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Perfluorooctane sulfonate induces apoptosis of hippocampal neuron in rat offspring associated with calcium overload

Yu Wang[a], Huimin Zhao[a], Qian Zhang[a], Wei Liu[*a], and Xie Quan[a]

The purpose is to investigate the effects of perfluorooctane sulfonate (PFOS) on neuronal apoptosis in hippocampus of rat offspring, and to elucidate the underlying mechanisms associated with calcium homeostasis. A cross-fostering model was established, enabling the evaluation of prenatal and postnatal exposure. Internal exposure was measured via PFOS concentration analysis in serum and hippocampus. Cell apoptosis of hippocampus neuron was identified along with the measurement of intracellular free calcium concentration ($[Ca^{2+}]_i$). Continuous PFOS exposure in both prenatal and postnatal period induced an increasing apoptosis in hippocampus neurocyte. Meantime, $[Ca^{2+}]_i$ increased in a dose dependent manner in the continuous exposure groups and prenatal exposure groups. Furthermore, expression of apoptosis-related genes serves for the mechanistic analysis of the apoptotic effects induced by PFOS. Both apoptosis-linked gene-2 (*alg-2*) and death-associated protein kinase (*dapk2*) genes were up-regulated, especially in prenatal exposure groups on postnatal day (PND) 35. *Bcl-2* was also significantly up-regulated both on PND7 and PND35. Overall results indicated that PFOS exposure caused increasing of apoptosis in hippocampus, where $[Ca^{2+}]_i$ overload acted as a potential mechanism. Moreover, prenatal exposure results in long-lasting effects on calcium homeostasis and the genes expression regulating calcium signaling and apoptosis of rat offspring, highlighting the developmental neurotoxicity risk of fetal PFOS exposure.

Introduction

Perfluorooctane sulfonate (PFOS), a typical perfluorinated chemical, is widely used for industrial and consumer applications, such as pesticides, fire retardants, and textiles because of its extremely stable carbon-fluorine bonds.[1-2] PFOS bioaccumulates through the food chain to higher trophic levels, and are now globally present in various environmental media, wildlife, and humans.[3-5] PFOS contamination was even gradually worsening in recent years in China and Japan.[6-7]

PFOS is capable of crossing the placental barrier and the blood-brain barrier,[8] and poses serious impact on brain tissue.[9] Unlike adult exposure, gestation and breast-feeding are considered important routes of infants and toddlers exposure.[10] Therefore, it is essential to explore the developmental neurotoxicity of PFOS. It has been reported that neonatal exposure to PFOS during a period of rapid brain development caused changes in spontaneous behavior,[11] markedly increased proteins involved in neuronal survival, growth, and synaptogenesis,[12-13] and affected the neuroendocrine system in rats.[14] Embryonic exposure and two generations breeding experiments showed that PFOS caused increased mortality and developmental delay in mice pups.[15] But the mechanisms underlying the PFOS-induced developmental neurotoxicity remain unclear. Moreover, the evaluation of prenatal and postnatal exposure is warranted due to some previous observations that prenatal exposure posed substantial impact on the pups.[8, 15]

As a sensitive indicator in neurotoxicity, apoptosis of neuronal cells is closely associated with a variety of neuronal diseases, especially during developmental period of synaptogenesis.[16] Many of the PFOS-induced toxicity in liver, lung, and immune organs are related to apoptosis.[17-20] Furthermore, neurotoxicity studies have shown that PFOS can cause abnormally high intracellular free calcium concentration ($[Ca^{2+}]_i$) in the hippocampus.[21] As an important second messenger in nerve cells, calcium homeostasis is the essential requirement to maintain the nerve physiological functions.[22] Calcium mediates the release of neurotransmitters, the formation of nerve spinous process and growth through the transmission of information.[22] Calcium also participates in a variety of biological processes, including cell proliferation, differentiation, and apoptosis.[23]

The present study aims to investigate the effects of prenatal and postnatal PFOS exposure on the neuron apoptosis, to compare the prenatal and postnatal effects, and to elucidate the potential mechanisms related to calcium homeostasis. A cross-fostering model was built to modulate prenatal and postnatal PFOS exposure. $[Ca^{2+}]_i$ and apoptosis were analyzed during the developing period on postnatal days (PNDs) 1, 7, and 35 in hippocampus. Gene expression associated with apoptosis were also determined to further confirm the apoptotic effects and elucidate underlying molecular mechanisms.

