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Haematological Parameters Induced by Benzo(a)pyrene Exposure as a Toxicity Biomarker in the Farmed Red Sea Bream, *Pagrus major*

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Farmed red sea breams, *Pagrus major*, were fed for 60 days with pellets containing different concentrations of benzo(a)pyrene (0, 0.2, 2, 20 ㎎/kg) to generate a biomarker of the chemical toxicity in the fish. The fish exposed to the chemical concentrations did not show any significant difference in the weight gain, conditioning factor, and hepatosomatic index. However, some haematological parameters, such as glucose, calcium, magnesium, GOT (glutamic oxalate transaminase), and GPT (glutamic pyruvate transaminase) were influenced by the chemical exposure. Of them, two enzymes, GOT and GPT, increased significantly 60 days after the exposure in a way of concentration dependence ($P<0.05$). In the study of ecotoxicological biomarker, sensitivity to adverse environments is one of the key available factors. The fish changes in GOT and GPT were an earlier and reliable sign of the fish response against the chemical exposure, rendering the two enzymatic factors as a useful biomarker at least to benzo(a)pyrene exposure in the farming waters.

Keywords: Benzo(a)pyrene, GOT, GPT, Biomarker, *Pagrus major*

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a class of toxic environmental pollutants which have accumulated in the environment due to a variety of anthropogenic activities. One of such high molecular weight PAHs is benzo(a)pyrene (BaP), a five-ring compound. BaP has been classified as a priority pollutant: a compound selected on the basis of its known or suspected carcinogenicity, teratogenicity or acute toxicity.

Fish blood analysis has been used as a diagnosis to assess its physiological state and the effect of hazardous substances, to determine the nonspecific resistance of progenitors and its descendants, and to establish the quality of food, genetic variability, and effects of stress (Svobodova and Vysukova, 1991). Haematology is used as an index of fish health status in a number of fish species to detect physiological changes following different stress sources: exposure to variety of environment pollutants, diseases, hypoxia, and so on (Blaxhall, 1972; Duthie and Tort, 1985; Morgan and Iwama, 1997).

Growth of a fish is generally used as a reliable method in chronic toxicological investigations (De Boeck et al., 1997). However, the growth parameter is still remained as an end-point coming from the toxicological response. Biomarker parameters representing earlier and more sensitive responses are necessary for more reliable assessment of the animal physiology. Glutamate oxaloacetate (GOT) and glutamate pyruvate (GPT) transaminases are enzymes frequently used in the diagnosis of damage caused by pollutants in various tissues such as liver, muscle, and gill (de la Torre et al., 1999).

The red sea bream, *Pagrus major*, is an economically important fish commonly cultured in marine-based cages in Korea. Because the most of the cages are located in the embayed waters to keep them from physical damage, the fish in the cages have an increasing potential to be exposed to the environmental pollutants, including BaP. Despite of the increased potential to the chemical exposure, little information on the chemical effects on the caged fish is available. The specific objective of this study is to evaluate the effects of BaP on some haematological parameters resenting earlier responses from the chemical exposure in the cultured red sea bream.

Materials and Methods

Diet preparation

Commercial fish diets were crumbled, supplemented with 0, 0.2, 2, and 20 mg/kg feed of BaP (Sigma) in acetone, and

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re-pelleted. After the processing, the pellets were packed into small bags and stored at -20 until being served to the test fish.

Experimental fish and treatment

Red sea breams weighing 306.32 g on average were acclimated in a 3000 L aerated running seawater tank for a month. A month after the acclimation in captivity, the fish selected randomly were individually transferred to 50 L container with running water and continuous aeration. The fish contained individually in the 50 L container were further acclimated in captivity for 2 weeks prior to the commencement of the experiment. The fish were fed the pellets containing different doses of BaP for 60 days.

