R-cis-αS)-CP and (−)-(1S-cis-αR)-CP, which are two of the four cis-isomers in cypermethrin (Fig. 1). It is considered to be 2- to 3-fold more toxic than cypermethrin.[6] Many studies about α-CP accumulation, metabolism, excretion, and toxicity in soil, water, sediment, and plants were carried out,[6–8] and the toxicity of α-CP to aquatic organisms were mostly focused on by researchers.[9,10] As for mammals, the major detoxification pathways of α-CP were hydrolysis by esterases and hydroxylation by cytochrome P450s.[11–13] In spite of its low toxicity to mammals with repeated daily oral doses of α-CP at 1/10 LD_{50}, it still altered the antioxidant status, decreased cytochrome P450 content, and resulted in histopathological changes in rats.[14] In addition, cis-DCCA and 3-PBA, metabolites of α-CP, were detected in Egyptian agriculture workers who were occupational exposed to α-CP.[3]

In fact, it cannot be ignored that α-CP contained of a pair of enantiomers which when used in the form of a racemic mixture might express stereoselectivity in different biological models like other chiral drugs. With the in-depth research of the separation method of chiral compounds, increasing studies based on different actions of enantiomers were involved. Liu et al.[15] separated eight diastereoisomers of cypermethrin successfully on two chained Chirex00G-3019-DO columns and discovered that the 1R-cis-αS-CP and 1R-trans-αS-CP contributed to almost all the toxicity to the aquatic invertebrates *Ceriodaphnia dubia* or *Daphnia magna*. As for environmental samples, the enantiomers of α-CP possess significant stereoselectivity in degradation in soil and sediment.[16] A similar phenomenon was also found in earthworm, with preferentially accumulation of the (−)-(1S-cis-α R)-enantiomer. Furthermore, an obvious difference in toxicity to earthworm between two enantiomers was observed.[5]

However, the information of biological mechanisms and metabolism or degradation trend of α-CP in enantiomeric levels in mammals are still insufficient. To supplement the

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Received for publication 6 May 2015; Accepted 27 August 2015
DOI: 10.1002/chir.22538
Published online 8 October 2015 in Wiley Online Library (wileyonlinelibrary.com).

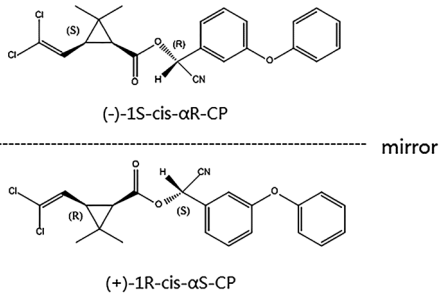

Fig. 1. The chemical structure of α-CP enantiomers.

information and explore the stereoselective behaviors of α-CP, herein we investigated the stereoselective degradation of α-CP enantiomers and the interaction of two enantiomers in vitro utilizing rat liver microsomes that mainly contained cytochrome P450s for xenobiotics metabolism. This research will provide a more thorough understanding of chiral pyrethroid pesticides and could contribute to the risk assessment of α-CP.

MATERIALS AND METHODS

Chemicals and Reagents

Rac-α-CP standard (95%) was obtained from the Institute for the Control of Agrochemicals, Ministry of Agriculture (Beijing, China). Optically pure (−)- (1S-cis-αR)-CP and (+)-(1R-cis-αS)-CP were separated by high-performance liquid chromatography (HPLC) with a cellulose-tris (3, 5-dimethylphenylcarbamate)-based chiral stationary phase (CDMPC-CSP). A stock solution of *rac*-α-CP and enantiomer monomers of α-CP standard were prepared in *n*-hexane and stored at 4 °C. Working standard solutions were obtained by dilutions of the stock solution in n-hexane. Water was purified by a Milli-Q system (Millipore, Bedford, MA). β-Nicotinamideadenine dinucleotide phosphate (NADPH) was purchased from Sigma-Aldrich (St. Louis, MO). All the mobile phase reagents were chromatographically pure from Sinopharm Chemical Reagent (Beijing, China), filtered through a 0.45-μm filter.

