

RESEARCH ARTICLE

Meoruh wine suppresses mast cell-mediated allergic inflammation

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Abstract

In this study, we investigated the effect of the Meoruh wine (MW), a Korean traditional wine made by the fruits of *Vitis amurensis* Rupr. (Vitaceae) on the mast cell-mediated allergic inflammation and studied the possible mechanism of action. Mast cell-mediated allergic disease is involved in many diseases such as anaphylaxis, rhinitis, asthma, and atopic dermatitis. MW inhibited compound 48/80-induced systemic anaphylaxis and serum histamine release in a dose-dependent manner in mice. MW decreased immunoglobulin E-mediated local allergic reactions, passive cutaneous anaphylaxis. MW dose-dependently reduced histamine release from human mast cells activated by phorbol 12-myristate 13-acetate and calcium ionophore A23187 (PMA). The inhibitory effect of MW on histamine release was mediated by suppression of intracellular calcium. In addition, MW attenuated the PMA-stimulated expression of pro-inflammatory cytokines such as tumor necrosis factor- α , interleukin-1 β (IL-1 β), IL-6, and IL-8 in human mast cells. The inhibitory effect of MW on these pro-inflammatory cytokines was nuclear factor (NF)- κ B dependent. Our findings provide evidence that MW inhibits mast cell-derived allergic inflammatory reactions and involvement of calcium, pro-inflammatory cytokines and NF- κ B in these effects.

Keywords: Meoruh wine, allergic inflammation, mast cells, histamine, pro-inflammatory cytokines.

Introduction

Mast cells are broadly distributed throughout mammalian tissues and play a critical role in a wide variety of biological responses. Typically, mast cells have been considered not only in the association of immediate type hypersensitivity, but also in late reactions like inflammatory responses.^(1,2) Immediate type hypersensitivity is mediated by histamine released in response to the antigen cross linking of immunoglobulin E (IgE) bound to Fc ϵ RI on the mast cells.⁽³⁾ After activation via the Fc ϵ RI, the mast cells start the process of degranulation which results in the releasing of mediators, such as products of arachidonic acid metabolism and an array of inflammatory cytokines.^(4,5) Among the inflammatory substances released from the mast cells, histamine is one of the best characterized and most potent vasoactive mediators implicated in the acute phase of immediate hypersensitivity.^(6,7)

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)- κ B and the release of inflammatory cytokines. Activated mast cells can produce histamine, as well as a wide variety of other inflammatory mediators such as eicosanoids, proteoglycans, proteases, and several pro-inflammatory and chemotactic cytokines, such as tumor necrosis factor- α (TNF- α), interleukin-6 (IL-6), IL-8, IL-4, IL-13 and the transforming growth factor- β .^(3,4) The transcription factor NF- κ B has important activities as mediators of cellular responses to extracellular signals and is thought to play an important role in the regulation of pro-inflammatory molecules on cellular responses, especially TNF- α , IL-6, and IL-8.^(8,9)

Meoruh wine (MW) is fermented from the fruits of *Vitis amurensis* Rupr. (Vitaceae) which has been used for

centuries as traditional oriental medicine.⁽¹⁰⁾ This crude drug contains polyphenols mainly catechin, procyanidin, amurensin.^(11,12) It is native to Korea, China, and Japan. It has been reported that the extract of *V. amurensis* have anti-inflammatory and anti-tumor activity.^(13–16) The aim of this study is to evaluate the anti-allergic inflammatory effect of the MW and to understand the mechanism of effect.

Materials and methods

Animals

The original stock of male ICR mice (20–30g) was purchased from the Dae-Han Biolink Co., Ltd (Daejeon, Korea). The animals were maintained in the College of Pharmacy, Woosuk University. The animals were housed 5–10 per cage in a laminar air flow room (conventional condition), maintained at a temperature of $22 \pm 2^\circ\text{C}$, 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 (PDTTC), phorbol 12-myristate 13-acetate (PMA) and calcium ionophore A23187 were purchased from the Sigma Chemical Co. (St. Louis, MO). rTNF- α and rIL-6, and anti-TNF- α , and anti-IL-6 antibodies were purchased from R&D Systems Inc. (Minneapolis, MN). 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) and 2 mM glutamine at 37°C in 5% CO_2 . Passages 4–8 of cultures were used in all experiments.

