Chemosphere 129 (2015) 225–231

Contents lists available at ScienceDirect

Chemosphere

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

Complement activation is involved in the hepatic injury caused by high-dose exposure of mice to perfluorooctanoic acid

Salomé Calado Botelho [a], Maryam Saghafian [b], Svetlana Pavlova [b], Moustapha Hassan [b], Joseph W. DePierre [a], Manuchehr Abedi-Valugerdi [a,b,c,*]

[a] Department of Biochemistry and Biophysics, Arrhenius Laboratories for the Natural Sciences, Stockholm University, SE-106 91 Stockholm, Sweden
[b] Experimental Cancer Medicine, Clinical Research Center, Novum, Karolinska University Hospital, Huddinge, 141 86 Stockholm, Sweden
[c] ImmunoBioTox (IBT) AB, 16954 Solna, Sweden

ARTICLE INFO

Article history:
Received 28 February 2014
Received in revised form 17 June 2014
Accepted 19 June 2014
Available online 6 August 2014

Handling Editor: I. Cousins

Keywords:
Complement system
Complement factor C3
Hepatotoxicity
Liver injury
Perfluorooctanoate
Peroxisome proliferator-activated receptor alpha

ABSTRACT

High-dose exposure of mice to perfluorooctanoate (PFOA) induces both hepatotoxicity and immunotoxicity. Here, we characterized the effects of 10-day dietary treatment with PFOA (0.002–0.02%, w/w) on the liver and complement system of male C57BL/6 mice. At all four doses, this compound caused hepatomegaly and reduced the serum level of triglycerides (an indicator for activation of the peroxisome proliferator-activated receptor-alpha (PPARα)). At the highest dose (0.02%, w/w), this hepatomegaly was associated with the hepatic injury, as reflected in increased activity of alanine aminotranferase (ALAT) in the serum, severe hepatocyte hypertrophy and hepatocellular necrosis. PFOA-induced hepatic injury was associated with *in vivo* activation of the complement system as indicated by (i) significant attenuation of the serum activities of both the classical and alternative pathways; (ii) a marked reduction in the serum level of the complement factor C3; and (iii) deposition of the complement factor C3 fragment (C3a) in the hepatic parenchyma. PFOA did not activate the alternative pathway of complement *in vitro*. At doses lower than 0.02%, PFOA induced hepatocyte hypertrophy without causing liver injury or activating complement. These results reveal substantial involvement of activation of complement in the pathogenesis of PFOA-induced hepatotoxicity.

1. Introduction

Perfluorooctanoate (PFOA, $C_7F_{15}COO^-$) belongs to the large family of perfluoroalkyl acids (PFAAs) that contain a fully fluorinated carbon chain and a hydrophilic head group (primarily carboxylate, sulfonate, or phosphonate group) (Kissa, 2001; Buck et al., 2011). PFOA has been produced either synthetically or as a breakdown product/metabolite of other perfluorinated compounds for several decades now (Kissa, 2001). This compound possesses pronounced surfactant properties and is uniquely resistant to chemical, thermal and biological degradation (Kissa, 2001; Lehmler, 2005). As a result, polymers of PFOA have been used as processing aids for a range of industrial and consumer applications, e.g., coating of non-stick Teflon pans, paper, cardboard, food packaging materials and electronic devices; impregnation of leather, carpets and textiles; and the monomers as components of shampoos and fire-fighting foams (Kennedy et al., 2004; OECD, 2002, 2005).

This extensive use and extraordinary resistance to degradation have resulted in detection of PFOA in humans, wildlife and the general environment worldwide (Giesy and Kannan, 2001; Houde et al., 2006; Lau et al., 2007; Olsen et al., 2007; Butt et al., 2010). Thus, there are both scientific and public concerns about its potential toxicity towards humans and other living organisms (Andersen et al., 2008; Domingo, 2012). The major adverse effects of high-dose, sub-acute exposure of rodents to PFOA include a reduction in food consumption; loss of body weight; liver enlargement and hepatic peroxisome proliferation; alterations in hormonal status; developmental deficits; and immunotoxicity (Lau et al., 2004, 2007; Andersen et al., 2008; Dewitt et al., 2011). Furthermore, prolonged high-dose treatment with this fluorochemical increases the

Abbreviations: ALAT, alanine aminotransfrase; PFAAs, perfluoroalkyl acids; PFOA, perfluorooctanoic acid.

* Corresponding author at: Department of Biochemistry and Biophysics, Arrhenius Laboratories for the Natural Sciences, Stockholm University, SE-106 91 Stockholm, Sweden. Tel.: +46 8 164240; fax: +46 8 153679.

E-mail addresses: salomeb@stanford.edu (S.C. Botelho), maryam.saghafian@ki.se (M. Saghafian), sve177@hotmail.com (S. Pavlova), Moustapha.Hassan@ki.se (M. Hassan), joe@dbb.su.se (J.W. DePierre), abedi@dbb.su.se (M. Abedi-Valugerdi).

http://dx.doi.org/10.1016/j.chemosphere.2014.06.093
0045-6535/

incidences of benign liver, pancreatic acinar cell and testicular Leydig cell tumors in rats (Biegel et al., 2001).

We have previously shown that sub-acute (10 days) dietary exposure to a moderate dose of PFOA (0.002% w/w) that apparently does not cause liver damage induces hepatomegaly and alters the relative sizes of different populations of hepatic immune cells, while attenuating hepatic levels of both inflammatory and non-inflammatory cytokines (Qazi et al., 2010). In this connection, we recently found that sub-acute pre-exposure of mice to the same dose of PFOA aggravates the hepatitis caused by concanavalin A (Con A) in mice (Qazi et al., 2013). The exact mechanisms underlying the hepatotoxic effects of PFOA have not yet been fully characterized.

