Bull. Environ. Contam. Toxicol. (1990) 44:189-196

Bulletin of Environmental Contamination and Toxicology

Effects of the Environmental Pollutants on Heme Oxygenase Activity and Cytochrome P-450 Content in Fish

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

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

It is well established that fish have the ability to metabolize foreign compounds in a similar manner to that of mammalian species (Adamson 1967). In addition, it is well known that many environmental and hereditary factors cause changes in the activity of microsomal drug metabolizing enzymes in mammals (Conney and Burns 1962, Remmer 1962, Fouts 1963, Conney 1967). However, very little is known in fish about the effects of environmental contaminants on the relationship between the activity of heme oxygenase, a rate-limiting enzyme in heme degradation, and the content of hemoprotein namely cytochrome P-450 (P-450).

It is of interest to clarify the nature of hepatic heme oxygenase because if that activity alters by environmental pollutants, this may have serious effects on physiological changes of hemoprotein P-450, which is known to play a major role in the metabolism and toxicity of xenobiotics. Furthermore, it has been suggested that the extent of induction of P-450-linked mixed function oxidases in fish may be a useful indicator of water pollution (Payne and Penrose 1975).

Therefore, in the present study, we have investigated the activities of heme oxygenase as well as other drug metabolizing enzymes and the content of P-450 in the hepatopancreas of fish as the biochemical indicator, and thereby have made an attempt to clarify the range of pollution in the aquatic environment.

MATERIALS AND METHODS

D-Glucose-6-phosphate, D-glucose-6-phosphate dehydrogenase and NADPH were purchased from Boehringer Mannheim-Yamanouchi Co., Ltd., (Tokyo, Japan), and NADP and ATP were obtained from Oriental Yeast Co., Ltd.,(Tokyo, Japan). Cadmium chloride ($CdCl_2$), sodium n-dodecylbenzenesulfonate (LAS), o-sec-butylphenyl N-methylcarbamate (BPMC) and other chemicals used were of the highest grade quality purchased from Wako Pure Chemical Industries Ltd., (Osaka, Japan). Red carps (Cyprinus carpio Linne) weighing 280-340g obtained from a fish farm in Nagasaki were used for all experiments.

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

After an adaptation period of 3-5 days fish were placed into a 45 L each aquarium (4-5 fish/aquarium, 4 aquariums used) with aerated, filtered and recirculated water at a temperature of 23±2°C. Fish were fed commercially available diet once daily.

Fish were intraperitoneally injected with chemicals dissolved in 0.9% saline ($CdCl_2$ and LAS) or in corn oil (BPMC) at 1ml ($CdCl_2$ and BPMC) or 2ml (LAS) per dose per kg. Control fish were injected with saline or corn oil alone at respective corresponding volume per kg. A daily half-volume change of water was performed. Three days post injection of chemicals fish were killed and hepatopancreas were immediately removed, washed and weighed. Hepatopancreas were homogenized with 4 volumes of 0.25M sucrose in a Potter-Elvehjem homogenizer with a teflon pestle. Preparation of 105000xg soluble fraction and microsomes were carried out by procedures described by Arizono et al. (1982) and Ariyoshi et al. (1970), respectively.

Hemoprotein P-450 was estimated by the method 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. Heme oxygenase activity was calculated from the amount of bilirubin formed by using an extinction coefficient of $40\ mM^{-1}\ cm^{-1}$ between 464 and 530nm as discribed by Maines and Kappas (1976). 7-Ethoxycoumarin 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). UDP-Glucuronyltransferase (UDPGT) activity (p-nitrophenol used as a substrate) was measured colorimetrically the disappearance of p-nitrophenol caused by glucuronidation as described by Storey (1965). Phenol sulfate conjugating (PSC) activity (p-nitrophenol as a substrate) was measured colorimetrically a methylene blue complex according to the method of Roy (1956). P-450 content and all enzymatic activities were reported on a per mg microsomal protein (Lowry et al. 1951) based on using bovine serum albumine as a standard.

RESULTS AND DISCUSSION

P-450 exists in multiple forms which differ with respect to substrate specificity and degree of inducibility by drugs and other chemicals in mammals (Ryan et al. 1975, Haugen et al. 1975). In addition, Elcombe and Lech (1979) reported that benzo[a]pyrene hydroxylase activity in trout liver is induced by polycyclic aromatic hydrocarbons-type inducers, while ethylmorphine N-demetylase activity which is known to be associated with phenobarbital-inducible forms of P-450 in the rat, is not affected. Although it is well known that P-450 and P-450-linked enzymes in fish are present in very low amounts than that of mammals, little information is available on the conjugating ability of enzymes in fish treated with pollutants. Therefore, we investigated the P-450 content and the activities of heme oxygenase as well as drug metabolizing enzymes in fish by the treatment with typical polluted chemicals such as heavy metal ($CdCl_2$), pesticide (BPMC) and surfactant (LAS).