Preparation of MW

MW was purchased from Chateau Muju Co. (Muju-Gun, South Korea). MW was concentrated at 50°C in water bath. The dried extract was lyophilized. The dried extract was dissolved in saline or Tyrode buffer A (10 mM HEPES, 130 mM NaCl, 5 mM KCl, 1.4 mM CaCl_2 , 1 mM MgCl_2 , 5.6 mM glucose, 0.1% bovine serum albumin) before use.

Compound 48/80-induced systemic anaphylaxis

Compound 48/80-induced systemic reaction was carried out as previously described.⁽¹⁷⁾ Briefly, the mice ($n = 10/\text{group}$) were given an intraperitoneal injection of 8 mg/kg body weight (BW) of the mast cell degranulator, compound 48/80. MW was dissolved in saline and administered intraperitoneally at doses of 10–1000 mg/kg BW 1 h before the compound 48/80 injection. Mortality was monitored for 1 h after induction of anaphylactic shock.

PCA

Passive cutaneous anaphylaxis (PCA) reaction was carried out as previously described.⁽¹⁸⁾ Briefly, mice were injected intradermally with 0.5 μg of anti-DNP IgE. After 48 h, each mouse ($n = 10/\text{group}$) was received an injection of 100 μg of DNP-HSA in PBS containing 4% Evans blue (1:1) via tail vein. MW (10–1000 mg/kg BW) was intraperitoneally administered 1 h 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 M KOH and 9 mL of a mixture of acetone and phosphoric acid (5:13). The intensity of the absorbent was measured at 620 nm in a spectrophotometer (Shimadzu, UV-1201, Kyoto, Japan).

Histamine assay

Histamine content was measured by the enzyme immunoassay kit (Oxford Biomedical Research, Oxford, MI) according to manufacturer manual. HMC-1 were pre-incubated with MW for 30 min, and then incubated for 8 h with PMA and calcium ionophore A23187 (PMACI). The cells were separated from the released histamine by centrifugated at 400g for 5 min at 4°C .

Measurement of calcium

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

RT-PCR

Reverse transcription-polymerase chain reaction (RT-PCR) was used to analyze the expression of mRNA for TNF- α , IL-1 β , IL-6, IL-8, and β -actin (internal control) with MW in PMACI-stimulated HMC-1 cells. The conditions for RT-PCR were similar to ones previously described.^(5,19) Amplified PCR products were separated by 2% agarose gels and digitized using UN-SCAN-IT software (Silk Scientific, Orem, UT).

Assay of TNF- α and IL-8 secretion

Secretion of TNF- α and IL-6 was measured by modification of an enzyme-linked immunosorbent assay (ELISA) as previously described.⁽²⁰⁾ HMC-1 cells were sensitized with PMACI for 16 h in the absence or presence of MW. The ELISA was performed by coating 96-well plates with 6.25 ng/well of monoclonal antibody with specificity for TNF- α and IL-6. Before use and between subsequent steps in the assay, the coated plates were washed twice with PBS containing 0.05% Tween-20 and twice with PBS alone. For the standard curve, rTNF- α and rIL-6 were added to

the serum which was previously determined to be negative to endogenous TNF- α and IL-6. After exposure to the medium, the assay plates were exposed sequentially to biotinylated anti-human TNF- α and IL-6 and 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) tablet substrates. Optical density readings were made within 10 min of the addition of the substrate with a 405 nm filter.

