Mutation Research 754 (2013) 51–57

Contents lists available at SciVerse ScienceDirect

Mutation Research/Genetic Toxicology and Environmental Mutagenesis

journal homepage: www.elsevier.com/locate/gentox
Community address: www.elsevier.com/locate/mutres

PFOS-induced apoptosis through mitochondrion-dependent pathway in human–hamster hybrid cells

Xiaofei Wang[a], Guoping Zhao[a], Junting Liang[a], Jiang Jiang[a], Ni Chen[b], Jing Yu[b], Qisen Wang[a], An Xu[a], Shaopeng Chen[a,*], Lijun Wu[a,b,*]

[a] Key Laboratory of Ion Beam Bioengineering, Hefei Institutes of Physical Science, Chinese Academy of Sciences, Hefei, Anhui, PR China
[b] School of Nuclear Science and Technology, University of Science and Technology of China, Hefei, Anhui, PR China

ARTICLE INFO

Article history:
Received 21 September 2012
Received in revised form 5 April 2013
Accepted 16 April 2013
Available online 22 April 2013

Keywords:
PFOS
Apoptosis
Mutagenicity
mtDNA-depleted (ρ^0) A_L cells
Oxidative stress

ABSTRACT

Perfluorooctane sulfonate (PFOS) was listed as one of the persistent organic pollutants (POPs) in Stockholm Convention in 2009. Recent evidence showed that PFOS could induce apoptosis both *in vivo* and *in vitro*. However, the apoptotic mechanisms induced by PFOS as well as the possible relationship between apoptosis and other PFOS-induced endpoints, remain unclear. In the present study, normal human–hamster hybrid (A_L) cells and mtDNA-depleted (ρ^0 A_L) cells were exposed to PFOS, and assayed for cytotoxicity, mutagenicity, and apoptosis (caspase-3/7, caspase-9 activities). Our results showed that PFOS decreased cell viability in a time- and concentration-dependent manner in A_L cells, but not in ρ^0 A_L cells. However, long-term exposure to PFOS failed to induce the mutagenic effects at the *CD59* locus in A_L cells. Exposure to 200 μM PFOS significantly increased the activities of caspase-3/7 and caspase-9 in A_L cells, but the activities of these caspases were not affected in ρ^0 A_L cells. In addition, PFOS increased the levels of reactive oxygen species (ROS), superoxide anion ($O_2^{\bullet -}$), as well as nitric oxide (NO), and decreased mitochondrial membrane potential (MMP) at the concentrations of 100 and 200 μM in A_L cells. On the other hand, exposure to PFOS had no effect on intracellular ROS, $O_2^{\bullet -}$, and NO production in ρ^0 A_L cells. Caspase-3/7 activity, which was increased by 200 μM PFOS, could be suppressed by ROS/$O_2^{\bullet -}$ scavengers and nitric oxide synthases (NOSs) inhibitors in A_L cells. These results implicate that PFOS-induced apoptosis and oxidative stress is mediated by a mitochondrion-dependent pathway and that the induction of apoptosis might be a protective function against mutagenesis in A_L cells exposed to PFOS.

1. Introduction

Perfluorooctane sulfonate (PFOS) was classified as one of the persistent organic pollutants (POPs) at the Stockholm Convention in 2009 because of its property of being potentially harmful to human health and difficult to be degraded in the natural environment. PFOS-related chemicals are widely used in industrial, commercial and consumer applications. So far, PFOS has been detected in a variety of biological samples, such as terrestrial food webs consisting of lichen and plants, wildlife including wolves from two remote northern areas in Canada [1] and bird eggs from representative German ecosystems [2,3], environmental media like water, sludge and sediment [3], and human serum [4].

