Toxicology and Applied Pharmacology 304 (2016) 18–29

Contents lists available at ScienceDirect

Toxicology and Applied Pharmacology

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

The role of hepatocyte nuclear factor 4-alpha in perfluorooctanoic acid- and perfluorooctanesulfonic acid-induced hepatocellular dysfunction

Kevin M. Beggs [a], Steven R. McGreal [a], Alex McCarthy [a], Sumedha Gunewardena [b], Jed N. Lampe [a], Christoper Lau [c], Udayan Apte [a,*]

[a] Department of Pharmacology, Toxicology and Therapeutics, University of Kansas Medical Center, 3901 Rainbow Blvd, 4052 HLSIC, Kansas City, KS 66160, United States
[b] Department of Molecular and Integrative Physiology, University of Kansas Medical Center, 3901 Rainbow Blvd, 2027 HLSIC, Kansas City, KS 66160, United States
[c] Developmental Toxicology Branch, Toxicity Assessment Division, National Health and Environmental Effects Research Laboratory, Office of Research and Development, US Environmental Protection Agency, Research Triangle Park, NC 27711, United States

ARTICLE INFO

Article history:
Received 20 January 2016
Revised 21 April 2016
Accepted 1 May 2016
Available online 3 May 2016

Keywords:
Perfluorooctanoic acid
Perfluorooctanesulfonic acid
Hepatocyte nuclear factor 4-alpha
Human hepatocyte
Liver

ABSTRACT

Perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS), chemicals present in a multitude of consumer products, are persistent organic pollutants. Both compounds induce hepatotoxic effects in rodents, including steatosis, hepatomegaly and liver cancer. The mechanisms of PFOA- and PFOS-induced hepatic dysfunction are not completely understood. We present evidence that PFOA and PFOS induce their hepatic effects via targeting hepatocyte nuclear factor 4-alpha (HNF4α). Human hepatocytes treated with PFOA and PFOS at a concentration relevant to occupational exposure caused a decrease in HNF4α protein without affecting HNF4α mRNA or causing cell death. RNA sequencing analysis combined with Ingenuity Pathway Analysis of global gene expression changes in human hepatocytes treated with PFOA or PFOS indicated alterations in the expression of genes involved in lipid metabolism and tumorigenesis, several of which are regulated by HNF4α. Further investigation of specific HNF4α target gene expression revealed that PFOA and PFOS could promote cellular dedifferentiation and increase cell proliferation by down regulating positive targets (differentiation genes such as CYP7A1) and inducing negative targets of HNF4α (pro-mitogenic genes such as CCND1). Furthermore, in silico docking simulations indicated that PFOA and PFOS could directly interact with HNF4α in a similar manner to endogenous fatty acids. Collectively, these results highlight HNF4α degradation as novel mechanism of PFOA and PFOS-mediated steatosis and tumorigenesis in human livers.

1. Introduction

Perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS) are structurally similar anthropogenic compounds that are used in various consumer products to provide soil, oil and water resistance to materials used in clothing, upholstery, and food packaging (Lindstrom et al., 2011, Suja et al., 2009). They are two of the most commonly used compounds in a larger class of chemicals known as perfluoroalkyl acids. The multitude of carbon-fluorine bonds present in both PFOA and PFOS make these compounds chemically stable, promoting resistance to environmental degradation and biotransformation (Giesy et al., 2010, Kudo and Kawashima, 2003). Both PFOA and PFOS are persistent organic pollutants and are present in detectable levels in humans and wildlife worldwide (Kudo and Kawashima, 2003, Suja et al., 2009). Excretion of these compounds is exceptionally slow, with an average half-life in humans on the order of 3–5 years (Kudo and Kawashima, 2003, Olsen et al., 2007). The stable chemical structures, as well as the slow rate of elimination, make PFOA and PFOS persistent organic pollutants with the potential to bioaccumulate and induce long-term health effects.

