Sparassis crispa suppresses mast cell-mediated allergic inflammation: Role of calcium, mitogen-activated protein kinase and nuclear factor-κB

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Abstract. Allergic inflammatory disease such as food allergy, asthma and atopic dermatitis are increasing worldwide. In this study, we investigated the effect of water extract of *Sparassis crispa* (WESC) Fr. (Aphyllophoromycetideae) on mast cell-mediated allergic inflammation and the possible mechanisms of action. WESC inhibited compound 48/80-induced systemic anaphylaxis and serum histamine release in mice. WESC decreased immunoglobulin E (IgE)-mediated passive cutaneous anaphylaxis. Additionally, WESC reduced histamine release and intracellular calcium in human mast cells activated by phorbol 12-myristate 13-acetate (PMA) and calcium ionophore A23187. WESC decreased PMA and A23187-stimulated expression of pro-inflammatory cytokines, such as tumor necrosis factor (TNF)-α, inlerleukin (IL)-6 and IL-1β. The inhibitory effect of WESC on pro-inflammatory cytokines was nuclear factor-κB, extracellular signal-regulated kinase and p38 mitogen-activated protein kinase-dependent. Our results suggest potential therapeutic application of WESC in allergic inflammatory diseases.

Introduction

Mushrooms are good food materials whose immunomodulatory potential has been investigated. *Sparassis crispa* (Wulf.) Fr. (Aphyllophoromycetideae) is an edible mushroom used for a natural medicine that recently became cultivatable in Korea and Japan (1). Recently, *S. crispa* has been reported to have many biological effects such as antitumor, anti-angiogenic, anti-metastatic, and wound healing (1-3). The major component of *S. crispa* is β-D-glucan, a polysaccharide of D-glucose comprising a β-(1→3)-D-glucan backbone (3,4). According to previous research, the β-glucan content of *S. crispa* was confirmed to be immense, up to 43.6% of the dry weight (4). *S. crispa* derived β-glucan has been known for several beneficial effects, however its allergic responses have not been clarified.

Mast cells are broadly distributed throughout mammalian tissues and play various functions as regulators of allergic inflammation, such as asthma, atopic dermatitis, eczema, and sinusitis. Mast cells have been considered not only in the association of immediate type hypersensitivity but also in late reaction, like inflammatory responses (5,6). Immediate type hypersensitivity is mediated by histamine released in response to the antigen cross-linking of immunoglobulin E (IgE) bound to FcεRI on the mast cells (7). After activation of mast cells, the process of degranulation is triggered which results in the releasing of mediators, such as products of arachidonic acid metabolism, cytokines, proteases and histamine (8,9). In mast cell-mediated inflammatory responses, histamine is one of the most characterized and important mediators implicated in the acute phase of immediate hypersensitivity (10,11). Mast cell activation is initiated by phosphorylation of tyrosine kinase which leads to activation of protein kinase C, mitogen-activated protein kinases (MAPKs), nuclear factor (NF)-κB, and expression of proinflammatory cytokines (8,12).

In the present study, we investigated the effect of the water extract of *S. crispa* (WESC) on the systemic and local allergic reaction and histamine release from mast cells. The intracellular calcium content was investigated to clarify the mechanism by which WESC inhibited histamine release from mast cells. In addition, the effect of WESC on phorbol 12-myristate 13-acetate (PMA) and calcium ionophore A23187 (PMACI)-induced gene expression and production of proinflammatory cytokines, and the role of NF-κB and MAPKs in this effect were investigated using human mast cells (HMC-1). In order to determine the amount of active compounds of WESC, we confirmed the contents of β-glucan in WESC.

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Materials and methods

Animals. The original stock of male imprinting control region (ICR) mice (6 weeks) and male Sprague-Dawley rats (8 weeks) were purchased from the Dae-Han Experimental Animal Center (Daejeon, Korea). The animals were housed 5 per cage in a laminar air flow room maintained under a temperature of 22±2˚C and relative humidity of 55±5˚C throughout the study. The care and treatment of the mice were in accordance with the guidelines established by the Public Health Service Policy on the Humane Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee.

