



## ***Fritillaria ussuriensis* Extract Inhibits the Production of Inflammatory Cytokine and MAPKs in Mast Cells**

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***Fritillaria ussuriensis* (FU, derived from the bulbs of various species of the genus *Fritillaria*, including *Fritillaria thunbergii* Miq.) is used in herbal medicine to treat conditions such as eczema, skin burns, and frostbite. In this study, we investigated the mechanism of the anti-allergy effect of FU. FU extract (80 mg/kg), orally administered to Sprague-Dawley (SD) rats, significantly inhibited the passive cutaneous anaphylaxis (PCA) reaction. It inhibited the compound 48/80-induced release of histamine from rat peritoneal mast cells in a concentration-dependent manner. Significant inhibitory effects of the FU extract on IL-6, IL-8, and TNF- $\alpha$  (1, 10, and 100  $\mu$ g/mL) were observed in HMC-1 cells. Treatment with FU attenuated PMA plus A23187-induced phosphorylation of all three MAPKs, especially at concentrations of 10 and 100  $\mu$ g/mL. Further, it (80 mg/kg) led to significant inhibition of mast-cell accumulation in ear tissue at the chronic phase. These results indicate that it inhibits allergic reactions.**

**Key words:** *Fritillaria ussuriensis*; HMC-1 cell; passive cutaneous anaphylaxis; interleukin; MAPKs

*Fritillaria ussuriensis* (Chinese name, Beimu), derived from the bulbs of various species of the genus *Fritillaria* (Liliaceae), is used as an anti-tussive and expectorant in traditional Chinese medicine.<sup>1)</sup>

Mast cells have been implicated in acute and chronic inflammatory responses and in many diseases characterized by inflammation.<sup>2)</sup> Mast cell-derived pro-inflammatory cytokines play important roles in the development of acute- and late-phase allergic inflammatory reactions. Hence inhibition of the production and secretion of these mediators is likely to provide a major mechanism by which the efficacy of the treatment of inflammatory diseases can be enhanced.<sup>3)</sup> Human mast cell line HMC-1 originated in a patient with mast-cell leukemia. The cells express several features of mature human mast cells, making them a valuable model for studies of human mast-cell biology.<sup>4)</sup> HMC-1 cells can serve as a useful mast-cell system for examining the expression of chemokines during inflammation.<sup>5)</sup>

In the inflammatory process, several pro-inflammatory cytokines, including tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin (IL)-1 $\beta$ , IL-4, IL-6, and IL-8, recruit activated immune and inflammatory cells to the sites of lesions, thereby amplifying and perpetuating the inflammatory condition.<sup>6,7)</sup>

Rats with passive cutaneous anaphylaxis (PCA) serve as animal models for anti-Ig mediated immediate allergic reactions, induced by mediators such as histamine secreted from mast cells.<sup>8,9)</sup>

Mitogen-activated protein kinase (MAPK) signaling pathways are activated by various extracellular stimuli, resulting in a wide range of cellular responses, including apoptosis, proliferation, and inflammation. In mammals, three major factors that mediate MAPK pathways have been identified: extracellular signal-regulated kinases (ERK), p38, and stress-activated protein kinases (SAPK)/c-Jun-N-terminal kinase (JNK).<sup>10-12)</sup>

Contact hypersensitivity (CH) is a cutaneous immune reaction that occurs in individuals sensitized to subsequent contact with a sensitizing hapten. Local endothelial cell activation plays a critical role in it. Repeated application of a contact-sensitizing agent in mice induces chronic CH responses that are clinically similar to human skin allergic diseases.<sup>13)</sup> Repeated epicutaneous application of such an antigen (Ag) results in a shift in time course from a typical delayed-type hypersensitivity to an immediate-type hypersensitivity response followed by a late-phase reaction. This immediate-type hypersensitivity response is site-restricted and Ag-specific.<sup>13,14)</sup>

In the present study, we investigated the effects of FU on compound 48/80-induced release of histamine from rat peritoneal mast cells, and on anti-dinitrophenyl (DNP) IgE antibody-induced PCA. We found that FU inhibited phorbol 12-myristate 13-acetate (PMA) and A23187-induced production of cytokines (IL-6, TNF- $\alpha$ , and IL-8). In a further investigation, we focused on the effects of FU on the production and expression of components of the MAPK pathways (ERK, JNK, and p38) in a human mast-cell line. Mice with 2,4-dinitrofluoro benzene (DNFB)-induced contact hypersensitivity were used for a comparative study.

