Toxicology 293 (2012) 97–106

Contents lists available at SciVerse ScienceDirect

Toxicology

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

Perfluorooctanoic acid induces developmental cardiotoxicity in chicken embryos and hatchlings

Qixiao Jiang[a], Robert M. Lust[b], Mark J. Strynar[c], Sonia Dagnino[c], Jamie C. DeWitt[a,*]

[a] Department of Pharmacology and Toxicology, Brody School of Medicine, East Carolina University, Greenville, NC 27834, United States
[b] Department of Physiology, Brody School of Medicine, East Carolina University, Greenville, NC 27834, United States
[c] Human Exposure and Atmospheric Sciences Division, Methods Development and Application Branch, National Exposure Research Laboratory, U.S. Environmental Protection Agency, Research Triangle Park, NC 27709, United States

ARTICLE INFO

Article history:
Received 5 October 2011
Received in revised form 4 January 2012
Accepted 10 January 2012
Available online 18 January 2012

Keywords:
Perfluorooctanoic acid
Chicken embryo
Immunohistochemistry
Cardiac ultrasound
Cardiac myofibril ATPase

ABSTRACT

Perfluorooctanoic acid (PFOA) is a widespread environmental contaminant that is detectable in serum of the general U.S. population. PFOA is a known developmental toxicant that induces mortality in mammalian embryos and is thought to induce toxicity via interaction with the peroxisome proliferator activated receptor alpha (PPARα). As the cardiovascular system is crucial for embryonic survival, PFOA-induced effects on the heart may partially explain embryonic mortality. To assess impacts of PFOA exposure on the developing heart in an avian model, we used histopathology and immunohistochemical staining for myosin to assess morphological alterations in 19-day-old chicken embryo hearts after PFOA exposure. Additionally, echocardiography and cardiac myofibril ATPase activity assays were used to assess functional alterations in 1-day-old hatchling chickens following developmental PFOA exposure. Overall thinning and thinning of a dense layer of myosin in the right ventricular wall were observed in PFOA-exposed chicken embryo hearts. Alteration of multiple cardiac structural and functional parameters, including left ventricular wall thickness, left ventricular volume, heart rate, stroke volume, and ejection fraction were detected with echocardiography in the exposed hatchling chickens. Assessment of ATPase activity indicated that the ratio of cardiac myofibril calcium-independent ATPase activity to calcium-dependent ATPase activity was not affected, which suggests that developmental PFOA exposure may not affect cardiac energetics. In summary, structural and functional characteristics of the heart appear to be developmental targets of PFOA, possibly at the level of cardiomyocytes. Additional studies will investigate mechanisms of PFOA-induced developmental cardiotoxicity.

1. Introduction

Perfluoroalkyl acids (PFAAs) are fluorinated compounds used to manufacture materials for myriad consumer and industrial products, including nonstick, stain-repellant, water repellant, and fire-retardant coatings. Perfluorooctanoic acid (PFOA) is a PFAA that is a polymerization aid used to manufacture fluorinated polymers and elastomers, the most well known being polytetrafluoroethylene (PFTE). According to the current major manufacturer of PFOA, a minimal amount of PFOA is still present in end-products (DuPont, 2011), which can leach out and may contribute to exposures. PFOA also is a breakdown product of certain fluorinated telomer alcohols and other precursor compounds of fluorinated polymers (Wang et al., 2005). PFOA has become a public health concern because it is present in environmental media and biota.

Increasing reports of PFOA-induced toxicity led to a stewardship program between major flourochemical manufacturers and the U.S. Environmental Protection Agency (USEPA). The goal of the stewardship program is to eliminate PFOA and precursor products that can break down to PFOA by 2015 (USEPA, 2011). However, as PFOA does not bio-degrade, it persists in the environment; it is ubiquitously found in environmental samples and in serum of the world population. The median serum concentration in the U.S. population reported in 2007–2008 was 4.3 ng/mL (CDC, 2011). In an area of the Mid-Ohio Valley contaminated with PFOA by a manufacturing plant, epidemiological studies of exposed populations reported a median PFOA serum concentration of 26.6 ng/mL, with a high value of 17556.6 ng/mL (Steenland et al., 2009).

