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Inhibition of human and rat 3β-hydroxysteroid dehydrogenase and 17β-hydroxysteroid dehydrogenase 3 activities by perfluoroalkylated substances☆

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ABSTRACT

Perfluoroalkylated substances (PFASs) including perfluorooctane acid (PFOA) and perfluorooctane sulfonate (PFOS) have been classified as persistent organic pollutants and are known to cause reduced testosterone production in human males. The objective of the present study was to compare the potencies of five different PFASs including PFOA, PFOS, potassium perfluorooctane sulfonate (PFOSK), potassium perfluorohexane sulfonate (PFHxSK) and potassium perfluorobutane sulfonate (PFBSK) in the inhibition of 3β-hydroxysteroid dehydrogenase (3β-HSD) and 17β-hydroxysteroid dehydrogenase 3 (17β-HSD3) activities in the human and rat testes. Human and rat microsomal enzymes were exposed to various PFASs. PFOS and PFOSK inhibited rat 3β-HSD activity with IC_{50} of 1.35 ± 0.05 and 1.77 ± 0.04 μM, respectively, whereas PFHxSK and PFBSK had no effect at concentrations up to 250 μM. All chemicals tested weakly inhibited human 3β-HSD activity with IC_{50}s over 250 μM. On the other hand, PFOS, PFOSK and PFOA inhibited human 17β-HSD3 activity with IC_{50}s of 6.02 ± 1.02, 4.39 ± 0.46 and 127.60 ± 28.52 μM, respectively. The potencies for inhibition of 17β-HSD3 activity were determined to be PFOSK > PFOS > PFOA > PFHxSK = PFBSK for human 17β-HSD3 activity. There appears to be a species-dependent sensitivity to PFAS-mediated inhibition of enzyme activity because the IC_{50}s of PFOS(K) for inhibition of rat 17β-HSD3 activity was greater than 250 μM. In conclusion, the present study shows that PFOS and PFOSK are potent inhibitors of rat 3β-HSD and human 17β-HSD3 activity, and implies that inhibition of steroidogenic enzyme activity may be a contributing factor to the effects that PFASs exert on androgen secretion in the testis.

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1. Introduction

Perfluoroalkylated substances (PFASs) are polyfluoro compounds that have been widely used in the manufacture of several products since the 1950s for their unique characteristics of extreme stability and surface activity. These chemicals are used as coatings of textiles, paper and upholstery and as reaction additives in various processes [1–3]. Some PFASs, including perfluorooctane acid (PFOA, 8-carbon perfluoroalkyl chain), perfluorooctane sulfonate (PFOS, 8-carbon perfluoroalkyl chain) and perfluorohexane sulfonate (PFHxS, 6-carbon perfluoroalkyl chain) have been classified as persistent organic pollutants in the environment and are present in tissues of the general population [4]. The levels of PFOA, PFOS and PFHxS measured in blood of human subjects are related to the exposure level and duration. For example, serum levels of PFOA, PFOS and PFHxS in the United States in 2006 averaged 3.4, 14.7 and 1.5 ng/ml, respectively [5]. However, blood levels may exceed 100 μg/ml in factory workers [2]. In particular,

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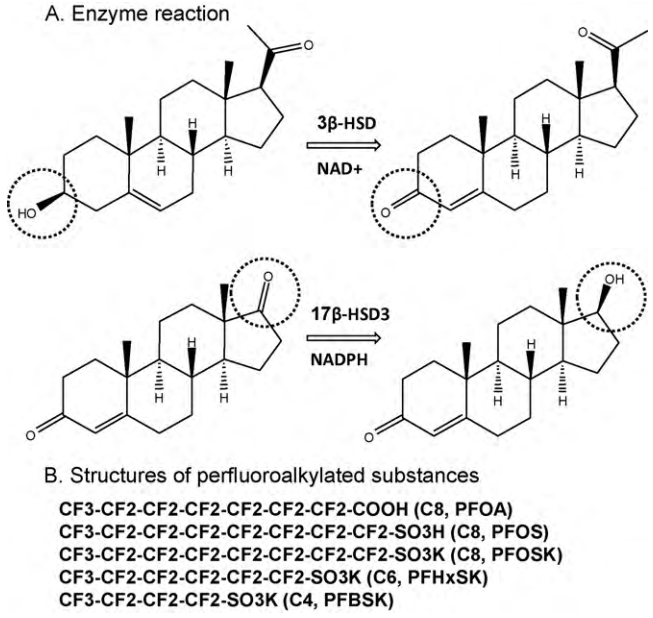

Scheme 1. The reactions of 3β-hydroxysteroid dehydrogenase (3β-HSD) and 17β-hydroxysteroid dehydrogenase 3 (17β-HSD3) and the structures of perfluoroalkylated substances (PFASs). (Panel A) The reactions of 3β-HSD and 17β-HSD3; (Panel B) structures of PFASs.

