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Acute Effect of Organotin Compounds to Red Sea Bream and Red Carp Using Biological Parameters

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Since organotin compounds were widely used for antifouling paints and biocides for marine nets, high levels of organotin compounds have been detected in fish and sediments. Reports on organotin toxicity include thymus atrophy(Seinen and Willems 1976), T-cell suppression(Seinen et al. 1979) and their inhibitory action on mammalian platelet aggregation(Manabe et al. 1983).

We have also reported that a good reciprocal relationship exists between increases in DT-diaphorase activity and atrophy of the thymus by organotin compounds in rats(Ariyoshi et al. 1991). There are very limited reports regarding the effects of organotin compounds on the hepatic drug metabolizing enzymes in fish, although the pollution of the aquatic environment and marine products by these chemicals is well known. Therefore, in this study we have investigated the effects on drug metabolizing enzymes, hemoproteins and related components both in salt water fish (red sea bream) and in fresh water fish (red carp) after organotin compounds treatment.

MATERIALS AND METHODS

D-Glucose-6-phosphate, D-glucose-6-phosphate dehydrogenase and NADPH were purchased from Boehringer Mannhein-Yamanouchi Co., Ltd., (Tokyo, Japan), and NADP was obtained from Oriental Yeast Co., Ltd., (Tokyo, Japan). Tributyltin chloride(TBTC) and triphenyltin chloride(TPhTC) were purchased from Tokyo Kasei Co., Ltd.,(Tokyo Japan). 7-Ethoxycoumarin and 2,6 dichlorophenol indophenol(DCPIP) were obtained from Aldrich Chemical Co., Inc.,(Milwaukee, USA) and E. Merck A.G.(Darmstadt, Germany) respectively and other chemicals used which were of the highest grade and were purchased from Wako Pure Chemical Industries Ltd., (Osaka, Japan).

Wild red sea bream and cultured red sea bream, *Pagrus major* (mixed sex, body weight of 800 to 1300 g) were obtained alive from a public fish market, after having been caught in the sea near Nagasaki, Japan. Red sea bream were held in flowing, filtered salt water in a 500 L tank(2 tanks used) in Seikai National

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Red carps(*Cyprinus carpio Linne*) weighing 60 - 100 g were obtained from a fish farm in Nagasaki. After an adaption period of 3-5 days the fish were placed into 4.5 L aquariums(4 - 5 fish/aquarium, 4 - 5 aquariums were used) containing aerated, filtered and recirculated water. Water temperature was controlled between 21 to 26°C. Fish were fed daily a commercial diet containing no detectable tin.

Red sea bream and red carp were given a single intraperitoneal injection of each organotin(12.5 µmole of TBTC/ 1.0 ml of corn oil / kg of body weight or 25.0 µmole of TPhTC/ 1.0 ml of corn oil / kg of body weight). Control fish were administered 1.0 ml of corn oil /kg of body weight. After treatment with organotins the fish were killed by decapitation and liver(sea breams) or hepatopancreas(red carp), kidney and muscle were immediately removed, washed and weighed. A part of fish backbone(vertebra and rib) was also removed and washed with hot water. The liver or hepatopancreas was homogenized with 4 volumes of 0.25 M sucrose in a Potter-Elvehjem homogenizer with a teflon pestle. Preparation of the 105000xg soluble fraction and microsomes were carried out by the procedures previously described by Arizono et al.(1982) and Ariyoshi et al. (1970), respectively.

Cytochrome P450(P450) and b_5 contents were determined according to the methods of Omura and Sato(1964) using an extinction coefficient of $91 mM^{-1}cm^{-1}$ between the absorbance spectra at 450 and 490nm following carbon monoxide bubbling and using an extinction coefficient of $185 mM^{-1}cm^{-1}$ between the absorption spectra at 424 and 409nm. Heme oxygenase activity was calculated from the amount of bilirubin formed using an extinction coefficient of $40 mM^{-1}cm^{-1}$ between 464 and 530nm as described by Maines and Kappas(1976). 7-Ethoxy- coumarin O-deethylase(7-EC) activity was measured by recording the fluorescence increase due to the formation of 7-hydroxycoumarin as reported by Ullrich and Weber(1972). Quinone reductase(QR) activity was measured spectrophotometrically by the reduction of DCPIP using procedure of Ernster(1967). The concentration of metal binding proteins(metallothioneins, MT) was determined by the cadmium-heme method of Onosaka et al.(1978). Protein concentration was determined according to Lowry et al.(l951) using bovine serum albumin as a standard. Concentration of organotin compounds was determined according to the method of Ishizuka et al.(1989).

