Ecotoxicology and Environmental Safety 164 (2018) 604–610

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Toxic effect of the novel chiral insecticide IPP and its biodegradation intermediate in nematode *Caenorhabditis elegans*

Tingting Bian[a], Xiaolin Zhu[a], Jing Guo[a], Ziheng Zhuang[a], Zhiqiang Cai[a,*], Xiubo Zhao[a,b,**]

[a] Laboratory of Applied Microbiology and Biotechnology, School of Pharmaceutical Engineering & Life Science, Changzhou University, Changzhou 213164, China

[b] Department of Chemical and Biological Engineering, University of Sheffield, Mappin Street, Sheffield S13JD, UK

ARTICLE INFO

Keywords:
Paichongding
Biodegradation intermediate
Toxic effect
Nematode
Caenorhabditis elegans

ABSTRACT

Caenorhabditis elegans, a kind of model organism, was used to investigate biodegradation pathway of IPP and M1 in nematodes, *in vivo* toxicity from IPP and M1 and the possible underlying molecular mechanism. The results showed that both IPP and M1 could decrease lifespan, locomotion behavior, reproductive ability and AChE activity. During IPP biodegradation process, three intermediates (M1–M3) were monitored and identified. Based on the identified metabolites and their biodegradation courses, a possible biodegradation pathway was proposed. IPP was probably transformed to different three metabolites in nematodes through oxidation and elimination of methyl and propyl etc. Under the same concentration, IPP had more severe toxicity than M1 on nematodes. IPP and M1 might reduce lifespan and decrease reproductive ability through influencing insulin/IGF signaling pathway and TOR signaling pathway. They could decrease expression levels of *daf*-16, *sgk*-1, *aak*-2, *daf*-15 and *rict*-1 genes, which involved in IGF and TOR signaling pathway.

1. Introduction

Paichongding (IPP, 1-((6-chloropydidin-3-yl) methyl)-7-methyl-8-nitro-5 -propoxy-1,2,3,5,6,7-hexahydroimidazo[1,2-α-]-pyridine), is a novel chiral insecticide with independent intellectual property rights developed in China (Cai et al., 2015a, 2015b, 2016a; Fu et al., 2013). It has higher insecticidal activity (40–50 times) compared to imidacloprid-resistance pests, and also has low toxicity to human. In China IPP was used for pesticide control and sprayed for almost 3.3 million hectares (Cai et al., 2016a; Chen et al., 2017).

Insecticides residue in environment was accumulated and accelerated with the increase of insecticides application. Many studies reported that insecticides and their residue in soil environment have potential risk to the soil balance (soil biochemical properties and fertility, *etc*), they also can be transferred through food chain and make ecosystems deterioration (Zabaloy et al., 2012; Zhang et al., 2014). Previous studies have emphasized on the biodegradation pathway of IPP, its behavior in soils, and its effect on microbial community, soil enzyme activity *etc*. (Cai et al., 2016a, 2016b, 2016c). However, little information is available on the toxicity of IPP and its biodegradation intermediates on protozoan in soils.

The nematodes are a diverse animal phylum and live in complex microbial environments that present many potential challenges to their heath and viability. *Caenorhabditis elegans* is a kind of nematodes and the model organism, which has widely been used in drug discovery, drug toxicity, ageing mechanism research etc., due to its relatively short lifespan and conserved mechanisms for regulation of antioxidant response (Avila et al., 2012). *C. elegans* has been widely accepted and utilized as an important alternative animal model for toxicity testing (Avila et al., 2012; Leung et al., 2008; Sprando et al., 2009). Many studies used *C. elegant* for toxicological research including organic compounds, drugs and nanomaterials etc., and found that toxicity is similar to that observed in mammals (Ju et al., 2013; Li et al., 2013; Zhao et al., 2013). In this study, we first studied and compared the toxicity of IPP and its metabolites, M1, to nematodes *Caenorhabditis elegans*, and IPP and M1 degradation pathway in nematodes were also studied and proposed. Moreover, there are limited information about toxicological mechanism for IPP and M1, we examined the relative gene expression for toxicity of IPP and M1. The results of this study will be useful for further understanding IPP degradation characteristics and its toxicity to animals.

