ASSOCIATION OF AHR- AND ESR1-RESPONSIVE GENE VARIATIONS WITH SUSCEPTIBILITY TO ENDOCRINE-DISRUPTING CHEMICALS IN RISK OF MALE GENITAL DISORDERS

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Abstract

Recently, the role of genetic susceptibility to estrogenic endocrine-disrupting chemicals (EDCs) in the development of male genital disorders has raised lots of concerns. In our previous study, the association of male genital disorders (hypospadias, cryptorchidism and micropenis) with genetic variants in 5 ESR1 related genes (AHR, ARNT2, CYP17A1, CYP1A2, NR1I2 (PXR)) was found. To further determine whether these genetic variants affect the susceptibility to estrogenic EDCs, we reanalyzed several EDCs' estrogenicity using a human reproductive cell system and examined their regulations on CYP17A1 and ARNT2 expression. As the results, OP, BPA, BBP and *o, p'*-DDT elicited the most strong potency and DBP, TCDD and *p, p'*-DDT (weakly) significantly induced the ERE-luciferase activity. The expression of CYP17A1 was significantly down-regulated by E2, BBP, DBP, DEHP, TCDD and 4OH-PCB187. The expression of ARNT2 was significantly up-regulated by BPA and BBP, and down-regulated by DBP, *o, p'*-DDT, *p, p'*-DDT, TCDD, 4OH-PCB107 and 4OH-PCB187. Our findings suggest the possible role of sexual steroid hormone synthesis and ER-AHR signaling responsiveness in the development of male genital disorders.

Introduction

Increases in the prevalence of hypospadias and cryptorchidism have been reported in various countries in the past few years[1]. It is hypothesized that exposure to environmental factors (i.e. endocrine-disrupting chemicals (EDCs)) affecting androgen homeostasis during the early life may cause these diseases[2]. Several epidemiological studies have suggested the association of perinatal exposure to environmental chemicals, such as organochlorine pesticides, PCBs, phthalates and xenoestrogens with male genital disorders[3, 4]. Although the underlying mechanism is not very clear, it is usually believed that EDCs can bind to the nuclear receptors and activate various genes. Some studies imply that the male genital disorders related genetic variants may increase the susceptibility to estrogenic EDCs[5]. In our previous study, we had systematically selected SNPs in the nuclear receptor genes interacting with ESR1 and examined the association of certain SNPs with male genital disorders[6]. Case-control analysis revealed

differences in 5 genes (AHR, ARNT2, CYP17A1, CYP1A2 and NR1I2 (PXR)), which may be candidates as modifiers of those diseases. In order to further determine whether these genetic variants affect the susceptibility to estrogenic EDCs, in the present study, we reanalyzed several EDCs' estrogenicity using a human reproductive cell system and examined their regulations on CYP17A1 and ARNT2 expression.

Materials and Methods

Test chemicals and Cell lines

All the test chemicals are shown in Table1. The recombinant BG1Luc4E2 human ovarian cancer cell line, a gift from Dr. M. Denison (University of California, Davis, CA), was used in estrogen bioassay. 30 peripheral lymphocytes cell samples, supplied by Dr. Tsutomu Ogata, National Research Institute for Child Health and Development, Japan, are collected from children aged 1-13 yrs (9 cryptorchidism, 7 hypospadias and 14 controls).

Estrogenic chemically activated luciferase gene expression (E-CALUX) bioassay

An ERE-luciferase reporter gene system (E-CALUX bioassay) was used to measure the estrogenic activity of EDCs. The ER-positive BG1Luc4E2 ovarian cancer cells were maintained in estrogen-stripped media for a week before they were plated to 96-well plates at 40,000cells/well and allowed to attach for 24 hr. Cells were then incubated with EDCs for 24 hr at 37°C. Luciferase induction was measured by ATTO AB-2100.

