Original Research

Suppression of mast-cell-mediated allergic inflammation by *Lindera obtusiloba*

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Abstract

Allergic disease is a consequence of exposure to normally innocuous substances that elicit the activation of mast cells. Mast-cell-mediated allergic response is involved in many diseases such as anaphylaxis, allergic rhinitis, asthma and atopic dermatitis. The discovery of drugs for the treatment of allergic disease is an important subject in human health. In this study, we investigated the effect of *Lindera obtusiloba* water extract (LOWE) on the mast-cell-mediated allergic inflammation and possible mechanism of action using *in vitro* and *in vivo* models. LOWE reduced histamine release from various types of mast cells activated by immunoglobulin E (IgE) or phorbol 12-myristate 13-acetate and calcium ionophore A23187 (PMACI). The inhibitory effect of LOWE on histamine release was mediated by calcium signal. LOWE decreased the PMACI-stimulated gene expression of proinflammatory cytokines such as tumor necrosis factor-α and interleukin-6 in human mast cells. The inhibitory effect of LOWE on the proinflammatory cytokines was nuclear factor (NF)-κB dependent. In addition, LOWE suppressed compound 48/80-induced systemic allergic reaction and serum histamine release in mice and IgE-mediated local allergic reactions. Our results indicate that LOWE inhibits mast-cell-derived allergic inflammation and involvement of calcium, histamine, proinflammatory cytokines and NF-κB in these effects.

Keywords: *Lindera obtusiloba*, allergic inflammation, mast cell, histamine, proinflammatory cytokine

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Introduction

Allergies are the result of an inappropriate reaction against innocuous environmental substances. The prevalence and severity of allergic diseases has increased during the last decade in developed countries.[1] Mast cells have long been recognized for their role in the genesis of allergic inflammation and more recently for their participation in innate and acquired immune responses.[2]

Mast cells have a broad impact on many physiological and pathological processes such as wound healing, tissue remodeling and homeostasis.[3] Typically, mast cells have been considered not only to be associated with immediate-type hypersensitivity but also with late reactions like inflammatory responses.[4,5] 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.[6] On activation of mast cells via cross-linking of the high-affinity IgE receptor (FcεRI) through complement receptors, mast cells can release a variable spectrum of proinflammatory mediators, such as histamine, serotonin, leukotriens, prostaglandins and cytokines/chemokines.[2]

The activation of mast cells leads to the phosphorylation of tyrosine kinase and the mobilization of internal calcium. This is followed by the activation of protein kinase C, an increase of mitogen-activated protein kinases, nuclear factor (NF)-κB and the release of inflammatory cytokines. Activated mast cells can produce histamine, as well as several proinflammatory 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.[6] The transcription factor NF-κB plays a pivotal role in inflammation by virtue of its ability to induce transcription of an array of inflammatory genes, especially to the regulation of proinflammatory molecules.[7,8]

The traditional Korean medicine, *Lindera obtusiloba*, is used for the treatment of inflammation and improvement of blood

circulation, and herbal infusions of *L. obtusiloba* are applied to treat chronic liver disease.[9] Extracts of *L. obtusiloba* contain lignans and butanolides, and were shown to exert antitumor activity by exhibiting cytotoxicity against various tumors.[10,11] The aim of this study is to evaluate the antiallergic inflammatory effect of *L. obtusiloba* water extract (LOWE) and to understand the underlying mechanism.

Materials and methods

Animals

The original stock of male ICR mice (20–30 g) and male Sprague–Dawley rats (200–300 g) were purchased from the Dae-Han Biolink Co, Ltd (Daejeon, Korea). The animals were maintained in the School of Medicine, Kyungpook National University, Daegu, South Korea. The animals were housed 5–10 per cage in a laminar air flow room, maintained at a temperature of $22 \pm 2^{\circ}C$, with a relative humidity of $55 \pm 5\%$ 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), pyrrolidine dithiocarbamate (PDTC), phorbol 12-myristate 13-acetate and calcium ionophore A23187 (PMACI) were purchased from the Sigma Chemical Co. (St Louis, MO, USA). Anti-TNF-α and -IL-6 antibodies were purchased from R&D Systems Inc. (Minneapolis, MN, 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 (FBS) and 2 mmol/L glutamine at 37°C in 5% CO_2. The rat-originated mast cell line, RBL-2H3, was maintained in RPMI-1640 medium (Invitrogen, Carlsbad, CA, USA) with 10% FBS and 2 mmol/L glutamine at 37°C in 5% CO_2. Passages 4–8 of cultures were used in all experiments.

