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Effects of Dietary Benzo[a]pyrene on Growth and Hematological Parameters in Juvenile Rockfish, *Sebastes schlegeli* (Hilgendorf)

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Abstract Experiments were conducted to evaluate the effect of dietary benzo[a]pyrene (B[a]P) on the growth and hematological parameters of rockfish, *Sebastes schlegeli*, after they were fed with subchronic dietary B[a]P for 30 days. The weight growth rate of the rockfish was significantly different from that of the control group at dietary B[a]P concentration of 1.5 and 2.0 mg/kg. Significant reduction in red blood cells, hematocrit, and hemoglobin were observed during 30 days of exposure to the highest concentration. Over 30 days, serum AST increased at a B[a]P concentration of 1.5 mg/kg and LDH increased at a B[a]P concentration of 1.0 mg/kg concentration. However, no changes were observed in ALT, total protein, magnesium or calcium.

Keywords Dietary benzo[a]pyrene · *Sebastes schlegeli* · Growth · Hematological parameter

Marine ecosystems are increasingly threatened by a wide variety of toxic chemicals. Although many toxic chemicals have been banned, many chemicals such as polycyclic aromatic hydrocarbons (PAHs) are still present in marine ecosystem, threatening the health of marine organisms including humans (van der Oost et al. 1997). Benzo[a]pyrene (B[a]P) is considered an indicator of such contamination because it usually occurs in mixtures of PAHs, and is a potentially toxic contaminant and an potent carcinogen. PAHs are widely distributed in the sediments of aquatic environments. It has been widely demonstrated in the laboratory and the field that sediment is a source of pollutants for marine organisms via water and the food web (Boleas et al. 1998). Marine fish readily take up lipophilic organic contaminants such as B[a]P from the marine environment, with a variety of physiological effects (Walker and Livingstone 1992). However, B[a]P acts as a genotoxicants in marine organisms, which are the biochemical and molecular toxic effects most studied in fish, and relatively few studies have been conducted on the physiological effects of dietary B[a]P.

The measurement of biochemical and physiological parameters is a diagnostic tool commonly used in aquatic toxicology and biomonitoring. Hematological parameters are more often used when clinical diagnoses of fish physiology are used to determine subchronic concentrations of pollutants (Wedemeyer and Yasutake 1977). Changes in serum asparate aminotransferase (AST), alanine aminotransferase (ALT), and lactate dehydrogenase (LDH) enzyme activities fish have been used frequently as indicators of intoxication and water pollution (Nelson and Cox 2000). However, little information is available about their application to the assessment of B[a]P exposure.

The rockfish *Sebaste schlegeli* is an economically important food fish in Korea, which is commonly cultured in marine-based cages. Despite its importance, relatively little information is available on the effect of B[a]P on the fish, particularly through dietary exposure. Therefore, the aims of present study were to evaluate the effects of subchronic dietary B[a]P exposure on the growth and to estimate changes in the hematological parameters of juvenile rockfish.

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Materials and Methods

The fish food was supplemented with 0 (control, solvent control), 0.5, 1.0, 1.5 or 2.0 mg B[a]P/kg feed of B[a]P ($C_{20}H_{12}$, ≥97%, Aldrich). It was dissolved in a small amount of acetone and mixed well with the other food ingredients before pelleting. All ingredients were mixed with distilled water, and were pelleted with a laboratory pellet machine without heating using a 2 mm diameter module. After processing, the food was packed into small bags and stored at −20°C until it was fed to the fish. Proximate analysis of the food indicated: crude protein 48%, crude lipid 4%, carbohydrate 4%, ash 15%, calcium 1% and phosphorous 1%.

