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Perfluorooctanoic acid exposure triggers oxidative stress in the mouse pancreas

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ABSTRACT

Perfluorooctanoic acid (PFOA) is used in the manufacture of many industrial and commercial products. PFOA does not readily decompose in the environment, and is biologically persistent. Human epidemiologic and animal studies suggest that PFOA exposure elicits adverse effects on the pancreas. While multiple animal studies have examined PFOA-mediated toxicity in the liver, little is known about the potential adverse effects of PFOA on the pancreas. To address this, we treated C57Bl/6 mice with vehicle, or PFOA at doses of 0.5, 2.5 or 5.0 mg/kg BW/day for 7 days. Significant accumulation of PFOA was found in the serum, liver and pancreas of PFOA-treated animals. Histopathologic examination of the pancreas revealed focal ductal hyperplasia in mice treated with 2.5 and 5.0 mg/kg BW/day PFOA, while inflammation was observed only in the high dose group. Elevated serum levels of amylase and lipase were observed in the 2.5 mg/kg BW/day PFOA treatment group. In addition, PFOA exposure resulted in a dose-dependent increase in the level of the lipid peroxidation product 8-iso-$PGF_{2\alpha}$ and induction of the antioxidant response genes Sod1, Sod2, Gpx2 and Nqo1. Our findings provide additional evidence that the pancreas is a target organ for PFOA-mediated toxicity and suggest that oxidative stress may be a mechanism through which PFOA induces histopathological changes in the pancreas.

1. Introduction

Perfluoroalkyl and polyfluoroalkyl substances (PFASs), such as perfluorooctanoic acid (PFOA), are widely used in consumer and industrial applications due to their unique hydrophobic properties. PFASs do not readily decompose in the environment and have been detected in air, soil, surface water, sediments, ice caps and wildlife worldwide [1]. Humans are exposed to PFOA by drinking water, dust in homes, food products or migration from food packaging and cookware [1,2]. Detectable levels of PFOA are found in 98% of the American population, with mean serum levels measured at 3.9 ng/ml [3]. PFOA is readily absorbed, but poorly eliminated with a predicted half-life of 3.8 years in humans.

Based on its biological persistence, it has been postulated that exposure to PFOA has the potential to contribute to development of chronic diseases in humans. Epidemiologic studies have shown an association between PFAS exposure and adverse health effects in humans [1,4]. Studies of occupationally-exposed workers, community residents exposed to contaminated drinking water, as well

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as general population studies have identified a positive association between PFOA exposure and increased total serum cholesterol and non-HDL cholesterol [5–7]. A general population study revealed an increase in obesity, as well as serum insulin and leptin levels in 20-year-old female offspring exposed to PFOA *in utero* [8]. PFOA was also associated with increased mortality due to diabetes in an occupationally-exposed cohort [9]. In addition, a weak association was seen between PFOA levels and pancreatic cancer in a general population study [10].

In rodents, exposure to PFOA results in reduced body weight, liver enlargement, decreased triglycerides, and hepatic peroxisomal proliferation [1,11]. The hepatic effects of PFOA have been attributed in large part to activation of the nuclear receptor peroxisome proliferator-activated receptor alpha (PPARα), although PPARα-independent effects have also been reported [12]. More recent studies have shown that PFOA disrupts the endocrine and immune systems and exerts multiple developmental effects in mice [13–15]. Female mice exposed to low levels of PFOA *in utero* displayed elevated serum leptin and insulin levels and increased body weight [14]. Chronic exposure to PFOA has been shown to induce a "tumor triad" in Sprague-Dawley rats, consisting of liver, Leydig cell and pancreatic acinar cell tumors (PACTs) [16]. While liver tumor formation is proposed to be mediated predominantly through PPARα activation [17], the mechanism by which PFOA induces PACTs is not well understood [16]. Due to the fact that pancreatic ductal adenocarcinoma (PDAC) exhibits a ductal morphology, it was previously thought that PDAC arises from ductal epithelial cells [18]. However, more recent studies have shown that targeting expression of oncogenic KRas to adult mouse acinar cells leads to development of PDAC, firmly establishing the acinar cell as a cell of origin for pancreatic cancer [19–21].