The primary sources of human exposure to PFOA and PFOS are consumption of food and water contaminated with either compound (D'Hollander et al., 2010, Domingo, 2012, Vestergren and Cousins, 2009). A recent study revealed that more than 98% of human serum samples examined in the United States contain detectable levels of PFOA/PFOS, which were dose dependently associated with positive liver function tests (Gleason et al., 2015). The general population of the United States has both PFOA and PFOS present in their blood at concentrations ranging from approximately 10–100 nM (Chang et al., 2014,

Abbreviations: ADH1B, alcohol dehydrogenase 1B; AKR1B10, aldo-keto reductase 1B10; ALT, alanine aminotransferase; CCND1, cyclin D1; CLDN1, claudin 1; CYP7A1, cytochrome P450 7A1; DMSO, dimethyl sulfoxide; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; HNF4α, hepatocyte nuclear factor 4-alpha; IPA, Ingenuity Pathway Analysis; PFOA, perfluorooctanoic acid; PFOS, perfluorooctanesulfonic acid; PLIN2, perilipin 2; PPAR, peroxisome proliferator-activated receptor; TAT, tyrosine aminotransferase.

* Corresponding author at: University of Kansas Medical Center, 3901 Rainbow Blvd, 4087 HLSIC, Kansas City, KS 66160, United States.
E-mail addresses: kbeggs2@kumc.edu (K.M. Beggs), smcgreal@kumc.edu (S.R. McGreal), sgunewardena@kumc.edu (S. Gunewardena), jlampe@kumc.edu (J.N. Lampe), lau.christopher@epa.gov (C. Lau), uapte@kumc.edu (U. Apte).

http://dx.doi.org/10.1016/j.taap.2016.05.001
0041-008X/

Gleason et al., 2015, Lau, 2012) (Fig. 1). As expected, residents of the areas surrounding cities including Decatur, Alabama and Cottage Grove, Minnesota, where the 3M company manufactured PFOA and PFOS, had higher concentrations in their blood, as a consequence of watershed contamination. Similar results were found for residents surrounding Parkersburg, West Virginia where DuPont manufactured the fluorinated compounds (Chang et al., 2014, Emmett et al., 2006, Landsteiner et al., 2014, Lau, 2012). Occupational workers that came in direct contact with PFOA or PFOS had the highest concentrations present in their blood, approaching 10 μM or higher (Chang et al., 2014, Lau, 2012, Olsen et al., 2007). Although concerns of significant public health issues induced by PFOA and PFOS have caused 3M and DuPont to cease production of these compounds in the United States (Kudo and Kawashima, 2003), they continue to be manufactured and utilized worldwide (Suja et al., 2009).

In rodents, both compounds distribute primarily to the liver and plasma (Kennedy et al., 2004, Kudo and Kawashima, 2003). The phenotypic results of exposure to PFOA or PFOS include immunotoxicity, developmental toxicity and tumorigenesis (Lau et al., 2003, Lau et al., 2004, Lau et al., 2006, Qazi et al., 2010). A multitude of effects are observed in the liver, including hepatomegaly, steatosis and hepatocellular carcinoma (Butenhoff et al., 2012, Kennedy et al., 2004, Qazi et al., 2010). Initial studies identified the nuclear receptor peroxisome proliferator-activated receptor alpha (PPARα) as a potential target for PFOA- and PFOS-induced liver dysfunction (Bjork et al., 2008, Elcombe et al., 2012). However, the hepatomegaly induced by both fluorocarbons was still observed in studies using PPARα-null mice (Abbott et al., 2007, DeWitt et al., 2009, Qazi et al., 2009). These PPARα-independent effects are attributed to other nuclear receptors, including the constitutive androstane receptor (CAR) and PPARγ (Rosen et al., 2008). Furthermore, the expression of PPARα in rodents is much greater than it is in humans (Cohen et al., 2003, DeWitt et al., 2009, Klaunig et al., 2003). These findings suggest other factors are involved in the hepatic effects that occur in response to PFOA and PFOS.

Hepatocyte nuclear factor 4-alpha (HNF4α) is considered the master regulator of hepatic differentiation (Bonzo et al., 2012, Hwang-Verslues and Sladek, 2010, Parviz et al., 2003). It regulates various hepatocyte specific processes including liver development, transcriptional regulation of liver specific genes, regulation of lipid metabolism and maintaining hepatocellular quiescence and differentiation (Watt et al., 2003). In mice, conditional hepatocyte specific deletion of HNF4α results in hepatomegaly and hepatic steatosis, a liver phenotype similar to that observed in rodents administered PFOA or PFOS (Bonzo et al., 2012, Walesky et al., 2013b). A recent study indicated that PFOA exposure might reduce HNF4α expression in hepatocytes (Scharmach et al., 2012). These results suggest that HNF4α could be a relevant target of PFOA and PFOS in the liver. The goal of this study was to investigate the effects of occupationally relevant concentrations of PFOA and PFOS on HNF4α and its signaling network, and to determine whether HNF4α down regulation could be a mechanism of PFOA- and PFOS-induced hepatic steatosis.