Sampling and analysis

Five fish were sacrificed from each tank in the 60 days of experiment. The fish were starved for 24 h prior to sampling to allow all feed and digestive metabolites to be excreted. Fish weight and total length were recorded for each individual. Diets were fed to all group fish twice a day. The following formulae were used to evaluate the effect of dietary exposed BaP on growth factors of red sea bream:

Weight gain (WG)={[final body weight–initial body weight]/initial body weight}×100

Condition factor (CF)=(wet weight/total length^3)×10^3

Hepatosomatic index (HSI)=(liver weight/total weight)×100

Blood samples were obtained with a haparinized syringe from the caudal vein of the fish. The blood was centrifuged, and the plasma was frozen until required for analysis. The frozen samples were analyzed for glucose (Asan Pharm, Co., Ltd.), calcium (Asan Pharm, Co., Ltd.), magnesium (Asan Pharm, Co., Ltd.), GOT (Asan Pharm, Co., Ltd.), and GPT (Asan Pharm, Co., Ltd.).

Statistics

Statistics were performed with SPSS, using one-way analysis of variance (ANOVA) followed by LSD multiple comparison test.

Results and Discussion

The growth of a fish is generally used as a sensitive and reliable endpoint in chronic toxicological investigations (De Boeck et al., 1997). The results of average WG, CF, and HSI of red sea bream are shown in Table 1. No significant differences were observed among WG, CF, and HSI of the fish exposed to concentrations of BaP, compared with control. Recent laboratory studies (Kubin, 1989; Rice et al., 2000) showed reduced growth in juvenile English solely exposed to PAHs through contaminated sediment or diet. Slow growth rates have been associated with increased juvenile mortality in several fish species (Lorenzen, 1996; McGurk, 1996). In our study, however, WG, CF, and HSI decreased without statistical difference.

In the biomarker studies of fish to a specific chemical pollution, finding an early physiological sign is a crucial concern. Haematological parameters of fish are suitable biomarkers for evaluating the potential risk of chemicals (Roche and Boge, 2000). Many investigators have also identified changes in several haematological variables as an indicator of toxic chemicals (Wepender et al., 1992; Jee et al., 2004). The present findings indicated that in the red sea bream, subchronic dietary exposure to BaP caused a significant induction ($P<0.05$) in GOT, GPT (Table 2). de la Torre et al. (2000) showed that both liver GOT and GPT activities increased by the metal by 63 and 98%. Yoshida et al. (2005)

Table 1. Effects of benzo(a)pyrene on weight gain, condition factor, and hepatosomatic index of *Pagrus major*

Parameters	Exposure Time (day)	Benzo(a)pyrene concentration (mg/kg)			
		Control	0.2	2	20
WG[a]	30	38.9 ± 11.4	35.5 ± 1.7	31.45 ± 7.9	29.1 ± 8.0
	60	57.6 ± 12.1	46.3 ± 5.8	48.0 ± 2.2	36.9 ± 1.7
CF[b]	30	18.14 ± 0.3	17.5 ± 0.1	16.5 ± 1.5	17.42 ± 1.1
	60	17.1 ± 0.5	16.3 ± 0.2	16.7 ± 0.6	16.7 ± 0.3
HSI[c]	30	2.1 ± 0.2	2.2 ± 0.3	2.0 ± 0.1	2.0 ± 0.2
	60	2.2 ± 0.2	2.3 ± 0.4	2.0 ± 0.1	2.3 ± 0.2

[a]Weight gain (WG): [(final body weight – initial body weight)/initial body weight]×100
[b]Condition factor (CF): (wet weight/total length^3)×10^3
[c]Hepatosomatic index (HSI): (liver weight/total weight)×100
*Values are means±SE.

