



Perfluorooctanoic acid induces mast cell-mediated allergic inflammation by the release of histamine and inflammatory mediators

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ABSTRACT

Perfluorooctanoic acid (PFOA) has unique physical and chemical characteristics, water and oil repellency, thermal stability, and surfactant properties. PFOA has been regularly found in the blood of animals and humans worldwide, and has become an increasing concern because of its adverse effects in immune system. However, the role of PFOA in the allergic inflammation is not well-known. To further extend the immunotoxicity of PFOA, we examined the role of PFOA on the mast cell-mediated allergic inflammation and studied the possible mechanism of action. PFOA dose- and time-dependently increased histamine release from mast cells and serum histamine by the induction of intracellular calcium. PFOA exacerbated the IgE-dependent local allergic reaction in the mouse allergy model. PFOA induced gene expression of pro-inflammatory cytokines such as tumor necrosis factor (TNF)- α , interleukin (IL)-1 β , IL-6, and IL-8 in mast cells. The inducing effect of PFOA on the pro-inflammatory cytokines was nuclear factor- κ B, p38 mitogen-activated protein kinase, and caspase-1 dependent. Furthermore, the activation of cyclooxygenase-2 by PFOA suggests the induction of allergic inflammatory mediators by the PFOA. Our findings provide evidence that PFOA, the known immunotoxic agent, induces mast cell-derived allergic inflammatory reactions by histamine release and expression of pro-inflammatory cytokines.

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1. Introduction

Perfluorooctanoic acid (PFOA) is one of the most studied and prevalent worldwide perfluorinated compounds, along with perfluorooctane sulfonate. PFOA has been used in the manufacture of Teflon® and other fluoropolymers. The primary sources of PFOA to the environment are direct emissions from the manufacturing facilities to air and water, indirect emissions from landfill leaching to groundwater, and further long-range transport via ocean current and atmospheric dispersion (McMurdo et al., 2008; Shin et al., 2011). Other potential sources of PFOA for human intake are assumed to be food and beverages, which are either primarily contaminated or secondarily contaminated by food packaging materials (Begley et al., 2005).

Allergic inflammation is a combination of early-phase immediate hypersensitivity following late-phase inflammation due to the allergen exposure (Galli et al., 2008). Mast cells are effector cells displaying various functions during immune responses and regulators of allergic inflammation such as asthma, atopic dermatitis, and

sinusitis. Allergen provocation, cross linkage of immunoglobulin E (IgE) bound on mast cells, via the high affinity receptors triggers the release of allergic inflammatory mediators including histamine, eicosanoid, and several pro-inflammatory cytokines/chemokines (Galli et al., 2005). Using these products, mast cells regulate not only immediate type hypersensitivity but also late reaction, like inflammatory responses. In the immediate phase of an allergic response such as anaphylaxis and asthma, histamine released from degranulation of mast cell acts as a major mediator (Galli et al., 2008). Mast cell degranulation can be elicited by nonimmunologic stimulators such as compound 48/80, phorbol 12-myristate 13-acetate (PMA), and calcium ionophore (Bae et al., 2011; Ennis et al., 1980). Thus an appropriate amount of those chemicals has been used as a direct and convenient reagent to study the mechanism of allergic inflammation.

The signaling pathway leading to degranulation of mast cells has been extensively characterized. Crosslinking of Fc ϵ RI leads phosphorylation of Src family kinases (Lyn, Syk, and Fyn) (Galli et al., 2008). Calcium mobilization occurs after phosphorylation of Lyn and phospholipase C- γ induces mast cell degranulation through the granule membrane fusion (Castle et al., 2002). This is followed by activation of protein kinase C, mitogen-activated protein kinase (MAPKs), nuclear factor (NF)- κ B, and the release of inflammatory cytokines/chemokines (Gwack et al., 2007; Kim et al., 2006). Cyclooxygenase (COX)-2 is also known responsible for activating mast cells in allergic inflammation and the hypersensitive

Abbreviations: PFOA, perfluorooctanoic acids; COX-2, cyclooxygenase-2.

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response. Caspase-1 and NF- κ B play a crucial role in the regulation of cytokines. Caspase-1, a member of cysteine protease also known as IL-1 β converting enzyme, is found predominantly in the cytoplasm of cells, where it proteolytically convert pro-IL-1 β into mature form, that is involved in inflammation (Thornberry et al., 1992). NF- κ B is an important transcriptional factor which regulates expression of pro-inflammatory cytokines in mast cells (Kim et al., 2006; Kim and Shin, 2005).

