J. Fish. Sci. Tech. 5(1), 28~31, 2002

JOURNAL OF
FISHERIES SCIENCE
AND TECHNOLOGY

Effects of Dietary Cimetidine, a Cytochrome P450 Inhibitor, on the Benzo[a]pyrene-induced Lipid Peroxidation of Liver in Olive Flounder, *Paralichthys olivaceus*

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(Received November 2001, Accepted March 2002)

Effects of cimetidine, a cytochrome P450 inhibitor, on the benzo[a]pyrene (BaP)-mediated cytochrome P450 induction and lipid peroxidation of liver in olive flounder, *Paralichthys olivaceus*, were investigated. Fish were fed either a cimetidine-supplemented diet or a cimetidine-free control diet once daily to satiation for 3 days. After 6 hrs of last feeding, the fish received intraperitoneal (i.p.) injection of BaP (20 mg/kg of body weight) dissolved in sterile corn oil (100 μL) or received only a corn oil i.p. injection. At 1, 2, 3, and 7 days after the injection, hepatic cytochrome P450 and thiobarbituric acid reactive substances (TBARS), an indicator of lipid peroxidation, were analyzed. BaP injection resulted in an increase of hepatic cytochrome P450, and the fish fed the cimetidine-supplemented diet before injection of BaP showed delayed increase of hepatic cytochrome P450 compared to the fish fed a cimetidine-free diet and BaP injected. Injection of BaP clearly induced hepatic lipid peroxidation, and consistently higher TBAR values were shown in the fish fed a cimetidine-supplemented diet before injection of BaP than the fish injected with BaP alone.

Key words: Benzo[a]pyrene, Cimetidine, Olive flounder (*Paralichthys olivaceus*), Cytochrome P450, Lipid peroxidation, TBARS

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous in the environment, and are readily taken up from the water column, sediments and food sources into the liver and other tissues of fish (Lackner, 1998). Some evidence has indicated that contaminant-stimulated reactive oxygen species (ROS) production and resulting oxidative damage may be a mechanism of toxicity in aquatic organisms exposed to pollutants (Livingstone, 2001). Fish possess metabolic pathways capable of transforming the chemical structure of organic pollutants. Enzyme-mediated oxidation of chemicals (Phase I mechanism) is an important biotransformation pathway in fish and other vertebrates, and binding of chemicals to large biomolecules (Phase II mechanism) is another important biotransformation process (Metcalfe, 1998).

Benzo[a]pyrene (BaP), a well-known carcinogen, is an important and extensively studied member of PAHs (Shabad, 1997). BaP undergoes a metabolic activation to form reactive intermediates before it is capable of inducing its mutagenic and carcinogenic effects in biological systems. The cytochrome P450 system participates in the bioactivation of BaP to its reactive intermediates (Gelboin, 1980). It has been reported that ROS such as superoxide anion radicals (O_2^-) and hydrogen peroxide (H_2O_2) are produced during the metabolic processing of BaP (Lesko and Lorentzen, 1985; Penning et al., 1996). Among the known biological molecules, lipids are considered to be extremely susceptible to the presence of ROS. In particular, unsaturated fatty acids located in the cellular membranes are prone to ROS attack, resulting in lipid peroxidation (Gutteridge and Halliwell, 1990).

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Many cytochrome P450 isoforms have been characterized in various fish species, including cytochrome P450-1 (CYP1), CYP2, CYP3, CYP4, CYP11, CYP17, CYP19 and CYP26 (Nelson et al., 1996). The H_2-histamine receptor blocker cimetidine has been shown to differentially inhibit a variety of P 450 isoforms (Knodell et al., 1991).

Given the inhibitory actions of cimetidine on several P450 isoforms, and in light of the importance of P450 isoforms in catalyzing the metabolic activation of BaP, the present study was undertaken to examine the effects of cimetidine on the BaP -mediated cytochrome P450 induction and lipid peroxidation of liver in cultured olive flounder, *Paralichthys olivaceus*.

Materials and Methods

Diet preparation

The experimental diets were prepared by mixing 980 g of the commercial powder feed (Woosung feed Co., Korea) with 20 g of cimetidine (Sigma Chemical Co., USA). The cimetidine-free diet was used as a control diet. After pelleting the powder with a meat grinder, the experimental diets were stored at −20℃ until needed. Small portions were kept at 4℃ for the convenience of feeding.

