Effects of aromatase inhibitors and estradiol-17 β on gonadal sex differentiation of the red seabream, *Pagrus major*

B.-S. LIM^{1*}, H. KAGAWA², K. GEN³, AND K. OKUZAWA³

¹Tokyo University of Fisheries, Konan 4-5-7, Minato-ku, Tokyo 108-8477, Japan (badang@fra.affrc.go.jp), ²National Research Institute of Aquaculture, Fisheries Research Agency, Nansei, Mie 516-0193, Japan, ³Inland Station of National Research Institute of Aquaculture, Fisheries Research Agency, Tamaki, Mie 519-0423, Japan

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INTRODUCTION

Since Yamamoto¹⁾ demonstrated that sex steroid hormones can induce sex differentiation in medaka, it has been hypothesized that endogenous sex steroids hormones regulate sex differentiation in teleosts. However, the fundamental mechanisms of these steroids on sex differentiation are not well understood. Recent studies have demonstrated that cytochrome P450 aromatase (P450arom), which is steroidogenic enzyme responsible for conversion of androgen to estrogen, is detected prior to ovarian differentiation of fish²⁾. Suppression of P450arom was observed during sex reversal of genetically females to phenotypic males by rearing at a high water temperature in flounder³⁾. Moreover, treatment of aromatase inhibitor induced sex reversal of genetically females to phenotypic males in Nile tilapia⁴⁾. These results suggest that P450arom is essential to sex differentiation of fish. However, current knowledge concerning endocrine regulation of gonadal sex differentiation has been obtained from gonochoristic fish.

Red seabream have bisexual gonads during the juvenile stage (juvenile hermaphroditism): ovarian cavity formation and subsequent oocytes and spermatogonia appear in the gonad of all fish, and finally the sex ratio of male to female becomes 1:1^{5,6)}. To elucidate the physiological implication of sex steroid hormones in gonadal sex differentiation of the red seabream, *Pagrus major*, we investigated effects of aromatase inhibitors (fadrozole and ATD), estradiol-17 β (E₂) and methyltestosterone (MT) on ovarian cavity formation and testicular differentiation.

MATERIALS AND METHODS

Experiment 1: Effects of aromatase inhibitors and steroids on ovarian cavity formation

This experiment was designed to examine involvement of estrogen in the process of sex differentiation, especially ovarian differentiation. Histological preparations of gonadal cross-sections from control and treated groups were classified into three stages by the following morphological

characteristics. Stage I: somatic cells proliferate and protrude into the dorsal side of body cavity from both edges. Stage II: completion of ovarian cavity formation. Stage III: presumptive testis containing many seminiferous tubules. The percentages of the various gonadal stages are shown in Fig.1.

Fish were divided into six groups, control, fadrozole (3 mg/kg BW; nonsteroidal aromatase inhibitor), ATD (1.5 mg/kg BW; steroidal aromatase inhibitor), fadrozole+E₂ (3+0.03 mg/kg BW), E₂ (0.03 mg/kg BW) and MT (0.03 mg/kg BW) groups. Each chemical was orally administrated to juvenile red seabream during a period from 60 to 160 days after hatching (DAH). They were reared in tanks with 100 l and at a water temperature of 19~20 °C. At the end of experiment, 30 fish per group were randomly collected and histologically investigated the gonadal development.

Experiment 2: Effects of aromatase inhibitors and steroids on testicular differentiation

To clarify the involvement of estrogen in testicular differentiation from bisexual gonads, aromatase inhibitors were orally administrated to juvenile red seabream. Histological preparations of gonads were classified into the following types (Fig. 2). Type I: Ovary containing cavity. Type II: Ovary containing oocytes. Type III: Bisexual gonad. Type IV: Testis showing active spermatogenesis. Type V: Testis with many seminiferous tubules and spermatogonia.

Fish were divided into five groups, control, fadrozole (3 mg/kg BW), fadrozole+E₂ (3+0.03 mg/kg BW), E₂ (0.03 mg/kg BW) and MT (0.03 mg/kg BW) groups. Each chemical was orally administrated to juvenile red seabream during a period from 140 to 214 DAH. They were reared in tanks with 100 l and at a water temperature of 22~28 °C. At the end of treatment, 30 fish per group were randomly collected, and sacrificed for gonadal histology.

Histological Analysis

A piece of the central part of the gonad was fixed in Bouin's solution and embedded in paraffin. Samples were

Fig. 1. Effects of oral administration of aromatase inhibitors(fadrozole and ATD), estradiol-17 β and methyltestosterone on gonadal development of juvenile red seabream. Sampling was done at 160 DAH.

sectioned longitudinally (6 μ m), and stained with haematoxylin and eosin. Morphological observations were performed under a light microscope.

RESULTS

Experiment 1: Treatment of fish with fadrozole (3 mg/kg·BW), but not ATD (1.5 mg/kg·BW), during 60–160 DAH inhibited the formation of ovarian cavity. Administration of E₂ (0.3 mg/kg·BW) reversed fadrozole-inhibited ovarian cavity formation. Treatment of fish with MT (0.3 mg/kg·BW) inhibited ovarian cavity formation, and induced formation of seminiferous tubules in all fish region of the gonad.

Experiment 2: Fadrozole induced the degeneration of ovarian tissue and active spermatogenesis. E₂ inhibited fadrozole-induced spermatogenesis and maintained the bisexual gonads. In half of MT-treated fish, the gonads consisted of well-developed seminiferous tubules and spermatogonia. Treatment of fish with E₂ alone did not affect the gonadal development.

DISCUSSION

In the present study, ovarian cavity formation is inhibited by treatment of fadrozole, nonsteroidal aromatase inhibitor, which blocked estrogen production by inhibiting the aromatization of endogenous androgens. The present data are consistent with the previous findings, indicating that treatment of aromatase inhibitor induced sex reversal of genetically females to phenotypic males in Nile tilapia⁴⁾. These results suggest that endogenous estrogen may be necessary for normal ovarian differentiation in red seabream. This hypothesis is supported by evidence showing that enzymes essential for steroidogenesis, including P450_{arom} appeared prior to the ovarian cavity formation of tilapia²⁾.

The present experiment further showed that oral administration of fadrozole induced testicular differentiation in the bisexual gonads. This is the first evidence showing the effects of aromatase inhibitor on testicular differentiation in

Fig. 2. Effects of oral administration of fadrozole, estradiol-17 β and methyltestosterone on gonadal development in juvenile red seabream at during 140–214 DAH.

fish with hermaphroditic gonad. Thus, it seems that estrogen may play an important role on maintenance of bisexual gonad, and that decrease of aromatase activity, resulting in decrease of amount of estrogen synthesis, may induce differentiation of testis in fish with bisexual gonad. Further studies are necessary to clarify whether 11-hydroxylase, a key enzyme for androgen synthesis of fish, are involved in the process of testicular differentiation in red seabream, as reported in previous studies⁷⁾.

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