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ENANTIOSELECTIVE INDUCTION OF OXIDATIVE STRESS BY PERMETHRIN IN RAT ADRENAL PHEOCHROMOCYTOMA (PC12) CELLS

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Abstract—Synthetic pyrethroid (SP) insecticides are chiral compounds with multiple asymmetric positions. Several recent studies have focused on the effect of enantioselectivity of SPs in acute aquatic toxicity, endocrine-disrupting activities, and immunotoxicity. However, the relevant molecular mechanisms are still unknown. The potential relationship between ecotoxicological effects and oxidative stress could contribute to SP-induced enantioselective cytotoxicity, but this requires further investigation. Therefore, this study was undertaken to evaluate the role of oxidative stress in enantiomer-specific permethrin (PM)-induced cytotoxicity in rat adrenal pheochromocytoma (PC12) cells. The study demonstrated that PM induced enantioselective oxidative stress and cytotoxicity. The reactive oxygen species (ROS) generation and lipid peroxidation production of malondialdehyde (MDA) were obviously increased, whereas the activity of antioxidant enzymes (superoxide dismutase [SOD] and catalase [CAT]) and glutathione (GSH) content had declined after exposure in 1*R-trans*-PM at a concentration of 30 mg/L. Meanwhile, the result of the cytotoxicity assay showed a clear, dose-dependent growth-inhibition effect of PM in an enantioselective manner. The most toxic effect on PC12 cells was shown by 1*R-trans*-PM and was approximately 1.6 times higher than that with 1*S-cis*-PM, which exhibited only a slightly toxic effect at a concentration of 20 mg/L. These results suggested that PM exhibited significant enantioselectivity in oxidative stress, which may be one of the initial events in PM-induced enantioselective cytotoxicity. The present study also improved understanding of enantiomer-specific, SP-induced cytotoxicity. The enantioselectivity should be taken into consideration when assessing ecological effects and development of new chiral pesticides. Environ. Toxicol. Chem. 2010;29:683–690. © 2009 SETAC

Keywords—Chiral pesticide Permethrin Enantioselectivity Oxidative stress Cytotoxicity

INTRODUCTION

A compound is chiral if it cannot be superimposed on its mirror image. Approximately 40% of the currently used pesticides in China have chiral structures, and the main classes include synthetic pyrethroids (SPs) and organophosphates as insecticides and metolachlor and diphenyl ethers as herbicides [1]. Chirality in pesticides has become a challenge for environmental scientists, because enantiomers of chiral pesticides not only have different insecticidal activities on target organisms but also have different toxicities to nontarget organisms [2]. Hence, it is important to evaluate their enantioselectivities with respect to environmental safety and ecotoxicologically related health risks. Several recent studies have shown that the environmental fate [3], acute aquatic toxicity [4,5], embryo development [6], endocrine-disrupting activities [7–10], and immunotoxicity [11,12] of chiral pesticides are enantioselectivity. Although much attention has been given to the differences among the enantiomers of chiral pesticides, much less attention has been given to the relevant toxic mechanisms of these compounds.

Synthetic pyrethroids are among the most commonly used pesticides for controlling agricultural and indoor pests. The liberal use of SPs has increased the risk of intoxication in nontarget species, such as birds, humans, fishes, and organisms present in soil and water [13]. Permethrin (PM) belongs to the family of SPs and functions as a neurotoxin, affecting neuron membranes by prolonging sodium channel activation. It is widely used as an insecticide, acaricide, and insect repellent. Permethrin has two asymmetric positions and contains two pairs, including four enantiomers [1*R-cis*-(+), 1*S-cis*-(−), 1*R-trans*-(+), and 1*S-trans*-(−); 5]. A previous study reported that PM concentrations were approximately 15.5 ng/g dry weight collected from the lower Missouri River in the United States [14]. In recent years, pesticide toxicity and ecosafety of racemates of PM have been extensively investigated using in vivo and in vitro models. Our previous studies have shown acute toxicity [2] and developmental toxicity to zebrafish (*Danio rerio*) [9] by PM in an enantioselective manner. However, the molecular mechanism of enantioselectivity in ecotoxicity of PM was poorly understood. Thus, research on the mechanism involved is necessary for proper assessment of ecotoxicity and health risk of SPs.

