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EFFECTS OF BROMINATED FLAME RETARDANTS ON TRANSCRIPTIONAL ACTIVATION MEDIATED BY THYROID HORMONE RECEPTOR

Haruya Sakai[1], Toshiko Yamada-Okabe[1], Yuji Kashima[1], Mitsuaki Matsui[1], Takashi Aono[1], Mitsuhiro Aoyagi[2] and Jun Hasegawa[3]

[1]Department of Hygiene, Yokohama City University School of Medicine
3-9 Fukuura, Kanazawa-ku, Yokohama 236-0004, Japan
[2]Graduate School of Environment Information & Sciences, Yokohama National University
79-7 Tokiwadai, Hodogaya-ku, Yokohama 240-8501, Japan
[3]Department of Environmental Conservation, Ehime University
3-5-7 Tarumi, Matsuyama , Ehime 790-8566, Japan

Introduction

Production of Brominated flame retardants (BFRs) has been increased in recent years. These compounds have been detected in environmental samples such as sediments[1], fish[2], birds[3] and marine mammals[3], furthermore, even in human breast milk[4] and blood[5, 6]. Because these compounds have some structural similarity to thyroid hormone (T4 and T3), it was speculated that they may disrupt thyroid hormone systems in mammals. Some studies indicates that the exposure of certain BFRs to the laboratory animal alters thyroid hormone level in blood[7]. It also showed that some BFRs are potent competitors of T4 for binding to human transthyretin *in vivo*[8]. T3, which acts as thyroid hormone biologically, transmits its signals through binding to thyroid hormone receptors (TRs). TRs are ligand-induced transcription factors that regulate target genes and are expressed in various organs. However, whether BFRs interfere thyroid hormone action at transcriptional level has not been clarified. Recently we established the cell line, HeLaTR which overexpressed human TRα_1 and could response to T3 because the expression level of TRs are very low in available cell lines derived from human[9]. In this study, we report the establishment of the HeLaTRDR4-luc cell line to determine the transcriptional activation mediated by TR and thyroid response element (TRE), and the effects of BFRs using HeLaTRDR4.

Material and Methods

Plasmid and Cell: Characterization of HeLaTR cells, in which human TRα_1 was overexpressed under CMV promoter and responded to T3, was described elsewhere[9]. One copy of thyroid hormone response element was inserted into upstream of the firefly luciferase gene in pGL2-promotor vector and designated as DR-4(TRE)-Luc. HeLaTR cells were cotransfected with DR-4(TRE)-Luc and pSV2hph and selected with hygromycin B. Among stable transfectants, HeLaTRDR4-luc showed elevated level of luciferase activity in the presence of T3 and were routinely maintained in Dulbecco's MEM (DMEM) supplemented with 5% fetal bovine serum (FBS) for further assays[9].

Luciferase Assay: Cells were pretreated with phenol red free DMEM without FBS for 3 days, then plated on 24-well plate and maintained in phenol red free DMEM supplemented with 5% charcoal-stripped FBS (assay medium) for 12 hours. Cells were cultured in fresh assay medium containing indicating concentrations of BFRs in the presence or absence of T3 for further 36 hrs. Cells were harvested and lysed using lysis buffer (Promega). After brief centrifugation, crude cell extract was recovered and luciferase activities were determined using luciferase assay system (Promega) and protein concentrations using BCA protein assay reagent (Pierce).

