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Enantioselective apoptosis induced by individual isomers of bifenthrin in Hep G2 cells

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ABSTRACT

Bifenthrin (BF) has been used in racemate for agricultural purposes against soil insects, leading to increased inputs into soil environments. However, most of the studies about the toxicology research on BF were performed in its racemic form. The aim of the present study was to evaluate the enantiomer-specific *cis*-BF-induced apoptosis and intracellular reactive oxygen species (ROS) generation on human hepatocarcinoma cells (Hep G2). The results of cell viability assay and cytoflow assay indicated an obvious enantioselective hepatocyte toxicity of 1*S*-*cis*-BF in Hep G2 cells. 1*S*-*cis*-BF also induced ROS production, up-regulated Bax protein expression and down-regulated Bcl-2 expression levels. The present study suggested that enantioselective toxicity should be evaluated on currently used chiral pesticides, such as synthetic pyrethroids.

1. Introduction

Synthetic pyrethroids (SPs) are widely used in most sectors of agricultural production to ensure high crop yields for decades (Tyler et al., 2000). Some pyrethroid pesticides have played a key role in providing reliable supplies of agricultural production at prices affordable to consumers and ensuring high profits to farmers. Recent works point out this ubiquity in the environment, even in vegetables (Velki and Hackenberger, 2013; Hall and Anderson, 2013; Phyu et al., 2013). Many SPs used as pesticides are chiral compounds (Carlsson et al., 2014). In recent years, people have become increasingly concerned about chiral pesticides use and particularly about their impact on human health and environmental quality (Liu et al., 2005).

Bifenthrin is effective against a wide range of insect and has been used for plant protection (Solomon et al., 2001). Previous study indicated that 1*S*-cis-BF presented more toxic effects than 1*R*-cis-BF on human amnion epithelial (FL) cell lines. FL cells incubated with 1*S*-*cis*-BF exhibited a dose-dependent accumulation of ROS (Liu et al., 2008).

Many studies reported that SPs have been associated with many human health hazards, ranging from short-term impacts such as allergies, and headaches to chronic impacts like reproductive disorders and neurological disorders (Wang et al., 2009; Guvenc et al., 2013). SPs are also known to be hepatotoxic and carcinogenic (Giray et al., 2001). Many of the toxic effects elicited by exposure to pesticides are mediated by the regulation of apoptosis (Park et al., 2013).

It has been demonstrated that pyrethroid insecticides have an apoptotic effect on different organisms (Casco et al., 2006). Apoptosis can be induced by a wide range of chemical, physical and genetic factors. However, most of these studies were performed in their racemic form of these chiral pesticides. Hence, in the present study, we aim to evaluate the apoptosis and ROS generation induced by

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individual isomers of *cis*-BF on human hepatocarcinoma cells (Hep G2).

2. Materials and methods

2.1. Chemicals

Racemic *cis*-BF (analytical standard, 99.5%, 2-methylbiphenyl-3-ylmethyl-(Z)-(1RS)-cis-(2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropane carboxylate was from Sigma (St. Louis, MO, USA). Minimal essential medium (MEM) was purchased from Gibco (Paisley, UK), and fetal bovine serum (FBS) was from Shijiqing Reagent Company (Hangzhou, China). Thiazolyl blue (MTT) solution (5 mg/mL in phosphate-buffered saline (PBS)) was from Amresco (Solon, OH, USA). 2′7′-dichlorofluorescin diacetate (DCFH-DA) was from Sigma (St. Louis, MO, USA).

