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Toxic effects of albendazole on adenosine triphosphatase activity and ultrastructure in *Eisenia fetida*

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Abstract

Veterinary drug may influence soil fauna through manure excretion and subsequent application to agricultural field. The aim of this study is to determine the toxicity of albendozale (ABZ) on the whole earthworm as well as its different regions. Earthworms of *Eisenia fetida* were exposed to ABZ at 0, 100, 200, 400, and 600 mg kg^{-1} concentrations; samples were taken at days 2, 7, and 14 exposure for determination of two adenosine triphosphatase (Na^+–K^+- and Mg^{2+}-ATPase) activities and survival and growth rate. In addition, the ultrastructure of intestinal epithelium of the earthworms was examined after 14-day exposure. The survival and growth rate were reduced as compared to the control at the two highest concentrations (400 and 600 mg kg^{-1}) after 7- and 14-day exposure. With increasing ABZ concentration, ATPase activities were inhibited significantly in the mid-part after 7 and 14 days and the posterior after 14 days. In particular, the inhibition effect was significant even at the lower treatment levels (100 and 200 mg kg^{-1}) after 14 days. Both ATPase activities, however, were increased significantly in the anterior of earthworms at the highest concentration (600 mg kg^{-1}) after 14 days. Ultrastructure observation in intestine epithelium in three concentrations (control, 100, 600 mg kg^{-1}) revealed that mitochondria and smooth endoplasmic reticulum were damaged with increasing ABZ concentration. Some mitochondria was exhibited the damage of inner membrane at 100 mg kg^{-1} and vacuolization at 600 mg kg^{-1}, which is consistent with ATPase activities inhibition. The investigation of enzymatic activities in different regions of earthworms and pathological alterations in the intestinal epithelium can provide important information in terms of toxic effects of soil contamination and be used as early warning systems.

Keywords: Earthworms; Albendazole; Adenosine triphosphatase; Ultrastructure

1. Introduction

Veterinary drugs are often used to help maintain the health condition of domestic animals and ensure their well-being (Boxall et al., 2003). However, incorrect or excessive use in recent decades has caused substantial amounts of drugs and their metabolites being released to the environment through manure application onto agricultural land. One potential adverse effect is harming some non-target organisms utilizing the excrement (Yoshimur et al., 2005; Jjemba, 2006). To date, only limited investigation has been conducted on the ecotoxicological effects of veterinary drugs Baguer et al., 2000; (Liguoro et al., 2003; Halley et al., 2005) despite of a growing interest in this area.

Biochemical reaction studies mostly focus on evaluation of possible adverse effects of chemicals on aquatic organisms (Wollenberger et al., 2000; Ghorpade et al., 2002; Robillard et al., 2003; Rajalakshmi and Mohandas, 2005; dos Santos Miron et al., 2005). The severity of soil contamination caused by veterinary drugs is commonly assessed with standard acute and reproduction tests. These standard tests are unable to evaluate the biochemical responses of selected organisms to the given drug exposure. These biochemical responses, generally called early warming signals, can provide valuable

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information in assessing the potential risk factors of soil contamination.

Earthworms are preferred bioindicators to be used for assessing chemical contamination to soils (Venkateswara and Kavitha, 2004). As important species in decomposer communities, earthworms have a great impact on decomposition activity, nutrient mineralization and primary production. In particular, earthworms can perceive a diverse range of chemical stimuli and response through certain reactions accordingly (Lukkari et al., 2004, 2005). For example, when exposed to chemicals, earthworms are capable of reducing potential toxic effects by adjusting their internal biochemical responses even before their growth and reproduction are affected. Thus, early biochemical reaction of earthworms on chemicals could provide important information in assessing potential adverse effects on soil environment (Svendsen et al., 2002). *Eisenia fetida*, because of its low cost, easy culturing and the standardization of the acute and subchronic ecotoxicological tests, is considered as a suitable biomonitor model species to determine the ecological hazard of heavy metals and chemicals contaminated soil (Xiao et al., 2006).