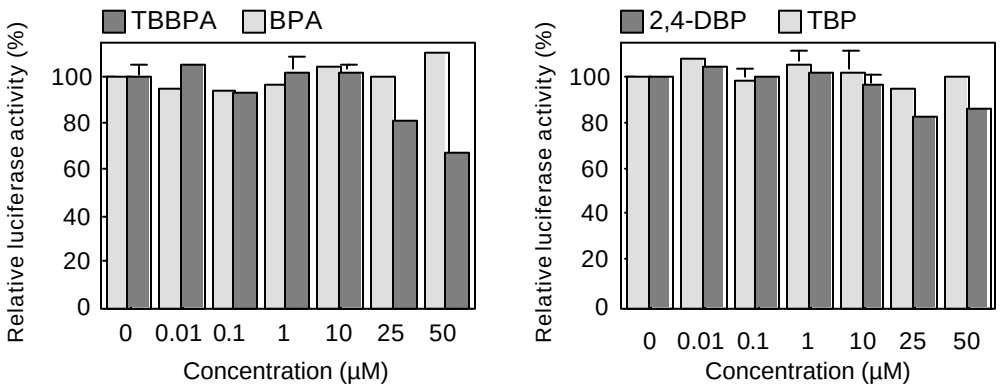

Fig. 1. TBBPA (left) and 2,4-DBP (right) suppressed TRE mediated transcriptional activation on HeLaTRDR4-luc. Cells were grown in the presence of T3 (60 nM) and indicated amount of BFRs or their related chemicals.

Results and Discussion

Four brominated flame retardants (2,4,6-Tribromophenol [TBP], Decabromodiphenyl ether [DBDE], Hexabromocyclododecane [HBCD], Tetrabromobisphenol A [TBBPA]) and their related chemicals (2,6-Dibromophenol [DBP], 2,4-DBP, Bisphenol A [BPA]) were tested for transcriptional activation through TR and TRE using HeLaTRDR4-luc. We found the antagonistic effect of TBBPA and 2,4-DBP. The presence of over 25 µM TBBPA or 2,4-DBP suppressed TRE mediated transcriptional activation by T3 (Fig. 1), and suppression by TBBPA is higher than 2,4-DBP. In Norway TBBPA have been detected in human serum under 1.3 pmol/g lipids[5], and in Sweden 2,4-DBP in human blood plasma[6]. In contrast to TBBPA and 2,4-DBP, BPA, TBP and 2,6-DBP did not inhibit transcriptional activation by T3. These results suggest that hydroxylation at the *para* position with one bromine atom adjacent to carbon, and *para*- position of opposite dibrominated phenol ring or bromine are essential for this inhibition.

HBCD which doesn't have phenol structure showed agonistic effect. Even 5 µM HBCD stimulate TRE mediated transcriptional activation. And much higher activation was observed in the presence of T3 (Fig. 2). Few studies have reported that environmental pollutants including BFRs could activate TRE mediated transcriptional activation. Further studies are needed to investigate the structural similarity between HBCD and T3.

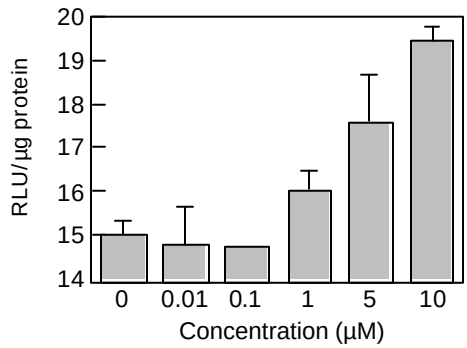

Fig. 2. HBCD stimulate TRE mediated transcriptional activation on HeLaTRDR4. Cells were grown in the presence of T3 (60 nM) and indicated amount of HBCD. RLU, Relative light units.

TBBPA and polybrominated diphenyl ethers are used for BFRs most widely, while TBP and HBCD are also used recently[10]. BFRs are extensively applied for a large varieties of electronic household equipment such as television and computer. There is a possibility that the

BFRs might be released into environment during production, usage, and disposal from the flame retarded products, and could be detected in wildlife[1, 2, 3] and humans[4, 5, 6]. The concentrations of TBBPA in serum from Norway has been increasing during 1977 to 1999. These results suggests that BFRs (TBBPA and HBCD) and its related compound (2,4-DBP) may disrupt thyroid hormone status, in part, by affecting TRE mediated transcriptional activation, which may influence growth and development. Further studies are necessary to monitor BFRs in biological sample especially from human samples for toxicological assessments.

Acknowledgements

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