DETERMINATION OF ESTROGEN RECEPTOR β-MEDIATED ESTROGENIC POTENCIES OF HYDROXYLATED PCBS BY A YEAST TWO-HYBRID ASSAY

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Introduction

Several environmental phenolic chemicals such as Nonylphenol and Bisphenol A (BPA) have been previously shown to possess estrogenic properties. In the previous paper, we have investigated the estrogenic activity of a series of hydroxylated PCBs (OH-PCBs) by a yeast two-hybrid assay (estrogen receptorα (ERα) -TIF2), in which the expression of estrogenic activity is based on the interaction of chemicals with ERα, and demonstrated that 4'-OH-CB30 and 4'-OH-CB61 are more estrogenic than BPA, one of the environmental estrogens [1,2]. We have showed that one chlorine substitution adjacent to 4-OH at 3- or 5-position significantly reduces the ERα-mediated estrogenic activity of 4-OH-PCBs[1,2]. Thus, 4'-OH-CB25 and 4-OH-CB56 showed a very weak estrogenicity. We have also showed that 4-OH-PCBs with two chlorine substitutions adjacent to 4-OH at 3- and 5-position such as 4'-OH-CB79 (hydroxylated metabolite of CB77) and persistent 4-OH-PCBs retained in human blood (4-OH-CB107, 4-OH-CB146 and 4-OH-CB187) have no ERα-mediated estrogenic activity [1,2].

ER is known to have two subtypes, namely ERα and ERβ and it is reported that ligand, some agonist and antagonist have a different binding affinity for ERα and ERβ. However, there is limited information on ERβ-mediated endocrine disrupting potency.

In this study, we examined the ERβ-mediated estrogenic activity of a series of OH-PCBs, including environmentally relevant 4-OH-PCBs by a yeast two-hybrid assay (ERβ-TIF2).

Methods and Materials

All OH-PCBs used in this study were synthesized as reported previously except for 4-OH-CB39 [3]. 4-OH-CB39 was purchased from AccusStandard. The chemical structures tested in the yeast two-hybrid assay are presented in Figure 1.

The ERβ-mediated estrogenic activity of OH-PCBs was examined using a yeast two-hybrid assay system with the ERβ and the coactivator, transcriptional intermediary factor 2 (TIF2), as described previously[4]. Briefly, the yeast cells were preincubated overnight at 30°C in SD medium free from tryptophan and leucine. The culture (250 µl) was then mixed in a small test tube with a DMSO

solution (2.5 µl) containing test chemicals and incubated for 4 hr at 30°C. After collecting the cells by centrifugation, the cells were digested enzymatically by incubation with 200 µl of Zymolyase (1 mg/ml) at 37°C for 15 min. The lysate was mixed with 40 µl of 2-nitrophenyl-β-D-galactoside (4 mg/ml) and reacted at 30 until development of the yellow color before the reaction was stopped by addition of 1 M Na_2CO_3 (100 µl). β-Galactosidase activity was determined as described previously.

