An agonist for membrane progestin receptor (mPR) induces oocyte maturation and ovulation in zebrafish in vivo

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学 位 論 文 要 旨

Abstract of Doctoral Thesis

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論文題目：

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Abstract：

Considering the near proximity of oocyte maturation and ovulation in fish, it's possible that these processes are intertwined and controlled by the same hormones. After being ovulated or released from the follicular layer, mature oocytes are being able to be spawned and fertilized. Luteinizing hormone (LH), its product maturation-inducing hormone (MIH), and several locally derived peptide growth factors play significant role in oocyte maturation and ovulation.

The effects of externally applied endocrine-disrupting chemicals (EDCs) or steroid hormones in zebrafish were investigated by simply adding the agents into water to resolve the effects of EDCs *in vivo* in zebrafish. As a result, a method for inducing fish ovulation *in vivo* was developed, which involved simply adding the maturation-inducing steroid 17a, 20b- dihydroxy-4-pregnen-3-one (DHP) to the water. In zebrafish, adding steroids to the ambient water causes oocyte maturation within a few hours. *In vivo* assays, oocyte maturation was induced in a matter of hours (2 hours by DHP and 3 hours by Diethylstilbestrol (DES). Unlike *in vitro* studies, steroids penetrate the fish body quickly and induce nongenomic action in the ovary. As a result, we concluded that the effects of EDC hormones on nongenomic activities via the membrane progestin receptor (mPR) could be assessed using oocyte maturation in an *in vivo* zebrafish assay, as well as genomic actions via the nuclear progesterone receptor (nPR) via ovulation scoring.

In the present study, as a selective agonist for mPR, the effect of Org OD 02-0 (Org OD 02) on fish oocyte maturation and ovulation was evaluated through both *in vitro* and *in vivo* assays. At first, the female wild type *roy* zebrafish was collected by paring the male and female zebrafish as the only

female fish was required for this experiment. Female fish with full-grown immature oocytes were gathered from a mixed group of 10-50 males and females. Later, females were placed in a glass tank with 100 ml of water per fish and added 10µl of 1 mM DHP into each of the 100 ml water and incubated at 28.5°C for 4 hours. Through this process all full-grown immature oocytes were ovulated those were observed by their posterior portion of the ovary by naked eye. These types of females were finally selected for proceeding the further experiment. All the confirmed females kept into same tank for 5-10 days prior to using for the experiment. After 5-10 days later, *in vitro* oocyte maturation assay was conducted. Gravid females were selected those are not contain ovulated eggs. Ovaries were segregated and washed in fresh zebrafish ringer solution from sacrificed female fish. Then ovaries were manually dissected into ovarian fragments. Fragments were treated with 4 ml of zebrafish ringer solution containing experimental agents in *in vitro* condition and incubated with a light agitation. Afterward the oocytes were observed under binocular microscope as transparent due to the germinal vesicle breakdown (GVBD) occurrence. Conversely, *in vivo* oocyte maturation and ovulation assay were also performed to evaluate the maturation and ovulation rate. In this portion, gravid female zebrafish was selected with full-grown immature oocytes those were not contain ovulated eggs from the group of confirm females. Selected females were placed in a glass tank with 100 ml water per fish and added 10µl of 1 mM agent into each of the 100 ml water and incubated at 28.5°C for 4 hours. Then, by following the same procedure as described for *in vitro* methods, the oocyte maturation of the zebrafish was also observed. The oocytes that were transparent was determine GVBD and the presence of a transparent fertilization membrane was determine ovulation. To observed more briefly, we conducted *in vitro* ovulation assay also. Females were chosen by same way as describe previously. Oocytes were put in fresh 90 percent L-15 medium pH 9.0 supplemented with 0.5 percent bovine serum albumin (BSA) before being washed with the same solution. Fully grown immature oocytes were exposed *in vitro*. 4,6-diamidino-2-phenylindole (DAPI) was applied to the medium to determine ovulation processes, and blue-fluorescent follicle cell nuclei were studied under a fluorescent binocular microscope. Ovulation was evaluated when the follicle cell layers were absent, and GVBD also when translucent.

These results indicated that Org OD 02 triggered physiological ovulation in live zebrafish. In summary, we have demonstrated the effect of Org OD 02 on fish oocyte maturation and ovulation *in vitro* and *in vivo*. The results suggested that Org OD 02 acted as an agonist not only of mPR but also of nPR.