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Correlation between mast cell-mediated allergic inflammation and length of perfluorinated compounds

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ABSTRACT

Perfluorinated compounds (PFC) have widely been used in numerous applications including clothing, food packaging, and nonstick coating. With the widespread use of PFC, concerns regarding potential adverse health effects in humans and wildlife have increased. In spite of the known PFC-mediated immunotoxicity, correlation with PFC and allergic inflammation still requires elucidation. The aim of this study was to examine the effect of four types of PFC (perfluoroheptanoic acid [PFHpA], perfluorononanoic acid [PFNA], perfluorodecanoic acid [PFDA], and perfluoroundecanoic acid [PFUnA]) on mast cell-mediated allergic inflammation in the presence of high-affinity immunoglobulin (Ig) E receptor (FcεRI) cross-linking. Among PFC family, long-chain PFDA and PFUnA increased release of histamine and β-hexosaminidase by up-regulation of intracellular calcium levels in IgE-stimulated mast cells. In addition, PFDA and PFUnA enhanced gene expression of pro-inflammatory cytokines, such as tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6, and IL-8 by activation of nuclear factor-κB in IgE-stimulated mast cells. In ovalbumin (OVA)-induced model of systemic anaphylaxis in the presence of hypothermia, PFNA, PFDA, and PFUnA exacerbated allergic symptoms accompanied by elevation in serum histamine, TNF-α, IgE, and IgG₁. Our data indicate that some PFC aggravated high-affinity IgE receptor (FcεRI)-mediated mast cell degranulation and allergic symptoms. Consequently, the results demonstrated that carbon-chain length of PFC may serve as a factor in allergic inflammation.

Introduction

Allergic inflammation is an abnormal reaction of the body evidenced as over-reaction of the immune system (Hainsworth 2003). Mast cells are known to play a key role in allergic inflammation, and release various inflammatory mediators, including histamine, cytokines, chemokines, and proteases (Amin 2012). Histamine is released by the degranulation of mast cells and is one of the predominant mediators leading to immediate-type hypersensitivity (Amin 2012). Mast cell degranulation is induced by calcium (Ca) mobilization through granule membrane fusion (Nishida et al. 2005). Subsequently, this induces activation of transcription factor nuclear factor (NF)-κB and release of inflammatory cytokines and chemokines (Liu et al. 2017). NF-κB regulates expression of pro-inflammatory cytokines and chemokines known to produce increased vascular permeability, inflammation, and tissue remodeling (Kempe et al. 2005).

Pro-inflammatory cytokines play an essential role in regulation of intracellular communications in

immune response (Lacy and Stow 2011). Degranulation of mast cells is induced by binding of Ig E to high-affinity IgE receptor (FcεRI) on the surface of mast cells (Rivera and Gilfillan 2006). FcεRI is necessary to induce for IgE-mediated allergic inflammation (Galli and Tsai 2012). Cross-linking of FcεRI generates release of several inflammatory factors, which produce allergic inflammation (He et al. 2013). Previously, human mast cell line (HMC-1) stimulated by phorbol 12-myristate 13-acetate and Ca ionophore were used to study activation of mast cells (Lee et al. 2017; Singh et al. 2012). However, HMC-1 are immature and do not express FcεRI (Laidlaw et al. 2011). Daron et al. (1995) examined IgE-FcεRI binding in degranulation of mast cells using rat basophilic leukemia cell line (RBL-2H3). Thus in the present study RBL-2H3 cells expressing FcεRI binding with IgE were employed to determine activation of mast cells.

Perfluorinated compounds (PFC) are a class of chemicals containing only carbon-fluorine bonds and carbon-carbon bonds. PFC has been used in

numerous industrial and home products, such as carpets, clothing, food packaging, and nonstick coatings (Corsini et al. 2014). As PFC is extremely stable and slowly degraded, these chemicals were detected in environment and may lead to potential adverse health effects in humans and wildlife (Lei et al. 2015). Previous investigators demonstrated that PFC produce immunotoxicity, hepatotoxicity, developmental toxicity, and metabolic abnormalities (Berntsen et al. 2017; Grandjean and Budtz-Jørgensen 2013; Zhou et al. 2016). Among PFC family, perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) have been widely used in large quantities in a number of applications including surface coatings and water- and oil resistance since the 1950s (Wu et al. 2015). However, the use of PFOA and PFOS has been reduced in the last years. As a consequence of indiscriminate use and potential adverse effects, PFOA and PFOS were substituted with other PFC, such as perfluoroheptanoic acid (PFHpA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), and perfluoroundecanoic acid (PFUnA). Consequently, human levels of the latter compounds have been found to be increasing (Lindh et al. 2012).

Previously, Lee et al. (2017) noted the effects of PFOA on mast cell-mediated allergic inflammation. Data demonstrated that allergic inflammation was exacerbated by exposure of PFOA by mechanisms involving increased histamine release (Lee et al. 2017). As the influence of other PFC currently in use on allergic inflammation are not known, this study aimed to investigate the relationship between PFHpA, PFNA, PFDA, and PFUnA and mechanisms underlying allergic inflammation. Therefore, effects of these PFC on degranulation of RBL-2H3 cells in the presence of FcεRI cross-linking were determined.

