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cis-Bifenthrin enantioselectively induces hepatic oxidative stress in mice

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ABSTRACT

Bifenthrin (BF), as a chiral synthetic pyrethroid, is widely used to control field and household pests. In China, the commercial *cis*-BF contained two enantiomers including 1R-*cis*-BF and 1S-*cis*-BF. However, the difference in oxidative stress induced by the two enantiomers in mice still remains unclear. In the present study, 4 week-old adolescent male ICR mice were orally administered *cis*-BF, 1R-*cis*-BF or 1S-*cis*-BF daily for 2, 4 and 6 weeks at doses of 5 mg/kg/day, respectively. We found that the hepatic reactive oxygen species (ROS) levels, as well as the malondialdehyde (MDA) and glutathione (GSH) content both in the serum and liver increased significantly in the 4 or 6 weeks 1S-*cis*-BF treated groups. The activities of superoxide dismutase (SOD) and catalase (CAT) also changed significantly in the serum and liver of 1S-*cis*-BF treated mice. More importantly, the significant differences in MDA content and CAT activity both in the serum and liver, and the activities of total antioxidant capacity (T-AOC) and SOD in serum were also observed between the 1S-*cis*-BF and 1R-*cis*-BF treated groups. Moreover, the transcription of oxidative stress response related genes including *Sod1*, *Cat* and *heme oxygenase-1(Ho-1)* in the liver of 1S-*cis*-BF treated groups were also significant higher than those in 1R-*cis*-BF treated group. Thus, it was concluded that *cis*-BF induced hepatic oxidative stress in an enantiomer specific manner in mice when exposed during the puberty, and that 1S-*cis*-BF showed much more toxic in hepatic oxidative stress than 1R-*cis*-BF.

1. Introduction

Synthetic pyrethroids (SPs) comprise a class of universal pesticides, of which their usage is expected to increase. Bifenthrin (BF), one of the most common SPs, has been widely used for pest control for two decades, and its use has continued to grow. As a SP, BF, characterized by great photostability, insecticidal activity and low mammalian toxicity, is used widely for agricultural applications [1,2]. More importantly, BF has also been used to control household pests [3,4]. Thus, humans suffering potential exposure to this compound is expected [5]. Unfortunately, more and more of studies have suggested that BF and other SPs are carcinogenic, neurotoxic and have immunosuppressive potential as well as reproductive toxicity in different non-target organisms, even in humans [6–11].

Generally, SPs contain one to three asymmetric positions, making them a family of chiral insecticides with one to four pairs of enantiomers [12]. In China, the commercial BF is a mixed compound containing *cis* isomers (*cis*-BF) including two enantiomers (1R-*cis*-BF and 1S-*cis*-BF), correspondingly. Recently, more and more concerns regarding the enantioselectivity of chiral pesticides, including BF and other pyrethroids, in aquatic toxicity, endocrine disruption, oxidative stress, immunotoxicity and biodegradation have been focused by the scientists. For example, Liu et al. found that the (+)-*cis* isomer of *cis*-BF or *cis*-permethrin was 17–38 times more active than the (−)-*cis* enantiomer for the aquatic invertebrates *Ceriodaphnia dubia* or *Daphnia magna* [13]. In field sediments, the (−) enantiomer of *cis*-BF or *cis*-permethrin was preferentially degraded, which resulted in the relative enrichment of the more toxic (+) enantiomer [14]. More recently, our study also suggested that, among the four permethrin enantiomers, (+)-*cis* enantiomer showed the greatest endocrine disruption activities in mice [10]. Thus, the effects of the enantiomers of chiral pesticides on environmental systems need to be investigated independently [15–17]. With regard to BF, the enantioselectivity of the two *cis*-BF enantiomers in aquatic toxicity and disrupting the endocrine systems has been previously suggested by Liu and his group [6,13,18]. However, whether the two enantiomers of BF could enantioselectively cause their oxidative stress in mice remains unclear yet.

Oxidative stress has become an important subject in mammalian toxicity [10,19], and pesticides including BF may be directly involved in this process. Exposure to xenobiotics could produce an imbalance between these endogenous and exogenous reactive oxygen species (ROS), and can subsequently induce a decrease in antioxidant defenses or cause oxidative damage outright in organisms [20]. Although it is likely that Sps may influence the antioxidant system of different organisms, the molecular mechanism has only

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received limited attention. In the present study, to better understand the oxidative stress process, the racemic *cis*-BF and two enantiomers of *cis*-BF were orally administered to adolescent male mice (4 weeks old) for 2, 4 or 6 weeks. The hepatic ROS levels and the related parameters indicating the oxidative stress including total antioxidant capacity (T-AOC), malondialdehyde (MDA), glutathione (GSH), catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPX) in the liver and serum were examined. Furthermore, the transcription of oxidative stress related to genes including *Sod1, Sod2, Cat, Gpx and heme oxygenase-1(Ho-1)* were also determined to elucidate the potential mechanism of oxidative stress induced by different *cis*-BF enantiomers. All the information is intended to provide new insights into how the BF enantiomer selectively affects oxidative stress in mice.

