Bull Environ Contam Toxicol (2012) 89:723–726
DOI 10.1007/s00128-012-0775-8

Effects of Paclobutrazol Exposure on Antioxidant Defense System in *Sebastiscus marmoratus*

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Received: 23 June 2012 / Accepted: 3 August 2012 / Published online: 12 August 2012

Abstract This study was conducted to detect the effects of paclobutrazol (PBZ) at environmental concentration on the antioxidant defense system in *Sebastiscus marmoratus*. Fish were exposed to concentrations of PBZ (10, 100, 1,000 ng/L) for 50 days. The results showed: (1) The glutathione contents in the liver and brain were significantly decreased in a dose-dependent manner. (2) The activities of glutathione S-transferases (GST) and catalase in liver and brain were inhibited as the increase of PBZ concentration. A highest 2.16-fold ($p = 0.05$) and 11.54-fold ($p < 0.001$) reduction of GST activity in the liver and brain respectively was observed in 1,000 μg/L group. (3) The activities of glutathione peroxidase in liver were inhibited. These results suggest that the exposure of PBZ would influence the antioxidant ability of *S. marmoratus*.

Keywords Pesticide · Paclobutrazol · Antioxidant defense system · Marine fish

Triazole-containing fungicides, a class of current pesticides, are widely used in agriculture. The continued extensive use of these pesticides has created a corresponding increase in the concern for potentially adverse effects to aquatic organisms (Konwick et al. 2006; Li et al. 2010a). Triazole fungicides, such as triadimefon, propiconazole (PCZ) and paclobutrazol (PBZ), have been detected in the aquatic environment. For instance, PCZ and ketoconazole are detected in groundwater at concentrations ranging from ng to μg per litre of influent (Van De Steene and Lambert 2008). The concentration of PCZ in surface water from a banana plantation in Limon, Costa Rica is 0.15–13 μg/L (Castillo et al. 2006). Realistic concentrations of PCZ after run-off waters from agricultural fields may be in the range 0.1–10 μg/L (Egaas et al. 1999). The concentration of PBZ in the surface water from the Jiulong River Estuary and Western Xiamen Sea, China was ND ~119.6 ng/L (unpublished data).

The adverse effects of many chemicals upon animals are related to their capacity producing reactive oxygen species and causing oxidative damages. Antioxidant defense systems can scavenge chemical reactive intermediates produced by xenobiotic metabolism. Glutathione S-transferase (GST) and glutathione peroxidase (GPx) decrease xenobiotic reactivity via catalyzing the conjugation of reduced glutathione (GSH) with a xenobiotic. Antioxidant defense systems are impacted by exposure of many chemicals.

It has been reported that the median lethal concentration value (LC_{50}) at 96 h of PBZ in zebrafish (*Danio rerio*) is 20.55 mg/L (Ding et al. 2009). In rainbow trout (*Oncorhynchus mykiss*) exposed to PCZ (0.2, 50 and 500 μg/L) for 20 and 30 days, the levels of oxidative stress indices (reactive oxygen species, lipid peroxidation, and carbonyl protein) are elevated significantly in the brain (Li et al. 2010a) and liver as well as gill (Li et al. 2010c), the activity of antioxidant enzyme (superoxide dismutase, catalase, GPx, glutathione reductase) and reduced

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glutathione content are inhibited significantly in the brain (Li et al. 2010a) and gill, but not in the liver (Li et al. 2010b). However, limited information on the effects of triazole pesticides at environmental concentrations on antioxidant defense systems in marine fish is available. The objective of this study was to investigate the effects of PBZ on antioxidant defense systems in *S. marmoratus*, which is distributed throughout the coastal areas of China.

Materials and Methods

Paclobutrazol was provided by the Sangon Company (Shanghai, China), with a purity of greater than 95 %. It was dissolved in dimethyl sulfoxide to produce stock solutions of 10, 100 and 1,000 μg/mL. All other chemicals were of analytical grade and were obtained from commercial sources.

Sebastiscus marmoratus weighing 21–32 g, were captured from a coastal area of Xiamen City, Fujian Province, China in November 2010. All animal experiments were conducted according to the research protocols approved by the Xiamen University Institutional Animal Care and Use Committee. Before the exposure experiment, the fish were acclimated in tanks (25 fish in each tank) containing 50 L of aerated sand-filtered static seawater, with a natural photoperiod for 7 days. Twenty-five fish per group were exposed to the nominal concentrations (10, 100 and 1,000 ng/L) of PBZ, and the control group received an equal volume of the solvent dimethyl sulfoxide (1 μL/L). Half the water containing the different concentrations of PBZ was changed every day. The fish were fed daily with commercial fish pellets at 1 % total body weight, and uneaten food was removed. The water temperature was maintained at 14 ± 2°C and salinity at 22 ‰–24 ‰. During the experimental period, the sand-filtered seawater (the reference water) was collected randomly several times and detected according to our previous method (Li et al. 2012). The results showed that the range of PBZ concentrations in the reference water ranged from ND-2.5 ng/L.