Nuclear protein extraction

Preparation of nuclear extract was basically as described elsewhere.⁽²¹⁾ Briefly, after cell activation for the times indicated, cells were washed in 1 mL of ice-cold PBS, centrifuged at 1200 rpm for 5 min, resuspended in 400 μ L of ice-cold hypotonic buffer (10 mM HEPES/KOH, 2 mM MgCl₂, 0.1 mM EDTA, 10 mM KCl, 1 mM DTT, 0.5 mM PMSE, pH 7.9), left on ice for 10 min, vortexed, and centrifuged at 15,000g for 30 sec. After several times of washing, pelleted nuclei were resuspended in 50 μ L of ice-cold saline buffer (50 mM HEPES/KOH, 50 mM KCl, 300 mM NaCl, 0.1 mM EDTA, 10% glycerol, 1 mM DTT, 0.5 mM PMSE, pH 7.9), left on ice for 20 min, vortexed, and centrifuged at 15,000g for 5 min at 4°C. Aliquots of the supernatant that contained nuclear proteins were frozen in liquid nitrogen and stored at -70°C.

Western blot analyses

HMC-1 cells were washed with PBS and resuspended in lysis buffer. Samples were electrophoresed using 8% sodium dodecyl sulfatepolyacrylamide gel electrophoresis, as described elsewhere⁽²²⁾ and then transferred to a nitrocellulose membrane. The NF- κ B was assayed using anti-NF- κ B antibody (Santa Cruz Biotech, Santa Cruz, CA). Immunodetection was done using an enhanced chemiluminescence detection kit (Amersham Pharmacia, Piscataway, NJ).

Transient transfection and luciferase activity assay

For transient transfections, HMC-1 cells were seeded at 2×10^6 in a 6-well plate 1 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; STANTAGEN, Grand Island, NY) were transfected with serum- and antibiotics-free Iscove's medium containing 8 μ L of Lipofectamine 2000 reagent (Invitrogen, Carlsbad, CA). 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 20 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 used to indicate significance.

Results

Effect of MW on compound 48/80-induced systemic anaphylaxis

To determine the effect of MW on allergic reaction, an *in vivo* model of a systemic reaction 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. As shown in Table 1, injection of compound 48/80 into mice induced fatal shock in 100% of animals. When MW was intraperitoneally administered at a concentrations ranging from 10 to 1000 mg/kg BW for 1 h, the mortality with compound 48/80 was dose-dependently reduced. In addition, the mortality of mice administered intraperitoneally with MW (1000 mg/kg) 5, 10, 15, and 20 min after compound 48/80 injection increased time-dependently (Table 2). We evaluated the effect of MW treatment on compound 48/80-induced serum histamine release. Our results indicated that MW dose-dependently decreased the compound 48/80-induced serum histamine release (Figure 1).

Effect of MW on IgE-mediated PCA

Another way to test the allergic reactions is to induce PCA. A local extravasation was induced by a local injection of

Table 1. Effect of MW on compound 48/80-induced systemic anaphylaxis

MW treatment (mg/kg BW)	Compound 48/80 (8 mg/kg BW)	Mortality (%)
None (saline)	+	100
10	+	100
50	+	100
100	+	80
500	+	10
1000	+	0
1000	-	0

Groups of mice ($n = 10$ /group) were intraperitoneally pre-treated with 200 μ L of saline or MW 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/total number of experimental mice.

Table 2. Time-dependent effect of MW on compound 48/80-induced systemic anaphylaxis

MW treatment (mg/kg BW)	Time (min)	Compound 48/80 (8 mg/kg BW)	Mortality (%)
None (saline)	-	+	100
1000	5	+	0
	10	+	50
	15	+	90
	20	+	100

Mice ($n = 10$ /group) were intraperitoneally pre-treated with 200 μ L of saline or MW (1000 mg/kg) 0, 5, 10, 15, and 20 min after 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/total number of experimental mice.

IgE followed by an antigenic challenge. Intraperitoneal injection of MW dose-dependently inhibited PCA reaction (Figure 2).