Experimental procedure

Olive flounder (*Paralichthys olivaceus*) with an average body weight of 50 g were obtained from a local fish farm and maintained in sea water aquaria at 20 ± 1℃ prior to experiment for 2 weeks. A total of 96 fish were stocked into 4 tanks (24 fish/tank) containing 100 L seawater. Two groups of fish (tanks 1 and 2) were fed a cimetidine-supplemented diet and the other two groups (tanks 3 and 4) were fed a cimetidine-free control diet once daily to satiation for 3 days. After 6 hrs of last feeding, the fish from tanks 1 and 3 received intraperitoneal (i. p.) injection of BaP (20 mg/kg of body weight) dissolved in sterile corn oil (100 μL), while olive flounder from tanks 2 and 4 received only a corn oil i.p. injection. At 1, 2, 3, and 7 days after the injection, 6 fish were randomly selected from each tank for the following assays.

Cytochrome P450 assay

Fish were anaesthetized with 200 mg/L tricaine methanesulfonate (MS-222, Sigma), and the livers were immediately removed, rinsed in 150 mM KCl solution and homogenized in five volumes of sucrose 250 mM, Hepes 10 mM buffer (pH 7.4) with a tissue homogenizer. The homogenates were centrifuged at 12,000 g for 15 min and the resulting supernatants centrifuged again at 100,000 g for 60 min. Microsomes were resuspended in the above Hepes buffer. Microsomal preparations were frozen in a deep freezer at −70℃. The contents of cytochrome P450 in the liver microsomes were determined by the method of Omura and Sato (1964).

Thiobarbituric acid reactive substances (TBARS) assay

TBARS in liver homogenates were determined by mixing a 100 μL aliquot of the homogenate with 200 μL of sodium dodecyl sulfate (8.1%, Sigma) and 1.5 mL of 20% acetic acid (pH 3.5). Then, 1.5 mL of 0.8% (w/v) thiobarbituric acid (TBA, Sigma) in water containing 0.025% 2,6-di-*tert*-butyl-4-methylphenol (BHT, Sigma) was added. The mixture was incubated in a boiling water bath for 60 min, centrifuged at 5,000 g for 5 min, and its absorbance was read at 535 nm.

Statistical analysis

One-way analysis of variance (ANOVA), followed by the Tukey multiple comparisons test was employed to evaluate the level of significance and the difference was considered significant when $P<0.05$.

Results

Cytochrome P450

At 24 h post injection, the group of fish injected with BaP without pre-treatment of cimetidine showed the highest induction of hepatic cytochrome P 450 compared to other experimental groups (Fig. 1). However, the group of fish pre-administered cimetidine and injected with BaP did not induce cytochrome P450 at 24 h. In contrast, at 48 h post injection, the fish pre-administered cimetidine and injected with BaP showed significantly higher cytochrome P450 induction than groups injected with BaP-free corn oil. At 72 h and 7 day post injection, there were no significant differences in cytochrome

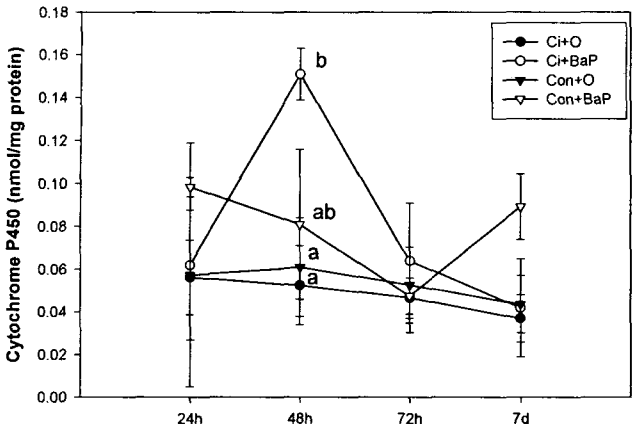

Fig. 1. Induction of hepatic cytochrome P450 in olive flounder (*Paralichthys olivaceus*) by intraperitoneal (i.p.) injection of benzo[a]pyrene (BaP). Different letters on each symbol represents statistically significant differences at $P<0.05$. (Ci+O, the group of fish pre-fed a cimetidine-supplemented diet and i.p. injection of only corn oil; Ci+BaP, the group of fish pre-fed a cimetidine-supplemented diet and i.p. injection of BaP; Con+O, the group of fish pre-fed a cimetidine-free diet and i.p. injection of only corn oil; Con+BaP, the group of fish pre-fed a cimetidine-free diet and i.p. injection of BaP).

