COPLANAR POLYCHROLINATED BIPHENYL (CO-PCB) AFFECTS STEROIDGENIC ENZYME mRNA EXPRESSION IN THE NEONATAL MOUSE TESTIS *IN VITRO*

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Introduction

Many researchers have reported that *in utero* and lactational exposure to dioxins including 2,3,7,8-tetrachlorodebenzo-*p*-dioxin (TCDD) resulted in a variety of adverse effects on the male reproductive system, i.e., reduced sperm count (1, 2, 3), reduced size of reproductive organs (4, 5). Among them, the reduced sperm count is the most important effect in terms of male infertility. The present study, however, effects of TCDD on spermatogenesis of offspring maternally exposed to TCDD are still controversial. Peterson and coworkers reported a series of extensive, carefully designed studies with male Holtzman rat offspring born from dams that were administered a single oral dose of TCDD (0, 64, 160, 400, or 1000 ng/kg bw) on Gestational Day (GD) 15 (1). Statistically significant reduction in the weight of testes and that in daily sperm production (DSP) were detected by administration of as low as 64 ng TCDD/kg bw. Some of the animals showed reduced fertilities at higher doses (400 and 1000 ng TCDD/kg bw), suggesting that maternal TCDD exposure induces defects in sperm production and causes male infertility. Gray and colleagues (3) also reported that maternal TCDD exposure (50, 200, or 800 ng TCDD /kg bw) on GD15 induced changes in the reproductive system of male Long Evans (LE) rats. They detected reduction in the epididymis and ejaculated sperm numbers, but in contrast, neither testicular weight nor daily sperm production (DSP) was affected at any of the doses used. Faqi *et al.* (6) gave female Wistar rats an initial loading dose of 25, 60, or 300 ng TCDD/kg bw at 2 weeks prior to mating, followed by a weekly maintenance dose of 5, 12, or 60 ng TCDD/kg bw, and reported a slight decrease of DSP with no changes in testicular weight. In other studies, no reduction of testicular weight was observed following maternal exposure to low-dose TCDD (2). We also used the experimental protocol of Mably *et al.* (1), and detected severe reduction of ventral prostate by TCDD, but there were no changes in testicular weight or DSP by TCDD administration even at a

dose as high as 800 ng/kg bw (7). Taken together, it is difficult to draw a conclusion regarding the consistency of effects of dioxins on sperm production.

In this study, we used 3,3',4,4',5-pentachlorobiphenyl (co-planar PCB: co-PCB) as a TCDD-like PCB congener to study its effects on mammalian spermatogenesis when the testis tissue has a direct contact with the compound. Thus, in order to investigate whether co-PCB directly affects prespermatogenesis and the other supporting cell differentiations in testis or not, we employed an organ culture system by using the neonatal mouse testis which seems to be at the most sensitive stage in the *in utero* and lactational dioxin-exposure.

Materials and Methods

Chemicals: 3,3',4,4',5-pentachlorobiphenyl (IUPAC PCB126) was a gift from Dr. M. Morita and Dr. Y. Aoki (NIES).

Animals and Organ cultures: Animal experiments were performed according to the guideline on animal welfare at NIES. Male neonatal mice were killed by cervical dislocation just after birth (postnatal day 0; PND0), and their testes were removed and immediately placed on the nucleopore filter (pore size: 0.45 μm) floating on Dulbecco's modified Eagle's medium (DMEM) containing 10% calf serum (Gibco) and penicillin-streptomycin (100 μg/ml; from Sigma). The medium was supplemented with 0, 10, 100, 1000 nM co-PCB. And then the testes were incubated at 37°C in a humidified atmosphere containing 95% air: 5% CO_2. After 48 hr incubation with co-PCB containing medium, the fresh co-PCB minus medium was changed every 48 hr. The culture was continued for 4, 8, or 12 days. At the end of the culture period, 5-bromo-2'-deoxyuridine (BrdU) was added to the media to make a final concentration of 50 μg/ml and incubated for the last 1 hr of the culture.

