Bull. Environ. Contam. Toxicol. (1990) 44:643-649

Bulletin of Environmental Contamination and Toxicology

Profile of Metal-binding Proteins and Heme Oxygenase in Red Carp Treated with Heavy Metals, Pesticides and Surfactants

Toshihiko Ariyoshi, Seiichi Shiiba, Hiroyuki Hasegawa, and Koji Arizono

Department of Hygienic Chemistry and Toxicology, Faculty of Pharmaceutical Sciences, Nagasaki University, 1-14 Bunkyo-cho, Nagasaki, 852, Japan

The increased use of various chemicals, including heavy metals, pesticides and surfactants, is known to contaminate the environment. The presence of contaminants in the aquatic environment is a major problem in fish, since these substances seem to produce many physiological and biochemical changes by alterations in the activities of several enzymes and the contents of essential metals.

Metallothioneins (metal-binding proteins(MBP)) are low molecular weight proteins which are considered to play an important role in the metabolism of essential or toxic metals. These metallothioneins have been extensively studied in mammals(Kägi and Nordberg 1979; Kägi and Kojima 1987) and observed also in a variety of marine invertebrates(Roesijadi 1980).

On the other hand, a family of hemoproteins known as cytochrome P-450, which is known to perform a major role in the metabolism of various agents, has been also investigated in fish as a criterion for monitoring water pollution. In addition, this enzyme is well known to be induced by various chemicals in fish as well as mammals (Elcombe and Lech 1979; Stegeman et al. 1981). However, very little information is available concerning the effects of environmental pollutants on the activity of heme oxygenase, the first and rate-limiting enzyme for heme degradation. To investigate the nature of heme oxygenase is of particular interest in that if heme oxygenase activity is altered by contaminants, that may contribute to the effect on physiological changes of heme and hemoprotein P-450.

In this study we investigated the effects of heavy metals, pesticides and surfactants on the MBP and the heme oxygenase in the hepatopancreas and kidney of a fresh water red carp(Cyprinus carpio Linné).

MATERIALS AND METHODS

Cadmium chloride($CdCl_2$), lead acetate($Pb(CH_3COO)_2 \cdot 3H_2O$), O,O-diethyl- O-(2-isopropyl-6-methyl-4-pyrimidinyl) phosphorothioate

---

Send reprint requests to T. Ariyoshi at the above address.

(diazinon), o-sec-butyl-phenyl-N-methylcarbamate(BPMC), sodium n-dodecyl-benzenesulfonate (LAS) and other chemicals used were of the highest grade quality purchased from Wako Pure Chemical Industries Ltd.,(Osaka, Japan). Polyoxyethyleneglycol nonylphenyl ether(Emulgen 913) was obtained from Kao Co., Ltd.,(Tokyo, Japan). D-Glucose-6-phosphate and D-glucose-6-phosphate dehydrogenase were purchased from Boehringer Mannheim-Yamanouchi Co., Ltd.,(Tokyo, Japan), and NADP was obtained from Oriental Yeast Co., Ltd.,(Tokyo, Japan). Red carp weighing 280-340g obtained from a fish farm in Nagasaki were used for all experiments. After an acclimation period of 3-5 days, fish were placed into each 45 L aquarium(4-5 fish/aquarium, 4-5 aquariums used) with aerated, filtered and recirculated water at a temperature range of 21 to 29 °C. Four to five fish per each dosage group were intraperitoneally injected with chemicals dissolved in 0.9% saline ($CdCl_2$, LAS and Emulgen 913), in distilled water ($Pb(CH_3COO)_2$) and in corn oil(diazinon and BPMC) at 1 ml($CdCl_2$, $Pb(CH_3COO)_2$, diazinon and BPMC) and 2 ml(Emulgen 913 and LAS) per dose per kg, respectively. Control fish were injected with either saline, distilled water or corn oil alone at their respective corresponding volume per kg of fish. Fish were fed a commercially available diet once daily during experiments. A daily half-volume change of water was performed. Three days after the injection of chemicals fish were killed and the hepatopancreas and kidney immediately removed, washed and weighed. The hepatopancreas and kidney were homogenized with 4 volumes of 0.25M sucrose in a Potter-Elvehjem homogenizer with a teflon pestle. Preparation of the 105000xg soluble fraction and microsomes were carried out by the procedures described by Arizono et al.(1982) and Ariyoshi et al.(1970).

