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Research Paper

Pyeongwee-San extract (KMP6): a new anti-allergic effect

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Keywords
histamine; histidine decarboxylase; KMP6; mast cell; passive cutaneous anaphylaxis; Pyeongwee-San

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Abstract

Objectives The prevalence of allergic diseases is increasing due to rapid industrialization and changes in lifestyle. Pyeongwee-San (KMP6) is a traditional Korean medicine that has been used as a basic prescription for digestive disorders. This study investigated the efficacy of KMP6 and its component hesperidin on experimental allergic models.

Methods The anti-allergic effect of KMP6 was studied against a compound 48/80-induced systemic anaphylactic reaction and the ear swelling response. In addition, a human mast cell line (HMC-1) was used to analyze the activity of histidine decarboxylase. Passive cutaneous anaphylaxis (PCA) from immunoglobulin E (IgE) was used.

Key findings KMP6 and hesperidin inhibited the compound 48/80-induced systemic anaphylactic reaction and the ear swelling response as well as histamine release, intracellular calcium levels and tryptase release from rat peritoneal mast cells. KMP6 inhibited histidine decarboxylase activity in stimulated HMC-1 cells and macrophages. In addition, KMP6 inhibited the PCA reaction induced by IgE as well as the levels of IgE, interleukin (IL)-4, IL-5, IL-6 and IL-13 in serum from mice.

Conclusions These results suggest that KMP6 may exert an anti-allergic effect through not only the inhibition of mast cell degranulation but also the inhibition of histamine synthesis.

Introduction

Allergies are a significant health problem affecting modern people. Allergic diseases have developed from complex interactions between genes and the environment. Digestive tract diseases have become a more common problem these days and are often blamed on lifestyle choices and poor eating habits. It has been reported that delayed cutaneous hypersensitivity reactions are induced by intragastrically administered hapten in mice.[1] It has also been asserted that ulcerative colitis is closely associated with atopic dermatitis.[2] Recently, it was reported that *Helicobacter pylori* infections play a role in the pathogenesis of a variety of skin diseases.[3]

Mast cells are strongly associated with immediate hypersensitivity, an immune reaction resulting from the release of chemical mediators and cytokines after immunoglobulin E (IgE)/FcεRI-mediated activation.[4,5] Activated mast cells can produce histamine, as well as a wide variety of other inflammatory mediators such as proteases and tryptase, and several proinflammatory cytokines such as tumor necrosis factor (TNF)-α, interleukin (IL)-1β, vascular endothelial growth factor (VEGF) and IL-4, IL-5, IL-6 and IL-13.[6] The formation of histamine from its precursor, L-histidine, is catalyzed in a single step by the enzyme histidine decarboxylase (HDC).[7] HDC is expressed in a variety of inflammatory cells, including mast cells and macrophages.[8]

During cell activation, Ca^{2+} acts as a second messenger.[9] Compound 48/80 or the aggregation of IgE receptors increases intracellular calcium levels in mast cells. The increase of intracellular Ca^{2+} levels has been proposed as an essential trigger for mast cell activation and degranulation.[10]

Pyeongwee-San (KMP6) is a traditional Korean medicine that has been used as a basic prescription for digestive disorders for centuries. Allergy is related to gastrointestinal tract disease. KMP6 has frequently been used for the treatment of atopic dermatitis in clinical care. Oh *et al.* have reported that KMP6 alleviates an ovalbumin (OVA)-induced allergic rhinitis reaction.[11] However, the effect of KMP6 in mast-cell-mediated allergic reactions has not been elucidated. To investigate precisely the effect and mechanism of KMP6 and its component hesperidin in mast-cell-mediated allergic

reactions, the anti-allergic effects of KMP6 and hesperidin were analyzed using in-vivo and in-vitro models.

Materials and Methods

Materials

Compound 48/80, anti-dinitrophenyl (DNP) IgE, DNP-human serum albumin (HSA), phorbol 12-myristate 13-acetate (PMA), A23187 (calcimycin; $C_{29}H_{37}N_3O_6$), Evans blue, dexamethasone, BAPTA-AM, Fura-2/AM, hesperidin and lipopolysaccharide (LPS) were purchased from Sigma Chemical Co. (St Louis, MO, USA). Isocove's Modified Dulbecco's Medium (IMDM) and Dulbecco's modified Eagle's medium (DMEM) were from Gibco BRL (Grand Island, NY, USA). Fetal bovine serum (FBS) was from Life Sciences (Grand Island, NY, USA); IgE, IL-4, IL-6, and TNF-α antibodies were from BD Pharmingen (Torreyana Road, San Diego, CA, USA). Recombinant interferon (IFN)-γ and IL-1β, IL-5, IL-13 and VEGF antibodies were from R&D Systems (Minneapolis, MN, USA); HDC and actin antibodies were from Santa Cruz Biotechnology (Santacruz, CA, USA), Talion was from Dong-A Pharmaceutical Co. (Seoul, Republic of Korea); thioglycollate was from Difco Laboratories (Detroit, MI, USA).