Materials and methods

Reagents and cell culture

o-Phthaldialdehyde, Ca ionophore A23187, dimethyl sulfoxide (DMSO), anti-dinitrophenyl (DNP) IgE, DNP-human serum albumin (HSA), ovalbumin (OVA), PFHpA, PFNA, PFDA, and PFUnA were purchased from Sigma (St. Louis, MO). Alum adjuvant was purchased from Thermo Scientific

(Waltham, MA). For *in vitro* and *in vivo* experiments, PFHpA, PFNA, PFDA, and PFUnA were dissolved in DMSO to make a 1 M solution and then diluted with Dulbecco's Modified Eagle's Medium (DMEM; GIBCO, Grand Island, NY) or phosphate-buffered saline (PBS, pH 7.4). Rat basophilic leukemia cells (RBL-2H3) were grown in 5% CO₂ at 37°C in DMEM supplemented with 100 U penicillin/ml, 100 µg streptomycin/ml, and heat-inactivated 10% fetal bovine serum (all from GIBCO).

Animals

Six-week-old male ICR mice weighing 27–32 g were purchased from Dae-Han Biolink (Daejeon, Korea), and housed in a laminar airflow room that was maintained at 22 ± 2°C and with 55 ± 5% relative humidity and 12 h light:dark cycle throughout the study. All mice had *ad libitum* access to standard rodent chow and filtered water during the study. All care and treatment of mice were performed in accordance with guidelines established by the Public Health Service Policy on the Humane Care and Use of Laboratory Animals. The study protocol was approved by the Institutional Animal Care and Use Committee of Kyungpook National University.

Cell viability

RBL-2H3 cells were seeded in 96-well plates at a density of 2×10^4 cells/well (100 µl/well) and allowed to rest overnight to attach to the plates prior to experimentation. Cells were then treated with PFC for 24 h. Cell viability was evaluated using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (Yang et al. 2016). After incubation with PFC, 20 µl MTT solution (5 mg/ml in PBS [pH 7.4]) was added to each well, and plates incubated at 37°C for 2 h. After the supernatant was removed from the plate, formazan cellular by-product was dissolved in 100 µl DMSO added to each well, and absorbance measured at 570 nm using a VersaMax automatic ELISA Microplate Reader (Molecular Devices, Sunnyvale, CA). The absorbance of the untreated control wells was set as 100% viability for all comparisons thereafter.

Determination of mast cell degranulation

To determine mast cell degranulation, levels of histamine in serum and culture media were measured. After mouse blood was clotted at room temperature, it was centrifuged at 400 g for 15 min at 4°C, and serum isolated. RBL-2H3 cells (5×10^5 cells/well [500 µl/well] in 12-well plates) were sensitized by treatment with anti-DNP IgE (50 ng/ml). After overnight incubation, cells were pre-treated with PFC for 30 min and then challenged with DNP-HSA (1 ng/ml) for 4 h. To separate histamine from serum and media, 0.1 N HCl and 60% perchloric acid were added. After centrifugation, the supernatant was transferred to a tube containing 5 N NaOH, 5 M NaCl, and *n*-butanol, and vortexed. The organic phase was removed, mixed thoroughly with 0.1 N HCl and *n*-heptane, and centrifuged. Histamine in the aqueous phase was assayed using *o*-phthalaldehyde spectrofluorometric procedure as previously described by Kim, Bae, and Kim (2013). Fluorescent intensity was detected using a fluorescence plate reader (Molecular Devices) at excitation wavelength of 360 nm and emission wavelength of 440 nm.

β-Hexosaminidase release is also widely used as a marker for mast cell degranulation (Fukuishi et al. 2014). Cell-free culture medium taken from each well (40 µl) was combined with 40 µl β-hexosaminidase substrate buffer (100 mM sodium citrate, 1 mM 4-nitrophenyl *N*-acetyl-β-D-galactosamine [pH 4.5]) and incubated for 1 h at 37°C. The reaction was terminated by adding 200 µl stop solution (0.1 M Na₂CO₃ and NaHCO₃) to each well, and absorbance of recovered supernatant measured in the automated microplate reader at 405 nm (Molecular Devices).

Active systemic anaphylaxis (ASA)

Mice ($n = 5$ /group, total five groups) were sensitized with OVA (500 µg/ml OVA and 10 mg/ml alum adjuvant in 200 µl PBS) by intraperitoneal (ip) injection on Day 0 and Day 7. PFC was orally administered three times (on Days 9, 11, and 13 of experiment). A dosage of 100 mg/kg in a volume of 100 µl was selected for use. On Day 14, 200 µg OVA were ip-injected, and then rectal temperature measured every 10 min for 70 min using a rectal thermometer (Testo, Alton, UK). After 70 min,

each mouse was euthanized with CO₂ inhalation, and blood obtained from the abdominal artery to permit subsequent measures of serum histamine, TNF-α, IgE, and IgG₁.

Intracellular calcium (Ca)

Intracellular Ca was measured using a Fluo-3/AM fluorescence indicator (Invitrogen, Carlsbad, CA) (Kim et al. 2014). In brief, RBL-2H3 cells (2×10^4 cells/well in 96-well plate) were sensitized with anti-DNP IgE (50 ng/ml). After an overnight incubation, cells were incubated with Fluo-3/AM for 1 h at 37°C and then washed with Tyrode's buffer B (137 mM NaCl, 5.5 mM glucose, 12 mM NaHCO₃, 2.7 mM KCl, 0.2 mM NaH₂PO₄, 1 mM MgCl₂, 1.8 mM CaCl₂) to remove the dye from the cell surface. Cells were then treated with PFC for 30 min and challenged with DNP-HSA (1 ng/ml). Fluorescent intensity was then measured in a fluorescent plate at 485 nm excitation wavelength of and 510 nm emission wavelength. Intracellular Ca levels in untreated control cells were calculated and designated as "1.0 relative absorbance". In these studies, Ca ionophore A23187 (1 µM) was used as a positive control.

