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Effect on metabolic enzymes and thyroid receptors induced by BDE-47 by activation the pregnane X receptor in HepG2, a human hepatoma cell line

XinNan Hu [a,b], JianQing Zhang [a,*], YouSheng Jiang [a], YiXiong Lei [b,*], LinGeng Lu [c], Jian Zhou [a], HaiYan Huang [a], Daokui Fang [a], GongHua Tao [d]

[a] Shenzhen Center for Disease Control and Prevention, Shenzhen 518055, PR China
[b] School of Public Health, Guangzhou Medical University, Guangzhou 510182, PR China
[c] Department of Chronic Disease Epidemiology, Yale School of Medicine and School of Medicine, Yale University, New Haven, CT 06520-8034, USA
[d] Shanghai Center for Disease Control & Prevention, 200336, PR China

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ABSTRACT

2,2′,4,4′-Tetra-bromodiphenyl ether (BDE-47), an important congener among polybrominated diphenyl ether (PBDE) compounds, has been predominantly in environmental samples and human tissue. Thyroid disruption is the most sensitive endpoint effect among a number of health effects of exposure to BDE-47 in animals and humans. However, the detailed underlying mechanisms in humans are not well understood. In the present study, human pregnane X receptor (hPXR)-overexpressing HepG2 cell model and a dual-luciferase reporter assay system were constructed to investigate the role of hPXR in BDE-47–induced alterations of expression of metabolic enzymes and TR in vitro. The results showed that hPXR was significantly activated by BDE-47, and expression levels of both mRNA and protein of the thyroid receptor (TR) isoforms TRα1 and TRβ1 were decreased in hPXR-overexpressing HepG2 cells after BDE-47 treatment. However, the increased expression of hepatic microsomal phase I enzyme CYP3A4 and phase II enzymes, UGT1A3 and SULT2A1 were also found. Taken together, the results indicated that BDE-47 was a strong hPXR activator, activation of hPXR played an important role in BDE-47–induced down-regulation of TR, and up-regulations of CYP3A4, UGT1A3, and SULT2A1 participated in the process, which may provide more toxicological evidence on mechanisms of disruption of thyroid hormone induced by BDE-47.

1. Introduction

The polybrominated diphenyl ethers (PBDEs) are extensively used as flame retardants in potentially flammable materials, such as plastics, rubbers, electronic insulation, and textiles (Alaee et al., 2003; Darnerud et al., 2001; de Wit, 2002; Sjödin et al., 2003). The varieties of PBDEs usages across the world have brought extensive hazard deposition to the environment, delivering harmful effects to ecologic system, botanic system and human body. With increasing use of amounts and higher concentrations of PBDEs, hazardous effects arouse great attention world widely over the past 30 years (Britta et al., 2005; Ikonomou et al., 2002). Among the 209 PBDE congeners, 2,2′,4,4′-tetra-bromodiphenyl ether (BDE-47) is the predominant congener appearing at increasing concentrations and frequencies in animal and human subjects, not only in blood plasma but also in breast milk (Law et al., 2006;

Abbreviations: PXR, pregnane X receptor; hPXR, human pregnane X receptor; PBDE, polybrominated diphenyl ethers; BDE-47, 2,2′,4,4′-tetra-bromodiphenyl ether; CYP3A4, cytochrome P450 3A4; TRs, thyroid receptors; TREs, thyroid hormone response elements; UGTs, uridine diphospho-glucuronosyltransferases; SULTs, sulfotransferases; RIF, rifampicin; T3, triiodothyronine; HepG2, human hepatocellular liver carcinoma cell line; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; QPCR, quantitative real-time polymerase chain reaction; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GFP, green fluorescent protein; RXR, retinoic acid receptor.

* Corresponding authors. Address: Shenzhen Center for Disease Control and Prevention, No. 8 Longyuan Road, Shenzhen 518055, Guangdong, PR China. Tel./fax: +86 755 25531955 (J. Zhang). Address: School of Public Health, Guangzhou Medical University, 195 Dongfengxi Road, Guangzhou 510182, PR China. Fax: +86 20 81340196 (Y. Lei).
E-mail addresses: zhjianqing95@gmail.com (J. Zhang), gz-leizeng@163.com (Y. Lei).

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Sjodin et al., 2003). A number of health effects of exposure to BDE-47 have been observed, and thyroid disruption was the most sensitive known endpoint of toxicity (Suvorov et al., 2011). A solid body of evidence has been accumulated in recent years demonstrating that BDE-47 interfered with thyroid function and altered thyroid hormone homeostasis (Darnerud et al., 2008; Suvorov et al., 2008). In particularly, the level of thyroid hormone (TH) thyroxine (T4) was reduced significantly in plasma when rats, mice, and fish exposed to PBDE (Hallgren et al., 2001; Tomy et al., 2004; Zhou et al., 2002). Furthermore, thyroid hormones are known to play a relevant role in brain development (Chan and Rovet, 2003; LaFranchi et al., 2005; Dingemans et al., 2011), and hypothyroidism has been reported that there were the association with a large number of neuroanatomical and behavioral effects (Zoeller and Crofton, 2005). However, the exact toxicological mechanisms on the disruption in thyroid hormone balance induced by PBDEs still remain unclear.

In vertebrates, thyroid hormones play key roles in regulating neural development, and their signaling is mediated through thyroid receptors (TRs) derived from two genes, TRα and TRβ. The gene-regulating activity of TRs is mediated by their binding to specific DNA sequences known as thyroid hormone response elements (TREs), which are located in the promoter regions of thyroid hormone target genes (Yen, 2001). Recent studies indicated that the transcriptional activity of TRs was further modulated via interactions with nuclear hormone receptors (Huang et al., 2006).