Animals

The original stock of male ICR mice (4 weeks old) and male Sprague–Dawley rats (7 weeks old) were purchased from the Dae-Han Experimental Animal Center (Eumsung, Chungbuk, Republic of Korea). The animals were maintained under conventional conditions and experiments were performed under approval from the animal care committee of Kyung Hee University (KHUASP(SE)-11–009).

Preparation of KMP6

KMP6 was provided by the Korea Medi Inc. (Seoul, Republic of Korea). A prescription of KMP6 (46.6 g) consists of *Atractylodes japonica* (13.3 g), *Machilus thunbergii* (10 g), *Citrus sunki* (10 g), *Zizyphus jujube* (6.7 g), *Glycyrrhiza uralensis* (3.3 g), and *Zingiber officinale* (3.3 g). An extract of KMP6 was prepared by decocting with distilled water for approximately 3 h. The decocted extract was filtered and lyophilized. The KMP6 powder was dissolved in distilled water and then filtered through a 0.22-μm syringe filter. We have previously reported that the contents of hesperidin and glycyrrhizin in KMP6 determined by HPLC analysis are about 5.26 and 2.60 mg/g.[12] In this study, hesperidin was used as a major compound. To compare the effects of KMP6, we obtained Han Shin Pyeongwee-San (HS-PS, an over-the-counter drug) from Han Kook Shin Yak Pharmaceutical Co., Ltd (Nonsan, Republic of Korea). The HS-PS granules were prepared by dissolving in distilled water and autoclaving for sterilization, afterwards being kept at 4°C. The dose of HS-PS (2 g/kg) was prepared to be twice as strong as KMP6 (1 g/kg) because HS-PS granules (3.5 g) contain some excipients (1.7 g). Dilutions were made in saline then filtered through a 0.45-μm syringe filter.

Mast cell culture

Human mast cell line HMC-1 cells were grown in IMDM and supplemented with 100 U/ml of penicillin, 100 μg/ml of streptomycin and 10% FBS at 37°C in 5% CO_2 with 95% humidity.

Peritoneal macrophages culture

Peritoneal macrophages culture was performed as previously described.[13]

Compound 48/80-induced systemic anaphylactic reaction

Mice were given an intraperitoneal injection of the mast cell degranulator compound 48/80 (8 mg/kg). Drugs were administered orally, with sonde 1 h before the injection of compound 48/80. The period for observation of mortality was based on the control mice, which had died within 20 min of receiving compound 48/80. Mortality was monitored for 1 h after the induction of anaphylactic shock.

Compound 48/80-induced ear swelling response

Compound 48/80 was injected intradermally (100 μg/site) into the dorsal side of a mouse ear ($n = 5$) using a microsyringe with a 28-gauge hypodermic needle. Ear thickness was measured with a digimatic micrometer (Mitutoyo, Japan) under mild anesthesia. The ear swelling response corresponded to an increase of thickness above baseline control values and was determined 40 min after compound 48/80 or saline injection. KMP6 (0.01–1 g/kg) was administered orally 1 h before compound 48/80-injection. The values obtained would appear to represent the effect of compound 48/80 rather than the effect of saline injection (physical swelling), since the ear swelling response evoked by physiologic saline returned almost to baseline thickness within 40 min.

Preparation of rat peritoneal mast cells

Rat peritoneal mast cells (RPMCs) were isolated as previously described.[14]

Histamine assay

The histamine assay was performed as previously described.[15]

Fluorescent measurements of intracellular calcium levels

Purified RPMCs suspensions (2×10^5 cells/ml) were pre-incubated with the drugs for 40 min, and then incubated for 15 min with compound 48/80 (6 μg/ml). Cells were harvested and treated with 4 μM of Fura-2/AM in DMEM for 30 min at room temperature. After washing twice with DMEM (without phenol red), intracellular calcium levels were measured using a spectrofluorometer.

Tryptase assay

Purified RPMC suspensions (2×10^5 cells/ml) were preincubated with the drugs for 40 min and then incubated for 15 min with compound 48/80 (6 μg/ml). Tryptase from culture supernatants was assayed by using a mast cell degranulation assay kit (Millipore Co., Billerica, MA, USA).