Preparation of Rat Liver Microsomes

Adult male Sprague–Dawley rats (200–250 g) were provided by the Vital River Laboratory Animal Technology Company (Beijing, China), housed in solid-bottom cages with hardwood chips, and acclimatized (1 week) in a humidity- and temperature-controlled room with a 12-h light/dark cycle before use. The rat liver microsomes were prepared as described in the literature[17]. The rat liver was quickly removed after the rats were anesthetized and placed in an ice-cold 1.15% KCl solution. Tissue was minced with scissors after being washed with 1.15% KCl solution to remove blood. After draining the 1.15% KCl solution, individual liver was homogenized in an ice-cold SET solution (1 mM ethylen-ediamine tetra-acetic acid [EDTA] and 50 mM Tris–HCl, pH7.4). The homogenate was centrifuged at 9700 rpm for 20 min at 4 °C and the pellet was discarded. The supernatant was centrifuged at 33,000 rpm for 60 min at 4 °C. The supernatant (cytosol) was decanted. The pellet was washed with 50 mM Tris–HCl and the homogenate was centrifuged at 33,000 rpm for 60 min at 4 °C again. The pellet was resuspended in 50 mM Tris–HCl buffer (pH 7.4) containing 20% glycerol. This procedure was used for the preparation of microsomes from Sprague–Dawley rats (male, $n = 6$). The protein concentration was determined by the method of Bradford[18] with bovine serum albumin (BSA) as the standard, and microsomes were stored at −80 °C until use.

Chromatographic Condition

Chromatography was performed using an Agilent (Palo Alto, CA) 1200 series HPLC equipped with a cellulose-tris- (3, 5-dimethylphenylcarbamate) (CDMPC)-based chiral stationary phase to separate enantiomers. G1311A pump, G1322A degasser, G1328A injector, a 20-ml sample loop, and G1314BVWD were equipped. The mobile phase was a mixture of 97% n-hexane and 3% isopropanol with a flow rate of 0.5 ml/min. Chromatographic separation was conducted at 20 °C and UV detection at 230 nm.

Methods of Experiments

A stock solution of α-CP was dried under a stream of nitrogen and dissolved with alcohol, and the volume of alcohol added to each incubate was less than 1.0% v/v. The final incubation concentration of α-CP was changed from 10 μM to 100 μM (*rac*-α-CP) or 5 μM to 40 μM (enantiomer monomers of α-CP) to obtain the Michaelis constants and the optimum concentration of enantiomers' degradation. Substrate-depletion studies in vitro were performed by incubation of α-CP in rat liver microsomes with 1 mg microsomal protein in 50 mM Tris–HCl buffer (pH 7.4) with 5.0 mM $MgCl_2$. All reaction mixtures were preincubated in a heated water bath at 37 °C for 5 min before the addition of NADPH with a concentration of 1 mg/ml (the final reaction volume was 1.0 ml). After incubation in a water bath (37 °C) for 10 min, the reactions were terminated by adding 4 ml of ice-cold ethyl acetate and the sample was whirled for 5 min. After centrifugation at 4000 rpm for 5 min, the supernatant was conveyed and aqueous solution was extracted with another 4 ml ethyl acetate again. The two-time supernatant was collected after the second centrifugation to a clean tube and evaporated to dryness under a stream of nitrogen at 35 °C, then the residue was reconstituted in 200 μl of isopropanol and filtered through a 0.22-μm filter for HPLC analysis.

According to the Michaelis constants, 10 μM of *rac*-α-CP and 5 μM of (−)-(1S-cis-αR)-CP and (+)-(1R-cis-αS)-CP were incubated in a water bath (37 °C) for 0–40 min under the same condition as the kinetic assays. Assays to assess the microsomal hydrolysis of the α-CP were conducted as described above but in the absence of NADPH.

To assess α-CP enantiomers recovery, the rat liver microsomes were inactivated by hot water (100 °C) before incubation. All of the assays were carried out in microsomes by measuring the remaining concentration of parent compounds.

Data Analysis

The degradation of *rac*-α-CP appeared to follow a first-order kinetic reaction, and the degradation rate constants were derived from "C versus t" plots by curve fitting through Origin8.0 software for the experiment. The starting point was the maximum concentration. The elimination, $t_{1/2}$, was determined by the following:

$$t_{1/2} = \frac{\ln 2}{k} \tag{1}$$

Where k is speed constant which is obtained from Eq. (2) fitted by regression analysis

$$C = C_0 e^{-kt} \tag{2}$$

The enantiomer fraction (EF) was used as a measure of the stereoselectivity of the α-CP enantiomers in vitro:

$$EF = \text{peak areas of the } \frac{(+)}{[(+)+(-)]} \tag{3}$$

where (+) and (−) are the first and second eluting enantiomers determined by the polarimeter in reference.[5] A racemic EF = 0.50, whereas preferential degradation of the (+) or (−) yields EF < 0.50 and > 0.50, respectively.

