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Plasma biomarkers in juvenile marine fish provide evidence for endocrine modulation potential of organotin compounds

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ABSTRACT

Organotin compounds, such as tributyltin (TBT) and triphenyltin (TPT), have been widely used to control marine fouling. Here, we show that organotin stimulation reduces the hormone levels in the plasma of two economically important aquaculture fish. Blood plasma samples were collected from juvenile red seabream and black rockfish exposed to environmentally realistic concentrations of TBT and TPT for 14 days. The levels of two plasma biomarkers, namely the yolk protein precursor vitellogenin (VTG) and the sex steroid 17β-estradiol (E2), were measured to determine the endocrine disrupting potential of the organotin compounds. Both organotin compounds were dose-dependently accumulated in the blood of two fish. Exposure to waterborne TBT and TBT significantly decreased the plasma VTG levels in both the juvenile fish in a dose-dependent manner. In contrast, the treatment with E2, a well-known VTG inducer, significantly increased the plasma VTG levels in both the fish. In addition, the mRNA levels of *vtg* were also downregulated in the liver tissues of both the fish at 100 and/or 1000 ng L^{-1} of TBT or TPT exposure. The plasma E2 titers were significantly suppressed at 100 and/or 1000 ng L^{-1} of TBT or TPT exposure for 14 days compared to their titer in the control. Since estrogen directly regulates *vtg* gene expression and VTG synthesis, our results reveal the endocrine disrupting potential of organotin compounds, and subsequently the endocrine modulation at early stage of fish can trigger further fluctuations in sexual differentiation, maturation, sex ration or egg production. In addition, the results demonstrate their effects on non-target organisms, particularly on animals reared in aquaculture and fisheries.

1. Introduction

Organotin compounds are used as toxicants in antifouling products to control biofouling. Among these, tributyltin (TBT) and triphenyltin (TPT) have been applied for preventing the adhesion of microorganisms, fungi, algae, mollusks, and crustaceans because of their strong biocidal activities (Fent, 1996; Dafforn et al., 2011). However, various adverse effects of waterborne organotin compounds have been recognized since the 1970s (Alzieu et al., 1981) owing to which their use in antifouling products is banned in many countries. One of the representative detrimental effects of TBT and TPT is the induction of imposex (penis in females) in mollusks even when these chemicals are dissolved at very low concentrations (1 ng L^{-1}) in water bodies (deFur et al., 1999; IPCS, 1999a, 1999b). The environmental concentrations of TBT and TPT are in the nanogram to microgram range (7.1 µg L^{-1} in freshwater; 1.58 µg L^{-1} in seawater), particularly in bays where boats coated with organotin-based antifouling paints are present (IPCS, 1999a, 1999b).

Although the distribution of these organotin compounds is restricted, there are several reasons to study the negative effects of waterborne TBT or TPT. First, they can be easily absorbed into the tissues of aquatic animals with bioconcentration factors of 10^2–10^4 and are considerably persistent (IPCS, 1999a, 1999b; Konstantinou and Albanis, 2004; Thomas and Brooks, 2010). Particularly, organotin compounds

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can accumulate in sediments and their concentrations decrease very slowly (Traas et al., 1996). The existence of boats that were previously coated with organotin and the prolonged use of materials before restriction as well as their continued use despite the restriction could be the possible sources of pollution. The TBT concentration has been decreasing very slowly after its ban (National Committee for CICAD, 1997; Hall et al., 2000). Thus, the organotin compounds are still detected in coastal regions worldwide (Laitano et al., 2015: 150 ng Sn g^{-1}; Batista et al., 2016: 122.3 ng Sn g^{-1}; Abraham et al., 2017: TBT up to 380 ng Sn g^{-1}; Maciel et al., 2018: TBT up to 339 ng Sn g^{-1}). Particularly, TBT was detected up to 41 ng Sn g^{-1} in Korean coastal areas (Lee et al., 2015), and TBT was accumulated up to 19 ng Sn g^{-1} in fish that were sampled at southeast of Korea (T. Kim et al., 2017).

The second reason for the need of continuous studies on organotin compounds is the limited information about their adverse effects on aquaculture and fisheries species. The ecotoxicity and mode of action of organotin compounds have been highlighted in several aquatic models as well as in laboratory animals. The accumulation of TBT and TPT in seafood has been continuously reported (Cardwell et al., 1999; Keithly et al., 1999), and these compounds can potentially be transported into the human body through food chains and cause adverse effects, as observed in marine mammals (Iwata et al., 1995; Kannan et al., 1997). In fact, aquaculture and fisheries have been threatened by organotin compounds through discharges of wastes and runoff from inland to the coastal regions (Fitridge et al., 2012; Guardiola et al., 2012; Lee et al., 2017).