RESULTS AND DISCUSSION

Although we have already reported on the activities of hepatopancreas enzymes in the red carp(Ariyoshi et al.1990a, 1990b), the activities of hepatic enzymes in wild and cultured red sea breams(*Pagrus major*) are shown in Table 1. The tendency of liver hypertrophy was noted in cultured red sea bream when compared with wild one. This hypertrophy might be caused by an overload of feeding or a lack of exercise. Microsomal protein may be more readily induced in wild sea

Table 1. Activities of heme oxygenase and drug-metabolizing enzymes and contents of cytochrome and metallothionein in the liver of wild and cultured red sea breams (*Pagrus major*)

Activity and content	Red sea breams		Ratio
	wild	cultured	wild/cultured
Liver weight(g/100g b.w.)	0.68 ± 0.03	0.75 ± 0.05	0.91
Microsomal protein(mg/g)	22.5 ± 0.7	16.4 ± 0.5 ***	1.37
Heme oxygenase(nmole/mg protein/hr)	0.91 ± 0.16	1.45 ± 0.29	0.63
Cytochrome P-450(nmole/mg protein)	0.58 ± 0.07	0.41 ± 0.03*	1.41
Cytochrome b_5(nmole/mg protein)	0.25 ± 0.04	0.14 ± 0.02*	1.79
7-Ethoxycoumarin O-deethylase (nmole/mg protein/min)	0.26 ± 0.03	0.20 ± 0.01	1.30
Quinone reductase (nmole DCPIP reduced/mg protein/min)	3.77 ± 0.60	5.69 ± 1.10	0.66
Metallothionein(μg/protein)	0.88 ± 0.19	0.51 ± 0.09	1.73

Values are the mean ± S.E. of each 10 wild and cultured red sea breams.
Significantly different from corresponding mean of wild red sea breams (*P<0.05; ***P<0.01).

Table 2. Activities of heme oxygenase and drug-metabolizing enzymes and contents of cytochrome and metallothionein in the liver of cultured red sea breams 72 hours after a single treatment with tributyltin chloride(TBTC)

Activity and content	Control	TBTC(μmole/kg)	
		12.5	50.0
Liver weight(g/100g b.w.)	0.84 ± 0.06	1.45 ± 0.20	2.29 ± 0.31**
Microsomal protein(mg/g)	16.3 ± 0.4	16.7 ± 0.7	16.5 ± 0.9
Heme oxygenase(nmole/mg protein/hr)	1.18 ± 0.30	1.38 ± 0.61	1.72 ± 0.17
Cytochrome P-450(nmole/mg protein)	0.60 ± 0.04	0.64 ± 0.12	0.54 ± 0.03
Cytochrome b_5(nmole/mg protein)	0.11 ± 0.01	0.08 ± 0.01	0.06 ± 0.01*
7-Ethoxycoumarin O-deethylase (nmole/mg protein/min)	0.15 ± 0.02	0.11 ± 0.02	0.07 ± 0.01*
Quinone reductase (nmole DCPIP reduced/mg protein/min)	5.40 ± 0.94	3.98 ± 0.90	2.10 ± 0.32*
Metallothionein (μg/protein)	0.76 ± 0.03	1.30 ± 0.46	1.32 ± 0.14*

Values are the mean ± S.E. of 5 cultured red sea breams per group.
Significantly different from corresponding mean of control(*P<0.05; **P<0.02).

