INTRODUCTION

Organotin compounds, such as tributyltin (TBT) and triphenyltin (TPT), have been widely utilized as biocides, agricultural fungicides, and rodent repellents. These widespread uses have resulted in the release of increasing amounts of organotins into the environment. In aquatic invertebrates, particularly marine gastropods, organotin compounds, such as tributyltin (TBT) and triphenyltin (TPT), induce irreversible sexual abnormality in females which is termed “imposex” at very low concentrations. Although it has been theorized that these compounds act as potential competitive inhibitors of aromatase, which converts androgen to estrogen, and then increase levels of unconverted androgens in gastropods, their effective concentrations for aromatase inhibition are high. In addition to wildlife, organotins may have various undesirable effects on human health. Contrary to the theory of organotin-induced aromatase inhibition in gastropods, in human choriocarcinoma cells, these compounds markedly enhance estradiol biosynthesis along with the increase of both aromatase activity and 17β-hydroxysteroid dehydrogenase type I (17β-HSD I) activity, which converts low-activity estrogen estrone to the biologically more active form estradiol, at the same low concentrations. Although there are many reports describing the potential toxicity of organotins in human and mammals, the critical target molecules for the toxicity of organotin compounds remain unclear. Recently, organotin compounds including TBT and TPT were identified as nanomolar agonists for retinoid X receptor (RXR) and peroxisome proliferator-activated receptor (PPAR) γ, which are members of the nuclear receptor superfamily. Here, we review the potential genetics action and subsequent toxicity induced by organotins via these nuclear receptors.

Key words: Organotin, Retinoid X receptor (RXR), Peroxisome proliferator-activated receptor (PPAR) γ, Aromatase, Endocrine disruptor

ABSTRACT — Organotin compounds have been widely used as antifouling biocides for ships and fishing nets, agricultural fungicides and rodent repellents. These widespread uses have resulted in the release of increasing amounts of organotins into the environment. In aquatic invertebrates, particularly marine gastropods, organotin compounds, such as tributyltin (TBT) and triphenyltin (TPT), induce irreversible sexual abnormality in females which is termed “imposex” at very low concentrations. Although it has been theorized that these compounds act as potential competitive inhibitors of aromatase, which converts androgen to estrogen, and then increase levels of unconverted androgens in gastropods, their effective concentrations for aromatase inhibition are high. In addition to wildlife, organotins may have various undesirable effects on human health. Contrary to the theory of organotin-induced aromatase inhibition in gastropods, in human choriocarcinoma cells, these compounds markedly enhance estradiol biosynthesis along with the increase of both aromatase activity and 17β-hydroxysteroid dehydrogenase type I (17β-HSD I) activity, which converts low-activity estrogen estrone to the biologically more active form estradiol, at the same low concentrations. Although there are many reports describing the potential toxicity of organotins in human and mammals, the critical target molecules for the toxicity of organotin compounds remain unclear. Recently, organotin compounds including TBT and TPT were identified as nanomolar agonists for retinoid X receptor (RXR) and peroxisome proliferator-activated receptor (PPAR) γ, which are members of the nuclear receptor superfamily. Here, we review the potential genetics action and subsequent toxicity induced by organotins via these nuclear receptors.

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androgens to masculinize reproductive organs in not only invertebrates but also vertebrates.

POSSIBLE HUMAN EXPOSURE TO ORGANOTIN COMPOUNDS

Human exposure to non-point sources of organotins may occur mainly through contaminated dietary sources, such as seafood, shellfish and food crops. Daily intakes of TBT oxide (TBTO) determined in Japan by the duplicate-position method were 4.7 ± 7.0 μg/day in 1991 (n=39) and 2.2 ± 2.2 μg/day in 1992 (n=40). Using the market-based method, the daily intake was estimated at 6–9 μg/day in 1991 and 6–7 μg/day in 1992 (Tsuda et al., 1995). In Finland, TPT were detected as the predominant compounds at a level up to 1.11 ng/g fresh weights in fish and seafoods (Rantakokko et al., 2006). In addition, a variety of monoo- and dialkyltin, which include significant contaminating trialkyl species, are also prevalently used as heat stabilizers in the manufacture of polyolefin plastics, bringing them into closer contact with drinking water and food supplies (Takahashi et al., 1999).

