Natural Product Sciences
16(3) : 185-191 (2010)

The Extract of Gleditsiae Spina Inhibits Mast Cell-Mediated Allergic Reactions Through the Inhibition of Histamine Release and Inflammatory Cytokine Production

Tae-Yong Shin*

College of Pharmacy, Woosuk University, 565-701, Republic of Korea

Abstract – Mast cell-mediated allergic disease is involved in many diseases such as anaphylaxis, asthma and atopic dermatitis. The discovery of drugs for the treatment of allergic disease is an important subject in human health. In the present study, the effect of water extract of Gleditsiae Spina (WGS) (Leguminosae), on compound 48/80-induced systemic allergic reaction, anti-DNP IgE antibody-induced local allergic reaction, and histamine release from human mast cell line (HMC-1) cells were studied. In addition, the effect of WGS on phorbol 12-myristate 13-acetate (PMA) plus calcium ionophore A23187 (A23187)-induced gene expression and secretion of pro-inflammatory cytokines were investigated using HMC-1 cells. WGS was anally administered to mice for high and fast absorption. WGS inhibited compound 48/80-induced systemic allergic reaction. WGS dose-dependently decreased the IgE-mediated passive cutaneous anaphylaxis. WGS reduced histamine release from HMC-1 cells. In addition, WGS decreased the gene expression and secretion of pro-inflammatory cytokines in PMA plus A23187-stimulated HMC-1 cells. These findings provide evidence that WGS could be a candidate as an anti-allergic agent.
Keywords – Gleditsiae Spina, anaphylactic allergic reaction, tumor necrosis factor-α, interleukin-6, interleukin-1β.

Introduction

The water extract of Gleditsiae Spina (WGS) (Leguminosae) has the pharmaceutical functions of anaphylactic effect (Shin and Kim, 2000) and anti-inflammatory effect (Ha *et al*., 2008), and has been used for the management of swelling, suppuration and carbuncle. This crude drug contains saponins, flavonoids and monoterpenoid glucosides (But *et al.,* 1997).

Mast cells are important producers of mediators of inflammatory response such as allergy and immediate-type allergic reaction. (Kemp and Lockey, 2002; Galli *et al*., 2005a). Hypersensitivity, an acute systemic allergic reaction, is mediated by histamine released in response to the antigen cross-linking of immunoglobulin E (IgE) bound to FcεRI on the mast cells. After activation via the FcεRI, 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 (Metcalfe *et al*., 1981; Church and Levi-Schaffer, 1997; Kim *et al*., 2006). Among the inflammatory substances released from mast cells, histamine is one of the best-characterized and most potent vasoactive mediators implicated in the acute phase of immediate hypersensitivity (Galli *et al*., 2005b; Kim and Shin, 2005). Mast cell activation is initiated upon interaction of multivalent antigen with its specific IgE antibody attached to the cell membrane via FcεRI (Alber *et al*., 1991; Metzger *et al*., 1986). Anti-dinitrophenyl (DNP) IgE antibody and antigen have been established to induce passive cutaneous anaphylaxis (PCA) reactions as a typical *in vivo* model for immediate hypersensitivity. Mast cell degranulation also can be elicited by nonimmunologic stimulators such as neuropeptides, basic compounds, complement components, and certain drugs (Lagunoff *et al*., 1983; Shin *et al*., 2005). Compound 48/80 and polymers of basic amino acids, such as substance P, are some of the most potent stimulators of mast cells. Thus, an appropriate amount of compound 48/80 has been used as a direct and convenient reagent to study the mechanism of anaphylactic allergic reaction (Kim *et al*., 2005; Kim and Shin, 2005). 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,

