P-16

Effects of dietary benzo(a)pyrene exposure on hematological index and detoxification enzymes of the Juvenile Rockfish, *Sebastes schlegeli*

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Benzo(a)pyrene (BaP) is a ubiquitous envrionmental pollutant that is a product of incomplete combustion of fossil fuels. It has been identified in suface water, tap water, rain water, groundwater, waste water and sewage sludge. Also, BaP is a priority PAHs, is a representative ecotoxicant and has been reported bioaccumulative potential in many organisms resulting in endocrine disruption, reproductive disturbance and DNA damage. Metabolic activation may generate reactive oxygen species (ROS), including singlet oxygen, hydrogen peroxide, and superoxide anion radicals (Leadon et la., 1988). BaP generate a substantial amount of ROS during their metabolism. In addition, some BaP metabolites are capable of producing ROS. For example, the 6-oxo-BaP radical can produce BaP diones (Lorentzen and Ts'o, 1977), which can enter a redox cycle between diones and diols, producing ROS as a byproduct (Cerutti et al., 1978). The BaP-7,8-dione also generate ROS in the presence of NADPH and $CuCl_2$.

This study was conducted to assess the effect of dietary BaP on the hematological parameters and detoxification enzymes (Phase II) of juvenile rockfish, *Sebates schlegeli*. The time-dependent changes of BaP levels were also examined.

Juvenile Rock fish (11.1±0.03 ㎝, 21.6±0.2 g) were obtained from a fish farm in Gyeongnam. After acclimatization, the fish were separated into four experimental lots with 10 animals each. Diets were supplemented with 0, 0.5, 1 and 2 ㎎ BaP kg^{-1} feed of BaP (3 ㎕ of 95% ethanol; Aldrich Chem. Com. Inc.) to investigate the hematological fluctuation and phase II enzyme activities. After 15 and 30 days exposure, fish were anesthetized and examined. Blood sample were centrifuged at 3000 g for 15min at 4℃. Hematological parameters include the total number of erythrocytes, haemoglobin concentration, haematocrit, chloride, phosphate, magnesium, calcium, tatal protein, glucose, total cholesterol, GOT,GPT and LDH. Their assay were perfomed using a diagnostic kit (Asan Pharm, Co., Ltd).

Hepatic microsome were prepared by homogenizing in buffer with several passess of a teflon pestle (099C K4424, Glas-Col, USA). The hemogenate was centrifuged (9,000 g for 20min, MIKRO22R, Hettich, Germany) at 4℃, supernatant was collected, and ultracentrifuged at 100,500g for 60 min using a Centrikon T-1190 Ultracentrifuge (Kontron Instruments, Italy) at 4℃ to obtain a cytosol. Glutathione (GSH) was determined according to Sen et al. (1992)The activity of glutathione reductase (GR) was assayed by the method of Carlberg and Mannervik (1985). Glutathione peroxidase (GPx) was assayed by the method of Mohandas et al. (1984). The cytosolic glutathione S-transferase (GST) activity was analyzed by the kinetic method of Habig et al. (1974) with CDNB (1-chloro-2,4-dinitrobenzene) as the substrate.

BaP has been shown to cause the flucation of haematological index include RBC count ($\times 10^4$ mm_3^{-1}), Ht (%) level and Hb (g dL^{-1}) concentration by the increase of BaP concentration. There was no significant difference in serum level of phsphate (mM) as compared to control group. However, Mg (mM), Ca (mM), Choride (mM), glucose, GOT, GPT (KU) and LDH (WU) significantly increased in 2.0 mg kg^{-1} ($P<0.05$). Serum total protein (g dL^{-1}) and total cholesterol (mg dL^{-1}) level were significant decreased in 2.0 mg kg^{-1} after 15 and 30 days exposure. The activity of antioxidant enzymes, GST, GR, GPx and GSH was increased in 2.0 mg kg^{-1} at 15 days. But, GPx and GSH activities decreased compared to control after 30 days of exposure ($P>0.05$). Although the endocrine effects of BaP were not statistically significant in all of the parameters measured, the discriminant analysis showed an overall trend of endocrine and metabbolic changes due to extended BaP exposure.

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