ANTIESTROGENECITY OF POLYCHLORINATED BIPHENYLS (PCBS) IN IMMATURE FEMALE RAT

Soon Sun Kim[1], So Hee Kim[1], Gyu Seek Rhee[1], Seung Jun Kwack[1], Kyung Hee Sohn[1], Rhee Da Lee[1], Byung Ho Kim[1], Chul Hoon Park[1], Sang Mi An[1], Ki Eun Joung[2], Yhun Yhong Sheen[2], Bum Soo Ahn[3], Eui Bae Jeung[3], Kui Lea Park[1]

[1]National Institute of Toxicological Research, Korea FDA, Seoul 122-704, Korea,
[2]College of Pharmacy, Ewha Womans University, Seoul 120-750, Korea
[3]College of Veterinary Medicine, Chungbuk National University, Chung-Ju 361-736, Korea

Introduction

Based on the results of previous studies, exposures to environmental chemicals can be adversely affect reproductive systems of wildlife and mammals including human. Polychlorinated biphenyls (PCBs) are now widely contaminated and accumulated throughout the world due to their high lipophilicity and chemical resistance, although production of these chemicals are banned in many countries. Various studies about estrogenic/antiestrogenic activity of PCBs have been reported, but the results of these reports are controversal. The Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC) proposed hormone receptor mediated reporter gene assays as *in vitro* pre-screening tests to investigate the endocrine disrupting properties of chemicals in 1998 (1). Reporter gene assay technique using MCF-7 human breast cancer cell has been considered to be a valuable model for screening endocrine disrupting chemicals with estrogenic activity, because it can detect estrogen responsive elements (2). In addition, the Organisation for Economic Cooperation and Development (OECD) proposed the immature rat uterotrophic assay as one of the screening test methods for investigating the estrogenic properties of endocrine disrupting chemicals in 2001 (3). Calbindin-D_{9k} (CaBP-9k) gene, one of intracellular calcium binding proteins, is estrogen responsive in the uterus (4,5,6). In the present study, we examined antiestrogenicity of PCB-118, -138, -153 and -180 *in vitro* (luciferase reporter gene assay) and *in vivo* (immature rat uterotophic assay and calbindin-D_{9k} gene expression assay). In the luciferase reporter gene assay, we use MCF-7 cell lines stably transfected with pERE-luc construct, which consists of three estrogen response element (ERE) and luciferase reporter gene. All PCBs (5 mM) tested significantly inhibited the induction of luciferase activity by E2. In the uterotrophic assay using 18-day old SD rats, PCBs (0.1 ?/?) led to significant decreases in absolute and relative uterine wet weight, and statistical-significance at certain doses. Also, the PCBs inhibited increase of uterine wet weight by E2. Northern blot analysis showed reduction of the calbindin-D_{9k} mRNA expression in response to the PCBs as well as E2 treatment. Our results indicate that PCB 118,138,153 and 180 act as estrogen antagonist in immature female rat.

Methods and Materials

Animals

Immature female SD rats (18-day old) rats were provided by the National Institute of Toxicological Research, KFDA (Seoul, Korea). Animals used in this experiment were handled in accordance with NITR guide for the care and use of laboratory animals.

Reporter gene assay

MCF-7-ERE cells were maintained in 10% charcoal treated FBS-supplemented phenol red-free DMEM (assay medium) for four days before the plating in 96-well plate. Cells were plated in a 96-well

plate. After 24 hrs incubation, cells were treated with various chemicals for 17 hrs. After chemical treatment, cells were washed with PBS and then lysed in reporter lysis buffer. Cell lysate were mixed with luciferase assay reagent (luciferin). Light output was measured with the Luminometer.

Uterotrophic assay

Immature female rats (18-day old, about 61g) were randomly assigned to the control and treatment group, corn oil as a vehicle control, 17ß-estradiol (E2, 3 ?/?/day) as a positive control or PCBs (0.1 ?/?/day) was subcutaneously injected from postnatal day (PND) 19 to 21. The volume of corn oil contained in PCBs solution for subcutaneous injection was 5 ?/?. Animals were sacrificed by cervical dislocation 24hr after the last treatment. The uteri and vaginae were removed, trimmed free of fat, and then weighed.

Northern blot analysis of calbindin-D9k (CaBP-9k) mRNA

For Northern blot analysis of CaBP-9k mRNA, uterine tissues obtained from uterotrophic assay were snap-frozen in liquid nitrogen. 3 frozen uteri per group were homogenized individually, and total RNA was extracted using Trizol reagent (Life Technologies, Rockvill, USA).

Statistical analysis

Results regarding experimental data were presented as mean ± SD or mean ± SEM. The differences between control and treatment groups was performed by One Way Analysis of Variance (ANOVA), followed by Dunnett's Method. The statistical significance was evaluated at levels of 0.01 and 0.05.