Morphology

Purified RPMCs suspensions (1×10^4 cells/ml) were preincubated with the drugs for 40 min and stimulated with compound 48/80 (6 μg/ml). The morphological changes of the cells were observed with an inverted microscope. Basal RPMCs were circular in shape and no granules extruded from the cells. In RPMCs that were stimulated with compound 48/80, extensive degranulation could be clearly seen and multiple granules extruded from the cells. The degranulated cells showed swelling, disrupted boundaries, obvious irregular shapes and vacuoles, and granule release increased by exocytosis compared to the basal cells.[16] For statistical analysis, five sections were randomly selected and the number of degranulated RPMCs was counted by two blinded observers.

Histidine decarboxylase assay

The HDC assay was performed as previously described.[15]

Western blot analysis

The stimulated HMC-1 cells were lysed and separated through 10% SDS-PAGE. After electrophoresis, the protein was transferred to nitrocellulose membranes and then the membranes were blocked and incubated with primary and secondary antibodies. Finally, the protein bands were visualized by an enhanced chemiluminesence assay purchased from Amersham Co. (Newark, NJ, USA) following the manufacturer's instructions.

Passive cutaneous anaphylaxis reaction

The PCA reaction was performed as previously described.[15]

Enzyme-linked immunosorbent assay

We collected the sera from mice after the PCA reaction. These sera were assayed for IgE, IL-1β, IL-4, IL-5, IL-6, IL-13, TNF-α and VEGF protein levels by the ELISA method according to the manufacturer's specifications (R & D System and BD Pharmingen).

Statistical analysis

The results shown are a summary of the data from at least three experiments and are presented as the mean ± SEM. Statistical evaluation of the results was performed by the Kruskal–Wallis test and Dunn's post-hoc test. The results were considered significant at a value of $P < 0.05$.

Results

Effect of KMP6 on compound 48/80-induced systemic anaphylactic reaction

To assess the contribution of KMP6 in anaphylactic reactions, we first used the murine model of systemic anaphylactic reaction. As shown in Table 1, oral administration of saline as

Table 1 Effect of KMP6 on compound 48/80-induced systemic anaphylactic reaction

Treatment	*n*	Dose (g/kg)	Compound 48/80 (8 mg/kg)	Mortality (%)
None (saline)	10	–	+	100.00 ± 0.00
KMP6	8	0.01	+	25.00 ± 25.00*
KMP6	8	0.1	+	12.50 ± 12.50*
KMP6	12	1	+	12.50 ± 12.50*
HS-PS	8	2	+	12.50 ± 12.50*
Hesperidin	18	0.001	+	11.25 ± 1.25*
Talion	18	0.01	+	11.25 ± 1.25*

The groups of mice were orally administered with saline or drugs 1 h before compound 48/80 injection (*n* = the total number of mouse/group). The compound 48/80 solution was given intraperitoneally to the groups of mice. Mortality (%) is presented as the 'number of dead mice × 100/total number of experimental mice'. Each value was presented as the mean ± SEM of three independent experiments. KMP6, Pyeongwee-San; HS-PS, Pyeongwee-San from Han Kook Shin Yak Pharmaceutical Co. *$P < 0.05$.

control induced a fatal reaction in 100% of each group. We also tested the effect of KMP6, HS-PS (2 g/kg) and hesperidin (an active component of KMP6, 1 mg/kg). Talion (a histamine H1 receptor antagonist; 10 mg/kg) was used as a positive control. When the drugs were orally administered 1 h before compound 48/80 injection, KMP6, HS-PS, hesperidin and Talion significantly inhibited compound 48/80-induced mortality ($P < 0.05$; Table 1). In addition, hesperidin was shown to be the active compound of KMP6 from Table 1.

Effect of KMP6 on compound 48/80-induced ear swelling response

To additionally examine the inhibitory effect of KMP6 on anaphylactic reactions, we examined the ear swelling response. As shown in Table 2, when mice were pretreated with KMP6 for 1 h, the ear swelling responses to compound 48/80 were significantly inhibited (1 g/kg KMP6; $P < 0.05$).

Effect of KMP6 on degranulation of RPMCs

To clarify the effect of KMP6 on the degranulation of RPMCs, we measured the factors related to degranulation such as histamine release, intracellular calcium level and tryptase release from RPMCs. KMP6 and HS-PS significantly inhibited compound 48/80-induced histamine release, intracellular calcium levels and tryptase release from RPMCs ($P < 0.05$; Figure 1a–c). BAPTA-AM, a calcium chelator, was used as a reference drug on intracellular calcium levels. We then additionally examined the degranulation of RPMCs on the basis of morphology. These images provided the evidence that compound 48/80 induces the degranulation of RPMCs. RPMCs were treated with KMP6 (1 mg/ml), HS-PS, hesperidin, dexamethasone (an anti-inflammatory drug) or Talion for 40 min prior to stimulation with compound 48/80. We counted the number of degranulated RPMCs based on phenotype. As a result, we showed that KMP6, HS-PS, hesperidin and Talion inhibit the degranulation of RPMCs ($P < 0.05$; Figure 1d).