Table 1. Enzyme activities and cytochrome P-450 content in the hepatopancreas of red carps at 3 days after a single injection of cadmium chloride($CdCl_2$)

Activity and content	Control	$CdCl_2$(mg/kg)		
		1.5	3.0	4.5
Heme oxygenase (nmole/mg protein/hr)	0.36 ± 0.06	0.44 ± 0.02	1.11 ± 0.10***	1.25 ± 0.12***
Cytochrome P-450 (nmole/mg protein)	0.20 ± 0.01	0.17 ± 0.03	0.14 ± 0.01**	0.11 ± 0.01***
7-Ethoxycoumarin O-deethylase (nmole/mg protein/min)	0.33 ± 0.02	0.31 ± 0.02	0.31 ± 0.02	0.19 ± 0.04*
UDP-glucuronyltransferase (nmole/mg protein/30min)	14.7 ± 0.3	14.5 ± 0.4	11.7 ± 1.2	13.6 ± 0.9
Phenol sulfate conjugating (nmole/mg protein/hr)	4.98 ± 1.00	5.33 ± 0.65	5.31 ± 0.92	5.04 ± 0.74

Values are mean ± S.E. of 3 to 4 red carps per group. Significantly different from corresponding mean of control(*$P < 0.05$;**$P < 0.02$;***$P < 0.01$).

Table 2. Enzyme activities and cytochrome P-450 content in the hepatopancreas of red carps at 3 days after a single injection of o-sec-butylphenyl N-methylcarbamate(BPMC)

Activity and content	Control	BPMC(mg/kg)		
		50	75	100
Heme oxygenase (nmole/mg protein/hr)	0.44 ± 0.05	0.52 ± 0.04	0.44 ± 0.05	0.44 ± 0.05
Cytochrome P-450 (nmole/mg protein)	0.22 ± 0.02	0.17 ± 0.01*	0.18 ± 0.01*	0.17 ± 0.01*
7-Ethoxycoumarin O-deethylase (nmole/mg protein/min)	0.37 ± 0.03	0.40 ± 0.04	0.32 ± 0.01	0.40 ± 0.04
UDP-glucuronyltransferase (nmole/mg protein/30min)	13.5 ± 0.3	14.3 ± 0.6	10.4 ± 0.4**	11.0 ± 0.6*
Phenol sulfate conjugating (nmole/mg protein/hr)	5.01 ± 0.54	8.58 ± 1.47*	9.66 ± 0.98**	8.67 ± 1.06*

Values are mean ± S.E. of 3 to 4 red carps per group. Significantly different from corresponding mean of control(*$P < 0.05$; **$P < 0.02$).

Table 3. Enzyme activities and cytochrome P-450 content in the hepatopancreas of red carps at 3 days after a single injection of sodium n-dodecylbenzenesulfonate(LAS)

Activity and content	Control	LAS(mg/kg)		
		50	75	100
Heme oxygenase (nmole/mg protein/hr)	0.46 ± 0.06	0.42 ± 0.03	0.36 ± 0.04	0.58 ± 0.06
Cytochrome P-450 (nmole/mg protein)	0.20 ± 0.03	0.13 ± 0.01*	0.11 ± 0.02*	0.08 ± 0.01**
7-Ethoxycoumarin O-deethylase (nmole/mg protein/min)	0.33 ± 0.03	0.14 ± 0.01***	0.18 ± 0.02***	0.15 ± 0.01***
UDP-glucuronyltransferase (nmole/mg protein/30min)	13.1 ± 0.8	11.7 ± 1.1	13.5 ± 0.5	13.9 ± 0.9
Phenol sulfate conjugating (nmole/mg protein/hr)	5.93 ± 0.55	4.99 ± 1.81	9.57 ± 1.53	10.6 ± 1.26*

Values are mean ± S.E. of 3 to 4 red carps per group. Significantly different from corresponding mean of control(*$P < 0.05$;**$P < 0.02$;***$P < 0.01$).

The effects of the injection of $CdCl_2$ at a single dose of 1.5,3.0 and 4.5mg/kg to red carps on heme oxygenase activity, P-450 content and drug metabolizing enzymes activities in the hepatopancreas are summarized in Table 1. Heme oxygenase activity was increased with increasing dose of $CdCl_2$, whereas P-450 content was decreased with dose related when compared with that of respective control. A good reciprocal correlation was observed between heme oxygenase activity and P-450 content ($r=0.62$, $n=12$) in this experiment. This finding agrees with the observation of earlier study (Maines and Kappas 1976) which reciprocal relations between heme oxygenase activity and the depression of hepatic P-450 content in male rats were produced by a number of metals including Cr, Mn, Fe, Ni, Co, Cu, Zn, Cd, Hg and Pb. However, the activities of other drug metabolizing enzymes in fish except 7-EC in the highest dose remained at the control level throughout this dose used.

