



Anti-allergic inflammatory activity of the fruit of *Prunus persica*: Role of calcium and NF- κ B

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ABSTRACT

Mast cell-mediated allergic symptoms are involved in many diseases, such as asthma and sinusitis. In this study, we investigated the effect of ethanol extract of fruits of *Prunus persica* (L.) Batsch (FPP) on the mast cell-mediated allergic inflammation and studied the possible mechanism of action. FPP dose-dependently inhibited compound 48/80-induced systemic anaphylaxis and immunoglobulin E-mediated local allergic reactions. Histamine releasing from mast cells was reduced by FPP, which was mediated by modulation of intracellular calcium. In addition, FPP attenuated the phorbol 12-myristate 13-acetate and calcium ionophore A23187 (PMACI)-stimulated expression and secretion of pro-inflammatory cytokines in human mast cells. The inhibitory effect of FPP on pro-inflammatory cytokines was nuclear factor (NF)- κ B dependent. Our findings provide evidence that FPP inhibits mast cell-derived allergic inflammation and involvement of calcium and NF- κ B in these effects.

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1. Introduction

Mast cell-derived mediators play a critical role in the initiation and progression of the allergic inflammatory reactions associated with atopic asthma and other allergic disorders (Brown et al., 2008). Mast cells express the high-affinity Fc ϵ RI on the cell surface. Its cross-linking by immunoglobulin (Ig) E antigen initiates a cascade of intracellular signaling events. In IgE-dependent mast cell activation, mast cells rapidly secrete the pre-formed and *de novo* synthesized allergic mediators such as histamine, cytokines, proteases, and arachidonic derivatives (Galli et al., 2005). One of allergic mediators, histamine, plays an important role both in normal human physiology as well as in various pathology and regulates several essential events in the allergic inflammation response (Jutel et al., 2005).

Mast cell exocytosis is correlated with an increase of intracellular calcium. Calcium influx initiates the downstream activation

of signal transducing enzymes including phospholipase C, phosphoinositide 3-kinase, and phospholipase D (Pandey et al., 2004). This is followed by the activation of mitogen-activated protein kinases (MAPKs), nuclear factor (NF)- κ B and the release of inflammatory cytokines. Activated mast cells can produce histamine, as well as a wide variety of other inflammatory mediators such as eicosanoids, proteoglycans, proteases, and several pro-inflammatory and chemotactic cytokines, such as tumor necrosis factor (TNF)- α , interleukin IL-6, IL-8, IL-4, IL-13 and the transforming growth factor- β (Galli et al., 2005; Kim et al., 2006). The transcription factor NF- κ B has important activities as mediators of cellular responses to extracellular signals and is thought to play an important role in the regulation of pro-inflammatory molecules on cellular responses, especially TNF- α , IL-1 β , IL-6 and IL-8 (Azzolina et al., 2003; Shin et al., 2005).

Prunus persica seed is well known as a traditional medicine (Persicae Semen) in Korea, Japan, China and other Asian countries. However, the biological activities of *P. persica* fruit except its seed are poorly understood (Zhu, 1998). In this study, we evaluated the anti-allergic inflammatory effect of the ethanol extract of fruits of *P. persica* (FPP) using the mast cell-based in vivo models. FPP reduced systemic and local anaphylaxis, and histamine release from mast cells. The reduction of intracellular calcium level showed the mechanism by which FPP inhibited histamine release

Abbreviations: DNP-HSA, dinitrophenyl-human serum albumin; HMC-1, human mast cells; PCA, passive cutaneous anaphylaxis; PMACI, phorbol 12-myristate 13-acetate and calcium ionophore; RPMC, rat peritoneal mast cells.

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from mast cells. In addition, the effect of FPP on expression of pro-inflammatory cytokines and role of NF- κ B in this effect were investigated using human mast cells (HMC-1).

2. Materials and methods

2.1. Animals

The original stock of male ICR mice (20–30 g) was purchased from the Dae-Han Biolink Co. Ltd. (Daejeon, Korea). The animals were maintained in the College of Pharmacy, Woosuk University. The care and treatment of the mice were in accordance with the guidelines established by the Public Health Service Policy on the Human Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee.