Materials and methods

1. Ethical approval of the study protocol

The study protocol was approved by School of Environmental Science and Technology, Dalian University of Technology (Dalian, PR. China).

2. Reagents

The potassium salt of PFOS (hereafter referred to as PFOS, CAS number 2795-39-3, purity≥98%, Sigma-Aldrich, USA), was solubilized in 2% Tween 20 (purity≥97%, Amresco, USA) in deionized water to prepare solution at the concentration of 2.5 and 7.5 g/L. Then the PFOS stock solution were diluted 500-fold with drinking water, which equal to 5 and 15 mg/L, as the drinking water fed to rats in low dose and high dose exposure groups. Deionized water contained 2% Tween 20 was diluted 500-fold with drinking water to feed control group.
Tetrabutylammonium hydrogen sulfate (TBAHS) was purchased from Fluka (USA) and methyl tertbutyl ether (MTBE) were high-performance liquid chromatography grade purchased from Sigma-Aldrich (USA).

3. Animals treatment and samples collection

Wistar rats weighing 180-220 g, were purchased from the Experimental Animal Center of Dalian Medical University. After one week acclimation, female rats were paired and mated with male rats. The day on which sperms were detected in vaginal smears was considered as the first day of pregnancy. The ratio of 3:2:2 by pregnant rats were randomly divided into control group (drinking water containing 0.004% Tween 20), low dose PFOS exposure group (drinking water containing 5 mg/L PFOS) and high-dose PFOS exposure group (drinking water containing 15 mg/L PFOS). On PND1, pups in control group and exposed groups were cross-feeded, establishing different groups, including control group (CC) without exposure, continued exposure to PFOS at low dose of 5 mg/L and high dose of 15 mg/L (TT5, TT15), only postnatal exposure at low-dose (CT5) and high dose (CT15), only prenatal exposure at low dose (TC5) and high dose (TC15). In the present study, 5 and 15 mg/l PFOS concentration was chosen, equal to 2% and 6% of LD50 for rats in acute toxicity test, respectively. And our previous studies found that such PFOS dosage posed no significant general effects on rats, but exhibited substantial neurotoxicity.[24]
Pups were sacrificed on PNDs 1, 7, and 35. Dams were sacrificed on PNDs 7, and 35. The blood was collected and the serum was removed immediately for determination of PFOS concentrations. The hippocampus of pups were rinsed in physiological saline solution, and immediately frozen in liquid nitrogen, storing at -80 °C for western blot and Real-time quantitative RT-PCR analyses, or prepared to single cell suspensions for apoptosis and calcium concentration analysis. Pups used for test in the same exposure group were from three different litters.

4. Hippocampus and serum PFOS analysis

This study analyzes procedural blank to exclude sample pollution that sample collection, sample handling and analysis may cause. Serum samples of 50 μL were homogenized in 0.25 M Na_2CO_3 in 15 mL polypropylene centrifuge tube. Hippocampal tissue of 50 mg was accurately weighed, placed in 15 mL polypropylene centrifuge tube, digested in 2 mL of 0.5 M sodium hydroxide at 80 °C for 12 h, then hydrochloric acid was added to neutral. Hippocampus or serum homogenate aliquots were mixed with 1 mL of 0.5 M TBAHS (pH 10.0) and 5 mL of methyl tert-butyl ether (MTBE), and then was vortexed for 20 min followed by centrifugation at 3000 rpm for 10 min. The MTBE layer was transferred into a new polypropylene tube. The residual mixture was rinsed with MTBE and separated again. Two rinses were combined, evaporated under nitrogen, and then resuspended in 1 mL of acetonitrile. The solution samples were then passed through 0.45 μm nylon filter before instrumental analysis.
The concentration of PFOS in extracted solution was quantified by liquid chromatography-mass spectrometry (LC-MS, Shimadzu 2010 A, Japan), following a method described by Wang et al.[5] The limit of detection (LOD) and limit of quantification (LOQ) for PFOS were 0.2 and 0.5 μg/L in methanol extract, respectively. And the recoveries of PFOS in serum and hippocampus were 97.1±4.0%, 96.9±3.8%, respectively.