Oxidative stress can be defined most simply as the imbalance between the production of free radicals capable of causing peroxidation of the lipid layer of cells and the body's antioxidant defense system [15]. The balance between production of free radicals and antioxidant defenses in the body has important health implications: if there are too many free radicals or too few antioxidants for protection, a condition of oxidative stress develops, which may cause chronic and permanent damage [16]. Some pesticides may induce oxidative stress, leading to

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generation of oxygen and other free radicals and alteration in antioxidants, the scavenging enzyme system, and lipid peroxidation. These have been focal points of toxicological research over the last decade, as possible mechanisms of toxicity; many studies have demonstrated that pesticides induced oxidative stress [17,18]. Development of oxidative stress conditions in different tissues following pesticide exposure has been suggested as a main cause of ecotoxicity and cytotoxicity [19,20]. Although PM has been widely used in modern agriculture, little information about its enantioselectivity in the induction of oxidative stress and ecotoxicological effects is available.

In the present study, rat adrenal pheochromocytoma (PC12) cells were used as an in vitro model with which to investigate the effects of enantioselectivity of PM enantiomers in oxidative stress. Several endpoints were utilized based on the measurement of the stable peroxidation products (mainly lipid peroxidation products) or antioxidizing agents. Furthermore, the enantioselectivity of PM in cytotoxicity was also investigated in an effort to understand the role of oxidative stress in enantiomer-specific, PM-induced cytotoxicity in PC12 cells. Procedures developed in the present study may be useful for understanding the effects of enantioselectivity of chiral chemicals on oxidative stress.

MATERIALS AND METHODS

Chemicals

An analytical standard of racemic PM [99.2%, (3-phenoxyphenyl)methyl (1*RS*)-*cis-trans*-3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropanecarboxylate] was purchased from Sigma. Thiazolyl blue (MTT) solution (5 mg/ml phosphate-buffered saline [PBS]) was purchased from Amresco and fetal bovine serum (FBS) from Shijiqing Reagent. The reagent kits for measuring malondialdehyde (MDA), catalase (CAT), super oxide dismutase (SOD), glutathione (GSH), and lactate dehydrogenase (LDH) were purchased from Nanjing Jiancheng Bioengineering Institute. All test compounds were dissolved in ethanol, with the solvent content at $<0.1\%$ of the final volume. Other chemicals or solvents used in this study were of cell culture, high-performance liquid chromatography (HPLC), or analytical grade.

Permethrin enantiomer separation and quantitative analysis

The enantiomers of PM were also resolved on a Jasco LC-2000 HPLC system. The column used was Sumichiral OA-2500I (4.6 mm inside diameter $\times$ 250 mm; Sumika Chemical Analysis Service), and the chromatographic conditions were similar to the methods reported in previous studies [5]. The enantiomers eluted from the chiral column were manually collected and used in the following bioassays.

Cell culture and treatments

The PC12 cells were obtained from the cell bank at the Chinese Academy of Sciences, the original source being the American Type Culture Collection. Cells were cultured in Dulbecco's modified Eagle's medium (Hyclone) supplemented with 10% (v/v) FBS. Cells were cultured at 37°C with 5% CO_2 in 25-cm^2 flasks. The culture medium was refreshed every 2 d, and subculture at a ratio of 1:4 was performed with routine trypsinization every 4 to 5 d.

For measurement of cytotoxicity and oxidative stress effect, the cells were seeded in 24- or 96-well plates (Costar) at a density of 1×10^5 cells/ml and allowed to adhere for 24 h prior to assaying. The dosing medium (the experimental medium along with a range of concentrations of test compounds) was added for 24 h (except for the ROS generation, which required 6 h). Ethanol (0.1% v/v) was used as the negative control.

Assessment of the cellular ROS content

Intracellular ROS generation was assayed using a fluorescence probe; nonfluorescent 2′,7′-dichlorofluorescein diacetate (DCFH-DA; Sigma) is oxidized to its fluorescent form 2′,7′-dichlorofluorescein (DCF) by H_2O_2 and other ROS. 2′,7′-Dichlorofluorescein diacetate can enter the cell, where it is enzymatically hydrolyzed to a nonfluorescent analog, 2′,7′-dichlorofluorescene (DCF-H), which is trapped in the cell. Briefly, after treatment with PM or vehicle for 6 h, the treated cells were washed three times with ice-cold PBS and then incubated with 10 μmol/L DCFH-DA (100 mmol/L in dimethylsulfoxide [DMSO]) for 30 min at 37°C. The cellular free radical content was assayed by measuring DCF fluorescence with a fluorescent spectrophotometer (excitation at 485 nm/emission at 535 nm; Tecan Infinite M200).

