J. Mar. Biosci. Biotechnol. 2017, p. 01 - 07 Vol. 9, No. 1 [Research Paper]

http://doi.org/10.15433/ksmb.2017.9.1.001 ISSN 2383-5400 (Online)

Fucoidan Attenuates Perfluorooctane Sulfonate-induced Apoptosis of Neuronal Cells

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(Received 18 May 2017, Revised 16 June 2017, Accepted 19 June 2017)

Abstract Perfluorooctane sulfonate (PFOS) is one of the most widely distributed environmental pollutants and causes neurotoxicities. Fucoidan is a main bioactive constituent of the brown seaweed and has many functions in a variety of physiological conditions. The present study attempted to investigate the potential role of fucoidan as neuroprotective marine polypeptide in environmental pollutant-induced apoptosis of neuronal cells in culture. MTT assay showed that cell viability was significantly reduced to 68 % at 30 μM PFOS, which was recovered up to 77% and 92% in the presence of fucoidan 25 and 50 μg/ml, respectively. Cytotoxicity assay showed that LDH release was significantly increased to 160% at 30 μM PFOS but was reduced to 150% and 122% in the presence of fucoidan 25 and 50 μg/ml, respectively. Caspase-3 activity, a hallmark of apoptosis, was measured to determine the cytotoxicity of PFOS and the cytoprotective effects of fucoidan. PFOS induced a 250% increase of caspase-3 activity at 30 μM but the increase was dampened to 180% and 130% in the presence of fucoidan 25 and 50 μg/ml, respectively. PFOS 30 μM induced 180 % increase in ROS accumulation, which was effectively blocked by 50 μg/ml fucoidan (120% of control). Our results demonstrated that PFOS is a powerful neurotoxicant and fucoidan may be a protective marine bioactive polypeptide against the neurotoxic environmental pollutants. It may contribute to establishing the potential role of fucoidan as a neuroprotective polypeptide that prevents the risk of neurological disorders from the possible neurotoxic pollutants.

Keywords : Fucoidan, perfluorooctane sulfonate (PFOS), neuronal cells, apoptosis, neurotoxicity

Introduction

Perfluorinated alkyl compounds (PFAs) have been widely used in a variety of industry and consumer products. Major PFA-related products include specialty paper, packaging products, carpet, surfactants, and textile [1]. Among these PFAs, perfluorooctane sulfonate (PFOS) is one of the most widely distributed compounds. It is persistent in the environment and accumulated in both humans and wildlife [2,3]. Ubiquitous contamination of PFOS is problematic in ecosystem and bioaccumulative through food chain [4]. Due to its potential hazardous characteristics, PFOS has been among POPs list under the Stockholm Convention since 2009.

Early exposure to environmental chemicals increases the risk of neurodegenerative diseases such as Parkinson's disease and Alzheimer's disease in later life and induces permanent changes in neuronal function [5,6]. Neurological disorder associated with environmental pollutants has drawn a great concern since an average of human lifespan is ever extended.

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Among diverse etiologies, environmental factor is believed to play as an important risk factor for development of neurodegenerative diseases. While PFOS is mostly accumulated in the liver and blood, its levels in the brain gradually increase with time after exposure [7]. PFOS is reported to trigger opening of tight junction in brain endothelial cells [8]. Neonatal exposure to PFOS can cause neurotoxic effects in adult mice, manifested as changes in spontaneous behavior and habituation [9]. High dose of PFOS leads to neurologic delays and neonatal mortality.

Apoptosis of neuronal cells during developmental period of synaptogenesis, known as the brain growth spurt period, is a critical event to induce neurobehavioral disturbances expressed either in childhood or with delayed onset in adulthood [10]. Many environmental pollutants are known to induce apoptotic neurodegeneration and closely associated with a variety of neuronal diseases. Lee at al. [11] reported that PFOS induces apoptosis of cerebellar granule cells via ROS-dependent PKC activation.

Fucoidan is a main bioactive constituent of the brown seaweed, which is widely distributed in East Asia and is consumed as a marine vegetable. Fucoidan is also available as food supplement in several countries. While the outstanding physiological functions of fucoidan are well-established, only limited studies are available about its neuroprotective potentials. It is reported that fucoidan induces ROS-dependent apoptosis in 5637 human bladder cancer cells [12]. Gao et al. [13] reported that fucoidan inhibits hydrogen peroxide-induced apoptosis of PC12 cells by increasing the Bcl-2/Bax ratio and decreasing caspase-3 activity, suggesting the neuroprotective role of fucoidan.

Potentials of PFOS to elicit neurotoxicity are not only scientifically important but draw a public concern over the long-term health effects. In particular, because environmental pollutant such as PFOS is exposed to the general population regardless of age and sex throughout their entire life span, it is important to identify the ways to alleviate the potential risk of pollutants through non-provocative approaches.

