

Aqueous Extract of *Mosla chinensis* Inhibits Mast Cell-Mediated Allergic Inflammation

Hui-Hun Kim,^{*,a} Jin-Su Yoo,^{†,a} Tae-Yong Shin[†] and Sang-Hyun Kim^{*}

^{*}CMRI, Department of Pharmacology, School of Medicine

Kyungpook National University

Daegu 700-422, Republic of Korea

[†]College of Pharmacy, Woosuk University

Jeonju 565-701, Republic of Korea

Abstract: Allergic inflammatory diseases such as food allergy, asthma, sinusitis, and atopic dermatitis are increasing worldwide. In this study, we investigated the effects of aqueous extract of *Mosla chinensis* Max. (AMC) on mast cell-mediated allergic inflammation and studied the possible mechanism of this action. AMC inhibited compound 48/80-induced systemic and immunoglobulin E (IgE)-mediated local anaphylaxis. AMC reduced intracellular calcium levels and downstream histamine release from rat peritoneal mast cells activated by compound 48/80 or IgE. In addition, AMC decreased gene expression and secretion of proinflammatory cytokines such as tumor necrosis factor (TNF)- α , interleukin (IL)-6 and IL-8 in human mast cells. The inhibitory effect of AMC on cytokine expression was nuclear factor (NF)- κ B dependent. Our results indicate that AMC inhibits mast cell-mediated allergic inflammatory reaction by suppressing histamine release and expression of proinflammatory cytokines and the involvement of calcium and NF- κ B in these effects. AMC might be a possible therapeutic candidate for allergic inflammatory disorders.

Keywords: *Mosla chinensis*; Allergic Inflammation; Mast Cells; Histamine; Proinflammatory Cytokine; Nuclear Factor- κ B.

Introduction

Mast cells are broadly distributed throughout mammalian tissue and play various functions as a regulator of allergic inflammation such as asthma, atopic dermatitis, and sinusitis. Mast

Correspondence to: Professor Sang-Hyun Kim, Department of Pharmacology, School of Medicine, Kyungpook National University, 101 Dongin-dong Jung-gu, Daegu 700-422, Republic of Korea. Tel: (+82) 53-420-4838, Fax: (+82) 53-423-4838, E-mail: shkim72@knu.ac.kr or Dr. Tae-Yong Shin, College of Pharmacy, Woosuk University, 443 Samnye-ro Samnye-eup Wanju-gun Jeollabuk-do 565-701, Republic of Korea. Tel: (+82) 63-290-1572, Fax: (+82) 63-290-1567, E-mail: tyshin@woosuk.ac.kr

^aThese authors contributed equally to this work.

cells have been considered not only in the association of immediate type hypersensitivity but also in late reaction like inflammatory responses (Galli *et al.*, 2008; Caughey, 2011). Immediate type hypersensitivity is mediated by histamine in response to the antigen cross-linking of immunoglobulin E (IgE) bound to Fc ϵ RI on the mast cells (Galli *et al.*, 2005). In mast cell-mediated inflammatory responses, histamine is one of the most characterized and important mediators implicated in the acute phase of immediate hypersensitivity (Lee *et al.*, 2006; Tegen *et al.*, 2009). 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)- κ B, and expression of proinflammatory cytokines (Kim *et al.*, 2006; Gwack *et al.*, 2007).

Mosla chinensis Max. (Labiatae) is widely distributed in the south of China and Korea. The leaves of this plant are frequently used as a raw vegetable or as an additive in foods to give flavor. It was used in traditional medicine for treatment of the common cold, fever and exhaustion (Li *et al.*, 2009). Various biological effects of *M. chinensis* have been reported such as anti-inflammation and anti-infection (Chen *et al.*, 1989; Osawa *et al.*, 1990; Yan *et al.*, 2002). The chemical components of the crude drug or essential oils are reported to be carvacrol, thymol and cymene (Duan, 1986; Ji *et al.*, 2004; Li *et al.*, 2009). In spite of the known various biological effects, anti-allergic inflammatory effect of *M. chinensis* has not been studied yet. The objective of this study is to elucidate the effect of aqueous extract of *M. chinensis* (AMC) on the allergic inflammation and to define the underlying mechanisms of these effects using *in vivo* and *in vitro* models.