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Abbreviation: FU, *Fritillaria ussuriensis*

## Materials and Methods

**Reagents.** Compound 48/80, anti-DNP IgE, DNP-human serum albumin (HSA), PMA, A23187, and Evans blue were purchased from Sigma (St. Louis, MO). Recombinant IL-6 and IL-8, biotinylated IL-6 and IL-8, and antihuman IL-6 and IL-8 were from BD Biosciences (San Diego, CA). Recombinant TNF- $\alpha$ , biotinylated TNF- $\alpha$ , and antihuman TNF- $\alpha$  were from R&D Systems (Minneapolis, MN). MTT, cell culture media Iscove's Modified Dulbecco's Medium (IMDM), and fetal bovine serum (FBS) were from Gibco BRL (Grand Island, NY). A histamine kit was from Oxford Biomedical Research (Oxford, MS). ERK, JNK, and p-38 Abs were from Santa Cruz Biotechnology (Santa Cruz, CA).

**Preparation of FU.** A plant sample was purchased from Kyung Hee University Medical Center (Seoul, Korea). One hundred g of FU was extracted with 80% ethanol at RT over 2 weeks and filtered using Whatman filter paper no. 3. The ethanol extract was concentrated in a rotary vacuum evaporator, lyophilized, and stored at  $-20^{\circ}\text{C}$  until use. After ethanol treatment, 12% of FU extract was recovered.

**Animals.** Animal care and all experimental procedures were conducted in accordance with the Guide for Animal Experiments of the Korean Academy of Medical Sciences. The original stocks of Sprague-Dawley (SD) rats and BALB/c mice (6 weeks old, female) were purchased from Samtako Experimental Animal Center (Ossan, Gyeonggi-do, Korea), and the Orient Bio-Experimental Animal Center (Gapyeong, Gyeonggi-do, Korea) respectively. The animals were maintained at the College of Oriental Medicine at Kyung Hee University. They were housed four per cage in a laminar air-flow room maintained at  $22^{\circ}\text{C} \pm 1^{\circ}\text{C}$  at a relative humidity of  $55\% \pm 10\%$  throughout the study.

**Culture of HMC-1 cells.** HMC-1 cells were kindly provided by Prof. Hyung Min Kim (Department of Pharmacology, Kyung Hee University, Republic of Korea). HMC-1 was grown in IMDM with 10% FBS at  $37^{\circ}\text{C}$  in a 5%  $\text{CO}_2$  atmosphere at 95% humidity. The HMC-1 cells ( $3 \times 10^5$  cells) were pretreated with various concentrations of FU extract (1, 10, and  $100\mu\text{g/mL}$ ) for 30 min and then stimulated with 25 nM PMA plus  $1\mu\text{M}$  A23187. Subsequently, they were incubated at  $37^{\circ}\text{C}$  for 7 h.

**PCA reaction.** The IgE-dependent cutaneous reaction was induced by sensitizing the skin with an intradermal injection of anti-DNP IgE, followed 48 h later by an injection of DNP-HSA into the dorsal vein of the rat's penis. DNP-HSA was diluted in phosphate-buffered saline (PBS). The rats received intradermal injections of  $0.5\mu\text{g}$  ( $50\mu\text{L}$ ) anti-DNP IgE at each of the three dorsal skin sites, which were shaved 48 h earlier. The sites were then outlined with a water-insoluble red marker. Forty-eight h later, each rat received an injection of  $100\mu\text{g}$  ( $100\mu\text{L}$ ) of DNP-HSA in saline containing 4% Evans blue through the dorsal vein of the penis. One h before the injection, the FU extract was administered orally (80 mg/kg of body weight). The rats were euthanized 30 min after the intravenous challenge. The dorsal skin was removed and the pigment area was measured. The amount of dye was colorimetrically measured after extraction with formamide. The absorbent intensity of the extract was measured at 620 nm with a spectrophotometer (Beckman, IL), and the amount of dye was calculated using the Evans blue measuring line.