Content of MBP in 105000xg soluble fraction was estimated as described by Onosaka et al.(1978). Protein concentration was measured by the method of Lowry et al.(1951) using bovine serum albumin as a standard. Heme oxygenase activity in microsomes was determined by the method of Maines and Kappas(1976).

RESULTS AND DISCUSSION

In preliminary time-course experiments, i.p. injection of $CdCl_2$ in red carp revealed that a maximum concentration of cadmium(Cd) in tissues such as hepatopancreas, gills and bone was noted at 3 days after the injection. Therefore, in this study all fish used were sacrificed 3 days after the injection of chemicals.

Table 1 shows the results of experiments carried out at different doses of $CdCl_2$ or $Pb(CH_3COO)_2$. In a single dose of 3mg/kg of $CdCl_2$, MBP content showed a remarkable increase of 6.1- and 7.2-fold in the hepatopacreas and kidney when compared with controls. However , that content was reduced with increasing doses 4.5mg/kg of $CdCl_2$ or higher. This seems to be due to the development of acute toxicity at the high dose of $CdCl_2$, as we observed toxic phenomena in some fish such as decrease of ratio of hepatopancreas weight to body weight, abdominal inflation and weak necrosis of the kidney by that treatment. Administration of $Pb(CH_3COO)_2$

Table 1. Metal-binding proteins and heme oxygenase activity in the hepatopancreas and kidney of red carp 3 days after injection with cadmium chloride($CdCl_2$) or lead acetate($Pb(CH_3COO)_2$)

		Metal-binding proteins (μg/mg protein)		Heme oxygenase activity (nmole/mg protein/hr)	
		Hepatopancreas	Kidney	Hepatopancreas	Kidney
	Control	0.39 ± 0.06	0.37 ± 0.06	0.47 ± 0.08	1.29 ± 0.03
$CdCl_2$ (mg/kg)	1.5	1.58 ± 0.16***	2.35 ± 0.16***	0.53 ± 0.03	ND
	3.0	2.38 ± 0.27***	2.67 ± 0.27***	1.57 ± 0.15***	0.87 ± 0.06**
	4.5	1.65 ± 0.25***	2.01 ± 0.18***	1.44 ± 0.15***	0.89 ± 0.01***
	6.0	1.03 ± 0.14***	1.03 ± 0.03***	0.80 ± 0.14	0.77 ± 0.01***
	Control	0.50 ± 0.16	0.53 ± 0.04	0.50 ± 0.03	1.55 ± 0.13
$Pb(CH_3COO)_2$ (mg/kg)	50	0.78 ± 0.10	0.62 ± 0.05	0.60 ± 0.02*	1.57 ± 0.14
	75	1.18 ± 0.21*	0.77 ± 0.13	0.64 ± 0.03*	1.65 ± 0.32
	100	0.79 ± 0.05	0.65 ± 0.03	0.81 ± 0.10**	1.76 ± 0.21

Values are the mean ± S.E. of 3 to 4 red carp per dosage group. Significantly different from corresponding mean of control(*$P < 0.05$;**$P < 0.02$;***$P < 0.01$). ND:Not determined.

at a dose of 75mg/kg increased the MBP content 2.4-fold in the hepatopancreas as compared with the control. In the kidney there was no significant change in MBP content at any of the doses used. This difference in inducing potency for MBP by $CdCl_2$ or $Pb(CH_3COO)_2$ treatment may be attributed to the differences in the pharmacokinetics or specific organ affinity in both metals, although lead and other metals can induce the metallothionein in the liver of mammals(Suzuki and Yoshikawa 1976; Arizono et al. 1982).