Assessment of MDA, SOD, CAT, and GSH

We titrated MDA, SOD, CAT, and GSH indexes to investigate the oxidative stress effect caused by PM; these indexes were detected with an assay kit according to the manufacturer's protocol. Briefly, the PC12 cells were seeded into 24-well plates and were allowed to attach for 24 h. The medium was replaced with the experimental medium along with each concentration of the tested PM or vehicle. After 24 h, the medium was removed, and the cells were pooled in a 1.5-ml tube. These cells were centrifuged at 3,000 *g* at 4°C for 10 min, and the pellets were resuspended with 1,000 μl PBS, freeze-thawed twice at −20°C, and centrifuged at 10,000 *g* at 4°C for 15 min. The supernatant was collected for MDA, SOD, CAT, and GSH assays, according to the manufacturer's instructions (Nanjing Jiancheng).

Assessment of cell viability and LDH release

Cell viability was measured by quantitative colorimetric assay with MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide). The medium was replaced with the experimental medium plus one of four concentrations of the test compound. After 24 h, the medium was removed from the wells, and thiazolyl blue solution was added. After 4 h, the solution was removed and 150 μl DMSO was added to each well. After 10 min of shaking with a micromixer, the absorbance was measured at 490 nm with a Bio-Rad model 680 microplate reader. Results were calculated as the ratio of each exposure group to the vehicle control (0.1% ethanol, one fold) and represented as the mean $\pm$ standard deviation of five independent measurements performed in triplicate. The results were analyzed by *t* test in Origin software (OriginLab).

Lactate dehydrogenase activity in the cell-free extracellular supernatant was quantified as an index of cell death. After exposureof the cells to PM or vehicle for 24 h at 37°C in a 5% CO_2 incubator, the medium was collected. Lactate dehydrogenase released by cells was determined with an assay kit (Nanjing Jiancheng), according to the manufacturer's instruc-

tions. After the reaction, the absorbance of each sample was measured at a wavelength of 440 nm.

Statistical analysis

All experiments were repeated three times. Unless otherwise stated, all data were expressed as mean ± standard deviation. Comparison of the values among groups was performed by analysis of variance, and significant differences were assessed by Dunnett's test, and $p < 0.05$ was considered statistically significant.

RESULTS

Enantioselectivity of oxidative stress effect in PC12 cells

Oxidative stress and generation of ROS caused by some chemicals play an important role in toxicity. The relative levels of ROS were expressed as the fluorescence intensity ratio of groups treated with PM or its enantiomers relative to the negative control (untreated). As Figure 1A shows, PC12 cells incubated with 1*R-trans*-PM exhibited a dose-dependent accumulation of intracellular ROS compared with the control. The ROS production in cells exposed to 1*S-cis*-PM only slightly increased at a concentration of 30 mg/L. The fluorescence intensity in PC12 cells followed the order: 1*R-trans*-PM> 1*S-trans*-PM > *rac*-PM > 1*R-cis*-PM > 1*S-cis*-PM. The fluorescence intensity of PC12 cells treated by 1*R-trans*-PM was 1.31 times higher than that after 1*S-cis*-PM treatment at its highest concentration.

Oxidation of membrane lipids, one of the primary events in oxidative cellular damage, can be assessed by measurement of MDA, a breakdown product of lipid peroxides. The results indicated that PC12 cells incubated with individual stereoisomers or a racemate of PM showed a dose-dependent increase in intracellular MDA levels. At concentrations above 20 mg/L, the difference between the enantiomers was significant (Fig. 1B). The MDA levels in cells increased by 4.10-, 3.67-, 6.50-, 5.28-, and 3.90-fold at 30 mg/L of 1*R-cis*-PM, 1*S-cis*-PM, 1*R-trans*-PM, 1*S-trans*-PM, and *rac*-PM, respectively, compared with the negative control. In particular, the MDA content induced by 1*R-trans*-PM in PC12 cells was 1.44 times higher than that induced by 1*S-cis*-PM at 30 mg/L.

Superoxide dismutase and CAT play a very important role in protecting cells from oxidative damage. The activity of SOD in PC12 cells was decreased by the enantiomers and racemate of PM in a dose-dependent manner. Among the enantiomers of PM, the 1*R-trans*-PM displayed the most significant inhibition of SOD activity (45.1%) compared with the control (Fig. 2A).