Epidemiological studies of the effects of PFOA on human embryonic and fetal development of residents living in the Mid-Ohio Valley have not produced clear associations with birth outcomes. Stein et al. (2009) reported modest association of PFOA serum

* Corresponding author at: Department of Pharmacology and Toxicology, Brody School of Medicine, East Carolina University, 600 Moye Blvd., Greenville, NC 27834, United States. Tel.: +1 252 744 2474; fax: +1 252 744 3203.
E-mail address: dewittj@ecu.edu (J.C. DeWitt).

0300-483X/$ – see front matter © 2012 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.tox.2012.01.005

concentrations with preeclampsia and birth defects in the Mid-Ohio Valley population. Additional studies reported that elevated PFOA exposure in this population was not associated with increased risk of lowered birth rate or gestational age (Nolan et al., 2009) or with congenital anomalies, labor or delivery complications, or maternal risk factors such as eclampsia or diabetes (Nolan et al., 2010). Although Apelberg et al. (2007) reported PFOA concentrations in umbilical cord serum for the general U.S. population (0.3–7.1 ng/mL), Nolan et al. (2009, 2010) did not measure PFOA concentrations in umbilical cord serum, so no corresponding cord serum levels are available for the highly exposed Mid-Ohio Valley U.S. population. Regardless, given the PFOA serum concentrations reported for this population and the potential for PFOA to affect development, additional studies of more subtle developmental effects are necessary.

In laboratory models, PFOA induces multisystem toxicity and data from studies of such models suggest that it is an agonist of the peroxisome proliferator activated receptor alpha (PPARα). Effects in laboratory models include hepatic, pancreatic and testicular cancer (Biegel et al., 2001), endocrine disruption (Olsen et al., 1998), and immunotoxicity (DeWitt et al., 2008). In addition, PFOA has been reported to induce developmental effects, including retarded development, decreased fetal survival and increased deformities in animal models (Wolf et al., 2007). In humans, epidemiology studies indicate that PFOA exposure is associated with elevated serum cholesterol and uric acid levels (Steenland et al., 2010).

Measurable serum concentrations and accompanying changes in cholesterol levels in exposed human populations warrant investigation of additional health effects of PFOA, especially effects related to the cardiovascular system. In addition, reasons for increases in fetal mortality associated with developmental PFOA exposure (Wolf et al., 2007; Lau et al., 2004; DeWitt et al., 2009b) are unknown. As cardiovascular development is a complex and delicate process, with numerous signaling activities vulnerable to exogenous disruptions from exposure to pathogens, drugs and environmental pollutants, altered cardiovascular development may affect embryo survival. Several agents have been reported to alter cardiovascular development. Lipopolysaccharide exposure induces cellular hypertrophy in H9c2 myocardiac cells and alters the calcineurin/NFAT-3 signaling pathway (Liu et al., 2008). Adriamycin (doxorubicin), an antitumor drug used to treat leukemias, lymphomas and neoplasms, induces ventricular septal defects, dextroposition of the aorta, and aortic arch anomalies in a dose-related manner when topically administered to 5-day-old chicken embryos (Takagi et al., 1989). Adriamycin likely decreased embryonic cardiac blood flow and inhibited rapidly exchangeable calcium within cardiac cells (Takagi et al., 1989). In addition, adriamycin induces dose-dependent cardiomyopathy, likely via iron oxidation and oxygen free radical formation, which limits its therapeutic applications (Shi et al., 2011). Environmental contaminants also have been reported to induce cardiotoxicity. 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) application enlarged left and right ventricles, thickened ventricular septa and thinned left ventricle walls in chicken embryos (Walker et al., 1997) and 3,3′,4,4′,5-pentachlorobiphenyl (PCB-126) exposure decreased myocyte proliferation in zebra fish, possibly by affecting hemodynamics (Grimes et al., 2008). Taken together, these studies indicate that the developing heart is sensitive to exogenous perturbations. Given PFOA's developmental toxicity in laboratory models, presence in humans and wildlife, and possible effects on endogenous compounds associated with heart disease, we investigated PFOA's effects on developing hearts. As avian and mammalian cardiovascular development is similar and avians lack a direct maternal influence, avian embryos are ideal models for developmental toxicity studies. This is the first study to assess both morphological and functional changes in avian hearts developmentally exposed to PFOA.

