Comparison of *In Vitro* and *In Vivo* Cytochrome P450-1A Activities Induced by Structural Difference of TXDDs and DL-PXBs

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Introduction

Polybrominated chlorinated biphenyls (PXBs) belong to a class of structurally similar chemicals known as polyhalogenated aromatic hydrocarbons, which includes human homeostasis disruptors such as polychlorinated biphenyls (PCBs) and polybrominated biphenyls (PBBs). Recently, we firstly reported the occurrence of PXBs in market fish samples collected from various global regions [1]. However, there are only a few reports with PXBs contamination until present [2, 3]. One of the reasons is that there are a limited number of PXBs commercially available and those are all co-planar. The introduction of a second halogen into the polychlorinated biphenyl moiety increases the number of possible congeners from 209 to 9180. Therefore, little is currently known about PXBs contamination sources, and contamination levels of PXBs in environment, food and human [4], and there is no report with their toxicities.

The aromatic hydrocarbon (Ah) receptor is a basic mediator of toxic of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), which appeared to be one of the most dangerous environmental contaminants. The bound receptor regulates the transcription of specific genes involved xenobiotic metabolism, with cytochrome P450 1A1 and 1A2 the most extensively studied examples. Safe *et al.* [5] was reported the quantitative *in vivo* structure-activity relationships with AHH induction for 2,3,7,8-TeCDD, 2,3,7,8-TeBDD, 2,3-diBr-7,8-diCl-DD and 2-monoBr-3,7,8-triCl-DD, similar activity against four congeners was observed. Therefore, the exposure to Co-PXBs also may give rise to adverse effects for human health since these compounds have properties similar to PCDD/Fs, PBDD/Fs and PXDD/Fs.

In this paper, we investigated the cytochrome P450-1A activities of TCDDs, TBDD, DL-PCB #126 and three kinds of DL-PXBs #126, in comparison with their difference of activities with *in vitro* and *in vivo* experiments.

Material and methods

1) *Cell culture*

HepG2 cells, a human hepatocarcinoma cell line, and Caco-2 cells, a human intestinal cell line, were cultured in

Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS) at 37 ˚C.

2) *Chemicals*

2,3,7,8-TCDD, 2,3,7,8-TBDD, 3,3',4,4',5-PeCB (#126), 4'-Br-3,3',4,5-PCB (Co-PXB-1Br), 3,4-Br-3',4',5'-Cl-PXB (Co-PXB-2Br), 3'4'5'-Br-3,4- PCB (Co-PXB-3Br) and 3,3',4,4',5'-PBB purchased from Cambridge Isotope Laboratories (MA, USA).

3) *In vitro EROD activity*

Cell-mediated cytocrome P450-1A activities were determined by ethoxyresorufin-*O*-deethylase (EROD) activity. Confluent HepG2 cells and Caco-2 cells, in 96-well microplate, were exposed to various organic environmental pollutants for 24 h at the indicated concentration. The cells were washed with PBS and incubated for 1 h at 37 ˚C with 5 mM 7-ethoxyresorufin in DMEM supplemented with 10% FBS. Resorufin-associated fluorescence was measured at 550-nm excitation and 595-nm emission using a SPECTRA FLUOR (TECAN).The EROD activity was normalized to the cell protein content, determined with the Bradford regent.

4) *In vivo EROD activity*

Female C57BL/6 mouse (6-8 weeks old) were administered a single intraperitoneal injection of dioxins and their related compounds in the saline containing 1% ethanol and 10% Tween20. Day 2 after the administration, mouse was sacrificed under the anesthesia. Liver were homogenized in 0.1 M Tris-acetate buffer (pH 7.4). Homogenates were centrifuged at 9,000 ×*g* for 15 min at 4 ˚C, and the supernatant was centrifuged at 105,000 ×*g* for 45 min at 4 ˚C. The resulting pellet was homogenized in 0.1 M potassium pyrophosphate buffer (pH7.4). The homogenate was centrifuged at 105,000 ×*g* for 45 min at 4 ˚C. The microsomal pellet was homogenized in 10 mM Tris-acetate buffer (pH 7.4) and stored at -80 ˚C until use. Protein concentrations in microsomes were determined according to the Lowly method with bovine serum albumin as the standard. Diluted liver microsomes and the reaction mixture (50 mM KPO_4, 5 mM $MgCl_2$, 0.5 mM $NADP^+$, 1 IU/ml G-6-P dehydrogenase and 2.4 μM 7- ethoxyresorufin) were preincubated for 5 min at 37 ˚C. The reaction was initiated by adding 5 mM glucose-6-phosphate and carried out at 37 ˚C for 10 min. Resorufin production was measured a SPECTRA FLUOR.

Result and discussion

To examine whether the organic environmental pollutants have the activities on cytochrome P450-1A in a cell-based context, we performed *in vitro* EROD assay. The chemical were incubated with human hepatocarcinoma HepG2 cells and human intestinal Caco-2 cells. The EROD activities on HepG2 cells induced by 2,3,7,8-TCDD and 2,3,7,8-TBDD increased in a dose-dependent manner (Fig. 1). Although TCDD and TBDD were similar dose-response curves, EROD activity of TBDD was slightly lower than that of TCDD (Fig.

