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Reduced-glutathione concentrations in *Boleophthalmus pectinirostris* tissues exposed to benzo(a)pyrene

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Abstract: The concentrations of reduced-glutathione (GSH) in liver and ovary of *Boleophthalmus pectinirostris* are quantified. The concentrations of GSH in the ovary are much higher than that of GSH in the liver(nearly 3 times of the liver). The study also investigates the changes of GSH contents in the two organs while the fishes were exposed to benzo(a)pyrene(BaP) at concentrations of 0, 0.05, 0.2 and 0.5 mg/L respectively for up to a week. The concentrations of GSH in the liver of BaP-exposed fish increased significantly with dose, whereas the concentrations of GSH in the ovary decreased significantly compared to controls. The results suggested that both the liver and the ovary are the primary organ in BaP metabolism, and that the changes of GSH levels may represent an adaptive response or toxic effect to BaP exposure.
Key words: *Boleophthalmus pectinirostris*; benzo(a)pyrene; reduced-glutathione

Introduction

Being a typical polycyclic aromatic hydrocarbons(PAHs), benzo[a]pyrene(BaP) deserves serious study because PAHs are now recognized as major environmental pollutants. Exposure to PaHs is virtually unavoidable and is strongly suspended of being a causative factor in certain tumors in aquatic species(Kraybill, 1977; Martin, 1984; 1985) and cancer in humans (Harvey, 1982; Theriaut, 1984).

It is becoming apparent that PAHs, especially BaP, function through free-radical or related oxidant-mediated reactions (Bus, 1979; Mason, 1982). Reduced-glutathione(GSH) is an essential nonprotein antioxidant that acts either directly as are reductant or as a substarate for enzymes such as glutathione peroxidase and glutathione transferases. GSH ensures the reduction of oxidants, the quenching of free radicals, the neutralization of organic peroxides, and elimination of hydrocarbons by conjugation. So it can protect against oxidative stress resulting from redox reactions.

Teleosts, similar to mammals, possess high concentrations of GSH in their tissues. Liver is the most important organ in the metabolism of xenobiotics, whereas avary is an organ which have great effect on the healthy of offspring. Pollutants exposure have been shown to alter GSH status of fish in recent laboratory or field studies. But most of the research has been focus on the liver of fishes, whereas research on ovary has received reactively little attention.

The present study investigated the concentrations of GSH in the liver and the ovary of *Boleophthalmus pectinirostris* and the alterations of GSH level in the two organs while exposed to BaP at different concentrations. The main aim is to explore the toxic mechanism of BaP in this fish and investigated the possibility and organ-speciality of using GSH as environmental biomarker.

1 Material and methods

1.1 Chemicals

Analytical grade BaP was obtained from Sigma Chemical Co. Analytical grade reduced-glutathione(GSH) was obtained from Biochemical Reagent Co. of Shanghai. All other reagents were of analytical or higher grades.

1.2 Animals

Boleophthalmus pectinitostris(weight 12 - 18g; length 11 ± 5 cm) were collected from Fuqing Sea areas in Fujian Province. They were acclimated to laboratory conditions in tanks for several days prior to exposure to BaP and were fed algae. The tanks were supplied with clean sea water(40L, temperature 20 ± 2℃). Fish placed in the tank exhibited no signs of stress or physical damage due to confinement.

1.3 Experimental design and sample preparation

Boleophthalmus pectinirostris were exposed to BaP at concentration of 0, 0.05, 0.2 and 0.5 mg/L respectively for week. The carrier solvent for BaP was acetone(0.5 mg BaP/ml acetone). The same volume of acetone was added to the control aquaria(1 ml acetone/L sea water). There were two tanks for each concentration and 4 fishes for each tank. The water in tanks was replaced every day in the same concentration as before and was aerated. Livers and ovaries were dissected and

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frozen in liquid nitrogen at the end of the experiment. Six fishes were collected before BaP exposure.

The homogenized samples were centrifuged at 15000 r/min for 20 min at 4℃ on a Beckman J2 – MC centrifuge. The supernant was used for measurement of GSH.

1.4 Measurement of GSH

GSH was analysed according to Cohn and Lyle, with O-phthalaldehyde (OPT) as fluorescent reagent. 100 μl OPT and 100 μl sample were added to 2.8 ml of 0.1 mol/L sodium phosphate buffer, pH 8.0, containing 0.005 mol/L EDTA. After 15 min incubation at room temperature, fluorescence was measured on a Hitachi 850 spectrofluorometer, with excitation at 350 nm and emission at 420 nm. Each time, a standard curve with known amounts GSH was measured. GSH content was expressed as μg GSH/g tissue.

1.5 Statistics

All values quoted are mean ± *SD*. Differences between two means were determined with Student's *t* test. Comparisons of multiple means were done with analysis of variance(ANOVA). The level of statistical significance was set at $P = 0.05$.