Nonlinear regression of substrate concentration versus reaction velocity curves were analyzed using Origin8.0 software by fitting experimental data to the Michaelis–Menten equation. The degradation of the α-CP enantiomers by rat liver microsomes was fitted to Eq. (4), and the K_m and V_{max} values were calculated by the following equation:

$$V = \frac{V_{max} \times S}{K_m + S} \tag{4}$$

V, S, V_{max}, and K_m represent the velocity of metabolism, substrate concentration, maximum velocity of metabolism, and Michaelis constant, respectively.

Intrinsic metabolic clearance (CL_{int}) was calculated by:

$$CL_{int} = \frac{V_{max}}{K_m} \quad (5)$$

All data are expressed as the mean ± SD of three independent experiments. Significance of difference between groups was evaluated by Dunnett's *t*-test. Statistical differences were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

Method Validation

As Figure 2 shows, the enantiomers of α-CP were separated entirely, with no endogenous interference peaks eluted at retention times in any sample. In combination with the reference, the first eluted enantiomer was (+)-(1R-cis-αS)-CP.[5] The limit of detection (LOD) and limit of quantification (LOQ) of the method for α-CP enantiomers were 0.4 μM and 1.0 μM, respectively. To obtain the linear calibration curves, the α-CP with concentrations ranging from 1.0 μM to 100 μM were incubated in inactivated rat liver microsomes. The linear calibration curves of (+)-(1R-cis-αS)-CP and (−)-(1S-cis-αR)-CP were $y = 1.0374x - 0.9533$ ($R^2 = 0.9976$) and $y = 1.0195x - 0.4602$ ($R^2 = 0.9992$), respectively. The accuracy and precision of the assay for both enantiomers are shown in Table 1 ($n = 6$), ranging from 81.73% to 93.98% (accuracy) and 0.8% to 7.9% (RSD) over the entire calibration range. The method recovery rate ranged from 82.18% to 90.74% for α-CP enantiomers in rat liver microsomes and are summarized in Table 2 ($n = 3$).

Kinetic Degradation in Rat Liver Microsomes

The degradation curve of *rac*-α-CP (10 μM) for 40 min in rat liver microsomes is shown in Figure 3. The results demonstrated that the degradation of α-CP enantiomers in rat liver microsomes was in accordance with the first-order kinetics equation. Fitting the curve of the parent compound concentration with the reaction time of α-CP enantiomers, the regression equations of (+)-(1R-cis-αS)-CP and (−)-(1S-cis-αR)-CP were $y = 20.8046\ e^{0.0068x}$ ($R^2 = 0.9784$) and $y = 20.0628\ e^{0.0301x}$ ($R^2 = 0.9712$), respectively. The $t_{1/2}$ of (+)-(1R-cis-αS)-CP and (−)-(1S-cis-αR)-CP calculated by regression equations were 101.93 min and 23.03 min, respectively, indicating significant stereoselectivity in degradation rates between the two enantiomers of *rac*-α-CP. Consistently selective degradation of a single enantiomer monomer of α-CP (5 μM) for 40 min in rat liver microsomes was observed, as shown in Figure 4, while the regression equations of (+)-(1R-cis-αS)-CP and (−)-(1S-cis-αR)-CP were $y = 19.6398\ e^{0.0147x}$ ($R^2 = 0.9933$) and $y = 17.6710e^{0.0334x}$ ($R^2 = 0.9541$), respectively. The $t_{1/2}$ of single enantiomer (+)-(1R-cis-αS)-CP was 47.15 min, which revealed a remarkable discrepancy compared with (+)-(1R-cis-αS)-CP in *rac*-α-CP. However, the $t_{1/2}$ of optically pure (−)-(1S-cis-αR)-CP was 20.75 min, which was approximate to (−)-(1S-cis-αR)-CP in *rac*-α-CP. Consequently, the degradation rate of (−)-(1S-cis-αR)-CP was much faster than (+)-(1R-cis-αS)-CP in both *rac*-α-CP and optically pure monomers of α-CP. There might be some interaction between the pair of enantiomers of α-CP, leading to a noteworthy slower degradation of (+)-(1R-cis-αS)-CP with (−)-(1S-cis-αR)-CP exiting than (+)-(1R-cis-αS)-CP exiting solely in rat liver microsomes.

The EF of α-CP incubated with NADPH increased with time over 40 min (Fig. 5), whereas the EF incubated without NADPH maintained at 0.5, and the concentration of α-CP enantiomers was almost constant, which suggested that NADPH was the indispensable condition for the reaction. The reaction of NADPH-dependent means that the degradation of α-CP in rat liver microsomes was oxidation, consistent with the research of Scollon et al.[13] However, it is noteworthy that the cypermethrin was metabolized primarily by hydrolysis (NADPH-independent) in human hepatic microsomes in Scollon et al.'s research, which means that the species differences and enantiomers interaction in the metabolism of cypermethrin should be considered in the toxicity assessment and development of pharmacokinetic and pharmacodynamic models.