Materials and Methods

Animals

The original stock of male Imprinting Control Region (ICR) mice (six weeks) and male Sprague-Dawley rats (eight weeks) were purchased from the Dae-Han Biolink Co Ltd (Daejeon, Korea). The animals were housed five per cage in a laminar air flow room maintained under a temperature of $22 \pm 2^\circ\text{C}$ and 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), phorbol 12-myristate 13-acetate (PMA), calcium ionophore A23187 were purchased from Sigma Chemical Co (St Louis, MO). The human mast cell line (HMC-1) was grown in Iscove's media (Life Technologies, Grand Island, NY) supplemented with 10% fetal bovine serum (FBS) at 37°C in 5% CO_2 . The passage ranging 4–8 of HMC-1 was used throughout the study.

Preparation of AMC

The plant of *M. chinensis* was collected in Wanju, South Korean on August 18, 2010. The voucher specimen (WSP-10-44) was deposited in the Herbarium of the College of Pharmacy, Woosuk University. *M. chinensis* 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 plant sample (100 g) was extracted twice with purified water (500 ml) at 70°C for 5 h in a water bath. The extract was filtered through Whatman no. 1 filter paper and the filtrate was lyophilized using a 0.45 μ m syringe filter. The yield of dried extract from crude materials was about 8.3%. The dried extract was dissolved in saline or Tyrode buffer A (HEPES 10 mM, NaCl 130 mM, KCl 5 mM, CaCl₂ 1.4 mM, MgCl₂ 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. AMC was administered intraperitoneally at doses of 10–1000 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 (Bae *et al.*, 2011).

Passive Cutaneous Anaphylaxis (PCA)

An IgE-dependent cutaneous reaction was carried out as described previously (Kim *et al.*, 2011). Briefly, mice were injected intradermally with 0.5 μ g of anti-DNP IgE. After 48 h, each mouse ($n = 10$ /group) received an injection of 1 μ g of DNP-HSA containing 4% Evans blue (1:4) via the tail vein. Thirty minutes after the challenge, the mice were killed and the dorsal skin (diameter, 1 cm) was removed for measurement of the pigmented area. The amount of dye was then determined colorimetrically after extraction with 1 ml of 1 M KOH and 9 ml of mixture of acetone and phosphoric acid (5:13). The absorbent intensity of the extraction was measured at 620 nm in a spectrophotometer (Shimadzu, UV-1201, Japan).

Preparation of Rat Peritoneal Mast Cells (RPMC)

RPMC were isolated as previously described (Kim *et al.*, 2006). Briefly, rats were anesthetized with carbon dioxide and injected with 20 ml of Tyrode buffer B (137 mM NaCl, 5.6 mM glucose, 12 mM NaHCO₃, 2.7 mM KCl, 0.3 mM NaH₂PO₄ and 0.1% gelatin), into the peritoneal cavity and the abdomen was gently massaged for about 90 s. The peritoneal cavity was carefully opened, and the fluid containing peritoneal cells were aspirated by Pasteur pipette. The peritoneal cells were sedimented at 150 g for 10 min at room temperature and resuspended in Tyrode buffer B. Mast cells were separated from the major components of rat peritoneal cells, i.e. macrophages and small lymphocytes. Peritoneal cells were suspended in 1 ml of Tyrode buffer B, layered on 2 ml of metrizamide

(22.5 w/v%), and centrifuged at 400 *g* for 15 min at 4°C. The cells remaining at the buffer-metrizamide interface were aspirated and discarded; the cells in the pellet were washed and resuspended in 1 ml of Tyrode buffer A. Mast cell preparations were about 95% pure as assessed by toluidine blue staining. More than 97% of the cells were viable as judged by trypane blue exclusion.