Preparation of rat peritoneal mast cells

Mast cells were separated from the rat peritoneal cavity cells as previously described.[12] In brief, the peritoneal cells were suspended in Tyrode buffer, layered on 2 mL of metrizamide (22.5 w/v%) and centrifuged at 400*g* for 15 min at 4°C. The cells that remained at the buffer–metrizamide interface were aspirated and discarded; the cells in the pellet were washed and resuspended in 1 mL of Tyrode buffer. Mast cell preparations were about 95% pure as assessed by toluidine blue staining. More than 95% of the cells were viable as judged by trypane blue exclusion.

Preparation of LOWE

L. obtusiloba was collected in Daegu, and was identified by Professor Bae (Chungnam University, Daejeon, South Korea). *L. obtusiloba* was ground (400*g*, 30 s) at room temperature using a Micro Hammer-Cutter Mill (Culatti Co., Zurich, Switzerland). The particle size was 0.5–2 mm after grinding. The sample was extracted at 100°C for four hours in purified water. The extract was filtered through Whatman No. 1 filter paper, and the filtrate was lyophilized using a 0.45-μm syringe filter, and then freeze-dried.

Histamine assay

Histamine content was measured by the enzyme immunoassay kit (Oxford Biomedical Research, Oxford, MI, USA) according to the manufacturer's manual. HMC-1 was preincubated with LOWE for 30 min, and then incubated for eight hours with PMA (20 nmol/L) and A23187 (1 μmol/L). RBL-2H3 and rat peritoneal mast cell (RPMCs) were sensitized with anti-DNP IgE (10 μg/mL) for 16 h. The cells were preincubated with LOWE for 30 min prior to the challenge with DNP-HSA (1 μg/mL). The blood from the mice was centrifuged at 400*g* for 10 min and the serum was withdrawn to measure histamine content.

Measurement of intracellular calcium

To measure the intracellular calcium, we used the fluorescent indicator Fluo-3/AM (Molecular Probes, Eugene, OR, USA) as previously described.[13] HMC-1 were preincubated with Fluo-3/AM for 30 min at room temperature. After washing the dye from the cell surface, the cells were treated with LOWE for 10 min before adding PMACI. The fluorescent intensity was recorded using a flow cytometer (BD Biosciences Pharmingen, San Diego, CA, USA) at an excitation of 488 nm and an emission of 515 nm. The intracellular calcium was also visualized in a fluorescence microscope (Olympus BX51, Olympus, Center Valley, PA, USA).

Real-time polymerase chain reaction

Total cellular RNA was isolated from cells using TRIzol according to the manufacturer's protocol. The first-strand complementary DNA (cDNA) was synthesized using Superscript II reverse transcriptase (Invitrogen). For quantitative polymerase chain reaction (PCR), realtime PCR was performed in triplicate using 12.5 μL of SYBR Premix Ex Taq (Takara, Japan) and 2 μL of cDNA as a template in 25 μL of final volume. PCR amplification was preceded by incubation of the mixture for 15 min at 95°C, and 40 cycles of the amplification step, consisting of denaturation, annealing and extension. The denaturation was performed for 30 s at 95°C; annealing was performed in a transitional temperature range from 58 to 62°C, with an increase of 0.5°C per cycle; and the extension was performed for 30 s at 72°C with fluorescence detection at 72°C after each cycle. After the final cycle, melting point analyses of all samples were performed within the range from 65 to 95°C with continuous fluorescence detection. The expression level of GAPDH was used for normalization.

Western blot analyses

HMC-1 was washed with phosphate-buffered saline (PBS) and resuspended in lysis buffer. Samples were electrophoresed

using 8–12% sodium dodecyl sulfatepolyacrylamide gel electrophoresis, as described elsewhere,[14] and then transferred onto a nitrocellulose membrane. Immunodetection was done using an enhanced chemiluminescence detection kit (Amersham Pharmacia, Piscataway, NJ, USA).