Heme oxygenase activity was greatly enhanced in the hepatopancreas after the treatment with $CdCl_2$ at a single dose of 3.0 or 4.5mg/kg, and was also stimulated slightly but significantly by $Pb(CH_3COO)_2$ injection. This finding agrees with the results reported in the liver of mammals(Maines and Kappas 1976; Ariyoshi 1981). On the contrary, heme oxygenase activity in the kidney was depressed appreciably by $CdCl_2$ treatment, whereas no alterations were observed in that activity by $Pb(CH_3COO)_2$ injection.

The effects of the treatments with respect to two pesticides, diazinon and BPMC, and two surfactants, Emulgen 913 and LAS, on the MBP content and the heme oxygenase activity in red carp are summarized in Tables 2 and 3, respectively. Although phosphorothioate diazinon increased the MBP content in the kidney, there were no remarkable differences in that content in the hepatopancreas. In addition, no appreciable changes were found in the MBP content in both tissues by the injection of carbamate BPMC at all doses used. On the other hand, we observed that non-ionic surfactant Emulgen 913 decreased the MBP content in the hepatopancreas, whereas that content in the kidney showed the tendency to be increased. Furthermore, we obtained similar effects on the MBP content after the injection of LAS. In general, MBP is known to be induced by metal treatments or physiological stresses. It is unknown whether the effect of surfactant on MBP content in fish tissues is specific or not. Comparative studies in mammals will be needed.

Heme oxygenase activity in the hepatopancreas was depressed by the injection of diazinon at doses higher than 50mg/kg, whereas that activity in the kidney was enhanced at the highest dose of 100mg/kg. However, BPMC did not affect that activity in both tissues at the doses used. In contrast, both surfactants used suppressed the heme oxygenase activity in the kidney, while we observed no significant changes in the hepatopancreas. In a preceding paper(Ariyoshi et al. 1990), we observed a good reciprocal correlation between heme oxygenase activity and cytochrome P-450 content in the hepatopancreas of red carp by $CdCl_2$ treatment, whereas there are no close relations between that activity and P-450 content by the treatment with BPMC or LAS. These findings suggest that the changes of heme oxygenase activity by the chemicals used in this study, except for $CdCl_2$, are not linked to the degradation of P-450 heme. This difference in tissue responsiveness to chemical induction of heme oxygenase may reflect differences in chemical binding affinities and in cellular content

Table 2. Metal-binding proteins and heme oxygenase activity in the hepatopancreas and kidney of red carp 3 days after injection with diazinon or o-sec-butylphenyl N-methylcarbamate(BPMC)

		Metal-binding proteins (μg/mg protein)		Heme oxygenase activity (nmole/mg protein/hr)	
		Hepatopancreas	Kidney	Hepatopancreas	Kidney
Diazinon (mg/kg)	Control	0.42 ± 0.09	0.38 ± 0.09	0.68 ± 0.05	2.01 ± 0.10
	25	0.30 ± 0.08	0.80 ± 0.11*	0.69 ± 0.05	1.65 ± 0.13
	50	0.52 ± 0.06	0.79 ± 0.13*	0.52 ± 0.08*	2.04 ± 0.26
	75	0.36 ± 0.08	0.71 ± 0.04	0.43 ± 0.07*	2.01 ± 0.21
	100	0.26 ± 0.05	0.81 ± 0.06*	0.41 ± 0.06**	3.16 ± 0.24*
BPMC (mg/kg)	Control	0.38 ± 0.09	0.32 ± 0.02	0.48 ± 0.05	2.40 ± 0.18
	50	0.60 ± 0.11	0.35 ± 0.06	0.56 ± 0.05	2.48 ± 0.33
	75	0.41 ± 0.08	0.28 ± 0.04	0.49 ± 0.05	2.36 ± 0.28
	100	0.33 ± 0.10	0.34 ± 0.05	0.46 ± 0.06	2.71 ± 0.26

Values are the mean ± S.E. of 3 to 4 red carp per dosage group. Significantly different from corresponding mean of control(*$P < 0.05$;**$P < 0.02$). Diazinon:0,0-Diethyl-0-(2-isopropyl-6-methyl-4-pyrimidinyl)phosphorothioate.