Table 2 shows the influences of the treatment with BPMC at a single dose of 50, 75 and 100mg/kg to red carps on the biochemical parameters. Although P-450 content was significantly depressed by treatment, no appreciable differences were found in the activities of P-450 dependent 7-EC and of heme oxygenase. This discrepancy with above findings obtained from $CdCl_2$ injection might be caused by the differences in the metabolism and disposition in both BPMC and inorganic metal. However, it is of particular interest that UDPGT activity was decreased by the injection of BPMC at the dose of 75 and 100mg/kg, whereas PSC activity was significantly enhanced by all dose used when compared with that of respective control. This increase may be due to the adaptive induction of PSC enzyme in BPMC metabolism in vivo. Further study on the changes in this enzyme in fish after the treatment with various chemicals will be needed.

The effects of LAS, a commonly used anionic surfactant, on the P-450 content and the activities of above enzymes in the hepatopancreas of red carps are shown in Table 3. P-450 content and P-450 dependent 7-EC activity were remarkably depressed by LAS at all dose used in this experiment. This finding suggests that LAS may exert on the characteristic P-450 species for O-dealkylation reaction. On the other hand, the highest dose of LAS enhanced PSC activity as compared with control. However , no marked changes in the activities of heme oxygenase and UDPGT were noted.

In general, heme and P-450 heme are degraded normally or through the action of exogenous substances such as environmental chemicals (Schacter and Mason 1974, Guzelian and Elshourbagy 1979, Rosenberg et al. 1980, Ariyoshi et al. 1981) and trace metals (Maines and Kappas 1976, Kransny and Holbrook 1978, Wood et al. 1979). In this study, we observed no close relations between heme oxygenase activity and hemoprotein P-450 content by the treatment with BPMC and LAS except for $CdCl_2$. A more detailed studies on the relationship between some enzymes activities and environmental contaminants in fish are needed to elucidate the range of pollution in the aquatic environment.

REFERENCES

Adamson RH (1967) Drug metabolism in marine vertebrates. Federation Proc 26:1047-1055

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

Ariyoshi T, Eguchi M, Muraki Y, Yasumatsu H, Suetsugu N, Arizono K (1981) Effects of chlorinated benzenes on the activities of δ-aminolevulinic acid synthetase and heme oxygenase and on the content of hemoprotein in the liver of rats. J Pharm Dyn 4:69-76

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

Conney AH, Burns JJ (1962) Factors influencing drug metabolism. Advance Pharmacol 1:31-58

Conney AH (1967) Pharmacological implications of microsomal enzyme induction. Pharmacol Reviews 19:317-366

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

Fouts JR (1963) Factors influencing the metabolism of drugs in liver microsomes. Ann N Y Acad Sci 104:875-880

Guzelian PS, Elshourbagy NA (1979) Induction of hepatic heme oxygenase activity by bromobenzene. Arch Biochem Biophys 196: 178-185

Haugen DA, Van der Hoeven TA, Coon MJ (1975) Purified liver microsomal cytochrome P-450. Separation and characterization of multiple forms. J Biol Chem 250:3567-3570

Kransny HC, Holbrook DJ (1978) Effects of cadmium on heme oxygenase and hemoproteins in smooth and rough endoplasmic reticulum of rat liver. Biochem Pharmacol 27:364-366

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

Omura T, Sato R (1964) The carbon monoxide-binding pigment of liver microsomes. I. Evidence for its hemoprotein nature. J Biol Chem 239:2370-2378

Payne JF, Penrose WR (1975) Induction of aryl hydrocarbon (benzo-[a] pyrene) hydroxylase in fish by petroleum. Bull Environ Contam Toxicol 14:112-116

Remmer H (1962) Drugs as activators of drug enzymes. In:Brodie BB, Erdös EG(ed) Proceedings of the First International Pharmacology Meeting, vol.6, Pergamon Press, Oxford, pp235-256

Rosenberg DW, Drummond GS, Cornish HC, Kappas A (1980) Prolonged induction of hepatic haem oxygenase and decreases in cytochrome P-450 content by organotin compounds. Biochem J 190:465-468

Roy AB (1956) The enzymic systhesis of steroid sulphates. Biochem J 63: 294-300

Ryan D, Lu AYH, West S, Levin W (1975) Multiple forms of cytochrome P-450 in phenobarbital- and 3-methylcholanthrene-treated rats. J Biol Chem 250: 2157-2163

Schacter BA, Mason JI (1974) The effect of phenobarbital, 3-methylcholanthrene, 3,4-benzpyrene and pregnenolone-16α-carbonitrile

on microsomal heme oxygenase and splenic cytochrome P-450. Arch Biochem Biophys 160:274-278
Storey IDE (1965) The inhibition of the uridine diphosphatetransglucuronylase activity of mouse liver homogenates by thiol reagents. Biochem J 95:201-208
Ullrich V, Weber P (1972) The O-dealkylation of 7-ethoxycoumarin by liver microsomes. A direct fluorometric test. Hoppe-Seyler's Z Physiol Chem 353:1171-1177
Woods JS, Carver GT, Fowler BA (1979) Altered regulation of hepatic heme metabolism by indium chloride. Toxicol Appl Pharmacol 49:455-461

Received June 16, 1989; accepted August 2, 1989.