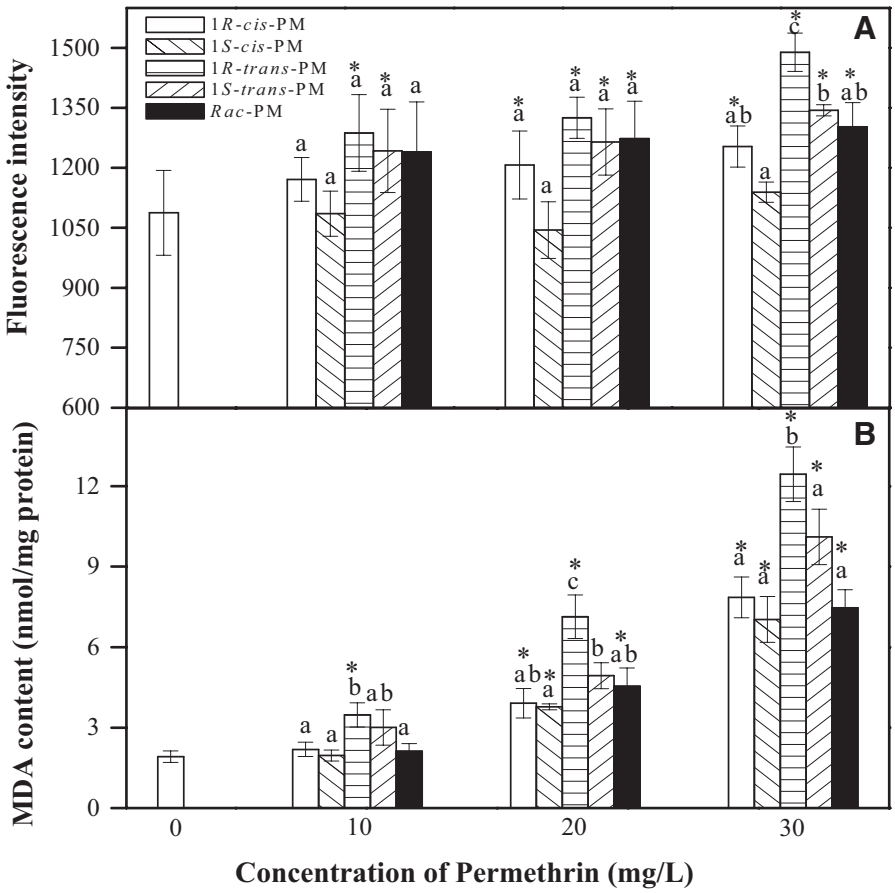

Fig. 1. Effect of individual stereoisomers and the racemate of permethrin (PM) on intracellular reactive oxygen species (**A**) and malondialdehyde (MDA; **B**) production. PC12 cells were exposed to different concentrations of individual stereoisomers and the racemate of PM for 6 h and 24 h, followed by ROS determination and MDA determination. Different letters above adjacent bars indicate a significant difference ($p < 0.05$, $n = 5$ for ROS, $n = 3$ for MDA) between individual enantiomers and racemate, whereas the same letter indicates no significant difference. The asterisk indicates a significant difference ($p < 0.05$, $n = 5$ for ROS, $n = 3$ for MDA) between cells treated with PM and negative control.

