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Effects of BPA and DES on longchin goby (*Chasmichthys dolichognathus*) *in vitro* during the oocyte maturation

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Abstract

This study was performed in order to assess whether bisphenol (BPA) and diethylstilbestrol (DES) had agonistic or antagonistic effects on oocyte maturation using marine fish. We tested the effects of these chemicals on *in vitro* maturation, germinal vesicle breakdown (GVBD), assay using oocytes from the longchin goby, *Chasmichthys dolichognathus*. During the maturation process, low concentrations of BPA and DES triggered GVBD depending on the stage of oocyte development; BPA at 0.044 nM and DES at 0.037, 0.37, and 3.73 nM induced GVBD in 0.82–0.88 mm diameter oocytes (germinal vesicle located near the center of oocytes). In 0.76–0.80 mm diameter oocytes (fully vitellogenic oocytes), BPA induced GVBD at relatively higher concentrations (4.38, 43.8, and 438 nM). In 0.86–0.90 mm diameter oocytes, BPA and DES had no observable effect on GVBD at the concentrations tested. Oocytes with diameters between 0.82 and 0.88 mm appeared to be more sensitive to these chemicals. Moreover, our results showed that BPA and DES did not inhibit GVBD.

Keywords: Bisphenol; Diethylstilbestrol; Estrogenic chemicals; GVBD; Longchin goby; Oocyte maturation

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1. Introduction

A number of chemicals released into the environment are estrogenic and can disrupt the endocrine system of wildlife and humans by binding to and activating the estrogen receptor. These environmental estrogens have the potential to perturb sensitive hormone pathways that regulate reproductive function. In fish, this may result in reduced gonad size or feminization of genetic male fish, skewed sex ratios, impaired gametogenesis or altered adult sexual maturity, delayed ovulation and spawning, decreased fertility and egg production (Arcand-Hoy and Benson, 1998; Jobling et al., 2003). Diethylstilbestrol (DES) is a synthetic estrogen. It has been used as an estrogen supplement before its carcinogenic effects was recognized (Herbst et al., 1971). Bisphenol A (BPA), a raw material of polycarbonate and epoxy resins (i.e. dental sealants and lacquer coating of food cans), is a widely used chemical proposed to have estrogenic activity (Krishnan et al., 1993). Estrogens play an important role in controlling fish reproductive processes. The induction of vitellogenin synthesis, which is controlled by estrogens, is a widely used end point for detecting the effects of estrogenic activity (Nichols et al., 2001). In addition, estrogens have been shown to inhibit gonadotropin-induced oocyte maturation and ovulation of intact follicles *in vitro* (Kime, 1998). Some studies have shown that estradiol-17β is generally not effective in inducing fish oocyte maturation (Young et al., 1982; Trant and Thomas, 1988).

In adult fish, exposure to xenoestrogens such as DES and BPA has been reported to result in increased or decreased vitellogenic productions in both male and female fish and in inhibited oocyte maturation (Thomas, 1999; Christiansen et al., 2000; Scholz and Gutzeit, 2000; Sohoni et al., 2001; Jobling et al., 2003;). In lower vertebrates, progestins induce oocyte maturation, which results in germinal vesicle breakdown (GVBD) (Nagahama, 1983; Das and Thomas, 1999). *In vitro*, estradiol inhibits progesterone-induced GVBD in *Rana pipiens* oocytes and 17α-ethinyl estradiol inhibits it in *Xenopus* oocytes, suggesting that amphibian oocyte maturation is sensitive to xenobiotics with estrogenic activity (Baulieu et al., 1978; Lin and Schuetz, 1983). Similarly, xenoestrogenic inhibition of progestin-induced GVBD has been demonstrated in fish oocytes, in Atlantic croaker, *Micropogonias undulates*, (Ghosh and Thomas, 1995).

Recently, Pickford and Morris (1999) demonstrated that the natural and synthetic estrogens estradiol and 17α-ethinyl estradiol had no observable effect on GVBD using *Xenopus* oocyte. However, their potencies vary: estradiol and 17α-ethinyl estradiol exhibited weak agonist activity at low micromolar concentrations, and at higher doses (33 uM), 17α-ethinyl estradiol was slightly antagonistic. Estrogenic EDC, BPA was also tested in this assay, has no observable effect on GVBD. However, Tokumoto et al. (2004) found that treatment of oocytes with one of the EDCs, DES, alone induces maturation in goldfish and zebrafish, suggested that DES and MIH (maturation-inducing hormone = 17α, 20β-dihydroxy-4-pregnen-3-one) are agonists to induce oocyte maturation. Additionally, they described that BPA, have structural features similar to DES, might also have the ability to induce oocyte maturation in fish.

In this respect, direct study was performed in order to assess whether BPA and DES had agonistic or antagonistic effects on oocyte maturation using marine fish. Therefore, we tested the ability of these chemicals in an *in vitro* maturation, GVBD, assay using oocytes from longchin goby, *Chasmichthys dolichognathus*. This fish are found at the tide pool of the coastal waters of Korea and Japan.