**Histamine assay.** Purified rat peritoneal mast cells (RPMCs) were resuspended in Tyrode buffer (137 mM NaCl, 5.6 mM glucose, 12 mM  $\text{NaHCO}_3$ , 2.7 mM KCl, and 0.3 mM  $\text{Na}_2\text{HPO}_4$ ) containing calcium and treated with compound 48/80. The suspended RPMCs ( $2 \times 10^5$  cells) were pre-incubated for 10 min at  $37^{\circ}\text{C}$  before addition of compound 48/80 for stabilization. The cells were pre-incubated with the FU extract (1, 10, and  $100\mu\text{g/mL}$ ) for 10 min, and then incubated with compound 48/80 ( $5\mu\text{g/mL}$ ) for 20 min. The reaction was quenched by cooling the tubes on ice. The cells were separated from the released histamine by centrifugation at  $400 \times g$  for 10 min at  $4^{\circ}\text{C}$ . The histamine content was measured by enzyme immunoassay performed using the histamine kit. OD was measured at 450 nm with a microplate

enzyme-linked immunosorbent assay (ELISA) reader (Molecular Devices, CA). Percentage inhibition was calculated as  $(A - B) \times 100/A$ , where A is the amount of histamine released without the FU extract, and B is the amount of histamine released with it.

**MTT assay for cell viability.** The viability of the cells was determined by MTT colorimetric assay, as described previously. In brief, suspended HMC-1 cells ( $3 \times 10^5$  cells) were cultured in 24-well plates with various concentrations of the FU extract (1, 10, and  $100\mu\text{g/mL}$ ). An MTT solution ( $5\text{mg/mL}$ ) was then added to each well, and the cells were cultured overnight. The supernatant was removed, and the insoluble formazan product was dissolved in DMSO. The OD value of the 96-well culture plates was measured at 540 nm with an ELISA reader. The OD value of the formazan that formed in the untreated control cells was considered to represent 100% viability.

**TNF- $\alpha$ , IL-6, and IL-8 assay.** The HMC-1 cells were grown in IMDM with 10% FBS at  $37^{\circ}\text{C}$  in an atmosphere containing 5%  $\text{CO}_2$  at 95% humidity. They were pretreated with various concentrations of the FU extract (1, 10, and  $100\mu\text{g/mL}$ ) for 1 h and then stimulated with PMA plus A23187. The various culture supernatants were then subjected to ELISA to determine the TNF- $\alpha$ , IL-6, and IL-8 levels. Cytokine levels were measured by modified ELISA method. Sandwich ELISA for TNF- $\alpha$ , IL-6, and IL-8 was performed in duplicate in 96-well ELISA plates (Nunc, Denmark), each coated with  $100\mu\text{L}$  aliquots of mouse anti-human TNF- $\alpha$ , IL-6, and IL-8 monoclonal antibodies in  $1.0\mu\text{g/mL}$  of PBS at pH 7.4. The plates were then incubated overnight at  $4^{\circ}\text{C}$ . They were washed with PBS containing 0.05% Tween-20 (Sigma, St. Louis, MO), and blocked with PBS containing 1% BSA, 5% sucrose, and 0.05%  $\text{NaN}_3$  for 1 h. After additional washings, the sample or recombinant TNF- $\alpha$ , IL-6, or IL-8 standard was added, and the plates were incubated at  $37^{\circ}\text{C}$  for 2 h. After incubation at  $37^{\circ}\text{C}$  for 2 h, the wells were washed and  $0.2\mu\text{g/mL}$  of biotinylated anti-human TNF- $\alpha$ , IL-6, or IL-8 was added, and then they were incubated at  $37^{\circ}\text{C}$  for 2 h. After the wells were washed, avidin-peroxidase was added, and the plates were incubated at  $37^{\circ}\text{C}$  for 30 min. The wells were washed again, and the tablet substrate avidin peroxidase, 2'-azino-bis [3-ethylbenzothiazoline-6-sulphonic acid] (ABTS, Sigma, St. Louis, MO) was added. Color development was measured at 405 nm with an automated microplate ELISA reader. A standard curve was generated on each assay plate using serially diluted recombinant human TNF- $\alpha$ , IL-6, or IL-8.