5. Flow cytometric analyses

FACSCalibur Flow Cytometer (Becton Dickinson, USA) was used to determine $[Ca^{2+}]_i$ and cell apoptosis. Cell suspension was prepared firstly. Hippocampus were washed in cold D-Hank's, and incubated in 3 mL of 0.25% trypsin at 37 °C for 30 min. Then the homogenate was centrifuged at 1000 rpm for 10 min at 4 °C to collect the hippocampus cells.
Percentage of apoptotic cells was determined with apoptosis kit (Invitrogen, USA). Briefly, cells were resuspended in cold binding buffer (1×), and the concentration was adjusted to 10^6 nucleated cells/mL. Annexin V-FITC (5 μL) was added into a 100 μL single-cell suspension. One μL of propidium iodide (PI) was added to the mixture later. The mixture was incubated at room temperature for 15 min, then 100 μL of binding buffer was added before analysis. At least 10^4 cells were assayed. Data were analyzed with Cell Quest software (Becton Dickinson, USA).

$[Ca^{2+}]_i$ in hippocampus was monitored by the calcium sensitive dye fluo-3 (Biotium, USA). Cell suspensions were washed with Ca^{2+}-free HANK's, and then stained with fluo-3 (final concentration of 0.1 M) and incubated at 37 °C for 30 min in the dark.

6. Real-time quantitative RT-PCR analyses

Table 1 Sequence of primers used for Real-Time RT-PCR amplification.

Target gene	GenBank accession no.	5' → 3' Primer sequences	Product size (bp)
β-actin	NM_031144.2	Forward: GGAGATTACTGCCCTGGCTCCTA	150
		Reverse: GACTCATCGTACTCCTGCTTGCTG	
ALG-2	AF192757.1	Forward: CACGGAAGACGGAAAGAGATG	86
		Reverse: TGGCGGAGGGATAGAAGGA	
DAPK2	NM_001013109.1	Forward: GTGCACTTGAGGACAAGTGAGGA	123
		Reverse: CAGGTGCCGGATCAGTTAGGA	
Bcl-2	NM_016993	Forward: ACAGAGGGGCTACGAGTGGGA	91
		Reverse:CTCAGGCTGGAAGGAGAAGATG	

RNA was isolated from individual samples of hippocampus, and was reverse-transcribed to cDNA using trans-script all-in-one first-strand cDNA synthesis supermix for qPCR kit (Transgene, China) according to manufacturer's instructions. Rat-specific primers were designed for the genes of *alg-2, dapk2, bcl-2* (Table 1) with the software Primer Premier 5.0 (PREMIER Biosoft International, USA). The housekeeping gene β-actin, which was assayed as described by Liu et al.,[25] was used as an internal control. The SYBR Green PCR Master Mix Reagent kit (Transgene, China) was used for the quantification of gene expression. The PCR amplification protocol was 30 s at 94 °C, followed by 40 cycles at 94 °C for 5 s and 60 °C for 30 s. Three randomly samples were selected from each dosage group for analysis, and each sample was carried out with three replicates. Melting curves analysis was performed, and only one peak was observed for each amplification, indicative for the specificity of the target gene. The fold change of the tested genes was analyzed by the $2^{-\Delta\Delta C_T}$ method.[26]

7. Statistical analyses

Data were analyzed via SPSS 16.0 software (SPSS, USA) and presented as mean±standard error (SE). One-way ANOVA was used to determine the differences between the control and treatment groups. $p<0.05$ was considered to be significant.

Results and Discussion

1. PFOS concentrations in serum and hippocampus of dam and pups

Table 2 Mean (±SE) PFOS concentrations in control and PFOS treated groups in serum of litter rats (μg/mL). n=3.

Postnatal Days	Groups						
	CC	TT5	TT15	TC5	TC15	CT5	CT15
PND1	nd[a]	35.7±8.9**	55.9±8.1**	—[b]	—	—	—
PND7	nd	21.7±1.7**	87.6±9.4**	8.2±0.8*	21.7±1.5**[#]	6.4±4.2	8.7±1.4*
PND35	nd	37.8±2.9**	121.0±7.1**	1.2±0.2[##]	2.7±0.5[##]	18.1±2.8**	61.3±1.1**

* Statistically significant ($p < 0.05$), ** Statistically significant ($p < 0.01$) difference from CC group. [#] Statistically significant ($p < 0.05$), [##] Statistically significant ($p < 0.01$) difference from TC group compared to CT group under the same PFOS level.

a: nd means not detectable.

b: — means this group does not exist.