Quantitative real-time polymerase chain reaction (qPCR)

Prior to isolation of total cellular RNA, RBL-2H3 cells (5×10^5 cells/well in 12-well plates) were sensitized with anti-DNP IgE (50 ng/ml). After overnight incubation, cells were treated with PFC for 30 min and challenged with DNP-HSA (1 ng/ml) for 1–2 h. RNAiso Plus reagent (Takara, Shiga, Japan) was then employed to extract total RNA according to manufacturer's protocol. After confirming purity/concentration of the isolated total RNA at 260/280 nm in a UV-Vis spectrophotometer (Thermo Scientific, Waltham, MA), complementary DNA (cDNA) was synthesized from 2 µg total RNA using a Maxime RT-Pre Mix Kit (iNtRON Biotechnology, Daejeon, Korea). qPCR was carried out using a Thermal Cycler Dice TP850 (Takara), according to manufacturer protocols. For qPCR, the total reaction mixture (25 µl) contained 1.5 µl cDNA (150 ng), 1 µl each of the forward and reverse primers (0.4 µM), 12.5 µl of SYBR Premix Ex Taq (Takara), and 9 µl of dH₂O.

The conditions for the PCR were similar to those previously described (Yang et al. 2017).

Enzyme-linked immunosorbent assay (ELISA)

Levels of TNF- α , IgE, and IgG₁ in RBL-2H3 cells and serum were measured by ELISA (Anlar et al. 2017). After mouse blood was clotted at room temperature, it was centrifuged at 400 g for 15 min at 4°C, and serum then obtained. RBL-2H3 cells (5×10^5 cells/well in 12-well plates) were sensitized with anti-DNP IgE (50 ng/ml). After overnight incubation, cells were treated with PFC for 30 min and challenged with DNP-HSA (1 ng/ml) for 6 hr. Each ELISA was performed in a 96-well Nunc Immuno plate using an ELISA kit (BD Biosciences, San Diego, CA) according to manufacturer's protocols.

Transient transfection and luciferase activity

NF- κ B luciferase reporter gene constructs (pNF- κ B-LUC, plasmid containing NF- κ B binding site; Stratagene, La Jolla, CA) were transiently transfected into cells with FBS and antibiotics free medium containing 8 μ l Lipofectamine 2000 reagent (Invitrogen) as previously described (Bae, Lee, and Kim 2011). After 6 h of incubation, medium was replaced with 10% FBS and antibiotic containing medium. Cells were allowed to recover for 18 hr and stimulated with DNP-HSA at 37°C for 2 h. PFC was pre-added to cells for 30 min prior to

stimulation. Cells were harvested and washed in ice-cold PBS before lysis in 100 μ l cell lysis buffer (Luciferase assay kit; Promega, Madison, WI), according to the manufacturer's protocol. Luciferase activity was determined using the luminometer.

Statistical analysis

All data presented in graphical format are means $\pm$ SD of independent experiments. Statistical analyses were performed using Prism5 software (GraphPad, San Diego, CA). Treatment effects were analyzed using a one-way analysis of variance (ANOVA) followed by a Dunnett's test. A p -value < 0.05 was accepted as indicative of statistical significance.

Results

Effects of PFHpA, PFNA, PFDA and PFUnA on cell viability, histamine release, and intracellular calcium in IgE-stimulated mast cells

Chemical structures of PFC are shown in Figure 1. Cytotoxicity was analyzed using the MTT assay following chemical exposure for 24 h. RBL-2H3 cells were exposed to PFC at concentrations ranging from 10 to 5,000 μ M. As illustrated in Figure 2(A), PFHpA and PFNA did not induce cytotoxicity up to 500 μ M. However, PFDA and PFUnA decreased cell viability after exposure to

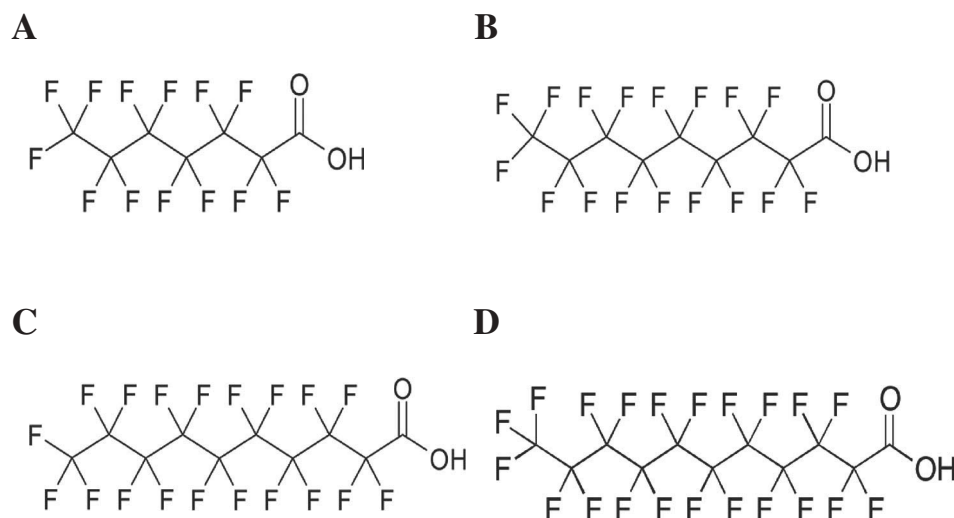


Figure 1. Chemical structure of PFC. (A) Perfluoroheptanoic acid, PFHpA. (B) Perfluorononanoic acid, PFNA. (C) Perfluorodecanoic acid, PFDA. (D) Perfluoroundecanoic acid, PFUnA.