**Western blotting analysis (MAPK).** The HMC-1 cells were pretreated with various concentrations of the FU extract (1, 10, and  $100\mu\text{g/mL}$ ) for 1 h and then stimulated with PMA plus A23187 for 30 min. They were harvested and washed twice with ice-cold PBS. The washed cell pellets were resuspended in a lysis buffer, and after immediate vortexing for 10 s, they were subjected to 20 min of incubation at  $4^{\circ}\text{C}$ . Protein fractions were obtained as supernatants after centrifugation at  $13,000 \times g$  at  $4^{\circ}\text{C}$  for 20 min. The protein concentration was determined using Bio-Rad protein assay reagent following the manufacturer's instructions. The various samples were separated on a 12% sodium dodecyl sulfate (SDS)-polyacrylamide gel. The proteins were then electrotransferred to a nitrocellulose membrane. The membrane was blocked with 1% skimmed milk and 1% BSA in PBS containing 0.5% Tween-20 (PBST) for 1 h at room temperature. It was washed lightly with PBST, and then incubated overnight at  $4^{\circ}\text{C}$  with anti-MAP Kinase Ab (1:1,000 dilution in 1% BSA in PBST) and then washed 3 times with PBST. Anti-mouse IgG, the secondary Ab (1:2,000 dilution in the blocking solution) was incubated for 1 h at room temperature, and the antibody-specific proteins were visualized by an enhanced chemiluminescence procedure with an enhanced chemiluminescence (ECL) detection reagent (Amersham Pharmacia, NJ). All samples were run in triplicate.

**Sensitization and elicitation procedure.** Allergic dermatitis was induced by repeated topical application of DNFB, as previously described. In brief,  $25\mu\text{L}$  of 0.15% DNFB in acetone/olive oil (3:1) was applied topically to the outer and inner sides of the right ears of BALB/c mice 6 times at 7-d intervals. The FU extract was orally administered (80 mg/kg) for 5 d after each application of DNFB during sensitization. The skin thickness of the ear was measured 0, 24, 48, and

72 h after each application using a thickness dial gauge, and the increase in skin thickness was determined by subtracting the skin thickness before the first application from that at each of the above-mentioned time points.

**Histological examination.** A central strip of the ear was fixed in 3.5% paraformaldehyde and then embedded in paraffin. Sections (5  $\mu$ m) were stained using hematoxylin and eosin (H&E) for general histological evaluation and with toluidine blue for mast cell staining. Mast cells were evaluated by averaging the number of mast cells in 12 high-power fields (0.07 mm<sup>2</sup>). Each section was independently examined in a blinded manner by three investigators, and the mean values were used for analysis.

**Statistical analysis.** Measured values are presented as mean  $\pm$  standard error. Statistical analysis was performed using SAS statistical software (SAS Institute, Cary, NC). Treatment effects were analyzed by one-way ANOVA, followed by Duncan's multiple-range test, and  $p < 0.05$  was considered significant.

## Results

### Effects of FU on cytotoxicity in HMC-1 cells

HMC-1 cells were incubated with FU at 37 °C. After they were incubated for 24 h, cell viability was measured by MTT assay. FU (1, 10, and 100  $\mu$ g/mL) had no cytotoxic effect over a 24-h period (Fig. 1).

### Effect of the FU extract on PCA

PCA is one of the most important *in vivo* models for anaphylaxis in a local allergic reaction. Orally administered FU extract (80 mg/kg) significantly inhibited the PCA reaction in the SD rats (Fig. 2).

### Effect of the FU extract on histamine release from RPMCs

The FU extract inhibited compound 48/80-induced histamine release at concentrations of 0.01–1 mg/mL ( $p < 0.05$ ) in a concentration-dependent manner (Fig. 3).

### Effect of the FU extract on proinflammatory cytokine secretion

HMC-1 cells were stimulated with PMA and the calcium ionophore, A23187. To assess the effect of FU on PMA plus A23187-induced TNF- $\alpha$ , IL-6, and IL-8 secretion, the cells were pretreated with various FU concentrations (1, 10, and 100  $\mu$ g/mL) for 1 h before the addition of the stimulators. The results indicate that pretreatment of the cells with the FU extract inhibited IL-6, IL-8, and TNF- $\alpha$  secretion, and that the inhibitory effect of the FU extract was significant at concentrations of 1, 10, and 100  $\mu$ g/mL (Fig. 4A, B, and C).