Effect of MW on histamine release from mast cells

We next evaluated the ability of MW to inhibit PMACI-induced histamine release from HMC-1 cells. Treatment with MW dose-dependently inhibited PMACI-induced histamine release at concentrations of 0.1–1 mg/mL (Figure 3). The concentration of MW treatment used in these studies had no significant effect on the viability of HMC-1 cells (data not shown).

Effect of MW on intracellular calcium in HMC-1 cells

Calcium movements across membranes of mast cells are critical to histamine release.⁽²³⁾ To investigate the mechanism of MW on the reduction of histamine release, we assayed intracellular calcium levels. Figure 4 shows the stimulation of intracellular calcium when mast cells are treated with PMACI. Preincubation of MW with cells decreased the intracellular calcium level induced by PMACI.

Effect of MW on the gene expression and secretion of pro-inflammatory cytokines from HMC-1 cells

TNF- α , IL-1 β , IL-6, and IL-8 are the most important pro-inflammatory cytokines. Therefore, we tested the effect of MW on the gene expression of TNF- α , IL-6, and IL-8 induced by PMACI in HMC-1 cells. HMC-1 cell line is a useful cell for studying cytokine activation pathway.^(5,24) Previously, we reported that gene expression of TNF- α , IL-1 β , IL-6, and IL-8 was peaked at 4 h after treatment of PMACI.⁽²⁵⁾ Therefore, stimulation of HMC-1 with PMACI was induced during 4 h, and the cells were pre-treated

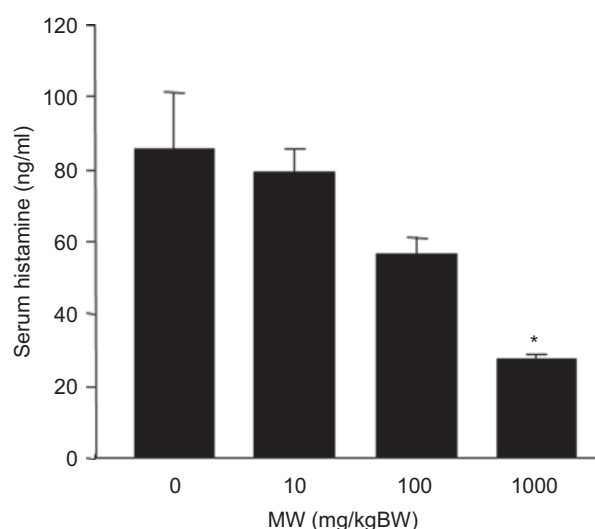


Figure 1. Effect of MW on serum histamine release. Groups of mice ($n=10$ /group) were intraperitoneally pre-treated with 200 μ L of saline or MW. MW was given at various doses 1 h before the injection of compound 48/80. The compound 48/80 solution was given intraperitoneally to the group of mice. The blood was obtained from the heart of each mouse and histamine content was measured. Each bar represents the mean $\pm$ SEM of three independent experiments. *Significant difference at $P<0.05$.

with MW for 30 min. MW dose-dependently inhibited PMACI-induced expression of TNF- α , IL-1 β , IL-6, and IL-8 (Figure 5). To confirm the effect of MW on the gene expression of pro-inflammatory cytokines, culture supernatants were assayed for TNF- α and IL-6 levels by ELISA methods. Stimulation of cells with PMACI during 16 h induced the secretion of cytokines. MW dose-dependently inhibited the secretion of TNF- α and IL-6 in PMACI-stimulated HMC-1 cells (Figure 6).

