Ecotoxicology and Environmental Safety 101 (2014) 31–35

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

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

Biochemical responses to the toxicity of the biocide abamectin on the freshwater snail *Physa acuta*

Junguo Ma, Chune Zhou, Yao Li, Xiaoyu Li*

College of Life Science, Henan Normal University, Xinxiang, Henan 453007, China

ARTICLE INFO

Article history:
Received 14 September 2013
Received in revised form
3 December 2013
Accepted 11 December 2013
Available online 8 January 2014

Keywords:
Abamectin
Physa acuta
Acetylcholinesterase
Nitric oxide synthase
Antioxidant enzymes
Lipid peroxidation

ABSTRACT

The toxic effects of abamectin (ABM), an anthelmintic drug, on the snail, *Physa Acuta*, and the biochemical responses to the exposure stress were evaluated. The activities of superoxide dismutase (SOD), catalase (CAT), glutathione S-transferase (GST), acetylcholinesterase (AChE), and nitric oxide synthase (NOS), and the contents of malondialdehyde (MDA) were determined in snail soft tissues (head, foot, visceral mass, and the mantle) for up to 96 h of exposure to 3.4, 9.6, 19.2, or 27.4 $\mu g\ L^{-1}$ of ABM. The results showed that SOD and GST activities were promoted by ABM-exposure at the earlier periods of treatment (12–48 h) while these activites were inhibited at the end of test. The tendency of CAT activity was similar to that of SOD, but it increased at the end of test. MDA levels of the snail soft tissues increased in all treatment groups, including the recovery group, indicating that lipid peroxidation occurred in snail soft tissues. ABM-exposure inhibited AChE activity. However, NOS activities increased by ABM-exposure. In addition, activities of antioxidant enzymes and AChE from the snail soft tissues resumed the normal levels after 96 h of recovery period, but MDA level did not attain the original level. This study provides information on the biochemical mechanism of ABM toxicity on the snail.

1. Introduction

Abamectin (ABM) is a kind of large ring lactone disaccharide compounds and the natural fermentation product of the soil-dwelling actinomycete *Streptomyces avermitilis* (Roth et al., 1993; Luo et al., 2013). It is highly lipophilic and used both as a biocide and as an anthelminthic drug. According to the classification standard by WHO, ABM belongs to highly toxic chemicals with neurological and developmental toxicity. The toxicological mechanism of ABM is believed to affect the γ-aminobutyric acid (GABA) system and Cl^- channels of animal cells (Maioli et al., 2013), in which the GABA receptor is responsible for regulating the neural basal tone (Turner and Schaeffer, 1989). Since twenty years ago, ABM had been widely used for preventing parasitic diseases from fish (Lorio et al., 1992; Johnson and Margolis, 1993). Therefore it may enter aquatic environment and pose environmental risks to aquatic animals and ecosystems. Toxicologically, ABM usually has only slight toxicity to earthworms and birds (Halley et al., 1993), but it is highly toxic to fish (Jenčič et al., 2006), representing a potential threat to aquatic animals.

Aquatic snail *Physa acuta* is an invasive species and now world widely distributed in freshwater body (Albrecht et al., 2009; Guo et al., 2009). Moreover, the snail is very sensitive to toxicants and thus appropriate for toxicity testing (Evans-White and Lamberti, 2009; Sánchez-Argüello et al., 2009; Lance et al., 2010; Musee et al., 2010; De Castro-Català et al., 2013; Hossain and Aditya, 2013; Seeland et al., 2013). Therefore it was adopted as experimental animal to determine ABM toxicity and biochemical alterations in the snail *P. acuta* by conducting an acute exposure of ABM in the present study.

2. Materials and methods

2.1. Chemicals and reagents

ABM as two percent microemulsions was obtained from Biogen Crop Science Limited, China. It was first dissolved in distilled water for stock solutions and then diluted to obtain the experimental concentrations.

The Superoxide dismutase (SOD), catalase (CAT), glutathione S-transferase (GST), acetylcholinesterase (AChE), and nitric oxide synthase (NOS) Diagnostic Reagent Kit were purchased from the Nanjing Jiancheng Bioengineering Institute (China). Bovine serum albumin and thiobarbituric acid (TBA) were obtained from Sigma (USA).