### Effect of the FU extract on the activation of MAPK

HMC-1 cells were stimulated with PMA (25  $\mu$ M) plus A23187 (1  $\mu$ M) for 1 h, and the cell lysate was analyzed by Western blot. FU was pretreated for 30 min prior to PMA plus A23187 stimulation in HMC-1. The various total forms of ERK (T-ERK), JNK (T-JNK), and p38 (T-p38) were used as loading controls. Treatment with

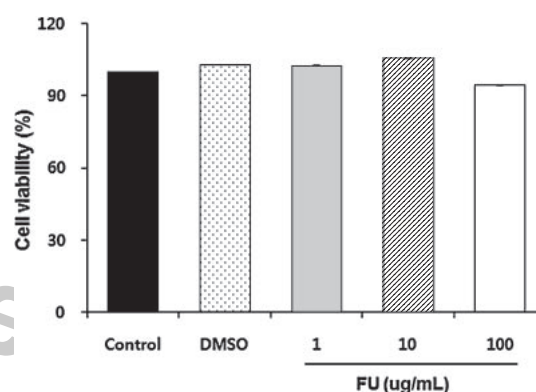


**Fig. 1.** Cytotoxicity of FU for Human Mast Cells (HMC-1 cells).

HMC-1 cell ( $2 \times 10^4$  cells/100  $\mu$ L) viability was evaluated by MTS colorimetric assay with 24 h of incubation after pretreatment with various concentrations of FU (1–100  $\mu$ g/mL). Data were compared with the data for untreated controls (% of control data). Data are presented as mean  $\pm$  SEM.

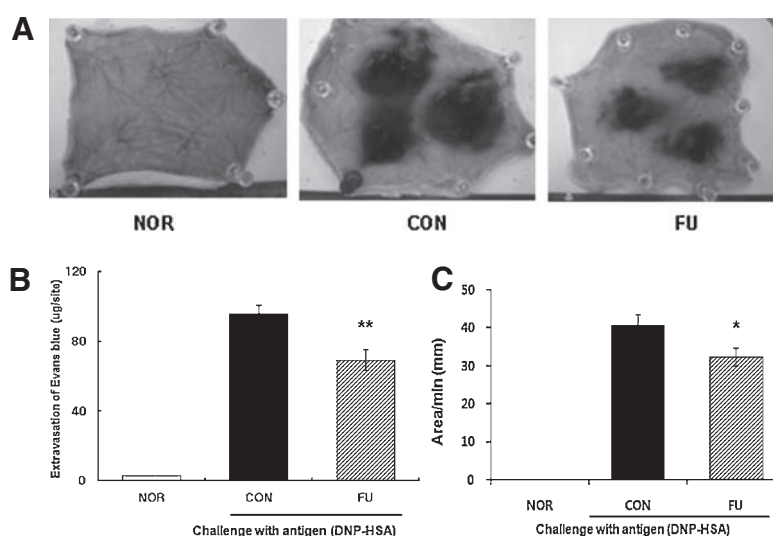


**Fig. 2.** Inhibitory Effects of FU on 48 h Passive Cutaneous Anaphylaxis in Rats.

Anti-DNP IgE was injected into dorsal skin sites. Samples (FU, 80 mg/kg) were orally administered 1 h before challenge with 2  $\mu$ g of DNP-HSA. A, Photogram of the internal surface of rat skin. B, Extravasation of Evans blue. Amounts of dye were extracted and measured by spectrophotometer. C, The patch of Evans blue was measured 30 min after antigen (DNP-HSA) injection. Each data point represents the mean  $\pm$  SEM for three independent experiments. \* $p < 0.05$ , \*\* $p < 0.01$  significantly different from the control value.

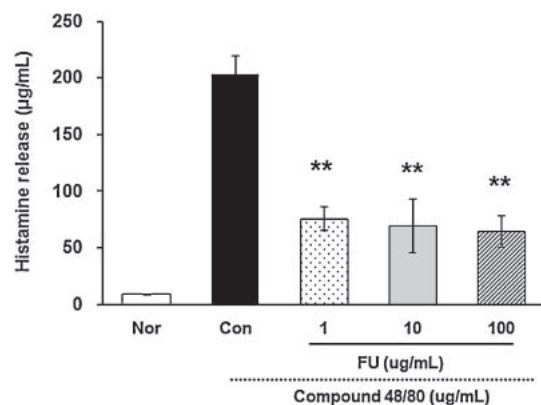


**Fig. 3.** Effects of FU on Compound 48/80-Induced Histamine Release from Rat Peritoneal Mast Cells.

Cells ( $2 \times 10^5$  cells) were pretreated with various concentrations of FU at  $37^\circ\text{C}$  for 10 min and then incubated with compound 48/80. Each data value represents the mean  $\pm$  SEM for three independent experiments. \*\* $p < 0.01$ , significantly different from the control value.

