Research Group 1
"The Effects of Exposure to Coplanar PCBs on the Progeny"

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Introduction

The inhibitory actions of PCB126 on steroid hormone synthesis and secretion were demonstrated in the previous study. *In utero* and lactational exposure to PCB126 decreased serum concentrations of E_2 and testosterone, and increased progesterone in immature female rat. In addition, these alterations of steroid hormone secretion were associated with ovarian gene expressions encoding the steroidogenic enzymes. In the same study, the author also observed that PCB126 exposed ovaries provide significantly small number of secondary follicles. Because E_2 play the important role in ovarian follicle development, these results hypothesized that PCB126 might alter the estrogen synthesis in the follicles, and that triggered modulation of follicle development; although so far the information available is insufficient to support this hypothesis.

This study aimed to examine whether the estrogen actions were involved in the modulation of follicle development induced by PCB126.

Materials and methods

Pregnant Sprague-Dawley rats were administered either corn oil, or 50 μg/kg of PCB126 orally at gestational day 15 (GD 15). The size of each litter was standardized to eight on postnatal day 1 (PND 1). The offspring from each dam was sacrificed on PNDs 10 and 15. The hemi-lateral ovary of each animal was frozen in liquid nitrogen and stored at −80°C for LMD analysis. Others were fixed by Bouin's solution for morphological analysis.

The author determined the number of growing follicles in primary and antral (secondary) follicles on PNDs 10 and 15, when these follicles were abundantly provided in the ovary. This time points were selected because estradiol likely to act on the transition from primary to secondary follicles. After fixation, ovaries were embedded in paraffin, sectioned throughout the entire ovary, and stained with hematoxylin-eosin. Morphologically growing follicles contained in every fifth sections were counted and classified as primary and secondary follicles according to the classification system (Pederson and Peters, 1968).

For laser microdissection (LMD), 20 primary (50–150 μm maximum diameter) and 10 secondary (151–250 μm diameter) follicles were selected in each ovary. The granulosa cell layers and oocytes of these follicles were dissected from each ovary. The amounts of mRNAs in these tissues were quantified by the real-time PCR using a PRISM 7700 Sequence Detector (Applied Biosystems). All PCR amplifications were carried out in duplicate for each sample, and the mean values of gene expression were calculated as the ratio to those of GAPDH.

Results of counting of follicle number were analyzed using a one-way analysis of variance (ANOVA).Subsequently, significant differences between the control and PCB-exposed groups were analyzed by Dunnett's test. Data of gene expression in the primary and secondary were represented as the mean ± SD from 5 animals. The significance of differences between primary and antral follicles was determined by Mann-Whitney's U test. A probability value less than 0.05 was considered to be significant.

Results

The female offspring in group treated with 100 μg/kg PCB126 showed significantly small number of primary follicles and secondary follicles at PND 10. The number of secondary follicle was also reduced by the exposure in the group treated with 50 μg/kg PCB126 at same time. At PND 15, the number of secondary follicle were also significantly reduced in both exposed groups, however there were no effects of PCB126 exposure on primary follicle number (Fig. 1). The P450aromtase, IGF-1 and FSH receptor mRNA expression in the granulosa cells isolated from primary and secondary follicles were determined using real-time PCR method. The amount of P450aromtase mRNA in the granulosa cells isolated from both primary and antral follicles were significantly reduced by the exposure to 50 μg/kg PCB126. The amount of ERβ in the granulosa cells isolated from secondary follicles was also reduced, whereas amount in the granulosa cells obtained from primary follicles did not alter. There were no effects on the amounts of FSH receptor and IGF-1 mRNA in the same samples (Fig. 2).

The author also determined the amounts of GDF-9 and BMP-15 in the oocytes isolated from primary follicles, and then

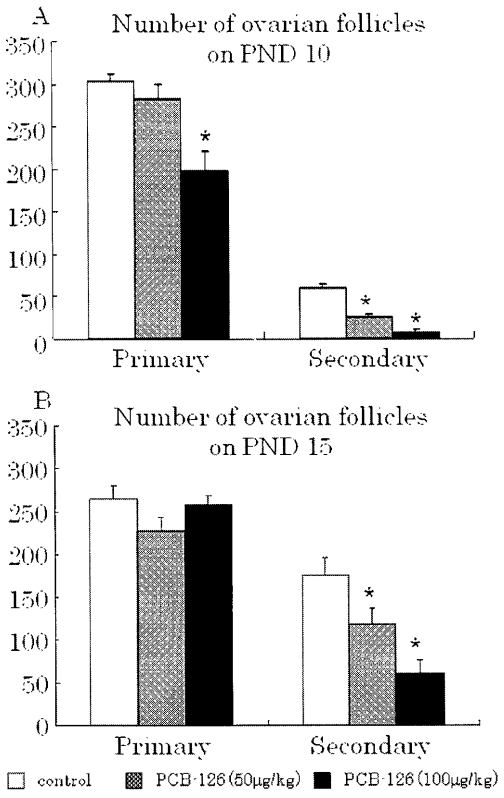

Fig. 1 The mean number of primary and secondary follicles on PND 10 (A) and on PND 15 (B) in the ovaries of offspring from dams given corn oil (control) or 50 or 100 μg/kg PCB126 at GD 15. Values represent the mean number of follicles ± SEM. * significant difference vs control at $p < 0.05$.