Table 3 Mean (±SE) PFOS concentrations in control and PFOS treated groups in hippocampus of litter rats (μg/g). *n* =3.

Postnatal Days	Groups						
	CC	TT5	TT15	TC5	TC15	CT5	CT15
PND1	nd[a]	123.3±22.5**	373.4±1.8**	—	—	—	—
PND7	nd	11.4±1.8**	32.30±1.8**	4.6±0.4**$^{\#\#}$	10.8±0.5**$^{\#\#}$	1.0±0.1	3.5±0.5**
PND35	nd	6.7±1.3**	14.66±1.0**	0.3±0.1$^{\#}$	0.3±0.0$^{\#\#}$	1.9±0.2**	5.7±0.7**

* Statistically significant ($p < 0.05$), ** Statistically significant ($p < 0.01$) difference from CC group. $^{\#}$ Statistically significant ($p < 0.05$), $^{\#\#}$ Statistically significant ($p < 0.01$) difference from TC group compared to CT group under the same PFOS level.

a: nd means not detectable.

b: — means this group does not exist.

In the maternal rats, both the serum and hippocampal PFOS concentrations increased with increasing dosage and longer exposure period (Table S1, S2). And hippocampal PFOS concentrations were lower than the respective serum PFOS concentrations in dams. Further, a remarkable PFOS transfer from dams to pups was observed in both the pups serum and hippocampus. Serum PFOS concentrations of litters in CT5, CT15 groups increased in a time-dependent manner after birth, with the highest concentration of 61.3 μg/mL in CT15 group on PND35. In contrast, serum PFOS concentration of litters in TC5, TC15 groups decreased as they aged (Table 2). Hippocampal PFOS concentrations of litters in CT5, CT15 groups also increased in a time-dependent manner after birth, but decreased as the pups aged in other groups (Table 3). The decrease of hippocampal PFOS concentrations in TC5 and TC15 groups is mainly attributable to the quick PFOS elimination through feces and urine.[27] And the decrease of hippocampal PFOS concentrations in TT5 and TT15 groups with continual exposure might be related to the blood-brain barrier developed after PND24.[5, 28] In addition, brain growth and the PFOS redistribution in brain may also contribute to the lower PFOS concentrations observed postnatally.

It is noteworthy that the hippocampal PFOS concentrations were even higher than the serum levels in the pups on PND1, while it is not the case in adults, as seen in the maternal rats, indicating the high risk of prenatal exposure on the neural system. Moreover, the hippocampal PFOS concentrations in TC groups were higher than in CT groups on PND7. Meanwhile, pups weight in TC groups was also lower than in CT groups on PNDs 1, 7, and 35 (data not shown), which strengthen the high risk of prenatal PFOS exposure on the neonatal growth. Pups weight in TT groups as low as TC group also confirmed this point. The prenatal and lactational exposure of PFOS in humans have been proved by the presence of PFOS in human cord blood and breast milk.[29-31] Furthermore, young people have the same or even higher PFOS levels in serum or blood, compared with older generations.[32] Therefore, it is crucial to evaluate the developmental neurotoxic effects induced by PFOS.