FU attenuated PMA plus A23187-induced phosphorylation of all three components of the MAPK pathway (P-ERK, P-JNK, and P-p38), especially at concentrations of 10 and  $100 \mu\text{g/mL}$  (Fig. 5).

#### *Histological measurements of ear thickness under repeated Ag exposure*

To assess the histological characteristics associated with ear thickness, ear biopsy specimens were examined at 0, 24, 48, and 72 h after the application of DNFB for 35 d. Histological study showed edema in the ear skin of the control group, but treatment with FU extract inhibited these inflammatory reactions (Fig. 6A and B). Mast-cell numbers were also calculated 35 d after challenge. The mast-cell numbers of the control group increased as compared to those of the untreated normal group. FU ( $80 \text{ mg/kg}$ ) led to significant inhibition of mast-cell accumulation in the chronic phase (Fig. 6C).

## Discussion

Mast-cell activation has been reported in a significant proportion of allergic inflammation sites. Mast cells are found throughout the human body, and their important function in the immediate-type allergic reaction is well recognized.<sup>15</sup> Compound 48/80 increases the permeability of the lipid bilayer membrane by causing a perturbation in it.<sup>16</sup> Thus, the increase in membrane permeability might be an essential trigger for the release of mediators from the mast cells. As indicated by previous studies, family of 48/80 basic compounds can induce histamine release from mast cells.<sup>17</sup>

Histamine is the best characterized and most potent vasoactive mediator implicated in the acute phase of type I allergic reactions.<sup>6</sup> When FU was orally administered to the rats, it potently inhibited the IgE-mediated PCA reaction. Rats with PCA are among the most important *in vivo* models for anaphylaxis in local allergic reaction.<sup>18</sup> This finding suggests that FU can be useful in the treatment of patients with allergic skin reactions.

Mast cells release inflammatory mediators, including histamine, heparin, tryptase, prostaglandins, and cytokines. In response to several stimuli, mast cells release

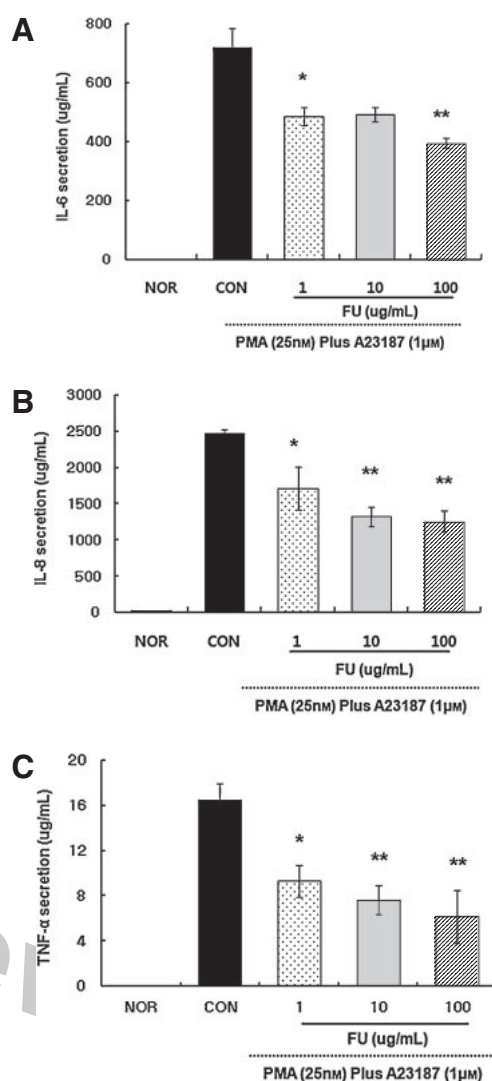


**Fig. 4.** Effects of FU on PMA Plus A23187-Induced IL-6, IL-8, and TNF- $\alpha$  Production.

HMC-1 cells ( $3 \times 10^5$  cells/mL) were pretreated with the sample for 1 h and stimulated by 7 h, incubation with PMA (25 nM) plus A23187 ( $1 \mu\text{M}$ ). IL-6 (A), IL-8 (B), and TNF- $\alpha$  (C) production significantly decreased in the PMA plus A23187-induced HMC-1 cells. Data represent mean  $\pm$  SEM for three independent experiments. \* $p < 0.05$ , \*\* $p < 0.01$ , significantly different from the control value.

