EFFECTS OF PRENATAL EXPOSURE OF 2,2',3,3',4,6'-HEXACHLOROBIPHENYL ON SPERM FUNCTION AND GENES EXPRESSION IN RATS

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Introduction

2,2',3,4',5',6-Hexachlorobiphenyl (PCB132) is stable non-dioxin like chlorinated biphenyls congeners and methylsulfonyl. PCB 132 and its metabolites can be detected in human tissues (Weistrand and Noren, 1997). Therefore, this congener is considered to be not persistent and attracted little attention in PCB toxicity studies. There are some limited evidences suggested that 2,3,6-substitued CB congeners possessing certain toxicities that were not identified before. Most of the male reproductive problems have been observed in rodents with *in utero* and lactational exposure to TCDD (Mably et al., 1992) or prenatal (Faqi et al., 1998a) and/or postnatal (Huang et al., 1998; Faqi et al., 1998b) exposure to dioxin-like CB congeners. Recently, our study showed postnatal exposure to PCB132 affected serum triiodothyronine (T3) levels, sperm motility, velocity and capability of penetration oocytes in rats (Hsu et al., 2003). Although these data are suggestive of probable toxicity at the sperm function, it is not clear how PCB132 affects testis function, and if so, through what mechanisms. The objective of this study is to determine whether treatment of prenatal non-dioxin congeners, PCB 132, affects the sperm function and expression of apoptosis-related genes including Fas, Bax, bcl-2, and p53 as well as apoptosis-related protein, caspase 3, 8, and 9 in testis.

Methods and Materials

Pregnant SD rats were randomly divided into control and PCB-treated groups. The animals were treated with intraperitoneal injection of single dose of 0, 1 or 10mg/kg corn oil or PCB132 at Day 15 of pregnancy. Male offsprings with age of 12 weeks were sacrificed. The right cauda epididymis was dissected from each male and transported in 1 ml 34 ^{o}C HTF buffer supplemented with 5 mg/ml bovine albumin. Sperm samples were held in a Makler chamber (Sefi Medical Instruments, Israel) for motility measurements. Computer assisted sperm analysis (CASA) was obtained for motility indices with a Hamilton Thorn Research motility analyzer (version HTM-IVOS Specification, Beverly, MA, USA) at a temperature of 37 ^{o}C. CASA was gained for sperm motility parameters: curvilinear velocity (VCL, μm/s), average path velocity (VAP, μm/s), straight line velocity (VSL, μm/s), amplitude of lateral head displacement (ALH, μm), and beat-cross frequency (BCF, Hz). The velocity parameters were adjusted for evaluating the levels of all sperm performance. Control female Sprague–Dawley rats were superovulated at age 63–70 d weighing 330–380 g. In the morning of day 1, the rats were injected with 25 IU of PMSG. On day 3, 52 h later, the animals were injected with 25 IU of hCG. Twenty hours later, the female rats were terminated and the oviducts dissected and placed into HTF–albumin buffer. The cumuli were dissected from the oviducts, collected in HTF–albumin buffer, and dissolved with 10 mg/ml of hyaluronidase. After adjusting to $10x10^6$ sperm/ml with HTF–albumin buffer, 10 μl of epididymal sperm suspension was added to 100 μl of HTF–albumin buffer containing 10–15 zona-intact rat ova. Each culture well of the sperm–ova preparation was overlaid with mineral oil and incubated at 37 ^{o}C in 95% air/5% CO_2. After 48 h of insemination, the number of oocytes penetrated by sperm was determined by phasecontrast microscopy at 400· magnification. Sperm–oocyte penetration rate (SOPR) was used to evaluate the sperm–oocyte penetration capacity. The left testis was fixed in Bouin's solution for at least 48 h. Each was processed in an automatic tissue processor and 276embedded in paraffin. Thin sections were cut at 3–4 μm thickness, stained with hematoxylin and eosin, and examined under a light microscope. Real time RT-PCR was used to detect testicular gene expression (Table 1) and western blot was measured to confirm the content of apoptosis-related protein, caspase 3, 8, and 9 in testis.

Table 1. Primers for apoptosis and housekeeping genes in real time RT-PCR.

