Health assessment of tetrabromobisphenol a as brominated flame retardants through peroxisome proliferator-activated receptor (PPAR)

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Introduction

Tetrabromobisphenol A (2,2-bis-(3,5-dibromo-4-hydroxyphenyl)-propane; TBBPA) is halogenated derivateves of bisphenol A (BPA), a typical xenoestrogen. This compound is used worldwide as a flame retardant in numerous products. TBBPA has been detected in sediment and mussels[1)], sewage sluge[2)] or air at the dismantling plant[3)]. Therefore, the human is highly exposed to TBBPA as it is used in consumer electronics as well as office and communication equipment; environmental TBBPA is an endocrine-disrupting chemical that can be potentially human adverse effect. Actually, previous studies have detected TBBPA in pooled human serum[4)], human milk[5)] and umbilical cords from mother-infant pairs[6)]. Thus, the studies about TBBPA as a human homeostasis disrupting factor are needed to better understand.

Peroxisome proliferator-activated receptor (PPAR) is a ligand-activated transcription factor that belongs to the nuclear hormone receptor superfamily. PPAR must form a heterodimer with RXR to bind DNA and activate transcription. PPAR has a large ligand binding pocket and is unique in its overall tertiary structure. That provides easier access for ligands[7)]. There are three isotypes of human PPAR, called α, δ and γ[8, 9)]. In particular, there are two isoforms of PPARγ denoted as PPARγ1 and PPARγ2. Until recently their actions were thought limited to specific tissue types, having roles in lipid catabolism, and peroxisome proliferation in the liver as PPARα [9)], and adipogenesis[10)] and macrophages[11)] as PPARγ. Although, PPARδ is almost ubiquitously expressed[12, 13)], due to the lack of any selective agonists or antagonists, its roles have yet to be ascertained.

The evidence that perinatal TBBPA exposure could lead to alter metabolic features was provided by Tada et al., who found that perinatal and postnatal exposure TBBPA in ICR mice resulted in an increase in serum concentrations of total-cholesterol and liver weights of treated dams and offspring[14)].

In this study, we hypothesized that TBBPA could be a ligand of PPARs or affect PPAR function. To accomplish our final purpose to elucidate the disruption of lipid and energy metabolism by this compound, as our first trial, we investigated the activities on PPARs (α, γ1, γ2, δ) in HepG2 cells by the additions of BPA and TBBPA. Further, we also measured the effect of TBBPA for expression of PPAR target genes.

Materials and methods

1) *Chemicals*

Bisphenol A, and tetrabromobisphenol A (TBBPA) purchased from Sigma-Aldrich (Prague, Czech Republic). GW7647 (PPARα agonist), GW501516 (PPARδ agonist), rosiglitazone (PPARγ1, γ2 agonist), purchased from WAKO (Japan).

Chemicals were dissolved as stock solutions in dimethyl sulfoxide (DMSO), and then the final concentration of the solvent in the culture medium was 0.1% v/v.

2) *Cell cultures*

HepG2 cells, kindly provided from Prof. T. Doi in Osaka University. The cells were maintained and cultured in a Dulbecco's modified Eagle's medium supplemented with 10% charcoal/dextran treated fetal bovin serum at 37 °C under 5% CO_2.

3) *Reporter gene assays*

HepG2 cells (3×10^4 cells/well) were seeded in 96-well plates 16–20 h before transfection. The cells were transfected with 80 ng of the reporter plasmid, 20 ng of phRL-TK (Promega, Madison, WI) and either 10 ng of pcDNA3-hPPARs expression vector. The cells were treated with the chemicals at twenty-four hours after transfection. After 24h, both firefly and *Renilla* luciferase activities were quantified using a Dual-Luciferase® Reporter Assay System (Promega).

4) *RNA isolation and cDNA synthesis*

HepG2 cells (3×10^5 cells/well) were seeded in 12-well plates 16–20h before transfection. The cells were transfected with pcDNA3-hPPARγ2 expression vector. The cells were treated with chemicals at twenty-four hours after transfection. After 48h, total RNA from HepG2 cells was isolated using isogen (WAKO). Purity and concentration of the isolated RNA was determined by measuring the absorbance ratio at 260/280nm and 230/260nm using a Nanodrop 2000 spectrophotometer (Thermo Scientific). RNA was reverse transcribed to complementary DNA (cDNA) using PrimeScript RT reagent Kit (TAKARA).

5) *Real time PCR*

Quantitative real-time PCR analyses were performed on a Thermal Cycler Dice Real Time System (TAKARA). The PCR master mixture contained SYBR Green supermix, 0,8 μM forward and reverse primer, and cDNA in a total volume of 20 μl. The following program was used for denaturation and amplification of the cDNA: 30sec at 95°C, followed by 40 cycles of 5s at 95°C and 30sec at 60°C. The expression levels of target genes were normalized using glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as an internal control.