* Corresponding author.
** Corresponding author at: Laboratory of Applied Microbiology and Biotechnology, School of Pharmaceutical Engineering & Life Science, Changzhou University, Changzhou 213164, China.
E-mail addresses: zhqcai@cczu.edu.cn (Z. Cai), Xiubo.Zhao@sheffield.ac.uk (X. Zhao).

https://doi.org/10.1016/j.ecoenv.2018.08.059
Received 4 April 2018; Received in revised form 19 July 2018; Accepted 17 August 2018
0147-6513/

2. Materials and methods

2.1. Chemicals and worm strain

Paichongding (IPP, 1-((6-chloropydidin-3-yl) methyl)-7-methyl-8-nitro-5-propoxy- 1,2,3,5,6,7-hexahydroimidazo [1,2-α-]-pyridine, chemical purity 98.3%) was obtained from Jiangsu Kesheng Company Ltd. The biodegradation intermediate of IPP, 1-((6-chloropydidin-3-yl) methyl)-7-methyl-8-nitro-5-hydroxyl- 1,2,3,5,6,7-hexahydroimidazo [1,2-α-]-pyridine (M1), was synthesized in our lab and its chemical purity was 97.2%. M1 was prepared and stored in the lab using a protocol described previously (Studziński et al., 2017). Briefly, IPP was dissolved in dichloromethane and added boron tribromide at − 78 °C, then reacted for overnight at room temperature. M1 was extracted by dichloromethane. Other chemical reagents are A.R. grade and purchased from Sinoreagent Company, China.

The wild type nematode, *Caenorhabditis elegans*, was used and maintained on nematode growth medium (NGM, pH 7.2) plates seeded with *Escherichia coli* OP50 at 20 °C as described previously (Sulston and Brenner, 1974; Zhang et al., 2015; Tissenbaum and Guarente, 2001). Age synchronous populations of *C. elegans* were obtained according to the previous reports (Zhao et al., 2013; Zhang et al., 2015). Different concentration of IPP and M1 (10 mg L^{-1}, 5 mg L^{-1} and 2.5 mg L^{-1}) were added to the NGM plates just before inocubation.

2.2. Lifespan and reproduction

During the lifespan assay, the worms transferred daily for the first several days of adulthood. The surviving worms were measured, and recorded every day and would be scored as dead when they did not respond to the stimulation of a platinum wire. The results were treated with three replicates. The reproduction was assayed by the brood size, which was determined as the number of offspring at all stages beyond the egg. Twenty replicates were performed.

2.3. Locomotion behavior

For the locomotion behavior assay, different concentration of IPP and M1 treatment were performed throughout the lifespan from L1-larvae. Head thrash and body bend were used as endpoints for locomotion behavior. Head thrashes are defined as a change in the direction of bending at the mid body. Body bends are defined as a change in the direction of the part of nematodes corresponding to the posterior bulb of the pharynx along they-axis, assuming that nematode was traveling along the x-axis.

During the locomotion behavior assay, the examined nematodes were transferred into the assay plate containing K medium on top of the agar. After a recovery time of 1 min, head thrashes, and body bends were counted for 1 min and 20 s respectively. Twenty replicates were performed for each experiment.

2.4. Acetylcholinesterase (AChE, EC 3.1.1.7) activity analysis

Inhibition of AChE in the LC50-treated worms was monitored after 24 and 48 h of exposure. Similarly, the normal worms were used to study the in vitro evaluation of AChE activity. 100 worms were chosen to estimate AChE activity (Ache, EC 3.1.1.7) and were ground with liquid nitrogen, then added 1.5 ml of 0.1 M phosphate buffer (containing 0.1% of TritonX-100, pH 7.5) and mixed thoroughly. The mixture was centrifuged at 12,000 rpm for 30 min at 4 oC. The supernatant was used for estimation of AChE activity and protein content. Protein was estimated by the method of Lowry et al. (1951). AChE was assayed as described by Ellman et al. (1961).

