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Gestational and lactational exposure to potassium perfluorooctanesulfonate (K^+PFOS) in rats: Toxicokinetics, thyroid hormone status, and related gene expression

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ABSTRACT

Perfluorooctanesulfonate (PFOS), a persistent and accumulative compound, is widely distributed in humans and wildlife. Human exposure can occur early in development, as evidenced by the detection of PFOS in umbilical cord blood and breast milk. As part of a developmental neurotoxicology study for which developmental endpoints, including those related to the developing nervous system, have been reported separately, groups of 25 pregnant Sprague Dawley rats were given daily oral doses of either vehicle control or potassium PFOS (K^+PFOS) at 0.1, 0.3, and 1.0 mg/kg-d from gestation day (GD) 0 (day positive for mating) through postnatal day (PND) 20. An additional 10 pregnant females per treatment group were treated through GD 19 and sacrificed on GD 20 in order to obtain maternal and fetal serum and tissue samples at the end of gestation. The present paper reports the results of samples of serum, liver, brain, and thyroid glands taken at various times to evaluate: (1) serum, liver, and brain PFOS concentrations by LC–MS/MS to establish the relationship between PFOS concentrations and study outcomes; (2) serum thyrotropin (TSH) concentrations by RIA; (3) thyroid follicular cell proliferation index by Ki-67 immunohistochemical staining; (4) thyroid follicle epithelial cell height and colloidal area by histomorphometric analysis; (5) selected liver mRNA transcripts by quantitative RT-PCR. PFOS concentrations in dam and pup serum, liver, and brain increased across treatment groups in approximate proportion to the proportional increases in maternal K^+PFOS dose, and sex differences in PFOS concentrations were not apparent in pups on PND 21. In pups from K^+PFOS maternal dose groups on PND 72, serum PFOS had decreased to about 3 and 11% of PND 21 concentrations in males and females, respectively, and liver PFOS had decreased to about 17% of PND 21 concentrations in both sexes. Liver PFOS concentrations were approximately 0.6–0.8 times serum PFOS in GD 20 fetuses, and increased to about 2–4 times serum concentrations on PND 4 and 21. GD 20 fetal and PND 4 pup brain PFOS concentrations were approximately 33% of the corresponding serum concentrations, dropping to approximately 10% by PND 21, in contrast to dam brain PFOS concentrations, which were approximately 4–9% of serum PFOS concentrations. Compared to controls, Cyp2b2 mRNA was increased (2.8-fold) in the 1.0 mg/kg-d treatment-group dams on GD 20. In male pups on PND 21, Cyp4A1, ACoA, and Cyp2b2 were increased 2.1-, 1.5-, and 1.8-fold, respectively, and Cyp7A1 was decreased 3.5-fold. Serum TSH and thyroid follicular morphology were not altered by K^+PFOS treatment. The mean number of proliferating thyroid follicular cells was increased 2.1-fold over control in GD 20 female fetuses from 1.0 mg/kg-d-treated dams, yet the highest individual count was similar to that of controls (116 versus 113 in controls).

1. Introduction

Perfluorooctanesulfonate (PFOS, $C_8F_{17}SO_3^-$) has been found to be widely distributed in samples from humans and wildlife [1–3]. PFOS is exceptionally resistant to environmental and metabolic degradation [4,5], and has been shown to bioconcentrate and biomagnify in the marine food web [6]. Due to evidence of widespread presence, persistence, and accumulation of PFOS in the environ-

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ment, 3M Company, the major manufacturer of PFOS and other compounds formed from perfluorooctanesulfonyl fluoride that may potentially degrade to form PFOS, announced on 16 May 2000 that 3M Company would discontinue manufacture of these materials by the end of 2002. There has been interest in the environmental and health properties of PFOS at an international level [7].