The information on human exposure to organotin compounds is limited. In a study of eight volunteers from Germany, TPT was detectable in serum at the concentration range of 0.17-0.67 μg/l (Lo et al., 2003). In a study of 38 volunteers from the USA, Kannan et al. reported that monobutyltin (MBT), dibutyltin (DBT) and TBT were detected in 53, 81, and 70% of the 32 blood samples tested. Blood concentrations of MBT, DBT and TBT were 8.17 ± 8.56, 4.94 ± 3.83, and 8.18 ± 15.4 ng/ml, respectively (Kannan et al., 1999). The toxicological significance of the concentrations of organotins measured in these studies is unknown. However, the potential exposure of humans to organotins has aroused great concern about their potential toxicity. Animal experiments suggested that the spectrum of potential adverse chronic systemic effects of organotins in humans is quite broad and includes primary immunosuppressive, endocrinopathic, neurotoxic metabolic, and enzymatic activity, as well as potential ocular, dermal, cardiovascular, upper respiratory, pulmonary, gastrointestinal, blood dyscrasias, reproductive/teratogenic/developmental, liver, kidney, bioaccumulative, and possibly carcinogenic activity. Although many reports have described the potential toxicity of organotin compounds, the critical target molecules for the toxicity of organotin compounds remain unclear.

ORGANOTINS AS ENDOCRINE-DISRUPTING CHEMICALS

The synthesis of sex steroids from cholesterol requires trafficking between mitochondria and smooth endoplasmic reticulum, and involves many enzymatic steps. Most of these steps use cytochrome P450 (CYP) haem-containing enzymes, and the genes coding for these enzymes are abbreviated to CYP (Fig. 1). Some organotins compounds are known as endocrine-disrupting chemicals to modulate steroid hormone biosynthesis. As mentioned above, these organotins have been suspected to masculinize reproductive organs in vertebrates because, in some gastropods, very low concentrations of these organotins induce "imposex" (Horiguchi et al., 1997; Matthiessen and Gibbs, 1998). Some evidences have theorized that these organotins act as a specific inhibitor of aromatase enzyme which converts androgen to estrogen (Bettin et al., 1996; Matthiessen and Gibbs, 1998). For example, exposure of the organotins increased testosterone levels in female gastropods and organotin-induced imposex can be mimicked by a specific inhibitor of aromatase (Bettin et al., 1996). In addition, TBT was reported to be catalyzed to dibutyltin, which is a metabolite of TBT, by aromatase enzyme (Lee, 1985). However, it has remained unclear whether organotin compounds especially inhibit catalytic activity of aromatase in vertebrates.

Actually, can organotin compounds inhibit the catalytic activity of aromatase? The answer to the question seems to be ‘Yes’. In in vitro experiments, butyltins are demonstrated to exhibit structure-related inhibition of the catalytic activity of human aromatase protein from human placenta (Heidrich et al., 2001) or transfected cells (Cooke, 2002). However, at concentrations effective (micromolar level) for the inhibition of aromatase, TBT and TPT are generally toxic to mammalian cells because

Fig. 1. Pathway of steroid hormone biosynthesis.