The procedures of enzyme assay were as follows: the blank consists of phosphate buffer (0.1 M, pH 8.0), substrate (0.075 M of acetylthiocholine iodide) and DTNB solutions (Dithiobisnitrobenzoic acid, 0.01 M, 39.6 mg of DTNB were dissolved in 10 ml pH 7.0 phosphate buffer and 15 mg of sodium bicarbonate were added). 0.1 ml of supernatant and 0.5 ml of substrate were mixed together and kept at 37 °C for 6 min, then 0.01 ml of DTNB solution and 4% of SDS solution were added in the reaction solution. The absorbance was measured at 421 nm. Enzyme activity was determined graphically using double-reciprocal plots of Leneweaver and Burk transformations

2.5. IPP/M1 degradation in nematodes and metabolite extraction

The wild type worms were grown for two generations on NGM plates seeded with OP50. The worms in six crowded plates were washed into 50 ml solution of NGM medium contained 10 mg L^{-1} of IPP or M1, grown at 22 °C and 220 rpm. Concentrated OP50 from 500 ml bacterial cultures were given on day 1, day 3 and day 5. The culture was harvested on day 7 by centrifugation at 5000 rpm. The supernatant was lyophilized and the residue was extracted with 95% ethanol for 10 h. the extraction was evaporated at RT to yield the crude extracts.

A Dionex U3000 HPLC system coupled with Bruker maXis 4G ion trap mass spectrometer with an electrospray ionization source (ESI) was used for LC-MS/MS analysis. The separate conditions were in accordance with those used for HPLC analysis. The ion source temperature was controlled at 250 °C, and the capillary voltage was − 4.5 kV. The analysis mode of ionization was electrospray ionization (ESI, positive). The operation conditions were as follows: collision energy, 10.0 eV; ISCID energy and ion energy, 5.0 eV; dry gas, 6 L min^{-1}; dry temperature, 180 °C; gas pressure, 1.5 bar. The continuous full scanning from m/z 50–500 Da was performed in positive ion mode.

2.6. RNA isolation and quantitative real-time PCR

Total RNA was isolated using Triozol RNA kit (Life technology) from worms treated with or without 5 mg L^{-1} of IPP or M1 for 48 h. Total RNA was then reverse-transcribed using PrimeScript 1st strand cDNA synthesis kit (Takara). Quantitative real-time-polymerase chain reaction (RT-PCR) was used to determine the relative quantification of the targeted genes (*sod*-2, d*af*-2, *sgk*-1, *aak*-2, *age*-1, *daf*-16 and *aak*-2 etc.) in comparison to the reference *act*-1 gene, and the results were expressed as the relative expression ratio. The primers used in this study were referenced the previous reports by Zhang et al. (2015, 2014).

3. Results and discussion

3.1. Effect of IPP and M1 on lifespan of C. elegans

Nematodes were treated with different concentration of IPP and M1 (2.5 mg L^{-1}, 5 mg L^{-1} and 10 mg L^{-1}) from L1-larvae stage in order to investigate IPP and M1's effect on lifespan of *C. elegans*. The results were shown in Fig. 1. Both IPP and M1 decreased nematodes lifespan significantly under the concentration of 10 mg L^{-1}, and IPP has higher toxicity to nematodes than M1 under the same condition. Lifespan was only 21 days in the 10 mg L^{-1} of IPP solution, while it reached 23 days in M1 solution. IPP and M1 both had gentle toxicity to *C. elegans* under the lower concentration (2.5 mg L^{-1}).

3.2. Effect of IPP and M1 on the locomotion and reproduction of C. elegans

C. elegans shows gradually impaired locomotion ability during its aging process. The effect of IPP and M1 on the locomotion ability of nematodes was investigated. Two important endpoints for locomotion ability, head thrash and body bend, were recorded every 1 min during its lifespan after treated with different concentration of IPP or M1 solution for 48 h. As shown in Fig. 2a and b, higher concentration of IPP and M1 solution significantly decreased locomotion ability during its aging process compared with untreated nematodes, which indicated that IPP and M1 can decrease life quality of nematodes. The body bent