Children are known to be exposed to PFOS [8,9], and these exposures can occur from gestational and lactational transfer of PFOS [10–17]. As reviewed by Olsen et al. [18 this issue], there are several groups of investigators that have studied the association of PFOS concentrations in human maternal and/or umbilical cord blood to birth outcomes, and one recent study followed developmental landmarks in infants through approximately 18 months of age [19].

The developmental toxicity of PFOS has been studied extensively in laboratory rats and mice [20–32], and one developmental study in rabbits has been reported [27]. In the developmental and reproductive studies with rats and mice, it has been demonstrated that neonates can be exposed to PFOS from *in utero* and lactational exposures [20,22]. Effects on gestation length, birth weight, postnatal growth and developmental delays, and neonatal survival have been noted [20,22–25,32]. Structural anomalies have been noted in fetal rats and mice at doses that affect maternal weight gain and food and/or water consumption [26,27] and include cleft palate, anasarca, ventricular septal defect, and enlargement of the right atrium. Structural defects were not observed in rabbits at maternally toxic dose levels [27]. Increased motor activity and decreased habituation have been noted in developmental neurotoxicological evaluations in rats and mice [28,29,31]; however, learning and memory do not appear to be affected [20,22,31].

Neonatal mortality and delayed postnatal growth in rat and mouse pups exposed to PFOS *in utero* are pronounced effects in these laboratory species that occur at doses not affecting the maternal rodents in an obvious manner. In exploring the potential etiology of these effects in laboratory rats and mice, several investigations have focused on lung development [20,23,24], cholesterol metabolism [25], the potential role of activation of the nuclear receptor peroxisome proliferator activated receptor α (PPARα) [32 this issue], and thyroid hormone status [22,25,26,31]. At the present time, neonatal mortality resulting from *in utero* exposure of rats to PFOS is hypothesized to result from alterations in lung function at birth, perhaps through a direct interaction of PFOS with components of pulmonary surfactant [23,33]. Alterations in cholesterol metabolism did not appear to relate to altered developmental outcomes [25]. Recent studies suggest that PFOS, unlike the eight-carbon perfluorinated carboxylate, perfluorooctanoate (PFOA), may not act via PPARα activation to produce the majority of observed developmental effects [32,34].

Thyroid hormones have numerous important roles in development [35–38] and are critical for mammalian brain development and maturation in that they control expression of genes involved in myelination, cell differentiation, migration, and signaling [35,36]. PFOS exposure has been associated with maternal and offspring hypothyroxinemia without a compensatory elevation of TSH in laboratory rats [22,25,26]. Although hypothyroxinemia was noted in a dose-dependent manner in pregnant mice on gestation day (GD) 6 by Thibodeaux et al. [26], it was not present on GD 12 or 18 in their study or in pregnant mice on GD 18 in a study reported by Fuentes et al. [39]. Neither was hypothyroxinemia observed in neonatal mice from PFOS-treated dams [22]. The ability of the pituitary to respond to hypothalamic thyrotropin-releasing hormone to release TSH in response to decreased thyroid hormone production after treatment with propylthiouracil was not altered in rats by co-treatment with PFOS [40]. The activity of choline acetyltransferase, an enzyme sensitive to thyroid hormone status, was marginally but statistically-significantly reduced in rat pups from PFOS-treated dams; however, activity in the hippocampus was unaffected [22]. PFOS-exposed rats appear to maintain a euthyroid state despite significant reductions in serum total thyroid hormones, likely due to competition for binding sites between PFOS and thyroid hormones in rat serum, leading to an adequate supply of free hormone while reducing the concentration of hormone carried on serum binding proteins [40–42].

The PFOS-induced hypothyroxinemia reported in previous developmental studies [22,25,26] led us to evaluate thyroid status and histomorphological factors associated with thyroid follicles during the course of a developmental neurotoxicological study in rats. The maternal, birth outcome, litter, and developmental neurotoxicity endpoints have been reported separately in a companion article to this [31 this issue]. Herein, we report the results of thyroid parameters, in addition to concentrations of PFOS in samples of serum, liver, and brain taken at various times during the study as well as and the results of quantitative evaluation of a select set of liver mRNA transcripts associated with liver hypertrophic modes of action, thyroid hormone and cholesterol metabolism, and liver cell proliferation.