Fish can respond to cellular and physiological stress. The analysis of plasma is one of the most widespread diagnostic tests in aquaculture and fisheries, and several plasma biomarkers have been used as early indicators of adverse effects that can help in predicting the health status of fish and for devising strategies for environmental protection. Among the plasma biomarkers, reproductive and endocrine parameters, such as sex-steroid hormones and vitellogenin are highly sensitive to endocrine disrupting chemicals (EDCs), organotin, and xenobiotics in fish (Hecker et al., 2002). Plasma 17β-estradiol (E2) is strongly associated with sexual behavior, reproduction, oocyte development, spawning, and sexual determination and differentiation in fish (Devlin and Nagahama, 2002). Thus, exogenous exposures of the natural (E2) and/or synthetic (17α-ethinylestradiol; EE2) estradiol are commonly used for artificial sex reversal and inducing hormonal fluctuation in fish. Vitellogenin (VTG) is the precursor of egg yolk proteins and is a nutritional source for developing embryos and larvae. It is synthesized by endogenous estrogen regulation in the hepatocytes of oviparous vertebrates (Mommsen and Walsh, 1988). The induction or inhibition of the levels of VTG in plasma and of its mRNA has been widely used as a promising biomarker for estrogenic contamination in the aquatic environment (Sumpter and Jobling, 1995; Marin and Matozzo, 2004). Although many studies have been suggested inhibitory effects of TBT on VTG synthesis or *vtg* mRNA in fish (Zheng et al., 2005; Lv et al., 2008; Zhang et al., 2013), only several reports highlighted similar potential of TPT as yet (Ishijima et al., 2005; Zhang et al., 2008). In addition, to our knowledge, there is no report for highlighting the modulatory effects of organotin on the estrogen-triggered VTG synthesis system in fish, while almost studies have focused VTG or E2 only. In fact, the secretion of VTG into the bloodstream and its incorporation into the growing oocytes are mainly regulated by estrogen (Arukwe and Goksøyr, 2003; Lubzens et al., 2010). Therefore, there is a reciprocal relationship between the levels of both the plasma biomarkers.

Most aquaculture and fishery systems are located within coastal regions where the ultimate sink for numerous pollutants including organotin and their metabolites are released from agricultural runoff, sewage, industrial outfall, or shipping port. In this study, juvenile stages of two economically important fish, red seabream (*Pagrus major*) and black rockfish (*Sebastes schlegeli*) were exposed to different concentration of TBT or TBT for 14 days. In teleost, the liver tissues of mature female have been considered as the only source for circulating plasma VTG, but extra-hepatic expressions of *vtg* genes and artificial induction of VTG in male fish have continuously been published (Islinger et al., 2003; Wang et al., 2005; Ma et al., 2009; Tingaud-Sequeira et al., 2012; Kim et al., 2016). Thus, study on the modulatory effect of organotin for the endocrine system of immature fish has some merits, as fluctuation in endocrine system would further interrupt the onset of sexual maturation in fish. After blood preparation, plasma VTG and E2 levels were measured to investigate their sensitivity as well as modulatory effects of the organotin compounds. Our results facilitate a better understanding on the following specific objectives such as 1) to determine biomarker potential of plasma VTG and its transcript with plasma E2 content upon waterborne organotin at early stage of marine fish, 2) to measure co-modulation of estrogen-triggered VTG synthesis system in organotin-exposed marine fish, and 3) to estimate potential non-target effect of waterborne organotin on aquaculture fish.

2. Material and methods

2.1. Fish

All animal handling and experimental procedures were approved by the Animal Welfare Ethical Committee and the Animal Experimental Ethics Committee of the Incheon National University (Incheon, South Korea) and the Korea Institute of Ocean Science and Technology (KIOST). The aquaculture fish, red seabream and black rockfish, used in this study were obtained from the enclosure aquaculture in Tongyeong, Gyungnam, South Korea. The two fish are neither endangered nor are they protected at this site.

Regarding sexual maturation status of red seabream, sex differentiation to ovary up to 93% of tested fish (N = 30) was measured at 241 days after hatching, as shown by a previous study that all fish tested had differentiated ovaries at 7 months after hatching (Kato et al., 2003). In the case of black rockfish, we observed that apparent differentiated gonad was measured at 95–105 day after hatching (N = 50; % female 53; male has dark gonad with high melanin content; female has white yellow gonad). Although there is no clear information on the beginning of sexual differentiation in black rockfish, a previous report showed that the process of ovary formation occurred from 40 to 110 days after hatching in the fish (Koya and Muñoz, 2007). In addition, sexual differentiation to ovary was observed in black rockfish at approximately 65 days after hatching and clear differentiated gonad was detected at 115 days after hatching (Lee et al., 1996). In this study, larvae (≈7 days after hatching) were moved from hatchery and maintained in an automated aquaculture system in artificial seawater (6.12 ± 0.84 mgO_2 L^{-1}) at 20 °C under lighting conditions of 14 h light:10 h dark. The fish were maintained in glass aquaria (60 L capacity) with each aquarium accommodating up to 10 juvenile fish (red seabream: ~6 months after hatching, 16.12 ± 1.92 cm in mean total length; black rockfish: ~7 months after hatching; 15.81 ± 1.97 cm in mean total length). They were fed *Artemia salina* (< 24 h after hatching) or frozen mosquito larvae twice a day until satiation.

2.2. Organotin exposure and blood sampling

Tributyltin chloride (TBTCl) and triphenyltin chloride (TPTCl) were purchased from Sigma-Aldrich, Inc. (St. Louis, MO, USA; 95% purity) and were dissolved in dimethyl sulfoxide (DMSO; Sigma-Aldrich, Inc., St. Louis, MO, USA). The juvenile red seabream (randomly sampled N = 20; ~58 days after hatching; 3.25 ± 0.29 cm in mean total length) and black rockfish (randomly sampled N = 20; ~52 days after hatching; 2.89 ± 0.28 cm in mean total length) were collected for organotin exposure. The juvenile red seabream (N = 50) and black rockfish (N = 50) were exposed to different concentrations (0, 10, 100, and 1000 ng L^{-1}) of TBT or TPT for two weeks at 20 °C. The organotin concentrations were chosen based on several studies conducted by other researchers on red seabream and fish (Dimitriou et al., 2003;