The incidence of asthma and allergic diseases has increased in the past several decades with a greater percent increase observed in children than any other group (Mannino et al., 2002). As one of the major uses of PFOA has been in carpet and fabric protectants, the potential exists for exposure of children through dermal as well as hand to mouth contact and adults through both environmental and occupational exposures (Begley et al., 2005; Kubwabo et al., 2005). Due to PFOA's potential for both environmental and occupational exposure, its long half-life, and known immunotoxic effects, our study was undertaken to determine if exposure to PFOA would exacerbate the mast cell-mediated allergic inflammatory reactions.

2. Materials and methods

2.1. Reagents and cell culture

Compound 48/80, *o*-phthalaldehyde, PMA, calcium ionophore A23187, and PFOA were purchased from Sigma (St. Louis, MO). For *in vitro* experiments, PFOA

was dissolved in dimethyl sulfoxide (DMSO) to make 1 M concentration and then diluted with PBS. Human mast cells (HMC)-1 were grown in IMDM (GIBCO, Grand Island, NY) and supplemented with 100 units/ml penicillin, 100 μ g/ml streptomycin, and heat-inactivated 10% fetal bovine serum in 5% CO₂ at 37 °C.

2.2. Animals

The 6 weeks old male ICR and female BALB/c mice were purchased from Dae-Han Experimental Animal Center (Daejeon, Korea). The animals were housed in a laminar air flow room maintained under a temperature of 22 $\pm$ 2 °C and relative humidity of 55 $\pm$ 5% throughout the study. The care and treatment of the animals were in accordance with the guidelines established by the Public Health Service Policy on the Humane Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee.

2.3. Histamine content

Histamine contents in serum and cells were measured by the *o*-phthalaldehyde spectrofluorometric procedure as previously described (Kim et al., 2007a). For PFOA-induced histamine release, cells were treated with PFOA in various concentrations for 12 h and 24 h. The cells were separated from the released histamine by centrifugation at 10,000 $\times$ g for 5 min at 4 °C. For serum histamine assay, PFOA and compound 48/80 (positive control, 8 mg/kg) were dissolved in PBS and intraperitoneally injected. The blood from the mice was centrifuged at 400 $\times$ g for 10 min and the serum was withdrawn to measure histamine content.

2.4. Passive cutaneous anaphylaxis (PCA)

An IgE-dependent cutaneous reaction was carried out as described previously (Bae et al., 2011) with slight modification. A schematic experimental procedure is

