IN UTERO EXPOSURE TO 3,3',4,4',5-PENTACHLOROBIPHENYL (PCB 126) INDUCES HYPOSPADIAS IN FEMALE RATS

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Introduction

In utero and lactational (IUL) exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) induces genital dysmorphogenesis, such as vaginal thread and cleft clitoris, in female rats[1,2]. While male hypospadias is known to be induced by disruption of androgen-signaling pathways[3-5], no mechanisms have been proposed to explan the gender-specific induction of external genital anomaly by IUL exposure to TCDD. Recently, we found that IUL exposure to 3,3',4,4',5-pentachlorobiphenyl (PCB 126), an aryl hydrocarbon (Ah) receptor agonist, delays the timing of the first ovulation at a dose of 10 μg/kg on gestational day (GD) 15 while slightly reducing the number of oocytes shed, and that it disrupts synchronization of the vaginal opening (VO) with the first ovulation at a dose of 100 μg/kg[6]. Since an equivalent treatment with TCDD also delayed puberty in female offspring[1], the mechanisms affecting female puberty might be similar between Ah receptor agonists. In order to assess how the Ah receptor-signaling pathway helps induce a genital dysmorphogenesis, the present study aimed to examine the potency and critical windows necessary for PCB 126 to induce the malformation of external genitalia in female progeny in rats.

Materials and Methods

PCB 126 (AccuStandard Inc., New Haven, CT, USA) was dissolved in corn oil to a constant volume of 2 ml/kg body weight, and was administered orally. Adult female Sprague-Dawley rats and their mating partners of the same strain were purchased from Charles River Japan, and were maintained under a lighting condition (lights on 08:00-20:00) in an animal husbandry facility. The animals were housed in a plastic cage with bedding materials, pellet chow (CE-2, Clea Japan Inc.), and water (tap water) *ad libitum*. In Exp. I, 4 (control) or 8 and 4 female rats copulated and conceived after daily administration for 2 weeks of corn oil (control) or 1 and 3 μg/kg/day of PCB 126, respectively. Daily administration continued through the gestational period until 20 days after delivery. In this experiment, cumulative maternal exposure to PCB 126 until weaning was estimated to be 57-68 μg/kg in the 1-μg/kg/day-exposed group and 171-186 μg/kg/day in the 3-μg/kg/day-exposed group. In Exp. II, three groups each containing 6 pregnant females, were single-administered corn oil (control) or 10 and 100 μg/kg of PCB 126 on GD 15 (GD 0 = day of sperm positive). In Exp. III, 3-8 (control), 5-9 and 5-10 pregnant females were single-administered corn oil (control) or 1 and 10 μg/kg of PCB 126, respectively on GD 8, GD 15 or 1 day after delivery. At 1 day after delivery, another 4 dams were also administered with 100 μg/kg of PCB

126. In all experiments, dams were allowed to deliver spontaneously, and the size of each litter was adjusted to 8 pups on postnatal day 1 (PND 1, day 0 = day of birth). At PND 21, the offspring were weaned from the dams. A gross examination of the external urogenitalia was performed on 1 to 5 female littermates from each dam on the day of VO. The abnormalities were categorized into mild (partial cleft formation from urethral orifice toward vaginal orifice), moderate or severe (more-intense cleft formation between urethral orifice and vaginal orifice, to a degree that is consistent with hypospadias). Tissue specimens including external genitalia were taken for histological examination from some offspring between the period PND 1 and the day of VO. The specimens were fixed with Bouin's solution, then dehydrated and embedded in paraffin. Serial sections (7 μm each) were made and stained with Mayer's hematoxylin and eosin.