Juvenile rockfish (*S. schlegeli*) were obtained from a rockfish nursery in Go-Seong Gun, Korea. The rockfish were acclimated in a 1,000 L aerated running seawater tank for 1 month under laboratory conditions (temperature, 16.6 ± 0.6°C; salinity, 32.2 ± 0.5‰). The fish were fed B[a]P-free pellets daily at a rate of 2% body weight per day during acclimation. After 1 month in the acclimating tanks, the fish were randomly transferred to 150 L tanks with running water (flow rate = 1.2 L/min) and continuous aeration. After transfer to the exposure tanks, the rockfish were acclimated to the experimental conditions. Fish with a total length of 11.1 ± 0.03 cm (n = 360), and a weight of 21.6 ± 0.21 g were selected for the exposure to dietary B[a]P experiment. The control group was fed B[a]P-free pellets and the solvent group was fed acetone-supplemented pellets. The experimental fish were maintained and tested under a 12 h light (0900–2100)/12 h dark illumination cycle.

Ten fish were removed from each tank every 10 days during the 30 days of the experiment. The fish were starved for 24 h before sampling to allow all the food to be excreted. The wet weight and total length of the fish and the wet weight of the liver were recorded for each individual. Blood samples were obtained with a heparinized syringe from the caudal vein of the fish. Hematocrit (Ht) values were determined after blood centrifugation (5 min, 2,000 × *g*) in glass capillaries, using a microhematocrit centrifuge and a micro-hematocrit reader (Hawksley & Sons, England). Hemoglobin (Hb) concentrations in the blood were measured with the cyano-methemoglobin method using a hemoglobin kit (Sigma, no. 525A). The red blood cell (RBC) count was determined in a 1:200 dilution of the blood sample in Hendrick's solution with a hemacytometer (Marienfeld, Germany). The remaining blood was centrifuged (1000 × *g* for 10 min at 4°C), and the serum was frozen (−20°C) until required for analysis. Frozen serum samples were analyzed for total protein, Ca (Sigma Diagnostic Kits no. 587-100P), and Mg (Sigma Diagnostic Kits no. 595-A). Enzyme activities in the serum were measured with a temperature-controlled spectrophotometer (DR/4000U, Germany). The assays were run in duplicate or triplicate. The activities of the enzymes AST and ALT were measured according to Reitman and Frankel (1957), and LDH was measured according to the method of the German Society for Clinical Chemistry (1972). Statistics were performed with SPSS (SPSS Inc., Chicago, USA), using one-way analysis of variance (ANOVA) followed by Duncan's multiple comparisons test of mean values if significant differences were found ($p < 0.05$). In brief, growth rate (GR) = [(end mass − start mass)/start mass] × 100, hepatic-somatic Index (HSI) = (liver wet weight/body wet weight) × 100, and condition factor (CF) = [wet weight/(total length)^3] × 100.

Results and Discussion

No mortality occurred during the subchronic exposure to dietary B[a]P in the experimental periods. The change in the growth and hematological parameters of rockfish exposed to dietary B[a]P are presented in Table 1. In this study, dietary B[a]P exposure resulted in a reduction in the rockfish weight growth, and an inverse relationship was observed between growth rate and the dietary B[a]P concentration. Moles et al. (1987) suggested that the slower growth of salmon exposed to oil was mainly attributable to the energy deficiency caused by the need to metabolize and detoxify PAH. Jee et al. (2004) showed an adverse exposure-dependent effect on the growth of juvenile olive flounder exposed to waterborne phenanthrene (1.0 and 2.0 μM) for 4 weeks. Heintz et al. (2000) showed that sublethal oil exposure affected the growth of pink salmon, with concentrations of 18.0–48.0 ppb significantly retarding their growth. This could be attributable to DNA damage impairing gene regulation, diverting energy to incipient carcinomas, or impairing the activity of the enzymes responsible for modulating growth or foraging. Toxicants interfere with energy-yielding reactions indirectly inhibiting the synthesis of RNA, DNA, and protein (Holbrook 1994). Thus, the reduced growth rate in the rockfish was probably the result of (a) an increased expenditure of energy to sustain their metabolism, allowing less energy for growth; (b) the metabolic costs associated with B[a]P detoxification.