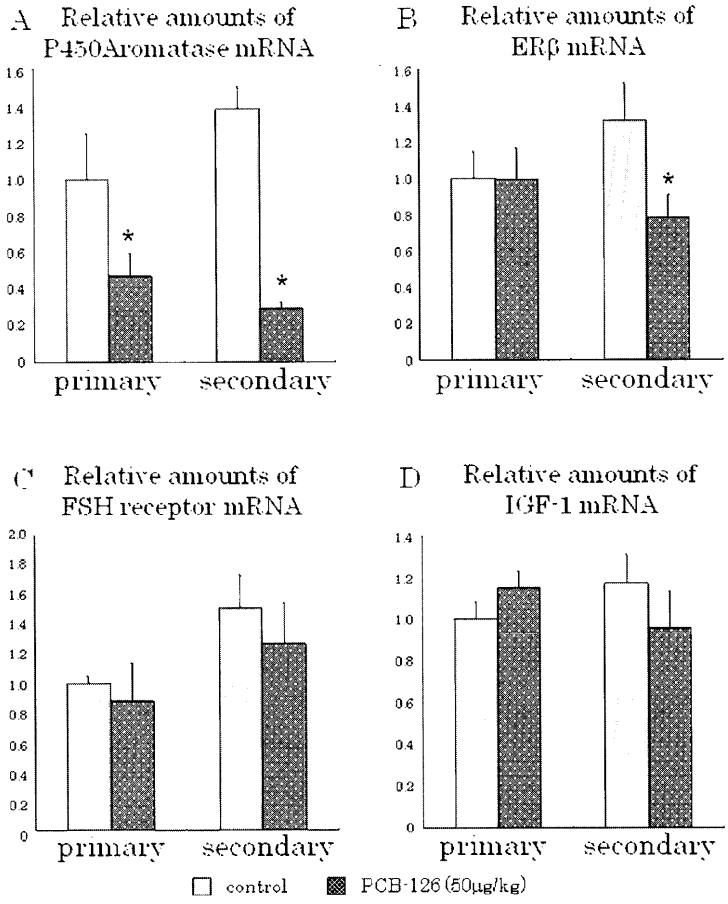

Fig. 2. Expression of mRNAs encoding P450aromtase (A), ERb (B), FSH receptor (C) and IGF-1 (D) in the granulosa cells isolated from primary and secondary follicles in the ovaries of offspring on postnatal day (PND) 15 from dams given corn oil (control) or 50 μ g /kg PCB126 at GD 15. The mRNA expression was evaluated by real-time PCR, the data were normalized by GAPDH mRNA level in each sample and expressed as values relative to the control level of primary follicles. The data are shown as mean ± SE of each group. * significant difference vs control at $p < 0.05$.

there were no significant differences of both GDF-9 and BMP-15 mRNA expressions between primary and secondary follicles.

Discussion

The ovarian expression of P450aromtase mRNA was reduced by exposure to PCB126 in previous study, although, such data reflect both the number of cells expressing the target gene in the ovary and the amount of transcript of the target gene in each cells. Estradiol-17β is synthesized in individual follicles, and then E_2 acts on own granulosa cells as the intra follicular factor through the paracrine and /or autocrine signaling; therefore it is important to investigate the intra follicular E_2 synthesis for good understanding of intrafollicular estrogen actions. Present study indicated that PCB126 reduced P450aromatase mRNA expression in each granulosa cells isolated from both follicles before or with formation in antral cavity individually. Furthermore, this reduction of P450arotamase mRNA expression might correlate with the E_2 synthesis. In the previous study, the serum concentration of E_2 and ovarian expression of P450aromtase were reduced by same exposure paradigm. The *in utero* and lactational exposure to TCDD reduced ovarian gene expression of P450aromtase, and this reduction followed enzymatic activity and serum concentration of E_2, suggesting the correlation between chemical induced reduction of P450aromtase mRNA expression and E_2 synthesis. Thus, whereas we did not evaluate the protein expression and enzymatic activity of P450aromtase, changes of gene expression might be reflected in the E_2 synthesis.