Transient transfection and luciferase activity assay

For transient transfections, HMC-1 was seeded at 2×10^6 in a six-well plate one 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 antibiotic-free Iscove's medium containing 8 μL of Lipofectamine 2000 reagent (Invitrogen). After five hours 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 assayed for luciferase activity using the Luciferase Assay System (Promega, Madison, WI, USA), according to the manufacturer's instructions.

Systemic allergic reaction

A compound 48/80-induced systemic reaction was carried out as previously described.[7] 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. LOWE was dissolved in saline and administered intraperitoneally at doses of 1–100 mg/kg BW one hour before the compound 48/80 injection. In the time-dependent experiment, LOWE (100 mg/kg) was administered 10, 20 and 30 min after compound 48/80 injection ($n = 10$/group). Mortality was monitored for one hour after induction of anaphylactic shock.

Passive cutaneous anaphylaxis

Passive cutaneous anaphylaxis (PCA) reaction was carried out as previously described.[15] Briefly, mice were injected intradermally with 0.5 μg of anti-DNP IgE. After 48 h, each mouse ($n = 10$/group) was given an injection of 1 μg of DNP-HSA in PBS containing 4% Evans blue (1:4) via the tail vein. LOWE (1–100 mg/kg BW) was intraperitoneally administered one hour 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 mol/L KOH and 9 mL of a mixture of acetone and phosphoric acid (5:13). The intensity of the absorbent was measured at 620 nm using a spectrophotometer (Shimadzu, UV-1201, Kyoto, Japan).

Statistical analysis

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

Results

Effect of LOWE on histamine release from mast cells

To determine the effect of LOWE on allergic reaction, histamine release from human mast cell line (HMC-1), rat mast cell line (RBL-2H3) and freshly isolated RPMC were evaluated. Treatment of LOWE dose-dependently inhibited PMACI-induced histamine release at concentrations of 1–100 μg/mL in HMC-1 (Figure 1). To confirm the inhibitory effect of LOWE on histamine release, RBL-2H3 and RPMCs were sensitized by anti-DNP IgE with DNP-HSA. LOWE inhibited IgE-mediated histamine release in a dose-dependent manner. The concentration and duration of LOWE used in these studies had no significant effect on the viability of mast cells (data not shown).

Effect of LOWE on intracellular calcium

Calcium movements across membranes of mast cells are critical to degranulation and histamine release in mast cells.[16] To investigate the mechanism of LOWE on the reduction of histamine release, we assayed intracellular calcium levels in HMC-1. Figure 2 shows the increase of intracellular calcium by the PMACI stimulation. Preincubation of LOWE with cells decreased the PMACI-induced intracellular calcium level.

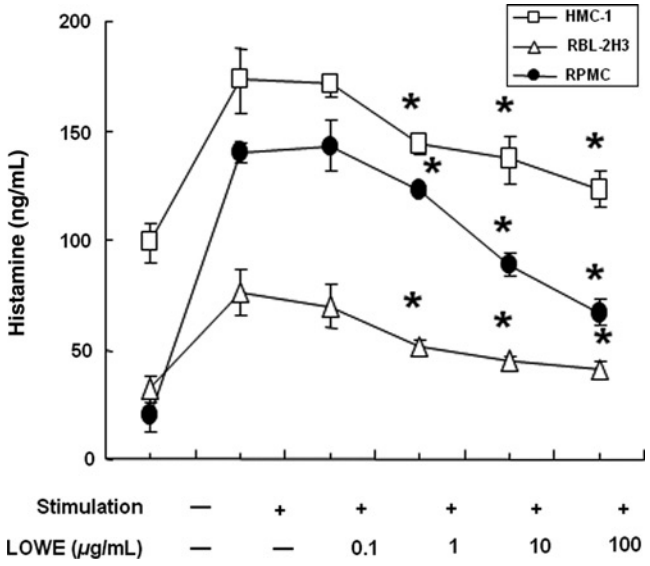

Figure 1 Effect of LOWE on histamine release from mast cells. HMC-1 (2×10^6 cells/mL) were preincubated with LOWE for 30 min and then incubated for eight hours with PMA (20 nmol/L) and A23187 (1 μmol/L). RBL-2H3 (1×10^6 cells/mL) and RPMCs (2×10^5 cells/mL) were sensitized with anti-DNP IgE (10 μg/mL) for 16 h. The cells were preincubated with LOWE for 30 min prior to the challenge with DNP-HSA (1 μg/mL). The cells were separated from the released histamine by centrifugation 400*g* for five minutes at 4°C. Each value represents the mean ± SEM of three independent experiments. *Significant difference at $P < 0.05$. LOWE, *Lindera obtusiloba* water extract; HMC-1, human mast cell 1; PMA, phorbol 12-myristate 13-acetate; RPMC, rat peritoneal mast cell; DNP, dinitrophenyl; HSA, human serum albumin