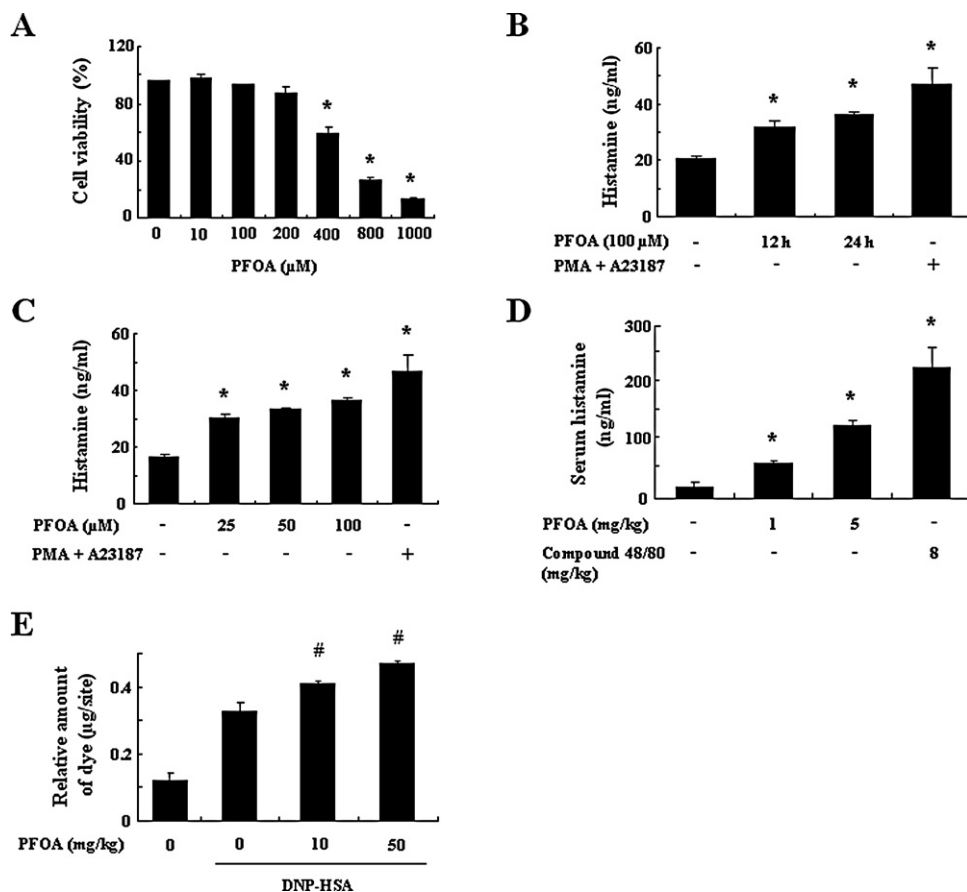


Fig. 1. Effect of PFOA on cell viability, histamine release, and allergic reaction. (A) Viability of cultured HMC-1 cells after exposure to various concentrations of PFOA (10–1000 μ M) for 24 h. The cell viability was determined using the MTT assay. (B) HMC-1 cells were treated with 100 μ M of PFOA for 12 h and 24 h. PMA (40 nM) plus A23187 (1 μ M) were used as positive control. Supernatant was used for histamine assay using the spectrofluorometric procedure. (C) HMC-1 cells were treated with 25–100 μ M of PFOA for 24 h. (D) Groups of mice (n = 5) were intraperitoneally injected with PFOA for 1 h. The mast cell degranulator, compound 48/80 (8 mg/kg), was used as a positive control. The blood was obtained from the heart of each mouse and histamine content was measured. (E) Groups of mice (n = 5) were exposed with PFOA (10 and 50 mg/kg) on dorsal surface of each ear for 4 days. On day 3, mice were intradermally injected with 0.5 μ g of anti-DNP IgE on dorsal skin of each ear. After 48 h, each rat received an injection of 1 μ g of DNP-HSA containing 4% Evans blue (1:4) via the tail vein. Each amount of dye was determined colorimetrically. The intensity of absorbance was measured at 620 nm using a spectrophotometer. Each bar represents the mean $\pm$ SEM of three independent experiments. *Significantly different from the control. #Significantly different from the DNP-HSA only.

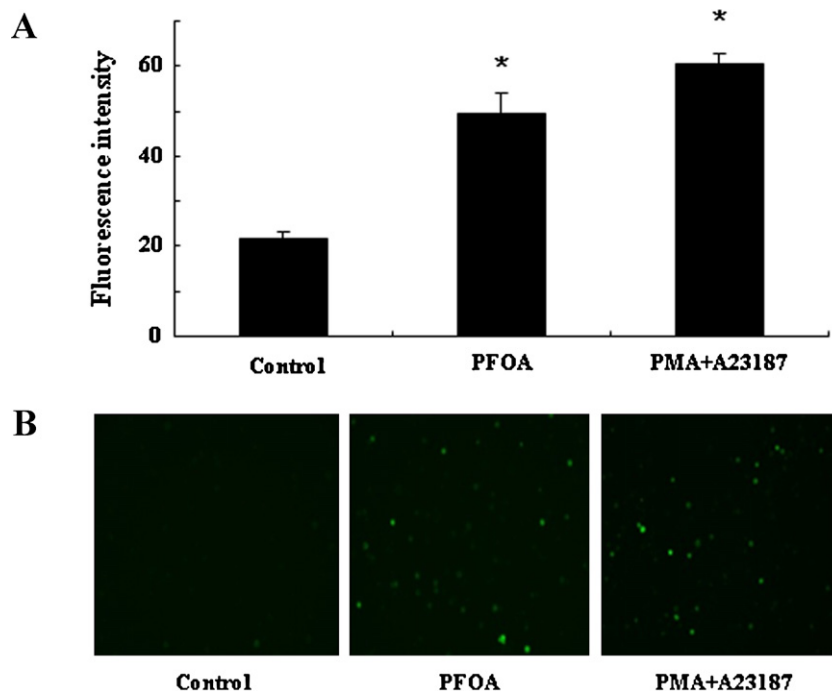


Fig. 2. Effect of PFOA on the intracellular calcium levels in mast cells. HMC-1 cells were treated with PFOA (200 μ M) for 10 min. PMA (40 nM) plus A23187 (1 μ M) were used as positive control. Intracellular calcium was measured by flow cytometer (A) and visualized by fluorescence microscope (B). Each bar represents the mean $\pm$ SEM of three independent experiments. *Significantly different from the control.