Reagents and cell culture. Compound 48/80, anti-dinitrophenyl (DNP) IgE, DNP-human serum albumin (HSA), phorbol 12-myristate 13-acetate (PMA), and calcium ionophore A23187 were purchased from Sigma Chemical Co. (St. Louis, MO, USA). The human mast cell line (HMC-1) was grown in Iscove's media (Life Technologies, Grand Island, NY, USA) supplemented with 10% fetal bovine serum at 37˚C in 5% CO_2. Passages 4-8 of HMC-1 cells were used throughout the study.

Preparation of WESC. The *Sparassis crispa* was purchased from the oriental drug store, Bohwa Dang (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. The *S. crispa* was ground (400 x g, 30 sec) at room temperature using Micro Hammer-Cutter Mill (Culatti Co., Zurich, Switzerland). The particle size was 0.5-2 mM after grinding. The plant sample (100 x g) was extracted twice with purified water (500 ml) at 70˚C for 5 h in a water bath. The extract was passed through filter paper and the filtrate was lyophilized using a 0.45 μm syringe filter. The dried extract was dissolved in saline or Tyrode buffer A (HEPES 10 mM, NaCl 130 mM, KCl 5 mM, $CaCl_2$ 1.4 mM, $MgCl_2$ 1 mM, glucose 1.4 mM, 0.1% bovine serum albumin) before use.

Compound 48/80-induced systemic anaphylaxis. Mice were given an intraperitoneal injection of 8 mg/kg body weight (BW) of the mast cell degranulator compound 48/80. WESC was administered intraperitoneally at doses of 10-1,000 mg/kg BW 1 h before the injection of compound 48/80 (n=10/group). Mortality was monitored for 1 h after induction of anaphylactic shock. After the mortality test, blood was obtained from the heart of each mouse to measure serum histamine contents.

Passive cutaneous anaphylaxis (PCA). An IgE-dependent cutaneous reaction was carried out as described previously (13). Briefly, mice were injected intradermally with 0.5 μg of anti-DNP IgE. After 48 h, each mouse (n=10/group) received an injection of 1 μg of DNP-HSA containing 4% Evans blue (1:4) via the tail vein. Thirty minutes after the challenge, the mice were sacrificed and the dorsal skin (diameter, 1 cm) was removed for measurement of the pigmented area.

Preparation of serum and histamine determination. Preparation of serum and determination of histamine contents were examined as previously described (14). Briefly, serum was withdrawn and the histamine contents were measured by the *o*-phthaldialdehyde spectrofluorometric procedure. The fluorescent intensity was measured at emission 438 nm and excitation 353 nm using a spectrofluorometer.

Determination of intracellular calcium. The intracellular calcium was measured with the use of the fluorescence indicator Fluo-3/AM (Molecular Probes, Eugene, OR, USA). HMC-1 cells were pre-incubated with Fluo-3/AM for 30 min at 37˚C. After washing the dye from the cell surface, the cells were treated with WESC for 10 min before adding PMACI. It was excited at 488 nm, and the emission was filtered with 515 nm by flow cytometer (BD Biosciences Pharmingen, San Diego, CA, USA) and visualized by a fluorescence microscope Olympus BX51 (Olympus, Center Valley, PA, USA).

RNA extraction and mRNA detection. The total cellular RNA was isolated from the cells ($1x10^6$/well in a 24-well plate) after stimulation with PMA (20 nM) and A23187 (1 μM) with or without WESC for 2 h using TRI reagent (Molecular Research Center, Cincinnati, OH, USA) according to the manufacturer's protocol. The first strand complementary DNA (cDNA) was synthesized using the Superscript II reverse-transcriptase (Invitrogen, Carlsbad, CA, USA). A reverse transcriptase polymerase chain reaction (RT-PCR) was used to analyze the expression of mRNA for TNF-α, IL-6, and β-actin (internal control). The conditions for the reverse transcription and PCR steps were similar to those described previously (13). The amplified products were separated by electrophoresis on a 2% agarose gel containing ethidium bromide, documented using a Kodak DC 290 digital camera and digitized with UN-SCAN-IT software (Silk Scientific, Inc., Orem, UT, USA). The band intensity was normalized to that of β-actin in the same sample.

Enzyme-linked immunosorbent assay (ELISA). The secretion of TNF-α and IL-6 was measured by the modification of an enzyme-linked immunosorbent assay (ELISA) as previously described (15). HMC-1 cells were cultured with media and resuspended in Tyrode buffer A. The cells were sensitized with PMACI for 8 h in the absence or presence of WESC. ELISA was performed by coating 96-well plates with 6.25 ng/well of monoclonal antibody with specificity for TNF-α and IL-6, respectively.