Although Eriksen et al. reported that plasma concentrations of PFOS were not associated with risk of liver, prostate, bladder, pancreas cancer in the general Danish population [5], several studies have shown its carcinogenic potential. For instance, a significant increase in the incidence of hepatocellular adenomas was found in male rats treated with high-dose PFOS [6]. Jacquet et al. found that PFOS induced Syrian hamster embryo (SHE) cell transformation ($p < 0.05$) at non-cytotoxic concentrations (0.2 and 2 μg/mL) ($p < 0.01$) [7]. Similarly, DNA damage studies regarding PFOS showed conflicting results as well. PFOS induced neither chromosomal aberrations in human whole blood lymphocytes with or without metabolic activation (S9), nor unscheduled DNA synthesis in rat liver primary cell cultures [8]. Florentin et al. also found that exposure to 5–300 μM PFOS could not induce DNA damage (DNA strand breaks and micronucleus) in HepG2 cells [9]. However, PFOS was recently found to strongly induce micronucleus frequency and

Abbreviations: PFOS, perfluorooctane sulfonate; POPs, persistent organic pollutants; MMP, mitochondrial membrane potential; NOSs, nitric oxide synthases; DMSO, dimethyl sulfoxide; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; JC-1, 5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzimidazolcarbocyanine iodide; CM-H_2DCFDA, 5-(6)-chloromethyl-2′,7′-dichlorodihydrofluorescein diacetate, acetyl ester; DHE, dihydroethidine; DAF-FM, diacetate, 4-amino-5-methylamino-2′,7′-difluorofluorescein diacetate; PEG-SOD, superoxide dismutase-polyethylene glycol; L-NMMA, N^G-methyl-L-arginine; D-NMMA, N^G-methyl-D-arginine; PFFAs, perfluorinated fatty acids.

* Corresponding author at: P.O. Box 1138, Hefei, Anhui 230031, PR China. Tel.: +86 551 65591602; fax: +86 551 65595670.
E-mail addresses: spchen035@ipp.ac.cn (S. Chen), ljw@ipp.ac.cn (L. Wu).

1383-5718/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.mrgentox.2013.04.004

DNA strand breaks in rat bone marrow [10], indicating its mutagenic potential [11].

PFOS-induced apoptosis was demonstrated by many studies. It was found that 50–200 μM PFOS induced expression of caspase-9 and production of ROS in HepG2 cells [12]. Lee et al. showed that PKC and ERK play pro-apoptotic roles in PFOS-induced apoptosis of cerebellar granule cells [13]. After PFOS exposure, significant apoptosis was observed in hippocampal cells of adult mice. Moreover, in the 10.75 mg/kg PFOS treatment group, apoptosis was accompanied with an increase of glutamate in the hippocampus and decreases of dopamine and 3,4-dihydrophenylacetic acid in Caudate Putamen [14]. The PFOS-induced apoptosis process was suggested to be related to mitochondrion-dependent pathway. Wallace and his colleagues reported that PFOS could induce calcium-dependent mitochondrial swelling and change mitochondrial inner membrane fluidity in rat livers [15]. The disruption of electron transport chain at the inner mitochondrial membrane can result in an increased production of intracellular reactive oxygen and nitrogen species (ROS/RNS) [16]. Therefore, investigation of the role of mitochondria in PFOS-induced apoptosis as well as the relationship between apoptosis and the mutagenic potential is essential for understanding the mechanisms of PFOS-related cells injuries.

In the present study, we detected the mutagenic and apoptotic effects of PFOS using human–hamster hybrid (A_L) cell line. This cell line, which contains a full set of hamster chromosomes and a single copy of human chromosome 11, is very sensitive in detecting mutagens such as ionizing radiation [17,18] and chemical agents [19,20]. In addition, mtDNA-depleted (ρ^0 A_L) cells were used for the study of the role of mitochondria in apoptotic process.

2. Methods and materials

2.1. Cell culture

Two human–hamster hybrid cell lines were used in these studies. Normal human–hamster hybrid (A_L) cells were maintained in Ham's F-12 medium supplemented with 8% heat-inactivated fetal bovine serum, 2×10^{-4} M glycine, and 25 μg/mL gentamicin at 37 °C in a humidified 5% CO_2 incubator. MtDNA-depleted (ρ^0 A_L) cells were generated by treating normal A_L cells with the chemotherapeutic drug ditercalinium over a period of 3–4 months to deplete the mtDNA by 95% [18]. ρ^0 A_L cells were cultured in F12/DMEM (1:1) medium supplemented with 15% heat-inactivated FBS, 2.7 g/L glucose, 584 mg/L glutamine, 50 μg/mL uridine, 25 μg/mL gentamicine, and 2×10^{-4} M glycine at 37 °C in a humidified 5% CO_2 incubator.

