APOPTOSIS OF CEREBELLAR GRANULE CELLS IS A KEY EVENT IN PERFLUOROHEXANESULFONATE (PFHxS) INDUCED NEUROTOXIC PATHWAY

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Introduction

Perfluorohexanesulfonate (PFHxS), one of the perfluoroalkyl compounds (PFCs) has been used for over 50 years in a variety of industrial and consumer applications[1]. PFCs are extremely persistent to degradation and have poor elimination from the body, which results in its accumulation in the environment and biomagnifications through food web[2]. Growing data on biomonitoring studies have shown the presence of PFHxS in serum from general population as well as in umbilical cord at birth and breast milk, indicating that its exposure occurs from fetus throughout the life span[3-5]. This raised a concern on its health related effects.

Compared to extensive studies on perfluorooctanesulfonpate (PFOS), the eight carbon congener (C8), little health outcomes of PFHxS have been documented. Although the shorter carbon chained PFCs including PFHxS (C6) have been suggested to be generally less toxic than PFOS, these compounds share many of common physical and chemical properties with PFOS. This suggests that PFHxS may produce similar biological effects to that by PFOS. Recent study has shown that a single neonatal exposure to PFHxS caused behavioral and cognitive disturbance in adult mice[6] and similar observations with PFOS have been reported[7].

Previously, we have reported that PFOS induced apoptosis of cerebellar granule cells from neonatal rats. Its neurotoxic effects were mediated by reactive oxygen species (ROS) and extracellular signal-regulated kinase (ERK) activation among mitogen-activated protein kinases (MAPKs)[8,9]. The apoptosis of neuronal cells is a critical neurological response to environmental toxicants, which is associated with various neurodegenerative diseases. Here, we examined the apoptotic effects of PFHxS using primary cerebellar granule cells (CGC) to determine its neurotoxic effects. The involvements of ROS and MAPKs activation were also investigated to compare the underlying mechanism responsible for PFHxS induced neurotoxicity to that by PFOS.

Materials and Methods

Cerebellar granule cell culture and exposure. Cerebellar granule cells were isolated from 7-day old Sprague-Dawley (SD) rat pups and cultured as described previously[8].

Western blotting. Western blot analysis was performed as described previously[9].

MTT assay. Cell viability was measured using tetrazolium salt 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT).

Caspase-3 activity assay. The caspase-3 activity was measured with colorimetrically labeled substrate, Ac-DEVD-pNA by using commercially available assay kits (Chemicon, Billerica, MA) as described previously[9].

ROS measurementy. Generation of ROS was measured with use of the fluorescent probe DCFH-DA. The fluorescence intensity was measured using microplate reader with excitation at 485 nm and emission at 520 nm as described previously[8] or detected under fluorescence microscope (Eclipse E600, Nikon).

TUNEL assay. DNA fragmentation was detected with terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end-labeling (TUNEL) assay kit (Promega, Madison, WI).

Results and Discussion

PFHxS has been reported to induce behavioral and cognitive disturbance in adult mice by a single neonatal exposure, suggesting its neurotoxicity. To evaluate its cytotoxic effects on CGC, cell viability was measured by MTT assay. Cells were treated with different concentration (0 ~ 500 μM) of PFHxS for 24 h. PFHxS did not cause any cytotoxicity up to 50 μM but caused significant reduction in cell viability at concentrations above 100 μM (Fig 1A).

As neurotoxicity of various environmental toxicants is attributable to the apoptosis of neuronal cells, the apoptotic effects of PFHxS on CGC were measured by casepase-3 activity and TUNEL assay. PFHxS exposure

of CGC for 24 h increased caspase-3 activity about by 20 %, 30 %, 340 % and 750 % at 100 μM, 200 μM, 300 μM and 500 μM, respectively (Fig 1B). These results were confirmed by TUNEL staining analyzed under fluorescent microscope (Fig 1C).

