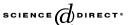


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Short communication

Anti-androgen flutamide affects gonadal development and reproduction in medaka (*Oryzias latipes*)

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Abstract

The effects of an anti-androgenic chemical on the reproduction of medaka (*Oryzias latipes*) were examined. Six mating pairs of medaka were exposed to flutamide (FLU) at measured concentrations of 0.101, 0.202, 0.397, 0.787, or 1.56 mg/L for 3 weeks. During the exposure period, one female died in the 1.56-mg/L treatment group, but no lesions or pathological abnormalities were observed. The fecundity and fertility of paired medaka were significantly decreased at 1.56 mg/L compared with those of the controls. Histological examination showed intersex gonads (testis-ova) in males exposed to FLU at 0.202, 0.397, and 0.787 mg/L. However, concentrations of hepatic vitellogenin (Vtg) in both sexes were not statistically different from those in the controls. These results demonstrate that FLU affected gonadal development in male medaka and reproductive capacity in paired medaka. This is the first report of testis-ova in male medaka exposed to FLU.

Keywords: Anti-androgenic compound; Medaka; Reproduction; Testis-ova; Vitellogenin

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In previous studies a variety of synthetic chemicals have been reported to disrupt the endocrine system of wildlife. Most studies have focused mainly on the effects of estrogenic compounds on sexual differentiation, growth, and reproduction of the wildlife. However, some chemicals, such as the DDT metabolite *p,p*-DDE, fungicide vinclozolin and anticancer agent flutamide (FLU) have been demonstrated to be antagonists of the mammalian androgen receptor (Ashby et al., 2002; Gray et al., 1994; Kelce et al., 1995). A few studies have evaluated the effects of anti-androgen on the reproductive abilities and biomarkers (e.g., gonadal development and Vtg levels) in fish.

We aimed to elucidate the effects of sublethal concentrations of FLU on the reproduction (fecundity and fertility), hepatic Vtg levels, and gonadal histology of adult medaka (*Oryzias latipes*).

Fifty-six mating pairs (3 months after hatching; body weight, \sim 300 mg; total length, ~33 mm) were acclimated for 3 weeks. Each pair was placed in a 1-L chamber filled with flow-through tap water at 24 ± 1 °C. The fish were kept under a constant 16 h light: 8 h dark photoperiod and fed with Artemia nauplii (<24 h after hatching) twice a day. After the acclimation period, fish were exposed to FLU (0 [control], 0.0938, 0.188, 0.375, 0.75, and 1.5 mg/L; 6 pairs/treatment) in a 2.5-L glass chamber flow through system. Test chamber was separated into two compartments, and one pair of medaka was placed in each compartment. The test solution in each test chamber was renewed 12 times daily. The dissolved oxygen (8.0–8.2 mg/L), pH (7.5–7.6), and temperature (23–24 °C) of the test solutions were maintained throughout the exposure period at the levels given. All spawned eggs were collected from female fish and the fecundity and percent fertility of each pair were checked daily for 3 weeks, as described previously (Kang et al., 2003). At the end of the exposure period, the gonads and livers of all fish were removed and weighed for determination of the gonadosomatic index (GSI) and hepatosomatic index (HSI), respectively. The gonads were subjected to histological examination, and the livers were stored at -70 °C until analysis of Vtg levels by enzyme-linked immunosorbent assay using a method described previously (Kang et al., 2003). The FLU concentrations of the test solutions were measured once a week using high-performance liquid chromatography with an L-column octadecyl silica (ODS; 150 × 4.6 mm). Briefly, the eluted samples at a flow rate of 1 L/min in a mobile phase of acetonitrile:water (6.5:3.5, v/v) were monitored at wavelength of 305 nm. The detection limit of FLU was 0.01 mg/L.

During the exposure period, the FLU concentrations in the test chambers were maintained at 101.4–105% of their nominal concentrations (Table 1). Therefore, we used the measured concentrations instead of the nominal concentrations in this section. Only one female died in the 1.56-mg/L treatment group; there were no apparent lesions or pathological abnormality.

The fecundity and fertility of paired medaka were significantly decreased at 1.56 mg/L in week 1 and 2, compared with those of the controls, and a decreasing tendency of their reproductive abilities was also found in week 3 at 1.56 mg/L, although this result was not significantly different (Table 1).

Male medaka exposed to FLU developed testis-ova (Table 1). However, no concentration-related increase in the incidence of testis-ova was observed, and normal spermatogenesis were found in their testes. We found that larger oocytes (maximum diameter: 100 μm) developed in the testes of male exposed to 0.787 mg/L (Fig. 1), than in those of male fish treated with estrogenic compounds (Kang et al., 2002, 2003). No histological

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