The pregnane X receptor (PXR), a member of the nuclear hormone receptor superfamily characterized by a ligand binding domain and a DNA binding domain, is a nuclear transcription factor that can be activated by a wide range of chemical ligands, such as pregnanes, rifampicin (RIF), and PBDEs (Ma et al., 2008). The role of PXR is known to regulate xenobiotic-metabolizing CYP enzymes after its binding to its ligand, translocation to the nuclei, and interacting with the response element of the substrate promoter to regulate the targeted gene expression. The genes regulated by PXR mainly include those encoding certain metabolizing enzymes, most notably cytochrome P450 3A4 (CYP3A) (Waxman, 1999). In addition, the phase II metabolism of xenobiotics by uridine diphospho-glucuronosyltransferases (UGTs) and sulfotransferases (SULTs) were also regulated by PXR (Karpeta et al., 2012; Tabb et al., 2004). Sanders et al. reported that CYP3A gene expression was significantly increased in C57BL6 mice administered with BDE-47 (Sanders et al., 2005). Another study indicated that UGTs and SULTs participated in the metabolism of BDE-47 (Falany et al., 2004). Recent emerging data evidenced PXR participated in the disruption of thyroid endocrine system induced by BDE-47, and such activation of PXR by BDE-47 was verified in other studies (Pacyniak et al., 2007). However, a full profile of BDE-47 on CYP enzymes and phase II enzymes has not been delineated and left a huge gap. Moreover the relationship between PXR and BDE-47–mediation disruption of the thyroid endocrine system was unclear. The present study aimed to bridge the gap via constructing a human PXR (hPXR)-overexpressing HepG2 cell model and a dual-luciferase reporter assay system to investigate the role of hPXR in BDE-47–induced alterations of CYP enzymes and phase II enzymes as well as TR expression in HepG2 cells. Therefore we can provide more toxicological evidence on mechanisms of disruption of thyroid hormone induced by BDE-47.

2. Materials and methods

2.1. Chemicals and cell culture

2,2',4,4'-Tetrabromodiphenyl ether (BDE-47) (its purity was 98%) was purchased from Cambridge Isotope Laboratories, Inc. (Andover, MA, USA). Rifampicin (RIF), G418, Dimethyl Sulphoxide (DMSO) and Triiodothyronine (T3) were all purchased from Sigma–Aldrich Corporation (Saint Louis, MO, USA).

100 μmol/L RIF was used as the positive control for activation of hPXR and its downstream genes, and 10 μmol/L T3 was used as the another positive control for BDE-47–induced alterations in TR expression.

The HepG2 cell line was provided by the Cell Bank of the Chinese Academy of Sciences, and these cells were cultured and expanded in high-glucose Dulbecco's minimum essential medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin, and 100 U/ml streptomycin at 37 °C and 5% CO2 in a humidified atmosphere.

2.2. Reporter gene assays

To examine the activation of human hPXR, HepG2 cells were transiently transfected with Lipofectamine 2000 (Invitrogen) and a cohort of purified plasmids, including *pGL3*-CYP3A4-*Luc* which contains the response elements of hPXR located in the proximal (−362/+53) and distal (−7836/−7208) promoter regions of the CYP3A4 gene linked to a firefly luciferase *pCI-neo Mammalian* expression vector (kindly provided by Professor Yanan Tian) and *Renilla* luciferase vector *pGL4.74 [hRluc/TK]* served as the internal control vector for transfection efficiency, HepG2 cells were transiently transfected with the above plasmids via Lipofectamine 2000 (Invitrogen) according to the instructions of Invitrogen. Briefly, HepG2 cells were seeded in 12-well plates, *pCI*-hPXR-*neo* or *pCI-neo Mammalian* were co-transfected into HepG2 cells with *pGL3*-CYP3A4-*Luc* and *pGL4.74 [hRluc/TK]*, respectively. The amount of each plasmid was added at 550 ng per well.

After cells exposed to BDE-47 (1,10,100 μmol/L) and RIF (100 μmol/L), the luciferase activity stimulated by *pGL3*-CYP3A4-*Luc* and renilla luciferase activity stimulated by *pGL4.74 [hRluc/TK]* was determined by a LUMINO chemiluminescence immune analyzer (Lumino, Germany) and the transfection efficiency was normalized by comparing to firefly and renilla luciferase activities. Then, luciferase activity detected from cells transfected with *pCI-neo Mammalian* was used to eliminate the influence of empty vector, and luciferase activity in cells transfected with *pCI*-hPXR-*neo* could reflect the induced effect of BDE-47 on hPXR. Folds showed in the results for each group to compare with the solvent control, in which luciferase activity set as 1. Each experiment was performed independently three times, and each dose was duplicated in an independent assay.

2.3. Construction of hPXR-overexpressing HepG2 cells

HepG2 cells were transfected using Lipofectamine 2000 according to the manufacturer's instructions. Briefly, HepG2 cells were pretreated with serum-free DMEM for 24 h after cell density reached at 90% in confluence. Then, Lipofectamine 2000 and diluted DNA plasmid were mixed together, and then added into cultured cells as the following three groups: (1) un-transfected group; (2) blank vector group (transfected with *pCI-neo Mammalia*); and (3) recombinant vector group (transfected with *pCI*-hPXR-*neo*). Afterward, 1 mg/ml G418 was used for screening and selection of the hPXR-overexpressed stable cell line, which the effect of transfection was identified by real-time PCR and western blotting.

2.4. Cell viability and chemical treatment

The viability of HepG2, HepG2-*pCI*-hPXR-*neo* and HepG2-p*CI-neo Mammalia* were measured using cell counting kit-8 (Dojindo, Japan). Cells were seeded in a 96-well plate at 103 cells/well, which