Thus, this study attempted to investigate the potential role of fucoidan as a neuroprotective marine polypeptide in environmental pollutant-induced apoptosis of neuronal cells in culture.

Materials and Method

PC-12 cell culture

PC12 cells (purchased from Korean cell line bank) were cultured in RPMI 1640 Medium supplemented with 5% heat-inactivated fetal bovine, 10% horse serum and 1% penicillin/streptomycin at 37℃ in a humidified atmosphere of 5% CO_2. The cells were used for experiments prior to passage 25.

MTT assay

Cell viability was measured using tetrazolium salt 3-[4,5-dimethylthiazol -2-yl]-2,5- diphenyltetrazolium bromide (MTT; Promega, Korea). PC12 cells were seeded in 96-well plate (3×10^4 cells/well) and allowed to attach overnight. Cells were treated with different concentrations of PFOS for 24 hr in presence or absence of fucoidan (Sigma-Aldrich, St. Louis) (25 or 50 μg/ml) and then, 10 μl of MTT (5 mg/ml) was added into each well. After 2hr of incubation at 37℃, the medium containing MTT was removed, and 100 μl of DMSO was added to dissolve the purple formazan. The absorbance was detected at 595 nm by a microplate reader (Bio-Rad).

LDH Assay

Cytotoxicity was evaluated by measurement of lactate dehydrogenase (LDH) activity released from the cytosol of damaged cells. Cells were treated with different concentrations of PFOS for 24 hr in presence or absence of fucoidan (25 or 50 μg/ml). The culture medium was collected and a Cytotoxicity Detection Kit (Roche, Penzberg, Germany) was used for the assay. The percentage of cell-mediated lysis was expressed according to the following formula: % cytotoxicity = (exp. value − low control)/ (high control − low control).

Intracellular ROS measurement

Fluorescent probe, 2',7'-dichlorofluorescin diacetate (DCFH-DA), was used to measure the formation of intracellular ROS as described previously [11]. PC12 cells were seeded in 96-well black plate (3×10^4 cells/well). After 24 hr, cells were treated with PFOS or H_2O_2 for 4 h and then treated with 5 μM of DCFH-DA for 30 min. After washing cells with PBS, the fluorescence intensity was measured using microplate reader (FLUOstarOPTIMA,BMGLABTECH) with excitation at 485 nm and emission at 520 nm.

Caspase-3 activity measurement

Cells were grown on 96- well plates (3×10^4 cells/well) and the caspase-3 activity was measured by using commercial assay kits (Promega, Madison, WI). The protocols were provided by the vendor. Briefly, cells were treated with a luminogenic substrate containing the DEVD sequence and the relative light units were measured using a Plus LB96V luminometer (Berthold Detection System, OakRidge, TN).

Statistics

Data are expressed as means ± SEM. Statistical analyses were made by the Student's *t*-test to compare values between two groups or by one way ANOVA followed by Tukey's post hoc test to compare values among more than three groups. A value of $P < 0.05$ was considered significant.

Results

The effects of fucoidan on viability of PC12 cells treated with PFOS

Cell viability and cytotoxicity were measured by MTT assay and LDH assay to evaluate the cytotoxic effects of PFOS and the protective effects of fucoidan on PC12 cells. Cells were treated with different concentration (0 ~ 30 μM) of PFOS for 24 h in presence or absence of fucoidan. Cell viability was significantly reduced to 68 % at 30 μM, which was recovered up to 77% and 92 % in the presence of fuoidan 25 and 50 μg/ml, respectively (Fig 1). In accordance with cell viability, cytotoxicity as measured by LDH release was significantly increased to 160% at 30 μM PFOS but was reduced to 150% and 122 % in the presence of fucoidan 25 and 50 μg/ml, respectively (Fig 2).

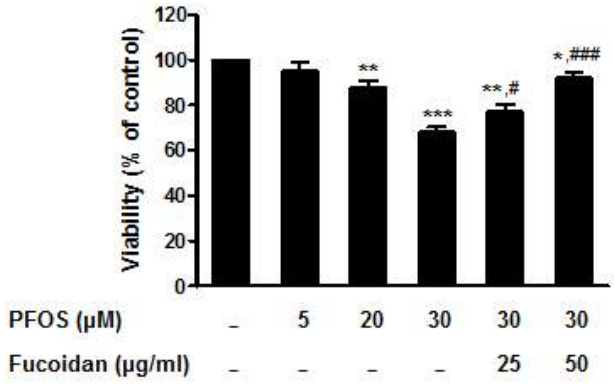

Figure 1. Effects of PFOS and fucoidan on cell viability. MTT assay in PC12 cells treated with DMSO as a vehicle control or PFOS (5, 20, and 30 μM), was conducted in the presence or absence of 25 and 50 μg/ml fucoidan as described in materials and method. All values are relative to the control cells. Values represent mean ± SEM of three replicate determinations.