*Author for correspondence
Tel: +82-63-290-1572; E-mail: tyshin@woosuk.ac.kr

such as TNF-α, IL-6, IL-8, IL-4, IL-13 and transforming growth factor-β (Galli, *et al*., 1991; Bradding *et al*., 1993). Therefore, modulation of secretion of these cytokines from mast cells can provide a useful therapeutic strategy for allergic inflammatory disease. Anal therapy is a drug delivery system through the anus. It is utilized in patients who have difficulty with oral administration. Absorption of a drug in the rectum avoids the first-pass effect in the liver and allows it to circulate directly in the whole body. The absorption rate in the rectum is faster than that in the gastrointestinal tract. The rate and total amount absorption through the rectum are not very different from those with venous administration (Shin *et al*., 2004; Shin *et al*., 2005). Thus, anal therapy is expected to have a better effect than oral therapy due to the increased absorption rate and the strong medical action. WGS was anally administered to mice for high and fast absorption.

In the present study, the effect of WGS on compound 48/80-induced systemic allergic reaction, anti-DNP IgE antibody-induced local allergic reaction, and histamine release from human mast cell line (HMC-1) cells were studied. In addition, the effect of WGS on phorbol 12-myristate 13-acetate (PMA) plus calcium ionophore A23187 (A23187)-induced gene expression and secretion of pro-inflammatory cytokines were investigated using HMC-1 cells.

Experimental

Animals – The original stock of male ICR mice were purchased from Dae-Han Biolink Co. Ltd. (Chungbuk, Korea). The animals were housed 5 per cage in a laminar air flow room maintained under a temperature of 22 ± 2 ºC and relative humidity of 55 ± 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 and calcium ionophore A23187 were purchased from Sigma (St. Louis, MO). rTNF-α, rIL-6, rIL-1β and anti-TNF-α, -IL-6, -IL-1β antibodies were purchased from R & D systemic Inc (Minneapolis MN, USA). HMC-1 was grown in Iscove's media (Life Technologies, Grand Island, NY) supplemented with 10% FBS and 2 mM glutamine at 37 ºC in 5% CO_2.

Preparation of WGS – Gleditsiae Spina was purchased from the oriental drug store Bohwa Dang (Jeonbuk, Korea). A voucher specimen was deposited at the Herbarium of the College of Pharmacy, Woosuk University. The sample was extracted with purified water at 70 ºC for 5 h (two times) in water bath. The extract was filtered and lyophilized. The yield of dried extract from starting crude materials was about 9.8%. The dried extract was dissolved in PBS before use.

Compound 48/80-induced systemic reaction – Compound 48/80-induced systemic reaction was examined as previously described (Shin *et al*., 2004). Mice were given an intraperitoneal injection of 8 mg/kg, body weight (BW), of the mast cell degranulator, compound 48/80. WGS was anally administered 1 h before the injection of compound 48/80 (n = 10/group). In the time dependent experiment, WGS (1000 mg/kg) was administered anally at 5, 10, and 20 min after injection of compound 48/80 (n = 10/group). Mortality was monitored for 1 h after induction of anaphylactic shock.

Passive cutaneous anaphylaxis (PCA) reaction – An IgE-dependent cutaneous reaction was examined as previously described (Shin *et al*., 2005). PCA reaction was generated by sensitizing skin with an intradermal injection of anti-DNP IgE followed 48 h later with an injection of DNP-HSA into the mouse tail vein. The mice were injected intradermally with 0.5 μg of anti-DNP IgE. After 48 h, each mouse was received an injection of 1 μg of DNP-HSA containing Evans blue via the tail vein. WGS (1 - 1000 mg/kg, BW) was anally administered 1 h before the challenge. Then 30 min after the challenge, the mice were killed and the dorsal skin was removed for measurement of 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) based on the previous report (Shin *et al*., 2005). The intensity of absorbance was measured at 620 nm in a spectrophotometer (Ultrospec 2100 pro, Amersham Biosciences).

Histamine Assay – Histamine content was measured by the enzyme immunoassay kit (Oxford Biomedical Research, Oxford, MI, USA) according to manufacturer's manual. HMC-1 cells were incubated with WGS (0.001 - 0.1 mg/ml) for 10 min at 37 ºC before the addition of PMA (20 nM) plus A23187 (1 μM) and incubated for an additional times. The cells were separated from the released histamine by centrifugation at 400 × g for 5 min at 4 ºC.