Effect of MW on activation of NF- κ B

NF- κ B is an important transcriptional regulator of inflammatory cytokines and plays a crucial role in immune and inflammatory responses. To investigate the intracellular

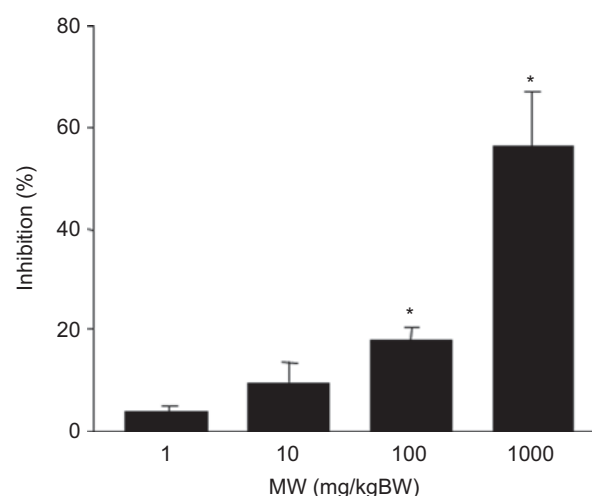


Figure 2. Effect of MW on PCA reactions. MW was intraperitoneally administered 1 h prior to the challenge with antigen. Each amount of dye was extracted as described in Materials and methods and measured by spectrophotometry. Each bar represents the mean $\pm$ SEM of three independent experiments. *Significant difference at $P<0.05$.

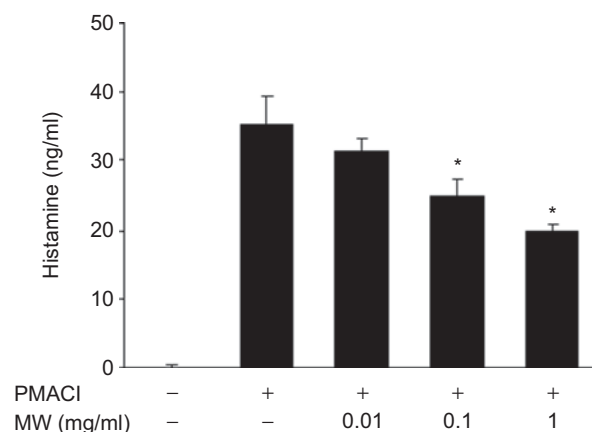


Figure 3. Effect of MW on PMACI-induced histamine release. HMC-1 cells (2×10^6 cells/mL) were preincubated with MW at 37°C for 10 min prior to incubation with PMA (20 nM) and A23187 (1 μ M). Each value represents the mean $\pm$ SEM of three independent experiments. *Significant difference from PMACI value at $P<0.05$.

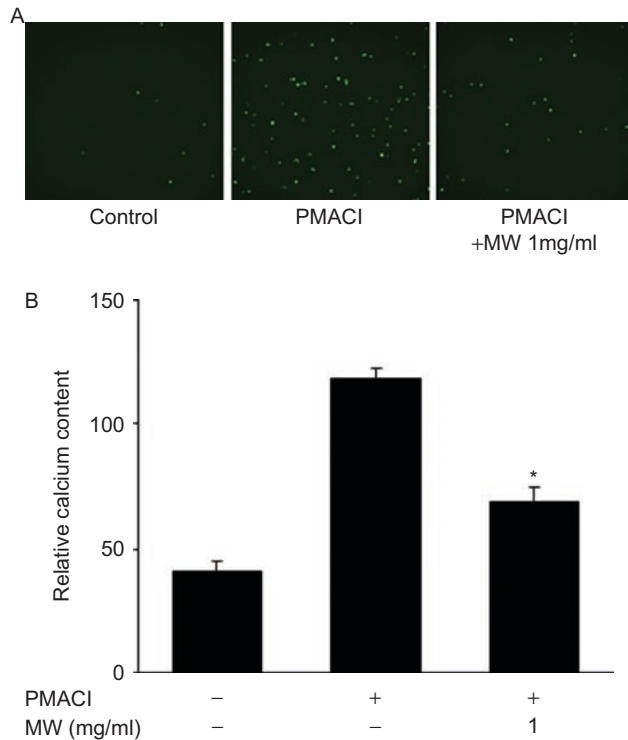


Figure 4. Effect of MW on intracellular calcium in mast cells. HMC-1 cells were stained with Fluo-3/AM and then cells were preincubated for 10min with MW before adding PMA (20nM) and A23187 (1 μ M), and then for another 10min with PMACI. Intracellular calcium was detected by fluorescence microscope (A) and flow cytometer (B). Each value represents the mean $\pm$ SEM of three independent experiments. *Significant difference from PMACI value at $P < 0.05$.