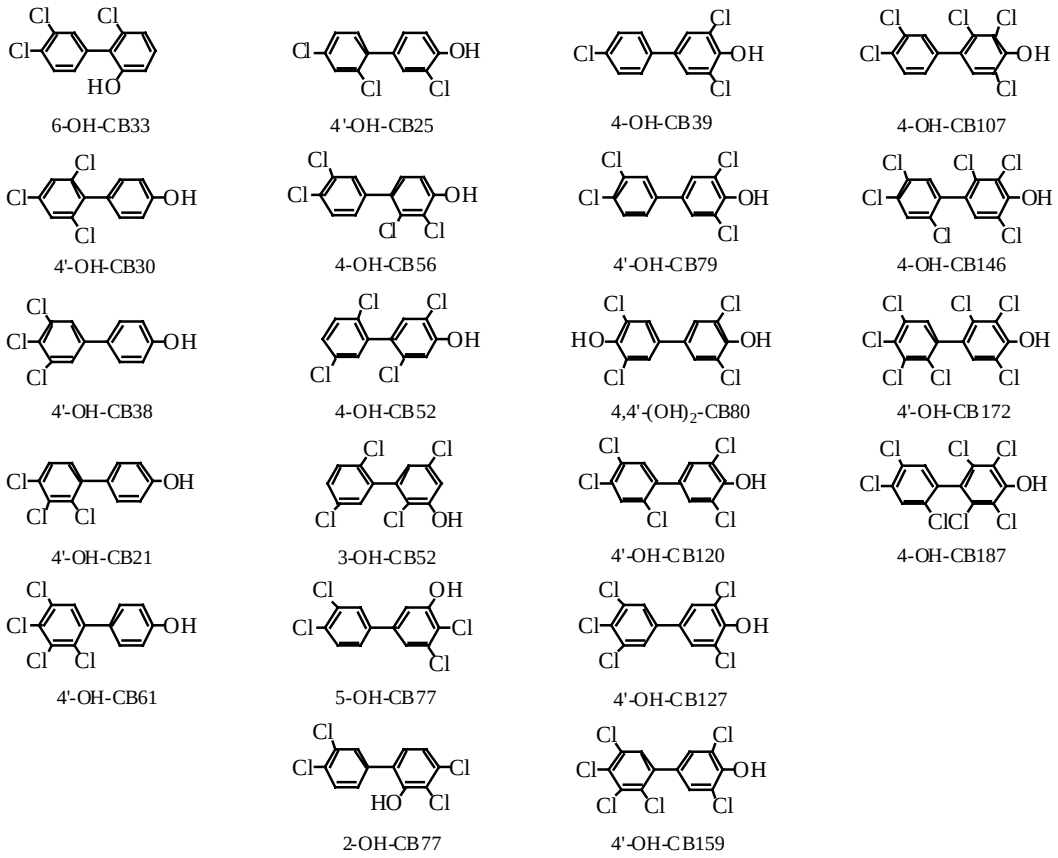

Figure1. Chemical structures of hydroxylated PCBs used in this study.

Results and Discussion

In this yeast two-hybrid assay system (ERβ-TIF2), 5×10^{-11} M 17β-estradiol (E2) caused a slight induction of β-galactosidase activity and this induction increased with E2 concentration (5×10^{-11} M ~ 1×10^{-7} M) as shown in Fig.2. The induced β-galactosidase activity was almost saturated at 1×10^{-8} M of E2.

4'-OH-CB30, 4'-OH-CB61, a mixture of 4'-OH-CB21 and 4'-OH-CB38 (1:1) and 4,4'-$(OH)_2$-CB80 (dihydroxylated metabolite of CB77) were found to show a significant ERβ-mediated activity. The relative estrogenic potencies were evaluated by the concentrations of test compounds showing 10% activity of 5×10^{-10} M E2, REC10 (10% relative effective concentration). The REC10 of E2 was 1.12×10^{-11} M ~ 2.15×10^{-11} M and the relative potencies of test compounds were determined based on each REC10s to that of E2 (Table 1, E2:100). The relative potencies of OH- and $(OH)_2$ -PCBs mentioned above were 1.35, 0.81, 0.19~0.47 and 0.035, respectively and were much higher than those of 4-Nonylphenol (0.012) and BPA (0.002). We have previously reported that these OH- and $(OH)_2$ -PCBs also exhibit a strong ERα-mediated estrogenicity, with the relative potencies within the range of 1.0~ 0.006 [1,2]. Especially 4'-OH-CB30 was found to be the most potent agonist for both ERα and ERβ. It is noteworthy to point out that 4-OH-PCBs with no chlorine substitutions on 4-OH phenyl ring possess high estrogenic activity except for 4,4'-$(OH)_2$-CB80.

