EFFECTS OF ESTROGEN-LIKE CHEMICALS ON THE INDUCTION OF CHORIOGENIN H GENE EXPRESSION IN MEDAKA, *Oryzias latipes*

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Introduction

The teleostean oocytes and eggs are surrounded by egg envelope which plays significant roles in reproductive and developmental processes by protecting the egg and the developing embryo. The inner layer of the fish egg envelope, called zona radiata, occupies most of the egg envelope and consisits of several glycoproteins. The synthesis of the egg envelope proteins in a number of teleost species has been studied using biochemical and immunochemical techniques. In medaka, it was reported that egg envelope proteins are produced in liver with response to estrogenic chemicals and released into the blood[1,2]. Zona radiata of the medaka, *Oryzias latipes*, consists of two major subunit groups, ZI-1,2 and ZI-3. The ZI-1,2 group glycoproteins with molecular weights from 74,000 to 76,000 were isolated by column chromatography and SDS-PAGE, and were known to be modified from the precursor protein, choriogenin H[3,4]. ZI-3 protein consists of a single protein with an Mw of 49,000 derived from choriogenin L gene[5]. Recently, some reports were published that zona radiata protein genes (in case of medaka called as choriogenin) was more responsive to environmental estrogens than vitellogenin in atlantic salmon and the gene expression could be possible sensitive biomarker of environmental estrogens[6].

Based on these reports, we tried to assess the expression of choriogenin mRNA of medaka exposed to estrogen-like chemicals using RT-PCR technique and to examine the possible usefulness of choriogenin mRNA expression as a biomarker for the monitoring of endocrine disrupting chemicals.

Materials and Methods

Experimental Animals and exposure

The orange-red variety of the medaka were fed on artemia daily and kept in 100L aquaria at 25℃ on 18hr light/8hr dark condition. Sexually matured male and spawning female with mean weight of 250mg were exposed to 17α-ethinylestradiol at nominal concentrations of 1, 5, 10, 20, 50, 100 and 200 μg/L for 144 hours. Nonylphenol and bisphenol A were treated with concentrations of 5, 50, 100, 200 and 500 μg/L for 144 hours, respectively. Ethanol was used as a

vehicle for the chemicals.

RT-PCR analysis of choriogenin H and β-actin expression

Total RNA was carefully extracted from the livers of treated males and spawning females with QIA-Shredder and RNeasy mini-kits(*QIAgene Co.*) according to the manufacture's instructions. Gene expressions of choriogenin H and β-actin expression were analyzed by reverse transcription PCR technique. The primers used for RT-PCR were selected from the combinations of the cDNA fragments based on the published sequences of choriogenin H cDNA[3]. Reverse transcription was performed using AMV reverse transcriptase and oligo dT primer at 42℃ for 30min. Twenty five cycles were then performed at 94℃for 30sec, 60℃for 30sec and 72℃for 1min.

Results and Discussion

For the selection of adequate choriogenin H primers, amplification of cDNA specific for the choriogenin H gene of spawning female medaka was performed using several pairs of primers based on the published sequences of choriogenin H cDNA[3]. Potential problems during RT-PCR are genomic DNA contamination in the RNA preparation and nonspecific amplified cDNA fragments in agarose gel electrophoresis. So we tried to choose primer sequences that are expected to be located on separate exons in choriogenin H. As a result, a pair of RT-PCR primers which showed critical single band on agarose gel and was distinguished from the products derived contaminating genomic DNA, was selected. The resulting chriogenin H cDNA fregment was 900bp long. The open reading frame of choriogenin H was also sequenced and identified as 2001bp that is 225bp longer than choriogenin H analyzed by Murata *et al* [3]. In this study, the ORF of choriogenin H contains a tandemly repetitive domain. The tandemly repetitive domain is composed of 15 amino-acid residue whose consensus sequence is PPQNPQVPQYPSKPQ.

All tested chemicals, 17α-ethinylestradiol(EE2), nonylphenol and bisphenol A induced chorogenin H mRNA expression in male medaka. The concentration for the induction of choriognin H gene expression was 20 μg/L for EE2, 100 μg/L for nonylphenol and 200 μg/L for bisphenol A and dose response reaction was appeared (Fig 1).

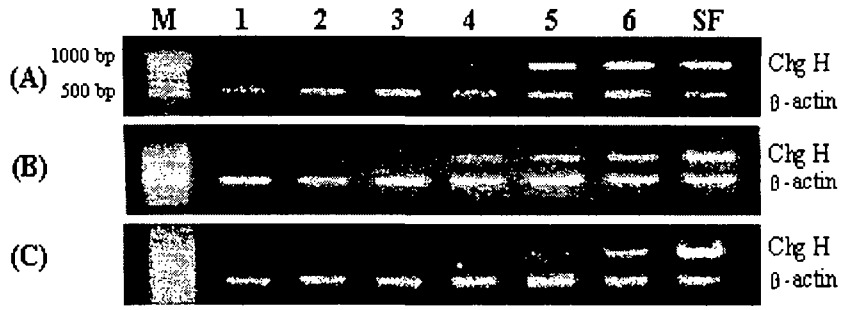

Fig 1. Choriogenin H gene expression in liver of male medaka exposed to EE2, Nonylphenol and Bisphenol A. Gene expression of choriogenin H and β-actin mRNA was analyzed by RT-PCR performed for 25 cycles. (A) EE2, M: markers, 1:control, 2: 10 μg/L, 3: 20 μg/L, 4: 50 μg/L, 5: 100 μg/L, 6: 200 μg/L, SF: spawning female (B) Nonylphenol, (C) Bisphenol A, M: markers, 1:control, 2: 5 μg/L, 3: 50 μg/L, 4: 100 μg/L, 5: 200 μg/L, 6: 500 μg/L, SF: spawning female

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*** Poster presentation**