Regarding the immunotoxic effects of PFOA, we and others have demonstrated that sub-acute exposure of mice to high doses impairs the adaptive immune responses to specific foreign antigens (Peden-Adams et al., 2008; Dong et al., 2009; Qazi et al., 2009b, 2012; Dewitt et al., 2011; Yang et al., 2002a; Zheng et al., 2009), while activating certain components of innate immune responses (Qazi et al., 2009a). It is well established that the complement system (a key factor in innate immunity) is rapidly activated in response to tissue damage, infection, or stress and that this activation interacts with other inflammatory responses, as well as the adaptive immunity (Markiewski and Lambris, 2007). Therefore, we hypothesized here that exposure to PFOA exerts adverse effects on the activities of the complement system and that these effects play a role in the hepatotoxicity caused by this perfluorochemical.

2. Materials and methods

2.1. Animals

Male C57BL/6 (H-2^b) mice (5–6 weeks old at the beginning of each experiment) were obtained from Nova-SCB (Nova-SCB AB Sweden, Sollentuna, Sweden). Male animals were used to avoid the hormonal fluctuations associated with cyclic ovulation and menstruation, which can influence the immune system. These animals were housed in the animal facility of the Wenner-Gren Institute, Stockholm University, at 22 °C with a 12-h light/12-h dark cycle, 50% humidity and access to the diets indicated below and tap water *ad libitum*. Upon arrival, they were housed individually in polycarbonate cages with bedding of heat-treated pine-shavings and nesting materials (white paper towels and toilet paper rolls). The mice were allowed to acclimatize for 7 days before dietary exposure to PFOA. All of these experiments were pre-approved by the Northern Stockholm Ethical Committee for Animal Experimentation (approval number N299/05).

2.2. Preparation of the diet

PFOA (the free acid, 96% purity, Sigma–Aldrich Sweden AB, Stockholm, Sweden) was dissolved in water and then mixed with powdered RMI (E) FG SQC diet (containing 2.71% fat, 14.38% protein and 61.73% carbohydrate; SDS, Special Diets Services, Essex, UK) to obtain the desired concentrations. Since mice scatter powdered food, this chow was mixed with water, shaped into cakes and allowed to dry, to obtain more reliable determination of food intake. All diets were stored at 4 °C prior to use.

2.3. Dietary exposure to PFOA

Groups of 4 mice each were supplied with chow containing no added xenobiotic (control diet) or PFOA at concentrations (w/w) of 0.002%, 0.005%, 0.01% or 0.02% for 10 consecutive days. The animals were weighed and their food consumption recorded daily.

2.4. Collection of blood and body organs

At the end of the feeding period, the mice were bled by retro-orbital puncture under light isoflurane anesthesia and thereafter sacrificed by cervical dislocation. The liver, spleen, thymus and epididymal fat were removed and weighed. Blood samples were centrifuged to obtain serum, which was stored at −20 °C until analysis.

2.5. The CH50 assay

Total serum hemolytic activity (CH_{50}), an indicator of the activity of the *classical pathway of the complement system*, was determined employing a commercially available ELISA kit (MyBioSource Inc., San Diego, CA, USA) in accordance with the manufacturer's instruction.

2.6. The AH50 assay

The AH50 assay of the total hemolytic activity of the alternative pathway of the complement system was performed as described previously (Giclas, 2001) employing unsensitized rabbit erythrocytes (Statens Veterinärmedicinska Anstalt, Sweden) as target cells. In brief, serum samples were diluted serially in Veronal-buffered saline containing gelatin, ethylene glycol bis(f-aminoethyl ether)-N,N,N′,N′-tetraacetic acid (EGTA) and magnesium chloride (GVB/MgEGTA buffer) and 100 µl of each diluted sample added to 50 µl rabbit erythrocytes and then incubated for 1 h at 37 °C. To stop the reaction, 1.2 mL ice-cold 0.15 M NaCl was added and the tubes centrifuged at 1250*g* for 10 min at 4 °C (GPR Centrifuge, Beckman, UK) to pellet the cells. The OD_{412} of each supernatant was determined and corrected for negative controls. The reciprocal of the dilution that caused 50% total lysis (the positive control) was taken as the AH50 value and expressed in U per mL serum.

2.7. Test for in vitro activation of the alternative pathway of the complement system

Fresh serum samples from C57BL/6 mice were pooled, mixed gently on ice with PFOA at concentrations ranging from 0.078 to 360 µg/mL, and then incubated at 37 °C for 60 min. Thereafter, AH50 was measured as described above.

2.8. Measurement of serum C3

The levels of the C3 component of the complement system in serum samples were quantitated employing a commercially available ELISA kit (Immunology Consultants Laboratory, Portland, OR, USA) in accordance with the manufacturer's instruction.

2.9. Measurement of serum alanine aminotransferase (ALAT) and triglycerides

The activity of alanine aminotransferase (ALAT) and the level of triglycerides in the serum were measured employing commercially available kits (ZiestChem Diagnostics, Tehran, Iran) in accordance with the manufacturer's instructions.

2.10. Histological examination of the liver

Livers (right lateral lobes) were fixed in 4% (v/v) formaldehyde in phosphate-buffered saline (PBS) for 24 h, placed in 70% (v/v) ethanol for an additional 24 h, and then embedded in paraffin. Sections (5–6 µm) were stained with hematoxylin and eosin (H&E) for