Determination of Histamine Level

Determination of histamine contents was examined as previously described (Kim *et al.*, 2007). The histamine contents of RPMC were measured by the *o*-phthalaldehyde spectrofluorometric procedure. The fluorescent intensity was measured at emission 438 nm and excitation 353 nm using spectrofluorometer. RPMC were preincubated with AMC, and then incubated for 10 min with compound 48/80 (5 µg/ml). RPMC suspensions (2×10^5 cells/ml) were also sensitized with anti-DNP IgE (10 µg/ml) for 16 h. The cells were preincubated with AMC at 37°C for 10 min prior to the challenge with DNP-HSA (1 µg/ml). The cells were separated from the released histamine by centrifugation at 400 *g* for 5 min at 4°C.

Determination of Intracellular Calcium

The intracellular calcium was measured with the use of the fluorescence indicator Fluo-3/AM (Molecular Probes, Eugene, OR). RPMC were preincubated with Fluo-3/AM for 30 min at 37°C. After washing the dye from the cell surface, the cells were pretreated with AMC for 10 min prior to the compound 48/80 (5 µg/ml) treatment. It was excited at 488 nm, and the emission was filtered with 515 nm by flow cytometer (BD Biosciences Pharmingen, San Diego, CA) and visualized in a fluorescence microscope (Olympus BX51, Center Valley, PA).

Cell Viability

In order to investigate the toxicity of AMC, cell viability was evaluated by a colorimetric assay based on the conversion of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). MTT is transformed by mitochondrial dehydrogenases into formazan, enabling cell viability to be assessed. The cells were cultured on 0.01 to 10 mg/ml AMC, placed in single layers in 96-well plates, at an initial seeding density of 2×10^4 cells per well and cultured for 24 h. Then 20 µl of MTT solution pre-warmed to 37°C was added to each well and culture continued for 3 h under the same conditions. The reaction was then stopped by adding 100 µl of dimethylsulfoxide to each well. The solution was transferred to new wells and the absorbance measured at a wavelength of 570 nm using an ELISA microplate reader.

RNA Extraction and mRNA Detection

The total cellular RNA was isolated from the cells (1×10^6 /well in a 24-well plate) after stimulation with PMA (20 nM) and A23187 (1 µM) with or without AMC for 2 h using

TRI reagent (Molecular Research Center, Cincinnati, OH) according to the manufacturer's protocol. The first strand complementary DNA (cDNA) was synthesized using the Superscript II reverse-transcriptase (Invitrogen, Carlsbad, CA). A reverse-transcriptase polymerase chain reaction (RT-PCR) was used to analyze the expression of mRNA for TNF- α , IL-6, IL-8 and β -actin (internal control). The conditions for the reverse transcription and PCR steps were similar to those described previously (Kim *et al.*, 2009). The amplified products were separated by electrophoresis on 2% agarose gel containing ethidium bromide, documented using a Kodak DC 290 digital camera and digitized with UN-SCAN-IT software (Silk Scientific, Orem, UT). The band intensity was normalized to that of β -actin in the same sample.

Enzyme-Linked Immunosorbent Assay (ELISA)

The secretion of TNF- α , IL-8 and IL-6 was measured by the modification of an enzyme-linked immunosorbent assay (ELISA) as described previously (Min *et al.*, 2007). HMC-1 cells were cultured with media and resuspended in Tyrode buffer A. The cells were stimulated with PMACI for 8 h in the absence or presence of AMC. The ELISA was performed by coating 96-well plates with 6.25 ng/well of monoclonal antibody with specificity for TNF- α , IL-8 and IL-6, respectively.

