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In utero perfluorooctane sulfonate exposure causes low body weights of fetal rats: A mechanism study

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ABSTRACT

Objectives: The objective of the present study is to investigate the mechanism of perfluorooctane sulfonate-induced low body weight of fetus by analysis of glucocorticoid metabolizing enzyme 11β-hydroxysteroid dehydrogenase 2 and gene expression profiling of the placenta after *in utero* PFOS exposure.
Study design: Pregnant Sprague–Dawley dams were gavaged with 0, 5, and 20 mg/kg body weight PFOS daily from gestational day 12–18. On gestational day 18, pregnant dams were euthanized, placentas, and fetuses were collected.
Main outcome measures: Body weights of fetuses and placentas were measured, the corticosterone levels in fetal serum, and 11β-hydroxysteroid dehydrogenase 2 as well as the placental gene profiling were analyzed.
Results: 20 mg/kg PFOS significantly reduced fetal body weight and placental weight. Both 5 and 20 mg/kg PFOS increased fetal serum corticosterone levels. PFOS potently inhibited placental 11β-hydroxysteroid dehydrogenase 2 activity. Of 21,910 genes, 45 genes were significantly downregulated ≥2 fold by 20 mg/kg PFOS, including extracellular matrix (*Slpi* and *Pi16*), growth factors and hormones (*Trh* and *Pdf*), ion transporters (*Aqp1*, *S100a4*, and *Abp1*), signal transducers (*Kap* and *Ampd3*), and structural constituents (*A2m* and *Des*).
Conclusions: PFOS exposure may alter placental development and function, causing intrauterine growth restriction via inhibiting placental 11β-hydroxysteroid dehydrogenase 2.

1. Introduction

Perfluorinated chemicals are environmentally stable compounds, and have been widely used in industrial, commercial, and consumer applications [1]. Perfluorooctane sulfonate (PFOS), one of mostly abundant perfluorinated chemicals, is present in the environment ubiquitously, because it is very stable and persistent in the environment. When absorbed, the elimination rate of PFOS in human beings is very long, with the elimination half-life of about 5 years [2]. Therefore, concern has been arisen about its potential effects on human health. In rodent models, it has been shown that PFOS has hepatotoxicity [3], carcinogenicity [3], immunotoxicity [4], reproductive system toxicity [5], and developmental toxicity [6].

PFOS can cross the placental barrier, therefore it has a potential to affect fetal growth. In rodents, the dam's toxicity and fetal developmental toxicities of PFOS have been evaluated in Sprague–Dawley rats and CD-1 mice [6]. Pregnant rats were orally administered PFOS with 1, 2, 3, 5, or 10 mg/kg/day from gestational day (GD) 2 to GD 20 and pregnant mice with 1, 5, 10, 15, and 20 mg/kg PFOS from GD 1 to GD 17 [6]. 3 mg/kg or above of PFOS (for rats)

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and 10 mg/kg or above (for mice) significantly reduced dam's body weights, while 10 mg/kg or above of PFOS also decreased fetal body weights [6,7]. Our previous study also showed that PFOS (5 and 20 mg/kg) by gavage from GD 11 to GD 19 significantly reduced male pup body weights and 20 mg/kg PFOS also reduced dam's body weights [8]. Epidemiological studies also point to the fact that the decreased body weights of neonates were associated with the cord serum level of PFOS [9]. A recent study using a physiologically based pharmacokinetic model to detect the associations of PFOS-related chemicals with lower birth weight also indicates that each 1-ng/ml elevation in prenatal PFOS levels was associated with 5.00 g decreases in birth weights [10].

Excess of glucocorticoids during the pregnancy has been linked with intrauterine growth restriction (IUGR) [11]. However, the placenta has a protective mechanism for the fluctuation of glucocorticoid, which is served by 11β-hydroxysteroid dehydrogenase isoform 2 (HSD11B2). HSD11B2 is a high-affinity NAD^+ dependent enzyme that inactivates active glucocorticoid (corticosterone, CORT, in rodents) into biologically inert 11keto steroid (11-dehydrocorticosterones, 11DHC) [12]. HSD11B2 is localized in the syncytiotrophoblast, which is the site of maternal–fetal exchange in the placenta [13]. The placental HSD11B2 plays a key role in pregnancy maintenance and fetal maturation. Thus, placental HSD11B2 is the critical enzyme that protects the fetus from overexposure to maternal glucocorticoid, which may check the fetal development [14]. In a mouse with *Hsd11b2* knockout, body weights of fetus and placental weights were significantly reduced, indicating that intrauterine restriction of fetuses after knockout of the enzyme is mediated partially by the altered placental transport of nutrients and structure as well as reduction in placental blood system [15,16]. Rats and mice exposed to PFOS had increased levels of the circulating corticosterone (CORT), an active glucocorticoid in rodents [4,17], indicating that in the placenta HSD11B2 protects the fetus from the high circulating levels of maternal glucocorticoid. However, whether PFOS inhibits placental HSD11B2 activity to increase CORT levels in fetal circulation and how it affects placental gene expression levels is unclear. The objective of the present study was to investigate the effects of PFOS on fetal serum CORT levels and to identify the important signaling pathways in the PFOS-mediated placental function using gene expression profiling.