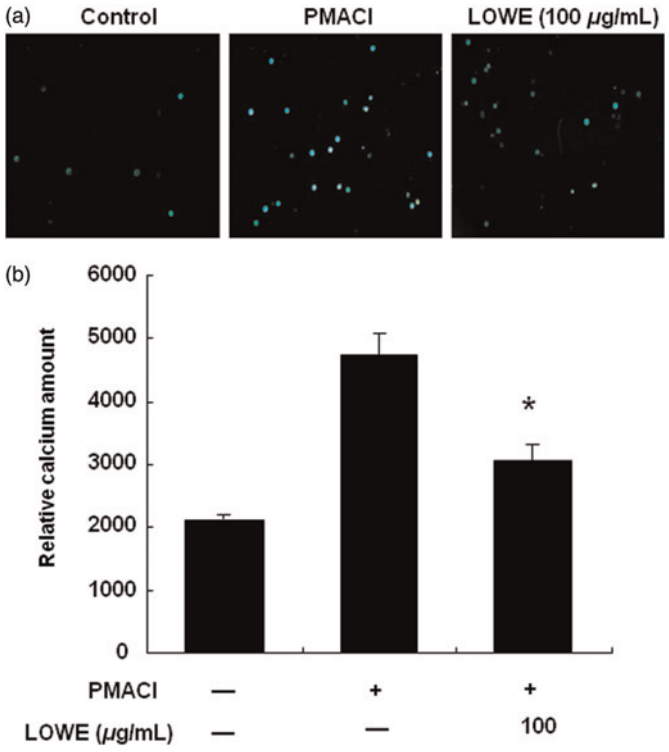

Figure 2 Effect of LOWE on intracellular calcium. HMC-1 were stained with Fluo-3/AM and then cells were preincubated for 10 min with LOWE before adding PMA (20 nmol/L) and A23187 (1 μmol/L), and then for another 10 min with PMACI. Intracellular calcium was detected by a fluorescence microscope (a) and a flow cytometer (b). Each value represents the mean $\pm$ SEM of three independent experiments. *Significant difference at $P < 0.05$. PMACI, phorbol 12-myristate 13-acetate and calcium ionophore A23187; LOWE, *Lindera obtusiloba* water extract; HMC-1, human mast cell 1; PMA, phorbol 12-myristate 13-acetate (A color version of this figure is available in the online jounral)