2.2. Exposure to PFOS

PFOS was purchased from the Accustandard Corporation (New Haven, CT, USA). A stock solution of PFOS at 250 mM was prepared by dissolving the powder in dimethyl sulfoxide (DMSO, Sigma-Aldrich, St. Louis, MO, USA). A working solution was prepared by diluting the stock solution with complete medium. The DMSO concentration was about 0.08% in 200 μM PFOS working solution. Exponentially growing A_L cells and ρ^0 A_L cells in 60-mm diameter Petri dishes were treated with 1–200 μM PFOS for 1–16 days. Cells were re-plated and re-exposed to PFOS every 4 days.

2.3. Cytotoxicity assay (MTT)

3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was used to determine the cytotoxicity of PFOS. This assay measures the conversion of MTT to insoluble formazan by succinate dehydrogenase enzymes of the intact mitochondria of living cells [21]. After cells were treated with 1–200 μM PFOS for 1, 4, 8, or 16 days, the growth medium was replaced with MTT working solution (1 g/L in medium), and cells were further incubated for 4 h at 37 °C. The medium was removed, and 1 mL of acidic isopropanol was added to dissolve the formazan crystals. The optical density (OD) was measured at 570 nm using a Spectra Max M2 fluorescence reader (Molecular Devices, Sunnyvale, CA, USA).

2.4. Mutant assay

After exposure to PFOS, cells were re-plated into 60 mm-diameter Petri dishes and subcultured for another 7 days. This expression period is needed to permit surviving cells to recover from the temporary growth lag caused by PFOS and to multiply sufficiently such that the progeny of the mutated cells no longer express lethal amounts of the *CD59* surface antigen. To determine mutant frequency, 5×10^4 cells per dish were plated into six 60 mm-diameter Petri dishes in a total of 2 mL of growth medium as described [20]. The cultures were incubated for 2 h to allow for cell attachment, after which 0.2% antiserum and 1.5% (v/v) freshly thawed complement were added to each dish. The cultures were incubated for an additional 8 days. Then the cells were fixed and stained, and the number of $CD59^-$ mutation colonies was scored. Identical sets of dishes containing complement alone, antiserum alone, or neither agent constituted the controls. The mutant frequency (MF) at each dose was calculated as the number of surviving colonies divided by the total number of cells plated after correction for any nonspecific killing due to complement alone, and was expressed as the number of mutants per 10^5 clonogenically viable cells.

2.5. Caspase assay

The Caspase-Glo assay kit (Promega, Madison, WI, USA) was employed to determine the activities of caspase-3/7 and caspase-9 [22]. The proluminescent substrate containing the amino acid sequence Asp-Glu-Val-Asp (DEVD) and Leu-Glu-His-Asp (LEHD) were cleaved by caspase-3/7 and caspase-9, respectively. After caspase cleavage, a substrate for luciferase (aminoluciferin) is released, which results in the luciferase reaction and the production of luminescent signal. Briefly, cells were cultured at 37 °C in medium containing PFOS. After exposure to PFOS for the specified durations, cells were mixed with CellTiter-Glo reagent and incubated for 60 min at ambient temperature to stabilize the luminescence signal. The luminescence was quantified by a GloMax™ 20/20 Luminometer (Promega, Madison, WI, USA).

2.6. Assay for mitochondrial membrane potential

Mitochondrial membrane potential ($\Delta\psi_m$) was determined by flow cytometry using J-aggregate forming lipophilic cationic probe, 5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzimidazolcarbocyanine iodide (JC-1, Molecular Probes, Eugene, Oregon, USA) as described [23]. Briefly, after treated with PFOS for 1 day, A_L cells were trypsinized and incubated with 2 μM JC-1 working solution in the dark for 20 min at 37 °C. The cells were washed twice with PBS and resuspended in 500 μL PBS. The stained cells were analyzed by flow cytometry to determine the change in fluorescence from red to green.

2.7. Reactive oxygen species (ROS), superoxide anion ($O_2^{\bullet -}$), and nitric oxide (NO) measurement

The level of ROS was assessed by a fluorescent probe, 5-(6)-chloromethyl-2′,7′-dichlorodihydrofluorescein diacetate, acetyl