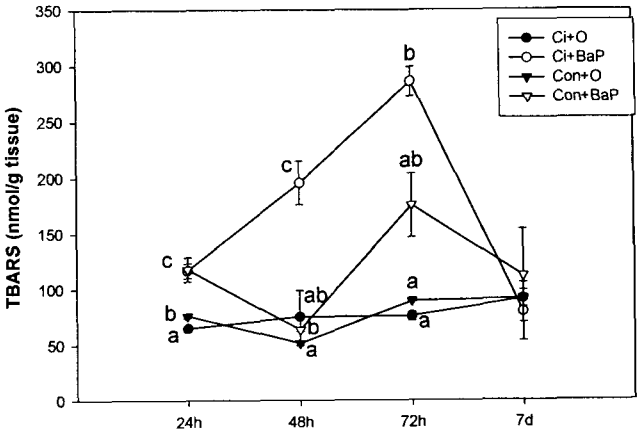

Fig. 2. The induction of thiobarbituric acid reactive substances (TBARS) of liver of olive flounder (*Paralichthys olivaceus*) by intraperitoneal (i.p.) injection of benzo[a]pyrene (BaP). Different letters on each symbol represents statistically significant differences at $P<0.05$. (Ci+O, the group of fish pre-fed a cimetidine-supplemented diet and i.p. injection of only corn oil; Ci+BaP, the group of fish pre-fed a cimetidine-supplemented diet and i.p. injection of BaP; Con+O, the group of fish pre-fed a cimetidine-free diet and i.p. injection of only corn oil; Con+BaP, the group of fish pre-fed a cimetidine-free diet and i.p. injection of BaP).

P450 among experimental groups. No increase of cytochrome P450 was observed in the groups injected with BaP-free corn oil.

Hepatic lipid peroxidation

At 24 h post injection, groups of fish injected with BaP showed significantly higher TBAR values than those injected with BaP-free corn oil (Fig. 2). The group of fish pre-fed a cimetidine-supplemented diet and injected with BaP showed significantly higher TBAR values than groups injected with BaP-free corn oil at 24, 48 and 72 h post injection. However, at 7 day post injection, there were no significant differences in TBAR among experimental groups.

Discussion

In the present study, i.p. injection of BaP increased hepatic cytochrome P450 in olive flounder, and the fish fed a cimetidine-supplemented diet before injection of BaP showed delayed increase of hepatic cytochrome P450 compared to the fish fed a cimetidine-free diet and BaP injected. BaP is metabolized mainly by the cytochrome P450-dependent aryl hydrocarbon hydroxylase (AHH) (Gelboin, 1980; Guengerich, 1991), and cimetidine has been shown to affect P450-dependent metabolism of compounds through direct interaction with P450s (Knodell et al., 1991; Agyeman and Sultatos, 1998). Although there is no information on the pharmacokinetics of cimetidine in fish, cimetidine is rapidly absorbed following oral administration, and about two thirds of the oral dose is excreted within 24 h in human (Kelly et al., 1995). The delayed increase of cytochrome P450 content resulting from pre-administration with cimetidine in this study suggests that, in the presence of cimetidine, the overall velocity of cytochrome P-450-dependent biotransformation of BaP was reduced significantly, but that this reduced metabolism resulted in a accumulation of BaP in the liver. Therefore, the high concentration of unmetabolized BaP in the liver and depletion of cime-

tidine in the fish might be the causes of considerably high induction of cytochrome P450.

Injection of BaP clearly induced hepatic lipid peroxidation in this study. BaP is known to induce the formation of reactive oxygen species (ROS) in organisms during biotransformation (Winzer et al., 2000). The most typical reaction during ROS-induced damage involves the peroxidation of unsaturated fatty acids (Kappus, 1987). The consistently higher TBAR values in the fish fed a cimetidine-supplemented diet before injection of BaP than the fish administered a cimetidine-free diet and BaP injected suggest that not only BaP but also higher cytochrome P450 were associated with the lipid peroxidation, since cytochrome P450 is able to influence lipid peroxidation during its catalytic cycle (Lambert et al., 1996).