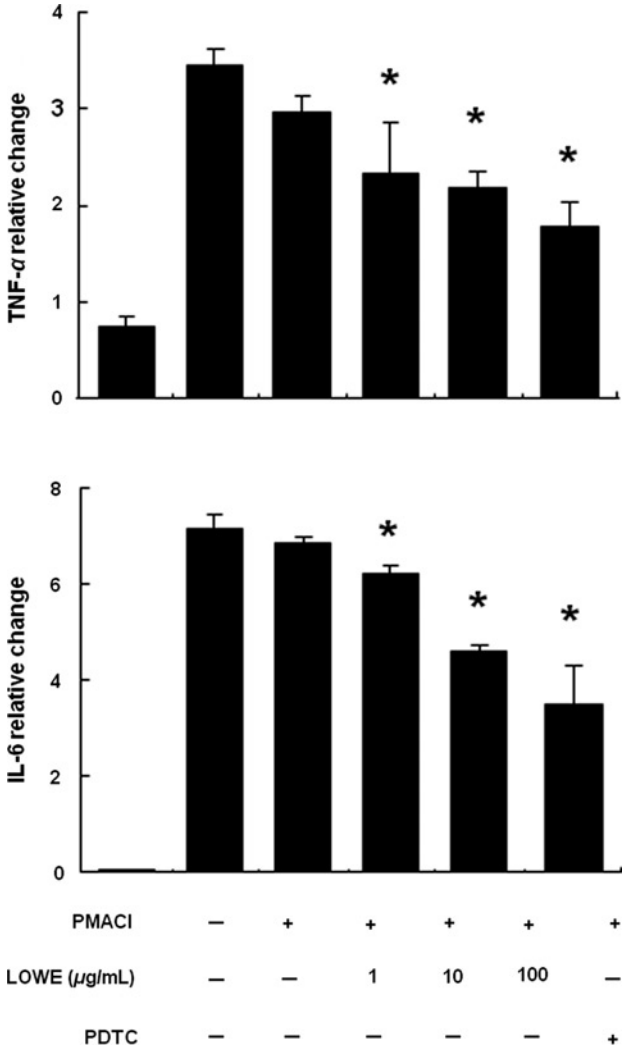

Figure 3 Effect of LOWE on the expression of proinflammatory cytokines. HMC-1 were pretreated with LOWE or PDTC (10 μmol/L) for 30 min prior to PMA (20 nmol/L) and A23187 (1 μmol/L) stimulation. After four hours of PMACI stimulation, the mRNA levels of TNF-α and IL-6 were determined by realtime PCR. Each value represents the mean $\pm$ SEM of three independent experiments. *Significant difference at $P < 0.05$. PDTC, pyrrolidine dithiocarbamate; PMACI, phorbol 12-myristate 13-acetate and calcium ionophore A23187; LOWE, *Lindera obtusiloba* water extract; HMC-1, human mast cell 1; TNF-α, tumor necrosis factor α; IL-6, interleukin 6; PCR, polymerase chain reaction

Effect of LOWE on the expression of proinflammatory cytokines

TNF-α and IL-6 are the most important proinflammatory cytokines. Therefore, we tested the effect of LOWE on the gene expression of TNF-α and IL-6 induced by PMACI in HMC-1. HMC-1 is a useful cell for studying the cytokine activation pathway.[17] Cells were stimulated with PMACI for four hours, and the gene expression of TNF-α and IL-6 was measured by realtime PCR. LOWE dose-dependently inhibited PMACI-induced expression of TNF-α and IL-6 (Figure 3). In addition, PMACI-induced expression of TNF-α and IL-6 was significantly blocked by the pretreatment of PDTC, a potent NF-κB inhibitor.

Effect of LOWE on activation of NF-κB

NF-κB is an important transcriptional regulator of inflammatory cytokines and plays a crucial role in inflammatory responses. Translocation of NF-κB to the nucleus requires IκBα phosphorylation and degradation, since this event is considered a key event that ultimately leads to the activation of NF-κB.[18] To investigate the intracellular mechanism responsible for the inhibitory effect of LOWE on the expression of proinflammatory cytokines, we examined the effect of LOWE on degradation of IκBα. Stimulation of HMC-1 with PMACI induced degradation of IκBα after two hours of incubation (Figure 4a). LOWE inhibited the PMACI-induced degradation of IκBα. To confirm the inhibitory effect of LOWE on NF-κB activation, we examined the effect of LOWE on the NF-κB-dependent gene reporter assay. Cells were transiently transfected with an 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 (Figure 4b). LOWE significantly reduced the PMACI-induced luciferase activity.

Effect of LOWE on systemic and local allergic reaction

To determine whether LOWE has antiallergic effects, an *in vivo* model of a systemic reaction was used.