Cytotoxicity assay – The 3(4,5-dimethyl thiazolyl-2)2,5-diphenyl tetrazolium bromide (MTT, Sigma) cytotoxicity assay was used to measure the cytotoxic response to WGS, as previously described (Shin *et al*., 2006; Kim and

Shin, 2009). HMC-1 cells were seeded at 1×10^5 cells/well in 96-well microplates (Falcon, Becton–Dickinson, Franklin Lakes, NJ). After 24 h of incubation with WGS, 20 µl of MTT (5 mg/ml) was added per well and incubated for 4 h. The formazan crystals dissolved in isopropyl alcohol with 0.04 M HCl and absorbance was read at 570 nm using a Power Wave$_x$ Microplate spectrophotometer (Bio-Tek Instrument, Inc., Winooski, VT).

Assay of TNF-α and IL-6 secretion – TNF-α and IL-6 secretion were measured by modification of an enzyme-linked immunosorbent assay (ELISA). HMC-1 cells were cultured with α-MEM plus 10% FBS and resuspended in Tyrode buffer A. The cells were sensitized with PMA (20 nM) plus A23187 (1 µM) for 6 h in the absence or presence of WGS. 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 respectively. 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, 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) tablet substrates. Optical density readings were made within 10 mins of the addition of the substrate with a 405 nm filter.

RNA extraction and mRNA detection (RT-PCR) – The total cellular RNA was isolated from the cells (1×10^6/well in a 24-well plate) after stimulation with PMA (20 nM) plus A23187 (1 µM) with or without WGS for 2 h using a TRI reagent (Molecular Research Center, Cincinnati, OH, U.S.A.) according to the manufacturer's protocol. The first-strand complementary DNA (cDNA) was synthesized using the Superscript II reverse transcriptase (Life Technologies). Reverse-transcriptase polymerase chain reaction (RT-PCR) was used to analyze the expression of mRNA for TNF-α, IL-6 , IL-1β and β-actin (internal control). The conditions for the reverse transcription and PCR steps were similar to those previously described (Kim and Shin, 2006). The primer sets were chosen with the Primer 3 program (Whitehead Institute, Cambridge, MA, U.S.A.). The cycle number was optimized to ensure product accumulation in the exponential range. 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, U.S.A.). The band intensity was normalized to that of β-actin in the same sample.

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

WGS treatment (mg/kg BW)	Compound 48/80 (8 mg/kg BW)	Mortality (%)
None (saline)	+	100
5	+	100
10	+	90
50	+	60
100	+	40
500	+	0
1000	+	0
1000	–	0

Groups of mice (n = 10/group) were anally pretreated with 200 µl of saline or WGS. Various doses of WGS were given 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 × 100/total number of experimental mice.

Statistical Analysis – Statistical analyses were performed using SAS statistical software (SAS Institute, Cary, NC). Treatment effects were analyzed using one way analysis of variance followed by Duncan's Multiple Range test. A value of $p < 0.05$ was used to indicate significant differences.

Results

Effect of WGS on compound 48/80-induced systemic reaction – Compound 48/80 (8 mg/kg BW) was used to induce 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 to mice induced fatal shock in 100% of animals. When WGS was anally administered at concentrations ranging from 5 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 with WGS (1000 mg/kg) 5, 10, and 20 min after compound 48/80 injection increased time-dependently (Table 2).