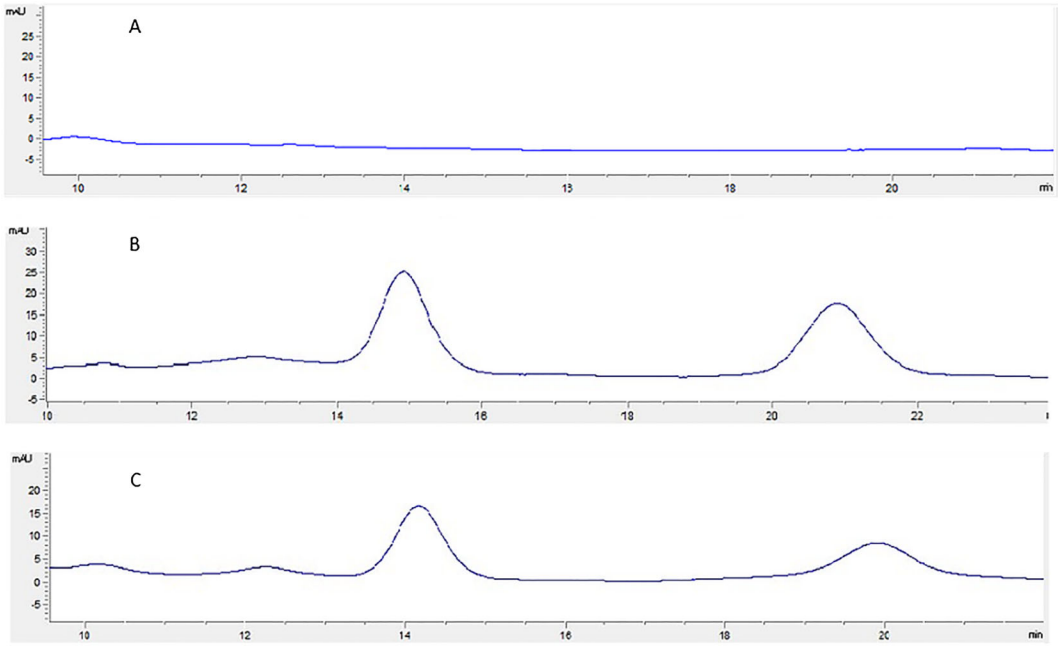

Fig. 2. HPLC chromatograms of α-CP enantiomers; (**A**) Extract from rat liver microsomes sample after incubation for 40 min without α-CP. (**B**) Standard sample of 10 μM rac-α-CP in rat liver microsomes. (**C**) Extract from rat liver microsomes sample after incubation for 30 min with 10 μM rac-α-CP.

TABLE 1. The accuracy and precision of α-CP enantiomers in rat microsomes ($n = 6$)

	(+)-(1R-cis-As)-CP			(−)-(1S-cis-αR)-CP		
Concentration (μM)	Concentration found	Accuracy (%)	RSD (%)	Concentration found	Accuracy (%)	RSD (%)
Within-day						
0.5	0.43 ± 0.09	85.32	4.6	0.41 ± 0.03	81.73	1.4
40.0	34.64 ± 2.71	86.61	1.6	35.85 ± 3.21	89.62	1.8
100.0	89.41 ± 6.59	89.41	1.5	91.39 ± 8.12	91.39	1.8
Day-to-day						
0.5	0.47 ± 0.10	94.3	4.4	0.45 ± 0.17	89.68	7.8
40.0	36.69 ± 6.18	91.72	6.7	37.59 ± 6.67	93.98	7.1
100.0	90.75 ± 3.79	90.75	4.2	92.55 ± 4.03	92.55	4.4

TABLE 2. Method recovery data for α-CP enantiomers in rat microsomes ($n = 3$)

	(+)-(1R-cis-As)-CP		(−)-(1S-cis-αR)-CP	
Concentration (μM)	Accuracy (%)	RSD (%)	Accuracy (%)	RSD (%)
0.5	87.98 ± 0.97	1.1	82.18 ± 1.24	1.5
40.0	87.05 ± 0.98	1.1	90.07 ± 1.40	1. 6
100.0	88.94 ± 0.72	0.8	90.74 ± 0.61	0.7