2.2. Reagents and cell culture

Compound 48/80, anti-dinitrophenyl (DNP) IgE, DNP-human serum albumin (HSA), pyrrolidine dithiocarbamate (PDTC), phorbol 12-myristate 13-acetate (PMA) and calcium ionophore A23187 (PMACI) were purchased from the Sigma Chemical Co. (St. Louis, MO). rTNF- α and rIL-6, and anti-TNF- α , and anti-IL-6 antibodies were purchased from R&D Systems Inc. (Minneapolis, MN). The human mast cell line (HMC-1) was grown in Iscove's media (Life Technologies, Grand Island, NY) supplemented with 10% FBS and 2 mM glutamine at 37 °C in 5% CO₂. Passages 4–8 of cultures were used in all experiments.

2.3. Preparation of FPP

The fruits of *P. persica* were purchased from the farm (the cultivator: T.K. Lee) of Wanju, Jeonbuk, Korea and identified by D.K. Kim, College of Pharmacy, Woosuk University. A voucher specimen was deposited at the Herbarium of the College of Pharmacy, Woosuk University. After removal of the cores of the fruits of *P. persica* (200 g), it was dried and extracted twice with 70% ethanol at 70 °C for 3 h in water bath. The extract was filtered through Whatman No. 1 filter paper and the filtrate was lyophilized using 0.45 μ m syringe filter. The yield of dried extract from starting crude materials was about 25.2%. The dried extract was dissolved in saline or Tyrode buffer A (10 mM HEPES, 130 mM NaCl, 5 mM KCl, 1.4 mM CaCl₂, 1 mM MgCl₂, 5.6 mM glucose, 0.1% bovine serum albumin) before use.

2.4. Compound 48/80-induced systemic anaphylaxis

As an in vivo model for the immediate-type allergic reaction, compound 48/80-induced systemic reaction was carried out as previously described (Kim et al., 2008). Briefly, the mice ($n = 10$ /group) were given an intraperitoneal injection of 8 mg/kg body weight (BW) of the mast cell degranulator, compound 48/80. FPP was dissolved in saline and administered intraperitoneally at doses of 10–1000 mg/kg BW 1 h before the compound 48/80 injection. In the time-dependent experiment, FPP (1000 mg/kg) was administered 5, 10, 15 and 20 min after compound 48/80 injection ($n = 10$ /group). Mortality was monitored for 1 h after induction of anaphylactic shock.

2.5. Passive cutaneous anaphylaxis (PCA)

PCA reaction was carried out as previously described (Kim et al., 2009). Briefly, mice were injected intradermally with 0.5 μ g of anti-DNP IgE. After 48 h, each mouse ($n = 10$ /group) was received an injection of 1 mg of DNP-HSA in PBS containing 4% Evans blue (1:1) via tail vein. FPP (10–1000 mg/kg BW) was intraperitoneally administered 1 h before the challenge. Thirty minutes after the challenge, the mice were killed and the dorsal skin (diameter, 1 cm) was removed in order to measure the pigment area. The amount of dye 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 the absorbent was measured at 620 nm in a spectrophotometer (Shimadzu, UV-1201, Kyoto, Japan).

2.6. Histamine assay

Histamine content was measured by the enzyme immunoassay kit (Oxford Biomedical Research, Oxford, MI) according to manufacturer manual. HMC-1 were pre-incubated with FPP for 30 min, and then incubated for 8 h with PMACI. The cells were separated from the released histamine by centrifuged at 400g for 5 min at 4 °C.

2.7. Measurement of calcium

The intracellular calcium was measured with the use of the fluorescent indicator Fluo-3/AM (Molecular probes, Eugene, OR). HMC-1 cells were preincubated with Fluo-3/AM for 30 min at 37 °C. After washing the dye from the cell surface, the cells were treated with FPP for 10 min before adding PMACI. It was excited at 488 nm,

and the emission was filtered with a 515 nm by flow cytometer (BD Biosciences Pharmingen, San Diego, CA) and visualized in a fluorescence microscope (Olympus BX51, Center Valley, PA).

2.8. Reverse transcription-polymerase chain reaction (RT-PCR)

RT-PCR was used to analyze the expression of mRNA for TNF- α , IL-1 β , IL-6, IL-8, and β -actin (internal control) with FPP in PMACI-stimulated HMC-1 cells. The conditions for RT-PCR were similar to ones previously described (Kim et al., 2006; Shin et al., 2006) and showed in Table 1. Amplified PCR products were separated by 2% agarose gels and digitized using UN-SCAN-IT software (Silk Scientific, Orem, UT).