TNF- $\alpha$ , IL- $1\beta$ , IL-4, IL-5, IL-6, IL-8, and IL-13.<sup>18,19</sup>

IL-6 and IL-8 are important inflammatory cytokines that are secreted from activated mast cells. IL-6 is a multifunctional protein. In innate immune responses, it stimulates the synthesis of acute-phase proteins by hepatocytes and thus contributes to the systemic effects of inflammation.<sup>20</sup> IL-8 is a potent neutrophil chemoattractant and activating factor. It serves as a chemical signal that attracts neutrophils at the site of inflammation.<sup>21</sup> TNF- $\alpha$  is a cytokine involved in systemic inflammation and is a member of a group of cytokines that stimulate the acute phase reaction.<sup>22</sup> In this study, we found that the expression of proinflammatory cytokines was inhibited by FU in HMC-1 cells.

ERK, p38, and JNK are activated in response to various extracellular stimuli and have different downstream targets, and therefore perform different functions, including mediation of apoptosis, proliferation, and inflammation.<sup>10–12</sup> The MAPK cascade is an important

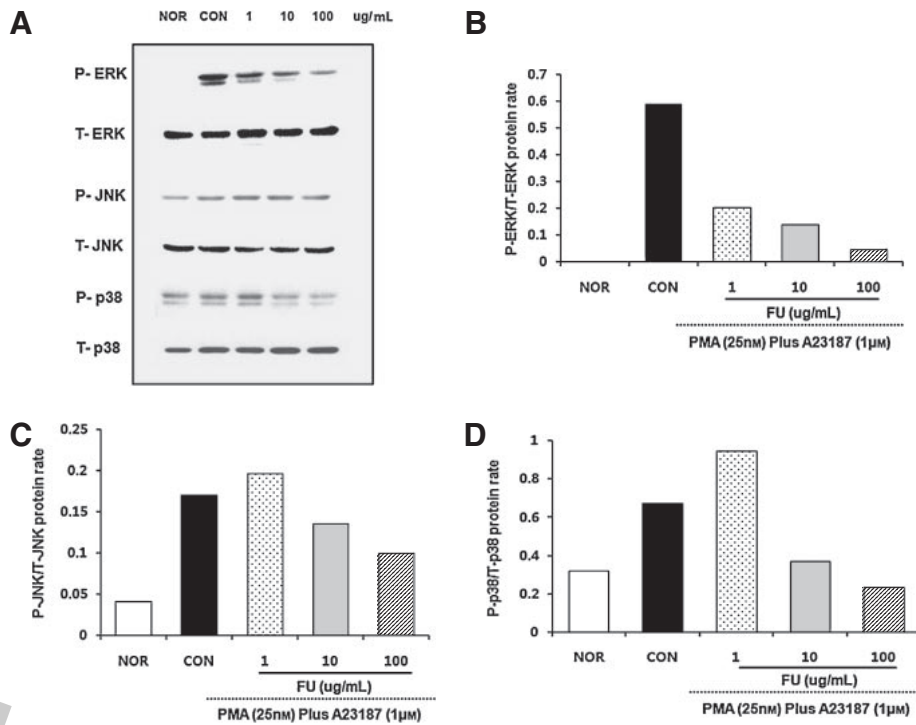


**Fig. 5.** Effect of FU on PMA Plus A23187-Induced MAP Kinase Activation.

HMC-1 cells ( $2 \times 10^6$  cells/5 mL) were pretreated with sample for 1 h and stimulated for 30 min by incubation with PMA (25 nM) plus A23187 (1  $\mu$ M). A, Phosphorylation (P-) and total (T-) of MAP kinase were analyzed by Western blot. B, P-ERK/T-ERK protein rate. C, P-JNK/T-JNK protein rate. D, P-p38/T-p38 protein rate. Data are mean percentages for three independent experiments. Total forms of ERK, JNK, and p38 were used as loading controls.