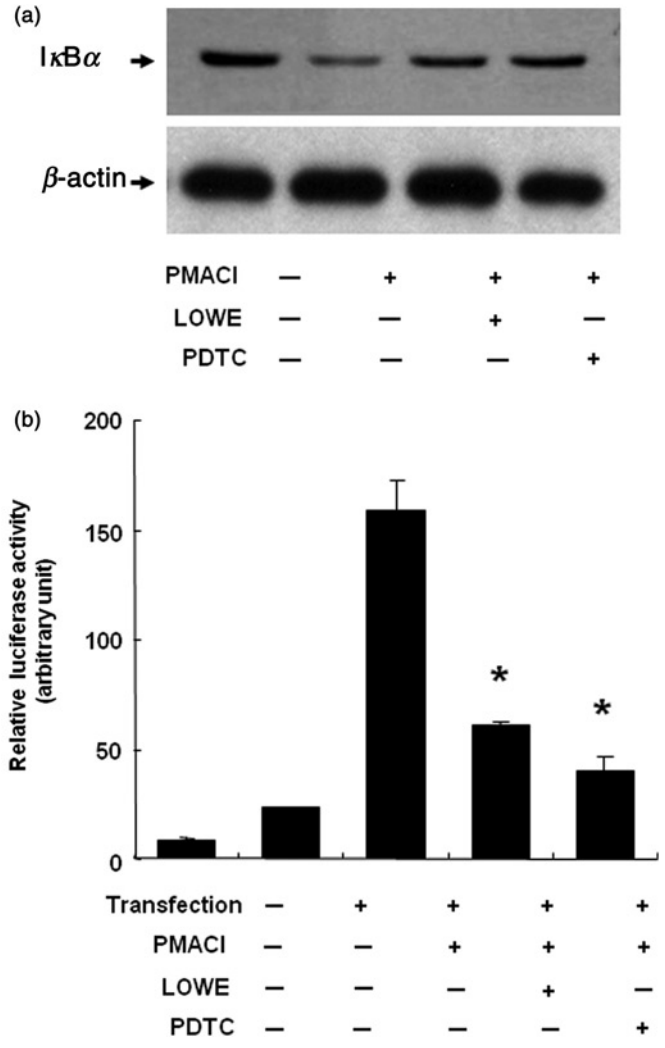

Figure 4 Effect of LOWE on the activation of NF-κB. HMC-1 were pretreated with LOWE (100 μg/mL) or PDTC (10 μmol/L) for 30 min prior to PMA (20 nmol/L) and A23187 (1 μmol/L) stimulation. (a) Degradation of IκBα was assayed by Western blot. β-Actin is a loading control. (b) Cells were transiently transfected with the NF-κB-luciferase reporter construct or empty vector. Then, the cells were incubated with PMACI with or without LOWE. NF-κB-dependent transcriptional activity was determined by luciferase activity assay. PDTC was used as a positive control. *Significant difference at $P < 0.05$. LOWE, *Lindera obtusiloba* water extract; HMC-1, human mast cell 1; TNF-α, tumor necrosis factor α; PDTC, pyrrolidine dithiocarbamate; NF-κB, nuclear factor κB; PMACI, phorbol 12-myristate 13-acetate and calcium ionophore A23187

Anaphylaxis is a serious allergic reaction that is rapid in onset and causes death.[19] 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 one hour. Injection of compound 48/80 into mice induced fatal shock in 100% of animals (Table 1). When LOWE was intraperitoneally administered ranging from 1 to 100 mg/kg BW for one hour, the mortality with compound 48/80 was dose-dependently reduced. In addition, the mortality of mice administered with LOWE (100 mg/kg) 10, 20 and 30 min after compound 48/80 injection increased time dependently (Table 2). To confirm the effect of allergic reaction *in vivo*, we evaluated the level of compound 48/80-induced histamine release in serum. Our results indicated that LOWE decreased the compound 48/80-induced serum histamine release (Figure 5). Another way to test the allergic reactions is to induce PCA. A local extravasation was induced by a local injection of IgE followed by an antigenic challenge. Intraperitoneal injection of LOWE inhibited PCA reaction (Figure 6).

Table 1 Effect of LOWE on compound 48/80-induced systemic allergic reaction

LOWE treatment (mg/kg BW)	Compound 48/80 (8 mg/kg BW)	Mortality (%)
None (saline)	+	100
1	+	90
10	+	50
100	+	0
100	−	0

Groups of mice ($n = 10$/group) were intraperitoneally pretreated with 200 μL of saline or LOWE at various doses one hour before the intraperitoneal injection of compound 48/80. Mortality (%) within one hour following compound 48/80 injection is represented as the number of dead mice × 100/total number of experimental mice. BW, body weight

Table 2 Time-dependent effect of LOWE on compound 48/80-induced systemic allergic reaction

LOWE treatment (mg/kg BW)	Compound 48/80 (8 mg/kg BW)	Time (min)	Mortality (%)
None (saline)	+		100
100	+	0	0
	+	10	10
	+	20	40
	+	30	100

