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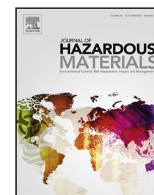

Potential hepatic toxicity of buprofezin at sublethal concentrations: ROS-mediated conversion of energy metabolism

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HIGHLIGHTS

- Liver was one of the major organs for buprofezin accumulation and toxicity.
- Buprofezin promoted the shunting of energy metabolism.
- ROS participated in buprofezin-mediated cytochrome c oxidase inhibition.

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ABSTRACT

Buprofezin is known for its broad-spectrum action and environmental safety. The popularity of buprofezin has raised concerns about its potentially adverse effects on human health and risk to the environment. In this study, we first identified the liver as one of the major organs in which buprofezin accumulated, and we detected a severe oxidative stress response. Next, we demonstrated that sublethal concentrations of buprofezin promoted the conversion of energy metabolism from the aerobic tricarboxylic acid (TCA) cycle and oxidative phosphorylation to anaerobic glycolysis. Importantly, reactive oxygen species (ROS) generation partially accounted for the shunting of the energy metabolism through the buprofezin-mediated inhibition of cytochrome c oxidase activity. ROS directly perturbed the activities of several key TCA cycle enzymes, stimulated glycolysis, and indirectly disturbed the activity of the respiratory chain complex by altering mitochondrial DNA (mtDNA). These findings clarify the potential mechanisms of buprofezin toxicity and provide biomarkers for buprofezin-mediated hepatotoxicity at sublethal concentrations.

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1. Introduction

Pesticides are widely used to control unwanted pests and disease vectors and to enhance food production in agriculture. Therefore, the potential adverse effects of environmental pesticide exposure on non-targeted organisms, particularly human beings, have become a major concern. According to a report from the World Health Organization (WHO) and the United Nations Environment Programme (UNEP), there are more than 26 million human pesticide poisonings worldwide per year, resulting in approximately 220,000 deaths [1]. In China, there are 0.5 million human pesticide poisonings per year, which are responsible for 0.1 million deaths [2]. Considering the environmental and health risks of pesticides, many traditional pesticides have been banned and replaced with lower-toxicity pesticides. Insect growth regulators such as chitin synthesis inhibitors appear to be promising because of their insect-specific mode of action and their lower toxicity in non-target organisms than conventional insecticides. However, chitin synthesis inhibitors at sublethal concentrations could have toxic effects on target and non-target organisms. Sublethal doses of hexaflumuron were shown to interfere with normal carbohydrate metabolism in certain instars of insects [3,4]. Diflubenzuron and flucycloxuron were shown to affect both glutathione levels and glutathione S-transferase activity in the adult females of a larvivorous fish [5]. Buprofezin (2-*tert*-butylimino-3-isopropyl-5-phenyl-1,3,5-thiadiazinan-4-one) is a typical inhibitor of chitin synthesis and molting. It acts specifically on immature developmental stages of homopteran pests by inhibiting the incorporation

Abbreviations: TCA cycle, tricarboxylic acid cycle; ROS, reactive oxygen species; mtDNA, mitochondrial DNA; SCO1, cytochrome c oxidase assembly protein; COX17, cytochrome c oxidase copper chaperone; CS, citrate synthase; OGDH, oxoglutarate (alpha-ketoglutarate) dehydrogenase (lipoamide); IDH2, isocitrate dehydrogenase 2 (NADP+); FH, fumarase; PFKFB3, 6-phosphofructo-2-kinase/fructose-2, 6-biphosphatase 3; LDHB, lactate dehydrogenase B; MMP, mitochondrial membrane potential; MRC, mitochondrial respiratory chain.

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of *N*-acetyl-[D-H3] glucosamine into chitin and interfering with cuticle formation, resulting nymphal mortality during molting [6,7]. It also exhibits larvicidal activity against the brown rice planthopper, *Nilaparvata lugens*, and the greenhouse whitefly, *Trialeurodes vaporariorum* [8,9]. According to the National Pesticide Toxicity Grading Standards Organization of China, buprofezin is graded as a low-toxicity pesticide [10]. However, current buprofezin production is approximately 4500 tons per annum, and due to its long half-life, buprofezin residues persist in plants and the environment [11]. According to the literature, the residues of buprofezin on oranges and lemons range from 0.05 to 0.69 mg/kg in Spain, Italy, Portugal, Australia and New Zealand, and the residues of buprofezin in rice samples range from 0.07 to 2.5 mg/kg in Korea, additionally, buprofezin residues are commonly detected in soil and water [12,13]. Notably, and in contrast to expectations, recent experimental evidence indicates that buprofezin in the aquatic environment, even at low concentrations (16 μM), causes detrimental effects to the early embryonic and larval development of African catfish [14]. Exposure to buprofezin at concentrations of 12.5–100 μM was shown to significantly increase the frequency of micronuclei in cultured Syrian hamster embryo cells [15]. Therefore, these findings raise concerns about the potentially adverse effects of buprofezin on human health at sublethal and even non-toxic concentrations.