Results and Discussion

In Exp. I, we started daily administration before conception to confirm PCB 126's potency in inducing external genital malformations in the rat offspring. In Exp. II, we followed the experimental schedule previously adopted in the study of TCDD[1, 2] to compare the potency of PCB 126 with that of TCDD. As shown in Table 1, daily exposure to PCB 126 induced macroscopic abnormalities of external genitalia in both groups of offspring. Also, a single, GD15 injection to the dams of 10 or 100 μg/kg of PCB 126 (equivalent in respective toxicity to 1 or 10 μg/kg of TCDD), induced the same abnormalities in all of the dam's female offspring. Vaginal threads frequently developed in association with hypospadias in the female offspring. These two experiments indicate that PCB 126 has the potency to induce external genital dysmorphogenesis, with dose-dependent increases in severity. While the cumulative doses of PCB 126 until GD 15 in the 1-μg/kg/day group in Exp. I were estimated to be much larger than 10 μg/kg, the severity of malformations seemed to be similar between Exps. I and II. However, it could not be concluded that daily exposure seemed to be less effective at inducing malformations, since the same treatment did not always induce malformations as severe as those found in the rats exposed to 10 μg/kg on GD 15 in Exps. II and III (Table 2).

Histopathology revealed that the cleft was lined by the urothelium. In the rats with hypospadias, continuity between the vaginal and urethral epithelia was confirmed.

Table 1. Incidence of external genital malformation in the female offspring in Exps. I and II

Treatment to dams (Number of dams)	Incidence of offspring Affected/examined (%)	Severity and incidence of malformation Number of offspring (%)		
		mild	moderate	severe
Experiment I (daily administration)				
Corn oil 2 ml/kg/day (4)	0/4 (0)	———	———	———
PCB 126 1 μg/kg/day (6)	8/8 (100)	0 (0)	5 (62.5)	3 (37.5)
PCB 126 3 μg/kg/day (2)	4/4 (100)	0 (0)	1 (25)	3 (75)
Experiment II (single administration on GD 15)				
Corn oil 2 ml/kg (5)	0/20 (0)	———	———	———
PCB 126 10 μg/kg (6)	24/24 (100)	24 (100)	0 (0)	0 (0)
PCB 126 100 μg/kg (6)	22/22 (100)	0 (0)	3 (13.6)	19 (86.4)

In Exp. III, we determined the critical window for exposure to induce external genital malformations. As shown in Table 2, none of the offspring exposed to PCB 126 on PND 1 onward

showed malformation of the external genitalia. The treatment on GD 15 induced more-severe abnormalities as compared with those induced by the exposure on GD 8. These results indicate that the critical window for exposure to induce external genital malformation in female rats is the prenatal period, which is also critical for the induction of hypospadias to male rats by androgen disruption[3]. Administration of 1 μg/kg of PCB 126 did not induce malformations. Since the morphological alterations of the female external genitalia seen in the present study were identical to those in the offspring of the dams exposed to a single oral dose of 1 μg/kg of TCDD on GD8 or GD15[1], female hypospadias induced by either TCDD or PCB 126 may be concerned with Ah receptor-signaling pathways.

Table 2. Incidence of external genital malformation in Exp. III

Treatment to dams (Number of dams)	Incidence of offspring Affected/examined (%)		Severity and incidence of malformation Number of offspring (%)	
			mild	moderate
Administration on GD 8				
Corn oil 2 ml/kg (3)	0/10	(0)	———	———
PCB 126 1 μg/kg (6)	0/29	(0)	———	———
PCB 126 10 μg/kg (8)	7/31	(22.6)	4 (57.1)	3 (42.9)
Administration on GD 15				
Corn oil 2 ml/kg (5)	0/11	(0)	———	———
PCB 126 1 μg/kg (9)	0/38	(0)	———	———
PCB 126 10 μg/kg (8)	10/28	(35.7)	0 (0)	10 (100)
Administration on 1 day after delivery				
Corn oil 2 ml/kg (5)	0/16	(0)	———	———
PCB 126 1 μg/kg (8)	0/33	(0)	———	———
PCB 126 10 μg/kg (10)	0/41	(0)	———	———
PCB 126 100 μg/kg (4)	0/7	(0)	———	———

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