Western blot analysis. HMC-1 cells were washed 3 times with PBS and resuspended in lysis buffer. Samples were electrophoresed using 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis, as described elsewhere (16), and then transferred to a nitrocellulose membrane. The nucleus and cytosolic p65 NF-κB and IκBα was assayed using anti-NF-κB (p65) and anti-IκBα antibody (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA). The phosphorylation of ERK and p38 was determined using anti-phospho-p38 and anti-phospho-ERK antibodies (Cell Signaling Technology, Inc., Beverly, MA, USA). Immunodetection was performed the using Supersignal West Pico chemiluminescent substrate (Thermo Fisher Scientific, Waltham, MA, USA).

Transient transfection and luciferase activity assay. For transient transfection, HMC-1 cells were seeded at $2x10^6$

Table I. Effect of WESC on compound 48/80-induced systemic anaphylaxis.

WESC treatment (mg/kg, BW)	Compound 48/80 (8 mg/kg, BW)	Mortality (%)
None (saline)	+	100
10	+	100
100	+	70
500	+	20
1000	+	0
1000	-	0

Groups of mice (n=10/group) were intraperitoneally pretreated with 200 μl of saline or WESC 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 x 100/total number of experimental mice.

Table II. Time-dependent effects of WESC on compound 48/80-induced systemic anaphylaxis.

WESC treatment (mg/kg, BW)	Compound 48/80 (8 mg/kg, BW)	Time (min)	Mortality (%)
None (saline)	+		100
1000	+	0	0
1000	+	5	0
1000	+	10	30
1000	+	15	50
1000	+	20	100

Mice (n=10/group) were intraperitoneally pretreated with 200 μl of saline or WESC. WESC (1000 mg/kg) was given 5, 10, 15 and 20 min after the intraperitoneal injection of compound 48/80. Mortality (%) with in 1 h following compound 48/80 injection is represented as the number of dead mice x 100/total number of experimental mice.

in a 6-well plate 1 day before transient transfection. The expression vectors containing the NF-κB luciferase reporter construct (pNF-κB-LUC, plasmid containing NF-κB binding site; Stantagen, Grand Island, NY, USA) were transfected with serum- and antibiotics-free Iscove's medium containing 8 μl Lipofectamine 2000 reagent (Invitrogen). After 5 h of incubation, the medium was replaced with Iscove's medium containing 10% FBS and antibiotics. Cells were allowed to recover at 37˚C for 30 h and subsequently were stimulated as indicated. Cell lysates were prepared and assayed for luciferase activity using the Luciferase Assay System (Promega, Madison, WI, USA), according to the manufacturer's instructions.

Measurement of β-glucan in WESC. β-glucan contents in WESC were determined using a mushroom β-glucan assay kit (K-YBGL; Megazyme International, Wicklow, Ireland) according to the manufacturer's protocol. The lyophilized extract of *S. crispa* (100 mg) and 1.5 ml of 37% hydrochloric acid (v/v, 10 N) were mixed vigorously, and incubated at 30˚C for 45 min. The materials were mixed with 10 ml distilled water and incubated at 100˚C for 2 h. After centrifugation at 1,500 x g for 10 min, 0.1 ml aliquots were combined with 0.1 ml of a mixture of exo-(1-3)-β-glucanase (20 U/ml) plus β-glucosidase (4 U/ml) in 200 mM sodium acetate buffer (pH 5.0) and incubated at 40˚C for 60 min. To measure total glucan content, 3 ml of glucose oxidase/peroxidase mixture (GOPOD) was added and incubated at 40˚C for 20 min. The absorbance of samples was analyzed spectrophotometrically at 510 nm against the reagent blank using a spectrophotometer (Shimadzu, UV-1201). To measure the α-glucan content, 2 ml of 2 M KOH was added, and the phytoglycogen and starch were dissolved by stirring for 20 min in an ice water bath. The suspension was added to 8 ml of 1.2 M sodium acetate buffer (pH 3.8), mixed with 0.2 ml of amyloglucosidase (16,300 U/ml) plus invertase (500 U/ml), and incubated in a water bath for 30 min at 40˚C with intermittent mixing on a vortex stirrer. Tubes were centrifuged (10 min, 1,500 x g), and 0.1 ml aliquots were combined with 0.1 ml of sodium acetate buffer (200 mM, pH 5.0) plus 3 ml of GOPOD reagent and incubated for at 40˚C for 30 min. The absorbance of samples was analyzed at 510 nm. The β-glucan content was determined by subtracting the α-glucan from the total glucan content.