2. Materials and methods

2.1. Animals

Fertile chicken (*Gallus gallus*) eggs were purchased from North Carolina State University Poultry Research Center (Raleigh, NC). Eggs were cleaned with 20% povidone iodine, candled to outline air cells in pencil on shells, weighed, given ID numbers, and evenly distributed by weight among doses. Due to incubator space limitations, eggs were incubated in eight batches of 40–58. Uninjected eggs were included in each batch as environmental controls.

2.2. Chemicals

Sunflower oil was purchased from Spectrum Organic Products, LLC (Boulder, CO). PFOA and other chemicals (if not otherwise mentioned) were purchased from Sigma–Aldrich (St. Louis, MO).

2.3. Egg injection and incubation

Injection procedures were as described in Henshel et al. (2003). Briefly, PFOA was suspended in sunflower oil and vortexed before injection into each egg. An awl was used to drill a 1 mm hole into the middle of air cells and PFOA doses of 0, 0.5, 1 and 2 mg/kg of egg weight were injected with a gel-loading pipette tip. Dose selection was based on both observations by O'Brien et al. (2009) that up to 10 mg/kg PFOA injection will not affect pipping success and previous work done in our lab. Stock solutions were prepared so that 0.1 μL of solution per gram of egg weight resulted in the appropriate mg/kg concentration. After injection, a drop of melted paraffin sealed injection holes. Eggs were incubated in a Lyon Roll-X incubator (Chula Vista, CA) set at 99.5–100 °F dry bulb and 87–88 °F wet bulb. Eggs were candled every 2–3 days; infertile/undeveloped/dead eggs were removed. After external pipping eggs were placed individually into containers large enough for the hatched chickens and transferred to a larger incubator (G.Q.F. Manufacturing Co., Savannah, GA). Hatchling chickens were kept in a warmed brood box until euthanasia. All procedures were approved by the East Carolina University Institutional Animal Care and Use Committee.

2.4. Histology

At embryonic day 19 (D19; 2 days prior to hatch), embryos were removed from eggs and quickly decapitated. Whole embryo, yolk, heart, and liver weights were recorded. Hearts were excised, rinsed in ice cold saline to fully dilate ventricles, fixed in 10% phosphate buffered formalin for 24 h, and cut transversely. Cuts were made approximately 60% of the length of the heart from the ventricular apex (Fig. 1A). Ventricular tissues were processed routinely (Thermo Scientific Shandon Citadel 1000, Waltham, MA), embedded in paraffin, and sliced at 6 μm per section on a rotary microtome (Thermo Scientific, HM 315 Waltham, MA). The septomarginal trabecula was used to maintain a relatively constant position to ensure that ventricular wall measurements were made at the same location within each heart (Fig. 1D–F). For routine histology, hearts were stained with Harris modified hematoxylin (Fisher Scientific, Fair Lawn, NJ) and Eosin Y solution (Harleco, Gibbstown, NJ) (H&E). Adobe Photoshop (San Jose, CA) and SPOT advance (SPOT imaging, Sterling Heights, MI) with rulers (made in Berkeley Logo, Berkeley, CA) was used to measure the thickness of the right ventricular wall (Fig. 1B). Average right ventricular wall thickness was normalized to whole heart weight to minimize potential PFOA-induced effects on body weight or developmental stage. The right ventricular wall was chosen as the histological target because endogenous variability in the size of the left ventricular wall (Walker and Catron, 2000) made it a less desirable histological target.

2.5. Immunohistochemistry

Immunohistochemistry was used to selectively stain sarcomere myosin. Antigen Unmask Solution, Vectastain ABC Kit (Mouse IgG), and Peroxidase Substrate Kit were purchased from Vector Labs (Burlingame, CA); MF-20 antibody against myosin was purchased from the Developmental Studies Hybridoma Bank (University of Iowa, Iowa City, IA). Sections were deparaffinized, blocked in serum albumin for 1 h, and incubated with 1:50–1:100 MF-20 in phosphate buffered saline with 0.5% tween-20 for 30 min at room temperature. Secondary antibody was applied at 1:75 and 3,3′-diaminobenzidine (DAB) with nickel solution was added for color development. Sections were counter-stained with hematoxylin for 20 s and cover slipped. Although myosin is present in all myocytes, a dense layer of myosin selectively stained in the right ventricular wall was used as the measurement target (Fig. 1C). Measurements similar to those on H&E stained sections were collected, with an additional measure to evaluate the myosin dense layer, which also was normalized to whole heart weight.