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Perfluorooctane sulphonate induces oxidative hepatic damage *via* mitochondria-dependent and NF-κB/TNF-α-mediated pathway

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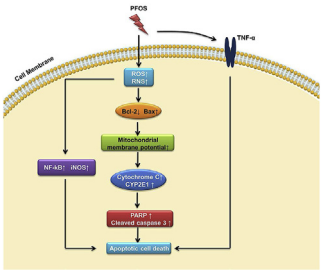
HIGHLIGHTS

- PFOS exposure is capable to cause liver dysfunction.
- PFOS exposure induces oxidative stress and apoptosis in liver.
- PFOS exposure induces hepatic apoptosis *via* mitochondria-dependent pathway.
- PFOS induced oxidative hepatic damage via NF-κB/TNF-α pathway.

GRAPHICAL ABSTRACT



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ABSTRACT

Perfluorooctane sulphonate (PFOS) has been reported to accumulate in liver and cause damage. The molecular mechanism of the PFOS-induced hepatotoxicity has not been completely elucidated. The aim of the present study was to investigate whether PFOS-induced oxidative stress plays an important role in liver damage, and if so, what pathway it undergoes for the mechanism of its toxicological action. Male Sprague-Dawley (SD) rats were orally administrated with PFOS at single dose of 1 or 10 mg/kg body weight for 28 consecutive days. Increased serum levels of liver enzymes and abnormal ultra structural changes were observed in the PFOS-exposed rats. Particularly, PFOS exposure significantly increased intracellular reactive oxygen species (ROS) and nitric oxide (NO) production, but weakened intracellular antioxidant defence by inhibiting catalase and superoxide dismutase activities. Signal transduction studies showed that PFOS exposure significantly elevated inducible nitric oxide synthase (iNOS), Bax, cytochrome *c*, cleaved caspase-9 and cleaved caspase-3, indicating the mitochondria-dependent apoptotic pathway was activated. On the other hand, significant alterations of the PFOS-induced

Abbreviations: ALT, alanine aminotransferase; AST, aspartate transaminase; APAP, acetaminophen; BSA, bovine serum albumin; CAT, catalase; Cyt *c*, cytochrome *c*; DCFDA, 2,7-dichlorofluorescein diacetate; DMSO, dimethyl sulfoxide; FITC, fluorescein isothiocyanate; GSH, glutathione; GSSG, glutathione disulphide; iNOS, inducible nitric oxide synthase; MDA, malondialdehyde; NF-κB, transcription factor nuclear factor-κB; NO, nitric oxide; PFOS, perfluorooctane sulphonate; PARP, poly(ADPribose) polymerase; PCNA, proliferating cell nuclear antigen; RNS, reactive nitrogen species; ROS, reactive oxygen species; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; SOD, superoxide dismutase; TBA, thiobarbituric acid; TBST, tris-buffered saline-Tween; TNF-α, tumor necrosis factor-α; TNFR1, TNF-α receptor 1; TRADD, TNF receptor 1-associated death domain protein.

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protein expression of NF-κB and IκBα in association with an enhanced level of TNF-α were observed. Taken together, these results indicate that mitochondria play an important role in PFOS-induced hepatotoxicity.

1. Introduction

Perfluorooctane sulphonate (PFOS), one of the most concerning polyfluoroalkyl chemicals (PFCs), has been widely used during the manufacture of carpet and apparel, paper coating, fabrics, paints and fire-fighting foams due to its special physical and chemical properties (Nakayama et al., 2005; Calafat et al., 2006). The increased manufacture and use are helping PFOS get an easy entry into the human body through the skin (Alexander et al., 2003), respiratory tract (Jogsten et al., 2012) and digestive tract (Kelly et al., 2004). It has been also found that PFOS predominantly accumulates in the mammalian liver (Hu et al., 2002). PFOS overload induces liver dysfunction (Cui et al., 2009; Wan et al., 2016), including alteration in the serum marker enzymes related to liver pathophysiology. Therefore, the potential harmful effect of PFOS on human health has received increasing concerns (Lau et al., 2004). It is well known that hepatic tissue play a major role in modulating xenobiotic metabolism (McClain et al., 1997; Fang et al., 2012). Oral administration of PFOS in animal models has been shown to cause adverse effects like hepatomegaly, hepatocellular hyperplasia, hepatic steatosis and hepatic peroxisome proliferation (Seacat et al., 2003; Lau et al., 2007; Qazi et al., 2010). After exposure and absorption, PFOS enters into the cells of different organs and then interacts with the macromolecules like protein, DNA etc., and cause a series of cytotoxic effects, such as oxidative damage and even cell death (Liu et al., 2007; Beesoon and Martin, 2015).