Effect of WGS on IgE-mediated local allergic reaction – PCA is one of the most important *in vivo* models of anaphylaxis in a local allergic reaction (Wershil *et al*., 1987). Local extravasation is induced by a local injection of anti-DNP IgE followed by an intravenous antigenic challenge. Anti-DNP IgE was injected into the right dorsal skin sites. As a control, the left dorsal skin site of these mice was injected with saline alone. After 48 h, all animals were injected intravenously with DNP-HSA and Evans blue dye. WGS was administered anally 1 h prior to the challenge with antigen. The administration of

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

WGS treatment (mg/kg)	Time (min)	Compound 48/80 (8 mg/kg BW)	Mortality (%)
None (saline)	0	+	100
1000	5	+	40
	10	+	80
	20	+	100

Groups of mice (n = 10/group) were anally pretreated with 200 μl of saline or WGS. WGS (1000 mg/kg) was given at 5, 10, 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 × 100/total number of experimental mice.

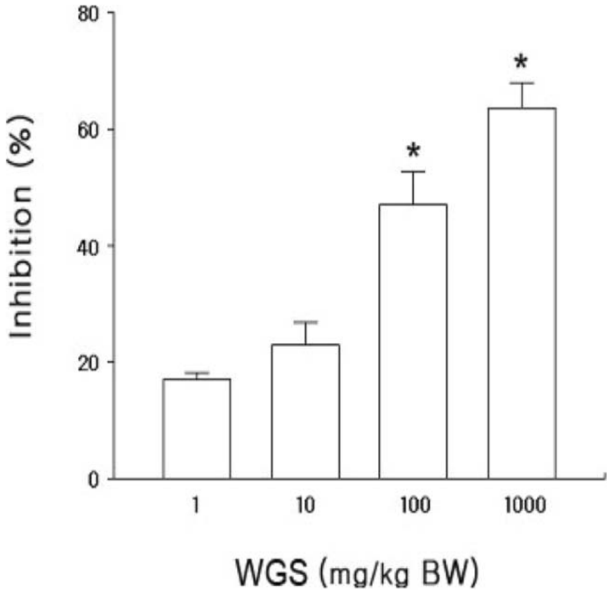

Fig. 1. Effect of WGS on the 48 h PCA. WGS was anally administered 1 h prior to the challenge with antigen. Each data represents the mean ± SEM of three independent experiments. *Significantly different from the saline value at $p < 0.05$.

WGS (1 - 1000 mg/kg, BW) showed a dose-dependent inhibition in the PCA reaction (Fig. 1).

Effect of WGS on histamine release from HMC-1 cells – Treatment with WGS dose–dependently inhibited PMA plus A23187–induced histamine release at concentrations of 0.001 - 0.1 mg/ml (Fig. 2). Up to 0.1 mg/ml of WGS did not show cytotoxicity (Fig. 3).

Effect of WGS on the gene expression and secretion of pro-inflammatory cytokines in HMC-1 cells – TNF-α, IL-6, IL-8 and IL-1β are the most important pro-inflammatory cytokines. Therefore, I examined the effects of WGS on the gene expression of TNF-α, IL-6 and IL-1β induced by PMA plus A23187 in HMC-1 cells. HMC-1 cell line is a useful cell for studying the cytokine

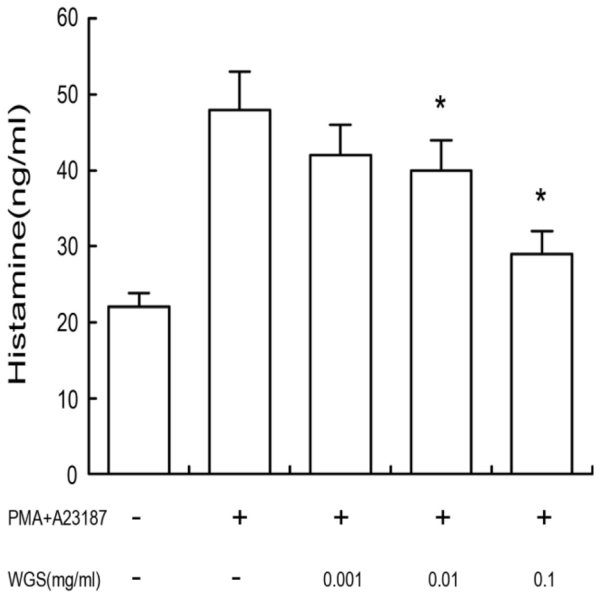

Fig. 2. Effect of WGS on PMA plus A23187-induced histamine release in HMC-1 cells. HMC-1 cells (2×10^6 cells/ml) were preincubated with WGS at 37°C for 10 min prior to incubation with PMA plus A23187. Each data represents the mean ± SEM of three independent experiments. *Significantly different from the PMA plus A23187 value at $p < 0.05$.