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ vs. DMSO control. #$P < 0.05$, ###$P < 0.001$ vs. PFOS 30 μM-treated cells.

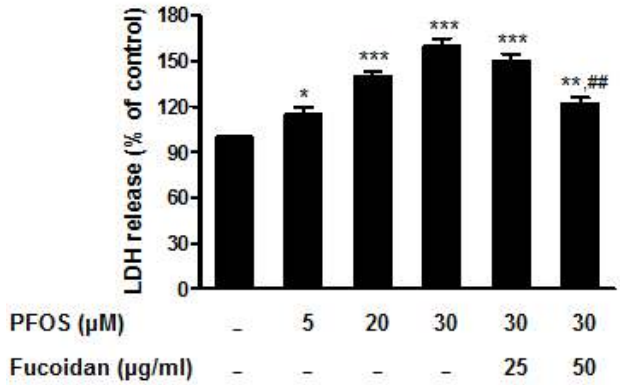

Figure 2. Effects of PFOS and fucoidan on cytotoxicity. LDH assay in PC12 cells treated with DMSO as a vehicle control or PFOS (5, 20, and 30 μM), was conducted in the presence or absence of 25 and 50 μg/ml fucoidan as described in materials and method. All values are relative to the control cells. Values represent mean ± SEM of three replicate determinations.

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ vs. DMSO control. ##$P < 0.01$ vs. PFOS 30 μM-treated cells.

The protective effect of fucoidan on PFOS-induced ROS production in PC12 cells

ROS plays a pivotal role in environmental pollutant-induced neuronal stress. The treatment of cells with 30 μM of PFOS for 4 h induced significant increase (170 % of control) in ROS accumulation, which was effectively blocked by pretreatment with fucoidan. While 25 μg/ml fucoidan did not significantly reduce ROS production, 50 μg/ml showed a significant reduction of ROS production (120% of control). H_2O_2 (100 μM) increased ROS generation about 280% and used as a positive control (Fig3).

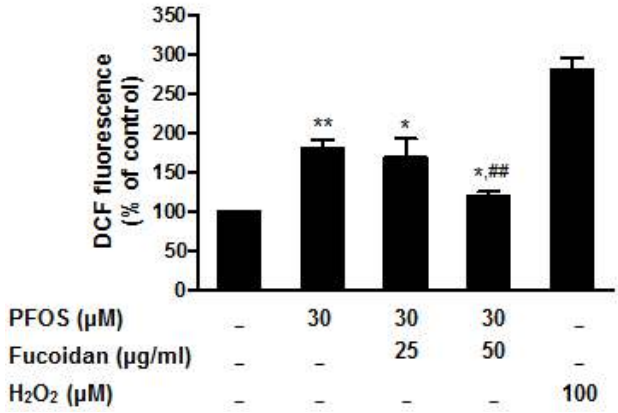

Figure 3. Effects of PFOS and fucoidan on ROS production. ROS production in PC12 cells treated with DMSO as a vehicle control, 30 μM PFOS or 100 μM H_2O_2 for 4 h, was measured in the presence or absence of 25 and 50 μg/ml fucoidan as described in materials and method. All values are relative to the control cells. Values represent mean ± SEM of three replicate determinations.

$*P < 0.05$, $**P < 0.01$ vs. DMSO control. $\#\#P < 0.01$ vs. PFOS 30 μM-treated cells.

The effects of fucoidan on PFOS-induced apoptosis of PC12 cells

Caspase-3 activity, a hallmark of apoptosis, was measured to determine the cytotoxicity of PFOS and the cytoprotective effects of fucoidan. PFOS exposure of cells for 24 h induced a significant increase in caspase-3 activity about 250 % of control at 30 μM. The increase was dampened to 180 % and 130% in the presence of fucoidan 25 and 50 μg/ml, respectively (Fig 4).

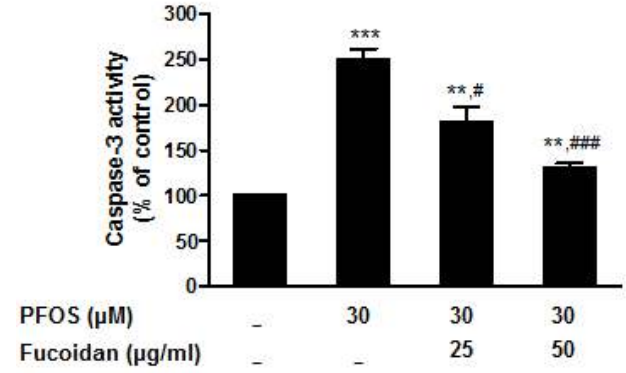

Figure 4. Effects of PFOS and fucoidan on caspase-3 activity. Caspase-3 activity in PC12 cellse treated with DMSO as a vehicle control or 30 μM PFOS, was measured in the presence or absence of 25 and 50 μg/ml fucoidan as described in materials and method. All values are relative to the control cells. Values represent mean ± SEM of three replicate determinations.

