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Chronic exposure to perfluorododecanoic acid disrupts testicular steroidogenesis and the expression of related genes in male rats

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ABSTRACT

Perfluorododecanoic acid (PFDoA), a synthetic perfluorinated chemical, has been detected in environmental matrices, wildlife, and human serum. Its potential health risk for humans and animals has raised public concern. However, the effects of chronic PFDoA exposure on male reproduction remain unknown. The aim of this study was to determine the effects of chronic PFDoA exposure (110 days) on testosterone biosynthesis and the expression of genes related to steroidogenesis in male rats. In this study, we examined the serum levels of sex hormones, growth hormone, and insulin in male rats. Testicular morphology and the expression of key genes and proteins in testosterone biosynthesis were also analyzed. Markedly decreased serum testosterone levels were recorded after 110 days of PFDoA exposure at 0.2 mg PFDoA/kg/day and 0.5 mg PFDoA/kg/day, and cast-off cells were observed in some seminiferous tubules in testes exposed to 0.5 mg PFDoA/kg/day. PFDoA exposure resulted in significantly decreased protein levels of steroidogenic acute regulatory protein (StAR) and cholesterol side-chain cleavage enzyme (P450scc), along with significantly reduced mRNA levels of insulin-like growth factor I (IGF-I), insulin-like growth factor I receptor (IGF-IR), and interleukin 1α (IL-1α) in rat testes at 0.2 mg/kg/day and 0.5 mg/kg/day. In addition, PFDoA exposure also affected the expression of some genes in the hypothalamo-neurohypophyseal system. However, PFDoA did not affect the expression of 5α-reductase, 3α-hydroxysteroid dehydrogenase, or aromatase in testis and liver. These findings demonstrate that chronic PFDoA exposure disrupts testicular steroidogenesis and expression of related genes in male rats. Multiple factors may be involved in the inhibition of testosterone by PFDoA.

1. Introduction

The perfluoroalkyl acids (PFAAs), including perfluorododecanoic acid (PFDoA, C12), perfluorodecanoic acid (PFDA, C10), perfluorooctanoic acid (PFOA, C8), and perfluorooctane sulfonic acid (PFOS, C8), are a family of perfluorinated chemicals that contain a carbon backbone. PFAAs are used as surfactants in industrial and consumer products, including firefighting foams, paint additives, and stain-resistant coatings for clothing (Renner, 2001). PFAAs have been detected in the environment, in wildlife and in human blood, testes, liver, and breast tissue in various countries (Lau et al., 2007). Studies determined that the total amounts of PFAAs transferred from nursing mothers to breast-feeding infants are approximately 200 ng/day and 23.5 ng/kg of bw/day in Sweden and the USA, respectively (Karrman et al., 2007; Tao et al., 2008). Although PFOA and PFOS are the two main PFAAs detected in samples worldwide, the existence of longer forms, such as PFDoA, has also been consistently reported in the recent scientific literature (Tao et al., 2008; Van de Vijver et al., 2007). For example, the highest PFDoA concentrations reported were 9.74 pg/ml in human milk in Massachusetts (Tao et al., 2008) and 9.5 ng/g (wet weight) in the liver of harbor porpoises from the Black Sea (Van de Vijver et al., 2007). The potential health risk of PFAAs to humans and wildlife has raised public concern.

PFAAs with chains of 8–10 carbons lead to hepatotoxicity, immunotoxicity, and lung toxicity in laboratory animals (Lau et al., 2007). In our previous study, PFDoA exposure also caused prominent hepatotoxicity in male rats (Zhang et al., 2008). Moreover, the reproductive toxicity of PFAAs has been reviewed in rodent models (Lau et al., 2007). PFDA targets the testes; exposure to PFDA results in obvious testicular toxicity, with decreased plasma testosterone and dihydrotestosterone levels in rats (Bookstaff et al., 1990; Olson and Andersen, 1983). PFOA exposure leads to decreased testosterone levels in serum and in testicular interstitial fluid, as well as increased serum estradiol levels in male rats (Biegel et al., 1995; Martin et al., 2007). Tumors in both Leydig cells and pancreatic acinar cells have been observed in male rats following chronic exposure to ammonium PFOA at 300 ppm in their diet (Biegel et al., 2001). An exclusive study examining PFDoA toxicological activity by our group demonstrated that 14 days of PFDoA exposure at a 5 mg/kg/day or 10 mg/kg/day dose resulted in testicular cell apop-

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tosis and a decline in serum testosterone (T) levels (Shi et al., 2007). Recently, PFAAs have been considered to be a potential endocrine disruptor, demonstrating effects on sex hormone levels (Jensen and Leffers, 2008).