Albendazole (ABZ), a derivative of benzimidazole, exhibits broad spectra of gastrointestinal anthelminthic activity and has been used widely in livestock (Martin, 1985). The anti-helminth mechanism of benzimidazole carbamate has been studied (Mottier et al., 2003; Daniel-Mwambete et al., 2004; Xiao et al., 2005). Previous reports indicated that ABZ binds to β-tubulin and inhibits its polymerization to form microtubules (Oxberry et al., 2001). ABZ also inhibits fumarat reductase and glucose transport, which is associated with adenosine triphosphate (ATP) synthesis (Grønvold et al., 2004). Therefore, ABZ is effective against nematodes, cestodes, and trematodes (McKellar and Scott, 1990). ATP hydrolysis is an essential reaction in providing energy for biomolecular synthesis and transport within the cell, and ATPase, as a group of enzymes, plays an important role in this process, and it is considered as a sensitive indicator of toxicity (Sancho et al., 2003). Because ATPase inhibition occurs before gross osmoregulatory dysfunction, measuring its activities can be used as early warning of pollutant-induced injury (Stagg et al., 1992).

Given orally, ABZ is partially metabolized in the gut or in the body of animals, with part of the absorbed drug is excreted unchanged in feces and urine. The main bioactive metabolite, ABZ-sulfoxide, an effective anthelmintic (Daniel-Mwambete et al., 2004), is also excreted to the environment. There is, however, no clarity of the potential effects of ABZ or its metabolites on soil organisms such as earthworms.

The main purpose of the present study was to assess the biochemical responses of earthworm upon exposure to ABZ by measuring adenosine triphosphatase (ATPase) activity in whole body and different regions. In addition, earthworms ingest large amounts of soil and therefore some portion of the chemical may be absorbed across their intestinal tract, which can cause direct toxic effects on the intestinal epithelium. Hence, the second purpose was to examine the morphological alteration of the intestinal epithelium of earthworms upon exposure to ABZ using electron microscopy.

2. Materials and methods

2.1. Experimental procedures

Earthworms *E. fetida* were obtained from an earthworm farm at China Agriculture University, kept in the test substrate (cattle manure) in laboratory for a week prior to the start of the experiment. The individual earthworms were adults with well-developed clitellum. Pre-treated urine- and pollution-free cattle manure (Helling et al., 2000) was used as test substrate in this study. The ABZ, purchased from Sigma with a purity above 98%, was first dissolved in acetone then mixed into the substrate with treatment rates at 0, 100, 200, 400, and 600 mg kg^{-1} dry weight. Distilled water was added to the mixture to reach a 75% weight increase. The substrates (pH 7.0 and C/N: 24) after being treated with ABZ were transferred to plastic containers (diameter 12 cm, depth 10 cm), which were placed in ventilated case to allow residual acetone to evaporate overnight. Water was added to compensate for the lost weight due to acetone evaporation, then earthworms were introduced. A total of 12 individually weighed earthworms (350 ± 20 mg each; served as 12 replicates) were placed into each container. The containers were maintained in an environmental chamber under 23 ± 1 °C and a lighting of about 1000 lx with a 12/12-h photo-period; water was supplemented to ensure appropriate water content of the substrate by weighing the containers (Jensen et al., 2003).

At days 2, 7 and 14 of exposure, earthworms of four replicates per treatment were removed from the substrate, washed in distilled water and dried on paper towels before using them for ATPase activity assay. Survival and growth rates were monitored at days 7 and 14 of exposure (Xiao et al., 2006). Some earworms from the three selected treatments (control, 100, 600 mg kg^{-1} ABZ) were used for the ultrastructure study of intestinal epithelium at the end of experiment (14 days).

2.2. Sample preparation and enzyme measurement

Prior to further examination and analysis after sampling, earthworms were incubated without feeding for 2 days on wet filter paper at room temperature to empty their gut content. Earthworm samples were collected at days 2, 7, and 14 and Na^+–K^+- and Mg^{2+}-ATPase activities were measured in the anterior region (mouth to 25 segments), mid-part (25–33 segments), posterior region (34 segments to anus) as well as in the whole earthworms. The whole earthworm or dissected parts from each replicate were pooled together, snap-frozen in liquid nitrogen and ground with liquid nitrogen, then homogenized in four parts (w:v) of Tris–HCl buffer (0.1 M, pH 7.6) containing 1 mM EDTA in 0.25 M sucrose. Then the homogenate was centrifuged at 3000*g* for 15 min and the supernatant was snap-frozen in liquid nitrogen and stored at −80 °C until analysis. All enzyme preparations were carried out at 4 °C. ATPase activity was measured spectrophotometrically using the method from Jena and Patnaik (1995) and protein content was measured according to Bradford (1976) with BSA as the reference substance.

2.3. Electron microscopy observations

Earthworms for ultrastructure examination were put on humid filter paper at room temperature to empty their gut content. Individuals were anesthetized by placing them on moist cold filter paper in petri dishes for ten minutes, and then in cold mineral water for five minutes, which helped to relax muscles for dissection (Sorour and Larink, 2001). The dissection