Dev. Reprod. Vol. 4, No. 2 (2001)

Histological Observation of Gonadal Differentiation and Development in SONGSARI, *ORYZIAS LATIPES* Exposed to Bisphenol A

OH SOO NA [1], YOUNG DON LEE[1], HAE JA BAEK[2] and HYUNG BAE KIM[3]
[1] Marine Research Institute, Cheju National University
[2] National Fisheries Research and Development Institute
[3] Department of Fisheries Development, Kangwon Province University

Introduction

A variety of synthetic compounds have been shown to mimic steroid hormones. Among these compounds, bisphenol A (BPA) has been identified as endocrine disruting chemical. BPA is widely used as the primary products of polycarbonated plastic and epoxy resins. The polycarbonates are used in the drink packs and earthen vessels, and the epoxy resins are used in the coating of cans, bottles, and water pipes. BPA released to the environment is generally dissolved in water and may be partly adsorbed to the soil and sediment. BPA has been reported to exert estrogenic activity in mammalian. In the male offspring of female wistar rats exposed to BPA, evaluation of reproductive organ development has been reported .The environmental toxicity of BPA has been investigated by numerous researchers and these data have been reviewed by Staples et al. (1998). However, only a few reports concerning the effects of BPA on gonadal differentiation and development in fish. Songsari (*Oryzias latipes*) is an ideal test organism for studies on the estrogenic effects of endocrine disruptors because songsari is characterized as a differentiation gonochorist and has no spontaneous intersex. Therefore, this study was conducted to evaluate the gonadal differentiation and development of the songsari exposed to BPA from newly hatched larvae to 70 days after hatching.

Materials and Methods

Chemical

The BPA was obtained from Aldrich Chemical Co. Inc., Milwaukee, Wisconsin, USA. A stock solution of 100 mg/mL was prepared by dissolving BPA in acetone.

Fish

Songsari used in this study were collected from an uncontaminated stream and transported to Marine Research Institute of Cheju National University (Korea). Breeding fish were maintained in recirculating Jeju Island ground water for over 3 months. During breeding, the fish (total length, 2.6±0.6 cm; body weight 0.21±0.1 g) were controlled to a light:dark photoperiod of 16:8 h and fed daily with commercial diet (Ewha oils & Fat Industry Co. Ltd., Korea). Water temperature of the breeding aquaria ranged from 22 to 24℃. Some water plants were put in the aquaria to attach the spawned eggs. Spawned eggs were collected from the aquaria, incubated in 10 L glass container, and checked for hatching.

Experimental conditions

In a chronic experiment, newly hatched larvae of songsari were exposed to nominal concentrations of BPA 50, 100, 200 μg/L in a static renewal system. BPA treatments took place in 1 L glass beakers filled with 900 mL of filtered Jeju Island ground water. The quality parameters of the ground water were pH 8.1 and COD 0.8 mg/L. The aqueous solutions of BPA were renewed every 72 h for the first month and every 48h thereafter, according to the methods described by Gray and Metcalfe (1997). There were five treatment groups, each with 40 fish in duplicate (total n= 400) at the start of the experiment. Nominal BPA concentration of 50, 100, 200 μg/L were maintained by adding appropriate volumes (4.5, 9.0, and 18.0 μℓ) of BPA stock solution to the water in the aquaria. In the acetone control treatment, acetone alone only the pure ground water was supplied dark photoperiod of 16:8 h and fed the commercial diet of three times

daily for the duration of the experiment.

Histology

The fish were then placed in tissue bottles and fixed in Bouins fixative. The fixed fish were prepared for histological examination. The fish were embedded whole in paraffin and sectioned (5 ㎛) with a microtome. The sections were stained using Hansens hematoxylin and 0.5% eosin and examined under a light microscope. Sagittal sections of the gonads were viewed with a microscope monitor system. We calculated the proportion of the sagittal sectional area of the gonad occupied by each germ cell type. The sex of quite a few fish could not be determined because the gonad was not sectioned properly during histological preparation.