Results and discussion:

To examine whether BPA and TBBPA have the activities on PPARs as agonists in a cell-based context, we performed reporter gene assays. The chemicals were incubated with HepG2 cells that had been co-transfected with each PPAR expression plasmid (pcDNA3-hPPARα, δ, γ1, γ2) and a PPAR-responsive reporter plasmid (PGV-P2-ACO). Although, the activity of these chemicals was different at two kind concentrations of 100 nM and 10 μM. Interestingly, TBBPA showed agonistic activity on specifically PPARγ1 and γ2 (Fig. 1). However, BPA didn't have activities of PPARs. In addition, this activity was dose-dependent increased with a one thirds of rosiglitazone as a PPARγ agonist (Fig. 2).

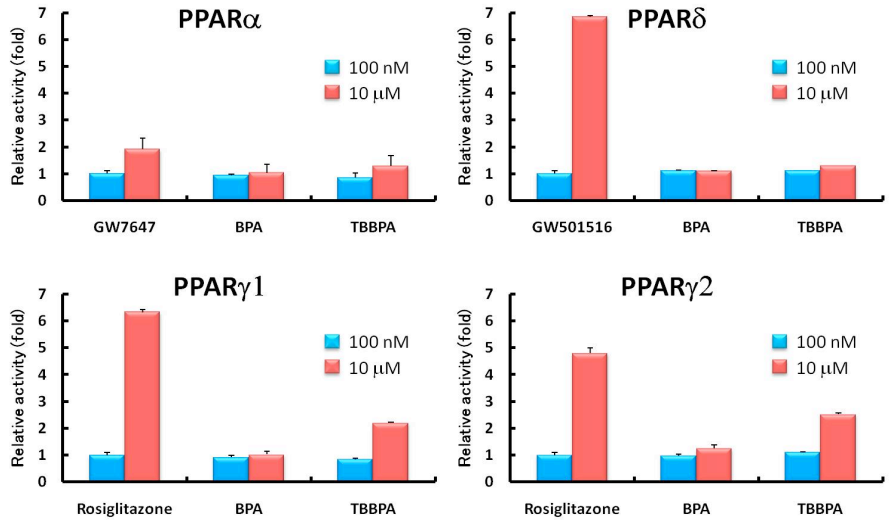

Fig. 1: Luciferase induction in HepG2 cells treated with BPA and TBBPA

Luciferase activities from reporter plasmids normalized to *Renilla* luciferase activities.

Values are expressed as fold induction of the control (DMSO). Value represent the mean ± SEM (n=3).

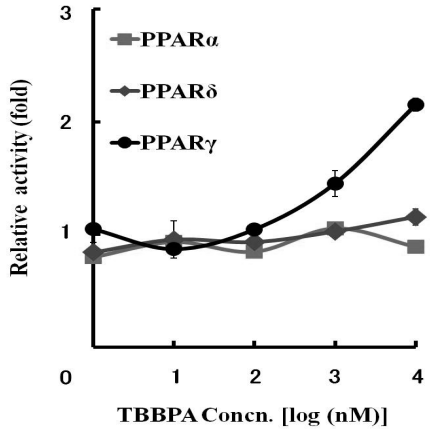

Fig. 2: Dose-response curves of luciferase induction in HepG2 cells treated with TBBPA

Luciferase activities from reporter plasmids normalized to *Renilla* luciferase activities.

Values are expressed as fold induction of the control (DMSO). Value represent the mean ± SEM (n=3).

PPAR-γ is a nuclear receptor that is activated to induce the expression of genes involved in lipid and glucose metabolism, thereby converting nutritional signals into metabolic consequences. PPARγ is the target of the thiazolidinedione (TZD) class of insulin-sensitizing drugs, which have been widely prescribed to treat type 2 diabetes mellitus. A common side effect of treatment with TZDs is weight gain. Then we measured hepatic change in expression of several metabolic genes regulated by PPARγ; angiopoietin-like protein 4 (ANGPTL4), liver-type-fatty acid binding protein (L-FABP) and phosphoenolpyruvate carboxykinase (PEPCK). We investigated in HepG2 cell expressed PPARγ. Hep G2 cells were exposed DMSO (0.1% v/v), 1 μM rosiglitazone, 100 nM or 10 μM TBBPA for 48h. Gene expression was determined by quantitative real-time RT PCR with GAPDH as reference gene. After exposure to TBBPA, mRNA levels of ANGPTL4, L-FABP and PEPCK were increasing trend at the 10 μM TBBPA (Table 1). However, these increasing degrees were less than that of rosiglitazone. That was also the same result of reporter gene assays.

In conclusion, we have found TBBPA having partial agonistic activity on PPARγ. TBBPA may be related in the disruption of lipid and glucose metabolism through PPARγ. Further study is needed to investigate toxic mechanism of TBBPA in adipose tissue and liver *in vivo*.

Table 1: Gene expression of PPARγ target genes in HepG2 cells after exposure to TBBPA

Target gene	Rosiglitazone	TBBPA 100 nM	TBBPA 10 μM
ANGPTL4	11.96	0.59	2.11
L-FABP	4.63	0.69	1.48
PEPCK	7.26	0.86	2.35

Values are expressed as fold induction of the control (0.1% DMSO).

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