Mice ($n = 10$/group) were intraperitoneally pretreated with 200 μL of saline or LOWE. LOWE (100 mg/kg) was given 10, 20 and 30 min after the intraperitoneal injection of compound 48/80. Mortality (%) within one hour following compound 48/80 injection is represented as the number of dead mice × 100/total number of experimental mice. BW, body weight

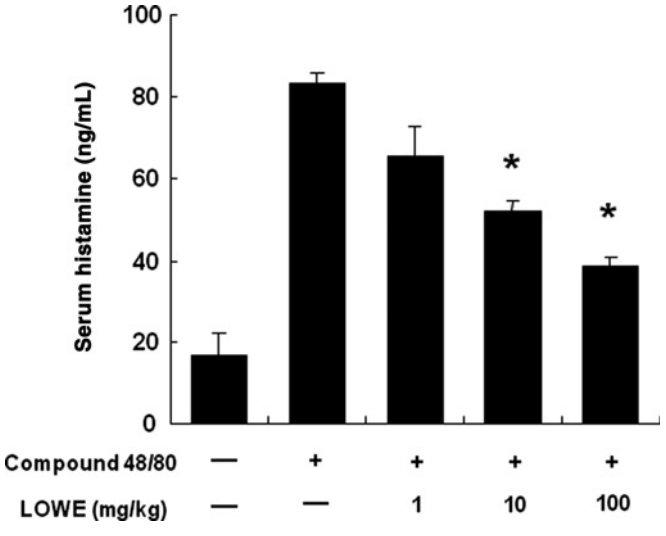

Figure 5 Effect of LOWE on serum histamine release. Groups of mice ($n = 10$/group) were intraperitoneally pretreated with 200 μL of saline or LOWE. LOWE was given at various doses one hour before the injection of compound 48/80. The compound 48/80 solution was given intraperitoneally to the groups of mice. The blood was obtained from the heart of each mouse and histamine content was measured. Each bar represents the mean ± SEM. *Significant difference at $P < 0.05$. LOWE, *Lindera obtusiloba* water extract

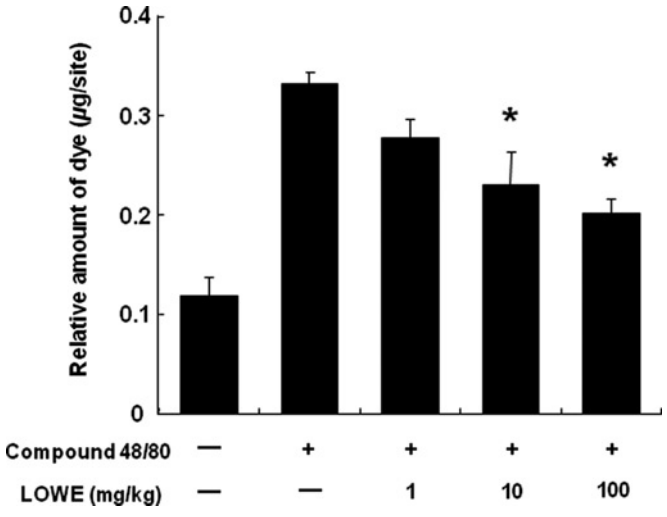

Figure 6 Effect of LOWE on PCA reactions. LOWE was intraperitoneally administered one hour prior to the challenge with antigen. Each amount of dye was extracted as described in Materials and methods and measured by spectrophotometry. Each bar represents the mean ± SEM of three independent experiments. *Significant difference at $P < 0.05$. LOWE, *Lindera obtusiloba* water extract; PCA, passive cutaneous anaphylaxis

Discussion

Immediate-type hypersensitivity (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.[5] Using an *in vitro* and *in vivo* model, we showed that LOWE reduces mast-cell-derived allergic inflammatory responses. In the present study, we clearly demonstrated that LOWE decreased histamine release and proinflammatory cytokine expression. In addition, LOWE inhibited systemic and local allergic reaction, the important *in vivo* models of anaphylaxis.