The activations of MAPKs including ERK1/2, JNK and p38 MAPK are known to play important roles in cytotoxic responses in CGC[10,11]. PFHxS increased the activation of all three MAPKs measured by Western blotting (Fig 2A). The inhibition of ERK by PD98059 significantly reduced PFHxS induced caspase-3 activity whereas JNK inhibition by SP98059 further increased casepase-3 activity. The treatment of cells with p38 MAPK inhibitor, SB253580 showed a tendency to decrease in casepase-3 activity, but the change was not significant. (Fig 2B). These results indicate that ERK and JNK play opposite roles, pro-apoptotic and anti-apoptotic, respectively, in PFHxS induced apoptosis of CGC. The TUNEL assay also showed the pro-apoptotic role of ERK1/2 (Fig 1C).

ROS has been considered as a major contributing factor in induction of apoptosis of neuronal cells. In previous study, PFOS induced apoptosis of CGC was ROS dependent. The formation ROS was determined by measuring DCF fluorescence. The significant increase (about 140 % of control) was observed at 6 h (Fig 3A). This increase was effectively blocked by pretreatment with antioxidants, 100 μM of Trolox and ERK inhibitor, PD98059 (Fig 3B). However, antioxidatns, Trolox and N-acetylcysteine (NAC) have no inhibitory effect on PFHxS-induced caspase-3 activation although AC-DEVD-CHO, a specific caspase-3 inhibitor, completely blocked the caspase-3 activation (Fig 3C). These results suggest that ROS may not play a key role in the process of apoptosis. Further studies are warranted to identify the downstream pathways of ERK activation responsible for PFHxS induced apoptosis of CGC.

Acknowledgment

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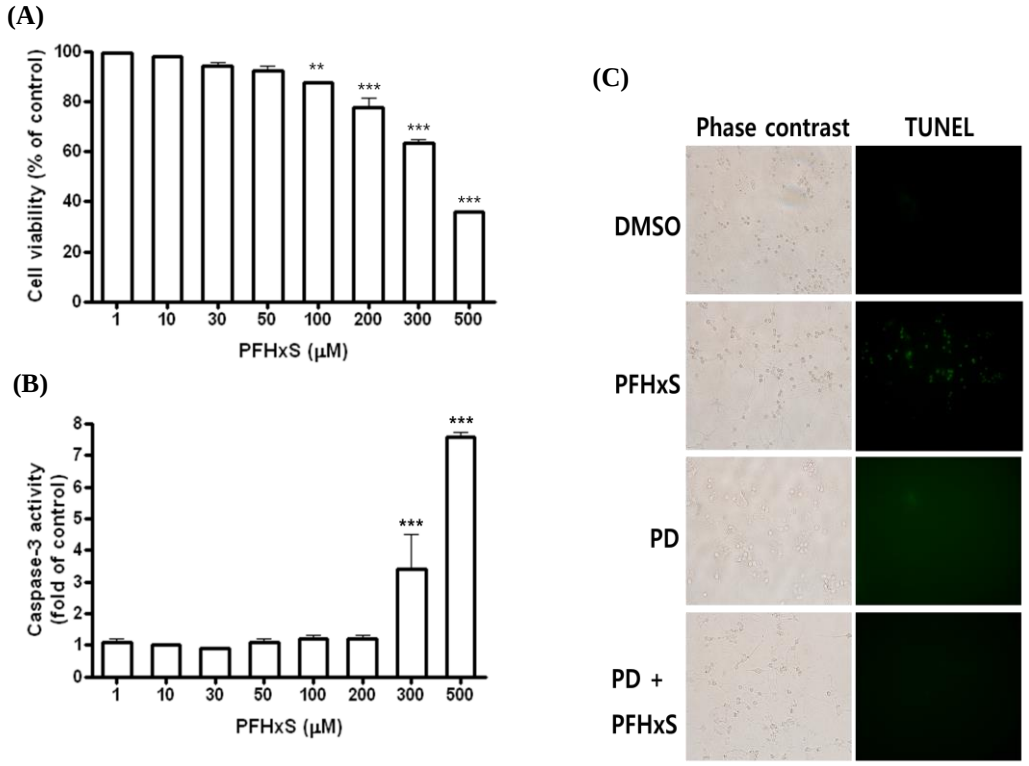

Figure 1. The effects of PFHxS on viability (A), Caspase-3 activity (B) and TUNEL assay (C) in CGC.