2 Cell apoptosis in hippocampus

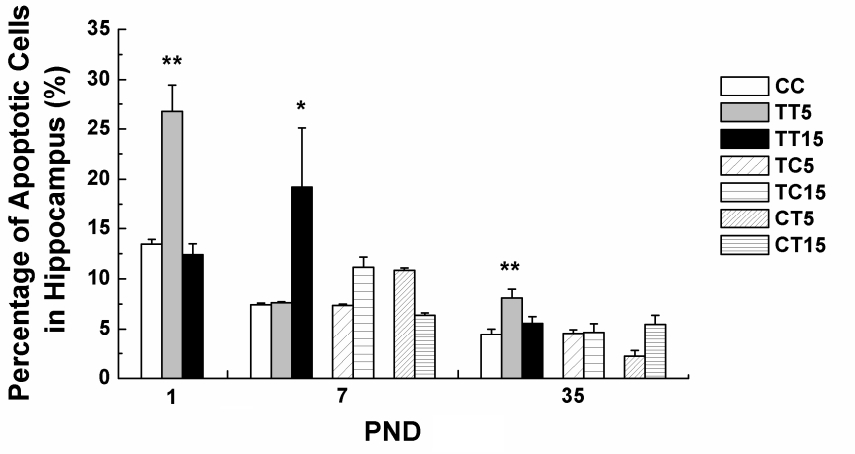

Fig. 1 Percentage of apoptotic cells in control and PFOS treated groups in hippocampus on PND1, 7, 35. n=3. * Statistically significant ($p < 0.05$), ** Statistically significant ($p < 0.01$) difference from CC group.

Apoptosis in hippocampus was shown in Fig. 1. On PND1, hippocampus apoptosis increased in TT5 group, but no significant change was observed in TT15 group compared with CC group, in a non-monotonic dose-response manner. On PND7, apoptosis in TT15 group was significantly higher than CC group by 90%. On PND35, apoptosis in TT5 and TT15 groups were higher than in CC group, with a significant change in TT5 group, similar to PND1, but different from PND7. No significant change was observed in CT and TC groups on PNDs 7 and 35, which is possibly due to relatively low hippocampal PFOS concentrations.

Previous reports have shown that PFOS induced apoptosis both *in vivo* and *in vitro*. Shi et al.[33] and Dong et al.[34] found that PFOS induced cell apoptosis in zebrafish embryos and immune organs, while Hu et al.[35] and Lee et al.[13] showed that the induction of apoptosis by PFOS detected in HepG2 cells and cerebellar granule cells, indicating that the PFOS has acted as a promoter of cell apoptosis. Chen et al.[36] reported that both intrinsic and extrinsic cell death pathways were involved in prenatal PFOS exposure-induced injuries in the lung of rat offspring, and adult mice exposed to PFOS caused apoptosis in hippocampal cells.[37] Different from cells and adult rodents, there is a "brain growth spurt (BGS)" in developing brain of offspring. In rat offspring, the BGS occurs in the neonate, spanning the first 3-4 weeks of life, and peaking around PND10.[8] The non-monotonic dose-response relationship of the PFOS-induced apoptosis may be attributable to the repression of apoptosis at higher PFOS dosage and leading to more serious adverse effects. Similar results were also reported in other studies concerning PFOS exposure during either developmental or mature stages. The early thyroid development related genes expression in zebrafish embryos were up-regulated in the low dose PFOS-treated groups, with no significant change in the high dose PFOS-treated groups.[33] Titanium dioxide nanoparticles induced higher apoptosis in lower exposure concentration in human glial cells.[38] It is worth noticing that the response to PFOS was inconsistent in different stages during the development stage. After embryonic exposure to PFOS, the heart rate of zebrafish decreased in 48-hour post-fertilization but increased in 84-hour post-fertilization.[33] Prenatal PFOS exposure led to the decrease on PND0 and increase on PND21 of *bcl-2* gene in the lung of rats offfspring.[36] Different glucose metabolism was also appeared on 10 and 15 weeks after weaning of rats when exposed to PFOS in prenatal period.[39] All these results illustrated the complexity of PFOS exposure in the developmental stages. Apoptosis during BGS period is a critical event to induce neurobehavioral disturbances occurred either in childhood or adulthood.[13] Long-lasting effects on adult behavior of mice have been reported when prenatally or postnatally exposed to PFOS. Johansson et al.[12] reported that neonatal exposure to 1.4 or 21 mmol/kg body weight of PFOS on PND10 caused neurotoxic effects on adult mice, manifested as changes in spontaneous behavior and habituation. The learning and memory ability of mice and rats was declined after prenatal PFOS exposure.[40,41] Apoptosis occurred in neurons during development of the nervous system may be responsible for neuronal deaths that occur in neurological disorders such as Alzheimer's and Parkinson's diseases. Therefore, the study on apoptosis related toxicological mechanism induced by PFOS is crucial for a better understanding of its neurotoxicity and the association with related clinical disease.

