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Morphological and functional deterioration of the rat thyroid following chronic exposure to low-dose PCB118

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Polychlorinated biphenyls (PCBs) are persistent environmental pollutants that can severely disrupt the synthesis and secretion of endocrine hormones. To investigate the effects of 2,3',4,4',5-pentachlorobiphenyl (PCB118) on thyroid structure and function, 40 male Wistar rats were divided into 4 equal treatment groups and administered vehicle or one of three doses of PCB118. The experimental groups received intraperitoneal (i.p.) injection of 10, 100, or 1000 µg/kg/day PCB118, 5 days per week for 13 weeks, whereas the control group was injected with corn oil (vehicle). Serum concentrations of free thyroxine (FT4), free triiodothyronine (FT3) and thyroid stimulating hormone (TSH) were measured by radioimmunoassays. Histopathological and ultrastructural changes in the thyroid were observed under light microscopy and transmission electron microscopy (TEM). The mRNA expression levels of the sodium-iodide symporter (NIS) and thyroglobulin (TG) were quantified by real-time PCR. Increasing doses of PCB118 resulted in progressively lower FT3, FT4 and TSH concentrations in serum. Injection of PCB118 at all doses led to histopathological deterioration of the thyroid characterized by follicular hyperplasia and expansion, shedding of epithelial cells and fibrinoid necrosis. Follicle cells exhibited swollen or vacuolated endoplasmic reticula, as revealed by TEM. Exposure to PCB118 also caused significant decreases in NIS and TG mRNA expression levels. Chronic exposure to low-dose PCB118 and other PCB congeners may be a significant risk factor for thyroid diseases.

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1. Introduction

The prevalence of thyroid diseases is increasing, possibly due to chronic exposure to environmental pollutants that target the thyroid gland like pesticides and certain industrial chemicals (Yard et al., 2011; Li et al., 2006). Polychlorinated biphenyls (PCBs) were widely used in industry as coolants or heat transfer agents until 1977 when use of these non-biodegradable compounds was restricted due to potential health consequences of environmental and tissue accumulation (Brucker-Davis, 1998). 2,3',4,4',5-Pentachlorobiphenyl (PCB118) is one of the most persistent PCB congeners and has been found in human tissues and detected in human breast milk (Tarkowski, 1996). Contamination is widespread in water and soil and among the aquatic organisms of the Yangtze River Delta region of China. PCB118 is also one of the nine PCB congeners most strongly linked to thyroid dysfunction (Bloom et al., 2003). Many studies have reported acute toxic responses induced by high doses of PCBs (Selvakumar et al., 2011; Martin and Klaassen, 2010), but few have examined the health consequences of chronic exposure to low-dose PCBs in humans or animal models.

Epidemiology studies have established an association between environmental PCB contamination and dysregulation of the peripheral thyroid hormones T3 and T4 (Hagmar et al., 2001; Persky et al., 2001; Morreale de Escobar et al., 2000; Crofton, 2004) and of thyroid stimulating hormone (TSH) (Morreale de Escobar et al., 2000; Crofton, 2004). The mechanisms of PCB–induced thyroid dysfunction are uncertain; therefore, we investigated the ultrastructure and function of the thyroid in Wistar rats exposed to relative low doses of PCB118 for 13 weeks.

2. Materials and methods

2.1. Animals and treatments

Forty adult male Wistar rats (200 ± 10 g, SLAC Laboratory Animal Co. Ltd., Shanghai, China) were acclimated for one week and
then randomly assigned to one of the four treatment groups comprising ten animals each. The animals were given standard rat pellet and tap water ad libitum. The animal room was kept on a 12-h light–dark cycle, with a temperature range of 22 ± 1 °C and a relative humidity of 55 ± 5% throughout the experimental period. PCB118 (New Haven, CT, purity >99.9%) was dissolved in corn oil. Rats in the three experimental groups were administered PCB118 at 10, 100, or 1000 µg/kg/day i.p. for 5 days per week for 13 weeks, whereas control rats were injected with corn oil (0.5 ml/kg) on the same treatment schedule. Body weights were recorded daily. At the end of treatment, all animals were exsanguinated for serum hormone analysis, and the thyroid glands were removed for histological analyses. All animal procedures were reviewed and approved by the Institutional Animal Care and Use Committee.

2.2. Determination of FT3, FT4 and TSH

Serum was isolated from whole blood and stored at −20 °C for further analysis. Serum concentrations of the hormones FT3, FT4 and TSH were determined by radioimmunoassays (Beijing North Institute of Biological Technology, China).

2.3. Morphological examination

2.3.1. Light microscopy

Thyroid glands were removed and fixed overnight in 4% paraformaldehyde, following paraffin-embedded thyroid sections were stained with hematoxylin and eosin (HE) and observed via light microscope.