2. Methods

2.1. Study design

The work presented in this article represents the analysis of biological samples collected during the course of a developmental neurotoxicity study involving gestational and lactational exposure to potassium PFOS (K^+PFOS, CASRN 2795-39-3, Lot number 217 (86.9% pure) 3M Company, St. Paul, MN) in rats. The details of treatment as well as results of birth outcomes, litter parameters, and neurological investigations have been reported in a companion article [31 this issue]. Briefly, groups of 25 pregnant Sprague Dawley rats (maternal rats) were given daily oral doses of either vehicle control (0.5% Tween 20 in water) or K^+PFOS (solublized in 0.5% Tween 20 in water due to limited water solubility of K^+PFOS) at 0.1, 0.3, and 1.0 mg/kg-d GD 0 (day positive for mating) through postnatal day (PND) 20. An additional 10 pregnant females per treatment group were treated through GD 19 and sacrificed on GD 20 in order to obtain maternal and fetal serum and tissue samples at the end of gestation. Pups were allowed to nurse until PND 21 and evaluated for developmental neurotoxicity landmarks specified per protocol throughout PND 72. Rats were euthanized by carbon dioxide asphyxiation. There was no evidence of treatment-related effect on postnatal growth and survival.

Aliquots of the dosing solutions were analyzed for concentration, stability, and homogeneity. The LC–MS/MS analyses indicated that the dosing solution aliquots were 97–100% of the target concentration and all were stable and homogeneous.

All experiments involving live animals were performed in laboratory accredited by Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC) and all procedures were reviewed and approved by facility's Institutional Animal Care and Use Committees (IACUC). Animal care and procedures were followed according to the US Department of Health and Human Services guide for the care and the use of laboratory animal guideline [43].

2.2. Determination of PFOS concentrations in serum, liver, and brain

Serum samples for PFOS analysis were obtained from dams on GD 20, PND 4, and PND 21, fetuses (pooled by litter) on GD 20, pups (pooled by litter) on PND 4, and individual male and female offspring on PND 21 and 72. Samples of livers for PFOS analysis were obtained from dams on GD 20, fetuses and pups (pooled by litter) on GD 20 and PND 4, respectively, and individual offspring on PND 21 and 72. Samples of brains for PFOS analysis were obtained from dams on GD 20, fetuses and pups (pooled by litter) on GD 20 and PND 4, respectively, and individual offspring on PND 21. Samples of serum, liver, and brain were snap frozen and remained frozen at approximately −80 °C until processing for analysis.

New Zealand newborn calf serum (Invitrogen, Carlsbad, CA) and liver and brain homogenates obtained from naïve Sprague Dawley rats (male and female, 10–12 weeks old) were used as the blank matrices to prepare the appropriate matrix-matched PFOS standard curves.

Due to limited sample volume and size, serum and liver samples from GD 20 fetus and PND 4 pups were pooled by litter. PFOS concentrations were determined by LC–MS/MS as described in Chang et al. [41]. Briefly, liver samples were homogenized in water (1 part liver and 4 parts water, w/w) with IKA® WERKE Ultra-Turrax T25 homogenizer at 20,000 rpm for ∼1.5 min followed by sonication in a water bath sonicator (30 min). After adding $^{18}O_2$-PFOS internal standard, 100 μL serum samples and/or liver homogenate aliquots were further treated with 1 mL of 1.0N formic acid, 100 μL of saturated ammonium sulfate (serum samples only), and 300 μL of water followed by solid phase extraction (SPE).

For the determination of PFOS concentrations in brain collected on GD 20 (maternal rats and their pooled fetuses), PND 4 (pups, pooled by litter), and PND 21 (male