Statistical analysis. Statistical analyses were performed using SAS statistical software (SAS Institute, Inc., Cary, NC, USA). Treatment effects were analyzed using analysis of variance, followed by Duncan's multiple range tests. $P<0.05$ indicated significance.

Results

Effect of WESC on systemic and local anaphylaxis. To determine the effect of WESC in allergic reaction, an *in vivo* model of a systemic anaphylaxis 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, the mice were monitored for 1 h, after which the mortality rate was determined. Injection of compound 48/80 into mice induced fatal shock in 100% of the animals. When WESC was intraperitoneally pretreated at doses ranging from 10 to 1,000 mg/kg for 1 h, the mortality was dose-dependently reduced. WESC completely inhibited compound 48/80-induced fatal shock at 1,000 mg/kg (Table I). In addition, the mortality of mice administered with WESC (1,000 mg/kg) 5, 10, 15 and 20 min after compound 48/80 injection time-dependently increased (Table II).

The effect of WESC on the compound 48/80-induced serum histamine release was investigated. The histamine level caused by compound 48/80 was decreased by WESC in a dose-dependent manner (Fig. 1A). To confirm the anti-allergic effects of WESC, we used a passive cutaneous anaphylaxis (PCA) model induced by anti-DNP-IgE and DNP-HSA. To compare to amount of dye with control, the left dorsal skin of these mice was injected with saline alone. WESC was intraperitoneally administered 1 h prior to the challenge with antigen. WESC dose-dependently inhibited PCA reaction (Fig. 1B).