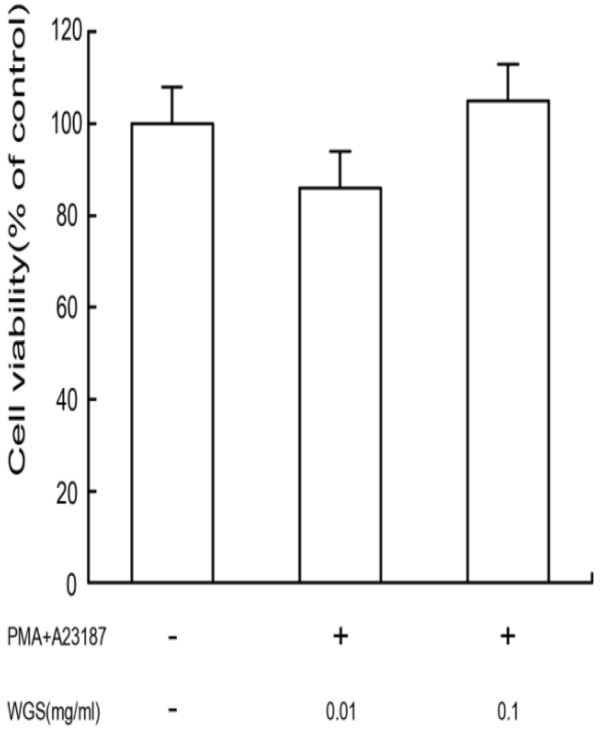

Fig. 3. Effect of WGS on HMC-1 cells viability. Cell viability was evaluated by MTT assay by incubation for 24 h after stimulation with PMA plus A23187 in the absence or presence of WGS (0.01 - 0.1 mg/ml). The percentage of viable cells was calculated using 100% for stimulation in the absence of WGS.

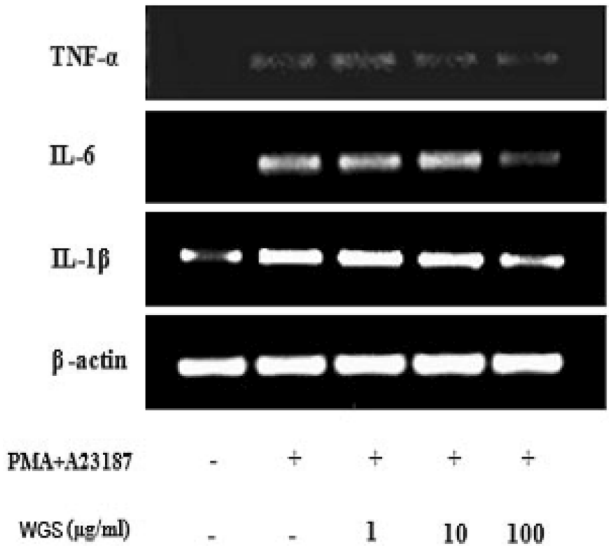

Fig. 4. Effect of WGS on the gene expression of pro-inflammatory cytokines in HMC-1 cells. HMC-1 cells were pretreated with WGS for 30 min prior to PMA plus A23187 stimulation. The levels of TNF-α, IL-6 and IL-1β were determined by RT-PCR.