500 μ M. In this study, 100 μ M PFC was selected and the effects of various PFC compared.

Histamine released by mast cells is a marker for degranulation of mast cells and is predominant mediator in allergic inflammation (Amin 2012). Thus the influence of PFC on histamine release from IgE-stimulated RBL-2H3 cells was examined. After sensitization with anti-DNP IgE, significantly higher histamine release levels were observed in PFDA and PFUnA-exposed groups compared with DNP-HSA-challenged groups. However, there was no significant change in histamine release in PFHpA and PFNA-treated groups compared with DNP-HSA-challenged groups (Figure 2(B)).

Levels of β -hexosaminidase are also widely utilized to demonstrate degranulation of mast cells (Qin et al. 2016). To confirm PFC exerted an effect on mast cells degranulation, levels of β -hexosaminidase in IgE-stimulated RBL-2H3 cells (4 h) were determined. While PFHpA and PFNA did not markedly affect degranulation of mast cells, PFDA and PFUnA elevated levels of β -hexosaminidase in sensitized RBL-2H3 cells compared with the DNP-HSA-challenged group (Figure 2(C)). Data suggest that PFDA and PFUnA exacerbated mast cell-derived histamine and β -hexosaminidase release from IgE-stimulated RBL-2H3 cells. However, there was no marked effect in degranulation of mast cells following PFHpA and PFNA incubation.

Ca influx of mast cells is a key factor for degranulation and release of histamine (Metcalf, Peavy, and Gilfillan 2009). To determine the influence of PFC on Ca influx, intracellular Ca levels were measured. In IgE-sensitized RBL-2H3 cells, PFDA and PFUnA increase intracellular Ca concentrations (Figure 2(D)). The levels of PFHpA and PFNA were not markedly altered.

Influence of PFHpA, PFNA, PFDA, and PFUnA on expression of pro-inflammatory cytokine and activation of NF- κ B in IgE-stimulated mast cells

Pro-inflammatory cytokines are important mediators of allergic inflammation, which act by enhancing recruitment and activation of immune cells (Angrish et al. 2016). The effect of PFC on expression of pro-inflammatory cytokines, such as TNF- α , IL-1 β , IL-6, and IL-8 in IgE-stimulated mast

cells, was assessed. While there was no marked change in expression of pro-inflammatory cytokines in PFHpA and PFNA-treated groups, PFDA and PFUnA significantly increased pro-inflammatory cytokine levels in IgE-stimulated RBL-2H3 cells (Figure 3(A)). In non-sensitized RBL-2H3 cells, all compounds did not significantly affect IL-1 β levels. The influence of PFC on secretion of TNF- α from IgE-stimulated RBL-2H3 cells was also confirmed by ELISA. PFDA and PFUnA enhanced secretion of TNF- α . However, PFHpA and PFNA did not markedly alter secretion of TNF- α (Figure 3(B)). Based upon these results, PFDA and PFUnA significantly increased expression and secretion of pro-inflammatory cytokines at both mRNA and protein levels. In contrast, treatment with PFHpA and PFNA failed to exert a marked response.

NF- κ B is known play a major role as a transcription factor regulating the pro-inflammatory response, cytokines expression, and adaptive and innate immune responses (Tak and Firestein 2001). In this study, the effect of PFC was investigated on NF- κ B activation to elucidate the signaling pathway for chemical-induced cytokine expression in IgE-stimulated RBL-2H3 cells. The result showed that PFDA and PFUnA significantly elevated luciferase activity in stimulated RBL-2H3 cells transfected with NF- κ B-luciferase reporter construct (Figure 3(C)). In contrast, treatment with PFHpA and PFNA did not produce a marked response.

Influence of PFHpA, PFNA, PFDA, and PFUnA on ovalbumin-induced active systemic anaphylaxis

The OVA-induced ASA model is employed widely and relevant for determination of mast cell functions and allergic inflammation (Ribeiro-Filho et al. 2014). The rectal temperature of mice in groups exposed to PFNA, PFDA, and PFUnA was significantly lower than in OVA-induced ASA mice at 70 min duration (Figure 4(A)). Animals in groups administered PFHpA did not markedly affect rectal temperature. Figure 4(B) shows the rectal temperature of mice at 50 min where in groups exposed to PFNA, PFDA, PFUnA exacerbation of hypothermia was noted.