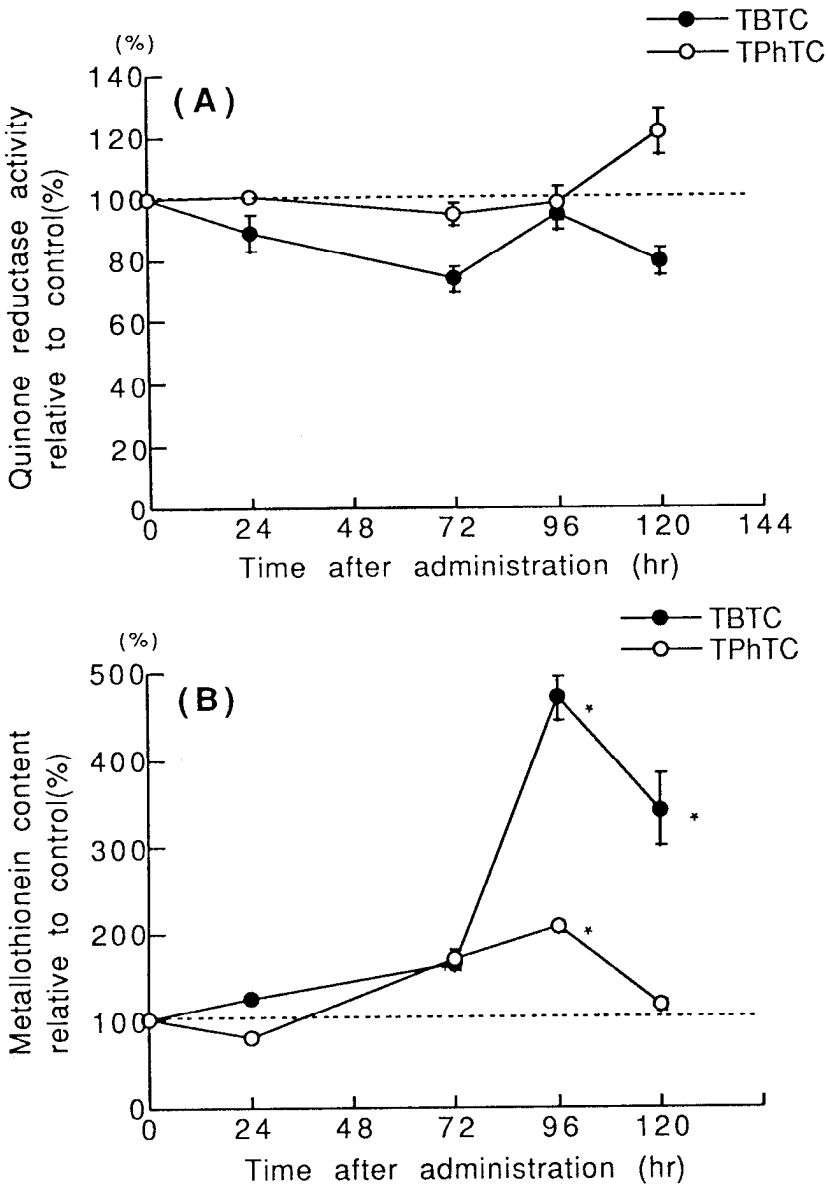

Fig 1 Time course of changes in the activity of quinone reductase (A) and the content of metallothionein(B) in the hepatopancreas of red carp after TBTC(12.5 μmol/kg B.W.) and TPhTC(25.0 μmol/kg B.W.) administrations. Both compounds were intraperitoneally administered in a single injection to red carp. Each value represents % to control and the mean ± S.E. of 4-6 red carp.(control value : quinone reductase 6.07 ± 0.04 nmol DCPIP reduced/ mg protein /min; metallothionein 0.66 ± 0.04 μg/mg protein). Significantly different from corresponding mean of control. *P<0.02

breams by chemicals than in cultured sea breams, consequently the hypertropy in the cultured sea breams. Consider environmental factors, genetics etc. Of interest, contents of both P450 and b_5 were markedly higher in wild one. HO activity, which is a rate limiting enzyme for heme degradation, was higher in cultured red sea bream. On the other hand, the higher concentration of MT, which relates the metal transport and storage, and which acts as scavenger of active oxygen, recognized in wild red sea bream. It is well known that both parameters in mammals and fish are induced by metals and other stresses(Maines and Kappas 1976, Kagi and Nordberg 1979, Roesijadi 1980, Ariyoshi et al. 1990a, 1990b). A part of the gene regulation elements of HO resemble that of MT(Yiangou et a1.1991). However it is not well establish whether the induction mechanism of HO is the same as MT genes.