Western Blot Analysis

HMC-1 were washed three times with PBS and resuspended in lysis buffer. Samples were electrophoresed using 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis, as described elsewhere (Lee *et al.*, 2008), and then transferred to a nitrocellulose membrane. The nucleus and cytosolic p65 NF- κ B and I κ B α was assayed using anti-NF- κ B (p65) and anti-I κ B α antibody (Santa Cruz Biotechnology, Santa Cruz, CA). Immunodetection was done by using supersignal west pico chemiluminescent substrate (Thermo scientific, Waltham, MA).

Transient Transfection and Luciferase Activity Assay

For transient transfection, HMC-1 cells were seeded at 2×10^6 in a 6-well plate one day before transient transfection. The expression vectors containing the NF- κ B luciferase reporter construct (pNF- κ B-LUC, plasmid containing NF- κ B binding site; Stratagene, Grand Island, NY) were transfected with serum- and antibiotics-free Iscove's medium containing 8 μ l Lipofectamine 2000 reagent (Invitrogen). After 5 h of incubation, medium was replaced with Iscove's medium containing 10% FBS and antibiotics. Cells were allowed to recover at 37°C for 30 h and subsequently were stimulated as indicated. Cell lysates were prepared and assay for luciferase activity using Luciferase Assay System (Promega, Madison, WI), according to the manufacturer's instructions.

Statistical Analysis

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

Results

Effect of AMC on Systemic and Local Anaphylaxis

To determine the effect of AMC in allergic reaction, an *in vivo* model of a systemic anaphylaxis was used. 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 (8 mg/kg) into mice induced fatal shock in 100% of animals. When AMC was intraperitoneally pretreated at doses ranging from 10 to 1000 mg/kg for 1 h, the mortality was dose dependently reduced. More than 1000 mg/kg of AMC completely inhibited compound 48/80-induced fatal shock (Table 1). In addition, the mortality of mice administered with AMC (1000 mg/kg) 5, 10 and 15 min after compound 48/80 injection increased time dependently (Table 2).

To confirm the anti-allergic effects of AMC, we used a passive cutaneous anaphylaxis (PCA) model induced by anti-DNP-IgE and DNP-HSA. To compare the amount of dye with control, the left dorsal skin of these mice was injected with saline alone. AMC (10 to 1000 mg/kg) was intraperitoneally administered 1 h prior to the challenge with antigen. AMC dose-dependently inhibited PCA reaction (Fig. 1).

Table 1. Dose-Dependent Effect of AMC on Compound 48/80-Induced Systemic Anaphylaxis

AMC Treatment (mg/kg BW)	Compound 48/80 (8 mg/kg BW)	Mortality (%)
None (saline)	+	100
10	+	100
50	+	60
100	+	30
500	+	10
1000	+	0
1000	–	0

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

Table 2. Time-Dependent Effect of AMC on Compound 48/80-Induced Systemic Anaphylaxis

AMC Treatment (1000 mg/kg BW)	Time (min)	Compound 48/80 (8 mg/kg BW)	Mortality (%)
None (saline)		+	100
+	5	+	20
+	10	+	40
+	15	+	100

Groups of mice ($n = 10/\text{group}$) were intraperitoneally pretreated with $200\ \mu\text{l}$ saline or AMC. AMC (1000 mg/kg) was given at 5, 10 and 15 min after the compound 48/80 injection. The compound 48/80 solution was intraperitoneally given to the group in mice. Mortality (%) within 1 h following compound 48/80 injection is represented as the number of dead mice $\times 100/\text{total number of experimental mice}$.