PFDoA is likely to be more toxic than PFOA and PFDA, since PFAAs with longer carbon chains persist longer in the body than shorter chain PFAAs (Kudo et al., 2001; Ohmori et al., 2003). PFDoA has been shown accumulate more than PFOA in wildlife species (Senthilkumar et al., 2007). However, little is known about the toxic effects and mechanism of PFDoA on animals. Although an acute study suggests that testosterone inhibition by PFDoA is associated with cholesterol transport and steroidogenesis in the testes (Shi et al., 2007), it is unknown whether other signaling mechanisms are affected by PFDoA. Moreover, the toxic characteristics that occur in acute exposure studies with high doses may not be the same as those present during chronic exposure at low doses. In addition, since humans and animals are exposed to this chemical on a prolonged basis in normal environmental, chronic exposure provides a more relevant model to study the effects of PFDoA on reproductive function.

In the present study, we exposed male rats to PFDoA for 110 days to identify any effects on testicular function after chronic exposure. We analyzed the resulting changes in serum levels of sex hormones, growth hormone (GH), and insulin in male rats treated with PFDoA for 110 days. Real-time quantitative polymerase chain reaction (qPCR) and Western blot analyses were used to characterize the changes in gene and protein expression associated with cholesterol transport and testosterone biosynthesis. In addition, the expression of hypothalamo-neurohypophyseal genes responsible for regulating testicular function was also evaluated by qPCR. This study reveals the toxic effects of PFDoA on chronically exposed male rats and a possible molecular mechanism by which it disrupts steroidogenesis.

2. Materials and methods

2.1. Animals

Three-week-old male Sprague–Dawley rats were purchased from Weitong Lihua Experimental Animal Center, Beijing, China. Animals were housed separately and maintained in a mass air-displacement room with a 12-h light–dark cycle at 20–26 °C and a relative humidity of 40–60%. Animals had access to food and water *ad libitum*. All rats were acclimatized for 1 week before the experiment began.

2.2. Study protocol

PFDoA (CAS No. 307-55-1, 95% purity, Sigma–Aldrich) was dissolved in 0.2% Tween-20. Rats were divided into one control and four treatment groups. Each group contained six rats. The rats in the treatment group were given PFDoA orally at doses of 0.02 mg PFDoA/kg weight body/day, 0.05 mg PFDoA/kg weight body/day, 0.2 mg PFDoA/kg weight body/day, and 0.5 mg PFDoA/kg weight body/day for 110 days in a volume of 6 ml/kg of body weight. Control animals were treated with 0.2% Tween-20 (vehicle) delivered orally for 110 days in a volume of 6 ml/kg of body weight.

At the end of the experiment, all of rats from each group were weighed and euthanized by decapitation. Blood was collected and centrifuged at 2000 × *g* at 4 °C for 15 min. Serum was stored at −20 °C until analysis. The testes, prostate, seminal vesicle, and vas deferens of each animal were immediately isolated and weighed. One part of each testis was fixed in modified Davidson's fluid (mDF) containing 30% formaldehyde (concentration 37–40%), 15% ethanol, 5% glacial acid, and 50% distilled water for histological evaluation. Another part of the testis was immediately frozen in liquid nitrogen and stored at −80 °C until analysis.

2.3. Histological analysis

After fixation in mDF for 48 h, testes were embedded in paraffin. Sections of 5 μm in thickness were serially cut from every testis and mounted on glass slides. Slides were stained with hematoxylin and eosin (H&E). Images were photographed using a BH2 microscope fitted with a DP-71 digital camera (Olympus, Tokyo, Japan).

2.4. Detection of serum hormone and total cholesterol levels

Serum testosterone concentrations were measured by the enzyme-linked immunosorbent assay (ELISA) using commercial rat ELISA kits (RapidBio Lab. Calabasas, CA, USA). Estradiol (E_2) concentrations were analyzed by chemiluminescence, while concentrations of follicle-stimulating hormone (FSH), luteinizing hormone (LH), growth hormone (GH), and insulin were determined by the radioimmunoassay (RIA) using commercial kits (Beijing North Institute of Biological Technology, China). Total cholesterol (TCHO) in serum was measured with a commercial TCHO kit (Biosino Bio-technology and Science Inc., Beijing, China). Total cholesterol was quantified by colorimetry (500 nm) using a UV1240 spectrophotometer (Shimadzu, Japan).

