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Effects of Cadmium and Environmental Pollution on Metallothionein and Cytochrome P450 in Tilapia

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Tilapia are widely distributed freshwater fish frequently used for environmental toxicology, comparative biochemistry and physiology studies. Tilapia can persist in a highly polluted habitat and have the potential for the development as a biological monitor of environmental pollution. Metallothioneins (MTs) are a group of small-molecular-weight cytoplasmic proteins induced in many animals including fish, following exposure to metals such as cadmium, copper, zinc, and mercury (Roesijadi 1992). An increasing number of reports have indicated that fish MT induction is a sensitive measure of metal contamination in the environment (Roesijadi 1992).

Fish cytochrome (P450)-dependent monooxygenases are inducible by many environmental pollutants including polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) (Stegeman 1989). Extensive studies have suggested that fish monooxygenase can serve as a biochemical marker for exposure to PAH- and PCB-types of pollutants (Payne et al. 1987). Tilapia P450 is highly responsive to the inductive effects of PAH and PCBs (Ueng et al. 1992). Tilapia collected from a polluted section of a river showed higher levels of P450 and dependent monooxygenase activities than tilapia collected from an unpolluted section (Ueng et al. 1992).

Previous studies showed that pretreatment with Cd decreased microsomal monooxygenase activities in fish such as plaice, bass, and trout (George and Young 1986; Fair 1986; Forlin et al. 1986). However, direct information regarding the effects of heavy metals on tilapia P450 are not available. Reports concerning the effect of heavy metal on tilapia MT are scarce. The purpose of the present study was to determine the ability of cadmium to modulate P450 and MT in tilapia liver and gill. In addition, we have extended our study to feral tilapia collected from Er-Jen Stream, a polluted river in Taiwan.

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MATERIALS AND METHODS

In the laboratory studies, male tilapia hybrids *(Oreochromis niloticus* female x *0. aureus* male) weighing 350-400 g were used. Fish were obtained from Da-Yuan fish farm (Tao-Yuan, Taiwan). Before the experiments began, fish were acclimated for at least one-month in the animal quarters with air conditioning and automatic 12-hr photocycle. The animals were fed *ad libitum* with a commercial fish diet. Atomic absorption spectrophotometric analysis of the diet showed no detectable level of Cd (data not shown). Thirty tilapia were used in the MT induction dose-response experiment. The fish were pretreated with 0.2, 1, 2, 3, 4 and 10 mg/kg of $CdCl_2$ in 1.15% KC1 intraperitoneally (i.p.) and control fish were treated with the KC1 solution only. Twelve tilapia were used in the MT and P450 modulation experiment, half of the fish were treated with 2 mg/kg of $CdCl_2$ and the other half served as the controls. The animals were killed 24 hr following the treatment. Liver and gills were removed, and cytosol and washed microsomes were prepared by differential centrifugation.

In the field study, tilapia were collected at the sampling site A, Bai-Sha-Kun, and site B, San-Ye-Gung-Se-Ko, of Er-Jen Stream which flows through an industrial area between Tainan and Kaoshiung Counties in the south of Taiwan. The river is heavily polluted by municipal wastes and discharges from industrial activities including reprocessing of used batteries, burning of used tires, and acid-wash operations for wire metal reclamation. Livers were removed at the sampling sites, frozen in liquid nitrogen immediately and transported to the laboratory where the cytosol and microsomes were prepared. Tilapia collected from Pu-Dai Aquaculture Pond (Chia-Yi, Taiwan) were used as the nonpolluted control for MT concentration. Benzo(a)pyrene hydroxylase activity of tilapia caught at Fe-Tsui Reservoir (Taipei, Taiwan) was taken from the data of Ueng et al. (1992) and used as the nonpolluted reference.

Microsomal P450 content was determined by the method of Omura and Sato (1964). NADPH-cytochrome P450 reductase was determined by following the reduction rate of cytochrome c by the method of Phillips and Langdon (1962). Benzo(a)pyrene hydroxylase activity was determined by measuring the formation of the phenolic metabolites according to the fluorimetric method of Nebert and Gelboin (1968) with 3-hydroxybenzo(a)pyrene as a standard. 7-Ethoxyresorufin O-deethylase activity was determined by measuring the fluorescence of the deethylated product, resorufin, following the procedures of Pohl and Fouts (1980). 7-Ethoxycoumarin O-deethylase activity was determined by measuring the formation of the fluorescent metabolite, 7-hydroxycoumarin, according to the method of Greenlee and Poland (1978). Protein concentration was determined by the method of Lowry et al. (1951) using bovine serum albumin as a standard. Cytosolic MT concentration was determined by the cadmium-hemoglobin and silver-saturation methods in the laboratory and field studies, respectively (Onosaka and Cherian 1982;

Scheuhammer and Cherian 1986). These two methods yielded comparable results in MT determination. Our preliminary results showed that untreated tilapia yielded MT values of 9.7 ± 1.2 and 9.9 ± 1.8 µg MT/g liver using the cadmium-hemoglobin and silver-saturation methods, respectively. Scheuhammer and Cherian (1986) showed an excellent correspondence in the measurement of hepatic MT of Cd-injected rats using these two methods. The statistical significance of differences between control and treated groups was evaluated by the student's t test. A p value of less than 0.05 was considered as statistically significant.