3. $[Ca^{2+}]_i$ in hippocampus

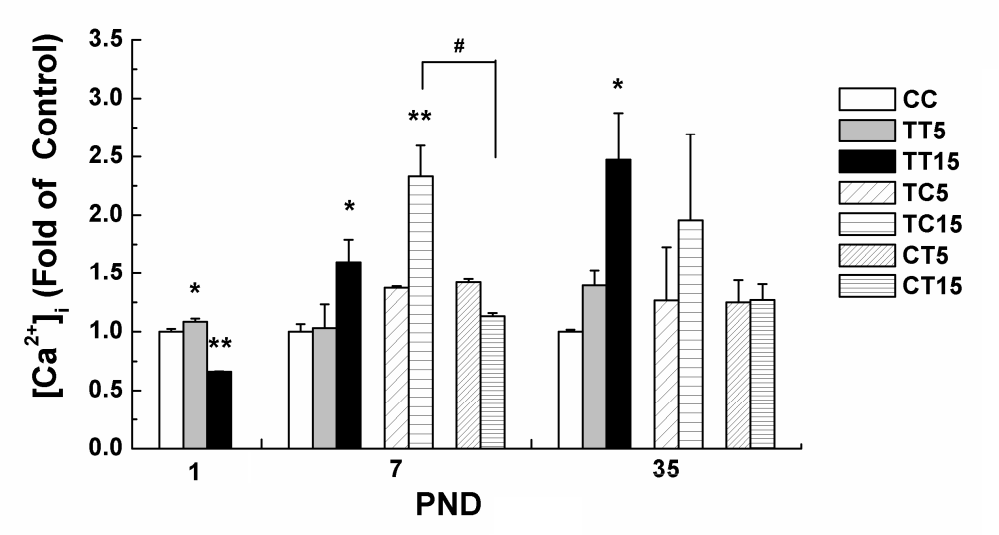

Fig. 2 $[Ca^{2+}]_i$ fold change in control and PFOS treated groups in hippocampus on PNDs 1, 7, 35. n=3. *, ** Significantly different from CC group at level of $p<0.05$, and $p<0.01$, respectively. # Statistically significant ($p < 0.05$) difference from TC group compared with CT group under the same PFOS level.

$[Ca^{2+}]_i$ was changed in hippocampal neurons by PFOS treatment. $[Ca^{2+}]_i$ on PND1 firstly increased and then decreased in TT5, TT15 groups compared with CC group, which had a similar pattern with apoptosis. On PND7 and PND35, $[Ca^{2+}]_i$ increased in a dose dependent manner in continuous exposure groups, with a significant change in TT15 groups. In cross-fostering groups, a dose dependent increase of $[Ca^{2+}]_i$ also occurred in TC groups, with significant change in TC15 group on PND7 when compared to both CC and CT15 groups. A moderate $[Ca^{2+}]_i$ increase was observed in CT groups on PND7 and PND35 with no significant change (Fig. 2).

Induction of calcium overload and the related molecular pathway have attracted extensive attention. It is reported that PFOS could induce activation of cell surface receptors, activate the L-type voltage gated calcium channels, and promote calcium release from intracellular calcium stores, resulting in calcium imbalance.[18,42] However, the role of calcium imbalance in regulating apoptosis process is not well understood related to the neurotoxicity mechanism of PFOS. In the present study, apoptosis and $[Ca^{2+}]_i$ changed concurrently in similar pattern, especially in continual PFOS exposure groups with relatively high dosage, demonstrating that PFOS-induced apoptosis is possibly related to the disturbance in calcium homeostasis. Furthermore, related studies showed that multiple signaling pathways mediating apoptosis are closely associated with calcium homeostasis. PFOS may induce apoptosis via a ROS-mediated protein kinase C signaling pathway[13] or by a mitochondrion-dependent pathway.[43] Protein kinase C, contributing to many kinds of neuronal processes, is activated by calcium. And mitochondria is one of the major intracellular calcium stores. These evidence further support that PFOS-induced apoptosis is associated with the increasing $[Ca^{2+}]_i$. Abnormal $[Ca^{2+}]_i$ increase induced by PFOS may lead to cell signaling system abnormalities, injure the calcium signal transduction pathways, then induce start-ups of apoptosis program, which would block the long-term potentiation induction, impact on synaptic plasticity, and eventually lead to the behavioral deficits. Some difference between changes in $[Ca^{2+}]_i$ and apoptosis were also observed, which may be related to the BGS. During the development of the central nervous system, a variety of feedback and compensation mechanisms are also involved in regulating the cell apoptosis besides the calcium homeostasis.