The liver is an important detoxification organ. It is a primary target organ of pesticides and is also the most sensitive organ to their effects [16]. Additionally, the liver exhibits high metabolic activity and energy demands and plays a central role in maintaining energy homeostasis. Energy metabolism disorders are important contributors to liver injury [17,18]. Furthermore, mitochondrial dysfunction and the subsequent abnormalities in energy metabolism elicited in response to external stimuli have been implicated in the regulation of hepatic toxicity and even liver-related diseases [19–23]. It has been reported that chlorpropham induces mitochondrial dysfunction in rat hepatocytes [24]; the organochlorine lindane impairs mitochondrial function and energy metabolism in HepG2 cells [25]; and atrazine leads to abdominal obesity and insulin resistance in rats by impairing mitochondrial function [26]. Although the liver has been identified as the main target of buprofezin toxicity [27], a limited number of studies have focused on buprofezin-mediated changes in cellular signaling, particularly in relation to mitochondrial function and energy metabolism processes.

The purpose of this study was to identify the target organ of buprofezin and elucidate the mechanism of buprofezin-induced energy metabolism disorder. Mice were treated with buprofezin, and substantial amounts of buprofezin accumulated in the mouse liver, resulting in an oxidative stress response. In addition, HepG2 cells were exposed to different concentrations of buprofezin, and ATP, pyruvate and lactate were measured, and the mRNA expression levels of critical genes in the TCA cycle and glycolysis were quantified by RT-PCR. Furthermore, indicators of oxidative phosphorylation, including the mitochondrial membrane potential (MMP) and mitochondrial respiratory chain (MRC) complex enzyme activities, were analyzed. Our research suggests possible mechanisms for evaluating, alleviating and preventing the adverse outcomes of chitin synthesis inhibitors from the perspective of energy metabolism.

2. Materials and methods

2.1. Oral exposure of animals

Male C57BL/6 mice (6–8 weeks of age) were obtained from Experimental Animal Center of the Academy of Military Medical Sciences of the Chinese PLA (Beijing, China). All mice were housed under standard conditions (24 ± 2 °C, 50 ± 5% humidity, with a 12:12 h light:dark cycle) and were acclimatized for 1 week before starting the treatments. The animals had free access to water and standard feed and were randomly divided into four groups. For the control group, 0.9% sterile NaCl (200 μL/mouse) was intragastrically administered every other day for a total of 9 administrations, and the other three exposure groups were given 0.0463, 0.139 or 0.417 g/kg (bw) buprofezin using the same protocol. The animals were sacrificed 18 h after the last treatment. The liver, brain, kidney, heart, spleen and stomach, were excised and quick frozen in liquid nitrogen, then stored at −80 °C.

2.2. Cell culture

The human hepatocellular carcinoma cell line (HepG2) was obtained from the Cell Bank of the Type Culture Collection (CAS, China). The cells were maintained in RPMI 1640 medium (Gibco, USA) supplemented with 10% fetal bovine serum (FBS, Gibco, USA), 100 U/mL streptomycin (Gibco, USA) and 100 U/mL penicillin (Gibco, USA), in a humidified culture at 37 °C and 5% CO_2.

2.3. Determination of buprofezin contents

Gas chromatography–mass spectrometry (GC–MS) (TRACE-DSQ, Thermo Scientific, America) was utilized to analyze the buprofezin contents of the different tissues. The tissues were homogenized through sonication with $CHCl_3$ on ice for 10 min. Next, the homogenates were concentrated in a rotary evaporator for 5 min. Subsequently, 1 mL $CHCl_3$ was added to dissolve the extracts, which were then concentrated again with N_2. Finally, the extracts were dissolved in 1 mL of $CHCl_3$, and 1 μL of the samples was used for the detection of buprofezin via GC–MS. The parameters were set as in our previous work [28].

2.4. Measurements of the methane dicarboxylic aldehyde (MDA) levels

Approximately 0.1 g of tissue was homogenized with 1 mL of 0.9% NaCl on ice. The homogenates were centrifuged for 30 min at 10,000 rpm at 4 °C. The supernatant was then transferred to a new tube for MDA determination according the manufacturer's instructions (Nanjing Jiancheng Bioengineering Institute, China).

2.5. Cytotoxicity determination

The 3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyl-2-H-tetrazolium bromide (MTT) assay was applied to determine cytotoxicity before and after buprofezin exposure. Cells (1.0×10^4/well) were plated in 96-well plates and treated with 0, 1, 3, 10, 30, 100, 300 or 1000 μM buprofezin for 24 h. After culture at 37 °C for 24 h, MTT was added to the culture media at a final concentration of 0.5 mg/mL, and the cells were cultured for an additional 4 h. Thereafter, the supernatant was removed, and 100 μL of dimethylsulfoxide (DMSO) was added to each well. The cultures were then shaken for 10 min in the dark, and the absorbance was detected at a test wavelength of 570 nm and a reference wavelength of 630 nm using a Thermo Scientific Varioskan Flash reader (Thermo Fisher Scientific, USA).

2.6. Apoptosis detection

Approximately 5×10^5 cells/mL were seeded in 35-mm petri dishes and treated with 0, 3, 10 or 30 μM buprofezin for 24 h. After exposure, the cells were collected and washed twice with cold PBS. Subsequently, the cells were resuspended in binding buffer and stained with 5 μL of annexin V and 1 μL of propidium iodide (PI) for

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