2. Materials and methods

2.1. Chemicals

[1,2,6,7-N-^{3}H]-Corticosterone (^{3}H-CORT, specific activity, 88 Ci/mmol) was purchased from Dupont–New England Nuclear (Boston, MA). [^{3}H]11 Dehydrocorticosterone (^{3}H-11DHC) was prepared from labeled ^{3}H-CORT as described earlier [18]. Unlabeled CORT and 11DHC were purchased from Steraloids (Newport, RI). PFOS potassium salt (purity $\geq$ 98%) was purchased from Sigma–Aldrich (St. Louis, MO, USA). Pregnant female Sprague–Dawley rats were purchased from Charles River Laboratories (Wilmington, MA).

2.2. Animal study

Rats were acclimated to the experimental environment for one week. Feed and water were provided ad libitum, and animals were kept on a 12L: 12D cycle. The adult pregnant dams were randomly divided into three groups (ten rats per group): control (vehicle, 0.5% Tween-20), 5, and 20 mg/kg PFOS. The doses were selected according to previous studies that PFOS had developmental toxicity when these doses were used [6,8]. PFOS was orally administered daily by gavage from gestational day (GD) 12 to GD 18 with 0, 5, or 20 mg/kg PFOS in 0.5% Tween-20. The pregnant dams were euthanized on GD 18.5 by CO2. The placentas and fetuses were collected. The body weights of each fetus and dam were weighed. Placentas were weighed, and stored at −80 °C until analysis. The body weights of fetuses per litter and the placental weights per dam were averaged before statistical analysis. All animal procedures were approved by the Rockefeller University's Animal Care and Use Committee.

2.3. Preparation of placental microsomal protein

Rat placental microsomes were prepared. Rat placenta was homogenized in 0.01 mM phosphate buffered saline (PBS, pH 7.4) containing 0.25 M sucrose, and nuclei and large cell debris were removed by centrifugation at 700×g at 4 °C for 30 min. The post-nuclear supernatants were centrifuged at 14,500×*g* at 4 °C for 30 min to remove mitochondria, and the resulting supernatants were further centrifuged twice at 105,000×*g* at 4 °C to collect microsomes. The resultant microsomal pellets were resuspended. Protein contents were measured by Bio-Rad Dye Reagent Concentrate according to manufacturer's instructions. The concentration of microsome protein was adjusted to 2 mg/ml. Microsomes were used for the measurement of HSD11B2 activities.

2.4. Enzyme activity assay

The oxidation of CORT by HSD11B2 was determined in a mixture containing 25 nM CORT (plus 30,000 cpm [^{3}H]-CORT), 0.2 mM NAD^+, 10 mM DTT, and 2% ethanol in 0.1 mM potassium phosphate buffer (pH 7.2, 250 μL total volume) at 37 °C as described previously [19]. Reactions were initiated by the addition of microsomes and NAD^+ and terminated by the addition of 2 mL ice-cold ether. The steroids were extracted by vigorous mixing for 1 min, and the organic layer was transferred and dried under nitrogen. The steroids were separated chromatographically on thin layer plates in chloroform and methanol (90:10, v/v), and the radioactivity was measured under a scanning radiometer (System AR2000, Bioscan Inc., Washington, DC). The percentage conversion of CORT to 11DHC was calculated by dividing the radioactive counts identified as 11DHC by the total counts associated with CORT plus 11DHC as previously described [19].

2.5. RIA of fetal serum CORT

The plasma CORT levels were measured using the method from Spencer et al. [20]. In brief, serum samples (20 μL) were diluted in 1 mL of 0.01 M phosphate buffer and were heated at 60 °C for 1 h in order to inactivate corticosteroid-binding globulin. The heat-inactivated samples (100 μL in triplicate) were incubated overnight with a mixture of rabbit CORT antiserum and ^{3}H-CORT. Bound steroid was separated from free steroid by mixing with dextran-coated activated charcoal followed by centrifugation. The bound supernatant was put into a bottle with scintillation cocktail, and the relative amount of radioactivity was determined by a liquid scintillation counter (Packard, Meriden, CT, USA). The interassay coefficient of variability was calculated, and it was 7.1% (n = 4).

2.6. RNA extraction from whole placenta tissues

Total RNA from each whole placental tissue was extracted using RNeasy kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. The quality and quantity of RNAs were evaluated at 260 nm and 280 nm spectrophotometrically using the NanoDrop 2000 spectrophotometer (Fisher Scientific, NJ) with absorbance ratio at 260 and 280 nm over 2.0, and integrity of the RNA was examined with an Agilent 2100 Bioanalyzer (Agilent, Santa Clara,