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Effects of Low Dose Tributyltin on Activities of Hepatic Antioxidant and Phase II Enzymes in *Sebastiscus marmoratus*

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The widespread industrial and agricultural application of organotin compounds gives rise to contamination of marine environments. Contamination of aquatic systems is mainly related to tributyltin (TBT) due to its use in antifouling paints. In recent years, environmental contamination and the high toxicity of TBT have been documented.

The adverse effects of many chemicals upon animals are related to their capacity for catalysing reactions producing reactive oxygen species (ROS) and lipid peroxidation. These reactive species, including superoxide radicals, hydroxyl radicals and hydrogen peroxide, can induce activities of antioxidant enzymes. Superoxide dismutase (SOD) destroys the superoxide free radical by converting it to hydrogen peroxide, which can in turn be destroyed by catalase (CAT) or glutathione peroxidase (GSH-Px) reactions. However, there is little information about effects of TBT exposure on antioxidant enzyme activity of marine organisms.

Chemicals may be biotransformed and detoxified by phase I and II enzyme systems. The majority of the phase II type enzymes catalyze synthetic conjugation reactions, thus facilitating the excretion of chemicals by the addition of more polar groups to the molecule (Commandeur et al. 1995). Glutathione *S*-transferases (GST) catalyze the conjugation of xenobiotics with glutathione, and eliminate lipid peroxidation (Stegeman et al. 1992). UDP-glucuronyltransferase (UDPGT) catalyze the conjugation of xenobiotics with glucuronic acid. It was suggested that these enzyme systems also play an important role in the metabolism of organotins (Lee 1993). Recently, it was demonstrated that both TBT and triphenyltin chloride (TPT) can strongly interact *in vitro* with hepatic microsomal cytochrome P450 (CYP) in marine and freshwater fish, leading to destruction of native enzymes and inhibition of enzyme activity (Fent and Bucheli 1994; Fent 1996). Similar effects were shown to occur with TBT *in vivo* (Fent and Stegeman 1993; Fent et al. 1998), and in a permanent fish hepatoma cell line (PLHC-1) in culture (Brüschweiler et al. 1996). Al-Ghais and Ali (1999) showed that organotin compounds inhibited *in vitro* glutathione s-transferase (GST) activities of liver and kidney in *Siganus canaliculatus* and *Sparus sarba*.

Inhibition of enzyme activities occurred at high concentrations of organotin

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compounds. Whether inhibition occurs at low concentrations is unclear.There are limited data on organotin sublethal effects on vertebrates at low concentrations, particularly at environmentally relevant concentrations. It was reported that reproductive function was impaired in *Danio rerio* exposed to environmentally realistic levels (0.01–100 ng /L) of TBT (McAllister et al. 2003). Purpose of the present work was to investigate the *in vivo* interaction of TBT at low doses with hepatic phase II enzymes and antioxidant enzymes of marine fish.

MATERIALS AND METHODS

Tributyltin chloride (TBT) was obtained from Fluka AG, Switzerland, with a purity of greater than 97%. TBT was diluted in 98% ethanol to concentrations of 5–50 μ mol/L as Sn, and used for injection. Uridine 5'-diphosphoglucuronic acid (UDPGA) and NADPH were supplied by Sigma Chemicals Co. All other chemicals were of analytical grade and were obtained from commercial sources.

Sebastiscus marmoratus weighing 28~48g, were captured from a coast in Xiamen city, Fujian province, China. The fish at first were maintained in tanks containing 60 L of aerated marine water, with natural photoperiod for 7 days. Twelve fish per dose group were injected with TBT intraperitoneally with single doses (1ml/kg) of 5, 25, 50 and 500 μ mol/L as Sn in 98% ethanol (1.9, 9.6, 19.3, 193 μ g/kg as Sn). Control fish received an equal volume of the solvent ethanol (injection volume in each case 1 ml/kg). At 2d and 4d six fish per dose group were killed by a sharp blow on the head. The liver was frozen in liquid N_2 immediately after collection and stored at -80 ℃ until analyzed.