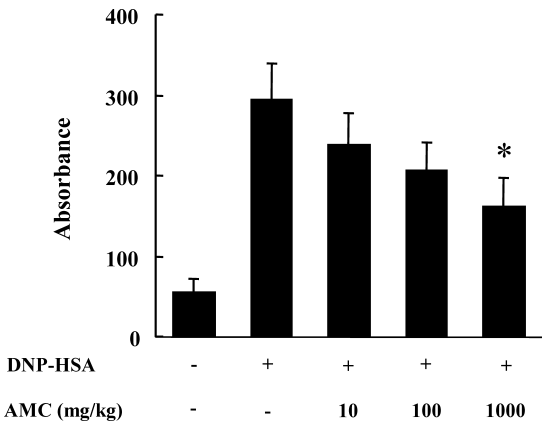


Figure 1. Effects of AMC on PCA reaction. AMC was intraperitoneally administered 1 h prior to the challenge with antigen. Each amount of dye was extracted as described in Materials and methods, and measured by spectrophotometry. Each bar represents the mean $\pm$ S.E.M. of three independent experiments. *Significant difference at $p < 0.05$.

Effect of AMC on Compound 48/80- or IgE-Stimulated Histamine Release from RPMC

We estimated the inhibitory effects of AMC on the compound 48/80- or IgE-mediated histamine release from RPMC. Very low levels of histamine were detectable in unstimulated cells; however, RPMC released a high level of histamine when stimulated with compound 48/80 ($5\ \mu\text{g/ml}$) or when anti-DNP IgE sensitized RPMC was challenged with DNP-HSA. When AMC was pretreated for 30 min, histamine level was dose-dependently inhibited in compound 48/80- or anti-DNP IgE sensitized RPMC (Fig. 2A). Up to 1 mg/ml of AMC did not show cytotoxicity (Fig. 2B).

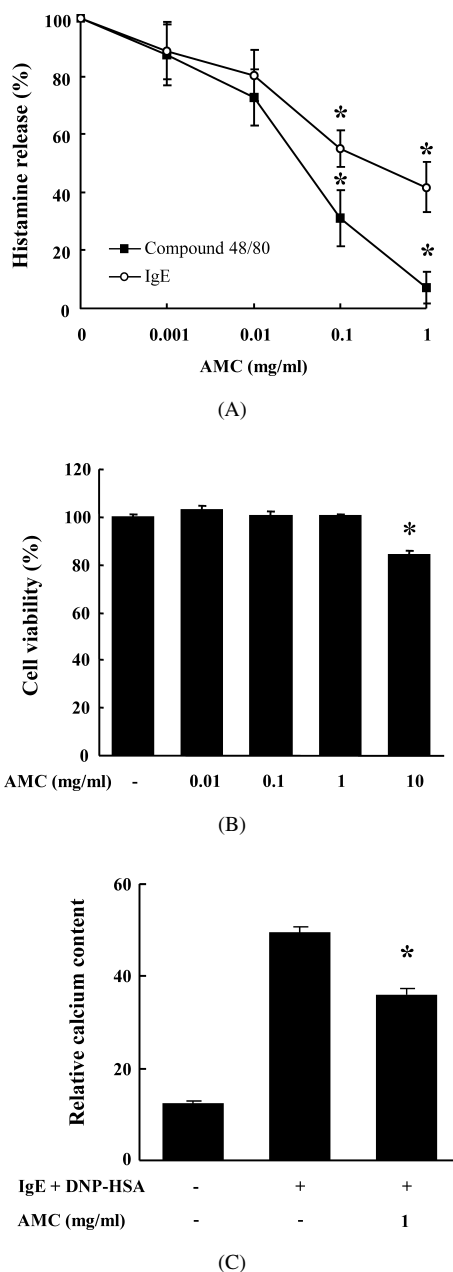


Figure 2. Effects of AMC on histamine release and intracellular calcium from RPMC. (A) The cells (2×10^5 cells/ml) were preincubated with various concentrations of AMC at 37°C for 10 min prior to incubation with either compound 48/80 (naive RPMC) or DNP-HSA (anti-DNP IgE sensitized RPMC). (B) Cells were treated with various concentrations of AMC (0.001 to 10 mg/ml) for 24 h. The cell viability was determined using the MTT assay. Cell viability was determined by the relative absorbance, compared to control. (C) Cells were preincubated with AMC (1 mg/ml) at 37°C for 10 min prior to incubation with DNP-HSA (anti-DNP IgE sensitized RPMC). Each data represents the mean $\pm$ S.E.M. of three independent experiments. *Significant difference at $p < 0.05$.