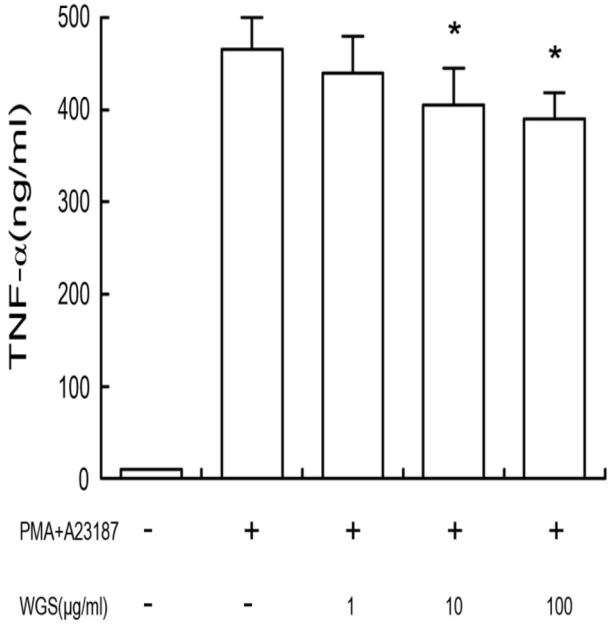

Fig. 5. Effect of WGS on secretion of TNF-α in HMC-1 cells. HMC-1 cells were pretreated with WGS for 30 min prior to PMA plus A23187 stimulation. TNF-α level in supernatant was measured using ELISA. Each data represents the mean ± SEM of three independent experiments. *Significantly different from the PMA plus A23187 value at $p < 0.05$.

activation pathways (Kim and Sharma, 2004; Kim *et al*., 2006). WGS dose-dependently inhibited PMA plus A23187-induced expression of TNF-α, IL-6 and IL-1β (Fig. 4). To confirm the effect of WGS on the gene expression

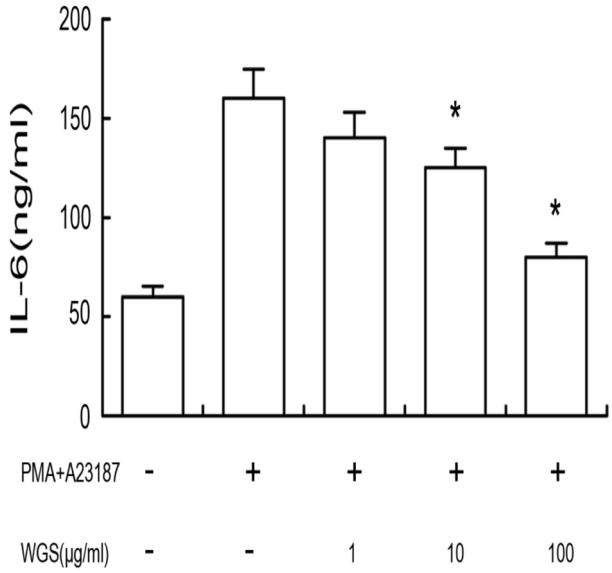

Fig. 6. Effect of WGS on secretion of IL-6 in HMC-1 cells. HMC-1 cells were pretreated with WGS for 30 min prior to PMA plus A23187 stimulation. IL-6 level in supernatant was measured using ELISA. Each data represents the mean ± SEM of three independent experiments. *Significantly different from the PMA plus A23187 value at $p < 0.05$.

of pro-inflammatory cytokines, culture supernatants were assayed for TNF-α and IL-6 levels by ELISA. The stimulation of cells with PMA plus A23187 for 16 h induced the secretion of cytokines. WGS dose-dependently inhibited the secretion of TNF-α and IL-6 in PMA plus A23187-stimulated HMC-1 cells (Fig. 5, Fig. 6).