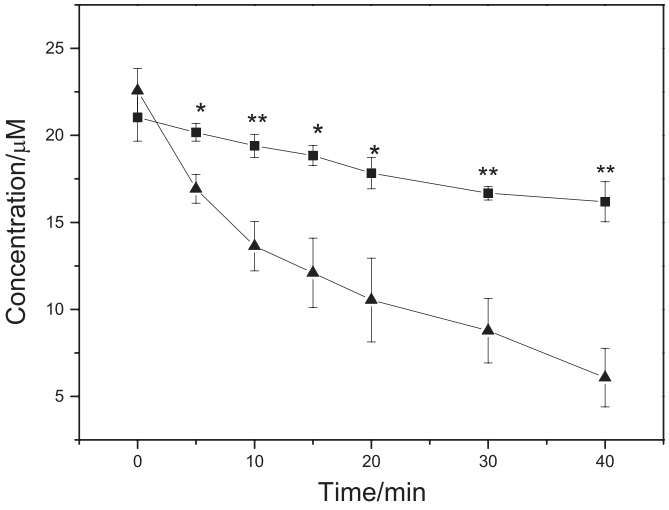

Fig. 3. The degradation curve of rac-α-CP (10 μM) for 40 min in rat liver microsomes ■ - the concentration-time of (+)-(1R-cis-αS) - CP, ▲ - the concentration-time of (−)-(1S-cis-αR)-CP. Significantly different from the values of (−)-isomer value at $*P < 0.05$ and $**P < 0.01$.

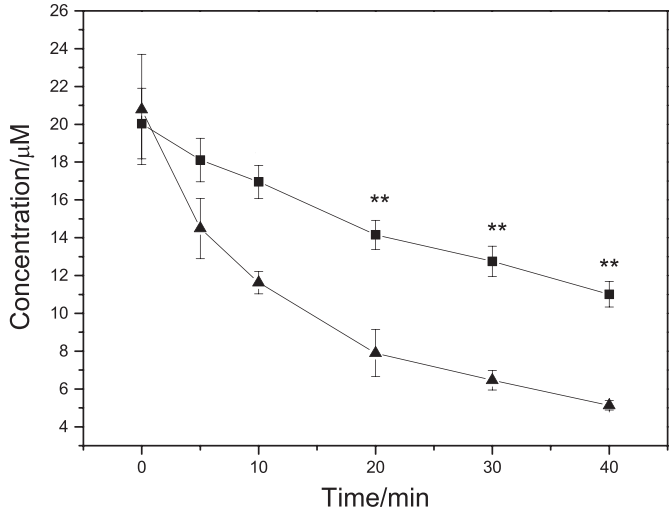

Fig. 4. The degradation curve of single enantiomer of α-CP (5 μM) for 40 min in rat liver microsomes. ■ - the concentration-time of(+)-1R-cis-αS-CP, ▲ - the concentration-time of (−)-(1S-cis-αR)-CP. Significantly different from the values of (−)-isomer value at $**P < 0.01$.

Enzyme Kinetic and Interaction Effects of α-CP Enantiomers

To elucidate the mechanism of stereoselective toxicokinetics and make a thorough inquiry about the variance of (+)-(1R-cis-αS)-CP between *rac*-α-CP and enantiomer monomers of α-CP, a Michaelis–Menten model was applied to different initial concentrations of *rac*-α-CP (5–100 μM) and a single enantiomer ((+)-(1R-cis-αS)-CP and (−)-(1S-cis-αR)-CP (2.5-40 μM)) in rat liver microsomes (Figs. 6 and 7). The enantiomers' enzymatic affinity (K_m), maximum reaction speed (V_{max}), and intrinsic clearance (CL_{int}) of *rac*-α-CP (Table 3) and the single enantiomer of α-CP (Table 4) were calculated via fitting the Michaelis-Menten equation.

The V_{max} of (+)-(1R-cis-αS)-CP (5105.22 ± 326.26 nM/min/mg protein) was about one-half of (−)-(1S-cis-αR)-CP (9308.57 ± 772.24 nM/min/mg protein) after the incubation of *rac*-α-CP enantiomers for 10 min in rat liver microsomes. The CL_{int} of (+)-(1R-cis-αS)-CP, which stands for an ability of an organism to eliminate a particular chemical had the same tendency with $V_{max.}$ Even though the K_m, which means enantiomers' enzymatic affinity expressed resembling closely in *rac*-α-CP, the results of other parameters indicated that the rat liver microsomes in vitro had a stronger potency to eliminate (−)-(1S-cis-αR)-CP than (+)-(1R-cis-αS)-CP.