Effect of AMC on Intracellular Calcium

Calcium movements across membranes of mast cells are critical to histamine release (Eisenhut and Wallace, 2011). To investigate the mechanism responsible for the reduction of histamine after AMC treatment, we assayed the level of intracellular calcium. Figure 2C shows the stimulation of intracellular calcium when anti-DNP IgE sensitized RPMC was challenged with DNP-HSA. Preincubation of AMC (1 mg/ml) with RPMC decreased the intracellular calcium level.

Effect of AMC on the Expression and Secretion of Pro-Inflammatory Cytokines

HMC-1 cell line is a useful cell for researching cytokine activation pathways (Kim *et al.*, 2010; Shin *et al.*, 2005). We investigated the inhibitory effect of AMC on the expression of proinflammatory cytokines such as TNF- α , IL-8 and IL-6. Previously, we reported that gene expression of TNF- α , IL-8 and IL-6 peaked at 4 h after treatment of PMACI (Park *et al.*, 2007). HMC-1 cells were stimulated by PMACI during 4 h, and the cells were preincubated with AMC for 30 min. As shown in Fig. 3A, AMC dose-dependently inhibited PMACI-induced gene expression of TNF- α , IL-6 and IL-8. To confirm the correlation of mRNA expression with protein secretion, we measured the level of TNF- α , IL-8 and IL-6 by ELISA. When the HMC-1 cells were stimulated with PMACI for 8 h, the secretion of cytokines was remarkably induced. Secretion of TNF- α , IL-8 and IL-6 was significantly inhibited by AMC (1 mg/ml) in PMACI-stimulated HMC-1 (Fig. 3B).

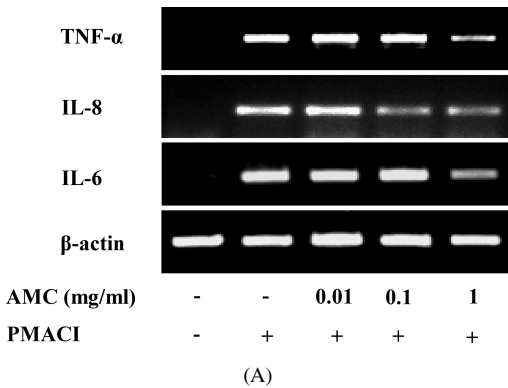


Figure 3. Effects of AMC on gene expression and secretion of pro-inflammatory cytokines in HMC-1. Cells (1×10^6 cells/ml) were preincubated with AMC for 30 min prior to incubation with PMA (20 nM) and calcium ionophore A23187 (1 μ M). (A) Extraction and analysis of mRNA was performed as described in Materials and methods. The gene expression of TNF- α , IL-8 and IL-6 were determined by RT-PCR. (B) The level of TNF- α , IL-8 and IL-6 in supernatant was measured using ELISA and represented as the mean $\pm$ S.E.M. of three independent experiments. *Significant difference from PMACI value at $p < 0.05$.