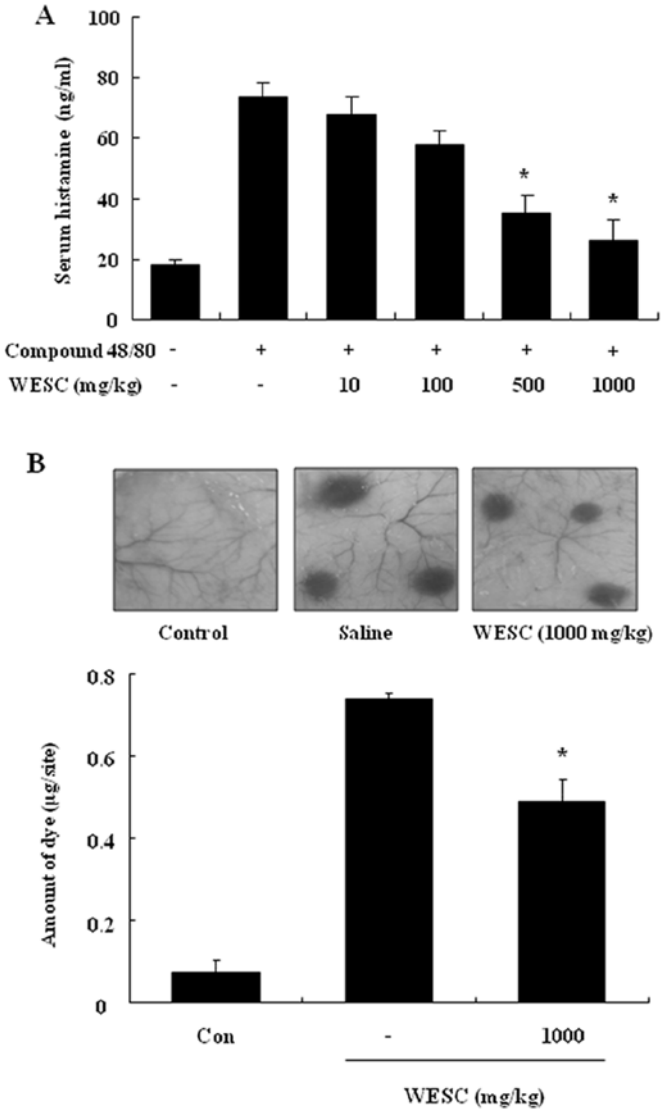

Figure 1. Effects of WESC on serum histamine and PCA reactions. (A) Groups of mice (n=10/group) were intraperitoneally pretreated with 200 μl of saline or WESC. WESC was given at various doses 1 h before the injection of compound 48/80. The compound 48/80 was given intraperitoneally to the group of mice. The blood was obtained from the heart of each mouse and histamine content was measured by a spectrofluorometer. (B) WESC was intraperitoneally administered 1 h prior to the challenge with antigen. Each amount of dye extracted as described in Materials and methods and measured by spectrophotometry. Each bar represents the mean ± SEM of three independent experiments. *Significant difference at P<0.05.

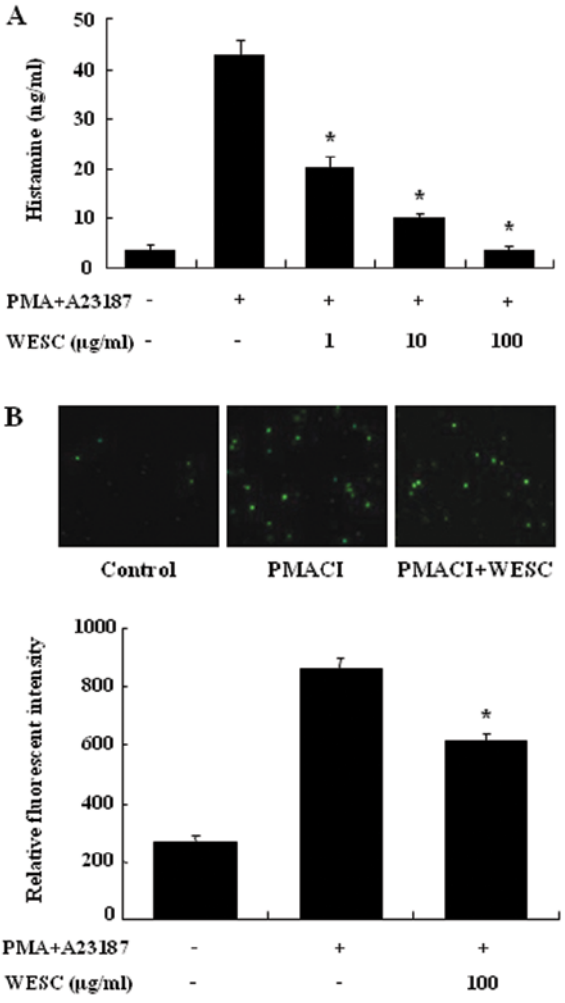

Figure 2. Effects of WESC on histamine release and intracellular calcium in mast cells. HMC-1 cells ($1x10^6$ cells/ml) were pre-incubated with WESC for 30 min prior to incubation with PMA (20 nM) and calcium ionophore A23187 (1 μM). (A) Histamine content was measured by a spectrofluorometer. (B) Intracellular calcium was detected by a fluorescence microscope and flow cytometer. Each bar represents the mean ± SEM of three independent experiments. *Significant difference from PMACI value at P<0.05.

Effect of WESC on histamine release and intracellular calcium. We estimated the reducing effects of WESC on the histamine release from PMACI-induced HMC-1 cells. Mast cells released a high level of histamine when simulated with PMACI (Fig. 2A). When WESC was pretreated for 30 min, histamine was dose-dependently inhibited in PMACI-induced HMC-1. Up to 1 mg/ml of WESC did not show cytotoxicity (data not shown). To investigate the mechanism responsible for the reduction of histamine after WESC treatment, we assayed the intracellular calcium levels. Calcium movements across membranes of mast cells are critical to histamine release (17). When HMC-1 cells were stimulated with PMACI, intracellular calcium levels were significantly elevated (Fig. 2B). WESC (100 μg/ml) decreased the intracellular calcium level. The levels of intracellular calcium were also assessed by the relative fluorescent intensity.