4 Apoptosis related gene expression in hippocampus

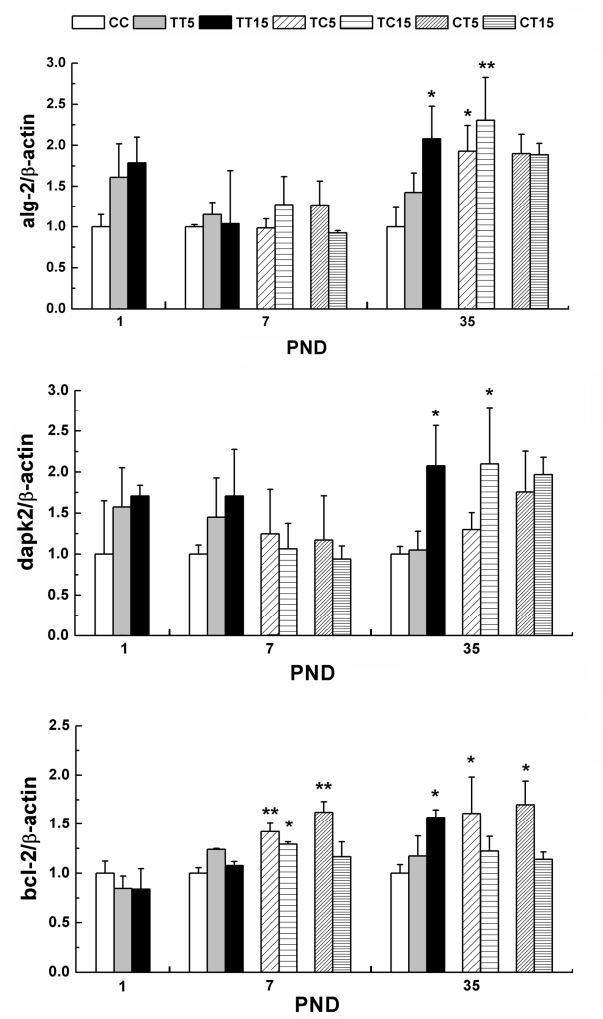

Fig. 3 Real-time quantitative RT-PCR analyses of hippocampus mRNA expression levels from control and PFOS-exposed rats on PNDs 1, 7, and 35. n=3. Gene expression levels represent the relative mRNA expression compared with the β-actin. * , ** Significantly different from CC group at p <0.05, and p < 0.01, respectively.

Both *alg-2* and *dapk2* gene expression exhibited an upward trend on PND1, with no significant change. Slight up-regulation of *alg-2* and *dapk2* gene was observed on PND7 in both TT and TC groups, but increased first and then decreased in CT groups, with no significant change. Significant up-regulation of *alg-2* was observed on PND35 in TT15, TC5 and TC15 groups. And *dapk2* gene expression exhibited significant up-regulation in TT15 and TC15 groups on PND35. *Bcl-2* gene expression was slightly down-regulated on PND1, and significantly up-regulated in CT5, TC5, and TC15 groups on PND7, and in CT5, TC5, and TT15 groups on PND35 (Fig.3).

The differential expression of multiple genes involved in the apoptosis process further confirmed the apoptotic effects caused by PFOS, and provided some evidence about the association between apoptosis and the interference on calcium homeostasis. ALG-2 was discovered as a pro-apoptotic calcium binding protein, and has been proposed that it is involved in calcium mediated signaling processes to regulate cell death.[44] DAPK2 is one calcium calmodulin-regulated serine/threonine protein kinase and associated with apoptotic signals.[45] In the present study, *alg-2*, *dapk2* and $[Ca^{2+}]_i$ changed concurrently in similar pattern, demonstrating again that PFOS-induced apoptosis is possibly related to the disturbance in calcium homeostasis. Both *alg-2* and *dapk2* mediate Ca^{2+}-regulated signals and play a role in apoptosis.[45,46] In the present research, *alg-2* and *dapk2* were over-expressed by PFOS. PFOS might induce the increasing of $[Ca^{2+}]_i$, leading to the over-expression of calcium-related apoptotic genes.