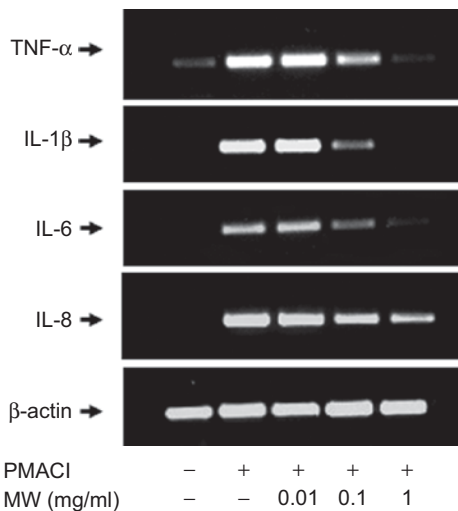


Figure 5. Effect of MW on the gene expression of pro-inflammatory cytokines in mast cells. HMC-1 cells were pre-treated with MW for 30min prior to PMA (20nM) and A23187 (1 μ M) stimulation. The levels of TNF- α , IL-1 β , IL-6 and IL-8 were determined by RT-PCR.

mechanism responsible for the inhibitory effect of MW on the secretion of pro-inflammatory cytokines, we examined the effect of MW on NF- κ B. Stimulation of HMC-1 cells with PMACI-induced nuclear translocation

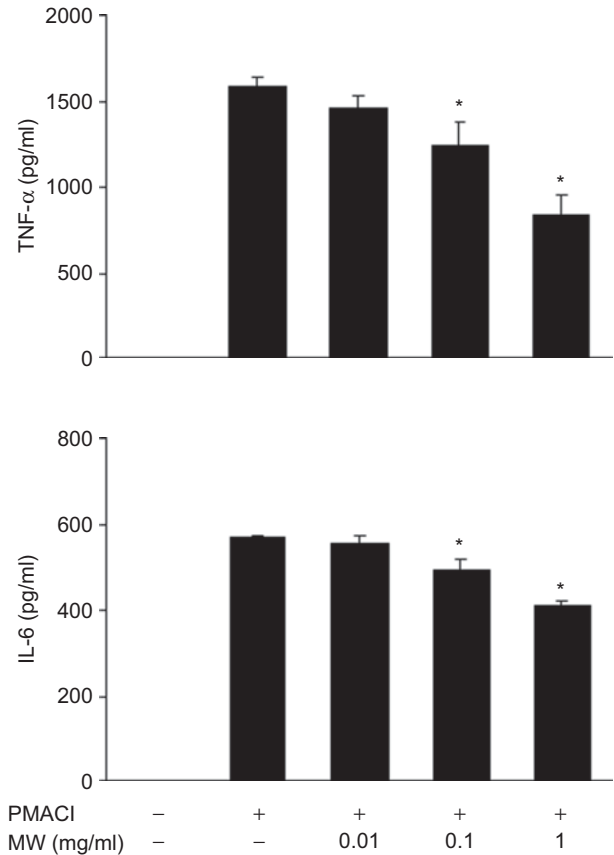


Figure 6. Effect of MW on the secretion of pro-inflammatory cytokines. HMC-1 cells were pre-treated with MW for 30min prior to PMA (20nM) and A23187 (1 μ M) stimulation, and incubated additional 16h. TNF- α and IL-6 level in supernatant was measured using ELISA and represented as the mean $\pm$ SEM of three independent experiments. *Significant difference from PMACI value at $P < 0.05$.

of p65 NF- κ B after 2h of incubation. (Figure 7A). MW inhibited the PMACI-induced nuclear translocation of p65 NF- κ B. To confirm the inhibitory effect of MW on NF- κ B activation, we examined the effect of MW on the 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 the NF- κ B luciferase reporter construct (Figure 7B). MW significantly reduced the PMACI-induced luciferase activity.