Oxidative stress can induce potential cell damage and is involved in many biological and pathological process, such as inflammation, carcinogenesis, and in the development of some liver diseases (Valko et al., 2006; Leung and Nieto, 2013). Oxidative stress is the result of a redox imbalance between the generation of reactive oxygen species (ROS) and the compensatory response from the endogenous antioxidant network (Limon-Pacheco and Gonsebatt, 2009). ROS are comprised of oxygen radicals like the superoxide anion ($O_2^{\cdot-}$), alkoxy radicals (RO·), peroxy radicals (ROO·), and hydroxyl radical (·OH) as well as hydrogen peroxide (H_2O_2). Molecular antioxidant defence can be triggered to eliminate ROS, including antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) as well as antioxidant molecules glutathione (GSH) and oxidative glutathione (GSSG) (Pamplona and Costantini, 2011). Therefore, these antioxidant enzymes and antioxidant molecules are playing important role in restoring cellular redox homeostasis in the presence of oxidative stress (Limon-Pacheco and Gonsebatt, 2009).

Apoptosis is a highly programmed physiological cell death and is involved in various physiological and pathological events (Thompson, 1995). In mammalian cells, two main apoptotic pathways have been delineated (Chen and Wang, 2002). The extrinsic pathway of apoptosis is initiated by the activation of tumor necrosis factor (TNF) family receptors and caspase-8-mediated activation of caspase-3, and then further induces cell apoptosis (Elmore, 2007). In the mitochondrial (intrinsic) pathway, mitochondrial stress, induced by factors such as DNA damage and heat shock, triggers the releasing of cytochrome *c* (Cyt *c*) from mitochondria into the cytoplasm. Cyt *c* in the cytosol forms a complex with ATP and Apaf-1 to activate caspase-9, and then further activated caspase-3 to induce apoptosis (Orrenius, 2004; Elmore, 2007). The release of Cyt *c* has been shown to be hallmark of mitochondria-dependent apoptosis (Wang et al., 2009). Both of these apoptotic pathways ultimately increase caspase-3 and caspase-7, which leads to the activation of poly(ADPribose) polymerase (PARP), a well-known substrate of caspase-3; this cleavage ultimately leads to the morphological and biochemical changes that are characteristic of apoptotic cells (Soldani and Scovassi, 2002).

Previous *in vitro* studies indicate that PFOS-induced oxidative stress can trigger apoptosis in human HepG2 cell line (Hu and Hu, 2009; Wan et al., 2016), mouse Leydig cells (Zhang et al., 2015), and fish primary cultured hepatocytes (Liu et al., 2007). The present study was designed to determine *in vivo* whether PFOS-induced oxidative stress plays an important role in liver damage and explore the potential signal pathway associated oxidative stress.

2. Materials and methods

2.1. Materials

Perfluorooctane sulphonate (potassium salt; 98% pure) was purchased from Fluka Chemicals (St. Louis, MO, USA). 2,7-dichlorofluoresceindiacetate (DCFH-DA), SOD and CAT were purchased from Sigma Aldrich (MO, USA). Glutathione assay kit was purchased from Promega (WI, USA). Anti-Parp-1 and anti-β-actin antibodies were purchased from Santa Cruz (CA, USA). ELISA assay kit for TNF-α was purchased from Peprotech (Rocky Hill, NJ, USA). All other chemicals and regents were analytical grade and were purchased from Sinopharm Chemical Reagent (Shanghai, PR China).

2.2. Animals and PFOS treatment

Male Sprague-Dawley rats, weighing approximately 100–120 g (6-week-old), were purchased from the Laboratory Animal Center of the Academy of Military Medical Sciences (Beijing, China). All experiments and protocols were approved by the Experimental Animal Centre, the First Hospital Affiliated to Chinese People's Liberation Army General Hospital. Rats were housed at the First Hospital Affiliated to Chinese People's Liberation Army General Hospital and maintained under standard conditions (12 h light/dark cycle, 24 °C) and had free access to standard rat chow and water. The animals were cared for in accordance with the principles of the Guide for Care and Use of Experimental Animals.

The animals were randomly divided into three groups, consisting of six rats each. PFOS was dissolved in dimethyl sulfoxide (DMSO, < 0.4%) before mixing with corn oil according to the methods by Wan et al. (2016). and Midgett et al. (2015). As the PFOS-treated group, rats were orally administrated with a single dose of PFOS at 1 or 10 mg/kg body weight for 28 d. As the non-treated group, rats only received DMSO (<0.4%) in corn oil. All of the solutions were freshly prepared prior to administration. During the experimental period, body weight and food and water consumption were monitored daily. At the end of the experiment, the rats were anesthetized using sodium pentobarbital (50 mg/kg body weight, intraperitoneal) and sacrificed after overnight fasting. Blood was collected from the abdominal vein with a microsyringe.

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