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they cause apoptosis or necrosis (Saitoh et al., 2001; Nakamichi et al., 2002; Watanabe et al., 2003; Nakamichi et al., 2006). In human choriocarcinoma cell lines, JAr, JEG-3 and BeWo, exposure to greater than 300 nM TBT or TPT markedly decreases DNA and protein synthesis (Nakanishi et al., 2002; Nakamichi et al., 2006). Concentrations under 1 μM of either organotin compound did not significantly affect aromatase activity in microsomes isolated from human choriocarcinoma cells (Nakanishi et al., 2002). In addition to aromatase, at above 1 μM, TBT inhibits the catalytic activity of human 5α-reductase I and II (Doering et al., 2002), rat 3β-hydroxysteroid dehydrogenase (3β-HSD) (McVey and Cooke, 2003) and pig 17β-HSD I (Ohno et al., 2005). At the same concentration ranges, TPT also inhibit the catalytic activity of human aromatase, 5α-reductase II 17β-HSD I and III (Lo et al., 2003). These observations suggest that these organotin compounds at micromolar level inhibit not specific to the catalytic activity of aromatase and we have to consider the toxicity of organotin compounds in distinguishing between nonspecific toxicity to cells and the specific inhibition of steroidogenic enzymes.

In addition to these, sex steroid receptors and steroidogenic enzymes for sex steroid hormones have not yet been identified in gastropods, and it remains unclear whether sex steroid hormones are critical factors for sexual development and reproduction in gastropods. Furthermore, homologues of both the estrogen receptor (ER) and androgen receptor (AR) have not been found in invertebrates (Escriva et al., 2000) and the composition of nuclear receptor family members is very different between vertebrates and invertebrates (Escriva et al., 1997; Escriva et al., 2000). Therefore, there is some doubt as to whether organotin compounds function as inhibitors of enzymes that metabolize androgens in gastropods, and this doubt led us to suspect that organotin compounds affect other target molecules in mammals.

ORGANOTIN COMPOUNDS AFFECT ENDOCRINE FUNCTIONS IN HUMAN PLACENTA AND OVARY

In a recent study, Nakamichi et al. investigated the effects of organotin compounds on aromatase activity (Nakanishi et al., 2002; Nakamichi et al., 2005) and 17β-HSD I, which converts low-activity estrone to high-activity estradiol (Nakanishi et al., 2006), in human choriocarcinoma cells. Both TBT and TPT increased the catalytic activity of aromatase and 17β-HSD I along with their mRNA expression in a dose-dependent fashion following exposure to non-toxic concentration ranges (3-100 nM). These results indicate that the observed organotin-induced alterations in human choriocarcinoma cells are due to the regulation of mRNA levels of both steroidogenic enzymes, not of the enzyme complex. In addition, these organotin compounds also markedly stimulated human chorionic gonadotropin (hCG) production in the same concentration ranges, along with its mRNA expression (Nakanishi et al., 2002; Nakamichi et al., 2005). These results suggest that organotin compounds are potent stimulators of human placental estrogen biosynthesis and hCG production in vitro and that the placenta represents a potential target organ in pregnant women for organotin compounds, the endocrine-disrupting effects of which might be the result of local changes in estrogen and hCG concentrations.

In opposition to the above results, however, Saitoh et al. reported that 20 ng/ml (about 60 nM) TBT and TPT suppressed both the activity and gene expression of aromatase in the human ovarian granulose-like cell line, KGN (Saitoh et al., 2001). This discrepancy in the action of organotins on the gene expression of human aromatase is due to the tissue-specific expression of aromatase, which is strictly regulated (Fig. 2). Human CYP19 is a single-copy gene composed of 10 exons; exons II to X encode the aromatase protein, as well as the 3’ untranslated region of mRNA common to all estrogen-producing tissues (Simpson et al., 1994). A number of variations of exon I exist. These encode the 5’ untranslated regions of various CYP19 mRNAs, which are selectively expressed in some tissues by alternative splicing (Simpson et al., 1994; Sebastian and Bulun, 2001; Bulun et al., 2003). The tissue-specific expression of CYP19 in humans appears to be mediated by tissue-specific promoters lying upstream of the respective exon I sequences, and by transcription

![Genomic organization of human CYP19 gene](image)