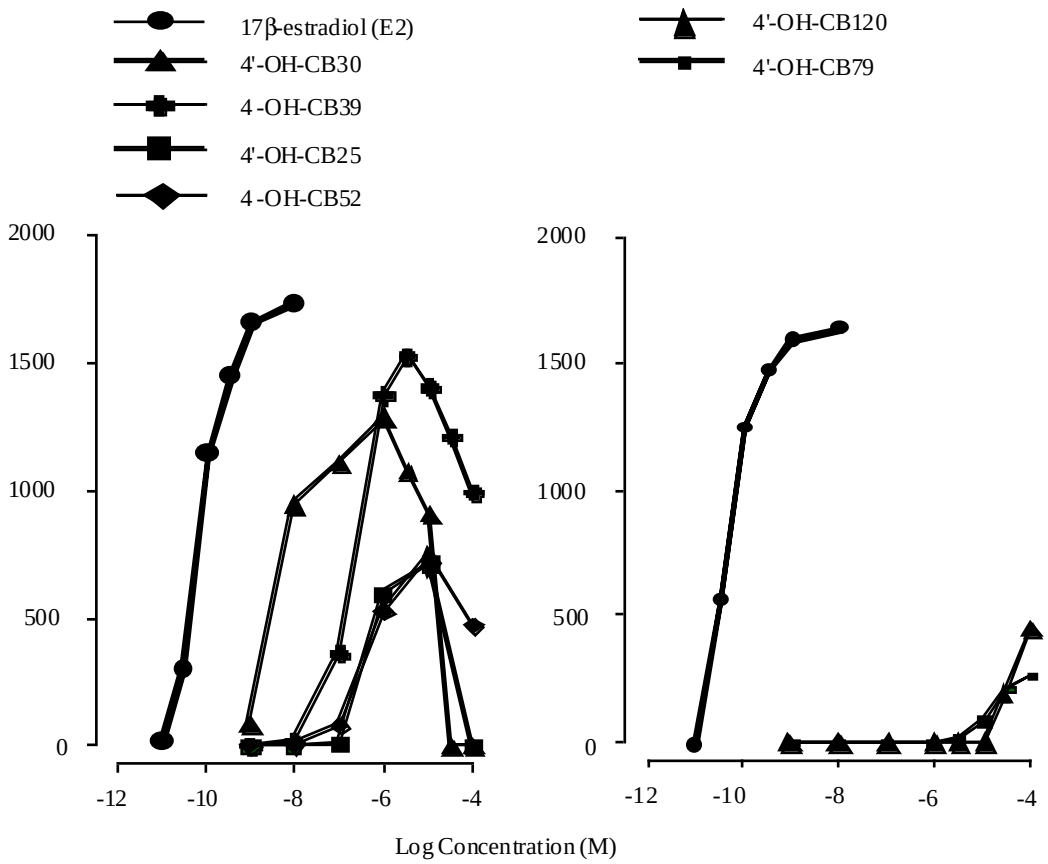

Figure 2. ERβ-mediated estrogenic activity of several OH-PCBs in the yeast two-hybrid assay.

Y axis : β -Galactosidase activity unit

4'-OH-CB25 and 4-OH-CB52 (4-hydroxylated metabolite of CB52) which have one chlorine substitution adjacent to 4-OH at 3- or 5-position (4-OH-3-chloro- type) showed a weak ERβ-mediated estrogenic activity, with relative potencies being 0.007 and 0.004~0.007, respectively, although the ERα-mediated estrogenic activity of 4-OH-CB52 was not detectable.

In the previous study, none of 4-OH-PCBs with two chlorine substitutions at 3- and 5-position (4-OH-3,5-dichloro- type) such as 4-OH-CB39, 4'-OH-CB79 (hydroxylated metabolite of CB77), 4'-OH-CB120, 4'-OH-CB127 (hydroxylated metabolite of CB126) and 4'-OH-CB159 showed the ERα-mediated activity [1,2]. On the other hand, in the present study, some of 4-OH-PCBs with 3,5-dichlorosubstituton were found to exhibit ERβ-mediated activity. 4-OH-CB39 was a significant estrogenic with relative potency 0.03~0.05, while 4'-OH-CB79 and 4'-OH-CB120, minor components of 4-OH-PCBs retained in human blood, showed a weak estrogenic activity, albeit at

concentrations exceeding 10μM. The relative potencies were in the range of 0.00003 ~ 0.00005. However, other 4-OH-PCBs with 3,5-dichlorosubstitution, 4'-OH-CB127 (hydroxylated metabolite of CB126) and 4'-OH-CB159, which were also detected in human blood, did not show the ERβ-mediated estrogenic activities.

Persistent 4-OH-PCBs (4-OH-2,3,5-trichloro- type) selectively retained in human blood as major components, 4-OH-CB146, 4'-OH-172 and 4-OH-CB187, were found not to be ERβ-mediated estrogenic, as well as the results previously obtained with ERα.

Any of 2-OH- or 3-OH-PCBs did not exhibit the ERα-mediated estrogenic activity in the previous study. In this study, most of the 2-OH- and 3-OH-PCBs including 2-OH-CB77 and 5-OH-CB77 (3-hydroxylated metabolite of CB77) also did not show the ERβ-inducing activity. However, several 2-OH- and 3-OH-PCBs such as 6-OH-CB33 and 3-OH-CB52 (3-hydroxylated metabolite of CB52) were found to be weakly ERβ-mediated estrogenic with relative potencies 0.001 and 0.0003 ~ 0.0007, respectively.

Table 1. ERβ-mediated estrogenic potency of hydroxylated PCBs in the yeast two-hybrid assay (ERβ-TIF2)

Congener	Relative potency *	Congener	Relative potency *
6-OH-CB33	0.001	4-OH-CB52	0.004~0.007
4'-OH-CB30	1.35	3-OH-CB52	0.0003~0.0007
4'-OH-CB38 + 4'-OH-CB21	0.19~0.47	4-OH-CB39	0.03~0.05
4'-OH-CB61	0.81	4'-OH-CB79	0.00003
4'-OH-CB25	0.007	4,4'-$(OH)_2$-CB80	0.035
4-OH-CB56	0.008	4'-OH-CB120	0.00005

* 17β-estradiol (E2): 100

In conclusion, a number of OH-PCBs, including several 2-OH- and 3-OH-PCBs, as well as 4-OH-PCBs, showed ERβ-mediated estrogenic activity with relative potencies varying 1.35 ~ 0.00003, and the estrogenic activity profile of OH-PCBs with ERβ was different from that of ERα. 4'-OH-CB30 is the most potent agonist for both ERα and ERβ. Chlorine substitution(s) adjacent to 2-OH-, 3-OH- or 4-OH- significantly reduced the ERβ-mediated estrogenic potency. Of the persistent OH-PCBs retained in human blood, only 4'-OH-CB79 and 4'-OH-CB120 exhibited the ERβ-mediated estrogenic activity at M concentrations.

References

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