Oxidative stress occurs when reactive oxygen species (ROS) production exceeds the capacity of the cells' detoxification mechanisms [22]. ROS can cause lipid, protein, and DNA damage and contribute to the pathology observed in several chronic diseases including cancer [22]. Previous experimental evidence exists demonstrating that PFOA induces oxidative stress. PFOA has been shown to stimulate ROS production in HepG2 cells [23–25] which led to oxidative DNA damage, assessed by the immunocytochemical detection of 8-hydroxydeoxyguanosine (8OHdG) [25], and activation of caspase-9 and apoptosis [24].

While the effects of PFOA in the liver have been extensively studied, few studies have evaluated the effects of PFOA on the pancreas. The goal of this study was to characterize the adverse effects of short-term exposure to PFOA in the pancreas.

2. Materials and methods

2.1. Chemicals

PFOA (96%), ammonium acetate, potassium hydroxide and ethyl acetate were purchased from Sigma–Aldrich (St. Louis, MO). Perchloric acid was purchased from ACROS (Fair Lawn, NJ). 8-iso-$PGF_{2\alpha}$ and 8-iso-$PGF_{2\alpha}$-d4 were purchased from Cayman Chemical (Ann Arbor, MI). n-Hexane was obtained from Baker Chemicals (Houston, TX). Water, acetone, methanol and acetonitrile were LC–MS grade and purchased from Fisher Scientific (Pittsburgh, PA).

2.2. Study design

Eight-week-old male C57Bl/6 mice were purchased from Harlan Laboratories (Indianapolis, IN), and were acclimated for 4 days prior to treatment. Mice were singly housed in polycarbonate cages with filter tops, and received LabDiet 5015 in pelletized form and de-ionized water *ad libitum*. The care and treatment of the mice were in accordance with the NIH Guide for the Care and Use of Laboratory Animals and was approved by the Indiana University Bloomington IACUC. Due to lack of gender-specific differences in elimination of PFOA [26], mice have been extensively used to evaluate the adverse effects of PFOA exposure [27]. Groups of 4 mice were treated with water (control) or PFOA (0.5, 2.5 or 5.0 mg/kg BW) *via* oral gavage (1×/day at a volume of 0.1 ml/10 g BW) for 7 days. These doses have previously been used in mouse studies evaluating the effects of PFOA [14]. An additional group of mice were treated with cerulein for 7 days (1×/day, 5 μg, i.p.) to stimulate pancreatitis. Mice were killed by CO_2 asphyxiation, and serum, pancreata and livers collected 24 h after the last treatment. Pancreata were divided in half along the longitudinal axis, from the head to tail of the pancreas. The top section was used for histology for all pancreata. Pancreas and liver sections were fixed in formalin for 48 h and then embedded in paraffin, sectioned and stained with H&E for histopathologic examination. Images were captured with an Aperio whole slide imaging system at 20× magnification. The remaining pancreata and liver were snap frozen in liquid nitrogen for further biochemical and gene expression analysis. For the quantitation of PFOA and 8-iso-$PGF_{2\alpha}$ in pancreas and liver, frozen tissues were homogenized in buffer containing 20 mM Tris buffer, 20 μM BHT at pH 7.4 on ice. Supernatants were collected following centrifugation at 16,000 × *g* for 5 min, and stored at −80 °C until use.

2.3. PFOA quantitation

One ml acetonitrile was added to serum or tissue homogenates, vortexed for 15 s and sonicated for 5 min. Samples were centrifuged at 16,000 × *g* for 3 min. An equal volume of supernatant was then mixed 1:1 with LC–MS grade water containing 3 mM ammonium acetate. PFOA was quantified using an Agilent 1260 UPLC system coupled with an Agilent 6430 triple quadrupole mass spectrometer. The samples were separated on an Agilent Zorbax Eclipse XBD-C18 column at 30 °C. Analytes were eluted in water containing 3 mM ammonium acetate and acetonitrile (50:50, v/v) at a flow-rate of 0.3 ml/min. The separated PFOA was detected by mass spectrometry with an electrospray ion source operating in the negative ion mode (ESI^-) using MRM. Precursor ion (*m*/*z* 413) and product ions (*m*/*z* 369 and *m*/*z* 169) were monitored at a fragmentation voltage of 66, cell acceleration voltage of 4, and collision energy of 1 and 13 for each product ion. PFOA levels were quantified from a standard curve prepared at final