2.3.2. Transmission electron microscopy (TEM)

Thyroid specimens were fixed in 2% glutaraldehyde, post-fixed in 2% osmium tetroxide and sectioned on an ultramicrotome for transmission electron microscopy using a JEOL, JEM-1010 system.

2.4. Quantitative real-time PCR analysis

Quantitative real-time PCR was performed to measure the expression levels of NIS and TG mRNAs in the thyroid. Total RNA was isolated using TRIzol reagent (Invitrogen, USA) according to the manufacturer’s protocol and reverse transcribed to complementary DNA using the PrimeScript® RT Master Mix kit (TaKaRa, China). Quantitative RT-PCR was performed on an ABI StepOnePlus™ Real-Time PCR system (Applied Biosystems, USA) using SYBR® Premix Ex Taq™ (TaKaRa, China). Expression of each mRNA species was normalized to that of the housekeeping gene β-actin. The primer sequences that were used for qualitative RT-PCR are shown in Table 1. All primers were synthesized by TaKaRa. Relative expression levels of target genes were calculated using the 2−ΔΔCT method.

2.5. Statistical analysis

Serum hormone concentrations are expressed as mean ± standard error of the mean (SEM). Means were compared by one-way ANOVA following transformation to normalized data sets with equal variance when necessary. Statistical significance was defined as P < 0.05, and the SPSS 13.0 statistical package (SPSS Inc., Chicago, IL, USA) was used for all statistical analyses.

3. Results

3.1. Effects of PCB118 on body weight

No sign of toxicity or morbidity was observed among the experimental rats during or following exposure to PCB118. Similarly, as shown in Fig. 1, no significant change in body weight was observed in PCB118 or vehicle-treated animals.

3.2. Serum levels of thyroid hormones

The serum concentrations of the thyroid hormones FT3, FT4 and TSH are plotted in Fig. 2 after 5 weekly doses of PCB118 (10, 100, or 1000 µg/kg/day) for 13 weeks. Serum FT3 only in the highest dosage group was significantly lower than that in controls (P < 0.05). Circulating concentrations of FT4 and TSH decreased progressively with increasing PCB118 dose and were significantly lower in all three dosage groups after 13 weeks compared with the control group. Compared with control rats, serum FT3, FT4 and TSH were reduced to 75%, 31% and 52%, respectively, at 1000 µg/kg/day PCB118.

3.3. Histopathological changes in the thyroid following PCB118 exposure

3.3.1. Light microscopy

Compared with the thyroid follicles, colloid and interfollicular areas in control rats (Fig. 3A), all PCB118-treated rats exhibited hyperplasia and expansion of the follicles, shedding of epithelial cells, deficient luminal colloid, collapsed follicles, mesenchymal fibrosis and interstitial vascular proliferation (Fig. 3B–D). Fibroinoid necrosis or even disappearance of the follicular structure was also observed in thyroid sections from the 100 and 1000 µg/kg/day dosage groups (Fig. 3C and D).

![Fig. 1](https://example.com/fig1.png) Growth curve of PCB118 and vehicle-treated animals. Data are presented as mean ± SEM.
3.3.2. Transmission electron microscopy (TEM)

Thyocytes in vehicle-treated control rats showed an abundance of neatly arranged microvilli on the apical membrane. The morphology of the mitochondria and rough endoplasmic reticulum (RER) was normal and the apical cell areas exhibited numerous secretory vesicles and colloid droplets (Fig. 4A). In PCB118-treated rats, dose-dependent changes in cellular and organelle ultrastructure were observed. In all PCB-treated groups, the follicular cells had many vacuoles and few microvilli, the perinuclear gap was wider than that in follicular cells from control rats, and secretory vesicles were sparse at the apical pole. Swollen mitochondria and dilated RER cisternae were present in the basolateral pole. There was a general loss of subcellular organization and of cellular contents (Fig. 4B–D). In rats exposed to 100 μg/kg/day of PCB118, swollen mitochondria were nearly round with indistinct cristae (Fig. 4C). At the 1000 μg/kg/day dosage (Fig. 4D), thyroid cells exhibited greater numbers of mitochondria, but mitochondrial cristae were fractured or even absent.

3.4. Detection of NIS and TG mRNA expression

Expression levels of NIS and TG mRNAs in the thyroid were significantly lower in the two higher PCB118 dosage groups (P < 0.05), but not in the lowest dosage group, compared with the vehicle-treated controls (Fig. 5).