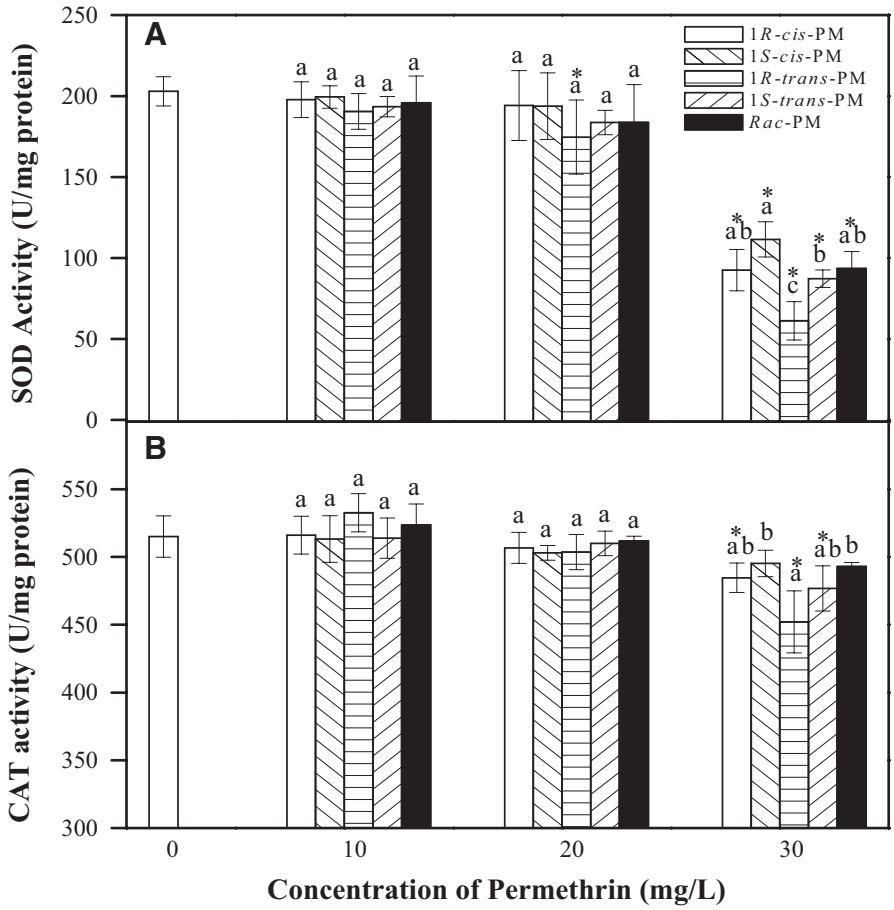

Fig. 2. Effect of individual stereoisomers and the racemate of permethrin (PM) on intracellular superoxide dismutase (SOD; **A**) and catalase (CAT; **B**) production. PC12 cells were exposed to different concentrations of individual stereoisomers and the racemate of PM for 24 h, followed by SOD and CAT determination. Different lowercase letters above adjacent bars indicate a significant difference ($p < 0.05$, $n = 3$) between individual enantiomers and racemate, whereas the same letter indicates no significant difference. The asterisk indicates a significant difference ($p < 0.05$, $n = 3$) between cells treated with PM and negative control.

As shown in Figure 2B, the CAT activity showed no obvious changes at 10 and 20 mg/L of PM. Although the CAT activity in PC 12 cells treated with 30 mg/L of PM was slightly decreased, the CAT activity decreased by 5.91, 3.85, 12.2, 7.42, and 4.28% when exposed to 1*R-cis*-PM, 1*S-cis*-PM, 1*R-trans*-PM, 1*S-trans*-PM, and *rac*-PM, respectively. There was a significant difference between the enantiomers at the 30 mg/L concentration.

The enantioselective effects on the GSH levels were also studied in PC12 cells. After 24 h of incubation with the enantiomers and racemate of PM, a significant decrease was observed in comparison with the negative control (Fig. 3). A concentration of 30 mg/L of 1*R-cis*-PM, 1*S-cis*-PM, 1*R-trans*-PM, 1*S-trans*-PM, and *rac*-PM led to decreases in GSH levels by 90.6, 95.1, 82.6, 88.1, and 92.2%, respectively, and the difference between the 1*R-trans*-PM and 1*S-cis*-PM was approximately 1.15 times. The results revealed that PM induced enantioselective, dose-dependent decreases in GSH. From the results of MDA, SOD, CAT, GSH, and ROS assays, the selectivity of these effects was consistently in the same direction, with 1*R-trans*-PM resulting in greater oxidative stress than the other enantiomers or racemate of PM. These data indicated that oxidative stress caused by PM could be attributed mainly to the 1*R-trans*-PM enantiomer.

Enantioselective cytotoxicity in PC12 cells

The cytotoxic effect of PM on PC12 cells after 24 h of incubation was measured by the MTT assay (Fig. 4A). From the cell viability assay, clear, dose-dependent enantioselectivity by PM was observed in the cytotoxic effects. The 50% mortality concentration of 1*R-trans*-PM (30.4 mg/L) was significantly lower than that for other enantiomers or *rac*-PM, whereas 1*R-trans*-PM was consistently more toxic to PC12 cells. Lactate dehydrogenase catalyses the interconversion of pyruvate and lactate, with concomitant production of nicotinamide adenine dinucleotide (NADH) and NAD^+. In cytotoxicity, LDH is often used as a marker of cell membrane damage. To substantiate the cytotoxic nature of PM, LDH release was measured. The results showed that a dose-dependent increase in LDH activity occurred following incubation with PM or its enantiomers (Fig. 4B). At the concentration of 30 mg/L, 1*R-trans*-PM induced extracellular LDH release that was greater than that with other enantiomers or *rac*-PM and approximately 1.13 times higher than that with 1*S-cis*-PM. Take together, the results of cell viability and LDH release demonstrated that PC12 cytotoxicity by PM could be due mainly to the 1*R-trans*-PM enantiomer, which is in accordance with the enantioselective, oxidative stress effect.