Nevertheless, when (+)-(1R-cis-αS)-CP and (−)-(1S-cis-αR)-CP were incubated individually for 10 min in rat liver microsomes, the V_{max} of (+)-(1R-cis-αS)-CP (30820.95 ± 5236.65 nM/min/mg protein) was about 2-fold of (−)-(1S-cis-αR)-CP (14488.03 ± 237.09 nM/min/mg protein), and the K_m of (+)-(1R-cis-αS)-CP was about 5-fold of (−)-(1S-cis-αR)-CP at the same time. These results suggested that the (−)-(1S-cis-αR)-CP had a more cohesive affinity with metabolic enzymes. As

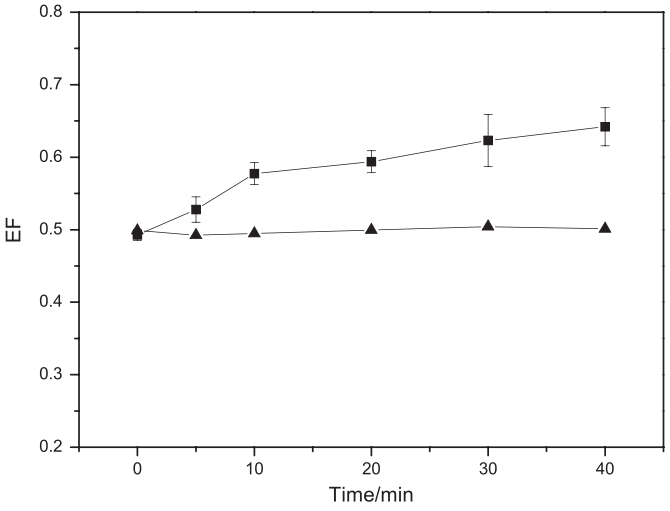

Fig. 5. The enantiomer fraction of rac-α-CP (10 μM) in rat liver microsomes after incubated for 40 min. ■ - incubation with NADPH, ▲ - incubation without NADPH.

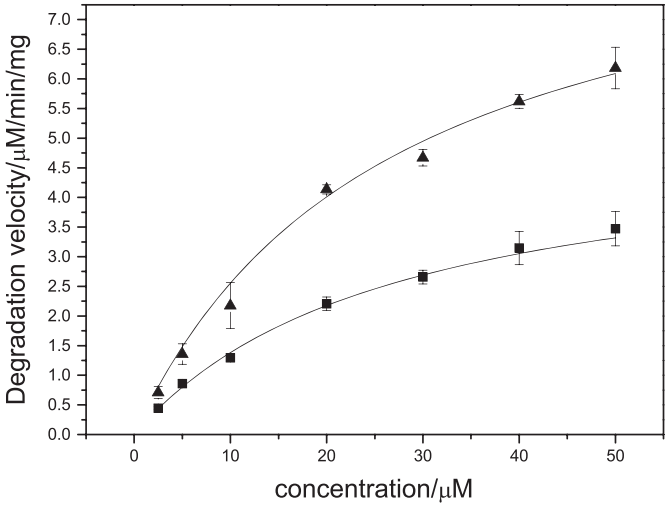

Fig. 6. Michaelis-Menten kinetic analyses of rac-α-CP enantiomers in rat liver microsomes. ■ - the degradation velocity of (+)-(1R-cis-αS)-CP, ▲ - the degradation velocity of (−)-(1S-cis-αR)-CP.

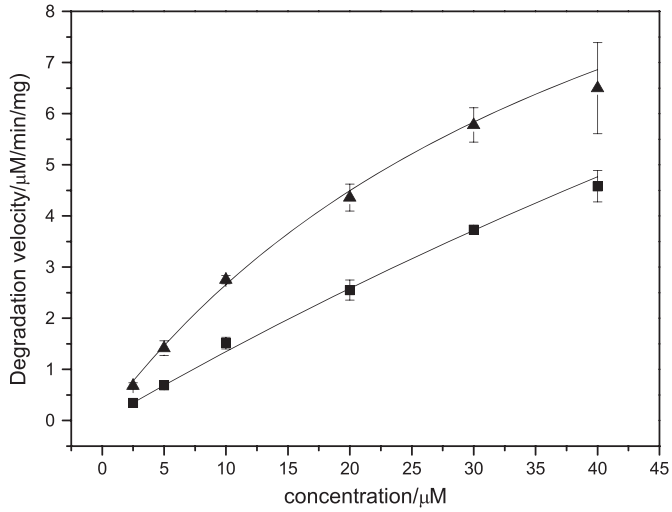

Fig. 7. Michaelis-Menten kinetic analyses of single enantiomer of α-CP in rat liver microsomes ■ - the degradation velocity of (+)-(1R-cis-αS)-CP, ▲ - the degradation velocity of (−)-(1S-cis-αR)-CP.