2.9. Assay of TNF- α and IL-8 secretion

Secretion of TNF- α and IL-6 was measured by modification of an enzyme-linked immunosorbent assay (ELISA) as previously described (Min et al., 2007). HMC-1 cells were sensitized with PMACI for 16 h in the absence or presence of FPP. The ELISA was performed by coating 96-well plates with 6.25 ng/well of monoclonal antibody with specificity for TNF- α and IL-6. Before use and between subsequent steps in the assay, the coated plates were washed twice with PBS containing 0.05% Tween-20 and twice with PBS alone. For the standard curve, rTNF- α and rIL-6 were added to the serum which was previously determined to be negative to endogenous TNF- α and IL-6. After exposure to the medium, the assay plates were exposed sequentially to biotinylated anti-human TNF- α and IL-6 and 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) tablet substrates. Optical density readings were made within 10 min of the addition of the substrate with a 405 nm filter.

2.10. Nuclear protein extraction

Preparation of nuclear extract was basically as described elsewhere (Kim et al., 2007). Briefly, after cell activation for the times indicated, cells were washed in 1 ml of ice-cold PBS, centrifuged at 1200 rpm 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 15,000g for 30 s. Pelleted nuclei were gently 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, and centrifuged at 15,000g for 5 min at 4 °C. Aliquots of the supernatant that contained nuclear proteins were frozen in liquid nitrogen and stored at –70 °C.

2.11. Western blot analyses

HMC-1 cells were washed with PBS and resuspended in lysis buffer. Samples were electrophoresed using 8% sodium dodecyl sulfatepolyacrylamide gel electrophoresis, as described elsewhere (Lee et al., 2008), and then transferred to a nitrocellulose membrane. The NF- κ B was assayed using anti-NF- κ B antibody (Santa Cruz Biotech, Santa Cruz, CA). Immunodetection was done using an enhanced chemiluminescence detection kit (Amersham Pharmacia, Piscataway, NJ).

2.12. Transient transfection and luciferase activity assay

For transient transfections, HMC-1 cells were seeded at 2×10^6 in a 6-well plate 1 day before transient transfection. The expression vectors containing the NF- κ B luciferase reporter construct (pNF- κ B-LUC, plasmid containing NF- κ B binding site; STANTAGEN, Grand Island, NY) were transfected with serum- and antibiotics-free Iscove's medium containing 8 μ l of Lipofectamine 2000 reagent (Invitrogen, Carlsbad, CA). After 5 h of incubation, medium was replaced with Iscove's medium containing 10% FBS and antibiotics. Cells were allowed to recover at 37 °C for 20 h and subsequently were stimulated as indicated. Cell lysates were prepared and assay for luciferase activity using Luciferase Assay System (Promega, Madison, WI), according to the manufacturer's instructions.

2.13. Statistical analysis

Statistical analyses were performed using SAS statistical software (SAS Institute, Cary, NC). Treatment effects were analyzed using ANOVA, followed by Duncan's multiple range tests. $P < 0.05$ was used to indicate significance.

3. Results

3.1. Effect of FPP on systemic and local anaphylaxis

To determine the effect of FPP on allergic reaction, an in vivo model of a systemic reaction was used. Compound 48/80 (8 mg/kg) was used as a model of induction for a systemic fatal allergic reaction. After the intraperitoneal injection of compound 48/80,

Table 1
Primers and PCR conditions for gene expression.

Gene	Primers	Annealing temperature (°C)	Amplified size (bp)
TNF- α	s 5'-cct acc aga cca agg tca ac-3' as 5'-agg ggg taa taa agg gat tg-3'	57	279
IL-1 β	s 5'-aaa cag atg aag tgc tcc tt-3' as 5'-tgg aga aca cca ctt gtt gc-3'	57	391
IL-6	s 5'-aaa gag gca ctg gca gaa aa-3' as 5'-atc tga ggt gcc cat gct ac-3'	59	412
IL-8	s 5'-aca gca gag cac aca agc tt-3' as 5'-ctg gca acc cta caa cag ac-3'	59	247
β -actin	s 5'-gga ctt cga gca aga gat gg-3' as 5'-agc act gtg ttg gcg tac ag-3'	54	234