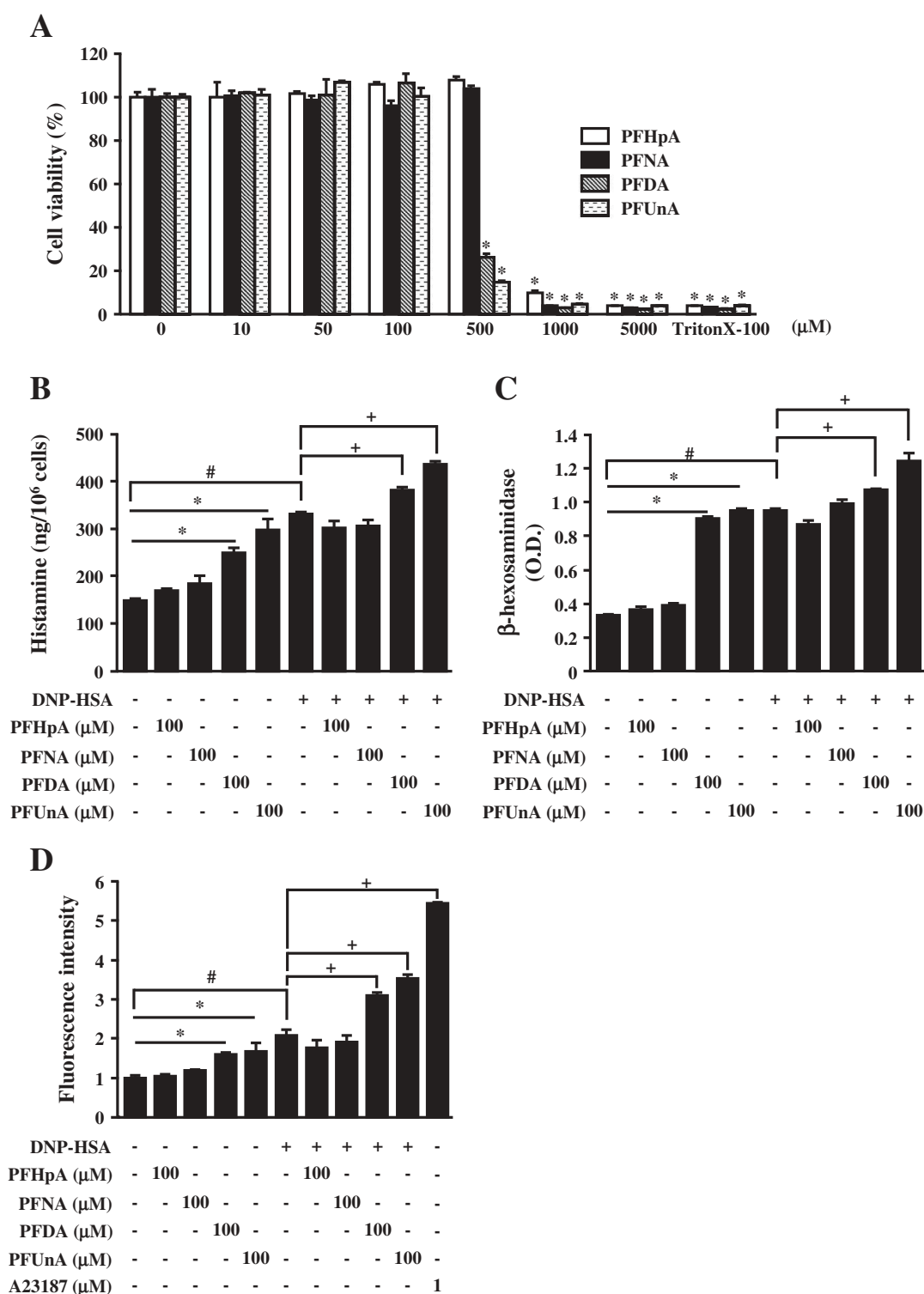


Figure 2. Effect of PFC exposure on cell viability, release of histamine and β -hexosaminidase, and intracellular calcium in RBL-2H3 cells. (A) Viability of RBL-2H3 cells after exposure to various concentrations of PFCs (10–5000 μ M) for 24 h. (B, C) Cells were sensitized with anti-DNP IgE (50 ng/ml). After overnight incubation, cells were treated with PFCs for 30 min and then challenged with DNP-HSA (1 ng/ml). (D) Cells were sensitized with anti-DNP IgE (50 ng/ml). After overnight incubation, cells were incubated with Fluo-3/AM for 1 h. Cells were treated with PFCs for 30 min and challenged with DNP-HSA (1 ng/ml). Data shown are means $\pm$ SD of three independent experiments. *Significantly different from the non-sensitized group ($p < 0.05$). #Significantly different from non-sensitized and IgE-sensitized mast cell groups ($p < 0.05$). +Significantly different from DNP-HSA-challenged and PFCs-treated group ($p < 0.05$).