Effect of WESC on the expression and secretion of pro-inflammatory cytokines. We investigated the inhibitory effect of WESC on the expression of proinflammatory cytokines such as TNF-α and IL-6. The HMC-1 cell line is a useful tool for researching the cytokine activation pathway (18,19). Previously we reported that gene expression of TNF-α and IL-6 peaked 4 h after treatment of PMACI (20). Consequently, HMC-1 cells were stimulated by PMACI during 4 h, and the cells were preincubated with WESC for 30 min. Fig. 3A shows that the expression of proinflammatory cytokines was inhibited by WESC. To confirm the correlation of mRNA expression with protein production, we measured the secretion of TNF-α and IL-6. When HMC-1 cells were stimulated with PMACI for 8 h, the secretion of cytokines was remarkably induced. WESC dose-dependently inhibited the secretion of TNF-α and IL-6 in PMACI-stimulated HMC-1 cells (Fig. 3B).

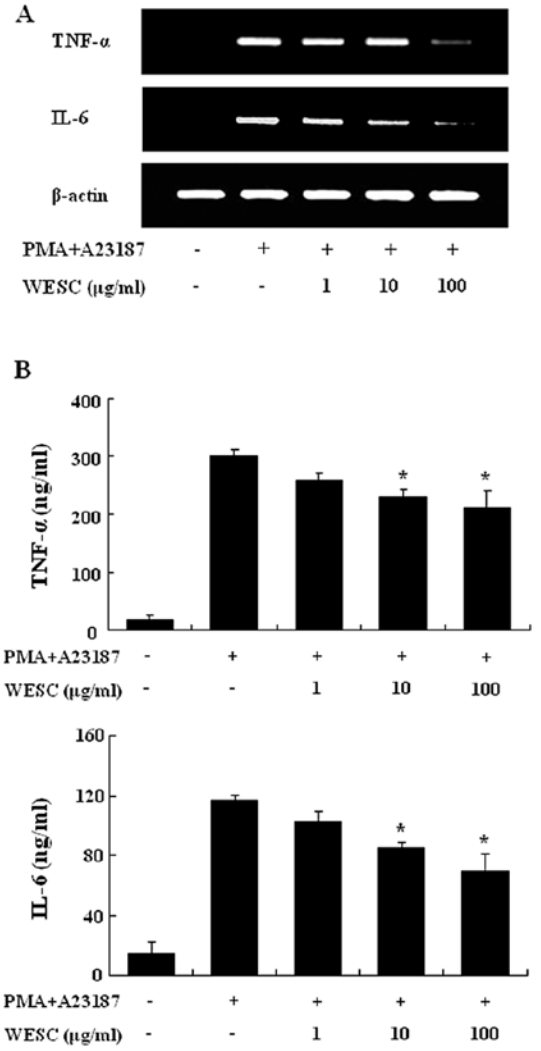

Figure 3. Effects of WESC on the gene expression and secretion of proinflammatory cytokines in mast cells. HMC-1 were treated with WESC for 30 min before being stimulated with PMA (20 nM) and ionophore A23187 (1 μM). (A) Extraction and analysis of mRNA was performed as described in Materials and methods. The gene expression of TNF-α and IL-6 were determined by RT-PCR. (B) The level of TNF-α and IL-6 in supernatant was measured using ELISA and represented as the mean ± SEM of three independent experiments. *Significant difference from the PMACI value at P<0.05.