2.2. Snails

The adult *P. acuta* with an average wet weight of 0.136 ± 0.035 g and an average shell length of 6.853 ± 1.438 mm were collected from a fishery pond in China and cultured in glass jars (3 L in volume) with aerated tap water (total hardness of

* Corresponding author. Fax: +86 373 3329390.
E-mail address: lixiaoyu65@263.net (X. Li).

0147-6513/$ - see front matter © 2014 Published by Elsevier Inc.
http://dx.doi.org/10.1016/j.ecoenv.2013.12.009

water 340 mg L^{-1}, pH 7.6, turbidity 1.5 nephelometric turbidity units, and total dissolved solid content 660 mg L^{-1}, which were detected according to the Standards for Drinking Water Quality, China, GB5479-2006) at a constant temperature (22 ± 1 °C) and a 16 h: 8 h light/darkness photoperiod under laboratory conditions for several generations prior to the experiment. Snails were fed on a mixture of commercial goldfish food (Wannong Fishery Company, China) at a rate of 0.5–1 percent of body weight/day and lettuce leaves. The water was changed weekly. The snail was handled according to the guidelines in the China Law for Animal Health Protection and Instructions for Granting Permits for Animal Experimentation for Scientific Purposes (Ethics approval No. SCXK (YU) 2005-0001).

2.3. ABM-exposure

The acute toxicity test was conducted according to the Spearman-Kärber method (Kärber, 1931) and the exposure concentrations were 3.4 μg L^{-1} (1/10 96 h LC_{50}), 9.6 μg L^{-1} (1/10 48 h LC_{50}), 19.2 μg L^{-1} (1/5 48 h LC_{50}), and 27.4 μg L^{-1} (2/7 48 h LC_{50}). These concentrations were designed based on the results of our previous report, in which the 96 h and 48 h LC_{50} of ABM to *P. acuta* was 34 and 96 μg L^{-1}, respectively (Ma and Li, 2011). Two hundred snails were randomly divided into five groups (40 snails in each group), out of which four groups were ABM-treated groups (3.4, 9.6, 19.2, and 27.4 μg L^{-1} of ABM) and exposed for 12, 24, 48, and 96 h. Another group was maintained as a control. The animals in each test group were placed in a 1000 mL beaker containing 800 mL of ABM solution, and the control snails were bred temporarily in a beaker with aerated tap water. The beaker was covered with cotton gauze to prevent snails from escaping. The cotton gauze must be on the surface of water in the beaker so that the snails cannot choke due to dryness when they attach to the cotton gauze. Each test was conducted in triplicate. Except snails were not fed, ABM exposure conditions were maintained as to those used for the breeding of the snails.

At the end of each exposure period (12, 24, 48, and 96 h), ten snails from each group were taken and placed in Eppendorf tube. After their shells were removed, all the soft tissues (head, foot, visceral mass, and the mantle) were homogenized (10 percent w/v) in 0.1 M phosphate buffer (pH 7.5). The homogenate was centrifuged at 12,000 g for 10 min at 4 °C and the supernatant obtained was stored at −20 °C for biochemical assays.

2.4. Recovery test

The recovery test was carried out to evaluate the surviving capacity of snails from the acute exposure of ABM. Sixty snails were randomly divided into three groups, in which two groups were the ABM-treatment groups (19.2 and 27.4 μg L^{-1} of ABM) and another as a control group. After 96 h of treatment, the snails were removed to the aerated tap water immediately and recovered in the ABM-free water for another 96 h. During the recovery test, snails were fed on commercial food at a rate of 0.5–1 percent of body weight/day. At 48 or 96 h time intervals, ten snails were collected and homogenized as described above for biochemical assays. Each test was conducted in triplicate.

2.5. Biochemical analysis

The activities of SOD, CAT, GST, AChE, and NOS in snail soft tissues were determined by using the Diagnostic Reagent Kit purchased from the Nanjing Jiancheng Bioengineering Institute (China) according to the manufacturer's instructions. The assay results were given in units of enzymatic activity per milligram of protein (U/mg protein), in which one unit was defined as the amount of enzyme producing 50 percent inhibition of the enzyme, as the amount of enzyme decomposing 1 μM H_2O_2 per second, as the amount of enzyme causing GSH concentration down 1 μM L^{-1} in 1 min, as the amount of enzyme decomposing 1 nM NO per minute, and as the amount of enzyme capable of converting 1 μM of acetylthiocholine iodide as a substrate per minute for SOD, CAT, GST, NOS, and AChE, respectively.