shown in [Supplementary Fig. S1](#). Female BALB/c mice were exposed on the dorsal surface of each ear with 25 μ l per ear of vehicle or PFOA (10 and 50 mg/kg, BW) for 4 days. PFOA was dissolved in ethanol. Animals were manually restrained until ethanol was evaporated. The mice were intradermally injected with 0.5 μ g of anti-dinitrophenyl (DNP) IgE on the dorsal skin of each ear on day 3. After 48 h, each mice received an injection of 1 μ g of DNP-human serum albumin (HSA) containing 4% Evans blue (1:4) via the tail vein. Thirty minutes after the challenge, the mice were killed. Spleen, thymus, liver and kidney were removed and weighed. The ears were cut off and the amount of dye in the pigmented area was determined colorimetrically after extraction with 1 ml of 1 M KOH and 9 ml of a mixture of acetone and phosphoric acid (5:13). The intensity of absorbance was measured at 620 nm in a spectrophotometer (UV-1201; Shimadzu, Kyoto, Japan).

2.5. Intracellular calcium

To determine intracellular calcium, Fluo-3/AM (Molecular Probes Inc., Eugene, OR) was used as previously described ([Bae et al., 2011](#)), a brief description of which follows. HMC-1 was pre-incubated with Fluo-3/AM for 30 min at 37°C. After washing the dye from the cell surface, PFOA was treated for 10 min. PMA (40 nM) plus A23187 (1 μ M) were treated as positive control. The fluorescent intensity was measured using flow cytometer (BD Biosciences, San Diego, CA) and visualized in fluorescence microscope (Olympus BX 51, Center Valley, PA).

2.6. Polymerase chain reaction (PCR)

HMC-1 cells (1×10^6 /well in 24-well plate) were treated with PFOA in different concentrations. PMA (40 nM) plus A23187 (1 μ M) were treated as positive control. The total cellular RNA was isolated using an RNAiso Plus (Takara Bio Inc., Shiga, Japan). The first strand complementary DNA (cDNA) was synthesized using RT Premix (iNtRON Biotech, Sungnam, Korea). The conditions for the reverse transcription steps were similar to those previously described ([Kim et al., 2007b](#)). The PCR products were separated on 1.8% agarose gel and visualized by staining with ethidium bromide. The gels were certificated using a Kodak DC 290 digital camera. Quantitative real-time PCR was also carried out to verify mRNA expression, using the Thermal Cycler Dice Real Time System (TP-800; Takara Bio Inc.) as previously described ([Bae et al., 2011](#)). Briefly, 2 μ l of cDNA (100 ng), 1 μ l each of sense and antisense primer solution (0.4 μ M), 12.5 μ l of SYBR Premix Ex Taq, and 9.5 μ l of dH₂O were mixed together to obtain a final 25 μ l reaction mixture in each reaction tube. The amplification conditions were 10 s at 95°C, 40 cycles of 5 s at 95°C and 30 s at 60°C, 15 s at 95°C, 30 s at 60°C, and 15 s at 95°C. Relative quantification of mRNA expression was performed using the TP850 software.

2.7. Nuclear protein extraction

After HMC-1 (3×10^6 in a 6-well plate) were treated with PFOA, cells were washed in 1 ml of ice-cold PBS, centrifuged at $1200 \times g$ for 5 min, resuspended in 400 μ l of ice-cold hypotonic buffer (10 mM HEPES/KOH, 2 mM MgCl₂, 0.1 mM EDTA, 10 mM KCl, 1 mM DTT, 0.5 mM PMSF, pH 7.9), left on ice for 10 min, vortexed, and centrifuged at $15,000 \times g$ for 30 s. After washing, pelleted nuclei were resuspended in 50 μ l of ice-cold saline buffer (50 mM HEPES/KOH, 50 mM KCl, 300 mM NaCl, 0.1 mM EDTA, 10% glycerol, 1 mM DTT, 0.5 mM PMSF, pH 7.9), left on ice for 20 min, vortexed, centrifuged at $15,000 \times g$ for 5 min at 4°C, and supernatant gathered.

2.8. Western blotting

Cell extracts were prepared by detergent lysis procedure as described elsewhere ([Kim and Sharma, 2004](#)). Samples of protein (50 μ g) were electrophoresed using 10% SDS-PAGE, and then transferred to nitrocellulose membrane. The nucleus and cytosolic p65 NF- κ B, I κ B α , and caspase-1 were assayed using anti-NF- κ B (p65), anti-I κ B α , and anti-caspase-1 antibodies (Santa Cruz Biotech., Santa Cruz, CA), respectively. Immunodetection was done using chemiluminescent substrate.

2.9. Statistical analysis

Data were analyzed with SPSS 17.0 program and differences were considered significant as $p < 0.05$. Statistical analysis of the data was performed by Turkey's HSD test to test the effect of different PFOA levels.