Six fish from each group were sampled after exposure for 50 days. Fish were killed by a sharp blow on the head, the liver and brain was frozen in liquid N_2 immediately after collection and stored at −80°C until analyzed.

Homogenate of the liver or brain was prepared in chilled buffered KCl (1.15 % KCl buffered with 0.01 mol/L Tris–HCl, pH 7.4) and centrifuged at 10,000 g for 20 min at 4°C to obtain post-mitochondrial supernatant, which was used as the source of enzyme (Livingstone 1988). GST activity was evaluated with 1-chloro-2,4-dinitrobenzene as substrate, following the formation of the conjugate with GSH at 340 nm ($\varepsilon = 9.6$/mMcm) according to Habig et al. (1974). GSH content was determined using a fluorometric assay according to the method of Hissin and Hilf (1976). GPx activity was measured according to Hafeman et al. (1973). One unit of GPx activity is defined as the amount of enzyme that oxidizes 1 mmol/L of GSH per min per mg of protein at 30°C. The catalase (CAT) activity assay, using the spectrophotometric measurement of H_2O_2 breakdown, was performed following the method of Beers and Sizer (1952). Protein concentrations in the supernatants were determined by the Bradford procedure (Bradford 1976) using bovine serum albumin as standard. All fluorometric assays were determined on a Hitachi F-4010 fluorescence spectrophotometer. The absorbance at UV and visible wavelength was monitored on a UV-2550 UV–VIS spectrophotometer (Shimadzu corporation, Japan) spectrophotometer.

Results are reported as mean ± SD. The data were statistically analyzed with one-way analysis of variance (ANOVA) followed by the Duncan post hoc test via SPSS 13.0 software. A value of $p < 0.05$ was used to indicate significant difference.

Results and Discussion

The GSH contents in the liver and brain were significantly decreased in a dose-dependent manner after the 50-day exposure compared to the control. A highest 1.68-fold ($p = 0.009$) and 1.78-fold ($p = 0.031$) reduction in the liver and brain respectively was observed in 1,000 μg/L group (Fig. 1a).

The GST activities in the liver and brain were significantly decreased in a dose-dependent manner after the 50-day exposure compared to the control. A highest 2.16-fold ($p = 0.05$) and 11.54-fold ($p < 0.001$) reduction in the liver and brain respectively was observed in 1,000 μg/L group (Fig. 1b).

The CAT activities in the liver and brain were significantly decreased in a dose-dependent manner compared to the control. A highest 2.51-fold ($p = 0.01$) and 1.96-fold ($p = 0.014$) reduction in the liver and brain respectively was observed in 1,000 μg/L group (Fig. 1c).

The GPx activity in the liver was significantly decreased in a dose-dependent manner after the 50-day exposure compared to the control. A highest 1.55-fold ($p = 0.034$) reduction was observed in 1,000 μg/L group (Fig. 1d).

Estuaries are particularly susceptible to anthropogenic pollution because they receive a lot of contaminants, especially pesticides. They are important nursery grounds for many marine species whose juvenile forms are often more sensitive to environmental contaminants. Triazole pesticides are generally less environmentally persistent having shorter half-lives, but durative input of them may cause detrimental effects on fish health. Very little research has been performed on the exposure of estuarine and offing species to triazole pesticides.