the mice were monitored for 1 h, after which the mortality rate was determined. As shown in Table 2, injection of compound 48/80 into mice induced fatal shock in 100% of animals. When FPP was intraperitoneally administered at concentrations ranging from 10 to 1000 mg/kg BW for 1 h, the mortality with compound 48/80 was dose-dependently reduced. In addition, the mortality of mice administered with FPP (1000 mg/kg) after compound 48/80 injection increased time dependently (Table 3). The effect of FPP on the compound 48/80-induced serum histamine release was investigated. FPP was administered at concentrations ranging from 10 to 1000 mg/kg 1 h before the compound 48/80 injection. Injection of compound 48/80 caused marked increase of serum histamine release, and this induction was inhibited by FPP treatment in a dose-dependent manner (Fig. 1A). To confirm the anti-allergic effects of FPP, we used IgE-mediated passive cutaneous anaphylaxis (PCA) model. PCA is one of the most important *in vivo* models of local allergic reaction. A local extravasation was induced by a local injection of IgE followed by an antigenic challenge. Intraperitoneal injection of FPP dose-dependently inhibited PCA reaction (Fig. 1B).

3.2. Effect of FPP on histamine release from mast cells

We next evaluated the ability of FPP to inhibit release of PMACI-induced histamine from HMC-1 cells. Treatment with FPP dose-dependently inhibited PMACI-induced histamine release at concentrations of 10–100 g/ml (Fig. 2). The concentration of FPP treatment used in these studies had no significant effect on the viability of HMC-1 cells (data not shown).

3.3. Effect of FPP on intracellular calcium in HMC-1 cells

Calcium influx of mast cells is critical to histamine release (Beaven and Metzger, 1993). To investigate the mechanism of FPP on the inhibition of histamine release, we evaluated intracellular calcium levels. PMACI induced the increase of intracellular

Table 2
Effect of FPP on compound 48/80-induced systemic anaphylaxis.

FPP treatment (mg/kg BW)	Compound 48/80 (8 mg/kg BW)	Mortality (%)
None (saline)	+	100
10	+	100
50	+	100
100	+	60
500	+	50
1000	+	0
1000	–	0

Groups of mice ($n = 10$ /group) were intraperitoneally pretreated with 200 μ l of saline or FPP at various doses 1 h before the intraperitoneal injection of compound 48/80. Mortality (%) within 1 h following compound 48/80 injection is represented as the number of dead mice $\times$ 100/total number of experimental mice.

Table 3
Time-dependent effect of FPP on compound 48/80-induced systemic anaphylaxis.

FPP treatment (mg/kg BW)	Time (min)	Compound 48/80 (8 mg/kg BW)	Mortality (%)
None (saline)	–	+	100
1000	5	+	20
	10	+	60
	15	+	100

Mice ($n = 10$ /group) were intraperitoneally pretreated with 200 μ l of saline or FPP (1000 mg/kg) 0, 5, 10 and 15 min after the intraperitoneal injection of compound 48/80. Mortality (%) within 1 h following compound 48/80 injection is represented as the number of dead mice $\times$ 100/total number of experimental mice.

calcium in mast cells (Fig. 3). Preincubation of FPP decreased the intracellular calcium level in PMACI-stimulated HMC-1 cells.

3.4. Effect of FPP on the gene expression and secretion of pro-inflammatory cytokines from HMC-1 cells

TNF- α , IL-1 β , IL-6 and IL-8 are the most important pro-inflammatory cytokines. Therefore, we tested the effect of FPP on the