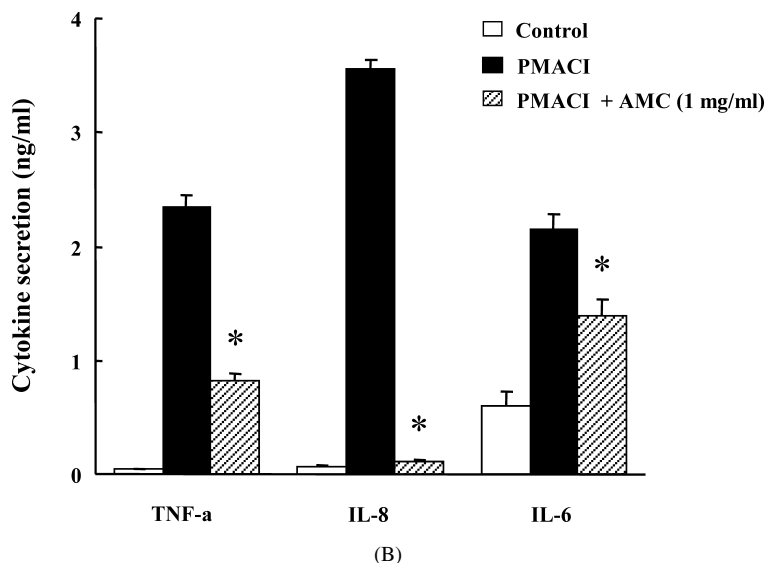


Figure 3. (Continued)

Effect of AMC on the Activation of NF- κ B

To investigate the intracellular mechanism responsible for the inhibitory effect of AMC on the expression of proinflammatory cytokines, we examined the effect of AMC on the activation of transcription factors, NF- κ B. NF- κ B is an important transcriptional regulator of inflammatory cytokines and plays a crucial role in immune and inflammatory responses (Kim *et al.*, 2006). Stimulation of HMC-1 with PMACI induced the nuclear translocation of p65 NF- κ B and degradation of I κ B α after 2 h of incubation. AMC inhibited the PMACI-induced nuclear translocation of NF- κ B and degradation of I κ B α (Fig. 4A). To confirm the inhibitory effect of AMC on the NF- κ B activation, we examined the effect of AMC on NF- κ B-dependent gene reporter assay. HMC-1 cells were transiently transfected with a NF- κ B-luciferase reporter construct or an empty vector. Exposure of cells to PMACI increased the luciferase activity in the cells transfected with NF- κ B-luciferase reporter construct (Fig. 4B). AMC (1 mg/ml) significantly reduced PMACI-induced luciferase activity.

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 (Boden and Wesley Burks, 2011). We showed that AMC reduces mast cell-mediated allergic inflammatory reactions using *in vivo* and *in vitro* models. AMC inhibited compound 48/80-induced systemic allergic reaction. In addition, AMC administered mice were

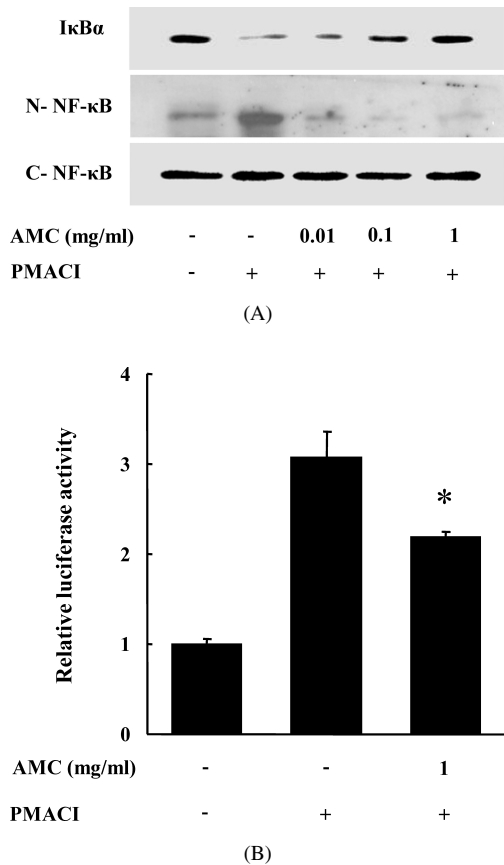


Figure 4. Effects of AMC on the activation of NF- κ B in HMC-1. Cells were pretreated with AMC for 30 min prior to PMA (20 nM) and A23187 (1 μ M) stimulation for 2 h. (A) Nuclear translocation of NF- κ B and I κ B α degradation were assayed by Western blot (N-NF- κ B, nuclear NF- κ B; C-NF- κ B, cytoplasmic NF- κ B). (B) Cells were transiently transfected with the NF- κ B-luciferase reporter construct or empty vector. Then, the cells were incubated with PMA and A23187 with or without AMC. NF- κ B-dependent transcriptional activity was determined by luciferase activity assay. Each bar represents the mean $\pm$ S.E.M. of three independent experiments. *Significant difference from PMACI value at $p < 0.05$.