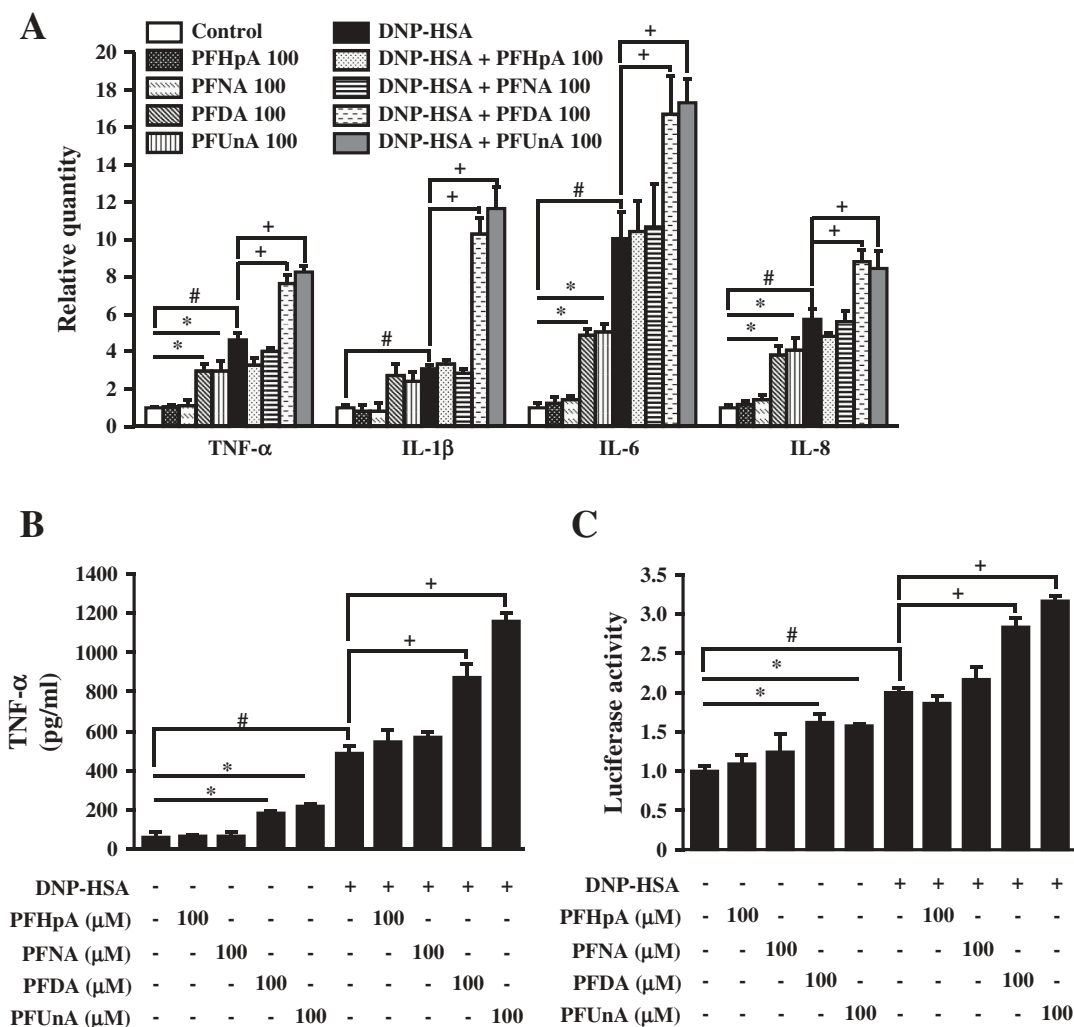


Figure 3. Influence of PFC exposure on expression and secretion of cytokines, and activation of NF- κ B in IgE-stimulated RBL-2H3 cells. Cells were sensitized with anti-DNP IgE (50 ng/ml). After overnight incubation, cells were treated with PFCs for 30 min and then challenged with DNP-HSA (1 ng/ml). Gene expression of pro-inflammatory cytokines was determined by (A) quantitative real-time PCR. (B) Secretion of TNF- α was measured by ELISA. Cells were sensitized with anti-DNP IgE (50 ng/ml). After overnight incubation, cells were treated with PFC for 30 min and then challenged with DNP-HSA (1 ng/ml). (C) Cells were transiently transfected with the NF- κ B-luciferase reporter construct gene. Then, NF- κ B dependent transcriptional activity was determined by luciferase activity assay. Data shown are means $\pm$ SD of three independent experiments. *Significantly different from non-sensitized mast cells ($p < 0.05$). #Significantly different from non-sensitized and IgE-sensitized mast cells ($p < 0.05$). +Significantly different from the DNP-HSA challenged and PFCs-treated group ($p < 0.05$).

Systemic anaphylaxis is initiated by IgE-dependent mast cell activation. IgE-dependent mast cells activation released allergic mediators including histamine and TNF- α (Finkelman et al. 2005). PFNA, PFDA, and PFUnA all significantly increased serum histamine and TNF- α levels compared with OVA-induced ASA mice (Figures 5(A) and 5(B)). PFHpA did not markedly affect release of histamine and TNF- α . Binding of IgE/IgG₁ with Fc ϵ RI/Fc γ R is a necessary step for activation of mast cells and consequent allergic responses especially in anaphylaxis (Finkelman et al. 2005). PFDA and PFUnA

were found to significantly elevate serum IgE and IgG₁ levels (Figures 5(C) and 5(D)). In contrast, treatment with PFHpA and PFNA did not produce a marked response.

Discussion

Histamine is an important mediator leading to immediate-type hypersensitivity and allergic inflammation by recruiting major effectors to the area of allergen exposure (Jutel, Blaser, and Akdis 2006). Histamine and β -hexosaminidase are markers for