In conclusion, the results of the present study suggest that oral administration of cimetidine can suppress BaP-mediated cytochrome P450 induction, resulting in reduction of Phase I biotransformation of BaP. However, when cimetidine is depleted in the fish, the cumulated parent BaP can induce higher magnitude of hepatic cytochrome P450, and these can induce highly elevated hepatic lipid peroxidation in olive flounder.

References

Agyeman, A.A. and L.G. Sultatos. 1998. The actions of the H_2-blocker cimetidine on the toxicity and biotransformation of the phosphorothioate insecticide parathion. Toxicology, 128, 207~18.

Gelboin, H.V. 1980. Benzo[a]pyrene metabolism, activation and carcinogenesis: role of regulation of mixed-function oxidases and related enzymes. Physiol. Rev., 60, 1107~1166.

Guengerich, F.P. 1991. Reactions and significance of cytochrome P-450. J. Biol. Chem., 226, 10019~10022.

Gutteridge, J.M. and B. Halliwell. 1990. The measurement and mechanism of lipid peroxidation in biological systems. Trends Biochem. Sci., 15, 129~135.

Kappus, H. 1987. Oxidative stress in chemical toxicity. Arch. Toxicol., 60, 144~149.

Kelly, M.T., D. McGuirk and F.J. Bloomfield. 1995. Determination of cimetidine in human plasma by high-performance liquid chromatography following liquid-liquid extraction. J. Chromatogr., 668, 117~123.

Knodell, R., D. Browne, G. Gwozdz, W. Brian and F. Guengerich. 1991. Differential inhibition of individual human liver cytochrome P-450 by cimetidine. Gastroenterology, 101, 1680~1691.

Lackner, R. 1998. "Oxidative stress" in fish by environmental pollutants. In Fish Ecotoxicology, T. Braunbeck, D.E. Hinton and B. Streit, ed. Birkhäuser Verlag, Basel · Boston · Berlin, pp. 203~224.

Lambert, N., S.J. Chambers, G.W. Plumb and G. Williamson. 1996. Human cytochrome P450s are pro-oxidants in iron-ascorbate-initiated microsomal lipid peroxidation. Free Radic. Res., 24, 177~185.

Lesko, S.A. and R.L. Lorentzen. 1985. Benzo[a]pyrene dione-benzo[a]pyrene diol oxidation-reduction couples; involvement in DNA damage, cellular toxicity, and carcinogenesis. J. Toxicol. Environ. Health, 16, 679~691.

Livingstone, D.R. 2001. Contaminant-stimulated reactive oxygen species production and oxidative damage in aquatic organisms. Mar. Poll. Bull., 42, 656~666.

Metcalfe, C.D. 1989. Tests for predicting carcinogenicity in fish. CRC Rev. Aqua. Sci., 1, 111~129.

Nelson, D., L. Koymans, T. Kamataki, J. Stegeman, R. Feyereisen, D. Waxman, M. Waterman, O. Gotoh, M. Coon, R. stabrook, I. Gunsalus and D. Nebert. 1996. P450 superfamily: update on new sequences, gene mapping, accession numbers and nomenclature. Pharmacogenetics, 6, 1~42.

Omura, T. and R. Sato. 1964. The carbon monoxide-binding pigment of liver microsomes. 1. Evidence for its hemoprotein nature. J. Biol. Chem., 239, 2370~2378.

Penning, T.M., S.T. Ohnishi, T. Ohiishi and R.G. Harvey. 1996. Generation of reactive oxygen species during the enzymatic oxidation of polycyclic aromatic hydrocarbon trans-dihydrodiols catalyzed by dihydrodiol dehydrogenase. Chem. Res. Toxicol., 9, 84~92.

Shabad, L.M. 1997. Carcinogenic action of polycyclic hydrocarbons in animals and in man. IARC Sci. Publ., 16, 257~269.

Winzer, K., W. Becker, C.J.F. Van Noorden and A. K hler. 2000. Short-time induction of oxidative stress in hepatocyte of the European flounder (*Platichthys flesus*). Mar. Environ. Res., 50, 495~501.