3. Results

3.1. Effect of PFOA on cell viability, histamine release, and local allergic reaction

A dose-dependent decrease in the cell viability of human mast cells (HMC-1) was observed following treatment of PFOA for 24 h ([Fig. 1A](#)). There was no significant reduction in cell viability up to 200 μ M of PFOA compared with the control. Therefore, 25–100 μ M of PFOA was chosen to determine the release of histamine from mast cells. PMA plus A23187 was used as a positive control for the histamine release. To check the effect of PFOA on histamine release in time-dependent manner, cells were treated with PFOA (100 μ M) for 12 h and 24 h ([Fig. 1B](#)). At 24 h of incubation, PFOA induced higher amount of histamine than 12 h. Next, cells were

treated with various concentrations of PFOA (25–100 μM) for 24 h (Fig. 1C). As a results, PFOA induced histamine release in a time- and concentration-dependent manner. To confirm the effects of PFOA on histamine release, we examined serum histamine in mice by the intraperitoneal injection of PFOA (Fig. 1D). The mast cell degranulator, compound 48/80, was used as a positive control for a systemic immediate hypersensitivity. After the intraperitoneal injection of PFOA (1 and 5 mg/kg, body weight) and compound 48/80 (8 mg/kg), the mice were monitored for 1 h. Administration of PFOA increased serum levels of histamine. These results suggest that PFOA could induce the mast cell-derived histamine release *in vitro* and *in vivo*.

To investigate the allergenic effect of PFOA *in vivo*, we induced PCA in the ears of mice. PCA is one of the most appropriate *in vivo* models of local allergic reaction (Kim et al., 2006). Local injection of anti-DNP IgE followed by an intravenous antigenic challenge induced local extravasation. After injection of 4% Evans blue mixed with antigen, the PCA reaction site showed blue color. Treatment of PFOA (10 and 50 mg/kg, BW) exacerbated IgE-dependent allergic reaction (Fig. 1E). After PCA reaction, the weight of major organs such as spleen, thymus, liver and kidney was measured. As expected, there was no change in the weight of spleen, thymus, and kidney. However the weight of liver was significantly increased in PFOA-treated groups (Supplementary Fig. S2). These results are consistent with our previous reports showing the liver-specific toxicity and immunomodulatory effect of PFOA in mice (Son et al., 2008, 2009).

3.2. Effect of PFOA on intracellular calcium

To investigate the mechanism of PFOA-induced histamine release, we assayed the intracellular calcium. Calcium movements across membranes of mast cells are critical to histamine release (Beaven et al., 1984; Kim et al., 2006). Fluo-3/AM was used for intracellular calcium assay. HMC-1 cells were treated with PFOA for 10 min, and the calcium influx was analyzed by flow cytometer (Fig. 2A) and visualized in fluorescence microscope (Fig. 2B). PFOA increased the intracellular level of calcium in HMC-1 cells.

3.3. Effect of PFOA on the expression of pro-inflammatory cytokines

We tested the effect of PFOA on the expression of pro-inflammatory cytokines such as TNF- α , IL-1 β , IL-6, and IL-8 in mast cells. We previously reported that gene expression of those cytokines was peaked at 4 h after treatment of PMA plus A23187 in HMC-1 (Park et al., 2007). However, in a time-dependent assay, gene expression of pro-inflammatory cytokines was peaked at 12 h by the PFOA treatment (data not shown). PMA plus A23187 was used as a positive control. The results showed that PFOA (50–400 μM) dose-dependently induced gene expression of all cytokines in HMC-1 at 12 h (Fig. 3A and B).

3.4. Effect of PFOA on the activation of p38, NF- κB , caspase-1, and COX-2

To find out the mechanism responsible for the induction of cytokines after PFOA stimulation, we investigated the effect of PFOA on MAPKs and NF- κB . Both transcription factors are known for the key regulator of pro-inflammatory cytokines (Kim et al., 2006). PMA plus A23187 was used as a positive control. When the HMC-1 cells were treated with PFOA (50–400 μM) for 1 h, phosphorylation of p38 is the only MAPK found to be activated by PFOA (Fig. 4A) leaving ERK and JNK (data not shown). In addition, stimulation of HMC-1 with PFOA induced translocation of p65