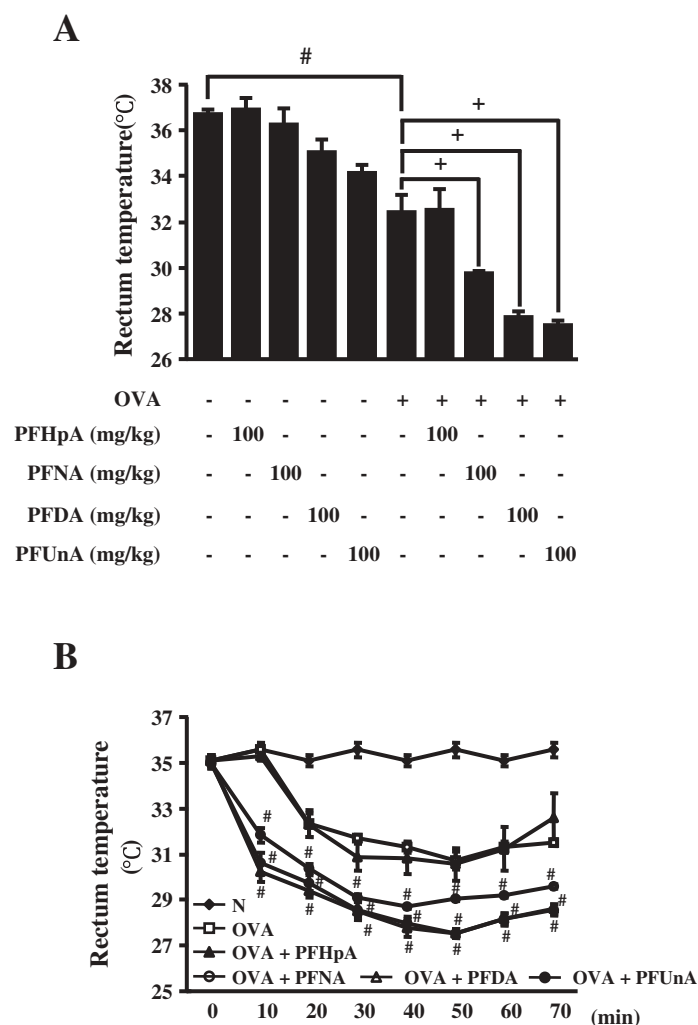


Figure 4. Effect of oral exposure of PFC on hypothermia (rectal temperature) in OVA-induced active systemic anaphylaxis. (A) Rectal temperature was measured every 10 min for 70 min. (B) Rectal temperature at 50 min. Data shown are means $\pm$ SD of three independent experiments. *Significantly different from non-sensitized mast cells ($p < 0.05$). #Significantly different from non-sensitized and IgE-sensitized mast cells ($p < 0.05$). +Significantly different from the DNP-HSA challenged and PFCs-treated group ($p < 0.05$).

the degree of mast cell degranulation (Fukuishi et al. 2014). Crosslinking of Fc ϵ RI on mast cells induces intracellular events, such as increase of intracellular Ca and activation of transcription factors (Filho et al. 2016). Ca mobilization is an essential and sufficient stimulus to induce mast cell degranulation (Law et al. 2011). Our findings demonstrated elevated levels of histamine, β -hexosaminidase, and intracellular Ca for mast cell-mediated allergic inflammation. In the present study, exposure to PFDA and PFUnA increased all three levels, but exposure of PFHpA and PFNA did not markedly affect these concentrations in IgE-stimulated mast cells. Production of pro-inflammatory cytokines from mast cells is also involved in allergic inflammation

and immediate-type hypersensitivity (Amin 2012). In IgE-stimulated mast cells, treatment with PFDA and PFUnA enhanced the expression of TNF- α , IL-1 β , IL-6, and IL-8. In contrast, there was no significant change following administration of PFHpA and PFNA. NF- κ B is a transcription factor regulating pro-inflammatory cytokines on mast cells (Turner et al. 2014). Evidence thus indicates that PFDA and PFUnA produced mast cell-derived allergic inflammation via activation of NF- κ B and downstream elevated pro-inflammatory cytokines in IgE-stimulated mast cells. Taken together, these results suggest that the number of carbon atoms in PFC was a factor in consequent mast cell-mediated allergic inflammation.