Calcium is the critical factor for the degranulation of mast cells. Calcium movements across the membranes of mast cells represent a major target for effective antiallergic drugs, as these are essential events that link stimulation to secretion.[6,16] The transduction pathways modulating intracellular calcium are modified by ADP-rybosylates G-protein-binding protein.[20] Our results, which show an attenuation of intracellular calcium in mast cells with LOWE treatment, are consistent with other reports. According to these observations, we strongly suggest that decreased intracellular calcium might be involved in the inhibitory effect of LOWE on histamine release, and LOWE might have a membrane stabilizing activity through G proteins.

Histamine is the principal mediator of allergy. Symptoms of allergy are supposed to be greatly influenced by changing the extent of histamine signaling. Numerous reports have established that stimulation of mast cells with PMACI, compound 48/80 or IgE initiates the activation of the signal transduction pathway, which leads to histamine release. Several studies have shown that compound 48/80 and other polybasic compounds are able, apparently directly, to activate G proteins.[21] Compound 48/80 increases the permeability of the lipid bilayer membrane by causing a perturbation in the membrane. This result indicates that the increase in membrane permeability may be an essential trigger for the release of the mediator from mast cells. In this sense, antiallergic agents having a membrane stabilizing action may be desirable. LOWE might stabilize the lipid bilayer membrane, thus preventing the perturbation being induced by mast cell stimulators.

The spectrum of cytokines produced by mast cells with PMACI stimulation supports the well recognized role of mast cells in immediate-type hypersensitivity. Proinflammatory cytokines, including TNF-α and IL-6, play a major role in triggering and sustaining the allergic inflammatory response in mast cells.[22,23] Mast cells are a principal source of TNF-α in human dermis. TNF-α has an important amplifying effect in asthmatic inflammation and potently stimulates airway epithelial cells to produce cytokines.[24] It is also a potent inducer of other inflammatory cytokines, including IL-1, IL-6, IL-8 and granulocyte–macrophage colony-stimulating factor. IL-6 is produced from mast cells and its local accumulation is associated with PCA reaction.[25] These reports indicate that the reduction of proinflammatory cytokines from mast cells is one of the key indicators of reduced allergic inflammatory symptoms. In our present study, LOWE inhibited the expression of TNF-α and IL-6 in PMACI-stimulated HMC-1. This result suggests that the antiallergic inflammatory effect of LOWE results from its reduction of TNF-α and IL-6 from mast cells.

Intracellular calcium plays an important role in the expression of inflammatory cytokines. Depletion of intracellular calcium blocked the expression of IgE-induced TNF-α and IL-6 in RBL-2H3 mast cells.[26] In our experiments, LOWE decreased the level of intracellular calcium in mast cells. We suggest that the inhibitory effect of LOWE on the inflammatory cytokines results from the reduction of intracellular calcium.

Expression of TNF-α and IL-6 gene is dependent on the activation of transcription factor NF-κB.[27] Activation of NF-κB requires phosphorylation and proteolytic degradation of the inhibitory protein IκBα, an endogenous inhibitor that binds to NF-κB in the cytoplasm.[8] In PMACI-stimulated mast cells, LOWE inhibited degradation of IκBα- and NF-κB-dependent gene transcription. We also previously reported that PDTC, the potent inhibitor of NF-κB, reduced PMACI-induced production of TNF-α, IL-6 and IL-8 in HMC-1.[15,28] These data demonstrate that LOWE attenuates activation of NF-κB and downstream TNF-α and IL-6 expression.

Since we used whole-water extract of *L. obtusiloba*, the active components that are responsible for the biological effect are not clear at this time. The effort to identify active components from the fruits of *L. obtusiloba* in the mast-cell-mediated allergic inflammation is ongoing in our laboratory. In the present report, we provide evidence that LOWE inhibits a model of mast-cell-mediated allergic inflammation and the possible mechanisms such as intracellular calcium for histamine release and NF-κB for proinflammatory cytokines. The results obtained in the present study show that LOWE contributes to the prevention or

treatment of mast-cell-mediated allergic inflammatory diseases.

Author contributions: All authors participated in the design, interpretation of the studies and analysis of the data and review of the manuscript. WMS and S-BP performed the major experiments and wrote the manuscript. SL, H-HK and HGC were responsible for the animal experiments. J-HS, KS and TKK have made substantial contributions to the conception and design of the study. S-HL and S-HK supervised the research and co-wrote the manuscript. WMS and S-BP contributed equally to this work.

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