Reproductive Toxicology 69 (2017) 159–166

Contents lists available at ScienceDirect

Reproductive Toxicology

journal homepage: www.elsevier.com/locate/reprotox

Maternal exposure to perfluorooctanoic acid inhibits luteal function via oxidative stress and apoptosis in pregnant mice

Yilu Chen[1], Ling Zhou[1], Jingjie Xu[1], Lu Zhang, Mo Li, Xingxing Xie, Yajuan Xie, Dan Luo, Dalei Zhang, Xiaochun Yu, Bei Yang*, Haibin Kuang*

Jiangxi Provincial Key Laboratory of Reproductive Physiology and Pathology and Department of Physiology, School of Medicine, Nanchang University, Nanchang, Jiangxi, China

ARTICLE INFO

Article history:
Received 15 September 2016
Received in revised form 9 February 2017
Accepted 15 February 2017
Available online 20 February 2017

Keywords:
Perfluorooctanoic acid
Reproductive toxicity
Luteal function
Oxidative stress
Apoptosis

ABSTRACT

Perfluorooctanoic acid (PFOA) is a synthetic perfluorinated compound, which has been reported to exert adverse effect on the pregnancy. However, whether it is associated with alteration of luteal function remains unknown. Mice were administered PFOA by gavage from gestational days (GD) 1–7 or 13. PFOA treatment did not significantly affect numbers of embryo implantation. Nevertheless, on GD 13, 10 mg/kg PFOA treatment significantly increased numbers of resorbed embryo. Furthermore, PFOA exposure markedly reduced serum progesterone levels but did not affect estradiol levels. Treatment also showed concomitant decreases in transcript levels for key steroidogenic enzymes, and reduced numbers and sizes of corpora lutea. In addition, PFOA administration inhibited activities of superoxide dismutase and catalase, and increased generation of hydrogen peroxide and malondialdehyde, and down-regulated level of Bcl-2 and up-regulated p53 and BAX proteins. In conclusion, PFOA exposure significantly inhibits luteal function via oxidative stress and apoptosis in pregnant mice.

1. Introduction

Perfluorooctanoic acid (PFOA), a synthetic perfluorinated eight-carbon organic chemical, has been used extensively in various consumer products for hydrophobic and lipophobic properties since the 1950s, including non-stick cookware, lubricants, polishes, paper and textile coatings, food packaging, and fire-fighting foams [1]. Due to its persistence and bioaccumulation, PFOA was classified as one of the persistent organic pollutants at the Stockholm Convention in 2009 [1,2]. Studies have indicated that food packaging, house dust, water and dietary intake were all potentially significant sources of PFOA [2,3]. PFOA is well absorbed following oral exposure, but poorly eliminated [3,4]. In humans, the average half-life for serum elimination of PFOA is estimated at 3.8 years (95% confidence intervals, 3.1–4.4) [5,6]. The arithmetic means of PFOA in nonoccupationally exposed human blood sera of industrialized countries range between 4.0 and 10 ng/mL [7], but occupational exposure can raise serum concentrations more than 200 times and the serum PFOA level could reach 691 ng/mL (range, 72–5100 ng/mL) in fluorochemical production workers [8].

In recent years, many reports have shown that PFOA had potential hazardous effects on animal and human health, such as hepatotoxicity, carcinogenicity, developmental toxicity and especially reproductive toxicity [9–12]. Epidemiology studies showed that high levels of PFOA were associated with a higher percentage of malformed sperm with coiled tails [5,13]. Animal experiments demonstrated that exposure to PFOA obviously disrupted seminiferous tubules and reduced sperm count in mice [5], and inhibited testosterone secretion of rat Leydig cells via downregulation of 3beta- and 17beta-hydroxysteroid dehydrogenase activities [14]. In addition, some studies demonstrated that PFOA also affected ovarian steroid hormone production as a novel means of endocrine disruption. In H295R cells, PFOA treatment could increase estradiol production [15]. Epidemiological studies indicated that delayed

Abbreviations: CAT, catalase; Cyp11a1, cytochrome P45011A1; GD, gestational days; H_2O_2, hydrogen peroxide; Hsd3b1, 3β-hydroxysteroid dehydrogenase; MDA, malondialdehyde; PFOA, perfluorooctanoic acid; ROS, reactive oxygen species; SOD, superoxide dismutase; StAR, steroidogenic acute regulatory protein; VTG, vitellogenin.