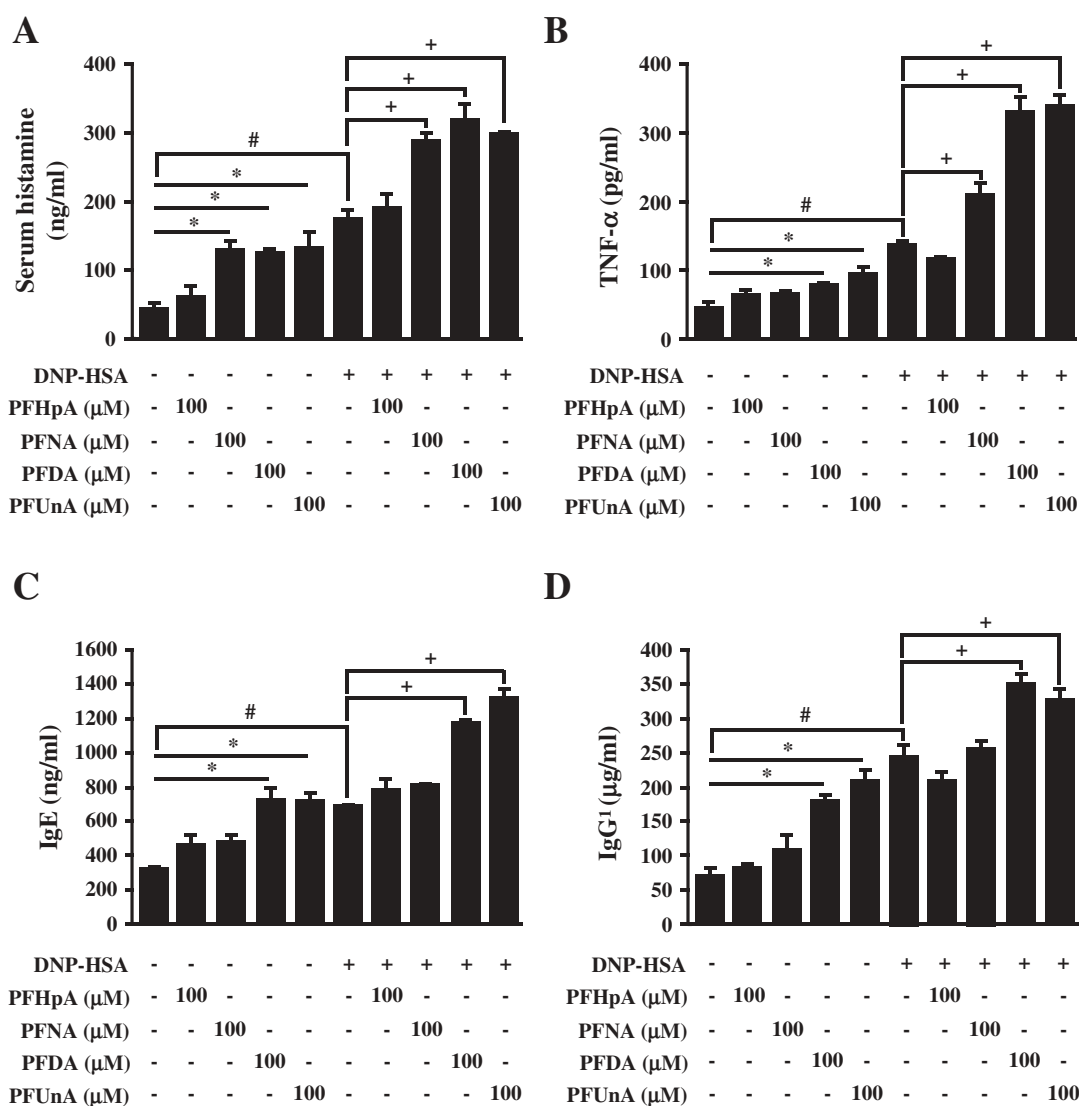


Figure 5. Influence of oral administration of PFC on cytokine levels in OVA-induced active systemic anaphylaxis. Blood was obtained from abdominal artery of each mouse and serum isolated. (A) Histamine levels assayed using *o*-phthaldialdehyde spectrofluorometric procedure. (B–D) Levels of TNF-α, IgE, and IgG₁ measured by ELISA. Data shown are means ± SD of three independent experiments. *Significantly different from non-induced group, #from non-induced and OVA-induced groups, and +from OVA-induced and PFCs-treated group (all $p < 0.05$).

Anaphylaxis is an IgE-mediated hypersensitivity induced by rapid release of allergic mediators including histamine and cytokines from mast cells. Histamine produces vasodilation and hypotension (Galli, Tsai, and Piliponsky 2008; Je et al. 2015). While IgE-dependent passive cutaneous anaphylaxis (PCA) was used to investigate the factors involved in mast cells responses, the PCA model has a shortcoming in that it is not spontaneous (Wex et al. 2014). ASA is closely related to natural allergic inflammation (Osterfeld et al. 2010). In prior studies, OVA-induced ASA was employed to

evaluate animal models for immediate-type hypersensitivity (Evans, Killoran, and Mitre 2014; Je et al. 2015). Because of a sudden rise in serum histamine levels, ASA induces a hypovolemic hypotension that subsequently led to hypothermia. Binding IgE and IgG₁ with FcεRI is an important step in anaphylactic symptoms (Ishikawa et al. 2010). Based on the results, it is postulated that all PFC except PFHpA deteriorated systemic anaphylaxis via increasing the release of histamine from IgE-stimulated mast cells, as well as enhanced production of IgE, IgG₁ and pro-inflammatory cytokines. It should be noted that

there were no significant alterations in rectal temperature in mice exposed only to PFC, compared with non-ASA. The possible reason account for the no change of rectal temperature in only PFC may come from the experimental procedure of ASA. In our experiment, PFC was administered until day 13. On day 14, mice were challenged with OVA and rectal temperature was measured. As mice are a homeothermic animal maintaining a stable body temperature (Devey, Festing, and Wigmore 2008), mice could recover from the altered body temperature. Thus, in this case, treatment with all PFC without OVA did not markedly affect body temperature change.

Chemical properties of PFC depend predominantly on the length of carbon chain (Cai et al. 2017). The chemical structure of PFC is a carbon-chain (4–16) surrounded by fluorine atoms. PFC with 8 or greater carbon atoms, including PFOA, PFOS, PFNA, PFDA, and PFUnA, are long-chain PFC (ATSDR 2015). Long-chain PFC (compounds with ≥ 8 carbon-fluorine bonds) break down slowly in the environment are characterized as persistent and bioaccumulate in animals (Stein and Savitz 2011). As the most common exposure route for PFC (>90%) is ingestion of agent-contaminated food and water (Kudo and Kawashima 2003), the present study utilized oral route exposure to determine if PFC augmented mast cell-mediated allergic inflammation with IgE activation. Treatment with PFNA, PFDA, and PFUnA exacerbated IgE-mediated allergic inflammatory symptoms. As PFCs, such as PFOA and PFOS are predominantly found in serum, serum chemical levels may be used as measures of body burden (Kudo and Kawashima 2003). At present there are no apparent data for serum levels of PFHpA, PFNA, PFDA, and PFUnA in mice. However, these PFCs were detected at 184 $\mu\text{g/ml}$ (PFHpA) and 185 $\mu\text{g/ml}$ (PFNA, PFDA) in drinking water (Ericson et al. 2009). In addition, D'Hollander et al. (2010) reported serum levels of PFHpA, PFNA, PFDA, and PFUnA in human at concentrations of 2.85, 2.08, 1.14, and 0.94 ng/ml, respectively.

This study used high doses in mice beyond levels recommended for humans. However, PFC has a potential to bioaccumulate/-bioconcentrate and levels are steadily increasing in environmental (Bartell et al. 2010). In addition, daily intake of PFC may be enhanced through various pathways, such as diet, drinking water, air, and dust (Kennedy et al. 2004).

Therefore, it is possible that humans are likely exposed to elevated PFC levels through several routes, such as contaminated foods and water (D'Hollander et al. 2010).

In conclusion, data demonstrated that long-carbon chain PFDA and PFUnA increased release of histamine via elevating intracellular Ca levels and expression of pro-inflammatory cytokines through activation of NF- κ B. In addition, PFNA, PFDA, and PFUnA but not PFHpA aggravated allergic reactions in the OVA-induced ASA model. Results demonstrated that PFC augmented mast cell-mediated allergic inflammation based upon compound chain length of carbon atoms number in PFC. Therefore, evidence suggests that individuals with allergic disease need to avoid PFC exposure especially to long-carbon chain compounds.

Declaration of interest

The authors declare that they have no competing interests.

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