Results and Discussion

Reporter gene assay

MCF-7 cells exhibited increase of luciferase activity on addition of 0.01nM estradiol (about 28-fold of the control). All PCB (5 mM) tested inhibited the induction of luciferase activity by E2, and this effect was statistically significant ($P<0.01$) except for PCB-180 (Fig. 1).

Uterotrophic assay

All PCB (0.1 ?/?) tested led to significant ($P<0.01$) decreases in absolute and relative uterine wet weight (Fig. 2) and also inhibited increase of uterine wet weight by E2 (Fig. 3).

Cabindin D_{9k} mRNA expression

Northern blot analysis showed reduction of the calbindin-D_{9k} mRNA expression in response to PCB-118 (Fig. 4) as well as E2 treatment (Fig. 5).

Acknowledgment

We greatly appreciate the National Institute of Toxicological Research, Korea Food and Drug Administration, for providing a grant for this work.

References

1. EDSTAC (1998), Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC). Final report.
2. Legler, J., van den Brink, C.E., Brouwer, A., Murk, A.J., van der Saag, P.T., Vethaak, A.D., van der Burg, B. (1999), Toxicol. Sci. 48, 55
3. OECD (2001), Organisation for Economic Cooperation and Development (OECD). Third meeting of the validation management group for the screening and testing of endocrine disrupters (mammalian effects). Joint meeting of the chemicals committee and the working party on chemicals, pesticides and biotechnology.
4. Krisinger J., Dann J.L., Currie W.D., Jeung E.B., Leung P.C..(1992) Endocrinology 86, 119
5. Darwish H., Krisinger J., Furlow JD, Smith C., Murdoch FE, DeLuca HF. (1991), J Biol Chem 266(1), 551
6. L'Horset F., Blin C., Colnot S., Lambert M., Thomasset M., Perret C. (1994), Endocrinology 134(4), 11

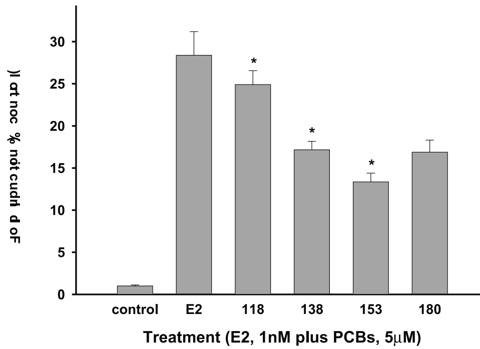
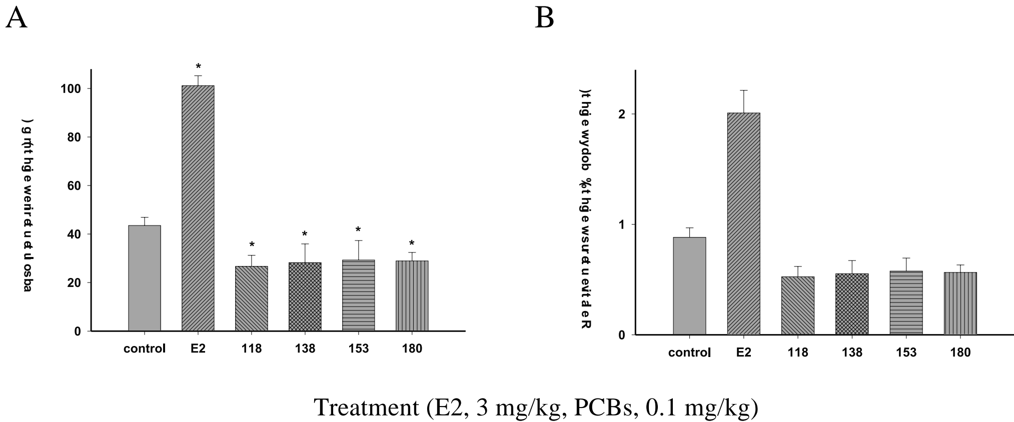
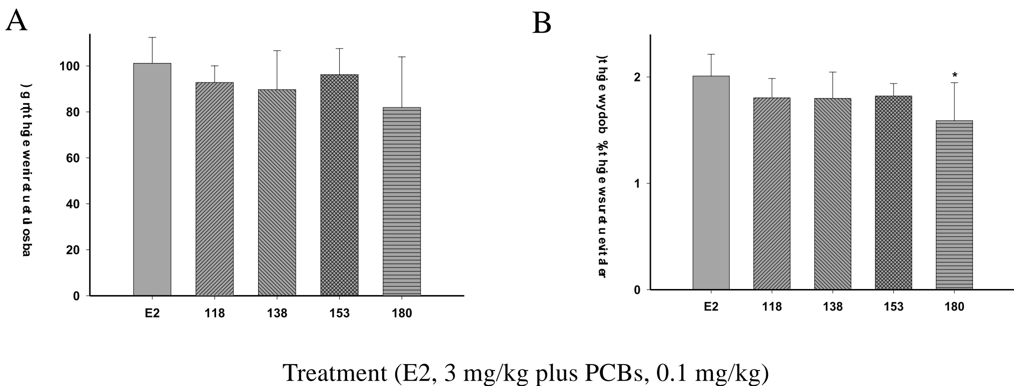