Hematological parameters are used more often when clinical diagnosis of fish physiology is used to determine subchronic concentrations of pollutants (Wedemeyer and Yasutake 1977). The present findings indicate that subchronic dietary B[a]P exposure causes a significant reduction in hemoglobin and hematocrit in the rockfish. Similar results were observed in common carp exposed to nonylphenol and ethinylestradiol (Schwaiger et al. 2000)

Table 1 Changes in the growth and hematological parameters of *Sebastes schlegeli* exposed to dietary benzo[a]pyrene for 30 days (mean ± SEM)

Day	Treatment	HSI (%)	Weight GR (%/day)	Hematocrit (%)	Hemoglobin (g/L)	RBC count ($\times 10^4$ mm^{-3})	T-P (g/dL)	Mg (mg/dL)	Ca (mg/dL)	AST[a]	ALT[a]	LDH[b]
10 days	Control	3.1 ± 0.1^{a}	10.1 ± 3.9^{a}	35.4 ± 0.7^{a}	8.6 ± 0.5^{a}	234 ± 12.2^{a}	3.2 ± 0.3^{a}	2.4 ± 0.2^{a}	11.2 ± 0.2^{a}	12.4 ± 1.3^{a}	3.5 ± 0.9^{a}	3.4 ± 0.9^{a}
	Solvent	3.1 ± 0.1^{a}	11.9 ± 2.7^{a}	35.8 ± 0.2^{a}	8.2 ± 0.4^{a}	236 ± 3.4^{a}	3.1 ± 0.2^{a}	2.8 ± 0.2^{a}	12.2 ± 0.4^{a}	13.8 ± 2.5^{a}	3.1 ± 0.4^{a}	4.0 ± 0.6^{a}
	0.5 mg/kg	3.4 ± 0.4^{a}	9.5 ± 2.9^{a}	36.3 ± 1.5^{a}	8.7 ± 0.5^{a}	232 ± 11.2^{a}	3.0 ± 0.2^{a}	2.7 ± 0.1^{a}	12.6 ± 0.4^{a}	12.7 ± 2.7^{a}	3.8 ± 0.9^{a}	3.3 ± 0.9^{a}
	1.0 mg/kg	3.4 ± 0.2^{a}	8.2 ± 2.5^{a}	33.4 ± 0.8^{a}	8.3 ± 0.4^{a}	211 ± 5.1^{ab}	3.0 ± 0.1^{a}	2.7 ± 0.1^{a}	11.5 ± 0.5^{a}	13.9 ± 2.7^{a}	3.7 ± 1.3^{a}	4.0 ± 0.1^{a}
	1.5 mg/kg	3.3 ± 0.1^{a}	8.3 ± 2.6^{a}	33.5 ± 1.7^{a}	7.8 ± 0.1^{a}	186 ± 14.2^{b}	2.3 ± 0.2^{a}	2.8 ± 0.2^{a}	11.7 ± 0.6^{a}	14.5 ± 2.3^{a}	3.3 ± 1.8^{a}	3.1 ± 0.7^{a}
	2.0 mg/kg	3.4 ± 0.2^{a}	7.2 ± 2.3^{a}	29.8 ± 1.7^{b}	8.0 ± 0.3^{a}	196 ± 6.2^{b}	3.3 ± 0.1^{a}	3.1 ± 0.2^{a}	12.2 ± 0.5^{a}	13.5 ± 3.2^{a}	3.6 ± 1.2^{a}	4.2 ± 0.7^{a}
20 days	Control	3.2 ± 0.1^{a}	20.9 ± 2.3^{a}	34.8 ± 0.5^{a}	8.6 ± 0.2^{a}	233 ± 5.9^{a}	3.3 ± 0.1^{a}	2.6 ± 0.2^{a}	11.6 ± 0.2^{a}	11.2 ± 2.3^{a}	4.1 ± 0.8^{a}	4.2 ± 0.8^{a}