Effect of KMP6 on activity of HDC from stimulated HMC-1 cells and macrophages

To evaluate the effect of KMP6 on histamine synthesis, we investigated the activity and expression of HDC from PMA plus calcium ionophore A23187-stimulated HMC-1 cells. Dexamethasone has been reported to inhibit the elevation of HDC mRNA, HDC activity and histamine levels,[17] therefore we used dexamethasone as a reference drug. We found that KMP6 significantly inhibited the activity ($P < 0.05$) and expression of HDC in PMA plus A23187-stimulated cells (Figure 2a and b). HS-PS also significantly inhibited the activity ($P < 0.05$) and expression of HDC. Furthermore, KMP6 (1 mg/ml) and hesperidin inhibited the activity of HDC in IFN-γplus LPS-stimulated macrophages (Figure 2c).

Effect of KMP6 on passive cutaneous anaphylaxis reaction

PCA is one of the most important murine models of anaphylaxis in allergic reactions.[18] When KMP6 and HS-PS were orally administered to the mice, the PCA reaction was significantly inhibited ($P < 0.05$; Figure 3). To further clarify the effect of KMP6 on the IgE-induced allergic reaction, blood was taken from mice with the PCA reaction. We measured the levels of histamine, IgE, IL-1β, IL-4, IL-5, IL-6, IL-13, TNF-α and VEGF in serum using the ELISA method (Table 3). KMP6 significantly inhibited the levels of serum IgE, IL-4, IL-5, IL-6 and IL-13 ($P < 0.05$). Moreover, HS-PS significantly inhibited the levels of serum IgE, IL-4, IL-5, IL-6 and TNF-α ($P < 0.05$). However, the levels of serum histamine, IL-1β and VEGF were not significantly inhibited by KMP6 or HS-PS.

Discussion

Mast cells are unique immune cells that release a spectrum of chemical mediators contributing to the inflammatory symptoms of allergic disorders. Compound 48/80, the condensed product of N-methoxyphenylamine with formaldehyde, is known to be one of the most potent mast cell secretagogues.[19] Compound 48/80, because of its potency, became an experimental exemplar of this category of secretagogues.[20] In addition, Compound 48/80 is a potent inducer of degranulation and the release of histamine and other chemical mediators responsible for anaphylactic symptoms from mast cells.[21,22] Compound 48/80 also increases intracellular calcium levels. The intracellular calcium pathways are

Table 2 Effect of KMP6 on compound 48/80-induced ear swelling response

Treatment	Dose (g/kg)	Thickness of ear (mm)	Inhibition rate (%)
None (saline)	–	0.142 ± 0.002	–
KMP6	0.01	0.125 ± 0.005	12.0 ± 0.0
KMP6	0.1	0.102 ± 0.008	28.2 ± 2.0
KMP6	1	0.070 ± 0.005	50.7 ± 1.0*

20 μl of compound 48/80 (100 μg/site) was applied intradermally (total $n = 10$/group). The mice were orally administered with various concentrations of KMP6 for 1 h before compound 48/80 application. Each value was presented as the mean ± SEM of three independent experiments. KMP6, Pyeongwee-San. *$P < 0.05$.