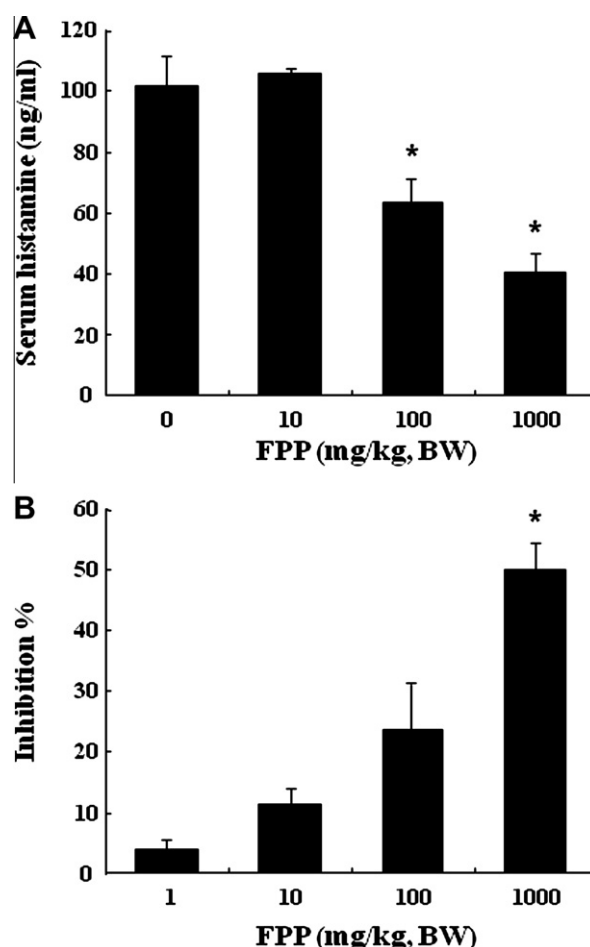


Fig. 1. Effect of FPP on serum histamine release and PCA reactions. (A) Groups of mice ($n = 10$ /group) were intraperitoneally pretreated with 200 μ l of saline or FPP. FPP was given at various doses 1 h before the injection of compound 48/80. The compound 48/80 solution was given intraperitoneally to the group of mice. The blood was obtained from the heart of each mouse and histamine content was measured by histamine assay kit. (B) FPP was intraperitoneally administered 1 h prior to the challenge with antigen. Each amount of dye was extracted as described in Materials and methods and measured by spectrophotometry. Each bar represents the mean $\pm$ SEM of three independent experiments. *Significant difference at $p < 0.05$.

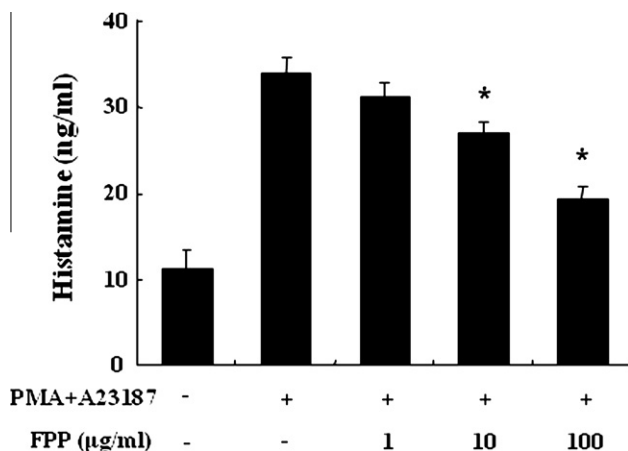


Fig. 2. Effect of FPP on histamine release. HMC-1 cells (2×10^6 cells/ml) were preincubated with FPP at 37 °C for 10 min prior to incubation with PMA (20 nM) and A23187 (1 µM). Each value represents the mean $\pm$ SEM of three independent experiments. *Significant difference at $p < 0.05$.

gene expression of these cytokines in PMACI-stimulated HMC-1 cells. HMC-1 cell is a useful cell for studying cytokine activation pathway (Kim et al., 2006; Sillaber et al., 1993). We previously reported that gene expression of TNF- α , IL-1 β , IL-6 and IL-8 was peaked at 4 h after treatment of PMACI (Park et al., 2007). Therefore, stimulation of HMC-1 with PMACI was induced during 4 h, and the cells were pretreated with FPP for 30 min. FPP dose-dependently inhibited PMACI-induced expression of TNF- α , IL-1 β , IL-6 and IL-8 (Fig. 4). To confirm the effect of FPP on the gene expression of pro-inflammatory cytokines, cultured supernatants were assayed for TNF- α and IL-6 levels by ELISA. Stimulation of cells

with PMACI during 16 h induced the secretion of cytokines. FPP inhibited the secretion of TNF- α and IL-6 in PMACI-stimulated HMC-1 cells (Fig. 5).

3.5. Effect of FPP on activation of NF- κ B

NF- κ B is an important transcriptional regulator of inflammatory cytokines and plays a crucial role in immune and inflammatory

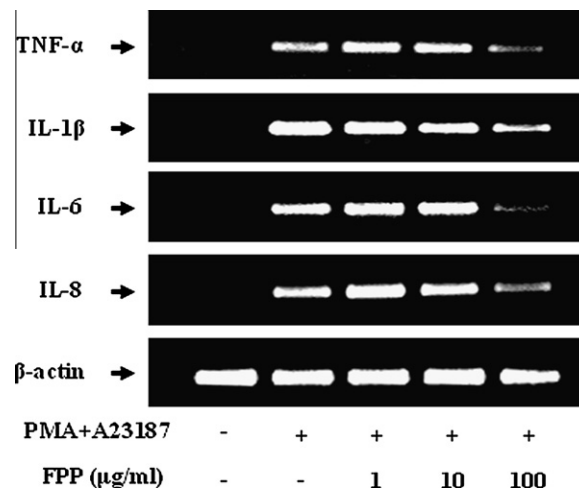


Fig. 4. Effect of FPP on the gene expression of pro-inflammatory cytokines in HMC-1 cells. Cells were pretreated with FPP for 30 min and stimulated by PMA (20 nM) and A23187 (1 µM) for 4 h. The levels of TNF- α , IL-1 β , IL-6 and IL-8 were determined by RT-PCR.