Taqman Real-time quantitative PCR

The responsiveness of male genital disorders related ER- and AHR-responsive genes CYP17A1 and ARNT2 to estrogenic EDCs exposure was examined by Taqman real-time PCR. The human ovarian cancer cell (BG1Luc4E2) was incubated with the EDCs for 24 hr at 37°C. Total RNA was extracted by the RNeasy kit (Qiagen, Chatsworth, CA) and cDNA was synthesized by High-capacity cDNA reverse transcription kits (Applied Biosystems) according to the manufacturer's protocols. The expressions of CYP17A1 and ARNT2 were detected using a Taqman® gene expression master mix (Applied Biosystems) with an ABI Prism 7000 sequence detection system.

Results and Discussion

Estrogenic activity of EDCs in human ovarian cancer cell line BG1Luc4E2

The agonistic effects of the test compounds on ER were analyzed in BG1 Luc4E2 ovarian cancer cells using the E-CALUX bioassay (Figure 1). OP, BPA, BBP and *o, p'*-DDT elicited the most strong potency and DBP, TCDD and *p, p'*-DDT (weakly) significantly induced the ERE-luciferase activity.

Regulation of EDCs on ER- and AHR-responsive genes CYP17A1 and ARNT2 expression

The expression of CYP17A1 was significantly down-regulated by E2, BBP, DBP, DEHP, TCDD and 4OH-PCB187 (Figure2a). CYP17A1 is a well known steroid hormone biogenesis gene. E2, BBP, DBP and TCDD, which elicited estrogenic effects in the E-CALUX bioassay, significantly inhibited CYP17A1

expression. It indicates a possible mechanism that estrogenic EDCs may affect the sexual steroid hormone biogenesis via the inhibition of CYP17A1 expression, and finally cause adverse effects on human reproductive health.

The expression of ARNT2 was significantly up-regulated by BPA and BBP, and down-regulated by DBP, *o, p'*-DDT, *p, p'*-DDT, TCDD, 4OH-PCB107 and 4OH-PCB187 (Figure2b). ARNT2 is a transcriptional regulator and member of the basic helix-loop-helix-PER-ARNT-SIM (bHLHPAS) protein family as well as ARNT, which is essential for the ligand-activated AhR signaling. In this study, TCDD had the highest potency to inhibit ARNT2 expression and weakly induced ERE-luciferase activity. It may imply that EDCs could adversely affect human health via a complicated ER-AhR crosstalk mechanism.

In summary, our findings imply the possible role of sexual steroid hormone synthesis and ER-AhR signaling responsiveness in the development of male genital disorders (Figure 3). More studies focusing on the susceptibility to EDCs are necessary to explore the mechanism that how environmental factors and genetic factors co-operated to affect human health via a complicated nuclear receptor signaling pathway.

Acknowledgments

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Table 1. Summary of test chemicals

Common name		Short name
Phenols	*p*-octylphenol	OP
	Bisphenol A	BPA
Phthalates	Butyl benzyl phthalate	BBP
	Di-n-butyl phthalate	DBP
	Diethyl phthalate	DEP
	Di(2-ethylhexyl) phthalate	DEHP
Persistent Organic Pollutants	2,3,3',4',5-pentachloro-4-biphenylol	4-OH-PCB107
	2,2',3,4',5,5',6-heptachloro-4-biphenylol	4-OH-PCB187
	2,3,7,8-tetrachlorodibenzo-p-dioxin	TCDD
Pesticides	1,1,1-Trichloro-2,2-bis(4-chlorophenyl)ethane	*p, p'*-DDT
	1,1,1-Trichloro-2,2-bis(2-chlorophenyl-4-chlorophenyl)ethane	*o, p'*-DDT