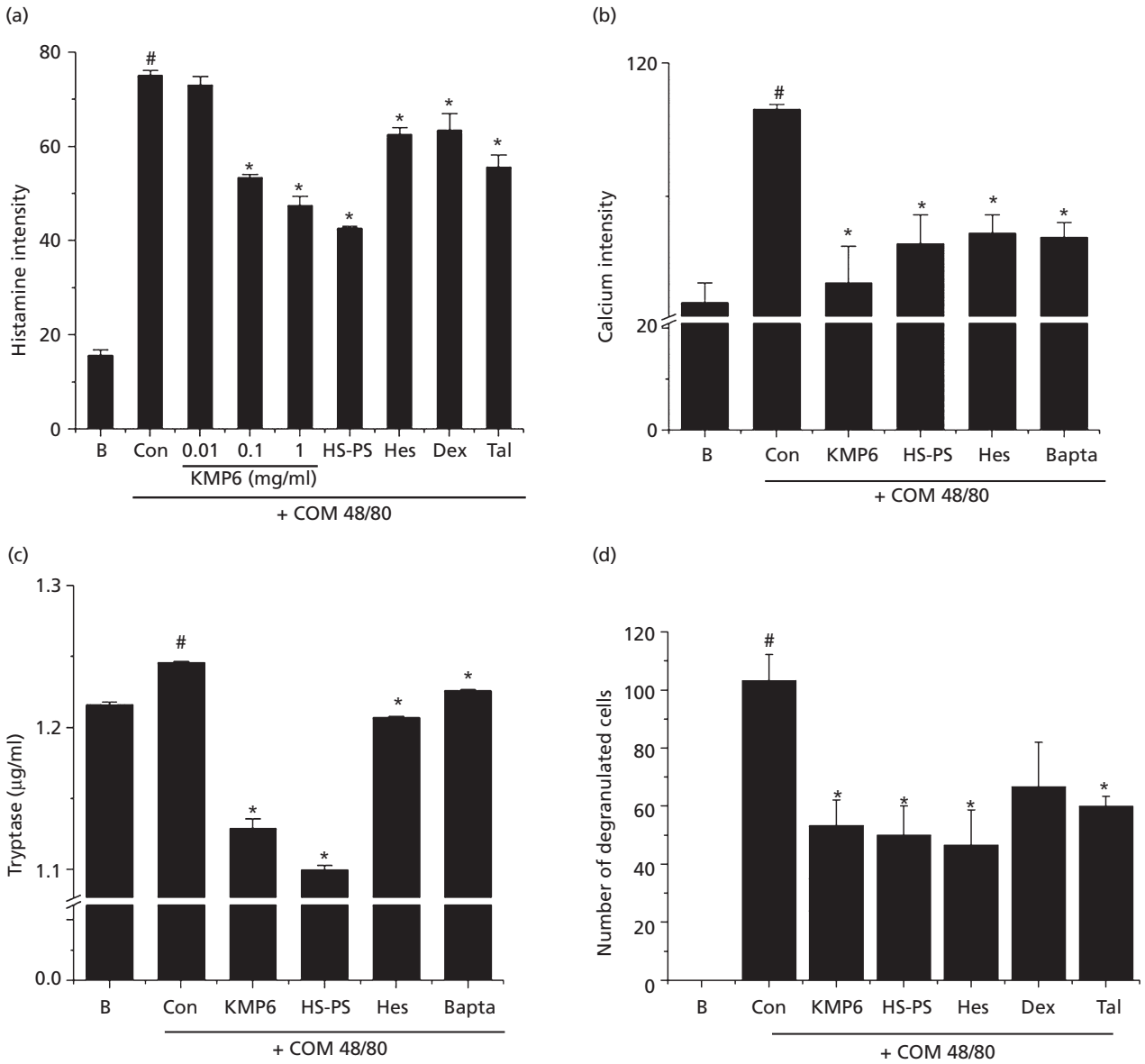

Figure 1 Effect of KMP6 on degranulation of RPMCs. RPMCs (2×10^5 cells/ml) were pre-incubated with KMP6 (0.01, 0.1, 1 mg/ml), HS-PS (2 mg/ml), Hes (1 μg/ml), Dex (100 nM), and Tal (0.01 mg/ml) or BAPTA-AM (10 μM) at 37°C for 10 min prior to incubation with compound 48/80. (a) Histamine release was measured from culture supernatants. (b) The intracellular calcium levels were measured from cells using the fluorescence. (c) Tryptase release was measured from culture supernatants by using a tryptase assay kit. (d) The number of degranulated mast cells was counted as the mean ± SEM. Each datum represents the mean ± SEM of three independent experiments. B, unstimulated cells; Con, compound 48/80-stimulated cells; COM 48/80, compound 48/80; KMP6 (b–d, 1 mg/ml), Pyeongwee-San; HS-PS, Pyeongwee-San from Han Kook Shin Yak Pharmaceutical Co.; Hes, hesperidin; Dex, dexamethasone; Tal, Talion; Bapta, BAPTA-AM. $^*P < 0.05$, significantly different from compound 48/80-stimulated cells. $^{\#}P < 0.05$, significantly different from the unstimulated cells.

critical to the degranulation of mast cells. Compound 48/80 has also been reported to increase the permeability of the lipid-bilayer membrane by causing a perturbation of the membrane. This means the increase of membrane permeability may be an essential trigger for the release of mediators from mast cells.[23] KMP6 or HS-PS significantly inhibit compound 48/80-induced systemic anaphylactic reactions (Table 1), the ear swelling response (Table 2) and intracellular calcium levels. Hence we can assume that KMP6 and HS-PS may exhibit a membrane-stabilizing action.