	Solvent	3.2 ± 0.2^{a}	17.8 ± 2.2^{ab}	35.1 ± 0.8^{a}	8.0 ± 0.1^{ab}	225 ± 5.4^{a}	3.8 ± 0.3^{ab}	2.4 ± 0.1^{a}	11.9 ± 0.6^{a}	13.5 ± 3.5^{a}	3.8 ± 0.5^{a}	4.8 ± 2.4^{a}
	0.5 mg/kg	3.4 ± 0.1^{a}	12.2 ± 3.2^{b}	34.6 ± 1.4^{a}	8.7 ± 0.2^{a}	219 ± 8.9^{ab}	3.7 ± 0.1^{ab}	2.5 ± 0.4^{a}	12.7 ± 0.4^{a}	10.9 ± 1.3^{a}	3.8 ± 0.3^{a}	4.9 ± 0.9^{a}
	1.0 mg/kg	3.2 ± 0.2^{a}	12.3 ± 3.9^{b}	33.2 ± 0.7^{ab}	8.7 ± 0.3^{a}	215 ± 1.9^{ab}	3.5 ± 0.2^{ab}	2.8 ± 0.3^{a}	12.8 ± 0.4^{a}	15.4 ± 2.8^{a}	3.4 ± 0.5^{a}	4.8 ± 0.3^{a}
	1.5 mg/kg	3.3 ± 0.2^{a}	11.7 ± 2.4^{b}	35.2 ± 0.7^{a}	8.4 ± 0.4^{ab}	209 ± 5.3^{b}	3.9 ± 0.3^{ab}	2.4 ± 0.2^{a}	13.1 ± 0.6^{a}	15.7 ± 2.2^{a}	3.3 ± 0.3^{a}	4.7 ± 1.5^{a}
	2.0 mg/kg	3.2 ± 0.1^{a}	3.5 ± 0.6^{c}	31.7 ± 0.3^{b}	7.8 ± 0.1^{b}	206 ± 7.8^{b}	4.2 ± 0.3^{b}	2.5 ± 0.2^{a}	13.3 ± 0.9^{a}	16.4 ± 1.0^{a}	3.7 ± 0.5^{a}	6.4 ± 0.9^{a}
30 days	Control	2.9 ± 0.1^{a}	20.9 ± 2.2^{a}	34.2 ± 0.7^{a}	8.8 ± 0.1^{a}	236 ± 9.9^{a}	3.9 ± 0.4^{a}	2.8 ± 0.2^{a}	11.7 ± 0.4^{a}	13.1 ± 1.3^{a}	3.7 ± 0.3^{a}	4.7 ± 0.5^{a}
	Solvent	2.9 ± 0.1^{a}	14.0 ± 2.6^{ab}	33.0 ± 2.0^{a}	8.5 ± 0.7^{a}	228 ± 9.0^{ab}	3.6 ± 0.3^{a}	2.9 ± 0.3^{a}	11.8 ± 0.4^{a}	16.0 ± 1.9^{ab}	3.8 ± 0.6^{a}	4.4 ± 0.1^{a}
	0.5 mg/kg	3.2 ± 0.2^{a}	11.9 ± 4.0^{b}	36.6 ± 0.9^{a}	8.6 ± 0.5^{a}	228 ± 4.5^{ab}	3.9 ± 0.2^{a}	2.9 ± 0.2^{a}	12.1 ± 0.1^{a}	19.1 ± 2.2^{bc}	3.6 ± 0.9^{a}	6.9 ± 0.6^{b}
	1.0 mg/kg	3.1 ± 0.2^{a}	11.8 ± 4.7^{b}	35.2 ± 1.0^{a}	8.2 ± 0.2^{a}	222 ± 9.6^{ab}	3.7 ± 0.5^{a}	2.7 ± 0.2^{a}	11.3 ± 0.5^{a}	20.2 ± 0.7^{bc}	3.7 ± 0.1^{a}	8.7 ± 0.1^{c}
	1.5 mg/kg	3.0 ± 0.1^{a}	12.5 ± 2.3^{b}	35.7 ± 2.4^{a}	8.8 ± 0.4^{a}	206 ± 3.1^{b}	3.8 ± 0.4^{a}	2.9 ± 0.4^{a}	12.2 ± 0.3^{a}	21.4 ± 2.0^{c}	3.8 ± 0.4^{a}	9.7 ± 0.3^{c}
	2.0 mg/kg	3.3 ± 0.2^{a}	3.8 ± 0.5^{c}	27.3 ± 2.7^{b}	6.9 ± 0.1^{b}	206 ± 3.5^{b}	3.7 ± 0.5^{a}	2.9 ± 0.3^{a}	12.0 ± 1.4^{a}	21.5 ± 0.9^{c}	5.7 ± 1.7^{a}	8.6 ± 1.1^{c}