* Corresponding authors at: Department of Physiology, School of Medicine, Nanchang University, Nanchang, Jiangxi 330006, China.
E-mail addresses: yilua_chen@163.com (Y. Chen), noradrenalin@126.com (L. Zhou), xjj0605@163.com (J. Xu), 13393733537@163.com (L. Zhang), 544182657@qq.com (M. Li), 1120434253@qq.com (X. Xie), 1454503826@qq.com (Y. Xie), thinker_20080502@126.com (D. Luo), zhangdalei@ncu.edu.cn (D. Zhang), physiology9909@163.com (X. Yu), yangbei@ncu.edu.cn (B. Yang), kuanghaibin@ncu.edu.cn (H. Kuang).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.reprotox.2017.02.010
0890-6238/

puberty in girls was associated with the highest levels of serum PFOA [16]. Furthermore, there are many reports that PFOA is developmentally toxic that includes dose-related full litter resorptions, reduced fetal weight, reduced prenatal and postnatal survival, fetal growth retardation in mice [4,17–19]. However, whether it is associated with the alteration of luteal function during pregnancy remains unknown. Therefore, our objective in the present study was to investigate the effect and mechanism of PFOA on the luteal function during early- and mid-pregnancy.

2. Materials and methods

2.1. Animals

Adult female Kunming mice (25–30 g) obtained from the Animal Facility of Jiangxi traditional Medical University were maintained in a consistent photoperiod (12 h light: 12 h dark cycle) with free access to water and standard feed ad libitum. All experimental procedures were performed in accordance with the Guidelines for Care and Use of Laboratory Animals and approved by the Animal Ethics Committee of Nanchang University.

2.2. Treatments

PFOA (96% purity, Sigma-Aldrich, USA) was dissolved in deionized water. After 3 days of acclimatization, adult virgin female mice were mated with fertile males of same strain in order to induce pregnancy. The following morning of finding a vaginal plug was regarded as gestational days (GD) 1. Pregnant mice divided into 4 groups (n = 12/group) were weighed and administered daily with different concentrations of PFOA (2.5, 5 or 10 mg/kg/day) by gavage from GD 1 until the day of sacrifice. Control group was given an equivalent volume of water (0.1 ml/10 g body weight). Treated mice were sacrificed through cervical dislocation for ovarian and uterine collection between 16:00 and 17:00 on GD 7 and 13. Uteri and ovaries from these mice were photographed by using digital camera (Nikon, Japan), and the implantation sites of treated mice were counted on GD 7 and GD13. The ovaries were weighed and frozen in liquid nitrogen for further research.

2.3. Hormone measurements

The levels of serum estradiol and progesterone were measured using specific radioimmunoassay kits (Jiuding Medicine Biotechnology Co, China). Samples were detected in duplicate. The intra- and inter-assay coefficients of variation using these kits did not exceed 10%. The cross-reactivities with other peptides and steroid hormones in these kits did not exceed 4%. The detection limits of estradiol and progesterone kits are 1 pg/mL and 0.25 ng/mL, respectively.

2.4. Ovarian histomorphology

Ovaries (one per mouse) free of oviduct, fat, and bursa were fixed in Bouin's solution for about 10 h, dehydrated in gradient ethanol (70% and 95% twice and 100% twice, at intervals of 1 h each), cleared in xylol/ethanol (1:1 v/v), infiltrated with paraffin wax, and then they were serially sectioned (thickness, 5 μm). Sections were mounted and stained with hematoxylin and eosin for morphological observation and histomorphology analysis. All ovaries were analyzed without treatment knowledge to avoid bias. Corpora lutea were classified based on their morphological appearances because, in the ovary, those units contain luteinized cells and are delimited by a layer of regressed theca cells. The numbers and sizes of corpora lutea were counted and measured in every 5th out of 30 midline serial sections of each ovary using a Nikon DS-Fi1 microscope (Nikon, Japan) at a 10× objective magnification. Mean number and area of corpora lutea were calculated using 6 selected sections for each ovary, and then for each group (6 mice per group). The ratio of corpora lutea to ovarian areas was measured and quantified using ImageJ software (v. 1.45s, NIH).