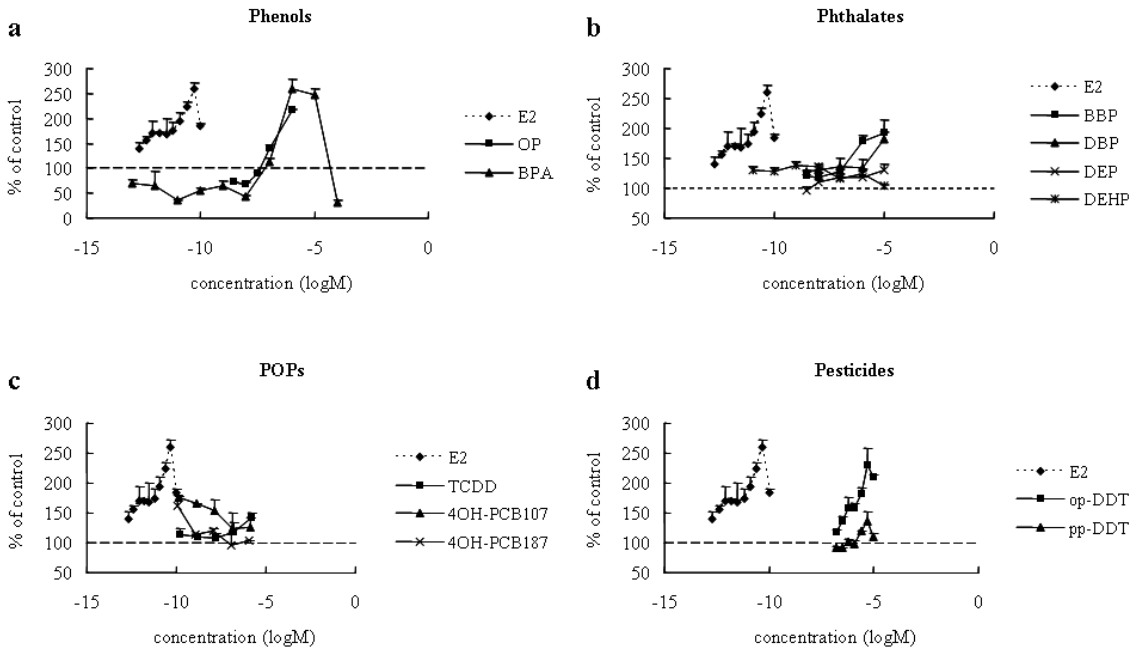

Figure 1. Dose-dependent estrogenic effect of phenols (a), phthalates (b), persistent organic pollutants (c) and pesticides (d) on induction of luciferase activity in the E-CALUX bioassay (results are expressed as mean ± S. E. M. from a representative experiment performed in triplicates).