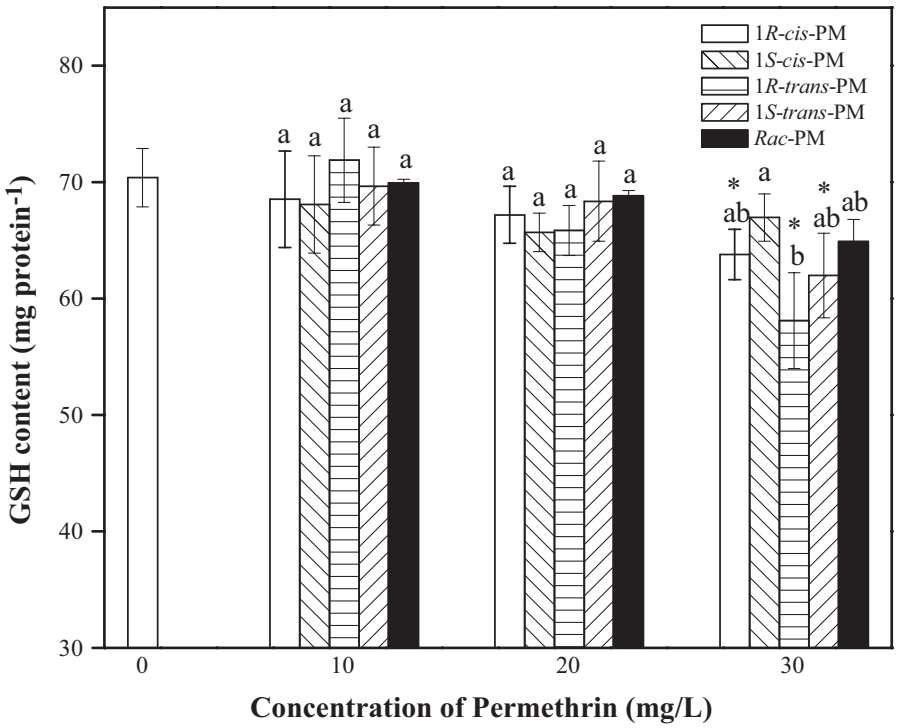

Fig. 3. Effect of individual stereoisomers and the racemate of permethrin (PM) on intracellular glutathione (GSH) production. The PC12 cells were exposed to different concentrations of individual stereoisomers and the racemate of PM for 24 h, followed by GSH determination. Different lowercase letters above adjacent bars indicate a significant difference ($p < 0.05$, $n = 3$) between individual enantiomers and racemate, whereas the same letter indicates no significant difference. The asterisk indicates a significant difference ($p < 0.05$, $n = 3$) between cells treated with PM and negative control.

DISCUSSION

Oxidative stress is a term commonly used to denote the imbalance between the concentrations of ROS and the antioxidative defense mechanisms of the body; cells deploy antioxidant defenses, activate damage removal and repair systems, and mount adaptive responses [21]. Oxidative stress played an important role in cytotoxicity in many in vivo and in vitro models [22]. To the best of our knowledge, this is the first report of enantioselectivity in oxidative stress resulting from chiral pesticides. The present study demonstrated the role of enantioselective oxidative stress from enantiomer-specific induction of cytotoxicity by PM in PC12 cells.

Permethrin was registered for use in a wide variety of agricultural and nonagricultural applications and has demonstrated several adverse effects in various in vivo and in vitro models [2,9]. At the same time, PM displayed enantioselectivity in acute toxicity [2], developmental toxicity [9], and environmental behavior [5]. The aim of the present study was to demonstrate whether enantioselective oxidative stress could be involved in the enantioselective cytotoxicity in PC12 cells. Thus, oxidative stress and cell viability were determined after treatment of PC12 cells with enantiomers and a racemate of PM.

The present study clearly showed that PM enantioselectively induced oxidative stress in PC12 cells, including increased generation of ROS and alteration of MDA, SOD, CAT, and GSH levels at concentrations from 10 to 30 mg/L, although there are differences in exposure levels of SPs in in vivo and in vitro models. However, the exposure levels of PM in the present study were lower than those in the human hepatocytes cell line (Hep G2). The cytotoxicity was noted in Hep G2 cells after 48 to 72 h exposure to 100 mM PM, and 3.125 mM PM could induce caspase-3/7 in Hep G2 cells. [23]. Reactive oxygen species, formed continuously in cells by oxidative biochemical reactions and external factors, are harmful when produced in excess; this is evident in abnormal conditions such as exposure to certain environmental pollutants. Although some reports have shown that the racemate of PM induced oxidative stress and generation of ROS in experimental systems [20]. Our data are the first to indicate that *trans*-PM (especially the 1*R*-*trans*-PM) induced more ROS generation than *cis*-PM (especially the 1*S*-*cis*-PM) in PC12 cells. The PC12 cells may be susceptible to oxidative damage resulting from *trans*-PM, which could act as a source of free radicals.

Reactive oxygen species, such as superoxide anions, hydroxyl radicals, and H_2O_2, enhance oxidative processes and produce lipid peroxidative damage of cell membranes. Malondialdehyde is a well-known byproduct of lipid preoxdation, and its formation indicates oxidative cellular damage following exposure to a xenobiotic compound. Previous studies showed that some chemicals elevated levels of MDA with a concomitant increase in ROS [24]. In this study, the enantioselective increase in membrane LPO production of MDA following PM exposure was dose dependent. Determining whether the enantioselective induction of MDA resulted from excess ROS requires further research.