On the other hands, this possible reduction of E_2 synthesis could also be resulted in modulation of follicle development. In present study, PCB126 reduced the number of secondary follicles, whereas that of primary follicles did not be altered at PND 15. Because primary and secondary follicle development has been associated with estrogen actions, it is possible that PCB126 reduced secondary follicles by the way of modulation of the estrogen actions. In addition, decrease of responsiveness to E_2 also may modulate the ovarian follicle development. The ERβ was localized in the granulosa cells of growing follicles, and high expression was also observed in primary and secondary follicles, whereas ERβ was not detected or weekly detected in the granulosa cell of preantral follicles; therefore estrogen may act on primary and secondary follicles through the ERβ. In the present study, ERβ mRNA expression in the granulosa cells isolated from secondary follicles was reduced by PCB126, and may be resulting in the inhibition of follicular transition from primary to secondary follicles.

However, we could not conclude the mechanisms of reduction of P450aromtase expression in present study. Since P450aromatase is induced by FSH, one mechanism for this possible reduction could be a decrease in responsiveness to gonadotropin, whereas the author believe this is not in case, because the amount of FSH receptor mRNA, which was also induced by FSH stimulation, did not be altered in present study. The AhR also plays a crucial role in female reproduction by regulating the expression of ovarian P450 aromatase, therefore there are several ways to alter the P450aromatase gene expression by the PCB126 exposure. In contrast to factors in the estrogen pathway PCB126 did not alter the FSH receptor and IGF-1 mRNA expressions, and also did not alter the GDF-9 and BMP-15 mRNA expressions. These factors were examined because of its potential to enhance the primary and secondary follicle development. Thus PCB126 might modulate follicle development through another way. In immature female rats, peaks in serum concentrations of FSH and E_2 were observed between PNDs 10 and 15 when the primary and secondary follicles were abundantly provided in the ovary, and that considered crucial for the induction of the growth of the primary and secondary follicles in the first follicular wave. The PCB126 reduced intra follicular E_2 synthesis at that time together with modulation of follicle development. Therefore PCB126 might modulate estrogen signaling pathway, and that triggered the impairment of reproductive development.

In summary, the present study suggested the possibility that *in utero* and lactational exposure to PCB126 altered intra follicular E_2 synthesis, and then reduced estrogen actions affected on primary to secondary follicle transition.

Abstract

Estrogen actions are involved in the ovarian follicle development, and then this study aimed to examine whether the estrogen actions were involved in the modulation of follicle development induced by PCB126. Pregnant Sprague-Dawley rats were orally administrated the PCB126 at dose levels of 0 (corn oil), 50 or 100 μg/kg on gestational day (GD) 15. The ovaries were collected on postnatal days (PNDs) 10 and 15 from the offspring, and growing follicle numbers of primary and secondary in each ovary were counted, respectively. Furthermore, granulosa cells in the primary (with 50 μm ~ 150 μm diameter) and secondary (with 151 μm ~ 250 μm diameter) follicles were then dissected by the Laser Microdissection (LMD), and mRNA expressions quantified by real-time PCR were compared between control and 50 μg/kg PCB126 group. The number of secondary follicles in the 50 and 100 μg/kg-exposed groups were decreased compared with that in age-matched control on PNDs 10 and 15 respectively, although that of primary follicles did not be altered at PND 15. The P450aromtase mRNA expressions in the granulosa cells isolated from both primary and secondary follicles were decreased in the group treated with 50 μg/kg PCB126. ERβ mRNA expression in the granulosa cells isolated from secondary follicles was also decreased by the PCB126 exposure at PND 15. In summary, the present study suggested the possibility that in utero and lactational exposure to PCB126 altered intra follicular E_2 synthesis by reducing the P450aromatase and ERβ mRNA expressions, and then reduced estrogen actions affected on primary to secondary follicle transition.

References

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研究サブ・グループ1
Co-PCBsの次世代に及ぼす影響

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要約：これまでの実験で，PCB126暴露により次世代動物の卵巣における発育卵胞数の減少，E_2産生の低下と共に，卵巣におけるArom遺伝子発現量の減少が認められた。そこで，本実験では妊娠15日にPCB126（50 μg/kg）暴露を受けた出生雌ラットの卵巣内発育卵胞を，発育段階により形態学的に分類してその数を数え，影響の認められた発育段階の卵胞からLMD法により顆粒層細胞を採取して遺伝子発現量の変化を検索した。その結果，暴露動物では15日齢において卵胞腔形成期の卵胞が減少しており，これら発育段階にある卵胞の顆粒層細胞ではAromとERβの遺伝子発現量が低下していた。いっぽう，顆粒層細胞のFSHRやIGF-1，あるいは卵細胞のGDF-9とBMP-15の遺伝子発現量には影響が認められなかった。これらの結果から，PCB126の卵胞発育の阻害は卵巣におけるestrogen作用の低下が関与していることが示唆された。