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Developmental perfluorooctane sulfonate exposure results in tau hyperphosphorylation and β-amyloid aggregation in adults rats: Incidence for link to Alzheimer's disease

Qian Zhang[a], Huimin Zhao[a], Wei Liu[a,*], Zhou Zhang[a], Hui Qin[a], Fang Luo[a], Song Leng[b]

[a] Key Laboratory of Industrial Ecology and Environmental Engineering (MOE), School of Environmental Science and Technology, Dalian University of Technology, Dalian 116024, China
[b] Health Management Center, The Second Hospital of Dalian Medical University, Dalian 116023, China

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ABSTRACT

With regard to the defects of the cognitive function observed after developmental exposure to perfluorooctane sulfonate (PFOS), and earlier studies on the developmental neurotoxicology, the aim of this study was to investigate the role of developmental PFOS exposure in neurodegenerative disorders in later life. Two pathological hallmarks of Alzheimer's disease (AD), Tau hyperphosphorylation and β-amyloid (Aβ) aggregation, were examined. SD rats were exposed to PFOS during only prenatal and/or postnatal period. *Tau* mRNA and protein levels were elevated by PFOS exposure. The phosphorylation of Tau at S199, T231 and S396 sites were also increased. Besides, PFOS exposure increased the Aβ1-42 levels, as well as the amyloid precursor protein (APP) regulation. The prenatal PFOS exposure caused alterations in the involved proteins at comparable levels with the postnatal and both prenatal and postnatal exposure. Thus, it has raised some evidence that early PFOS exposure can affect processes linked to neurodegeneration, enhancing the AD pathological risk. And PFOS exposures in early life may be of particular etiologic importance of neurodegenerative diseases.

1. Introduction

Perfluorooctane sulfonate (PFOS) is the most representative of perfluorochemicals, a rising class of persistent organic pollutants. Due to its unique physicochemical properties, including excellent surfactant capabilities, stability and amphiphilic properties, PFOS has found extensive applications. However, the properties of PFOS also cause it to be persistent and bioaccumulative in the environment. PFOS has become detectable in various environmental matrices, exposing humans to PFOS via drinking water, food, dusts, and air (Begley et al., 2005; Brooke et al., 2004; Wang et al., 2015). PFOS is not readily to be degraded and excreted, with elimination half-life in human serum at about 5 years (Olsen et al., 2007). Moreover, PFOS is capable of crossing the placental and blood-brain barrier during the early developmental stage, adversely impacting the nervous system (Mariussen 2012). The health risk posed by PFOS will still be a concern for a long period (Brooke et al., 2004; Wang et al., 2015). Besides, PFOS is being replaced by other poly- and perfluorinated chemicals, for which little is known.

The nervous system is susceptible to PFOS exposure, particularly in early developmental stage. It has been demonstrated that high doses of developmental PFOS exposure led to neonatal mortality and developmental delays in rodents (Lau et al., 2003; Luebker et al., 2005). And exposure to much lower dose of PFOS during a period of rapid brain development caused deficiency of learning and memory abilities, changes in spontaneous behavior and key proteins associated with synaptic plasticity and synaptic transmission (Johansson et al., 2009; Liao et al., 2008; Wang et al., 2015a). Moreover, *in vitro* models identified the developmental neurotoxicity effects of PFOS on neuronal growth and differentiation (Slotkin et al., 2008). It was also demonstrated that developmental PFOS exposure induced an alteration of Ca^{2+} homeostasis and mediated the molecules of calcium signaling pathway (Liu et al., 2010). Most recently, epidemiology studies suggested that prenatal exposure to perfluoroalkyl substances was associated with decreased IQ test scores in children (Wang et al., 2015b). These findings suggest the significance of the studies on the developmental neurotoxicity induced by PFOS.

Furthermore, increasing studies suggest that the pathogenesis of neurodegenerative diseases are influenced by early life

* Corresponding author at: School of Environmental Science and Technology, Dalian University of Technology, Dalian 116024, China.
E-mail address: liu_wei@dlut.edu.cn (W. Liu).

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exposures and argue for both an environmental trigger and a developmental origin (Charlet et al., 2012; Wu et al., 2008). With the prolongation of life span, more people are suffered from neurodegenerative diseases. World Health Organization estimated that around a billion people worldwide are affected by Alzheimer's and Parkinson's disease (World Health Organization, 2006). The study of occupational exposure to pesticides found that the relative risks of Alzheimer's disease were increased in French male elderly (Baldi et al., 2003). Alzheimer's disease is pathologically characterized by the presence of neurofibrillary tangles and senile plaques in the brain. The neurofibrillary tangles are composed of hyperphosphorylated Tau protein, which are incompetent to maintain the normal structure and the functions of microtubules, resulting in neuronal injury and eventual neuronal death (Cardenas et al., 2012). In addition, the senile plaques are composed of aggregates of β-amyloid (Aβ), which are generated from the sequential cleavages of amyloid precursor protein (APP) at N-termini by β-secretase (BACE-1), and subsequently, at the C-termini by γ-secretase (Bezprozvanny and Mattson 2008). Aβ aggregates typically reduce the numbers of synapses, and damage the neurites associated with the plaques (Mattson, 2004). Furthermore, it has demonstrated that Tau proteostasis and APP metabolism are coupled with each other in human-stem-cell-derived forebrain neurons with genetic forms of AD (Moore et al., 2015).