$**P < 0.01$, $***P < 0.001$ vs. DMSO control. $\#P < 0.05$, $\#\#\#P < 0.001$ vs. PFOS 30 μM-treated cells.

Discussion

PFCs have been found to be relatively high level in human blood compared to other environmental contaminants and of particular concern was their possible neurotoxic effects. PFOS is considered as a neurotoxicant in animal studies**.** Since there has been a public concern over the long-term health effects of neurotoxic pollutants, development of prophylactic approaches for its prevention is in a high demand.

Apoptosis of neuronal cells is a key element in determining neurotoxicity and apoptotic potential of compound is considered as one of key elements that distinguish neurotoxicants from other toxic materials [14]. While a wide spectrum of biological activities by fucoidan are reported, there is a lack of data that fucoidan may have a capacity to improve conditions of environmental pollutant-induced neurotoxicity. Thus, this study attempts to look into PFOS-induced neurotoxicity with respect to the apoptotic events and examine protective effects of readily available

marine bioactive polypeptide, fucoidan, from environmental pollutant-induced neurotoxicity.

In the present study, dose-dependent increase of cytotoxicity indicates the evidence that PFOS is a nuerotoxicant, as suggested in other studies. PFOS-induced cytotoxicity and apoptosis of neuronal cells may play a part in inducing a variety of neurological conditions. The protective effects of fucoidan on PFOS-induced cytotoxicity suggest that this marine polypeptide may be a material useful for the neurological disorders.

ROS is a key factor in the regulation of apoptotic processes in neurons. Elevated production of ROS is associated with induction of apoptotic death in many types of neuronal cells including cerebellar granule cells [14]. In accordance with previous studies that demonstrated an increased production of ROS by PFOS in several different cell types [15-18], our results also showed a dose-dependent increase of ROS production with exposure to PFOS. While antioxidant activity of fucoidan is well established, effectiveness of antioxidant activity for environmental pollutants remains to be ascertained. This study clearly demonstrated that fucoidan is an effective marine bioactive polypeptide for reducing PFOS-induced ROS stress in neuronal cells. The results further suggest that prophylactic therapy containing fucoidan may be a practical long-term approach that may prevent neurological diseases caused by the environmental neurotoxicant exposure.

In this study, PFOS generated a dose-dependent ROS production, which paralleled with apoptotic events. The loss of neuronal cells by apoptosis is the common final step of most neurodegenerative diseases. In particular, apoptosis during developmental period of synaptogenesis, known as the brain growth spurt (BSG) period, is a critical event to induce neurobehavioral disturbances expressed either in childhood or with delayed onset in adulthood [9,10]. Many environmental pollutants are known to induce apoptotic neurodegeneration and closely associated with a variety of neuronal diseases. However, studies on PFOS-induced apoptosis of neuronal cells are limited. PFOS induced increase of an apoptotic parameter such as caspase-3 activity. An effective blocking of apoptosis by fucoidan clearly demonstrates protective effects of fucoidan on PFOS-induced neuronal cell death.

Fucoidan exhibits various biological activities, such as an anti-inflammation in the brain [19] and neuroprotectant in cerebral ischemic-reperfusion injury [20]. Fucoidan has been shown to inhibit β-amyloid protein (Aβ) accumulation within microglia through its effects on multiple scavenger such as tumor necrosis factor-α, or nitric oxide [21]. Fucoidan also interacts with amyloid protein (Aβ) in the rat basal forebrain neurons and improves cognitive impairment [22]. Recently, it has been reported that fucoidan attenuates mitochondrial dysfunction [23] and improves neurological outcome in traumatic brain of aged rat and protects brain microvessel endothelial cells against diesel exhaust particle exposure- induced disruption [24]. In keeping with the recent series of reports that suggest protective effects of fucoidan in brain, the current study further supports the potential use of fucoidan in preventing or delaying the neurological disorders. Because one third of causes of diseases is from the environmental origin, it is very important to identify the non-provocative therapeutic or prophylactic ways to protect the humans from the ever-increasing environmental pollutants. Thus, the present study may contribute to establishing the potential role of fucoidan as a neuroprotective polypeptide that protects the risk of neurological disorders from the possible neurotoxic pollutants.

Acknowledgement

This work was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (2014R1A1A2056565), Republic of Korea.

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