Upon degranulation, a range of mediators are released from mast cells, including histamine, tryptase and some

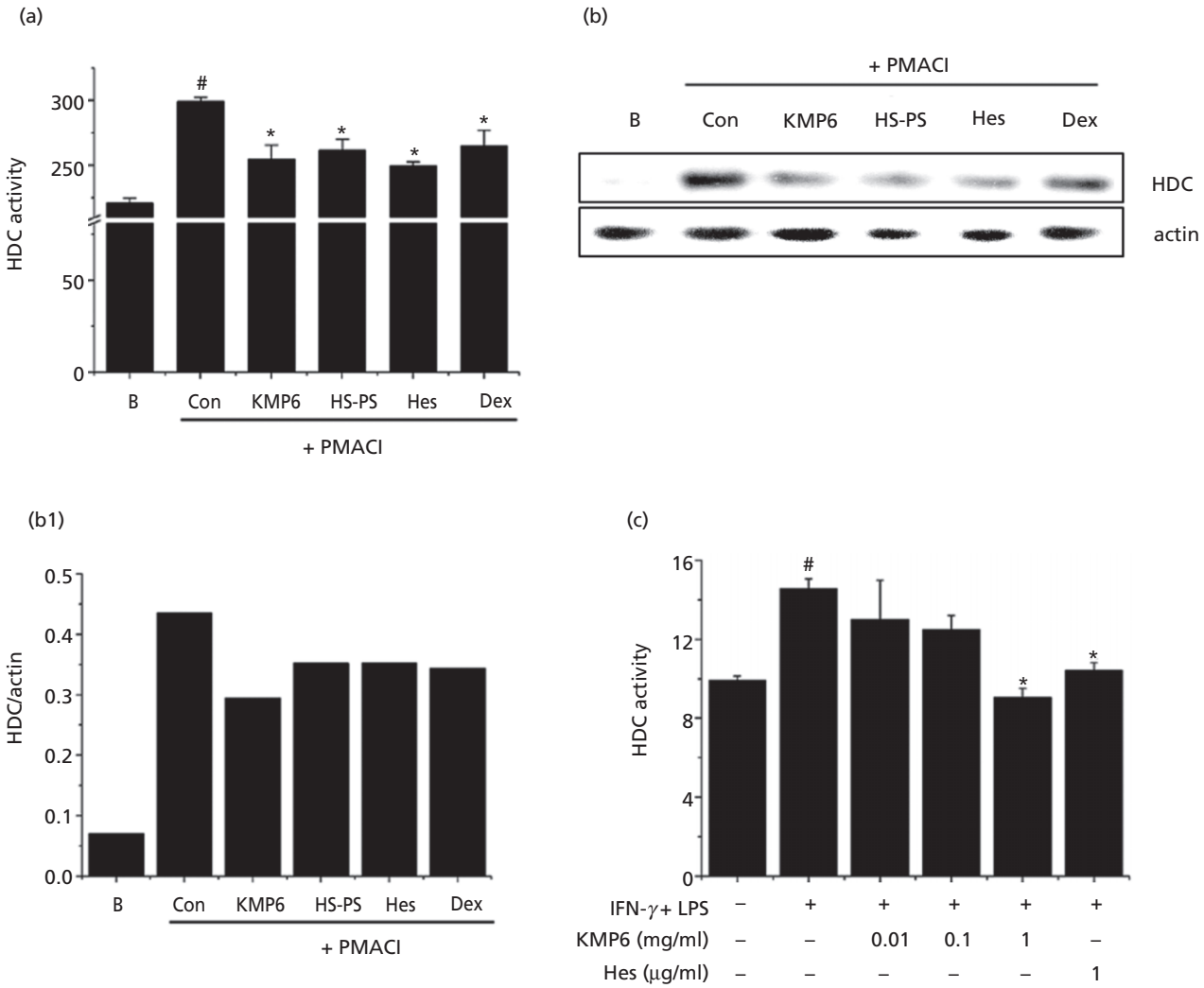

Figure 2 Effect of KMP6 on activity of HDC from stimulated HMC-1 cells and macrophages. HMC-1 cells were pretreated with or without drugs for 2 h before PMA plus A23187 stimulation. The activity (a) and expression (b) of HDC were evaluated by using HDC assay and Western blot analysis. (b1) Each protein level was quantified by densitometry. B, unstimulated cells; Con, PMA plus A23187-stimulated cells (PMACI); KMP6 (1 mg/ml), Pyeongwee-San; HS-PS (2 mg/ml), Pyeongwee-San from Han Kook Shin Yak Pharmaceutical Co.; Hes (1 μg/ml), hesperidin; Dex (100 nM), dexamethasone. (c) Macrophages were pretreated with various concentrations of KMP6 or Hes for 2 h before IFN-γ(10 U/ml) and LPS (100 ng/ml) stimulation. The HDC activity was evaluated by using the HDC assay. Each datum represents the mean ± SEM of three independent experiments. *$P < 0.05$, significantly different from stimulated cells. $^{\#}P < 0.05$, significantly different from the unstimulated cells.