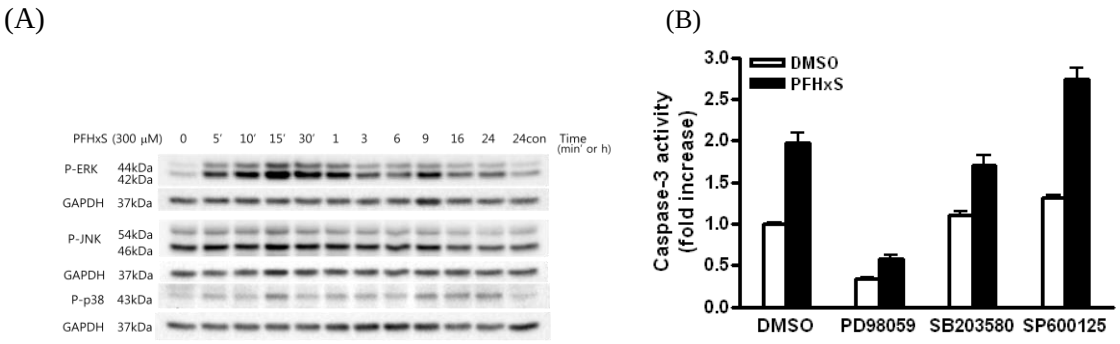

Figure 2. The effects of PFHxS on MAPKs activation (A) and roles of MAPKs in PFHxS-induced apoptosis of CGC (B).

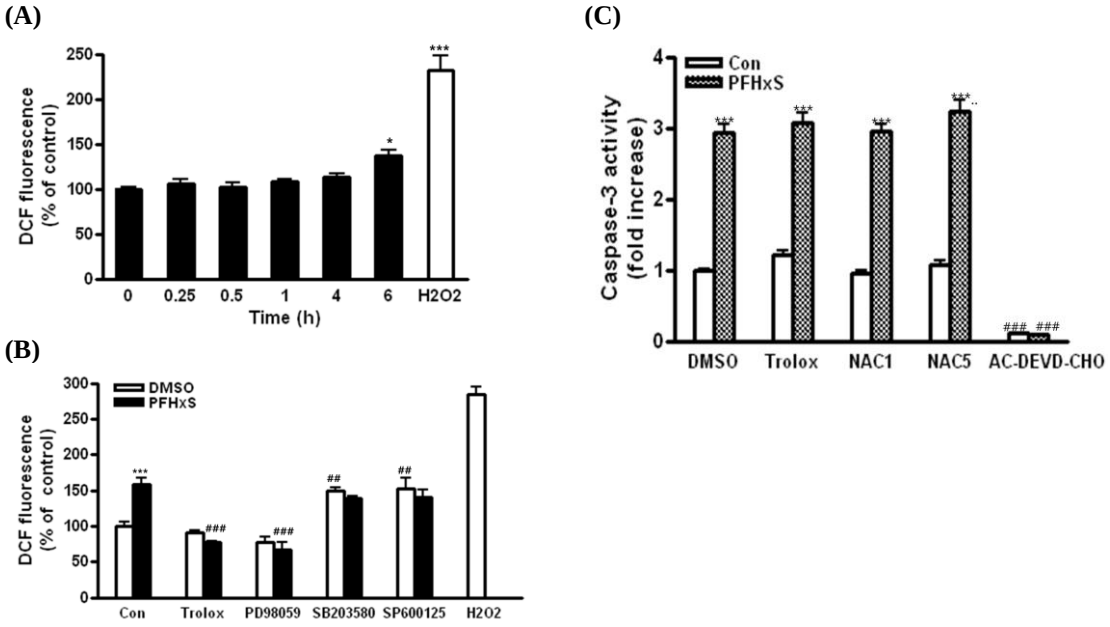

Figure 3. The effects of PFHxS on ROS generation (A), the role of MAPKs in ROS generation (B) and the role of ROS in PFHxS-induced apoptosis of CGC (C).