Table 2. Hemochemical changes induced by dietary exposure of benzo(a)pyrene in the cultured *Pagrus major*

Exposure day	BaP conc.	Glucose (mg/100 ml)	Calcium (mg/100 ml)	Magnesium (mg/100 ml)	GOT (Karmen Units)	GPT (Karmen Units)
0	0	35.8 ± 1.2	11.02 ± 0.40	4.97 ± 0.20	47.48 ± 2.45	20.87 ± 0.33
7	0	36.3 ± 3.3	11.44 ± 0.17	4.87 ± 0.10	46.46 ± 2.21	20.40 ± 0.32
	0.2	39.7 ± 2.3	11.02 ± 0.75	4.55 ± 0.05	50.05 ± 2.12	20.29 ± 0.13
	2	46.0 ± 4.0	11.28 ± 0.54	3.75 ± 0.55*	50.40 ± 1.65	20.56 ± 0.57
	20	43.2 ± 9.9	10.39 ± 0.39	3.06 ± 0.12*	53.66 ± 1.70*	21.20 ± 0.16
14	0	34.8 ± 1.3	10.36 ± 0.19	4.38 ± 0.11	45.85 ± 1.51	20.20 ± 0.16
	0.2	36.2 ± 2.8	10.34 ± 0.23	4.29 ± 0.86	51.00 ± 1.30	20.05 ± 0.32
	2	39.9 ± 2.9	10.66 ± 0.10	4.61 ± 0.25	50.25 ± 2.47	20.72 ± 0.10
	20	33.8 ± 0.4	10.23 ± 0.80	4.42 ± 0.02	53.06 ± 2.70*	21.56 ± 0.60*
30	0	34.8 ± 2.9	11.44 ± 0.17	5.32 ± 0.26	45.47 ± 1.01	20.64 ± 0.37
	0.2	36.7 ± 8.3	13.57 ± 0.98	4.97 ± 0.39	63.5 ± 6.43	22.1 ± 0.63
	2	39.6 ± 4.2	11.06 ± 0.64	5.68 ± 0.58	56.93 ± 2.53	22.12 ± 0.12
	20	40.5 ± 1.1	10.78 ± 0.51	6.18 ± 1.04	62.8 ± 7.72	24.4 ± 2.24*
60	0	34.9 ± 2.4	10.92 ± 0.17	5.32 ± 0.26	45.52 ± 0.74	21.06 ± 0.42
	0.2	39.3 ± 1.9	12.90 ± 0.90	6.14 ± 0.47	61.70 ± 0.75*	24.68 ± 0.30*
	2	45.6 ± 2.0	10.40 ± 0.40	5.80 ± 0.21	61.35 ± 1.26*	24.98 ± 0.34*
	20	45.0 ± 8.5	8.60 ± 1.20	5.97 ± 0.05	62.70 ± 2.10*	25.86 ± 0.47*

*Values are means±SE.

also found that an intraperitoneal administration of carbon tetrachloride to mice induced the increase in hydroxyoctadecadienoic acid in liver and plasma followed by an increase in plasma GOT and GPT.

The mitochondrial GPT form predominates in organs with intensive glycogenesis such as the liver. The traction catalyzed by the GOT is particularly important because the molecular rearrangement involves amino acids linked with the citric acid cycle at two points (oxaloacetic and ketoglutaric acids), being the most important mechanism for introducing reduction equivalents into the mitochondria. In the particular case of fish, the activity of liver GOT and GPT is extremely variable (Moon and Foster, 1995). In addition, the effects of Cd on GOT and GPT is also extremely variable. Gill et al. (1991) exposed rosy barbs, *Barbus conchonius*, to the metal observing a slight inhibition of GOT activities while GPT was strongly inhibited. Inhibition of these two enzymes was reported by Gill et al. (1990) in *Puntius conchonius* exposed to insecticides and Cd. Results indicated that the changes in GOT and GPT might be an early sign of the fish response against the chemical exposure, rendering the two enzymatic factors as a useful biomarker at least to BaP exposure. These results provide us with some insights for an employment of the two enzymatic parameters as a potential biomarker to the chemical pollution in the farming waters.

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