cytokines. Histamine and tryptase are regarded as the principal products of activated mast cells.[24] Histamine, which is formed from L-histidine by HDC, has been widely used as a marker of mast cell degranulation *in vitro*.[25] Numerous anti-allergic drugs such as bepotastine besilate (the principal component of Talion) are reported to be able to inhibit the release of histamine and tryptase from human mast cells.[24,26] Dexamethasone also suppresses histamine synthesis by repressing both the transcription and activity of HDC in allergic rats.[27] We showed that KMP6 and HS-PS significantly inhibit the release of histamine and tryptase from RPMCs (Figure 1). KMP6 and HS-PS also significantly inhibit the activity and expression of HDC from HMC-1 cells as well as macrophages (Figure 2). Therefore, we can speculate that KMP6 and HS-PS exert strong anti-allergic effects through not only the inhibition of mast cell degranulation but also the inhibition of histamine synthesis.

The secretory responses of mast cells can be induced by the aggregation of their cell surface-specific receptors for IgE by the specific antigen.[28,29] Mast cells stimulated by cross-linking with antigen-specific IgE and FCεRI promote the immediate release of preformed mediators (histamine and

tryptase) as well as the synthesis of delayed phase cytokines.[30] In the present study, mice administered KMP6 or HS-PS were protected from this IgE-induced allergic reaction (Figure 3 and Table 3). It is conceivable that KMP6 and HS-PS inhibit the initial phase of immediate-type allergic reactions, probably by regulation of mast cell degranulation. The levels of IgE and histamine in the serum of the asthma murine model were significantly increased compared with the control mice.[31] Levels of inflammatory cytokines, such as TNF-α and IL-6, were increased in the serum of mice of the atopic dermatitis model NC/Nga.[32] The levels of IL-4, IL-5 and IL-13 in serum from allergic rhinitis or dermatitis patients increased compared to normal healthy controls.[33] Our results also show that the levels of serum IgE, IL-4, IL-5, IL-6 and IL-13 increase in mice under the PCA reaction, whereas the levels of IgE, IL-4, IL-5, IL-6 and IL-13 decrease in KMP6-administered mice under the PCA reaction. Therefore, we can deduce that KMP6 inhibits mast-cell-mediated allergic reactions.

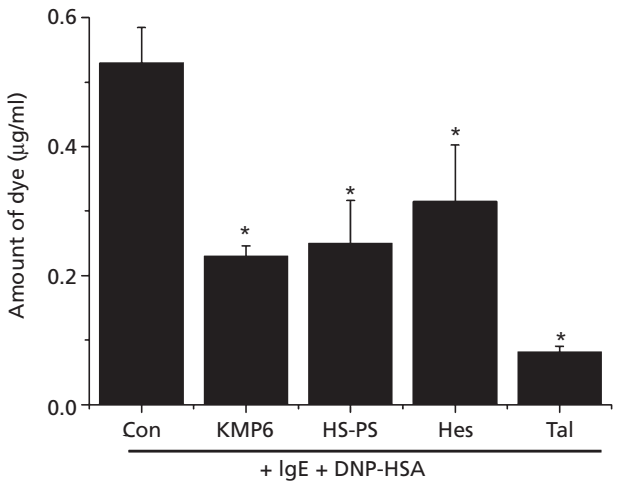

Figure 3 Effect of KMP6 on PCA reaction. KMP6 (1 g/kg), HS-PS (2 g/kg), Hes (0.001 g/kg), or Tal (0.01 g/kg) were administered orally 1 h before the challenge with antigen (DNP-HSA). Each amount of dye is presented as the mean ± SEM of three independent experiments. Con, saline-administrated mice (IgE + DNP-HSA); KMP6, Pyeongwee-San; HS-PS, Pyeongwee-San from Han Kook Shin Yak pharmaceutical Co.; Hes, hesperidin; Tal, Talion. *$P < 0.05$, significantly different from the saline-administrated mice.