RESULTS AND DISCUSSION

An initial experiment was carried out to determine the dose-response relationship of MT induction by Cd. Single doses of 0.2, 1 and 2 mg/kg of $CdCl_2$ caused 6-, 14- and 18-fold increases of liver MT, respectively (Fig. 1). Pretreatments with 3, 4 and 10 mg/kg of $CdCl_2$ resulted in 12- to 13-fold increases of MT. The data in Fig. 1 also show that the maximal induction of tilapia MT occurred near 2 mg/kg of $CdCl_2$ and that MT did not increase proportionately with doses greater than 2 mg/kg. The exact reason for this biphasic dose-response relationship remains unclear. Similar disproportionality of MT induction was observed with plaice treated with $CdCl_2$ exceeding 0.82 mg/kg (George 1989). These tilapia and plaice MT induction data indicate a possibility that administration of high doses of Cd led to excess cellular Cd which, in turn, decreased MT induction.

Based on the results of the dose-response experiment, 2 mg/kg of $CdCl_2$ was chosen as the dose to study the effects of the metal on MT and P450 of tilapia liver and gill. In the liver, pretreatment with $CdCl_2$ markedly increased cytosolic MT level (Table 1). P450 content of Cd-treated fish liver microsomes was similar to the hemoprotein content of control fish. The pretreatment had no effect on NADPH-cytochrome P450 reductase, benzo(a)pyrene hydroxylase, 7-ethoxyresorufin and 7-ethoxycoumarin O-deethylases activities. In the gills, pretreatment with $CdCl_2$ caused a 10-fold increase of MT. Gill NADPH-cytochrome P450 reductase and benzo(a)pyrene hydroxylase activities were not affected by the Cd pretreatment. These data show that Cd can induce MT in tilapia liver and gills, and suggest that Cd induction of MT protects the microsomal monooxygenases from the adverse effects of the metal. In order to further confirm MT induction in tilapia and to determine the partitioning of Cd between MT and other cytosolic fractions, Cd-treated tilapia liver cytosol was subjected to Sephadex G-75 column chromatography and the column elution fractions were analyzed by atomic absorption spectrophotometry. The elution profile showed a Cd-binding peak at Ve/Vo of 2.0 and no detectable Cd was associated with the Vo or Vo + Vi peaks, indicating that the majority of cytosolic Cd was bound to tilapia MT and little or no Cd was bound to the high and small molecular weight fractions (data not shown). The chromatographic result is in agreement with the result

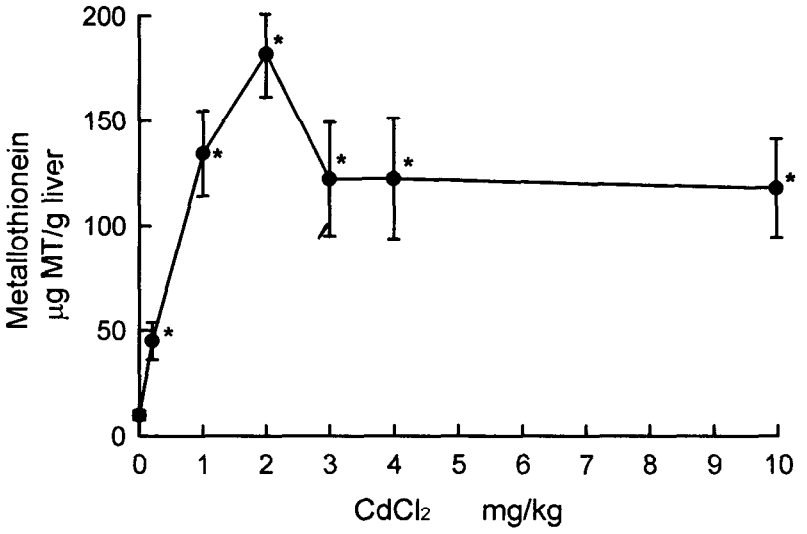

Figure 1. Dose response relationship in cadmium induction of metallothionein in tilapia liver. Male tilapia, *O. niloticus* x *0. aureus,* were administered $CdCl_2$ in 1.15 % KC1 i.p. at the doses indicated. Each value represents the mean ± S.E. for at least 3 fish.
* Significantly different from control value, $p < 0.05$.

of the monooxygenase study (Table 1), suggesting that MT has a protective role against Cd toxicity.