Cellular cytoprotective molecules may protect against oxidative stress. To investigate the effects of oxidative stress, several other methods, based on the measurement of antioxidizing agents such as SOD, CAT, and GSH, are available.

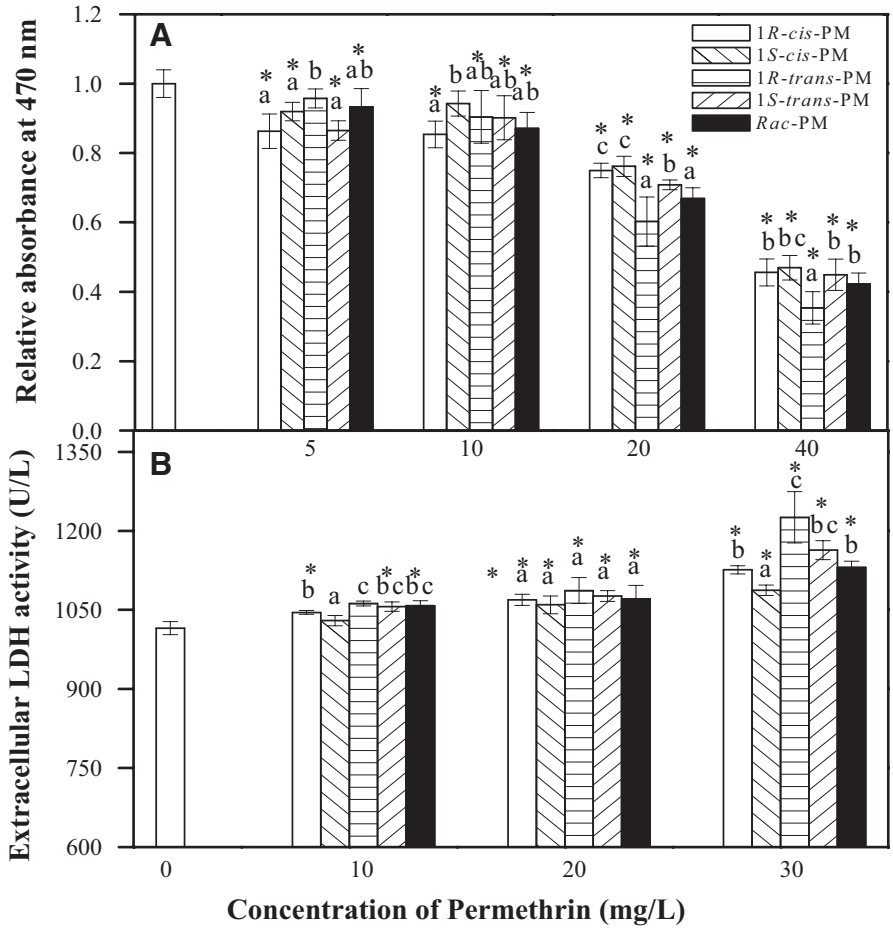

Fig. 4. Effect of individual stereoisomers and the racemate of permethrin (PM) on the viability and on extracellular lactate dehydrogenase (LDH) release. The PC12 cells were incubated with different concentrations of individual stereoisomers and the racemate of PM for 24 h, followed by the MTT (thiazolyl blue; **A**) assay and LDH determination (**B**). Different letters above adjacent bars indicate a significant difference ($p < 0.05$, $n = 5$ for MTT, $n = 3$ for LDH) between individual enantiomers and racemate, whereas the same letter indicates no significant difference. The asterisk indicates a significant difference ($p < 0.05$, $n = 5$ for MTT, $n = 3$ for LDH) between cells treated with PM and negative control.