Homogenates of liver were prepared in chilled buffered KCl (1.15% KCl buffered with 0.01M tris-HCl, pH7.4) and centrifuged at 10,000 g for 20 min at 4 ℃ to obtain post-mitochondrial supernatant, which was used as the source of enzyme. GST activity was quantitated using 1-chloro-2,4-dinitrobenzene (CDNB) as the substrate (Habig et al. 1974), on a Beckman DU-8B spectrophotometer. UDPGT activity was determined as described in Andersson et al. (1985) and Lemaire et al. (1992), with *p*-nitrophenol (PNP) as the substrate, by measuring the consumption of PNP at 405 nm. SOD activity was determined according to the method based on the autoxidation of epinephrine to adrenochrome (Misra and Fridovich, 1972). One unit of SOD (U) was defined as the quantity of enzyme producing inhibition of 50% in the rate of adrenochrome accumulation of at 480 nm, 30 ℃ and pH 10.0. CAT activity was determined according to Greenwald (1985) by the decrease in absorbance at 240 nm due to H_2O_2 consumption. Protein content was determined according to Lowry et al. (1951) using bovine serum albumin as the standard.

Results are reported as mean ± SDE. The data were processed by parametric statistical analysis (ANOVA) using SPSS 10.0 software followed by the t-test and $P<0.05$ was accepted as significant.

RESULTS AND DISCUSSION

Ethanol was used as the solvent in the present study according to Fent et al. (1998). Control fish received an equal volume of the solvent ethanol (injection volume in each case 1 ml/kg). The results showed that hepatic enzymes activities were not affected by ethanol. There were no significant differences between the groups.

The dose-effect curve of the SOD and CAT activity in the liver was a bell shape. Hepatic SOD activity was significantly induced at concentrations of 9.6, 19.3, 193 μ g/kg after 4d exposure (Fig.1). The highest SOD activity at 19.3 μ g/kg group was 4.3 times of that of the control group. The highest CAT activity was appeared at 19.3 μ g/kg group after 4d exposure, and was 2.8 times of that of the control.

The dose-effect curve of hepatic GST activity was a bell shape. Hepatic GST activity was induced at lower doses groups after 2d exposure, but there were no significant differences, as compared to the control. GST activity showed significant induction at 19.3 μ g/kg after 4d exposure. Hepatic UDPGT activity was not affected by exposure to TBT.

Induction of SOD and CAT activities in the present work indicates production of reactive oxygen species in the process of TBT metabolism, and shows that induction and inhibition of TBT to SOD and CAT activity exist simultaneously. The SOD and CAT activities were both lower at 193 μ g/kg group after 2d exposure than that of the control, though it was not significant. The induction of SOD and CAT activities was due to production of reactive oxygen species in the metabolism of TBT, while inhibition of the enzyme activity may be due to the destruction of native enzyme protein that occurs at higher organotin concentrations.

Phase II enzymes can play an important role in homeostasis as well as in detoxification and clearance of many xenobiotic compounds. The synthesis of glucuronides by microsomal UDPGT is a major pathway for the inactivation and subsequent excretion of both endogenous and xenobiotic organic compounds (Lech and Vodicnik 1985; George 1994). In the present work, however, the hepatic UDPGT activity was almost not affected by exposure of TBT. It was suggested that UDPGT would not be related directly with the biotransformation and metabolism of TBT. It was reported that TPT was shown to be a strong inhibitor of GST in fish (George and Buchanan 1990). Al-Ghais and Ali (1999) reported that organotin compounds inhibit GST activity of liver and kidney homogenates *in vitro*, and the enzyme inhibitory potency of organotins was in the order of TBT>DBT (dibutyltin). The IC_{50} values for liver GST of DBT was 2-3 times of that of TBT. Compared to the highly toxic biocides TBT and TPT, DBT and monobutyltin (MBT) have less biological activity in aquatic species evaluated (Fent 1996). The present work has shown that the effect of TBT at low doses on hepatic GST activity *in vivo* was different from that *in vitro* activity reported by Al-Ghais and Ali (1999). The present result suggests that GST activity of liver in *Sebastiscus marmoratus* would be simultaneously induced and inhibited by organotin compounds, the induction effect would dominate at low doses, as phase II enzymes play an important role in