Discussion

The results of this study demonstrated that MW has anti-allergic inflammatory properties. MW inhibited compound 48/80-induced systemic anaphylaxis and histamine release from mast cells. MW administered mice are protected from IgE-mediated local allergic reaction, PCA, which is one of the most important in vivo models of anaphylaxis. These results indicate that mast cell-mediated allergic reactions are inhibited by MW. In addition, MW suppressed PMACI-stimulated expression

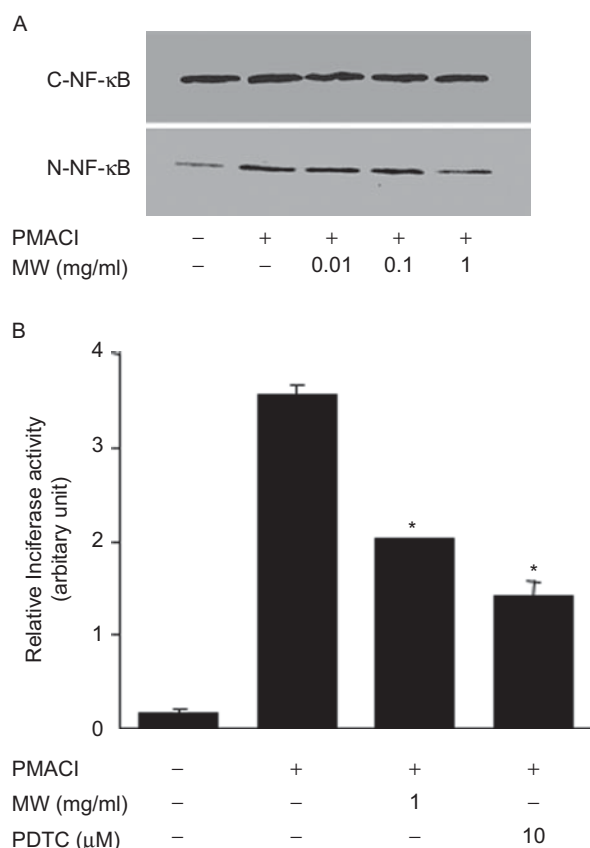


Figure 7. Effect of MW on the activation of NF- κ B. HMC-1 cells were pre-treated with MW or PDTC (10 μ M) for 30 min prior to PMA (20 nM) and A23187 (1 μ M) stimulation. (A) NF- κ B translocation was assayed by western blot (N-NF- κ B, nucleus NF- κ B; C-NF- κ B, cytoplasmic NF- κ B). (B) Cells were transiently transfected with the NF- κ B-luciferase reporter construct or empty vector. Then, the cells were incubated with PMACI with or without MW. NF- κ B-dependent transcriptional activity was determined by luciferase activity assay. PDTC was used as a positive control. *Significant difference from PMACI value at $P < 0.05$.

of pro-inflammatory cytokines. These finding suggests that MW might be useful in the treatment of allergic inflammatory diseases.

The intracellular calcium is the critical functional compartments in the degranulation of mast cells. Calcium movements across the membranes of mast cells represent a major target for effective anti-allergic drugs, as these are essential events that link stimulation to secretion.^(3,26,27) The transduction pathways modulating intracellular calcium are modified by ADP-ribosylates G protein binding protein.⁽²⁸⁾ Our results which show an attenuation of intracellular calcium in mast cells with MW treatment is consistent with other reports. According to these observations, we strongly suggest that decreased intracellular calcium might be involved in the inhibitory effect of MW on histamine release, and MW might have membrane stabilizing activity through G protein.