protected from IgE-mediated PCA, which is one of the most important *in vivo* models of anaphylaxis in a local allergic reaction. These results suggest that AMC 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. It has been reported that histamine affects chronic inflammation and regulates several essential events of immune response such as immune cell maturation, polarization, and lymphocyte responsiveness (Jutel *et al.*, 2002; Wu *et al.*, 2011). Histamine also induces actin polymerization and chemotaxis (Jutel *et al.*, 2005). 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. Intracellular calcium pathways are critical to the degranulation of allergic mediator such as histamine from mast cells (Kim *et al.*, 2006). In addition, calcium movements across membranes of mast cells represent a major target for useful anti-allergic drugs, as these are essential events linking stimulation to secretion (Beaven *et al.*, 1984). In this study, we speculated that decreased intracellular calcium might be involved in the inhibitory effect of AMC on histamine release.

The HMC-1 cell line is one of the useful cells for studying cytokine activation pathways (Kim *et al.*, 2006). 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 , IL-8 and IL-6, the known proinflammatory cytokines, play an important role in triggering and sustaining the allergic inflammatory response in mast cells (Walsh *et al.*, 1991). Mast cells are a principal source of cytokines in human dermis. TNF- α is a well-known inflammatory factor produced mainly by macrophages, and plays a critical role in systemic inflammation (Hu *et al.*, 1997). It is also a potent inducer of other inflammatory cytokines, including IL-1 β , IL-6, IL-8 and GM-CSF. IL-6 is one of the most important mediators of a multifunctional pro-inflammatory cytokines, and actually the major cytokine produced by activated mast cells (Burd *et al.*, 1989). These reports represent that the inhibition of pro-inflammatory cytokines is one of the main points of reduced allergic inflammatory symptom. In the present study, AMC inhibited the expression of TNF- α , IL-8 and IL-6 in PMACI-stimulated mast cells. This result suggests that the anti-allergic inflammatory effect of AMC results from its inhibition of TNF- α , IL-8 and IL-1 β generation from mast cells.

Expression of TNF- α and IL-1 β is regulated by the activation of transcription factor NF- κ B (Karin, 2009). NF- κ B regulates the expression of multiple inflammatory and immune genes and plays a critical role in chronic inflammatory diseases. Activation of NF- κ B required phosphorylation and proteolytic degradation of the inhibitory protein I κ B α , an endogenous inhibitor that binds to NF- κ B in the cytoplasm. In PMACI-stimulated mast cells, AMC decreased the degradation of I κ B α and nuclear translocation of NF- κ B. These results demonstrated that AMC attenuates activation of NF- κ B and downstream cytokine expression.

In summary, the present study demonstrates that AMC significantly reduced mast cell-mediated allergic inflammation in *in vivo* and *in vitro* models. We suggest that AMC reduces histamine release by the modulation of intracellular calcium. AMC inhibits expression of inflammatory cytokines by the suppression of NF- κ B activation. Because we used the whole aqueous extract of *Mosla chinensis* not a purified single compound, the biological effects of the individual active components are not clear at this time. The effort to identify the active components from AMC in allergic inflammatory symptom is ongoing in our laboratory. However, the results presented in this report give an insight into the mechanism responsible for anti-allergic inflammatory activity of AMC and evidence that AMC could contribute to the prevention or treatment of mast cell-mediated allergic inflammatory diseases.

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