1A). Mason *et al.* reported *in vivo* and *in vitro* structure-activity relationships of various polyhalogenated dioxins and furans, AHH activity induced with TBDD is higher than that with TCDD [6], however, it is presently unclear the reason of difference between their data and our data. As shown in Fig. 1B, it was observed the patterns of dose-response curve on 3',4',5'-Br-3,4-Cl-PXB (Co-PXB-3Br) and 3,3',4,4',5'-PBB were different from those on 3,3',4,4',5'-PCB, 4'-Br-3,3',4',5-Cl-PXB (Co-PXB-1Br) and 3,4-Br-3',4',5'-Cl-PXB (Co-PXB-2Br). Thus, the introduction of bromine into the polychlorinated biphenyl tends to their higher activity. The relative EROD activity at the concentration of 100 nM of PCB, Co-PXB-1Br, Co-PXB-2Br, Co-PXB-3Br, and PBB showed 6.36, 14.9, 14.7, 51.1 and 42.3 fold against the value of control, respectively. In the case of Caco-2 cells, similar tendency was observed.

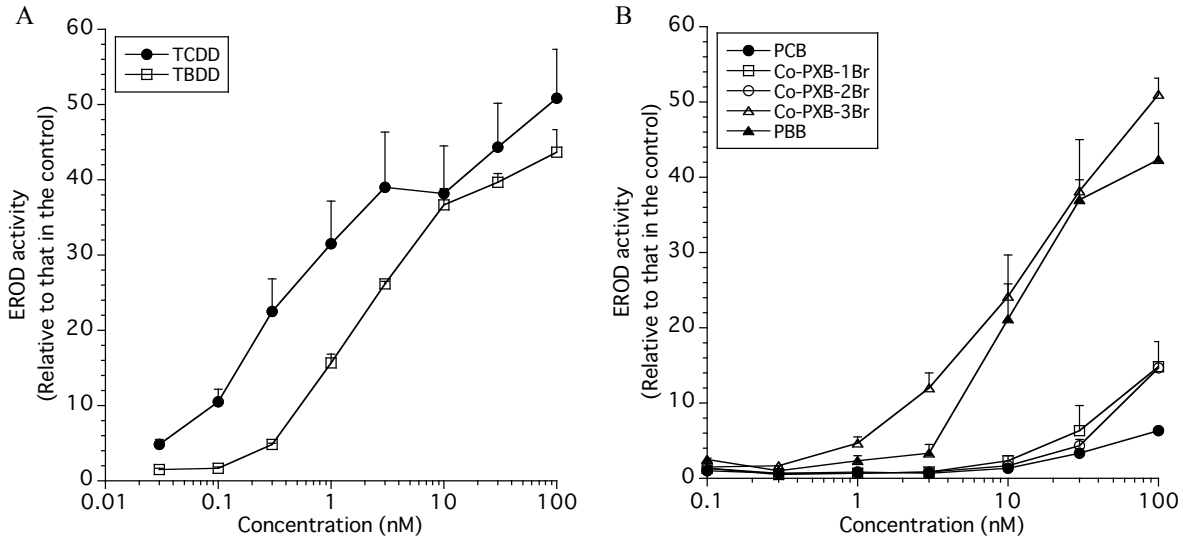

Fig. 1 *In vitro* EROD activities induced with (A) TXDDs and (B) Co-PCBs, Co-PBBs and Co-PXBs

The EROD activity was expressed as fold-induction of the control. Value represent the mean ± SD (n=3).

To ensure the difference of *in vitro* EROD activities by coplanar polychlorinated and/or brominated biphenyl, we performed *in vivo* EROD assay for C57BL/6 mouse, and EROD activities of their liver microsomes were measured. Similar activities were observed at 100 nmol/kg as high concentration of all congeners tested. On the other hand, at 10 nmol/kg as low concentration, PBB and Co-PXB-3Br were slightly decreased, however, other congeners was remarkably decreased. Their relative activity of PCB, Co-PXB-1Br, Co-PXB-2Br, Co-PXB-3Br and PBB indicated 1.6, 1.7, 2.2, 8.1 and 7.8 fold against control, respectively. From these results, it is clear that higher brominated congeners indicated higher EROD activity in *In* vitro and *In vivo* experiments.

Further study is needed to clarify their metabolism and the disruption of human homeostasis by Co-PXBs.

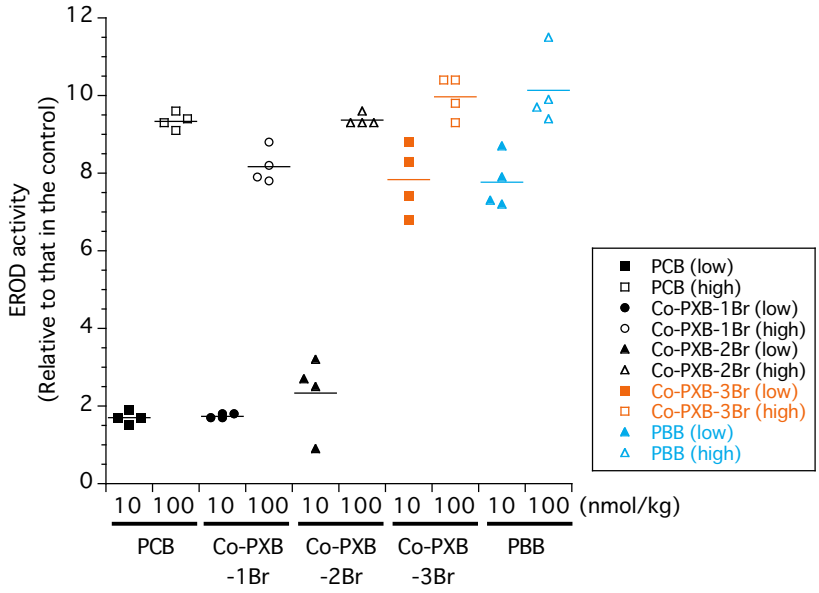

Fig. 2 *In vivo* EROD activities induced with Co-PCBs, Co-PBBs, and Co-PXBs at the oral administration of 10 (Low) or 100 (High) nmol/kg of each congeners

The EROD activity was expressed as fold-induction of the control. Data are from n=4 mice.

Reference

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