Gene	Forward primer	Reverse primer
Fas	5'TGTCAACCGTGTCAGC 3'	5'GGTCACAGAGAGAAGC 3'
Bax	5'ATGATTGCTGACGTGG 3'	5'CCACAAAGATGGTCACT 3'
bcl-2	5'TGGACAACATCGCTCT 3'	5'ACTGCTTTAGTGAACCT 3'
p53	5'CCGTATGCTGAGTATCT 3'	5'ACAAACACGAACCTCAA 3'
Gusb	5'CTAAAGCTACGACTACCT 3'	5'CCTTAGCCGGTAACCA 3'

Results and Discussion

There were no statistical differences in body weights, right or left testis weight, right or left epididymis weight, and cauda epididymis weights between control and treated groups (Table2). Sperm count was found to be significantly decreased in the groups prenatally exposed to 10 mg/kg of PCB 132. In addition, although there was a dose-dependent decrease in the SOPR in study group, there was no significant differences between these groups. There were also no significant differences in sperm VCL, VAP, VSL, AHH, and BCF between the groups exposed to PCB 132 and the controls (Table 3). To detect apoptotic gene expression in testis, we found that *Fas*, *Bax*, *bcl-2*, and *p53* were significantly decreased in 10 mg/kg of PCB132 prenatal exposure as compared with 1 mg/kg and controls, respectively ($P < 0.05$) (Table 4). However, *p53* was significantly induced in 1 mg/kg of PCB132 prenatal exposure as compared with controls ($P < 0.05$) (Table 4). Caspase activities of caspase 3 and 9 were significantly depressed in 10 mg/kg of PCB132 prenatal exposure as compared with 1 mg/kg and controls, respectively ($P < 0.05$). However, caspase 3 was increased in 1 mg/kg of PCB132 prenatal exposure as compared with controls ($P < 0.05$) (Figure 1). Histological examinations were performed on the testicular sections of PCB 132-treated groups and the control group (Figure 2). There were significant germ cell degeneration in rats exposed to 1 mg PCB 132/kg and 10 mg PCB 132/kg as compared with the controls.

Fas has been localized to germ cells, and *FasL* to Sertoli cells, within the rat testis (Lee et al., 1997). *Fas* and *FasL* genes and their protein products have been shown to be up-regulated in rats exposed to Sertoli cell toxicants that induce apoptotic germ cell death (Lee et al., 1997). Recent studies have provided some clues for understanding the underlying molecular mechanisms governing germ cell death in the testis. For example, *bcl-2* transgenic mice, in which a human *bcl-2* transgene, an antiapoptotic gene, is overexpressed in spermatogonia, have overpopulated spermatogonia and a decreased incidence of germ cell apoptosis (Furuchi et al., 1996). Targeted gene disruption of *bax*, a proapoptotic gene, in mice revealed hyperplasia of spermatogonia as well as massive death of early spermatocytes, suggesting *Bax* dependent and -independent apoptosis pathways in testis (Knudson et al., 1995). In addition, the tumor suppressor *p53*, a regulator for both cell proliferation and apoptosis, has been shown to play a role in the testis (Hasegawa et al., 1998; Yin et al., 1998). Mice deficient in *p53* exhibit a decreased or delayed onset of germ cell apoptosis induced after radiation exposure or experimental cryptorchidism (Hasegawa et al., 1998; Yin et al., 1998).

Table 2. Body and tissue weight between rats prenatally exposed to CB 132 (1 mg/kg or 10 mg/kg) and unexposed controls.

Parameters	Control (n=12)	Treatment of PCB 132		p value (ANOVA)
		1 mg/kg (n=12)	10 mg/kg (n=12)	
Body weight (g)	435±9	413±11	409±8	0.2026
Right testis weight (g)	1.63±0.13	1.53±0.16	1.54±0.14	0.2259
Left testis weight (g)	1.62±0.15	1.56±0.15	1.57±0.12	0.4609
Right epididymis weight (mg)	541.6±96.1	502.0±65.9	558.4±63.7	0.2206
Left epididymis weight (mg)	557.1±90.5	509.3±62.0	553.0±22.8	0.2293
Right cauda weight (mg)	244.1±31.6	204.8±31.4*	211.7±26.9*	0.0008
Left cauda weight (mg)	212.8±40.1	205.8±22.7	214.8±13.7	0.6941

*$P < 0.05$ as compared with control group.

Table 3. Sperm function between rats prenatally exposed to CB 132 (1 mg/kg or 10 mg/kg) and unexposed controls.

Parameters	Control (n=12)	Treatment of PCB 132		p value (ANOVA)
		1 mg/kg (n=12)	10 mg/kg (n=12)	
Sperm count (10^6/ml)	258.3±76.1	158.3±54.9*	153.3±26.4*	<0.0001
Motility (g)	64.6±16.8	60.2±11.1	60.9±18.1	0.7662
VCL (μm/s)	160.8±26.3	155.3±16.2	161.7±26.4	0.7700
VAP (μm/s)	107.6±21.7	93.8±13.3	100.5±19.8	0.2111
VSL (μm/s)	77.8±24.2	65.7±9.3	70.4±16.6	0.2618
ALH (μm)	5.6±2.5	6.1±1.7	6.2±2.3	0.7452
BCF (Hz)	20.4±11.5	20.0±2.1	20.8±6.3	0.9689
SOPR (%)	35.4±17.4	28.3±23.2	22.0±14.7	0.2323

*P < 0.05 as compared with control group.