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FIG. 2. Genomic organization of human CYP19 gene.
factors binding to specific regions of each promoter. In the placenta, \textit{CYP19} is driven by the placental major promoter (I.1), and the transcript contains exon I.1, located approximately 89 kb upstream from exon II. On the other hand, ovarian transcripts contain a sequence at the 5'-end immediately upstream of the translation start site, because gene expression in the ovary uses a proximal promoter (II). In ovarian granulosa cells, the expression of \textit{CYP19} is strongly regulated by the steroidogenic tissue-specific transcriptional factor, Ad4Bp/SF-1, via promoter II. In contrast, Ad4Bp/SF-1 is expressed at very low levels in the human placenta and may not play an important role in activation of the placental major promoter I.1 (Bamberger \textit{et al}., 1996; Simpson \textit{et al}., 1997). Saitoh \textit{et al}. suggest that the effects of organotin compounds in KGN cells are caused partly by association with Ad4Bp/SF-1 (Saitoh \textit{et al}., 2001). It is therefore likely that the action of organo-

have been associated with exposure to environmental pollutants capable of mimicking the action of natural hor-

min D receptor, which cannot be activated by RXR agonists regardless of the presence (or absence) of the agonist of its partner receptor; formation of the heterodimer is thought to actually preclude the binding of the ligand to RXR (Forman \textit{et al}., 1995; Thompson \textit{et al}., 1998). TBT and TPT simulated the transactivation of an RXR homodimer and PPAR\textgamma/RXR heterodimers at non-toxic concentration ranges (10-100 nM), whereas they had no effect on the transactivation of RXR/TR and RXR/ RAR heterodimers (Nakanishi \textit{et al}., 2005). Although the effects of organotin compounds on the transactivation of permissive RXR heterodimers other than PPAR\textgamma/RXR have not been determined, it is probably possible to stim-

ORGANOTIN COMPOUNDS ARE PPAR\textgamma AND RXR AGONISTS

Nuclear receptors play important roles in maintenance of the endocrine system, regulation of organ differentiation and fetal development. Reproductive abnormalities in wildlife can be associated with exposure to environmental pollutants capable of mimicking the action of natural hor-

mone, because it stimulates aromatase gene expression through promoter II (Michael \textit{et al}., 1995). The possible target of these organotin compounds may be a signaling pathway common to the gene expression of aromatase, 17\beta-HSD I and hCG in the human placenta and ovary.

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ulate the transactivation of other heterodimers because these compounds function as RXR agonists.

REGULATION OF AROMATASE GENE EXPRESSION BY ORGANOTIN COMPOUNDS THROUGH RXR OR PPARγ ACTIVATION IN HUMANS

Gene expression of human aromatase is regulated by the activation of PPARγ and/or RXR. In the human placenta, a selective RXR ligand stimulates aromatase gene expression; however, a selective PPARγ ligand has little or no effect on aromatase gene expression (Sun et al., 1998; Nakanishi et al., 2005). In addition, the PPAR ligand 15-deoxy-A12,14-prostaglandin J2 (24), FXR ligand chenodeoxycholic acid (Nakanishi et al., 2005) and LXR ligand T0901317 (Nakanishi et al., unpublished data), which are agonists of permissive heterodimer partners of RXR, all also failed to increase mRNA expression of aromatase in human choriocarcinoma cells. It is suggested that none of these permissive heterodimers are involved in aromatase expression in the human placenta and that RXR homodimer may be required for the regulation of aromatase expression (Fig. 3).

Unlike in the placenta, both RXR- and PPARγ-selective ligands suppress aromatase gene expression in the ovary (Mu et al., 2000; Mu et al., 2001; Fan et al., 2005). However, it was suggested that PPARγ/RXR may inhibit promoter II lying upstream of the ovarian major exon I (PII) by an indirect mechanism because of the absence of a PPARγ/RXR response element in promoter II of aromatase (Mu et al., 2001). A transcriptional factor, nuclear factor-kB (NF-kB), interacts with the ovarian promoter II sequence of aromatase and up-regulates its gene expression in the human ovary. In addition, activation of the PPARγ/RXR heterodimer interferes with the interaction between NF-kB and promoter II sequence of aromatase (Fan et al., 2005). PPARγ/RXR, in the ovary, may regulate aromatase gene expression via the NF-kB signaling pathway (Fig. 3).