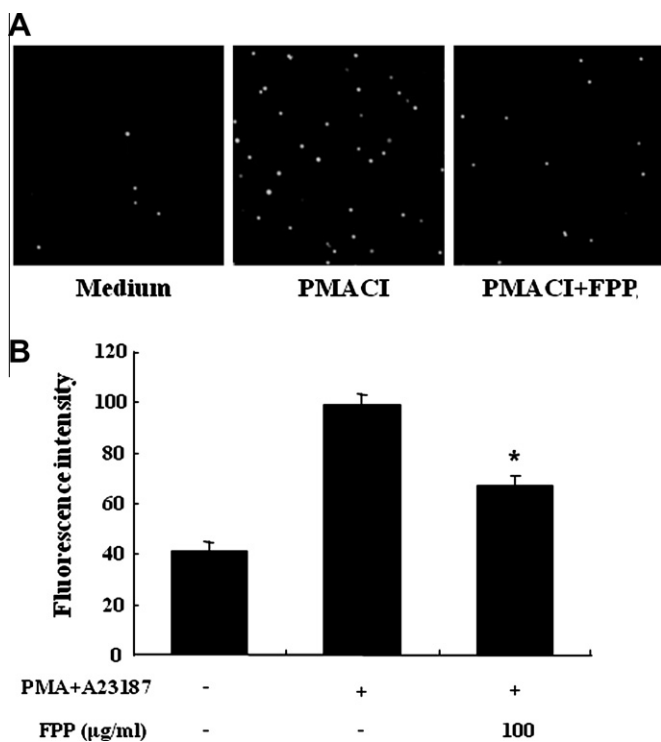


Fig. 3. Effect of FPP on intracellular calcium in mast cells. HMC-1 cells were stained with Fluo-3/AM. Cells were preincubated for 10 min with FPP before adding PMA (20 nM) and A23187 (1 µM), and then another 10 min with PMACI. Intracellular calcium was detected by fluorescence microscope (A) and flow cytometer (B). Each value represents the mean $\pm$ SEM of three independent experiments. *Significant difference at $p < 0.05$.

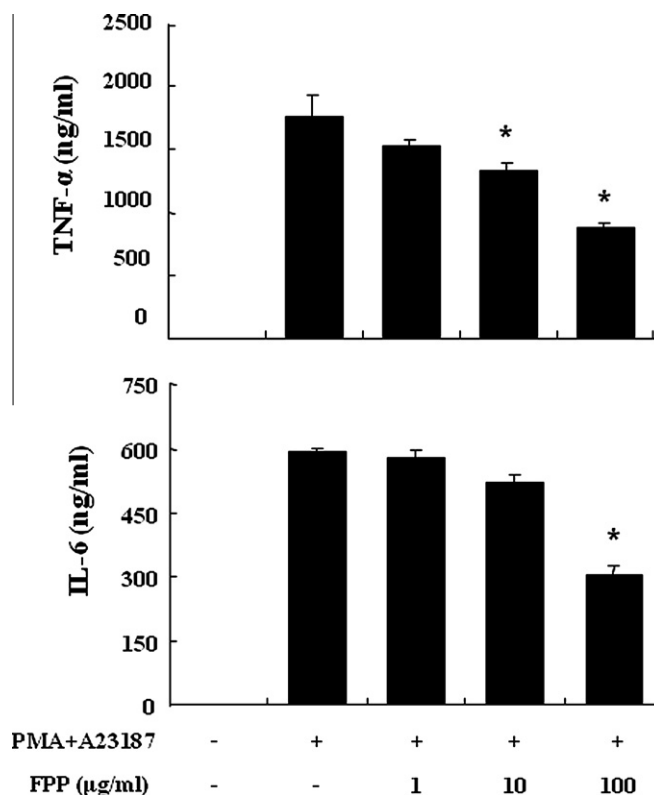


Fig. 5. Effect of FPP on the secretion of pro-inflammatory cytokines. Cells were pretreated with FPP for 30 min and stimulated by PMA (20 nM) and A23187 (1 µM) for 16 h. The levels of TNF- α and IL-6 in supernatant were measured using ELISA and represented as the mean $\pm$ SEM of three independent experiments. *Significant difference at $p < 0.05$.