The disagreement on the changes in the activity and content of these parameters between cultured fish and wild fish suggests the independence of induction mechanisms in each case. Further it may also indicate the limited ability of this fish as indicators of marine pollution. Wild to cultured ratio of these parameters are also presented in Table 1.

TBTC and TPhTC were detected in fish in Japanese sea and local waters. The daily intake of these compounds were estimated to be 5.29 µg(TBTC) and 9.36µg(TPhTC) by Baba et al. (1991a). Baba et al. (1991a) also reported that the content of TBTC in cultured fish is higher than that of wild fish. These findings indicate a serious problem because of the large amount of fish consumed in Japan. In order to provide an answer to this problem, the changes in the parameters described in the Methods section were investigated in detail. The results are summarized in Table 2. HO activity and MT content in cultured red sea bream showed a dose dependent increase, while QR(DT-diaphorase) activity decreased in a dose dependent manner. The time course of QR activity in red carp after TBTC and TPhTC administration is shown in Figure 1A and B. QR activities in the hepatopancreas of red carp decrease 72 hr after TBTC administration. Similar results were obtained with red sea bream. The induction of DT-diaphorase activity (DCPIP used as substrate) by organotin compounds such as TBTC, TPhTC, dibutyltinchloride(DBTC) and diphenyltinchloride was observed in rats(Ariyoshi et al. 1991), but not in sea breams or red carp. The reason for this disagreement may be species dependent. More detail studies using fish may be reqiured to resolve this issue.

TBTC concentrations in hepatopancreas, kidney, muscle and bone of red carp are shown in Table 3, 72 hr after TBTC administration. DBTC, a metabolite of TBTC, was detected in every tissue measured in this study. Considerable amounts of TBTC were found distributed in muscle after ip injection. The fact that both TBTC and DBTC were found in bone is interesting. The distribution of TBTC in bone has already been reported in the case of salt water fish. The TBTC concentration in bone was about 1/5 to 1/10 that of muscle(Baba et al. 1991b). The reason for the presence of TBTC in bone is unknown, but it may be related to

Table 3. Concentrations of tributyltin chloride (TBTC) and dibutyltin chloride (DBTC) in various tissues of red carps(*Cyprinus carpio Linne*) 72 hours after a single injection of TBTC

Tissues	Metabolites	Control(3) (μg/g)	TBTC(10) (μg/g)	Recovery(%)
Hepatopancreas	TBTC	0.05 ± 0.01	7.58± 0.64	4.12± 0.44
	DBTC	<0.02	0.27 ± 0.03	
Kidney	TBTC	0.06 ± 0.01	6.20± 0.67	0.63± 0.06
	DBTC	<0.05	0.22 ± 0.05	
Muscle	TBTC	0.03	2.00 ± 0.22	NC
	DBTC	<0.01	0.02 ± 0.01	
Bone	TBTC	< 0.01	0.69 ± 0.06	NC
	DBTC	<0.02	0.02 ± 0.01	

Values are the mean ± S.E. of 3 to 10 red carps whose number is shown in parenthesis.

Recovery value(%) of TBTC is calculated from total content in the tissues and administered dose of 12.5 μmole /kg.

NC: Recovery values of muscle and bone are not able to calculate owing to respective total tissue weight is unknown. The calculation of recovery value for DBTC in the tissues are impossible, DBTC is a metabolite of TBTC.

its distribution in bone marrow. Future work should be carried out with large fish so that bone and bone marrow may be analyzed from the same animal.

In the present study involving the administration of TBTC to sea bream, a decrease in P450 and b5 contents and 7-EC and QR activities were observed along with an increase in the activity of HO and the content of MT. These observations were similar to those obtained with red carp. Detailed investigations into the effects of low level chronic exposures of fish to organotin compounds are needed before these parameters may be used as an index of environmental pollution.

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