Immunohistochemistry for BrdU-Labeled cells: The cultured testis was fixed with Carnoy's solution and embedded in paraffin. Sections (5 μm in thickness) were incubated in 3% H_2O_2 in methanol for 30 min to eliminate endogenous peroxidase, and immersed in 1 N HCl for 60 min in order to denature the genomic DNA. After rinsing, the specimens were treated with 1% bovine serum albumin and incubated with peroxidase labeled anti-BrdU mouse monoclonal antibody (1:100, Boehringer Mannheim, Germany) for 1 hr. After rinsing, 3,3'-diaminobenzidine solution was applied to the sections. BrdU-positive germ or Sertoli cells were counted and divided by the numbers of all live germ cell or Sertoli cells.

Semiquantitative RT-PCR: Semiquantitative RT-PCR method used in this study was described previously (7). Total RNA from the cultured testis (n=5) was extracted by standard protocol. Reverse-transcribed samples were subjected to measure mRNA levels for cytochrome P450 1A1 (CYP1A1), cytochrome P450 side chain cleavage (P450scc), cytochrome P450 17a-hydroxylase/17,20-lyase (P450c17), 3β-, 17β-hydroxysteroid dehydrogenase (3β-, 17β-HSD), and spermatogenic cell specific genes (protamine-2, calnexin-t, Hsp70t). Relative amounts of target mRNA products were quantified by standardizing with PCR product of cyclophilin or

G3PDH using Scion Images software (Scion Co., USA).

Statistical analysis: All data were analyzed by one-way analysis of variance (ANOVA) followed by Dunnet test for comparison of means. Statistical difference was considered significant below $p < 0.05$.

Results and Discussion

In order to investigate how co-PCB affects the prespermatogenesis and other supporting cell differentiation in the neonatal testis, we used to an organ culture system. Semiquantitative RT-PCR analysis revealed that in this system, expression of CYP1A1 mRNA at 4 days of culture was increased by co-PCB in a dose-dependent manner. BrdU-labeling indexes of germ cells and Sertoli cells were not altered by any doses of co-PCB, suggesting that co-PCB does not affect mitotic activities of prespermatogenic cells and supporting cells in the neonatal testis. The mRNA levels of spermatogenic cell specific markers tested were not affected by co-PCB. RT-PCR analysis for steroidogenic enzymes (P450scc, P450c17, 3β-HSD, 17β-HSD) was also performed. Although mRNA levels of P450c17, 3β-HSD, and 17β-HSD were not changed by co-PCB, the P450scc mRNA level in all co-PCB-treated testes were significantly lower than that in control cultured testis (Fig. 1).

It has been reported that administration of TCDD to adult male rats decreased testosterone production in Leydig cells by inhibiting pregnenolone biosynthesis from cholesterol probably due to reduced activity of P450scc (8). There is a report that PCB congeners inhibited the activity of P450scc in the bull testis (9). It is plausible to speculate from the present finding that down-regulation of P450scc mRNA expression by co-PCB in the neonatal mouse testis is mediated by inhibition of steroidgenesis in the testis.

The present results also strongly suggest that dioxin and related compounds including co-PCB do not directly impair the proliferation and differentiation of prespermatogenic cells and Sertoli cells in the testis. Although this supposition is thought to be consistent with the report by Gray *et al.* (3), and our recent study (7), in which no effects were observed on testicular weight and sperm production in the rat testis perinatally exposed to dioxin, further work is needed to clarify the mechanism of direct effects of dioxin in the spermatogenesis.

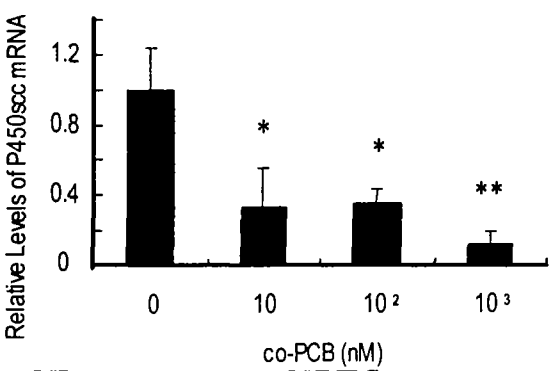

Fig 1. Semiquantitive RT-PCR analysis for P450scc mRNA level in the neonatal mouse testis in vitro. ($p < 0.01$, * $p < 0.05$)**

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