responses. To investigate the intracellular mechanism responsible for the inhibitory effect of FPP on the secretion of pro-inflammatory cytokines, we examined the effect of FPP on activation of NF- κ B. Stimulation of HMC-1 cells with PMACI induced nuclear translocation of p65 NF- κ B after 2 h of incubation (Fig. 6A). FPP inhibited the PMACI-induced degradation of I- κ B α and nuclear translocation of p65 NF- κ B. To confirm the inhibitory effect of FPP on NF- κ B activation, we examined the effect of FPP on the NF- κ B-dependent gene reporter assay. HMC-1 cells were transiently transfected with a NF- κ B-luciferase reporter construct or an empty vector. Exposure of cells to PMACI increased the luciferase activity in the cells transfected with the NF- κ B-luciferase reporter construct (Fig. 6B). FPP significantly reduced the PMACI-induced luciferase activity.

4. Discussion

This study showed that FPP has anti-allergic inflammatory properties via controlling calcium influx and NF- κ B signaling. Anaphylaxis is a life-threatening syndrome induced by the sudden systemic release of inflammatory mediators, such as histamine, heparin, and various cytokines from mast cells (Kemp and Lockey, 2002). FPP inhibited compound 48/80-induced systemic anaphylaxis and histamine release from mast cells. FPP administered mice

are protected from IgE-mediated local allergic reaction, PCA, which is one of the most important in vivo models of anaphylaxis. These results indicate that FPP inhibited mast cell-mediated allergic reactions. In addition, FPP decreased PMACI-stimulated expression of pro-inflammatory cytokines. These results suggest that FPP might be useful in the treatment of allergic inflammatory diseases.

Histamine originally considered as a mediator of acute inflammatory and immediate hypersensitivity responses. Recently, it has been reported that histamine affects chronic inflammation and regulates several essential events of immune response such as immune cell maturation, polarization, and lymphocyte responsiveness (Jutel et al., 2005). Histamine also induces actin polymerization and chemotaxis (Jutel et al., 2002). Compound 48/80, the synthetic mast cell stimulator, increases the permeability of the lipid bilayer membrane by perturbation in the membrane. These reports indicate that the increasing of membrane permeability may be an essential for the release of the mediator from mast cells. In our study, FPP dose-dependently reduced compound 48/80-induced histamine release from mast cells. Taken together, we attempt to speculate that FPP might stabilize the actin polymerization of membrane, thus preventing the compound 48/80-induced membrane perturbation.

Mast cells play a critical role in inflammation and protective immune responses by releasing pre-formed vasoactive amines, prostaglandins, cytokines and chemokines (Hu et al., 2007). Various inflammatory cytokines such as TNF- α , IL-1 β , IL-6 and IL-8, which act triggers or regulators in allergic inflammatory response, are crucial for mast cell development (Galli et al., 2005). TNF- α is a well-known inflammatory factor produced mainly by macrophages, and plays a critical role in systemic inflammation (Hu et al., 1997). It is also a potent inducer of other inflammatory cytokines, including IL-1 β , IL-6, IL-8 and GM-CSF. IL-6 is one of the most important mediators of a multifunctional pro-inflammatory cytokines, and actually the major cytokine produced by activated mast cells (Burd et al., 1989). IL-1 is known to stimulate PGE and INF- γ production and it promotes mast cell proliferation (Phipps et al., 1991). These reports indicate that the inhibition of pro-inflammatory cytokines is one of the critical point of reduced allergic inflammatory symptom. In our study, FPP inhibited gene expression and secretion of cytokines. These results imply that FPP has anti-allergic inflammatory effects by controlling pro-inflammatory cytokines in mast cells.

NF- κ B regulates the expression of multiple inflammatory and immune genes and plays a critical role in chronic inflammatory diseases (Kim et al., 2005). NF- κ B is a transcriptional factor with heterodimer or homodimer forms of rel family proteins, and sequestered in the cytoplasm, bound to I- κ B proteins. In PMACI-stimulated mast cell, FPP inhibited degradation of I- κ B and translocation of NF- κ B. Our previous reports, the potent inhibitor of NF- κ B, PDTC, reduced PMACI-induced cytokines in HMC-1 cells. According from these results, we suggest that FPP inhibits expression of inflammatory cytokines via inhibition of NF- κ B pathway.

