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The inhibitory effects of perfluoroalkyl substances on human and rat 11β-hydroxysteroid dehydrogenase 1

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ABSTRACT

Perfluoroalkyl substances (PFASs) are man-made polyfluorinated compounds that are widely used and persistent in the environment. PFASs have potential effects on many biological systems including the development of lung. Glucocorticoids have been reported to promote fetal and neonatal lung development at the late stage, and 11β-hydroxysteroid dehydrogenase 1(11βHSD1) in the lung is critical for the generation of local active glucocorticoid cortisol (human) or corticosterone (rodents) from biologically inert 11keto-steroids. The purpose of the present study is to study the direct inhibitory effects of PFASs on 11βHSD1 activities and action modes. Microsomal 11βHSD1 was subjected to the exposure to various PFASs, including perfluorooctane sulfonic acid (PFOS), perfluorooctanoic acid (PFOA), potassium perfluorohexanesulfonate (PFHxS) and potassium perfluorobutane sulfonate (PFBS). PFOS and PFOA inhibited neonatal rat lung 11βHSD1 activity with IC_{50}s of 3.45 μM (95% Confidence Intervals, CI_{95}: 1.97–6.37 μM) and 45.31 μM (CI_{95}: 27.64–74.26 μM), respectively, while PFHxS and PFBS did not inhibit the enzyme activity at 250 μM. PFOS and PFOA inhibited human 11βHSD1 activity with IC_{50}s of 7.56 μM (CI_{95}: 2.86–19.97 μM) and 37.61 μM (CI_{95}: 24.49–57.75 μM), respectively, while PFHxS and PFBS did not inhibit the enzyme activity at 250 μM. PFASs showed competitive inhibition on both human and rat 11βHSD1. In conclusion, the present study shows that PFOS and PFOA are the inhibitors of 11βHSD1.

1. Introduction

The perfluoroalkyl substances (PFASs) are man-made polyfluorinated compounds that are widely utilized in commercial and industrial products, including the coatings for textiles, paper, and leather; in wax, polishes, paints, varnishes, and cleaning products for general use [1]. Studies have shown that PFASs, such as perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA), are persistent in the environment, and have been found to be present in wildlife and humans [2].

Concerns have been raised for PFASs because they have been shown to be toxic to several animal models. PFOS and PFOA have liver and immune toxicity [3]. Furthermore, animal studies and human investigations showed that PFOS and PFOA had developmental toxicity including reduced birth weight, delayed development, and neonatal mortality [4–6]. The neonatal mortality of PFOS and PFOA has been found to be caused by the failure of lung function or lung maturity [7–10].

Our previous studies showed that PFASs possibly interfered with glucocorticoid metabolism [11]. Glucocorticoids are critical hormones for lung maturation [12]. The local level of glucocorticoid in the lung is controlled by 11β-hydroxysteroid dehydrogenase 1 (11βHSD1) [13]. In the lung, 11βHSD1 behaves primarily as a reductase, which generates active glucocorticoid cortisol (human) or corticosterone (CORT, rodents) from biologically inert cortisone and 11-dehydrocorticosterone (11DHC), respectively [14,15]. 11βHSD1 has been found to be critical for fetal and neonatal lung development, since the ablation of 11βHSD1 gene expression or inhibition of the enzyme in mice delayed the lung maturity [16]. We hypothesize that some PFASs have direct inhibition on 11βHSD1 activity in the lung or other tissues. In the present study, we examined the direct action and the modes of actions of the four different PFASs with various lengths of carbon and sulfur chains on 11βHSD1 activities (Fig. 1).

Abbreviations: 11βHSD1, 11β-hydroxysteroid dehydrogenase 1; 11DHC, 11-dehydrocorticosterone; CORT, corticosterone; G6P, glucose-6-phosphate; PFAS, perfluoroalkyl substance; PFOS, perfluorooctane sulfonic acid; PFOA, perfluorooctanoic acid; PFBS, potassium perfluorobutane sulfonate; PFHxS, potassium perfluorohexanesulfonate.

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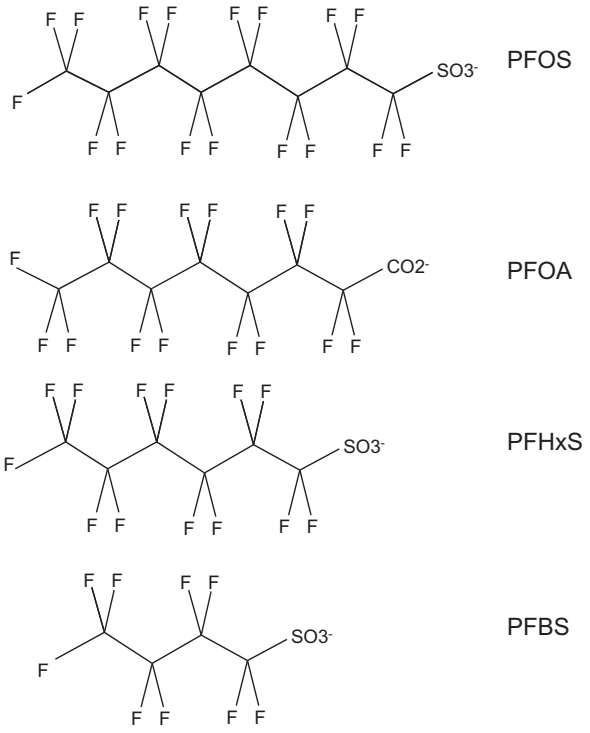

Fig. 1. The chemical structure of perfluoroalkyl substances. PFOS, perfluorooctane sulfonic acid; PFOA, perfluorooctanoic acid; PFBS, perfluorobutane sulfonate; PFHxS, perfluorohexanesulfonate.