2.5. RNA isolation and quantitative PCR

Total RNA was extracted from ovary tissues using RNAiso Plus solution (TaKaRa, China) and reverse-transcribed into single-stranded cDNA in a 25 μl reaction mixture (TaKaRa, China). Real-time PCR was then performed in a 20 μl reaction volume containing 10 μl of 2 x Brilliant SYBR Green Mix (TaKaRa, China), 2 μl of template cDNA, 0.5 μM primers, and 300 nM reference dyes using the ABI thermal cycler 7500. The thermal cycling conditions were 95 °C for 30 s, followed by 40 cycles at 94 °C for 5 s, 60 °C for 34 s. Melting curve analysis and agarose gel electrophoresis were conducted following the quantitative PCR assays to monitor PCR product purity. The results were analyzed using ABI Prism 7500 software (Applied Biosystems, USA). 18 S was used for normalization. The following primers were used: 18S (Accession: NR_003278): sense, 5′-AATCAG GGT TCG ATT CCG GA–3′; antisense, 5′-CCAAGA TCC AAC TAC GAG CT-3′. StAR (Accession: NM_011485): sense, 5′-CGC AGA GGT TCC ACC TGT GT-3′; antisense, 5′-TCC GGC ATC TCC CCA AA-3. Cyp11a1 (Accession: NM_019779): sense, 5′-CCG GAG CGG TTC CTT GT-3′; antisense, 5′-CCA ATG GGC CTC TGA TAA TAC TG-3′. Hsd3b1 (Accession: NM_013821): sense, 5′-GGA GGA AGC CAA GCA GAA AA-3′; antisense, 5′-CCC TGT GCT GCT CCA CTA GTG-3′.

2.6. Analysis of MDA, H_2O_2, SOD and CAT

The frozen ovarian tissues were homogenized in ice-cold PBS solution. The levels of malondialdehyde (MDA), hydrogen peroxide (H_2O_2), superoxide dismutase (SOD) and catalase (CAT) in ovary tissue were measured by specific commercial available kits (Jiancheng Institute of Biotechnology, Nanjing, China) according to the manufacturer's instructions. The intra and inter-assay coefficients of variation did not exceed 7.1% and 8.2%, respectively. Protein concentrations were determined using the bicinchoninic acid assay kit (Jiancheng Institute of Biotechnology, Nanjing, China)

2.7. Western blot analysis

The ovary tissues were homogenized in ice-cold radio immunoprecipitation assay buffer supplemented with a phosphatase inhibitor cocktail (Applygen Technologies, China) and phenylmethylsulfonyl fluoride, then centrifuged 16,099 *g* for 10 min at 4 °C. The proper volume protein extracts were subjected on the 15% SDS-polyacrylamide gel for electrophoresis, and transferred onto the polyvinylidene difluoride membranes (Millipore, USA). The membranes were blocked with 5% bovine serum albumin for 1 h at room temperature and incubated with the following primary antibodies at 4 °C overnight: rabbit anti-β-actin, p53 (Santa Cruz, USA), Bax and Bcl-2 (cell signaling, USA). The membranes were then incubated with horseradish peroxidase-conjugated secondary antibodies and visualized via enhanced chemiluminescence (Pierce, USA). The relative band intensity was acquired by using the Quantity One software. The data were corrected for background, normalized to β-actin expression.

2.8. Statistical analysis

The data are presented as the means ± SE. The results were analyzed by using one-way analysis of variance followed by least-significant difference's post-hoc test. A value of $P < 0.05$ was