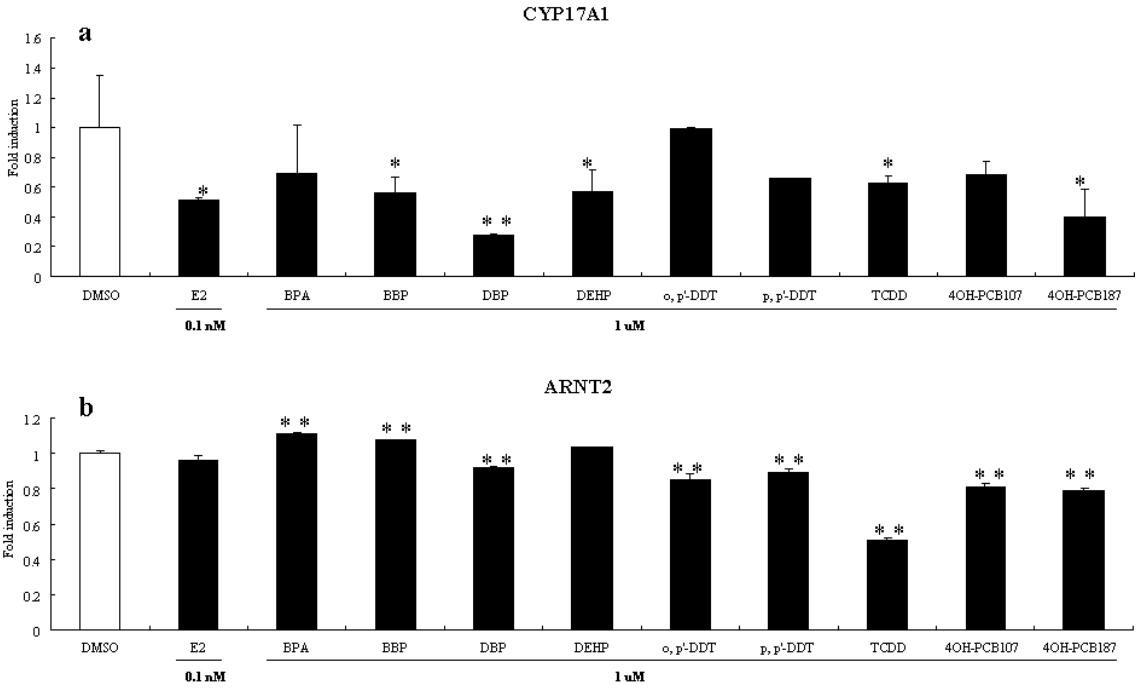

Figure 2. Expression of male genital disorders related genes CYP17A1 (a) and ARNT2 (b) regulated by EDCs. Results are expressed as fold inductions (mean ± S. E. M. from a representative experiment performed in triplicates. * $p < 0.05$; ** $p < 0.01$).

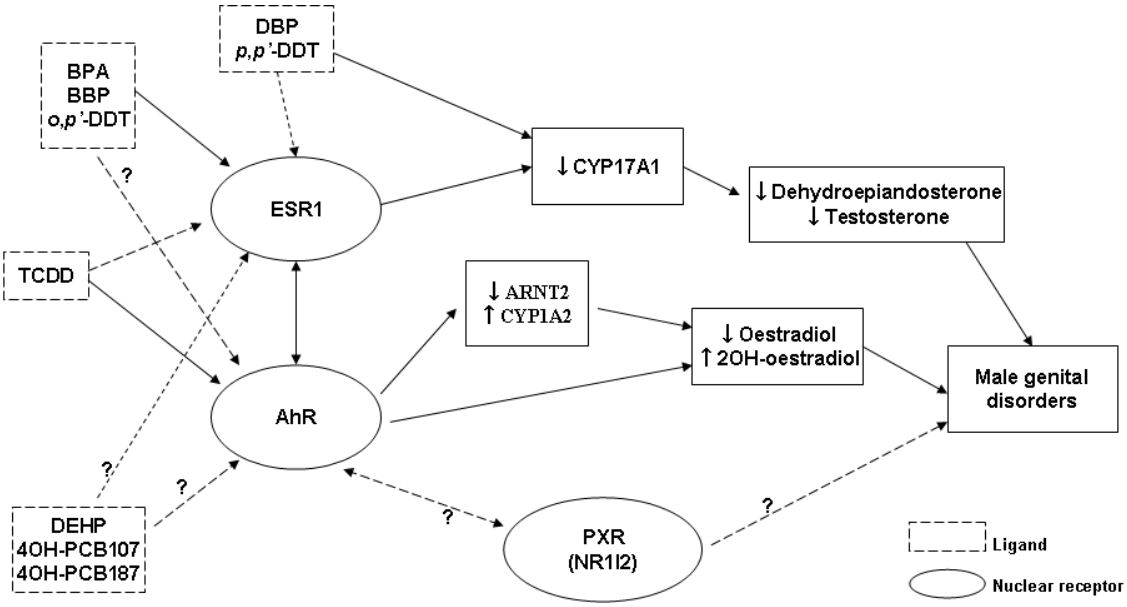

Figure 3. Potential mechanisms of reproductive effects of endocrine-disrupting chemicals (EDCs).