2. Materials and methods

2.1. Chemicals and enantiomer separation

Original racemic *cis*-BF (>95%, 2-methylbiphenyl-3-ylmethyl-(Z)-(1RS)-cis-3-(2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropane carboxylate, 1R-*cis*/1S-*cis* = 1/1) was obtained from the Nanjing Panfeng Chemical Co., Ltd (Nanjing, China). The different two BF enantiomers were separated by a Supercritical Fluid Chromatography MG II (Berger) with a chromegachiral CCJ column (3 cm I.D. × 25 cm) and supercritical CO_2/methanol (85/15) as the mobile phase. The flow rate was set at 70 ml/min and the column's temperature was kept at 35 °C. Injection volume was 1 ml and the UV wavelength for detection was 254 nm. Finally, the separated stereoisomers were quantified and identified according to a previous report [6].

2.2. Animals and experimental design

Three weeks old young male ICR mice (*Mus musculus*) were purchased from the China National Laboratory Animal Resource Center (Sungkiang, Shanghai, China). After one week, 72 mice were weighed and divided into 12 groups randomly. Then, the mice in each group were orally administered *cis*-BF, 1R-*cis*-BF or 1S-*cis*-BF (5 mg/kg) daily for 2, 4 or 6 weeks, respectively. They were kept in animal facilities (light-on at eight clock, light-off at twenty clock; illumination with strip lights, 200 lux at cage level; 22 ± 1 °C). Food was available *ad libitum* at night. Water was available *ad libitum*. The body weight of each mouse was recorded every other day.

The animals were maintained under a normal light cycle and sacrificed on the last day of treatment. The bodyweight were determined before sacrificing. Blood sera were collected, and livers were quickly removed and weighed, after which they were immediately frozen in liquid nitrogen and kept at −80 °C until use. Every effort was made to minimize animal suffering in each experiment. All experiments were performed in accordance with the Guiding Principles in the Use of Animals in Toxicology in Zhejiang University of Technology.

2.3. Hepatic ROS levels measurement

Livers were homogenized with 9 volumes of ice cold saline water. Then, the ROS levels were determined by the mouse ROS ELISA kit (RapidBio, USA) according to the manufacturer's instruction.

2.4. Determination of T-AOC, SOD, GPX and CAT activity, GSH and MDA content

Livers were defrosted and homogenized with 10 volumes of cold buffer consisting of 250 mmol L^{-1} sucrose, 5 mmol L^{-1} Tris–HCl, and 0.1 mmol L^{-1} EDTA–2Na (pH 7.5). The homogenate was centrifuged at 4000*g* at 4 °C for 15 min to obtain the supernatant for the enzyme activity assays. The serum samples were directly diluted by 10 volumes indicated cold buffer. The activities of T-AOC, SOD, GPX, and CAT, GSH and MDA contents in the liver or serum were determined using kits purchased from the Nanjing Jianchen Institute of Biotechnology (Nanjing, China) according to the manufacturer's instructions and our previous reports [10,21]. Protein concentration was determined using bicinchoninic acid (BCA) as a detection reagent for Cu^{+} following the reduction of Cu^{2+} by protein in an alkaline environment (BCA protein kit, Sangon Company, China). Measurements were made on a microplate reader (Bio-TEK, USA) by using micro well plate protocol A590 according to the manufacturer's instructions.

2.5. Quantification of mRNA

Total RNA was isolated from the livers using TRIzol reagent (Takara Biochemicals, Dalian, China), and then synthesized into cDNA using a reverse transcriptase kit (Toyobo, Tokyo, Japan) according to the manufacturer's protocol. 1-μL of each RT product was used directly for real-time quantitative polymerase chain reaction (RT-qPCR). RT-qPCR was performed on an Eppendorf MasterCycler® ep RealPlex^4 (Wesseling-Berzdorf, Germany). Oligonucleotide primers were used to detect the expression of *18sRNA, Sod1, Sod2, Cat, Gpx1, and HO-1* genes using the SYBR Green system (Toyobo, Tokyo, Japan). The detailed information of the primers is indicated in previous reports [10,22]. The 18sRNA transcript was used as housekeeping gene. The following PCR protocol was used with the Eppendorf MasterCycler® ep realPlex^4 (Wesseling-Berzdorf, Germany): denaturation for 1 min at 95 °C, followed by 40 cycles of 15 s at 95 °C and 1 min at 60 °C. PCR was performed three separate times. The PCR protocol and the relative quantification of gene expression among the treatment groups were analyzed according to our previous reports [21,23].

2.6. Data analysis

Data were evaluated by one-way ANOVA followed by Dunnett's or Fisher's Protected Least Significant Difference test using SPSS 13.0 (SPSS, Chicago, IL, USA). When necessary, data were transformed for normalization and to reduce heterogeneity of variance. Differences with p-values <0.05 were considered significant.

3. Results

3.1. Body and liver weights

During 2, 4 or 6 weeks of oral exposure to 5 mg/kg/day *cis*-BF and its two enantiomers, no obvious symptom of poisoning was found in male mice. Neither absolute nor relative weights of the liver in the groups treated with 5 mg/kg BF enantiomers for 2 and 4 weeks were significantly different from those of the control. While they increased significantly when the exposure period to BF was extended to 6 weeks (Table 1). In addition, no significant difference in the hepatic weight was found between the 1R-*cis* and 1S-*cis*-BF treated groups.

3.2. Enantioselective effects on hepatic ROS levels

The effects of *cis*-BF and its enantiomers during 2, 4 or 6 weeks of puberty exposure on hepatic relative ROS levels are presented in Fig. 1. Exposure to *cis*-, 1R-*cis*- and 1S-*cis*-BF for 2 weeks did not cause significant differences in the ROS levels between three BF-treated groups and the control or among the three BF-treated groups (data not shown). However, when the exposure period extended to 4 and 6 weeks, the significant increase of the hepatic