The lack of effect of Cd on tilapia monooxygenase (Table 1) is markedly different from the inhibitory effects of Cd on the microsomal enzymes of other fishes. Administrations with $CdCl_2$ i.p. at doses lower than 2 mg/kg decreased liver microsomal 7-ethoxyresorufin O-deethylase, benzo(a)pyrene hydroxylase, and 7-ethoxycoumarin O-deethylase activities in plaice, bass, and trout, respectively (George and Young 1986; Fair 1986; Forlin et al. 1986). These apparent variations in the effects of Cd on microsomal monooxygenases may be attributed partially to species and time-course differences. Another possible explanation is that the pollution-resistant tilapia have a more effective MT induction system against Cd toxicity than other fishes. In tilapia, pretreatment with Cd caused a marked induction of hepatic MT without affecting 7-ethoxyresorufin O-deethylase activity at 24 hr following pretreatment (Fig. 1 and Table 1). In plaice, Cd pretreatment did not cause apparent induction of MT but caused a marked decrease of 7-ethoxyresorufin O-deethylase activity at 24 hr following pretreatment (George and Young 1986). These contrasting tilapia and plaice results suggest that the time required to induce MT may be

an important determinant of susceptibility of fish monooxygenase to acute Cd toxicity.

Table 1. Effects of cadmium administration on metallothionein concentration and monooxygenase activity in tilapia liver and gill.

Assay	Control	$CdCl_2$
	Liver	
Metallothionein (µg MT/g liver)	8.0 ± 2.2	152.1 ± 17.9 *
Cytochrome P450 (nmol/mg protein)	0.324 ± 0.043	0.346 ± 0.032
NADPH-cytochrome P450 reductase (nmol cyt. c/min/mg protein)	36.0 ± 2.0	44.8 ± 11.2
Benzo(a)pyrene hydroxylase (pmol 30HBP/min/mg protein)	45.1 ± 10.2	48.8 ± 7.2
7-Ethoxyresourifn O-deethylase (pmol RF/min/mg protein)	47.2 ± 8.0	51.3 ± 7.7
7-Ethoxycoumarin O-deethylase (nmol HC/min/mg protein)	0.170 ± 0.021	0.153 ± 0.028
	Gill	
Metallothionein (µg MT/g gill)	0.8 ± 0.6	8.2 ± 0.9 *
NADPH-cytochrome P450 reductase (nmol cyt. c/min/mg protein)	3.8 ± 0.7	3.2 ± 0.2
Benzo(a)pyrene hydroxylase (pmol 30HBP/min/mg protein)	4.1 ± 2.3	3.6 ± 1.2

Male tilapia, *0. niloticus* x *0. aureus*, were administered i.p. $CdCl_2$ in 1.15 % KC1 at 2 mg/kg. Control fish received the KC1 solution only. Each value represents the mean ± S.E. for at least 5 fish.
* Significantly different from respective control value, $p < 0.05$.

The laboratory study shows that tilapia benzo(a)pyrene hydroxylase activity is insensitive to Cd inhibition. Therefore, the PAH- and PCB-inducible monooxygenase activity can possibly serve as a measure of exposure to the organic pollutants when the fish is also exposed to the heavy metal. The following field study was carried out to determine the effect of environmental pollution on tilapia MT and P450. Tilapia were caught in the waters at site A, Bai-Sha-Kun, and site B, San-Ye-Gung-Se-Ko, of Er-Jen Stream. The levels

of hepatic MT and benzo(a)pyrene hydroxylase of tilapia from site A were 10- and 9-fold higher than the respective levels of tilapia from a nonpolluted reference site (Table 2). MT level of tilapia collected from site B was 9-fold higher than the MT level in tilapia from the control site. In contrast, the aryl hydrocarbon hydroxylase activity in tilapia from site B was similar to controls. These environmental induction data indicate that tilapia from site A had previously been exposed to MT and P450 inducers of which heavy metals and PAHs are the respective prototypes and that tilapia from site B were exposed to heavy metals, among other possibilities. It will be of interest to carry out direct metal and chemical analyses using the livers of tilapia collected from these sampling sites.

Table 2. Hepatic metallothionein concentration and benzo(a)pyrene hydroxylase activity of tilapia collected from Er-Jen Stream and nonpolluted control sites.

Sampling Site	Metallothionein Concentration (μg MT/g liver)	Benzo(a)pyrene hydroxylase (pmol 30HBP/min/mg protein)
Controls	9.4 ± 1.8 (13)	31.3 ± 5.9 (23)
Er-Jen Stream		
Site A. Bai-Sha-Kun	89.8 ± 16.2* (16)	284.4 ± 34.4* (8)
Site B. San-Ye-Gung-Se-Ko	80.6 ± 9.0* (26)	38.2 ± 4.0 (14)

Tilapia were caught from the waters of Er-Jen Stream and the control sites as described in Materials and Methods. Each value represents the mean ± S.E. for the number of fish indicated in the parenthesis.
* Significantly different from respective control value, $p < 0.05$.

In conclusion, the present study demonstrates that tilapia liver and gill P45Os are resistant to the inhibitory effect of an acute cadmium dose. In contrast, MT in the tilapia tissues is markedly responsive to the inductive effect of cadmium. The concomitant induction of MT and benzo(a)pyrene hydroxylase in feral tilapia collected from Er-Jen Stream suggests that the river is possibly polluted with heavy metals, PAHs and PCBs.

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