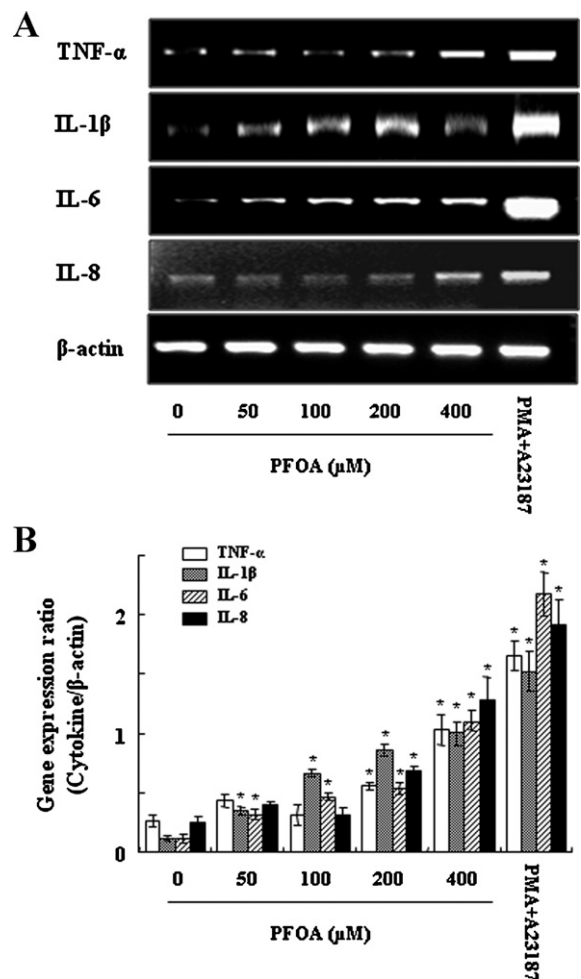


Fig. 3. Effect of PFOA on expression of pro-inflammatory cytokines in mast cells. HMC-1 cells were treated with PFOA (50–400 μM) for 12 h. PMA (40 nM) plus A23187 (1 μM) were used as positive control. The expression level of TNF- α , IL-1 β , IL-6, and IL-8 were determined by RT-PCR (A) and real-time PCR (B). Each bar represents the mean $\pm$ SEM of three independent experiments. *Significantly different from the control.

NF- κB to the nucleus following degradation of I $\kappa\text{B}\alpha$. PFOA induced degradation of I $\kappa\text{B}\alpha$ and translocation of p65 NF- κB (Fig. 4B).

Caspase-1 plays a key role in inflammatory responses by cleaving pro-IL-1 β into secreted pro-inflammatory cytokines (Bae et al., 2011). For the additional underlying mechanism responsible for the cytokine expression, we assayed activation of caspase-1 in mast cells. Caspase-1 cleaves precursor of IL-1 β to biologically active form. Active caspase-1 is derived from the cleavage of the 45 kDa pro-caspase-1. PFOA-treated mast cells showed a dose-dependent increase of active form of caspase-1 (Fig. 4C).

Among the pro-inflammatory mediators, COX-2 is responsible for the development of allergic inflammation of airways and skin (Kawata et al., 1995). To find out the effect of PFOA on allergic inflammatory mediators in mast cells, we investigated the effect of PFOA on COX-2 (Fig. 4D). PFOA showed a dose-dependent induction of COX-2, and this result suggests the induction of allergic inflammatory mediators by the PFOA.

4. Discussion

Mast cells are important in immediate-type allergy and play a pivotal role in initiating and maintaining a wide range of allergic inflammatory diseases. Anaphylaxis is a severe hypersensitivity induced by the mast cell degranulation and following the release