2.5. RNA extraction

Testicular RNA was isolated from the testis with TRIZOL reagent (Invitrogen Corp., Carlsbad, CA, USA), according to the manufacturer's instructions. Pituitary and hypothalamic RNA were extracted using the RNeasy kit (Qiagen, Germany). RNA was purified on an affinity resin (Qiagen, Germany). The concentration of total RNA was measured by absorbance at 260 nm using a UV1240 spectrophotometer (Shimadzu, Japan). The purity was estimated by the 260/280 nm absorbance ratio. The quality of total RNA was further confirmed based on the integrity of 28S and 18S rRNA using 1% agarose gel electrophoresis. All RNA samples were stored at −80 °C until analysis.

2.6. Real-time quantitative polymerase chain reaction (qPCR)

RNA samples from each rat were synthesized into cDNA by reverse transcription using an oligo-$(dT)_{15}$ primer (Promega, USA) and M-MuLV reverse transcriptase (Promega, USA), according to the manufacturer's instructions. Real-time PCR reactions were performed using SYBR Green PCR Master Mix reagent kits on the Stratagene Mx3000P qPCR system (Stratagene, USA). The detected genes included luteinizing hormone (LHR) (GenBank accession no. NM_012978), scavenger receptor class B type 1 (SR-B1) (GenBank accession no. AY451993), steroidogenic acute regulatory protein (StAR) (GenBank accession no. NM_031558), cholesterol side-chain cleavage enzyme (P450scc) (GenBank accession no. J05156), 3-beta-hydroxysteroid dehydrogenase (3β-HSD) (GenBank accession no. M38178), 17-beta-hydroxysteroid dehydrogenase (17β-HSD) (GenBank accession no. NM_054007), cytochrome P450c17 subfamily a (CYP17a) (GenBank accession no. NM_012753), aromatase (GenBank accession no. NM_017085), androgen receptor (AR) (GenBank accession no. NM_012502), insulin-like growth factor I (IGF-I) (GenBank accession no. NM_178866), insulin-like growth factor I receptor (IGF-IR) (GenBank accession no. NM_052807), insulin-like growth factor binding protein 3 (IGFBP3) (GenBank accession no. NM_012588), growth hormone receptor (GHR) (GenBank accession no. J04811), insulin receptor (IR) (GenBank accession no. M29014), interleukin-1 alpha

Table 1
Changes in body and reproductive organ weights in PFDoA-treated male rats.

	Doses (mg/kg/day)				
	0	0.02	0.05	0.2	0.5
BW (g)	556.33 ± 11.26	562.00 ± 11.80	571.33 ± 6.48	546.83 ± 1.47	525.50 ± 11.75*
TW (g)	3.65 ± 0.14	3.54 ± 0.09	3.75 ± 0.05	3.40 ± 0.11	3.15 ± 0.20
RTW (%)	0.65 ± 0.02	0.63 ± 0.02	0.66 ± 0.01	0.62 ± 0.02	0.61 ± 0.03
PW (g)	0.65 ± 0.03	0.58 ± 0.06	0.64 ± 0.02	0.65 ± 0.04	0.60 ± 0.02
RPW (%)	0.12 ± 0.01	0.10 ± 0.01	0.11 ± 0.01	0.12 ± 0.01	0.11 ± 0.01
SW (g)	1.47 ± 0.12	1.67 ± 0.16	1.75 ± 0.04	1.74 ± 0.07	1.83 ± 0.10
RSW (%)	0.26 ± 0.02	0.29 ± 0.03	0.31 ± 0.01	0.33 ± 0.02	0.30 ± 0.02
VW (g)	0.24 ± 0.01	0.22 ± 0.02	0.26 ± 0.01	0.25 ± 0.01	0.25 ± 0.01
RVW (%)	0.043 ± 0.002	0.039 ± 0.004	0.049 ± 0.002	0.045 ± 0.001	0.047 ± 0.001

Values represent the means ± S.E.M., $n = 6$. Asterisks indicate a statistically significant difference compared to the control, $p < 0.05$ (one-way ANOVA followed by Dunnett's post hoc two-sided *t*-test). BW, TW, PW, SW, and VW indicate body, testis, prostate, seminal vesicle, and vas deferens weights, respectively. RTW, RPW, RSW, and RVW indicate relative testis, prostate, seminal vesicle and vas deferens weights, respectively.