Values in columns with the same superscripts are not significantly different ($p < 0.05$)

[a] Karmen units

[b] Wroblewski units

and rainbow trout exposed to cadmium (Haux and Larsson 1984), with depressed Hb and Ht values. Anemia with reduced Ht and Hb is one of the common and most sensitive responses to toxicants in higher vertebrates and several fish species. Although the mechanism of this induced anemia is not clear, anemia results from a shortened erythrocyte lifespan and the impairment of heme synthesis (Haux and Larsson 1984). Gill and Epple (1993) suggested that the anemia observed in toxicant-exposed fish may be attributable to the inhibition of iron absorption, defective iron metabolism, shortened erythrocyte lifespan, and/or impaired erythropoiesis. Although respiratory impairment or iron metabolism defects arising from dietary B[a]P were not evident in the present study, B[a]P probably induced anemia with reduced Ht and Hb in the rockfish.

Generally, total serum protein is a potential indicator of changes in plasma volume reflecting changes in the plasma. Reduced serum protein in exposed fish can indicate possible nutritional imbalance, infectious disease, kidney damage, or inanition (Wedemeyer and Yasutake 1977). Although some studies have shown a reduction in total protein content during pollutant exposure, our findings indicate that subchronic dietary B[a]P exposure had no significant effect in the rockfish on total protein, Mg or Ca. This normal serum chemistry suggests that there was no osmotic disturbance during dietary B[a]P exposure.

Serum AST and ALT are important diagnostic tools in medicine and are used to detect the toxic effects of various pollutants (Nelson and Cox 2000). Our results show that dietary B[a]P significantly increased the activity of AST in the serum of rockfish after 30 days, at all the concentrations examined. However, ALT activity showed no significant variations in rockfish exposed to the lowest or highest level of B[a]P. Bálint et al. (1997) showed that serum AST and ALT increased in eels (*Anguilla anguilla*) exposed to the insecticide deltamethrin by 20% and 100%, respectively. Kwon and Chang (1996) demonstrated that both AST and ALT increased when black sea bream (*Acanthopagrus-schlegeli*) were exposed to high levels of ammonia, and both returned to their normal status during the recovery period. Vaglio and Landriscina (1999) also suggested that because the liver is rich in AST and ALT, damage to it could result in the liberation of large quantities of these enzymes into the blood. Therefore, increases in AST activity in the serum of the rockfish, are assumed to be the result of liver damage by B[a]P.

LDH is involved in cellular respiration and the production of the high-energy compound adenosine triphosphate (ATP) from glucose to lactic acid (Nelson and Cox 2000). LDH is a cytoplasmic enzyme present in numerous tissues, and an increase in serum LDH indicates cell lysis (Lemaire et al. 1991). Long et al. (2003) observed a significant increase in LDH activity in mussels exposed to PAH for four month, and suggested that LDH activity is a sensitive biomarker of petroleum hydrocarbon exposure. In the present study, the LDH activity in the serum of the rockfish increased at all the B[a]P concentrations tested. Therefore, an increase in the serum LDH may point to dysfunction of the injured tissue induced by B[a]P exposure.

In conclusion, we observed growth inhibition in the B[a]P-exposed groups and an inverse relationship between growth rate and the dietary B[a]P concentration. Dietary B[a]P exposure results in anemia and increased serum AST and LDH concentrations in the rockfish. These results indicate some damages to the blood-forming functions and the disruption of blood homeostasis in the rockfish during chronic exposed to B[a]P. Physiological stress indicators, such as some hematological parameters, could be useful in evaluating the effects of B[a]P in fish, but the application of these results to environmental diagnoses requires a more detailed investigation and should be evaluated in situ before these parameters are used as indicators. Future experiments should investigate not only changes in physiological parameters but also the bioavailability of the toxicant, which is important in determining the extent of toxicant toxicity in fish.

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