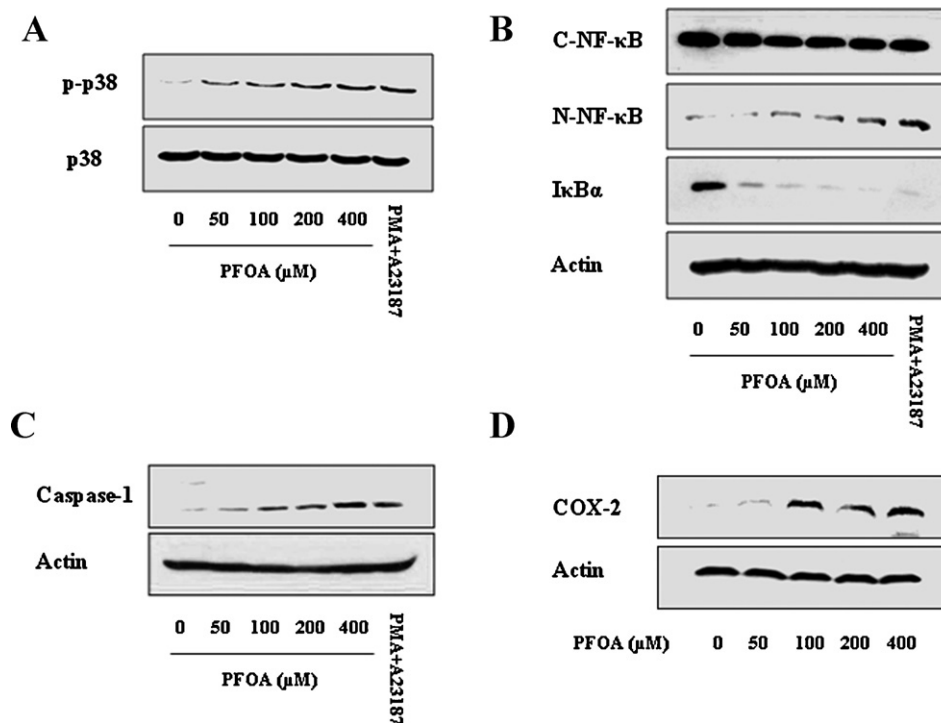


Fig. 4. Effect of PFOA on the activation of p38, NF-κB, caspase-1, and COX-2 in mast cells. Cells were treated with PFOA (50–400 μM) and the activation of each protein was assayed by Western blot. PMA (40 nM) plus A23187 (1 μM) were used as positive control. Actin was used as a loading control. (A) Phosphorylation of p38 was measured after 1 h of PFOA stimulation. (B) Nuclear translocation of NF-κB and degradation of IκBα were measured after 1 h of PFOA stimulation (C-NF-κB, cytoplasmic NF-κB; N-NF-κB, nucleus NF-κB). (C) Amount of caspase-1 was measured after 2 h of PFOA stimulation. (D) COX-2 was measured after 2 h of PFOA stimulation. The results are representative of three independent experiments.

of inflammatory mediators such as histamine, proteolytic enzymes and cytokines (Galli et al., 2005). We observed that PFOA induced mast cell-derived allergic reaction and serum histamine release, which is an index of mast cell degranulation. Mast cell degranulation is preceded by the calcium influx. Calcium leads the granule membrane fusion via the SNAP receptor protein and secretory carrier membrane protein finally granules are broken up (Castle et al., 2002). Our results showed that PFOA increased histamine dose- and time-dependently, and intracellular calcium in mast cells. These results suggest that PFOA induced the degranulation of mast cells by the increasing of intracellular calcium level.

HMC-1 is a useful cell for studying cytokine activation pathways (Kim et al., 2005; Moller et al., 1998). The spectrum of cytokines produced by HMC-1 supports the well recognized role of mast cells in immediate-type hypersensitivity. Pro-inflammatory cytokines, including TNF-α, IL-1β, IL-6, and IL-8, play a major role in triggering and sustaining the allergic inflammatory response in mast cells. TNF-α promotes inflammation, leukocyte infiltration, chemotaxis of neutrophils and T cells (Thomas, 2001). TNF-α is also involved in eosinophil survival, and thereby contributes to chronic inflammation (Levi-Schaffer et al., 1998). IL-1β induces expression of adhesion molecules on endothelium to enhance PMN transmigration (Lin et al., 2002). IL-6 is also produced from mast cells and its local accumulation is associated with anaphylaxis (Mican et al., 1992). These reports indicate that the induction of pro-inflammatory cytokines from mast cells by PFOA is one of the key indicators of inducing allergic inflammation. In the present study, PFOA induced the expression of TNF-α, IL-1β, IL-6 and IL-8 from mast cells. These results indicate that PFOA has allergic inflammatory effects by inducing pro-inflammatory cytokines from mast cells.

Intracellular calcium is crucially involved in gene transcription of inflammatory cytokines as well. Several reports have

demonstrated associations between intracellular calcium and inflammatory cytokine production from mast cells (Jeong et al., 2002; Tanaka et al., 2005). In our study, PFOA increased the level of intracellular calcium in mast cells. We suggest that the inducing effect of PFOA on the inflammatory cytokines results from the induction of intracellular calcium. Because of the inducing effect of PFOA on the intracellular calcium, we suggest that one possible pathway of the inducing effect of PFOA for the inflammatory cytokines is mediated by the induction of intracellular calcium in mast cells.

Among the pro-inflammatory cytokines, IL-1β is synthesized in cytoplasm as a 31 kDa molecule and is processed and released from cells by a mechanism involving an enzyme called IL-1β converting enzyme, known as caspase-1. Pro-IL-1β is biologically inactive and must be converted to the mature 17 kDa IL-1β to acquire the ability to bind to receptors and activate cells. Caspase-1 presents in a catalytically inactive 45 kDa proenzyme and is activated by cleavage into two non-identical subunits, p20 and p10. Active caspase-1 can also cleave other members of the IL-1 family, IL-18 and IL-33 (Thornberry et al., 1992). The administration of caspase-1 inhibitors attenuates the course of collagen-induced arthritis (Ku et al., 1996) and inhibits the pro-inflammatory responses of monocytes isolated from patients with familial cold auto-inflammatory syndrome (Stack et al., 2005). In our study, we observed that PFOA activated caspase-1 in mast cells. From that result we postulate that PFOA induced IL-1β via the regulation of caspase-1.