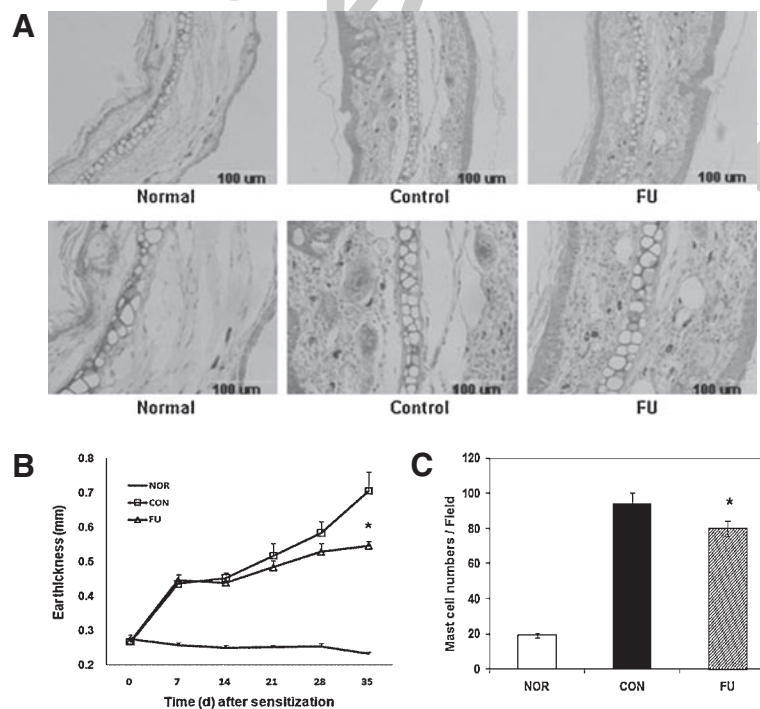


**Fig. 6.** Histopathological Examination of Ear Thickness under Repeated Ag Exposure.

A, Sections were stained with hematoxylin and eosin (upper panel, magnification 100 $\times$ ; lower panel, 400 $\times$ ), and then calculated under a light microscope. B, Time course of change in ear thickness and effects of FU (80 mg/kg). Dermatitis was elicited by repeated application of 0.15% DNFB (in acetone/olive oil) on the outer and inner sides of the mouse ear 6 times at 7 d intervals. The same volume of DNFB was applied to the control mice. C, Mast-cell accumulation in the ear on day 35. Data are mean  $\pm$  SEM. \* $p < 0.05$  as compared with the vehicle group.

signaling pathway in immune responses. MAPK pathways are appropriate targets for the pharmacological treatment of inflammatory disorders.<sup>23</sup> In this study, FU inhibited PMA plus A231867-induced 3 MAPK pathway components, especially ERK and p38, in HMC-1

cells. Thus it is evident that inhibition of MAPKs by FU plays a role in controlling the inflammatory process.

It is well-known that nuclear factor  $\kappa$ B (NF- $\kappa$ B) is a major factor downstream of ERK, JNK, and p38 MAPK.<sup>24–26</sup> Although we did not investigate the effects

of FU on NF- $\kappa$ B activation, our results indicate that the inhibitory effects of FU on cytokine release are related to the ERK, JNK, and p38 MAPK pathways, including NF- $\kappa$ B activation.

Mast cells play an integral role in the inflammatory response by accumulating at sites of inflammation and mediating the production of inflammatory cytokines.<sup>27)</sup> The chronic inflammatory response is dependent on the recruitment of mast cells, which is regulated by the expression of adhesion molecules on the cells.<sup>28)</sup> Mast-cell recruitment into tissues is thought to occur due to the release of immature mast cell precursors from the bone marrow into the peripheral blood, followed by the migration of these precursors into the tissues and their subsequent differentiation into mature mast cells.<sup>29)</sup> In the present study, FU significantly inhibited mast-cell recruitment induced by repeated application of DNFB. This suggests that FU regulates mast-cell recruitment at inflammatory sites.

FU has been known clinically for its anti-tussive expectorant effect in Chinese medicine. Peimine is known to a major component of *Fritillaria* herbs. Isoverticine, ebeiedine, and ebeiedinone have been also isolated from *Fritillaria* species,<sup>30)</sup> but the anti-inflammatory reactions due to FU and peimine are yet to be studied (Supplemental Fig. 1; see *Biosci. Biotechnol. Biochem.* Web site).

In conclusion, FU inhibited mast-cell derived inflammatory allergic reactions by blocking the release of histamine and proinflammatory cytokines and MAPKs. Furthermore, the *in vivo* and *in vitro* anti-allergic effects of FU play a critical role in the progression of inflammatory responses, and this suggests the potential therapeutic application of this agent to control inflammatory allergic diseases. Further study is needed to investigate the mechanisms of FU in detail, and to isolate the active compounds.

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