Mobilization of calcium plays a critical role in the degranulation and release of histamine in mast cells (Cho et al., 2004). Calcium movements across membranes of mast cells represent a major target for effective anti-allergic drugs, as these are essential events linking stimulation to secretion. Our results showing an attenuation of intracellular calcium in mast cells with FPP treatment might suggest the decreased intracellular calcium might be involved in the inhibitory effect of FPP on histamine release.

Following the increase of intracellular calcium, several Ca-dependent signaling proteins and their target transcription factors are activated, including the calcineurin and its target nuclear factor of activated T cells (NFAT) and calcium-calmodulin-dependent kinase. Intracellular calcium is sufficient to rapidly stimulate MAPK pathways (Oh-hora and Rao, 2008), and implicates in the

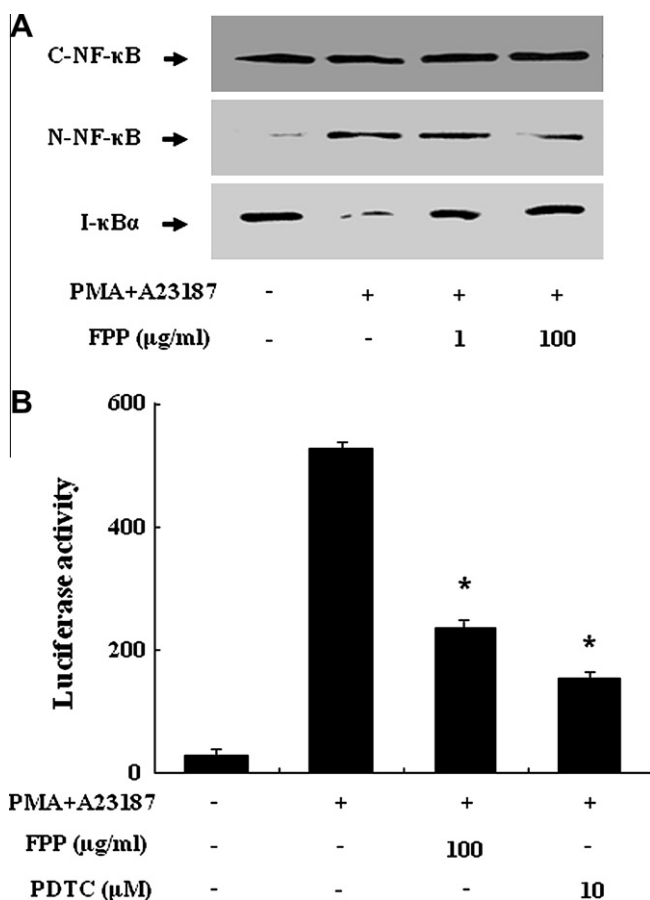


Fig. 6. Effect of FPP on the activation of NF- κ B. HMC-1 cells were pretreated with FPP or PDTC (10 μ M) for 30 min prior to PMA (20 nM) and A23187 (1 μ M) stimulation. (A) NF- κ B translocation was assayed by Western blot (N-NF- κ B, nucleus NF- κ B; C-NF- κ B, cytoplasmic NF- κ B). (B) Cells were transiently transfected with the NF- κ B-luciferase reporter construct or empty vector. Then, the cells were incubated with PMA and A23187 with or without FPP. NF- κ B-dependent transcriptional activity was determined by luciferase activity assay. *Significant difference at $p < 0.05$.

NF- κ B as well as in the up-regulation of cytokines (Kim et al., 2005). Taken together with our observation, we speculate that the inhibitory effect of FPP on the activation of mast cell is mediated by regulation of intracellular calcium. FPP might inhibit calcium-dependent transcription factors through inhibition of calcium mobilization.

Fruit of *Prunu sp.*, peach, composed of many anti-oxidant compounds including quercetin, catechin, and kaempferol (Jung et al., 2003). In mouse granulocytes, anti-radical activity and its protective effects against oxidative stress were evaluated (Bouayed et al., 2009). However, due to the using of whole ethanol extract of fruits of *P. persica* not a purified component, the active components that are responsible for the anti-allergic inflammatory effect are still unknown at this time. However, in this report, we provided sufficient evidence that FPP inhibits mast cell-mediated allergic inflammatory reaction and their possible mechanisms via their signaling pathways. FPP may act the prevention or inhibition of mast cell-mediated allergic inflammatory diseases.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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