2. Materials and methods

2.1. Chemical and animals

[1,2,6,7-^{3}H] Corticosterone (^{3}H-CORT) and [1,2,6,7-^{3}H] cortisol (^{3}H-cortisol) were purchased from Dupont-New England Nuclear (Boston, MA). ^{3}H-11-Dehydrocorticosterone (^{3}H-11DHC) and ^{3}H-cortisone were prepared from labeled ^{3}H-CORT or ^{3}H-cortisol as described earlier [17]. Cold CORT, 11DHC, cortisol and cortisone were purchased from Steraloids (Newport, RI). The following PFASs were purchased from Sigma–Aldrich Company (St. Louis, MO, USA): PFOS, PFOA, potassium perfluorohexanesulfonate (PFHxS) and potassium perfluorobutane sulfonate (PFBS). Sprague Dawley dams with male pups were purchased from Charles River Laboratories (Wilmington, MA). Human liver microsomes were purchased from Gentest (Cat.# 452156, Woburn, MA), which were prepared from 50 pooled livers and stored with final concentrations of 20 mg/mL.

2.2. Preparation of microsomal protein

Male neonatal pups (postnatal day 14) were euthanized by CO_2, and lungs were collected. Rat neonatal lung microsomes were prepared as described previously [18]. In brief, rat lungs were homogenized in 0.01 mM PBS buffer containing 0.25 M sucrose, and nuclei and large cell debris were removed by centrifugation at 1500*g* for 10 min. The post-nuclear supernatants were centrifuged twice at 105,000*g*, the resultant microsomal pellets were resuspended. Protein contents were measured by Bio-Rad Dye Reagent Concentrate (Cat.# 500–0006). The concentrations of rat lung microsomes were 20 mg/ml. Microsomes were used for measurement of 11βHSD1 activities.

2.3. 11βHSD1 assay

11βHSD1 activity assay tubes contained 1 μM substrate 11DHC (for rat) or cortisone (for human), spiked with 30,000 cpm of their respective ^{3}H-steriods. 11DHC or cortisone was used as the substrate to measure 11βHSD1 activity. The rat lung microsomes or human liver microsomes were incubated with substrate, 0.2 mM NADPH and 0.5 mM glucose-6-phosphate (G6P) and various concentrations of each PFAS at 37C for 15 min. The inhibitory potency of PFOS or PFOA was measured relative to control (only DMSO). Each PFAS was dissolved in dimethyl sulfonate (DMSO) with final concentration of 0.4%, at which DMSO did not inhibit this enzyme activity. At the end of the reaction, the reaction was stopped by adding 2 ml ice-cold ether. The steroids were extracted, and the organic layer was dried under nitrogen. The steroids were separated chromatographically on the thin layer plate in chloroform and methanol (90:10, v/v), and the radioactivity was measured using a scanning radiometer (System AR2000, Bioscan Inc., Washington, DC) as described previously [18]. The percentage conversion of 11DHC to CORT or cortisone to cortisol was calculated by dividing the radioactive counts identified as 11-OH-steroids by the total counts.

2.4. Determination of half maximum inhibitory concentrations (IC_{50}) and inhibitory mode

The IC_{50} was determined by adding 1000 nM substrate and 0.2 mM NADPH plus 0.5 mM G6P and various concentrations of each PFAS to 250 μl reaction buffer (0.1 mM PBS) containing 2–10 μg rat or human microsomal protein as describe previously [11]. The mode of inhibition was assayed by adding various concentrations of either 11DHC plus NADPH or cortisone plus NADPH.

2.5. Statistics

Each experiment was repeated two to four times. Data were subjected to nonlinear regression analysis by GraphPad (Version 4, GraphPad Software Inc., San Diego, CA) for IC_{50}. Lineweaver–Burk plot was used for the analysis of the mode of inhibition. Data were subjected to analysis by one-way ANOVA followed by ad hoc Tukey multiple comparison testing to identify significant differences between groups when three and more groups were calculated. All data are expressed as means ± SEM. Differences were regarded as significant at $P < 0.05$.

3. Result

3.1. Effects of PFASs on rat neonatal lung 11βHSD1 activity

The inhibition of PFASs on rat neonatal lung 11βHSD1 activity depends on their structure, with PFOS and PFOA potently inhibiting the enzyme activity and PFHxS and PFBS no effects at 250 μM (Fig. 2A). PFOS and PFOA inhibited neonatal rat lung 11βHSD1 activity with IC_{50}s of 3.45 μM (95% Confidence Intervals, CI_{95}: 1.97–6.37 μM) and 45.31 μM (CI_{95}: 27.64–74.25 μM), respectively (Fig. 2A). The potency of inhibition is: PFOS > PFOA > PFHxS = PFBS, indicating that the length of carbon plus sulfur chain of a PFAS is important for its potency.

3.2. Effects of PFASs on human liver 11βHSD1 activity

The inhibition of PFASs on human liver 11βHSD1 activity depends on their structure, with PFOS and PFOA potently inhibiting the enzyme activity and PFHxS and PFBS no effects at 250 μM (Fig. 2B). PFOS and PFOA inhibited human liver 11βHSD1 activity with IC_{50}s of 7.56 μM (CI_{95}: 2.86–19.97 μM) and 37.61 μM (CI_{95}: 24.49–57.75 μM), respectively (Fig. 3B). The potency of inhibition is: PFOS > PFOA > PFHxS = PFBS, indicating that the length of carbon plus sulfur chain of a PFAS is important for its potency.