HMC-1 cell line is a useful cell for studying cytokine activation pathways.⁽²⁹⁾ The spectrum of cytokines produced by HMC-1 cells with PMACI stimulation

supports the well recognized role of mast cells in allergic inflammation. Pro-inflammatory cytokines, including TNF- α , IL-1 β , IL-6, and IL-8, play a major role in triggering and sustaining the allergic inflammatory response in mast cells.^(30,31) 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.⁽⁴⁾ It is also a potent inducer of other inflammatory cytokines, including IL-1 β , 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.⁽³¹⁾ IL-8 from mast cells, acts on surrounding cells such as neutrophils, T-lymphocytes, and eosinophils and plays a role in activation of inflammatory effector cells.⁽³²⁾ These reports indicate that the reduction of pro-inflammatory cytokines from mast cell is one of the key indicators of reduced allergic inflammatory symptom. In our present study, MW inhibited the gene expression and secretion of TNF- α , IL-1 β , IL-6, and IL-8 in PMACI-stimulated HMC-1 cells. This result suggests that the anti-allergic inflammatory effect of MW results from its reduction of TNF- α , IL-1 β , IL-6, and IL-8 from mast cells.

Intracellular calcium plays an important role in the expression of inflammatory cytokines. Depletion of intracellular calcium blocked the IgE-induced TNF- α and IL-6 expression through NF- κ B signaling pathway in RBL-2H3 mast cells.⁽³³⁾ Because of the reducing effect of MW on the intracellular calcium, we suggest that one possible pathway of the inhibitory effect of MW on the inflammatory cytokines is mediated by the reduction of intracellular calcium in mast cells.

Expression of pro-inflammatory cytokines is dependent on the activation of transcription factor NF- κ B.⁽³⁴⁾ Activation of NF- κ B requires phosphorylation and proteolytic degradation of the inhibitory protein I- κ B α , an endogenous inhibitor that binds to NF- κ B in the cytoplasm.⁽⁹⁾ In PMACI-stimulated mast cells, MW inhibited nuclear translocation of NF- κ B and NF- κ B dependent gene transcription. We also previously reported that PDTC, the potent inhibitor of NF- κ B, reduced PMACI-induced production of TNF- α , IL-6, and IL-8 in HMC-1 cells.^(5,6) These data demonstrate that MW attenuates activation of NF- κ B and downstream TNF- α , IL-1 β , IL-6, and IL-8 production.

In our previous experiments, 750 mL of MW made by *V. amurensis* contains ~25.1 g of extract of *V. amurensis*. According from our animal study and calculation, 200 mL of *V. amurensis* wine might be effective to human. Of course this calculation is just assumption. However, this assumption could provide a general idea of the usefulness of MW in human health but rigorous longitudinal controlled studies are now needed to better define the benefits of the proposed supplements in a real-life setting.

Because MW is a crude extract, not a purified component, the active components that are responsible for the biologic effect are not clear at this time. The effort to identify active components of MW in allergic inflammation is ongoing in our laboratory. *V. amurensis* contains high

levels of polyphenols mainly catechin and procyanidin.⁽¹¹⁾ Polyphenols is well known to possess anti-oxidant, anti-allergic, and anti-inflammatory activities. We assume that the high amount of polyphenols in *V. amurensis* is responsible for the anti-allergic inflammatory activity of MW. Recently Kumazawa group showed that fermented grape marc (FGM) from *V. vinifera* inhibited degranulation of mast cells and type-I allergic reactions.^(35,36) On the other hand, non-FGM had no effect. Because MW is also a fermented material, these reports are consistent with our results. However, authors concluded that the anti-allergic activity of FGM is not due to the action of resveratrol or oligomeric procyanidin,⁽³⁵⁾ major components of MW. This discrepancy may come from the difference of grape or microbes used in fermentation.

In the present report, we provided evidence that MW inhibits mast cell-mediated allergic inflammatory reactions and their possible mechanisms such as intracellular calcium for histamine release and NF- κ B for pro-inflammatory cytokines. The results obtained in the present study shows that MW contributes to the prevention or treatment of mast cell-mediated allergic inflammatory diseases.

Declaration of interest

This work was supported by Woosuk University. The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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