Statistics

The data on sex ratios distinguished from the gonads were assessed by chi-square analysis. Differences were considered to be significant at the $P \leq 0.05$ level.

Results

Gonadal differentiation and development

Just after hatching: 10 individuals were examined. Average body length of these individuals was 4.4 mm. The germinal strand was located. Between the myotome and the gut gonia cells were visible in the posterior region of the primitive gonad. The gonia cells were nearly ovoid shape and average diameter of these cells was 8 ㎛.

20 days after hatching: In the female, the ovaries were composed of the oocytes at the chromatin-nucleolus stage and peri-nucleolus stage. Average diameter of the oocytes of the chromatin-nucleolus stage was 19 ㎛; and the oocytes of the peri-nucleolus was 25 ㎛ In the male, the testis contained gonia cells alone. The gonia cells were nearly round or ovoid and average diameter of these cells was 7~8 ㎛.

30 days after hatching: The ovaries were composed of the oocytes at the chromatin stage and peri-nucleolus stage increased in numbers. In the testis, a number of spermatogonia and spermatocytes were observed in the testicular lobule. In the process of the sex differentiation, gonadal development was not different in the controls and BPA treatment groups until 30 days after hatching.

70 days after hatching: In the controls, the ovaries were composed of the oocytes of the chromatin-nucleolus, peri-nucleolus and yolk vesicle stage. Otherwise in the BPA treatment groups, the ovaries were composed of the oocytes of the chromatin-nucleolus, peri-nucleolus, yolk vesicle, yolk globule and mature stag.

Sex ratio

More females than males were identified in the BPA 50 ㎍/L and 100㎍/L treatments in comparison to the controls and BPA 200㎍/L treatment. Fry of medaka exposed to 4.0 and 29.4 ㎍/L 17β-estradiol both exhibited 53% testis-ova or presumptive hermaphroditism, approximately 40% female and 5% male in each dose group.

Discussion

The developmental stage of the oocyte came to be a yolk vesicle stage in the controls and to be vitellogenic stage in the BPA treatment groups. Thus advanced development of oocytes in the ovary was observed from BPA treatment groups when compared to the controls. In sex differentiation of fishes, estradiol-17β also advanced ovarian development in comparison with untreated females as observed in coho salmon and in pejerrey (Strüssmann et al., 1996).

In these results, the testes of male songsari showed several morphologic changes, including less of the testicular structure with lobules clearly separated from each other. And inhibition of the development of spermatogenesis in the testis was observed in BPA treatment groups when compared to the controls Nonylphenol and estradiol-17β have severe effects on the testis and Sertoli cells in the eelpout. Estrogen inhibits the differentiation of Leydig cells, Sertoli cells and early formation of the spermatic duct in the European eel. In sexually developing fish, the pronounced effects on vitellogenin synthesis caused by exposure to various estrogenic chemicals were accompanied by concomitant significant decreases in testicular growth (Jobling et al., 1996).

Gray and Metcalfe (1997) reported that fry of medaka exposed to 100 ㎍/L of *p*-nonylphenol (NP) induced both the intersex state (i.e., testis-ova) in males as well as sex reversal (i.e., male to female), while exposure to a lower

concentration of NP (50μg/L) induced only testis-ova. But present histological analysis of 205 fishes uncovered an intersex individual.

The results indicated that BPA exposed fish enhances ovary development and deeply slows the development of testes. The mechanism underlying advancement of ovarian development and inhibition of testicular growth by BPA is unknown.

References

Gray MA and Metcalfe CD (1997) *Environ toxicol Chem* 16: 1082-1086.

Jobling S, Sheathan D, Osborne JA and Sumpter JP (1996) *Environ Toxicol Chem* 15:194-202.

Staples CA, Dorn PB, Klecka Gm, OBlock ST, Harris LR (1998) *Chemosphere* 36: 2149-2173.

Strüssmann CA, Takashima T, and Toda K (1996) *Aquaculture* 139: 31-45.