2.2. Preparation of BF enantiomers

The enantiomers of *cis*-BF were resolved on a Jasco LC-2000 series HPLC system (Jasco, Tokyo, Japan) equipped with a PU-2089 intelligent quaternary gradient pump, an AS-1559 autosampler with a 100 μL loop, a mobile-phase vacuum degasser, an UV-2075 ultraviolet-visible detector, a variable-wavelength CD-2095 circular dichroism (CD) detector, a CO-2060 column temperature-control compartment, and an LC-Net II/ADC data collector (Jasco, Tokyo, Japan). The mobile phase (β-cyclodextrin) was made of *n*-hexane fortified with ethanol. The Chiralcel OJ [250 mm × 4.6 mm i.d., cellulose tris (4-methyl benzoate) column (Daicel Chemical Industries, Tokyo, Japan)] was used. The detection wavelength of the CD detector was 230 nm. The resolved enantiomers were individually collected at the HPLC outlet, evaporated to dryness, and used in the following experiment. The enantiomers concentrations were tested on an Agilent 6890N gas chromatograph (GC) equipped with an electron capture detector (ECD), assuming the same response factor for enantiomers as for the racemate of *cis*-BF.

2.3. Cell culture and treatments

Hep G2 cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Thermo Scientific HyClone, San Jose, CA, USA) with 10% (v/v) fetal bovine serum (FBS) at 37 °C in a 5% CO_2 incubator. Cells were subcultured every 2–5 days and seeded in 96-well plates (Costar, Cambridge, MA, USA) at a density of 2×10^5 cells/mL and allowed to adhere for 24 h prior to assaying.

2.4. Assessment of cell viability

Cell viability was measured by quantitative colorimetric assay with MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide). Hep G2 cells were incubated with five different concentrations of individual *1S-cis*-BF, *1R-cis*-BF, or *cis*-BF for 12 h. MTT at 500 μg/mL (final concentration) was added to each well and the cells were incubated at 37 °C for 4 h. After removing MTT, the cells were lysed with dimethyl sulfoxide (DMSO) (150 μL each well). The absorbance at 490 nm was measured using a microplate reader (Bio-Rad Laboratories, Hercules, CA, USA). Control cells were treated with the vehicle (0.1% ethanol, onefold).

2.5. Assay of the cellular contents of ROS

Hep G2 cells were incubated with individual enantiomers or a racemate of *cis*-BF at concentrations of 0, 5, 10, 15, and 20 mg/L for 2 h. After this treatment, cells were washed three times with ice-cold PBS then incubated with 10 μM DCFH-DA for 30 min at 37 °C. A fluorescent spectrophotometer (Tecan Infinite M200, Switzerland) was used to assay the cellular ROS by measuring 2′7′-dichlorofluorescein fluorescence (excitation at 485 nm/emission at 535 nm). The resulting ROS levels were determined as relative fluorescence intensities.

2.6. Cell apoptosis assay

Hep G2 cells at 5×10^6 cells mL^{-1} were treated with individual or racemate *cis*-BF at 10 or 20 mg/L for 6 h, respectively. Then cells were harvested and washed twice with PBS. The treated cells were suspended in binding buffer at 3×10^6 cells mL^{-1}, and supplemented with 5 μL of FITC-Annexin-V and 10 μL of propidium iodide (PI) (Annexin-V-FITC Apoptosis Detection Kit, Sigma). Cell apoptosis was assessed by flow cytometry (Beckman Coulter EPICSXL, USA) after incubated for 20 min at room temperature in the dark. All experiments were repeated at least three times.

2.7. Western blot analysis

The lysate was cleared by centrifugation at 17,000 × *g* for 30 min at 4 °C after cells were homogenized on ice in lysis buffer. 50 μg protein from each sample were loaded on a 12% SDS-PAGE gel and transferred to PVDF membranes by electrophoretic transfer and blocked with 5% nonfat dry milk in TBST buffer. Then membranes were incubated with Bax and Bcl-2 (ProteinTech Group) overnight at 4 °C. Enhanced chemiluminescence (Pierce) was used to detect HRP-labeled secondary anti-rabbit antibody (Cell Signal). Blots were exposed to X-ray film for radiographic detection of the bands after immersion in ECL chemiluminescence reagents.

2.8. Statistical analysis

Statistical significance was determined by one-way analysis of variance (ANOVA). The statistical analysis of the data was performed with the statistical program package SPSS 11.0. A p-value <0.05 was considered significant.

3. Results and discussion

3.1. Enantioselective cytotoxicity in human Hep G2 cells

The results of MTT assay show that treatment with *1S-cis*-BF reduced cell viability in a dose-dependent manner (Fig. 1).