In light of these findings, human aromatase expression regulated by organotin compounds may involve the activation of PPARγ and/or RXR (Saitho et al., 2001; Nakanishi et al., 2002; Nakanishi et al., 2005), because the aromatase expression pattern induced in the human placenta and ovary by activation of PPARγ and/or RXR is similar to that induced by organotin compounds (Fig. 3). It has already been found, as supporting evidence, that organotin compounds stimulate the expression of a luciferase reporter construct containing the human placental promoter I.1 sequence of aromatase via a ligand-depend-
in coordinate regulation of lipogenic PPARγ/RXR target gene expression in adipose tissue and liver, and modulated adipocyte differentiation factors such as members of the CCAAT/enhancer binding protein family and sterol regulatory element-binding protein 1c (Grün et al., 2006). Furthermore, developmental exposure in utero led to a fatty liver (hepatic steatosis) phenotype and enhanced lipid staining of neonatal fat deposits, and resulting in a significant increase in the epididymal fat pad size of mice later in life (Grün et al., 2006). Whether this occurs through increased lipid storage, an increase in adipocyte number, or a combination of both is currently unresolved. However, activation of PPARγ/RXR induced by organotin compounds represents a compelling mechanistic example of a class of environmental pollutants that have the ability to impact key adipogenic factors, fat deposit size and function.

Exposure of rats in utero to TBT induces a dramatic increase in the incidence of low-birth-weight fetuses because of maternal hypothyroidism (Adeeko et al., 2003). On the other hand, the RXR agonist bexarotene causes clinically significant hypothyroidism in patients with cutaneous T-cell lymphoma (Duvic et al., 2001), and experimental exposure of rats to LG100268 (a selective RXR agonist) induces the acute phase of hypothyroidism (Liu et al., 2002). Similarities between the toxicity of TBT and selective RXR agonists suggest that at least some of the toxic effects of organotin compounds may be mediated by RXR.

Yamabe et al. reported that TBT and TPT enhance the proliferation of androgen-dependent human prostate cancer cells and the transactivation of AR (Yamabe et al., 2000). However, the AR antagonist flutamide cannot inhibit organotin-mediated AR transactivation (Yamabe et al., 2000), and these organotin compounds do not function as AR agonists in a yeast two-hybrid system (Nishikawa et al. unpublished data). Recently, RXR was found to function as a novel co-regulator of AR, and 9cRA was found to inhibit AR activity through the activation of RXR (Chuang et al., 2005). It remains unclear whether the co-regulators recruited by organotin-activated RXR are different from those recruited by 9cRA, but RXR activation by organotins might be involved in the AR transactivation induced by them.

Taken together, these compounds may cause adverse effects on mammals through the activation of PPARγ and/or RXR because of the above-described toxic effects of organotin compounds in human cells and experimental animals.

**CONCLUSIONS**

Although organotin compounds inhibit the enzymatic activity of aromatase, their effective concentration is toxic for mammalian cells. In this review, we have proposed the activation of PPARγ and/or RXR as a novel mechanism for organotin-induced toxic effects in mammals. In addition, RXR are recently reported to play an important role in the development of gastropod imposex, by showing the cloning of an RXR homolog from a marine gastropod, binding of organotins to that receptor, and imposex induction by injection of 9cRA (Nishikawa et al., 2004; Castro et al., 2007). These findings indicated that RXR activation is also a critical event for endocrine disruption of organotins in gastropods. However, it is possible that organotin compounds affect target molecules other than PPARγ and RXR. For instance, organotin compounds have been shown to enhance histone acetyltransferase activity (Osada et al., 2005). Further studies are needed to clarify the precise action mechanism of the toxicity of organotin compounds in mammals in vitro and in vivo, because they appear intricate.

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