Figure 1. Inhibition of E2-induced luciferase activity by PCBs in MCF-7 ERE cells. Each value represents mean ± SEM (N=4). *Significantly different from E2, P<0.01.



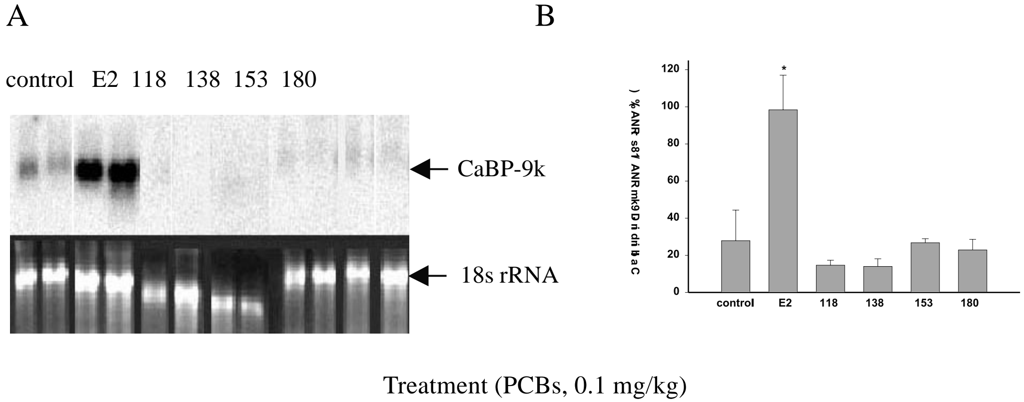
Treatment (E2, 3 mg/kg, PCBs, 0.1 mg/kg)

Figure 2. Effects of PCBs on absolute (A) and relative (B) uterine wet weights in immature female rats. Each value represents mean ± SD (N=6-8). *Significantly different from control, P<0.01.



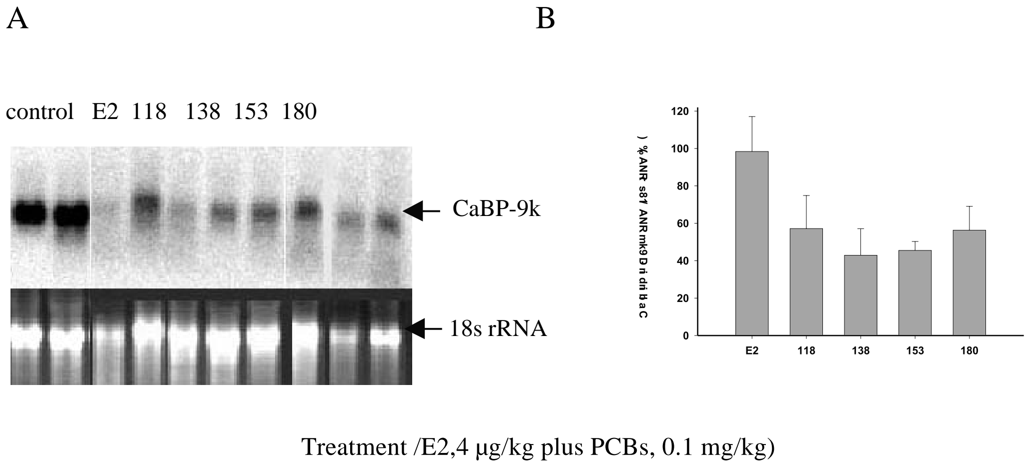
Treatment (E2, 3 mg/kg plus PCBs, 0.1 mg/kg)

Figure 3. Effects of PCBs on E2-increased absolute (A) and relative (B) uterine wet weights in immature female rats. Each values are mean ± SD. *Significantly different from E2, P<0.05



Treatment (PCBs, 0.1 mg/kg)

Figure 4. Effects of PCBs on calbindin-D9k mRNA expression in the uterus of immature female rats. Each values represents mean ± SD. *Significantly different from control, P<0.01



Treatment /E2,4 µg/kg plus PCBs, 0.1 mg/kg)

Figure 5. Effects of PCBs on E2-induced calbindin-D9k mRNA expression in the uterus of immature female rats. Mean ± SD. Significantly different from E2, *P<0.05, **P<0.01.