Table 3. Metal-binding proteins and heme oxygenase activity in the hepatopancreas and kidney of red carp 3 days after injection with Emulgen 913 or sodium n-dodecylbenzenesulfonate(LAS)

		Metal-binding proteins (μg/mg protein)		Heme oxygenase activity (nmole/mg protein/hr)	
		Hepatopancreas	Kidney	Hepatopancreas	Kidney
Emulgen 913 (mg/kg)	Control	0.95 ± 0.11	0.71 ± 0.20	0.55 ± 0.05	2.05 ± 0.12
	100	0.90 ± 0.14	0.84 ± 0.20	0.39 ± 0.03	1.06 ± 0.24**
	125	0.46 ± 0.15*	0.86 ± 0.14	0.50 ± 0.05	1.05 ± 0.11***
	150	0.35 ± 0.04***	0.98 ± 0.11	0.56 ± 0.04	0.84 ± 0.12***
	175	0.40 ± 0.06**	0.73 ± 0.15	0.52 ± 0.04	1.22 ± 0.22**
LAS (mg/kg)	Control	0.83 ± 0.11	0.65 ± 0.04	0.51 ± 0.05	2.12 ± 0.32
	50	0.80 ± 0.15	0.88 ± 0.02*	0.42 ± 0.03	1.47 ± 0.05
	75	0.52 ± 0.18	0.78 ± 0.05	0.43 ± 0.05	1.19 ± 0.18*
	100	0.44 ± 0.12*	0.77 ± 0.08	0.57 ± 0.06	0.77 ± 0.08**
	125	0.50 ± 0.07	0.72 ± 0.15	0.52 ± 0.03	0.95 ± 0.13*

Values are the mean ± S.E.of 3 to 4 red carp per dosage group. Significantly different from corresponding mean of control(*$P < 0.05$;**$P < 0.02$;***$P < 0.01$). Emulgen 913:Polyoxyethyleneglycol nonylphenyl ether.

of some functional groups which complex and block chemicals.

REFERENCES

Ariyoshi T(1981) Heavy metals and heme metabolism. Eisei Kagaku 27:257-270

Ariyoshi T, Shiiba S, Hasegawa H, Arizono K(1990) Effects of the environmental pollutants on heme oxygenase activity and cytochrome P-450 content in fish. Bull Environ Contam Toxicol (Accepted August 2, 1989 No3562)

Ariyoshi T, Takabatake E, Remmer H(1970) Drug metabolism in ethanol induced fatty liver. Life Sci 9(Part II):361-369

Arizono K, Ito T, Yamaguchi M, Ariyoshi T(1982) Induction of zinc metallothionein in the liver of rats by lead. Eisei kagaku 28:94-98

Elcombe CR, Lech JJ(1979) Induction and characterization of hemoprotein(s) P-450 and monooxygenation in rainbow trout(Salmo gairdneri). Toxicol Appl Pharmacol 49:437-450

Kägi JHR, Nordberg M(1979) Metallothionein. Experientia(Suppl) 34:41-124

Kägi JHR, Kojima Y(1987) Metallothionein II. Experientia(Suppl) 52:23-134

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ(1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193:265-275

Maines MD, Kappas A(1976) Studies on the mechanism of induction of haem oxygenase by cobalt and metal ions. Biochem J 154:125-131

Onosaka S, Tanaka K, Doi M, Okahara K(1978) A simplified procedure for determination of metallothionein in animal tissues. Eisei Kagaku 24:128-131

Roesijadi G(1980) The significance of low molecular weight, metallothionein-like proteins in marine invertebrates:current status. Mar Environ Res 4:167-179

Stegeman JJ, Klotz AV, Woodin BR, Pajor AM(1981) Induction of hepatic cytochrome P-450 in fish and the indication of environmental induction in scup. Aquatic Toxicol 1:197-212

Suzuki Y, Yoshikawa H(1976) Induction of hepatic zinc-binding proteins by various metals. Ind Health 14:25-31

Recieved July 10, 1989; accepted September 16, 1989.