TABLE 3. Metabolic parameters of *rac*-α-CP enantiomers incubated in rat liver microsomes

Sample in rat microsomes	V_{max}(nM/min/mg protein)	K_m (μM)	CL_{int}(mL/min/mg protein)
(+)-(1R-cis-αS)-CP	5105.22 ± 326.26[b]	26.92 ± 2.63[a]	189.64
(−)-(1S-cis-αR)-CP	9308.57 ± 772.24	26.43 ± 4.40	352.19

[a]Significantly different from the values of single (+)-isomer at $P < 0.05$.
[b]Significantly different from the values of (−)-isomer and single (+)-isomer at $P < 0.05$.

TABLE 4. Metabolic parameters of enantiomer monomers of α-CP incubated in rat liver microsomes

Sample in rat microsomes	V_{max}(nM/min/mg protein)	K_m (μM)	CL_{int}(mL/min/mg protein)
(+)-(1R-cis-αS)-CP	30820.95 ± 5236.65[a]	218.61 ± 39.62[a]	140.97
(−)-(1S-cis-αR)-CP	14488.03 ± 237.09	44.48 ± 9.37	325.72

[a]Significantly different from the values of (−)-isomer at $P < 0.01$.

for CL_{int} of the single enantiomer of α-CP, (+)-(1R-cis-αS)-CP was about half of (−)-(1S-cis-αR)-CP, which was almost similar to *rac*-α-CP.

The enzyme kinetic of (+)-(1R-cis-αS)-CP and (−)-(1S-cis-αR)-CP were different in the single enantiomer and *rac*-α-CP, which showed that some mutual effect on the degradation of the two enantiomers of α-CP may exist. The study of enantiomers interaction verified the mutual effect of (+)-(1R-cis-αS)-CP and (−)-(1S-cis-αR)-CP subsequently. A single (+)-(1R-cis-αS)-CP enantiomer (5 μM) with different concentrations (0–20 μM) of (−)-(1S-cis-αR)-CP were added into rat liver microsomes to study the inhibiting effect of (−)-(1S-cis-αR)-CP to (+)-(1R-cis-αS)-CP. For study of the inhibiting effect of (+)-(1R-cis-αS)-CP to (−)-(1S-cis-αR)-CP, (+)-(1R-cis-αS)-CP concentrations were set from 0 to 80 μM. The inhibitory effects of single α-CP enantiomers to its antipode are shown in Figure 8, the $IC_{50\ (-)/(+)}$ for (−)-(1S-cis-αR)-CP inhibiting (+)-(1R-cis-αS)-CP and the $IC_{50\ (+)/(-)}$ for (+)-(1R-cis-αS)-CP inhibiting (−)-(1S-cis-αR)-CP were calculated by EXCEL. The $IC_{50\ (-)/(+)}$ was 24.89 μM, which was lower than that of $IC_{50(+)/(-)}$ (40.73 μM). The results of the enantiomers interaction demonstrated that the inhibiting effect of (−)-(1S-cis-αR)-CP to (+)-(1R-cis-αS)-CP was more sensitive, and a lower concentration of (−)-(1S-cis-αR)-CP had a greater effect on the degradation of (+)-(1R-cis-αS)-CP in rat liver microsomes. Maybe the stronger inhibiting effects of (−)-(1S-cis-αR)-CP to (+)-(1R-cis-αS)-CP is one of the reasons that the differences in degradation rate and enzyme kinetic of (+)-(1R-cis-αS)-CP between single enantiomer and *rac*-α-CP were larger than (−)-(1S-cis-αR)-CP.

It has been shown that isomerization induced by light, heat, and organic solvents would occur for some pyrethroid insecticides,[19] while the isomerization of CP enantiomers was found to be relatively small in our experiments (<5%).