NF-κB is a transcription factor that induces the transcription of a variety of genes. Many of these genes encode molecules important in inflammatory processes, such as cytokines and adhesion molecules. The role of NF-κB and its regulation of cytokine production in allergic inflammation has been characterized (Kim et al., 2006). In our study, PFOA increased the nuclear translocation of NF-κB. These data demonstrate that PFOA induces mast cell-derived

allergic inflammation via the activation of NF- κ B and downstream pro-inflammatory cytokines. The MAPK cascade is also an important signaling pathway in immune responses and cross talks with NF- κ B to regulate inflammation signaling (Arbabi and Maier, 2002). The expression of pro-inflammatory cytokines is known to be regulated by all three types of MAPKs in mast cells (Kim et al., 2006). However, interestingly, PFOA induced the activation of p38 but not ERK and JNK. These data suggest that the inducing effect of PFOA on the pro-inflammatory cytokines is mediated by p38 and NF- κ B.

COX-2 is an inducible enzyme found at low concentrations in healthy tissues, but its expression becomes elevated in response to tissue damage during inflammation. Several studies have reported that PGD₂, the major COX-2 metabolite released from activated mast cells, stimulates the pathogenesis of eosinophilic airway inflammation (Bochenek et al., 2004). Among the pro-inflammatory mediators, COX-2 is responsible for the development of allergic inflammation of airways and skin and it catalyzes the synthesis of inflammatory prostaglandins, notably PGE₂. PGE₂ enhances mast cell degranulation and release of inflammatory mediators from mast cells (Stock et al., 2001). Our results showed that treatment with PFOA stimulates the expression of COX-2 in HMC-1 cells, indicating that PFOA stimulates mast cell-derived allergic inflammatory response via the enhancement of the COX-2 expressions.

The hydrolytic half-life of PFOA in the environment is estimated to be more than 97 years and the biological half-life in human is reported to be 4.37 years (Kudo and Kawashima, 2003). Median uptake of PFOA is 2–3 ng/kg/day with food being responsible for greater than 90% of this exposure (Fromme et al., 2009). It has been reported that dermal exposure of PFOA (25 mg/kg) for 4 days enhances airway hypersensitivity in mice (Fairley et al., 2007). In the present study, we used 1–50 mg/kg of PFOA for *in vivo* study. As a result, only 1 mg/kg of PFOA significantly elevated serum histamine level. Due to its long half-life and bioaccumulation, even low dose of PFOA exposure could be harmful to human.

Our previous reports showed that PFOA has an immunomodulatory effect by altering T lymphocyte phenotypes, induces inflammation by the increase of gene expression of pro-inflammatory cytokines, and provokes carcinogenesis resulting from chronic inflammation and subversion of immunity (Son et al., 2008, 2009). PFOA is known as a peroxisome proliferator and binds to peroxisome proliferators-activated receptor α (PPAR α) (Kennedy et al., 2004). On the other hand, the role of PPAR γ in PFOA-induced immunomodulation is not well known. PPAR γ acts as a transcription factor for genes containing PPAR response elements in their promoters, including COX-2 (Pawliczak et al., 2004; Pontsler et al., 2002). PPAR γ is one of master regulators on mast cells and plays a key role in allergic inflammation. Activation of PPAR γ induces PGD₂ in human mast cells (Paruchuri et al., 2008). Our findings suggest the involvement of PPAR γ in PFOA-induced mast cell-mediated allergic inflammation. Further study for the role of PPAR γ in PFOA-induced allergic inflammation is ongoing in our group using the proteomics and genomics technology.

5. Conclusion

In this study, for the first time, we carefully evaluated the role of PFOA on the mast cell-derived allergic inflammation, and defined underlying mechanisms of action. We showed that PFOA induced histamine release via the intracellular calcium and inflammatory cytokines through p38, NF- κ B, and caspase-1. We provide evidence that PFOA could induce the mast cell-mediated allergic inflammatory reactions by histamine release, pro-inflammatory cytokine expression. Due to the function of PPAR γ as a target of PFOA and master regulator of allergic inflammation, more detailed studies

regarding the role of PPAR γ on the PFOA-induced allergic inflammation will be needed.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.toxlet.2012.01.014.

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