2 Results

2.1 Difference of GSH level in the liver and the ovary

The GSH contents in the liver and the ovary are 75.85 μg/g and 234.12 μg/g respectively. The results of comparing the two means with Student's *t* test show the significantly difference($P < 0.05$). The GSH contents in the ovary are much higher than those in liver(nearly 3.1 times of the liver).

2.2 Effects of BaP exposure on GSH content in the liver

After the fishes were exposed to BaP at concentrations of 0, 0.05, 0.2 and 0.5 mg/L respectively for a week, the results with analysis of variance (ANOVA) in the liver showed that the GSH contents in exposure groups are significantly difference with those of control($P < 0.05$). GSH content elevated with the increasing of BaP concentrations(Fig.1). Analysis between exposure group and control with Student's *t* test indicted that GSH content in each of the three exposure group significantly differed from that of control($P < 0.05$).

2.3 Effects of BaP exposure on GSH content in the ovary

After the dishes were exposed to BaP at concentrations of 0, 0.05, 0.2 and 0.5 mg/L respectively for a week, the results with analysis of variance(ANOVA) in the ovary showed that the GSH contents in exposure groups are significantly difference with those of control($P < 0.05$). GSH content decreased gradual with the increasing of BaP concentrations(Fig. 2). Analysis between exposure group and control with Student's *t* test indicated that only the GSH content in 0.5 mg/L exposure group significantly differed from that of control($P < 0.05$).

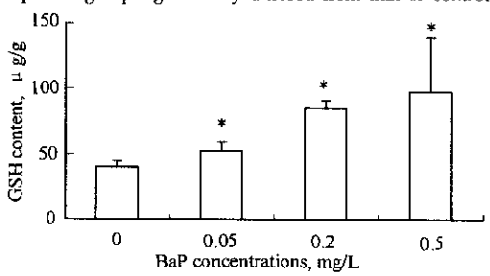

Fig.1 Effect of BaP in different concentrations on content of GSH in the liver of *Bolephthalmus pectinirostris* ($n = 6$)

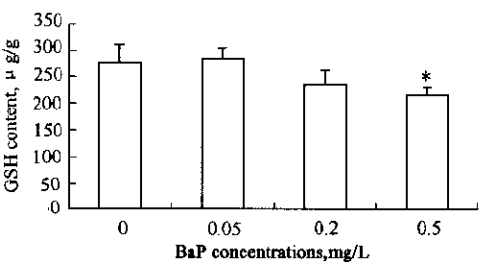

Fig.2 Effect of BaP in different concentrations on content of GSH in the ovary of *Boleophthalmus pectinirostris* ($n = 3 - 4$)

3 Discussion

GSH participates in a number of fundamental cellular processes, including the synthesis of proteins, transport of amino acids, enzyme activity and cell defense against a variety of internal and external stressors(Meister, 1983). Therefore, alterations in the level of GSH can be expected to give rise to a variety of functional changes, related either directly or indirectly to contaminant toxicity.

The results from this study provide evidence that although the liver is the primary organ in metabolism of BaP, the ovary might function in BaP metabolism for its much higher level of GSH than those in liver. The underlying mechanisms responsible for the high level of GSH in the ovary are not identified yet.

Recent studies with fish have shown that the levels of hepatic GSH are responsive to exposure to contaminated sediment, pulp mill effluent, and 0.2 fuel oil(Lindstrom-Seppa, 1990; Thomas, 1984). In the present study, elevated concentrations of hepatic GSH and decreased concentrations of ovarian GSH were observed in *Boleophthalmus pectinirostris* exposed to BaP at different concentrations compared to control, which indicate that the changes in GSH levels were a specific response to contaminant exposure.

The increased levels of GSH may represent an adaptive response to contaminant exposure (Kosower, 1978), whereas decreased levels of GSH have been related to a saturation of the cellular capacity to detoxicate reactive xenobiotic compounds or metabolites or a toxic effect to contaminant exposure(Ketterer, 1983). In our study, the increase of hepatic GSH levels and the decrease of ovarian GSH levels might indicate that the toxic effect has been produced in the ovary while an adaptive response is only exited in the liver. GSH levels in the liver were nearly the same in two of the higher concentration groups (0.5 mg/L and 0.2 mg/L, $P > 0.05$), which might indicate the saturation of the cellular detoxifying capacity with the increasing of BaP concentrations. Presently, it is unknown whether the increase in GSH levels is solely an adaptive response to contaminant exposure. Although further studies are needed to delineate the consequences of alterated GSH in fish, the results of the present study suggest that GSH represents a promising biomarker. Moreover, due to the role of GSH in the defense against toxicity of reactive oxygen species, alterations in GSH levels may be a sensitive biomarker of exposure to contaminants that perturb the flux of oxyradicals in fish.

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