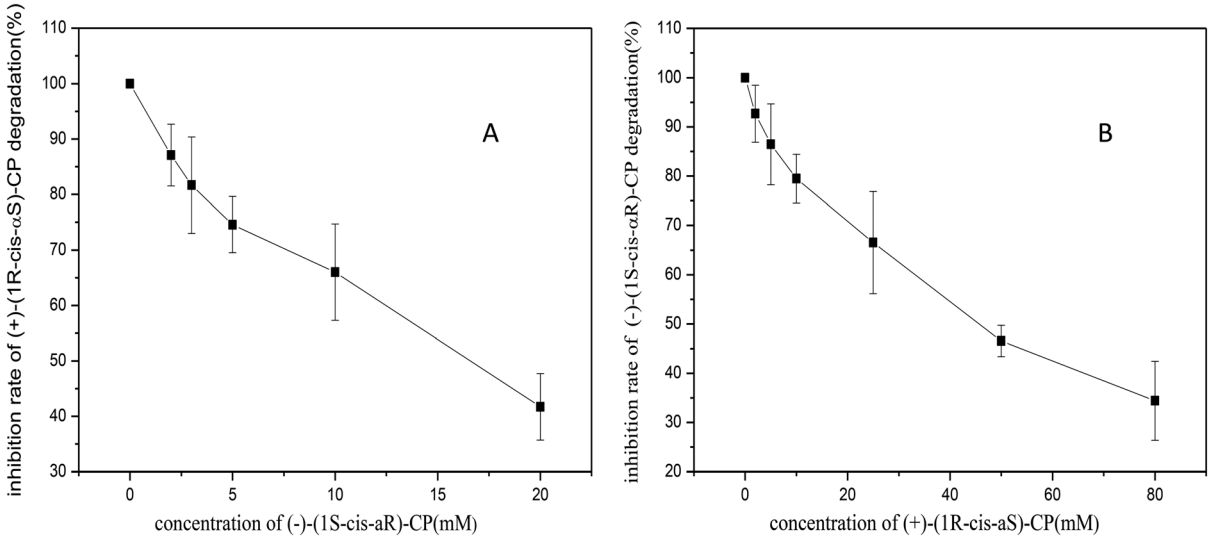

Fig. 8. Inhibitory effects of single α-CP enantiomer by its antipode in rat liver microsomes. Inhibitory effects of (−)-(1S-cis-αR)-CP to (+)-(1R-cis-αS)-CP (**A**) and inhibitory effect of (+)-(1R-cis-αS)-CP to (−)-(1S-cis-αR)-CP (**B**).

Considering that the isomerization would not affect the degradation kinetics of individual enantiomers, the effect of isomerization on enantiomeric compositions of the samples was not considered in this study.

The impurities and additives may influence the toxicological and ecotoxicological effects of a pesticide, and even compete with the active substance to affect the metabolism of a pesticide. In our study, the single enantiomers of α-CP obtained by HPLC with CDMPC-CSP has high purity of the active substance. However, the purity of *rac*-α-CP was 95%, which contained some impurities from raw materials, intermediate products, and by-product in the manufacturing process. In contrast to single enantiomers of α-CP, some impurities existing in *rac*-α-CP might be another reason for the difference between monomer and racemate, but it is not an important reason because of the low concentration of the impurities. More research should be done in the future to verify the hypothesis when we have highly purified impurities.

CONCLUSION

A chiral method for analysis of α-CP enantiomers in rat liver microsomes was established. Stereoselective degradation and significant differences in enzyme kinetics were discovered between *rac*-α-CP and enantiomer monomers. The results revealed that the (−)-(1S-cis-αR)-CP showed a faster degradation rate and stronger intrinsic clearance than (+)-(1R-cis-αS)-CP in both *rac*-α-CP and enantiomer monomers of α-CP. Some discordance of V_{max} and K_m were found between *rac*-α-CP and enantiomer monomers of α-CP, and inhibiting effects between the two enantiomers of *rac*-α-CP were confirmed by the study of enantiomers interaction in rat liver microsome. In addition, the inhibiting effects had a greater impact on (+)-(1R-cis-αS)-CP. The latent important message is that the selective degradation of α-CP may contribute to elucidating the mechanism of stereoselective toxicokinetics of a chiral pesticide and the safety of using pesticide with regard to public health.

ACKNOWLEDGMENTS

We gratefully acknowledge financial support of the National Natural Science Foundation of China (Contract grant number: 21207158) and Chinese Universities Scientific Fund (2012RC026) the National Science Foundation for Fostering Talents in Basic Research (J1210064),

ABBREVIATIONS

α-CP, Alpha-cypermethrin; *rac*-α-CP, Racemic alpha-cypermethrin; CDMPC-CSP, cellulose-tris (3, 5-dimethylphenylcarbamate)-based chiral stationary phase; V_{max}, Maximum velocity of metabolism; K_m, Michaelis constant; CL_{int}, Intrinsic metabolic clearance; EF, enantiomer fraction; IC_{50}, median inhibitory concentration.

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