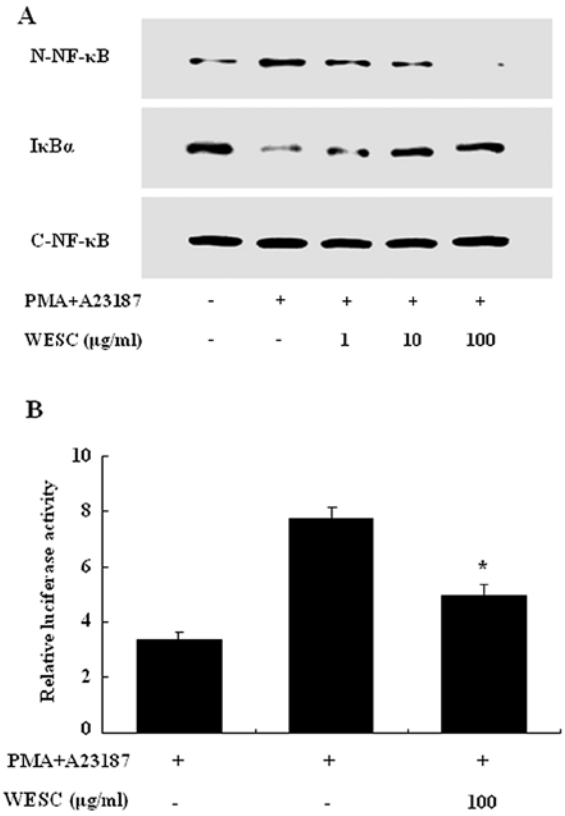

Figure 4. Effects of WESC on the activation of NF-κB in mast cells. HMC-1 were pretreated with WESC for 30 min prior to PMA (20 nM) and A23187 (1 μM) stimulation for 2 h. (A) Nuclear translocation of NF-κB and IκBα degradation were assayed by western blot analysis (N-NF-κB, nuclear NF-κB; C-NF-κB, cytoplasmic NF-κB). (B) Cells were transiently transfected with the NF-κB-luciferase reporter construct or empty vector. Then, the cells were incubated with PMA and A23187 with or without WESC. NF-κB-dependent transcriptional activity was determined by the luciferase activity assay. Each bar represents the mean ± SEM of three independent experiments. *Significant difference from the PMACI value at P<0.05.

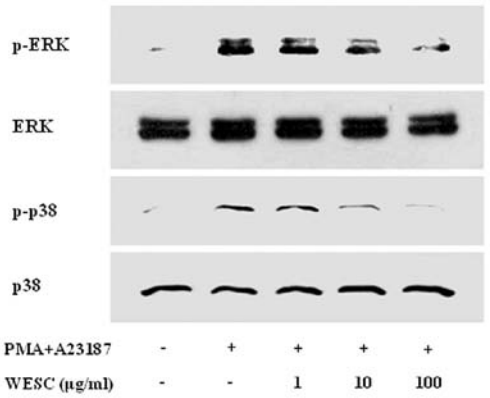

Figure 5. Effect of WESC on phosphorylation of MAPKs in mast cells. HMC-1 were pretreated with WESC for 30 min prior to PMA (20 nM) and A23187 (1 μM) stimulation for 30 min, and phosphorylation of p38 and ERK was assayed by western blot analysis.

Effect of WESC on the activation of NF-κB and MAPKs. To investigate the intracellular mechanism responsible for the inhibitory effect of WESC on the expression of proinflammatory cytokines, we examined the effect of WESC on the activation of transcription factors, NF-κB and MAPKs. NF-κB is an important transcriptional regulator of inflammatory cytokines and plays a crucial role in immune and inflammatory responses. Stimulation of HMC-1 with PMACI induced the nuclear translocation of p65 NF-κB and degradation of IκBα after 2 h of incubation. WESC inhibited the PMACI-induced nuclear translocation of NF-κB and degradation of IκBα (Fig. 4A). To confirm the inhibitory effect of WESC on the NF-κB activation, we examined the effect of WESC on the NF-κB-dependent gene reporter assay. HMC-1 cells were transiently transfected with a NF-κB-luciferase reporter construct or an empty vector. Exposure of cells to PMACI increased the luciferase activity in the cells transfected with the NF-κB-luciferase reporter construct (Fig. 4B). WESC significantly reduced PMACI-induced luciferase activity.

The MAPK signaling cascades also regulate important cellular processes including gene expression, cell proliferation, and cell survival and death (21). Previously we documented

that PMACI activates all three types of MAPKs such as p38, JNK, and ERK at 15-30 min in HMC-1 (22). In the present results, stimulation of cells with PMACI induced phosphorylation of p38, JNK, and ERK, and WESC markedly attenuated PMACI-induced phosphorylation of ERK and p38 MAPK (Fig. 5). However WESC did not affect the phosphorylation of JNK (data not shown).

Discussion

Anaphylaxis is a life-threatening syndrome induced by a sudden systemic release of inflammatory mediators, such as histamine, various cytokines and lipid-derived mediators (23). Using *in vitro* and *in vivo* models, we showed that WESC has anti-allergic properties. WESC inhibited compound 48/80-induced systemic allergic reaction (anaphylaxis) and serum histamine release in mice. These results indicate that mast cell-mediated immediate-type allergic reactions are inhibited by WESC. In addition, WESC-administered mice were protected from IgE-mediated PCA, which is one of the most important *in vivo* models of anaphylaxis in a local allergic reaction. This finding suggests that WESC might be useful in the treatment of allergic disease, particularly skin reactions.