Bcl-2 gene are key indicators to detect apoptosis. It is well known that Bcl-2 protein plays a dominant role in cell apoptosis.[47] *Bcl-2* family members affect cell death in either a positive or negative fashion. Specifically, *bcl-2* is a

negative regulator of apoptosis, serving to protect cells from apoptosis. Although some redundancy exists between family members, expression of certain family members is important during development in an organ-specific manner. *Bcl-2* tends to be highly expressed in the embryo and declines postnatally following differentiation and maturation.[48] In the present study, *bcl-2* gene significantly up-regulated on both PND7 and PND35, which demonstrated that an anti-apoptotic action was induced as a response to the PFOS-enhanced apoptosis in the hippocampus at the transcriptional level. Chen et al.[36] also found that prenatal PFOS exposure led to the up-regulation of *bcl-2* gene on PND21 in the lung of rat offspring. Other researchers have found the decreased expression of Bcl-2 protein in hippocampus and immune organs in adult mice after exposure to PFOS.[34, 37] The increase of *bcl-2* gene failed in TT15 group on PND35, which suggested that anti-apoptotic pathway was injured.

The changes in the genes expression reflected the toxic mode of action of PFOS in different developmental stages. In the present study, no statistically significant changes in the target gene levels were observed on PND1, with upregulation on PND7 and PND35. The feedback and compensatory response to the molecular damage or cellular dysfunction of PFOS exposure may be an important reason.[49] Similar results were found in the thyroid toxicity research of PFOS that gene changed on PND21 but not on PND0,[50] supporting the complex molecular regulation under the stress of the developmental PFOS exposure. The up-regulation of *alg-2, dapk2* and *bcl-2* in prenatal exposure groups on PND35 suggested that exposure to PFOS during gestation results in long-lasting adverse effect on apoptosis related genes of rat offspring. Moreover, despite the decreased PFOS concentration in TC15 group compared with the CT15 group, comparable impact occurred on the transcriptional level. These results demonstrated that prenatal exposure was more effective in altering expression of the apoptosis related genes and further stress the health risk of the fetal PFOS exposure.

The present cross-fostering study illustrated that exposure to PFOS during prenatal and postnatal periods caused the increasing apoptosis of hippocampal neural cells in rat offspring. The differential expression of apoptosis-related genes further confirmed the apoptotic effect in hippocampus caused by PFOS. Furthermore, the abnormal overload of $[Ca^{2+}]_i$ in hippocampus along with the increasing apoptosis, together with the roles of the differential expressed genes in the regulation of calcium homeostasis, suggested that the PFOS-induced apoptosis is possibly associated with the imbalance in calcium signaling. Moreover, prenatal exposure to PFOS results in long-lasting effects on calcium homeostasis and the genes expression regulating calcium signaling and apoptosis of rat offspring, which caused similar effects with postnatal exposure, highlighting the developmental neurotoxicity risk of fetal PFOS exposure. The study on apoptosis-related toxicological mechanism induced by PFOS contributes to a better understanding of its neurotoxicity and the association to related clinical disease.

The paper is to commemorate late Prof. Dr. Yihe Jin (1959-2013), who has devoted his whole life to the scientific research, and contributed greatly to the present research.

Conflict of interest

The authors have no conflicts of interest to declare.

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Notes and references

[a] *Key Laboratory of Industrial Ecology and Environmental Engineering (MOE), School of Environmental Science and Technology, Dalian University of Technology, Ganjingzi District, Linggong Road 2, Dalian 116024, China. Yu Wang, E-mail address: wy-986@163.com; Huimin Zhao, E-mail address: zhaohuim@dlut.edu.cn; Qian Zhang, E-mail address: zhangqian726@sina.com; Xie Quan, E-mail address: quanxie@dlut.edu.cn.*

[*] *Corresponding author: School of Environmental Science and Technology, Dalian University of Technology, Ganjingzi District, Linggong Road 2, Dalian 116024, China. Tel.: +86 411 84709160. E-mail address: liu_wei@dlut.edu.cn (Wei Liu)*

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