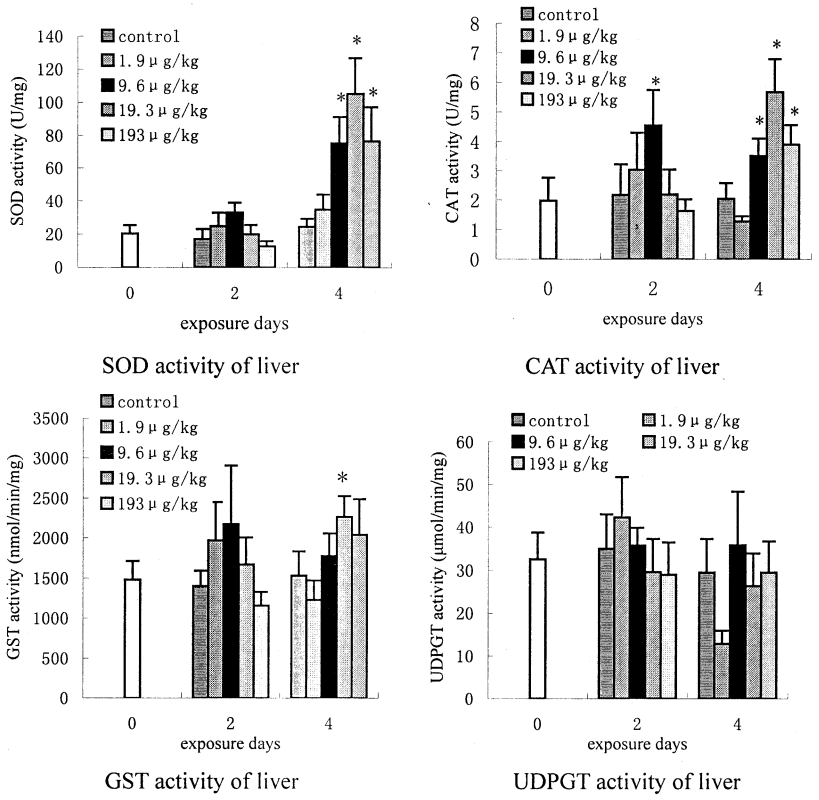

Figure 1. SOD, CAT, GST and UDPGT activity of liver in *Sebastiscus marmoratus,* during exposure to 0, 1.9, 9.6, 19.3, 193 μ g/kg of tributyltin chloride for 2 or 4 days. n=6 *, $P<0.05$ vs control

detoxification of organotin compounds. The hepatic GST activity appeared to be inhibited at the highest dose group (193 μ g/kg) after 2d exposure. With prolonged exposure, TBT was metabolized to DBT or MBT. Induction of the enzyme activity would appear and dominate, since the inhibitory potency of DBT was lower than that of TBT (Al-Ghais and Ali 1999). It is conceivable that the induction of hepatic GST activity perhaps mainly was due to the production of reactive oxygen species and lipid peroxidation. While inhibition of enzyme activity may be due to the destruction of native enzyme protein because of organotins exposure.

The present results suggest that GST and antioxidant enzyme activities in the liver of *Sebastiscus marmoratus* would be simultaneously induced and inhibited by organotin compounds. Induction of enzyme activity would dominate at lower concentrations, while inhibition of enzyme activity would be main the effect at higher concentrations of TBT. The present results suggest that there would be a threshold concentration of TBT to inhibit GST and antioxidant enzyme activity.

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