Evidence suggests the close association between the effects of environmental factors on Tau phosphorylation and Aβ aggregation, with the incidence of neurodegenerative disease (Brown et al., 2005; Demartini et al., 2014). The aggregation of the Aβ peptide and the acceleration of the amyloid plaque formation induced by metal ions, are considered to be a key event in the etiology of neurodegenerative diseases (Charlet et al., 2012; Viles 2012). Abnormalities of Cu, Zn, and Fe homeostasis in brain are recognized as a promotion of AD, where electrostatics might be the driven reason (Barnham and Bush 2008). In addition, aged monkeys with infantile Pb exposure elevated the expression of AD-related genes *App* and *Bace-1*, as well as the formation of amyloid plaques (Wu et al., 2008). And also, the exposure of rats to benzo[a]pyrene for 3 months induced Tau hyperphosphorylation, which was correlated with memory impairments (Nie et al., 2013). Cypermethrin exposure in weaning rats induced the detected early signs of AD-like pathology, and dose-dependent increases in both Aβ and phosphorylated Tau in frontal cortex and hippocampus (Maurya et al., 2016). Previous studies have demonstrated that PFOS exposure could impair the spontaneous behavior, decrease long-term potentiation (LTP) *in vivo* and disturb calcium homeostasis, which are similar with AD pathogenesis. Therefore, further study is warranted to evaluate the effects of PFOS on Tau hyperphosphorylation and Aβ accumulation, which is valuable to determine the association between PFOS exposure and potential AD pathogenesis.

With regard to the defects of the cognitive function observed after developmental exposure to PFOS, and earlier studies on the developmental neurotoxicology, the aim of this study was to investigate the role of developmental PFOS exposure in neurodegenerative disorders in later life. In the present study, SD rats were exposed to PFOS during only prenatal and/or postnatal period. Tau expression and its hyperphosphorylation at key sites, as well as the Aβ accumulation along with the associated genes in amyloidogenesis, were determined in adult rats.

2. Materials and methods

2.1. Chemicals

PFOS ($C_8F_{17}KO_3S$, CAS number 2795-39-3, purity ≥98%, Sigma-Aldrich, USA) stock solution was prepared using 2% Tween 20 in deionized water at the concentration of 1.7, 5 and 15 g/L. The stock solution was diluted 1000-fold to be used as the drinking water in 1.7, 5 and 15 mg/L exposed groups.

2.2. Animals and treatment

Sexually matured SD rats of clean grade weighing 180–200 g were provided by the National Institutes of Food and Drug Control (Beijing, China). Animals were acclimated for at least 7 days at room temperature (25 ± 2 °C) under a 12:12 light/dark cycle. The rats were paired and the day when sperms were detected in vaginal smears was denoted as the first day of exposure. Pregnant rats were randomly divided into control group and treatment groups with free accession to food and water. PFOS was administered by drinking water containing 1.7, 5, 15 mg/L PFOS, respectively. Control group was fed with 1000-fold diluted stock water containing 2% Tween 20. Litters were weaned at postnatal day (PND) 21 and then were given the same food and water as dams until PND 90. Besides, treated groups exclusively exposed in prenatal period (TC) or postnatal period (CT) were established by cross-fostering pups in control and 15 mg/L PFOS exposed group from PND1. The doses were administered according to previous study (Wang et al., 2015a), which showed no significant general effects on rats, but inhibited the learning and memory ability and reduced the synaptic proteins amount.

On PND 90, pups from each group were lightly anesthetized with diethyl ether. Blood was collected and centrifuged at 1200*g* for 20 min, and the supernatant serum was used for determination of PFOS concentrations. Hippocampus was dissected from brain, with residual blood washed off in physiological saline, and was immediately frozen at −80 °C before gene and protein analyses. For all the analysis, independent measurement for 3 or 6 individuals, from at least 3 litters of each group were conducted.

2.3. Serum PFOS analysis

The serum samples (N = 6) of each group were extracted following a method described in previous report (Wang et al., 2010). Briefly, serum samples were homogenized in 0.25 M sodium carbonate. Then, 0.5 M tetrabutylammonium hydrogen sulfate (TBAHS) and 5 mL of methyl *tert*-butyl ether (MTBE) were added for extraction, vortex mixed (20 min) and centrifuged (1260*g*, 10 min). Supernatant extractions were evaporated under nitrogen, and then resuspended in acetonitrile. After filtration by 0.45 μm nylon filter, the extracts were ready for PFOS concentrations analysis by liquid chromatography-mass spectrometry (LC-MC, Shimadzu 2010A, Japan) and following a method described by Liu et al. (2010). Ten μL of extracts were chromatographed by HPLC column (Zorbax XDB C-18, Agilent) at a flow rate of 0.2 mL/min. The mobile phase consisted of acetonitrile and 10 mM ammonium acetate buffer, and acetonitrile graded increased from 35% to 45%. Negative electrospray ionization mode of ion-spray mass spectrometer was employed, and N_2 flowed at 1.5 L/min. The selected-ion monitoring mode was 499 (*m*/*z*) for PFOS quantitation. The limit of detection and limit of quantification for PFOS were 0.2 and 0.5 μg/L in extracts, respectively.

2.4. Western blot and Elisa analysis

The levels of Tau and the phosphorylations in rat hippocampus were measured by Western Blot. Hippocampus was homogenized on ice using protein extraction reagent containing protease and protein phosphates inhibitors (CoWin Biotech, China), and then was centrifuged at 10,000*g* for 15 min. The supernatants were collected and assayed immediately. The total protein concentrations were measured with the BCA Protein Assay Kit (CoWin