Table 4. Apoptosis-related genes expression between rats prenatally exposed to CB 132 (1 mg/kg or 10 mg/kg) and unexposed controls.

Parameters	Control (n=12)	Treatment of PCB 132		p value (ANOVA)
		1 mg/kg (n=12)	10 mg/kg (n=12)	
Fas/*Gusb*	1.13±0.06	1.14±0.05	0.91±0.06*,#	0.0059
Bax/*Gusb*	1.07±0.08	1.17±0.08	0.65±0.06*,#	<0.0001
bcl-2/*Gusb*	1.21±0.09	1.28±0.06	0.88±0.05*,#	0.0008
p53/*Gusb*	1.02±0.05	1.19±0.04*	0.52±0.05*#	<0.0001

*P < 0.05 as compared with control group.
#P < 0.05 as compared with 1mg/kg group.

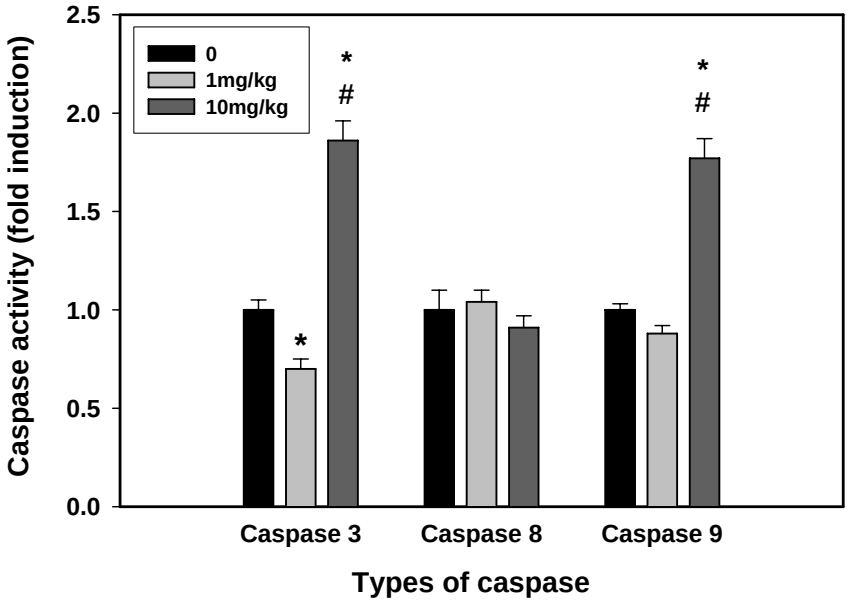

Figure 1. Caspase activity of caspase 3, 6, and 9 in rats prenatally exposed to PCB 132. *P < 0.05 as compared with control group; #P < 0.05 as compared with 1mg/kg group.

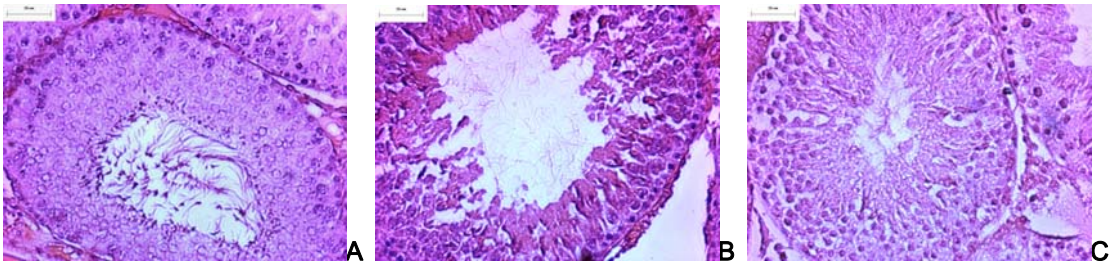

Figure 2. Histological assessment of testicular germ cell degeneration in rats prenatally exposed to 1 mg PCB 132/kg (A), 10 mg PCB 132/kg (B), and unexposed controls (C).

Conclusion

In conclusion, the present study demonstrated that there are adverse effects of PCB 132 prenatal exposure on sperm count and expression of apoptosis-related genes and caspase-related proteins in testes. The degeneration of germ cells resulted from PCB 132 exposure might cause decreased expression in apoptosis-related genes. Further efforts are required for a more complete understanding of the underlying molecular mechanisms governing the pathway of apoptosis and to identify the route of genetic and epigenetic in the role of endocrine disruptors in male reproductive disorders.

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