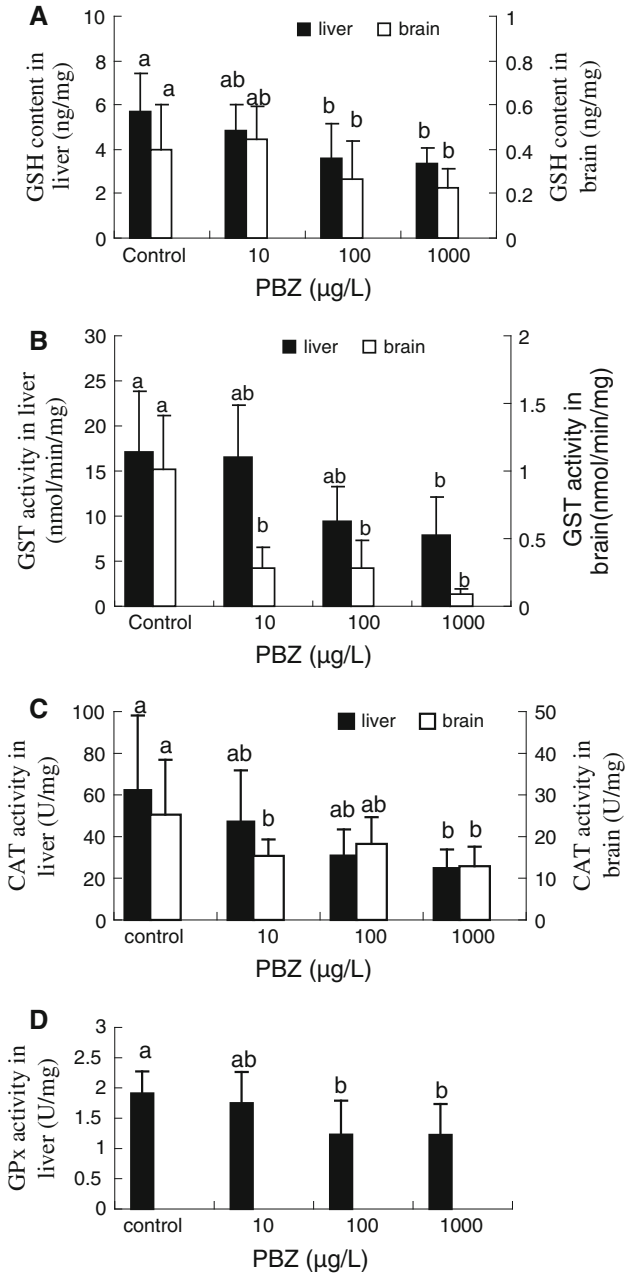

Fig. 1 Effects of paclobutrazol (0, 10, 100 or 1,000 ng/L) exposure for 50 days on the GSH contents (**a**), GST (**b**), CAT (**c**) and GPx (**d**) activities in *Sebastiscus marmoratus*. Data are presented as mean ± SD (n = 6). Means of exposures not sharing a common letter are significantly different at $p < 0.05$ as assessed by one-way ANOVA followed by the Duncan test

Many pollutants may exert toxicity related to oxidative stress. The conjugation of GSH with a xenobiotic, either done spontaneously or catalyzed by GST and GPx, decreases xenobiotic reactivity. GSH depletion may reduce the cellular ability to scavenge free radicals, raising the general oxidative potential in the cells. Lowering the intracellular GSH level and decreasing GSH-related antioxidant enzymes activity simultaneously lead to oxidative imbalance and induces oxidative processes, resulting in increased cell death (Pandey et al. 2008). In rainbow trout, hepatic GST and GPx activity is significantly induced in 50 and 500 μg/L groups after exposure for 20 or 30 days (Li et al. 2010c). A significant decrease in GSH levels in 50 and 500 μg/L groups is observed in liver after 20 days of exposure, while it resumes to control level in 500 μg/L after 30 days (Li et al. 2010c). Our results were different with the previous reports, which may due to the different exposure concentration and duration. In the present study, PBZ exposure resulted in a significant inhibition of GST and GPx activity and a significant reduction of GSH levels, which would reduce the capacity of fish to detoxify chemicals.

The activities of hepatic superoxide dismutase, CAT and GPx were induced significantly at higher concentrations (50, 500 μg/L) of PCZ after 20d and at 50 μg/L after 30d, there was a decreasing trend in those exposed to 500 μg/L after 30d exposure (Li et al. 2010a). But CAT and GPx activity in brain were significantly inhibited in the 50 and 500 μg/L group after exposure for 20 or 30 days (Li et al. 2010b). In the present study, the indices of antioxidant defense system were decreased in the brain, suggesting that the brain of fish would be a sensitive target of triazole pesticides.

In general, both induction and inhibition in antioxidant defense system are observed in fish after exposure to environmental pollutants. An undulation is often shown in the activity of antioxidant enzymes such as GST, GPx, SOD and CAT during exposure (reviewed by Van der Oost et al. 2003). Our results showed that the antioxidant defense system in fish liver and brain was affected after long-term exposure to PBZ, suggesting that PBZ exposure increases the vulnerability of the fish to oxidative stress. Chronic exposure to a contaminant at environmentally relevant concentrations could show steady-going and realistic effects.

Acknowledgments This work was supported by the Ph.D. Programs Foundation of the Ministry of Education of China (20100121110005).

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