4. Discussion

In the present study, we selected PCB118 as the PCB congener to assess relatively chronic effects of low-dose exposure on thyroid ultrastructure and function in Wistar rats. The U.S. Environmental Protection Agency (EPA) defined low-dose effect of PCBs as biological or physiological changes parallel to the range of human exposures, or at doses lower than those typically used in the standard testing paradigm of regulatory agencies for evaluating reproductive and developmental toxicity (Vandenberg et al., 2012). In our study, the adopted doses of PCB118 ranged from 10 to 1000 μg/kg/day, which were relatively low and similar to those used in previous studies on thyroid pathology and function following PCB exposure (PCB 118; NTP TR 559, 2010; Ness et al., 1993). Similarly, Chu et al. (1995) used PCB118 dosages of 0.85, 8.5, 85, 850 and 0.17, 1.7, 17, 170 μg/kg/day in male and female rats, respectively, but observed no adverse effect level (NOAEL) of PCB118 at 17 μg/kg/day. However, significant deterioration of thyroid structure and function were revealed in our study even at a minimal dosage of 10 μg/kg/day.

The results of significant structural transformation and decreased thyroid hormone release at 10–1000 μg/kg/day of PCB118 suggested that chronic PCB118 exposure posed a significant risk for thyroid dysfunction. It was also found that decreased serum FT3 and FT4 levels in PCB–treated rats. This finding was consistent with those of several previous studies (Boas et al., 2006; Kato et al., 2004; Hallgren et al., 2001; PCB 118; NTP TR 559, 2010). However, Klicic et al. (2005) reported no effect of PCBs on FT3 or FT4 levels, possibly due to the different PCB congeners tested or the dosing regimen. Furthermore, serum TSH levels in the study were significantly decreased following PCB118 exposure, yet an observation at odds with several studies showing no change or even a PCB-induced increase in circulating TSH. However, most of these studies assessed only short-term PCB exposure (van den Berg et al., 1988; Osius et al., 1999; Gu et al., 2009), which may signify hormonal homeostatic mechanisms rather than the damage of thyroid structure or disruption of the hypothalamus–pituitary–thyroid axis caused by PCBs. Primary hypothyroidism, due to thyroid disruption or dysfunction is characterized by decreased circulating FT3 and FT4 and increased circulating TSH, whereas secondary hypothyroidism manifests as decreased circulating FT3 and FT4 with decreased circulating TSH attributed to abnormalities of the pituitary and/or hypothalamus. Further analysis of our data revealed that FT3 and FT4 levels decreased concomitantly with decreasing TSH levels to some extent, which was possibly due to the direct effect of PCB118 through disruption of thyroid function and inhibition of the synthesis and secretion of thyroid hormones. Moreover, PCB118 may also break down the thyroid hormone equilibrium through the hypothalamus–pituitary–thyroid axis; however, such a mechanism will have to be explored in future studies. A recent study discovered that ortho-PCB95 and PCB101 decreased the response of pituitary gland to thyrotropin-releasing hormone (TRH) (Khan and Hansen, 2003), and another study demonstrated that short-term exposure to PCB153 decreased TRH in SD rats (Liu et al., 2012).

Thyroid follicles were the functional units of the thyroid gland, which secrete the thyroid hormones. In our study, hyperplasia and expansion of the follicles, shedding of epithelial cells, deficient
Fig. 3. Representative HE-stained photomicrographs (200×) of rat thyroid tissue from each treatment group after 13 weeks of i.p. vehicle (A) or PCB118 (B–D) administration. (A) Normal appearance of thyroid tissue and intact follicular structure in control rats. (B–D) Micrographs of the PCB118-treated rats. (B) Thyroid in a rat treated with 10 μg/kg/day of PCB118. Arrows indicate vascular proliferation in the interfollicular areas. (C and D) Thyroid tissue from rats treated with 100 μg/kg/day (C) or 1000 μg/kg/day (D) of PCB118. Arrows indicate mesenchymal fibrosis and fibrinoid necrosis.

Fig. 4. Ultrastructural changes in thyroid follicle cells induced by PCB118 as revealed by TEM. (A) Control cells. (B–D) Cells administered 10–1000 μg/kg/day of PCB118. Panel A showed an abundance of neatly arranged microvilli and the morphology of the mitochondria and rough endoplasmic reticula were normal. Panel B: the follicular cells contained many vacuoles and few microvilli. Panel C: swollen mitochondria were nearly round with indistinct cristae. Panel D: greater numbers of mitochondria were present, but the mitochondrial cristae were fractured or absent. In panels B–D, the perinuclear gap was wider than that in panel A. FL, follicular lumen; M, mitochondria; Mi, microvilli; N, nucleus; RER, rough endoplasmic reticulum. Scale bar: 2 μm (big panels); 1 μm (small panels). PCB: polychlorinated biphenyl.
the expression of synthesis-associated genes. Systematic studies in vitro may be required to distinguish all the possible cytotoxic mechanisms of PCB118.

In conclusion, we demonstrated that chronic low-dose PCB118 can destroy the structure of the thyroid gland and dramatically decrease serum FT3 and FT4 concentrations, possibly by reducing the expression of proteins involved in TH biosynthesis.

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