Histamine was 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 the immune response, such as immune cell maturation, polarization, and lymphocyte responsiveness (24). Many reports have established that stimulation of mast cells with compound 48/80 or IgE initiates the activation of signal transduction pathways, which lead to histamine release. Several studies have shown that compound 48/80 and other polybasic compound are able, apparently directly, to activate G-proteins (25). Compound 48/80 increases the permeability of the lipid bilayer membrane by causing a perturbation in the membrane. These reports indicate that the increase in membrane permeability may be an essential trigger for the release of the mediator from mast cells. In this sense, anti-allergic agents having a membrane-stabilizing action may be desirable (26). WESC might stabilize the lipid bilayer membrane, thus preventing the compound 48/80-induced membrane perturbation.

Intracellular calcium plays an important role in the release of histamine and the expression of cytokines. Calcium movements across the membranes of mast cells represent a major target for efficient anti-allergic drugs, as these are essential events linking stimulation to secretion. In our results, WESC decreased the intracellular calcium level in mast cells. We suggest that the decreased intracellular calcium levels may be involved in the inhibitory effect of WESC on histamine release.

The HMC-1 cell line is one of the useful cells for studying cytokine activation pathways (8). The various types of cytokines produced by HMC-1 with PMACI stimulation supports the well-recognized role of mast cells in immediate-type hypersensitivity. TNF-α and IL-6, the known proinflammatory cytokines, play an important role in triggering and sustaining the allergic inflammatory response in mast cells (27,28). Mast cells are a principal source of TNF-α in human dermis. TNF-α has major amplifying effect in asthmatic inflammation and potently stimulates airway epithelial cells to produce cytokines (29). It promote inflammation, leukocyte infiltration, chemotaxis of neutrophils and T cells (30). IL-6 is also produced from mast cells, and its local accumulation is associated with PCA reaction (31). These reports indicate that the reduction of proinflammatory cytokines from mast cells is one of the key indicators of reduced allergic symptoms. In the present study, WESC inhibited the expression of TNF-α and IL-6 in PMACI-stimulated mast cells. This result suggests that the anti-allergic effect of WESC results from its inhibition of TNF-α and IL-6 generation from mast cells.

Expression of TNF-α and IL-6 is regulated by the activation of the transcription factor NF-κB (32). NF-κB regulates the expression of multiple inflammatory and immune genes and plays a critical role in chronic inflammatory diseases. Activation of NF-κB required phosphorylation and proteolytic degradation of the inhibitory protein IκBα, an endogenous inhibitor that binds to NF-κB in the cytoplasm. In PMACI-stimulated mast cells, WESC decreased the degradation of IκBα and nuclear translocation of NF-κB. The data demonstrated that WESC attenuates activation of NF-κB and downstream cytokine expression such as TNF-α and IL-6. To further identify the mechanism of WESC, we evaluated the inhibitory effect of WESC on activation of MAPKs, such as p38, JNK and ERK in PMACI-stimulated mast cells. The MAPK cascade is one of the important signaling pathways in immune responses. The MAPK signaling cascades regulate important cellular processes including gene expression, cell proliferation, cell survival and death, and cell mobility (21). Precise signaling pathways in allergic diseases among three types of MAPKs such as ERK, JNK, and p38 are still unclear. However, the induction of inflammatory cytokine genes requires activation of the p38 MAPK and ERK (33). In our results, WESC decreased phosphorylation of ERK and p38 MAPKs in PMACI-stimulated mast cells. This data suggest that WESC may decrease cytokine production and activation of NF-κB via inhibition of ERK and p38 MAPK.

In summary, WESC significantly reduced mast cell-mediated allergic inflammation in *in vivo* and *in vitro* models. In the present study, we used the whole water extract of *S. crispa,* not a purified single compound. However β-glucan is already known to be the major compound of WESC. We examined the β-glucan content in WESC using a mushroom β-glucan assay kit. The β-glucan content in WESC was 39.3%. Therefore, we assume that β-glucan is responsible for the anti-allergic inflammatory effects of WESC. In conclusion, *S. crispa* could contribute to prevention or treatment of mast cell-mediated allergic inflammatory diseases.

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