concerns have been raised regarding DuPont workers in China, whose blood PFOA levels increased to 2.25 μg/ml in just 1 year [6]. Due to long elimination *t*1/2 for these chemicals and potential persistent hazards, the 3M Company automatically phased out the production of PFOA and PFOS in 2000 [5]. Subsequently, another PFAS with short carbon chain perfluorobutane sulfonate (PFBS, 4-carbon perfluoroalkyl chain) was introduced to replace PFOA, PFOS and PFHxS compounds (Scheme 1). The serum elimination of PFBS is expected to be more rapid than that of PFOA or PFOS. It was reported recently that the *t*1/2 of PFBS was 3.1 h in male rats and about 10 h in monkeys [7]. There is growing evidence to show that PFASs may act as endocrine disruptors on reproductive system. Workers in 3M company in Cottage Groove that produced PFOA had higher level of PFOA and decreased serum testosterone concentrations (Minnesota, USA) [8,9]. Laboratory animal studies showed that rats exposed to PFOA and related chemicals had lower testosterone levels [10,11]. The effects of PFASs on androgen secretion in rodents may be associated with their interference with Leydig cell function. In one study, ammonium perfluorooctanoate acted directly in rat Leydig cells to inhibit hCG-stimulated testosterone production [10]. Leydig cells utilize cholesterol as a substrate to produce testosterone. The conversion of the cholesterol substrate into testosterone occurs in a series of reactions catalyzed by four enzymes: cytochrome P450 cholesterol side-chain cleavage enzyme (CYP11A1), 3β-HSD, cytochrome P450 17α-hydroxylase/17-20 lyase (CYP17A1) and 17β-HSD3. Hydroxysteroid dehydrogenases (3β-HSD and 17β-HSD3) are localized to the smooth endoplasmic reticulum in Leydig cells. 3β-HSD catalyzes conversion of pregnenolone (PREG) into progesterone in the presence of the cofactor NAD^+, whereas 17β-HSD3 catalyzes conversion of androstenedione (DIONE) into testosterone in the presence of cofactor NADPH (Scheme 1). Exposure of adult rats to perfluorododecanoic acid, a PFAS, at 5 or 10 mg/kg body weight/day for 2 weeks inhibited gene expression for several proteins involved in androgen biosynthesis, including the cholesterol transport protein steroidogenic acute regulatory protein (*Star*), scavenger receptor class B member 1 (*Scarb1*), and the steroidogenic enzymes (*Cyp11a1*, *Hsd3b1*, *Cyp17a*1 and *Hsd17b3*) [11]. In our previous study, we determined that PFOA inhibited 3β-HSD and 17β-HSD3 in rat Leydig cells with the half maximal inhibitory concentration (IC_{50}) of 53.2 and 17.7 μM, respectively [12]. In the present study, we have compared the potencies of five PFASs for the inhibition of 3β-HSD and 17β-HSD3 activities in both human and rat testes.

2. Materials and methods

2.1. Materials

[1,2-^{3}H] androstenedione (^{3}H-DIONE), [^{3}H] pregnenolone (^{3}H-PREG), specific activity, 40 Ci/mmol were purchased from Dupont-New England Nuclear (Boston, MA). Unlabeled DIONE, PREG, progesterone and testosterone were purchased from Steraloids (Newport, RI). PFOA, PFOS, and the potassium salts of PFHxS (PFHxSK), PFOS (PFOSK) and PFBS (PFBSK) were purchased from Sigma–Aldrich (St. Louis, MO). PFASs were dissolved in dimethyl sulfoxide (DMSO). 90-day-old male Sprague–Dawley rats (250–300 g) were purchased from Charles River Laboratories (Wilmington, MA). All animal procedures were approved by the Rockefeller University's Animal Care and Use Committee (protocol #07080). Human testis samples were obtained from Wenzhou Medical College Affiliated Hospitals (Wenzhou, China). These testis biopsy samples were mixed testis samples from infertile patients with normal Leydig cell function or testosterone parameters. The use of human testis samples was approved by the Ethics Committee of Wenzhou Medical College.

2.2. Preparation of microsomal protein

Microsomal preparations of human and rat testes were done as described previously [13]. In brief, testes were homogenized in cold 0.01 mM phosphate buffered saline (PBS, pH 7.4) containing 0.25 mM sucrose and centrifuged at 700 × *g* for 30 min at 4C to remove cellular fragments. The supernatants were transferred to new tubes and were centrifuged at 10,000 × *g* for 30 min at 4C to remove mitochondria. The supernatants were centrifuged twice at 105,000 × *g* for 1 h at 4 °C to collect microsomal pellets. Pellets were resuspended in order to measure protein contents. The protein concentrations of samples were measured using the Bio-Rad Protein Assay Kit (cat# 500-0006, Bio-Rad, Hercules, CA) according to manufacturer's protocol. Microsomal protein concentrations were adjusted to 4 mg/ml and used for measurement of 3β-HSD and 17β-HSD3 activities.

2.3. 3β-HSD activity assay

3β-HSD activity in testis microsomes was measured as described previously [13]. In brief, 3β-HSD activity assay tubes contained 0.2 μM PREG plus 40,000 cpm [^{3}H] PREG and 0.2 mM NAD^+ in 250 μl 0.5% Tween-20 PBS. PFASs were dissolved in DMSO, and the final DMSO concentration was 0.5%, which had no effects on 3β-HSD activity. We determined the linearity of reactions using different concentrations of rat and human microsomes for 3β-HSD activity. The 30 min reactions were initiated by addition of 20 μg rat or 90 μg human testis microsome in the presence of different concentrations of PFASs to determine IC_{50}. To determine the inhibitory mode of PFASs, different concentrations of PREG (0.002–10 μM) plus 0.2 mM NAD^+ was added into reaction mixtures (0.5% Tween-20 PBS buffer) containing 20 μg rat or 90 μg human testis microsome and PFASs (different concentrations). To determine whether the inhibitory mode of PFASs occurred by competing with NAD^+, different concentrations of NAD^+ concentrations (0.002–100 μM) plus 0.2 μM PREG were added into 0.5% Tween-20 PBS reaction mixture containing 20 μg rat testis microsome and

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