Superoxide dismutase is the first enzyme of the antioxidant process, and it catalyses the conversion of the superoxide anion into oxygen and hydrogen peroxide, and the latter becomes oxygen and water via CAT activity. Catalase uses H_2O_2 as a source of electrons and is found in the peroxisomes of almost all eukaryotic cells [25,26]. Increasing evidence supports the important role of GSH in detoxification and protection from oxidative damage. Glutathione is the major antioxidant in the cell; because of its reducing and nucleophilic properties, it behaves as a free radical scavenger and helps in regenerating other antioxidants [27]. Results of the present study show that PM treatment resulted in an enantioselective decrease in SOD and CAT activities in PC12 cells. Additional enantioselective GSH depletion was further observed after PM incubation. Our previous studies revealed that the *cis*-bifenthrin (*cis*-BF) induced the enantioselective generation of ROS and cytotoxicity in human amnion epithelial cell lines (FL) [22] and human hepatocellular liver carcinoma (Hep G2) cell lines [28]. This study is the first to report that the enantioselective alteration in the activities of oxidative stress-related molecules, such as SOD, CAT, and GSH, may be attributed to enantioselective oxidative stress of chiral pesticides. Although we could not identify a clear mechanism of enantioselective oxidative stress by PM, results from other SPs provide some insights. The cleavage of cypermethrin and its ester metabolites releases cyanohydrins, which are unstable under physiological conditions and decompose to cyanides and aldehydes. We propose that the difference between the enantiomers of PM might result from the difference in the rate of biotransformation, especially insofar as some enzymes show stereospecificity or stereoselectivity in their hydrolysis of PM.

Nonspecific cellular oxidative damage is often observed during toxicity of chemicals. It is difficult to determine whether the oxidative stress response was the cause or the consequence of cytotoxicity. To assess further that PM induced enantioselective cytotoxicity, cell growth and LDH release were investigated. The MTT assay for detecting cytotoxicity was more sensitive than the other assay. The LDH leakage assay is based on the release of the enzyme into the culture medium after cell membrane damage, whereas the MTT assay is an indicator of the cell's energy metabolism [29,30] and mitochondrial activity [31,32]. Results of the present study indicate that 1*R-trans*-PM was the most toxic compound tested, in PC12 cells, compared with other enantiomers and the racemate of PM. These results are in agreement with the results of enantioselective oxidative stress induced by PM. Enantioselective oxidative stress was

consistent with the enantioselective cytotoxicity. Based on our previous studies, the enantioselective cytotoxicity induced by SPs contributes, at least in part, to the enantioselective activation of the mitogen-activated protein kinase signaling pathway and induction of ROS generation in vitro [28]. Although the possible molecular mechanism of PM-induced enantioselective cytotoxicity in PC12 cells is unknown, the enantiomer-specific activation of cellular signaling pathways and oxidative stress may be important factors.

We propose, based on our experimental results, a hypothetical model of PM-induced oxidative stress and cytotoxicity in PC12 cells. Permethrin induced enantioselective increases in intracellular ROS, probably triggering enantioselective oxidative stress in PC12 cells, subsequent enantioselective cytotoxicity, and altered activities of antioxidant enzymes. However, the molecular mechanisms of the proposed model warrants further investigation.

Interestingly, the enantioselective toxicity and activity of PM between the nontarget organisms (such as human cells and aquatic vertebrates) and the target organism (such as *Daphnia magna*) were different. Our previous studies showed only two enantiomers with the 1*R-cis* (or *trans*)-PM configuration were active, whereas the two enantiomers with the 1*S-cis* (or *trans*)-PM configuration were essentially ineffective in *D. magna* [5]. In zebrafish (*Danio rerio*), the 1*S-trans*-PM showed the greatest estrogenic activity [9]. In this study, we identified that the enantiomer of 1*R-trans*-PM was more toxic to PC12 cells than other enantiomers. The anther SPs *cis*-bifenthrin showed similar phenomena; 1*R-cis*-bifenthrin was 300 times more active than 1*S-cis*-bifenthrin on a target pest, whereas 1*S-cis*-bifenthrin was more toxic than its enantiomers to human amnion epithelial cell lines [22]. Based on these findings, the difference in enantioselectivity of PM, in target or nontarget organisms, might have arisen from any or all of the processes involved in the PM effects: transport to the site of action, storage in depots, interaction with protein and nucleic acids, metabolic events, and terminal transport in the excretory pathway. Given that different organisms are exposured to chiral pesticides, enantioselectivity may follow a specific direction that can be revealed via integrative assessments in adverse organisms. Chiral pesticides in future applications are also worth considering, because they are the biologically predominant form and their production is cost effective.

CONCLUSIONS

Permethrin is the most widely used SP that exhibits enantioselective induction of oxidative stress and cytotoxicity in PC12 cells. This study is the first to address the role of enantioselective oxidative stress resulting from PM-induced cytotoxicity. Our findings not only suggest that PM can produce potential enantioselective health risks but also advance our understanding of the possible molecular mechanisms of enantioselectivity in SP-induced cytotoxicity. Given the worldwide use of PM and the presence of other SPs in the environment, more comprehensive studies on the potential health risks of SPs are necessary. Furthermore, development of enantiomer-enriched pesticide products should consider the selection of the enantiomer with the highest potency to target organisms but with the fewest adverse effects on nontarget species.

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