The total protein contents of samples were measured by the method of Lowry et al. (1951) using bovine serum albumin as a standard. Lipid peroxidation was measured by the TBA method (Ohkawa et al., 1979). The assay was monitored for the appearance of the conjugated complex of TBA and malondialdehyde (MDA) at 532 nm. The concentration of MDA was expressed as nM MDA per mg protein.

2.6. Statistical analysis

Statistical analysis was performed using the SPSS 13.0 software (SPSS Inc.). All data were analyzed by one-way ANOVA and post-hoc pairwise comparisons among all groups using the Duncan's test ($p < 0.05$).

3. Results

3.1. Antioxidant enzymes

3.1.1. SOD activity

No change was found in SOD activity of the snail soft tissues between the treatment and control groups after 12 h of ABM-exposure, except for an increase in the group of 19.2 μg L^{-1} of ABM when compared to the control (Fig. 1A). However, at 24 h exposure, SOD activities in the groups of 9.6 and 19.2 μg L^{-1} of ABM were significantly higher than that of control, while it was remarkably inhibited in the highest concentration group (27.4 μg L^{-1}). Its activity was maintained unchanged in the lowest concentration group (3.4 μg L^{-1}). Then (48 h later), SOD activity in 19.2 μg L^{-1} of ABM group also decreased while still increased in 9.6 μg L^{-1} group. At the end of test (96 h), SOD activities in all treated groups (except for 3.4 μg L^{-1}) were lower than that of control (Fig. 1A).

3.1.2. CAT activity

The average change tendency of CAT activity was similar to that of SOD before 48 h of ABM-exposure, but it increased in all treated groups (except for 3.4 μg L^{-1}) at the end of test (Fig. 1B).

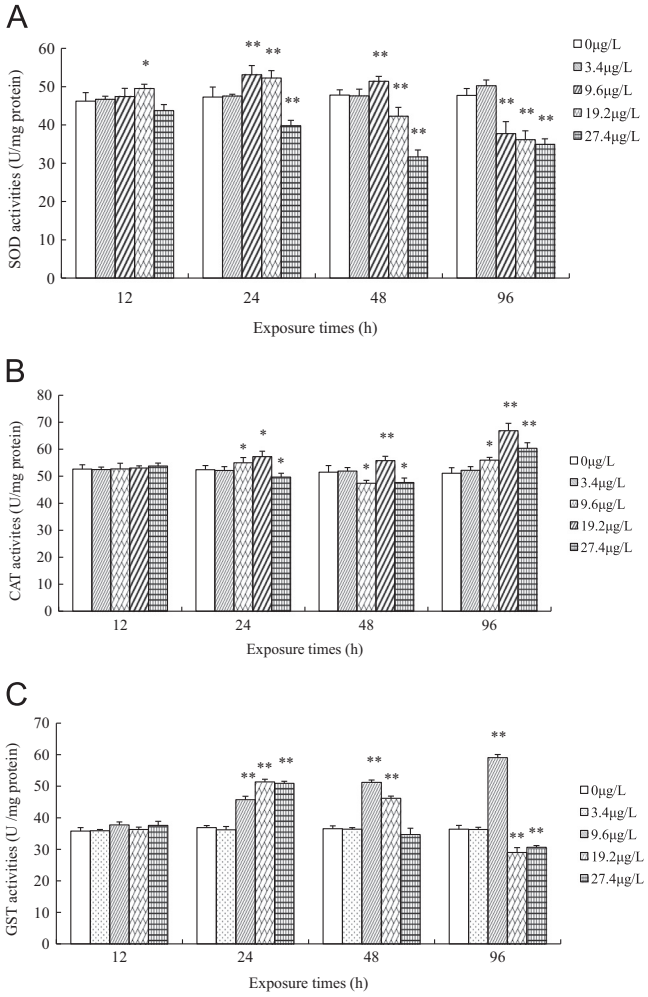

Fig. 1. Effects of ABM on activities of SOD (A), CAT (B), and GST (C) in the snail soft tissues after 12, 24, 48, or 96 h of exposure. Values represent the means and vertical bars indicate the standard deviation of three separate experiments. Asterisks denote a response that is significantly different from the control (*$p < 0.05$, **$p < 0.01$).