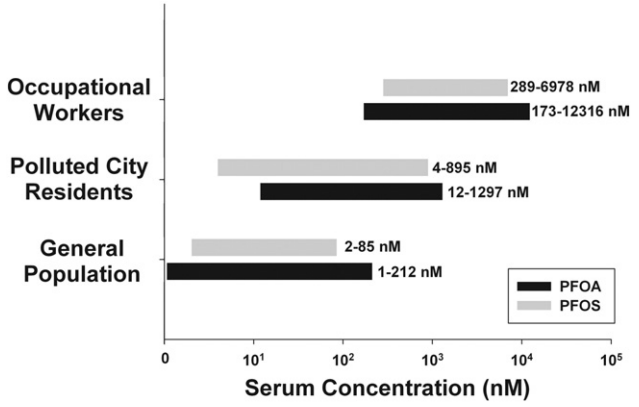

Fig. 1. Human serum concentrations of PFOA and PFOS within different subpopulations of exposure in the United States. A literature review was conducted to determine concentrations of PFOA and PFOS in human serum of United States residents (Chang et al., 2014, Emmett et al., 2006, Gleason et al., 2015, Landsteiner et al., 2014, Lau, 2012, Olsen et al., 2007). Populations were categorized into three different groups based on the potential for exposure to either compound. General population data was collected from human blood banks across the United States and National Health and Nutrition Examination Survey (NHANES) data. Concentrations reported in the polluted city residents category included data collected from residents in the surrounding areas of the 3M facilities (Cottage Grove, MN and Decatur, AL) and the DuPont facility (Parkersburg, WV) responsible for the production of PFOA and PFOS. Concentrations reported in the occupational exposure group were observed in the serum samples collected from workers of the 3M and DuPont facilities.

2. Materials and methods

2.1. Materials

Unless otherwise noted, all materials were purchased from Sigma Aldrich (St. Louis, MO). Phenol red-free Williams' Medium E and glutamine were purchased from Life Technologies (Grand Island, NY). For western blotting analysis, HNF4α antibody was purchased from R&D Systems (Minneapolis, MN). All other antibodies were purchased from Cell Signaling Technologies (Beverly, MA). Primers were ordered from Integrated DNA Technologies (Coralville, IA).

2.2. Isolation and culture of human hepatocytes

Primary human hepatocytes were isolated from liver explants by the Cell Isolation Core of the department of Pharmacology, Toxicology and Therapeutics at the University of Kansas Medical Center. All human tissues were obtained with informed consent from patients in accordance with ethical and institutional guidelines. The Institutional Review Board at the University of Kansas Medical Center approved this study. Hepatocytes were isolated using a standard multi-step collagenase procedure as described previously (Xie et al., 2014). At the time of isolation, cellular viability was 85% or greater. Cells were seeded to confluency in collagen-coated 6-well plates at a density of 1×10^6 cells per well, and were cultured with phenol red-free Williams' Medium E supplemented with 2 mM L-glutamine, 10 mM HEPES buffer, 100 nM insulin, 100 nM dexamethasone, 100 U/mL penicillin, 100 μg/mL streptomycin and 0.25 μg/mL amphotericin B. Cells were maintained in an incubator set to 37 °C and a humidified atmosphere of 95% air and 5% CO_2. Cells were treated following a brief period of cell attachment.

2.3. Hepatocellular PFOA and PFOS exposure protocol

PFOA (free acid) and PFOS (potassium salt) were dissolved in dimethyl sulfoxide (DMSO) at a stock concentration of 100 mM. Hepatocytes were exposed to concentrations ranging from 10 nM to 10 μM of either PFOA or PFOS, or their vehicle control (0.01% DMSO, referred to as Veh throughout). These concentrations were chosen based on the observed serum concentrations measured in humans (Fig. 1). Culture medium was changed every 48 h.

2.4. Assessment of PFOA- or PFOS-induced cytotoxicity

After exposure, cell death was assayed as described previously (Beggs et al., 2014). Briefly, culture supernatant was collected before 1% Triton in PBS was added for 30 min to lyse the cells. Both solutions were centrifuged at 600g for 5 min before ALT activity was measured as recommended by the manufacturer (Thermo Scientific, Marietta, OH). As a positive control for cell death, various concentrations of Triton dissolved in PBS were added to cells for 30 min before cytotoxicity was measured.