As already described in the Materials and Method section, KMP6 consists of six different herbs. Each of them or their components has already been reported to have an effect on the symptoms of allergy. Mice administered with *Atractylodes lancea* D.C extract experienced suppression of OVA-mediated allergic diarrhea by preferential stimulation of Th1-type immune responses.[34] Atractylenolide III, a component of *Atractylodes lancea* D.C, significantly decreases histamine release increased by PMA plus A23187 in HMC-1 cells.[35] The methanol extract of *Citrus unshiu* S. Marcov. shows a potent inhibitory activity against histamine release from basophils of patients suffering from seasonal allergic rhinitis to ceder pollen.[36] Magnolol and honokiol, the main components of *Magnolia officinale* Sieb. et Zucc., inhibit the contraction of porcine tracheal smooth muscle through the blockage of Ca^{2+} influx in the asthmatic model.[37] Magnolol also inhibits compound 48/80-induced histamine release in a dose-dependent manner from RPMCs.[38] Beta-glycyrrhetinic acid, a component of *Glycyrrhiza uralensis* Fischer, inhibits production of IgE in OVA-induced asthma mice.[39] In addition, glycyrrhizin inhibits the PCA reaction.[40] Jujubosides A1 and C and acetyljujuboside B, components of *Zizyphus jujuba var. inermis* (Bunge) Rehder, were found to inhibit histamine release from RPMCs induced by the antigen–antibody reaction.[41] IL-4 and IL-5 levels in the lungs as well as specific IgE in serum were clearly diminished in mice treated with *Zingiber officinale* Roscoe extract relative to their controls after allergen sensitization and challenge.[42]

Table 3 Effect of KMP6 on IgE-induced histamine and cytokine production

	B	Con	KMP6	HS-PS	Hes	Tal
Histamine	5.083 ± 0.249	6.259 ± 1.385	4.374 ± 0.062	4.300 ± 0.059	4.647 ± 0.225	4.626 ± 0.127
IgE (ng/ml)	24.214 ± 1.188	28.760 ± 1.352#	24.173 ± 0.850*	22.969 ± 0.452*	24.522 ± 0.682*	23.903 ± 0.926*
IL-1β (ng/ml)	0.256 ± 0.033	0.295 ± 0.031	0.265 ± 0.029	0.226 ± 0.018	0.211 ± 0.018	0.262 ± 0.029
IL-4 (ng/ml)	0.154 ± 0.005	0.234 ± 0.008#	0.194 ± 0.012*	0.200 ± 0.004*	0.208 ± 0.003	0.216 ± 0.007
IL-5 (ng/ml)	0.036 ± 0.003	0.082 ± 0.014#	0.030 ± 0.002*	0.025 ± 0.001*	0.030 ± 0.001*	0.028 ± 0.001*
IL-6 (ng/ml)	0.554 ± 0.013	0.770 ± 0.030#	0.625 ± 0.027*	0.538 ± 0.039*	0.679 ± 0.025	0.497 ± 0.026*
IL-13 (ng/ml)	0.342 ± 0.003	0.522 ± 0.022#	0.398 ± 0.013*	0.468 ± 0.008	0.477 ± 0.032	0.514 ± 0.027
TNF-α (ng/ml)	0.190 ± 0.005	0.243 ± 0.013#	0.194 ± 0.007	0.173 ± 0.013*	0.24 ± 0.020	0.201 ± 0.007
VEGF (ng/ml)	0.055 ± 0.006	0.064 ± 0.004	0.059 ± 0.005	0.060 ± 0.006	0.059 ± 0.004	0.055 ± 0.006

KMP6 (1 g/kg), HS-PS (2 g/kg), Hes (0.001 g/kg), and Tal (0.01 g/kg) were administered orally 1 h before the challenge with antigen (DNP-HSA). Serum was isolated from blood and then assayed using histamine assay and the ELISA method. Each datum represents the mean ± SEM of three independent experiments. B, normal mice; Con, saline-administrated mice (IgE + DNP-HSA); KMP6, Pyeongwee-San; HS-PS, Pyeongwee-San from Han Kook Shin Yak Pharmaceutical Co.; Hes, hesperidin; Tal, Talion. *$P < 0.05$, significantly different from the saline-administrated mice. #$P < 0.05$, significantly different from the normal mice. (total $n = 10$/group).

In this study, KMP6 showed a strong inhibitory effect on histamine release from RPMCs and PCA reaction, therefore KMP6 is helpful for the prevention of allergic reactions. We can deduce that KMP6 may contribute to their pharmacological effects by acting as an anti-allergic agent.

Conclusions

In conclusion, the results obtained in the present study provide evidence that KMP6 inhibits mast cell-mediated allergic inflammatory reactions. These results warrant further study of the anti-allergic mechanisms of KMP6 and suggest that it might be a candidate for prevention of atopic allergies.

Declarations

Conflict of interest

The authors declare that they have no conflicts of interest to disclose.

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