Discussion

The results of this study demonstrated that WGS has anti-allergic inflammatory properties. WGS inhibited compound 48/80-induced systemic allergic reaction (anaphylaxis) and IgE-mediated local allergic reaction. These results indicate that mast cell-mediated allergic reactions are inhibited by WGS. Numerous reports established that stimulation of mast cells with compound 48/80 or IgE initiates the activation of a 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 (Mousli *et al*., 1990). The evidence indicates that the protein is Gi-like and that the activation is inhibited by benzalkonium chloride (Bueb *et al*., 1990). Tasaka (Tasaka *et al*., 1986) reported that compound 48/80 increases the permeability of the lipid bilayer membrane by causing a perturbation of 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, anti-allergic agents having a membrane-stabilizing action may be desirable. WGS may act on the lipid bilayer membrane, thus preventing the perturbation induced by compound 48/80.

PCA is one of the most important *in vivo* models of anaphylaxis in local allergic reactions. The mice administered with WGS were protected from IgE-mediated PCA. This finding suggests that WGS might be useful in the treatment of allergic skin reactions.

Mast cell-derived cytokines, especially TNF-α, IL-6 and IL-1β have a critical biological activity in the allergic reaction. It has been reported that mast cells are a principal source of TNF-α in human dermis, and degranulation of mast cells in the dermal endothelium is abrogated by the anti-TNF-α antibody (Walsh *et al*., 1991). IL-6 and IL-1β are also produced from mast cells and its local accumulation is associated with a PCA reaction (Mican *et al*., 1992; Sillaber *et al*., 1993). These reports indicate that reduction of pro-inflammatory cytokines from mast cell is a one of the key indicator of reduced allergic symptom. The HMC-1 cell is a useful cell for studying cytokine activation pathways (Sillaber *et al*., 1993). WGS inhibited the expression and secretion of cytokines in PMA plus A23187-stimulated HMC-1 cells. These results suggest that WGS reduces the allergic responses through decreasing the expression of pro-inflammatory cytokines. The effect of WGS on cytokines production by mast cells *in vivo* and the relative importance of mast cells as a source of cytokines during inflammatory and immune responses are important areas for future studies. In the present study, I used the whole water extract of Gleditsiae Spina; hence the active components that are responsible for the biological effect are not clear at this time. The effort for identify active components from WGS in allergic reaction is ongoing in my laboratory. In conclusion, the results obtained in the present study provide evidence that WGS might contribute to the prevention or treatment of mast cell-mediated allergic inflammatory diseases.

Acknowledgements

"This work was supported by Woosuk University (2010)

References

Alber, G., Miller, L., Jelsema, C.L., Varin-Blank, N., and Metzger, H., Structure/function relationships in the mast cell high-affinity receptor for IgE. Role of the cytoplasmic domains and of the beta subunit. *J. Biol. Chem*. **266**, 22613-22620 (1991).

Bradding, P., Feather, I.H., Wilson, S., Bardin, P.G., Heusser, C.H., Holgate, S.T., and Howarh, P.H., Immunolocalization of cytokines in the nasal mucosa of normal and perennial rhinitic subjects. The mast cell as a source of IL-4, IL-5, and IL-6 in human allergic mucosal inflammation. *J. Immunol.* **151**, 3853-3865 (1993).

Bueb, J.L., Mousli, M., Bronner, C., Rouot, B., and Landry, Y., Activation of Gi-like proteins, a receptor-independent effect of kinins in mast cells. *Mol. Pharmacol*. **38**, 816-822 (1990).

But, P.P.H., Kimura, T., Guo, J.X., and Han, B.H., *International Collation of Traditional and Folk Medicine (2)*, World Scientific, New Jersey, 1997, pp.68-69.

Church, M.K. and Levi-Schaffer, F., The human mast cell. *J. Allergy Clin. Immunol*. **99**, 155-160 (1997).

Galli, S.J., Gordon, J.R., and Wershil, B.K., Cytokine production by mast cells and basophils. *Curr. Opin. Immunol*. **3**, 865-872 (1991).

Galli, S.J., Nakae, S., and Tsai, M., Mast cells in the development of adaptive immune responses. *Nat. Immunol*. **6**, 135-142 (2005a).

Galli, S.J., Kalesnikoff, J., Grimbaldeston, M.A., Piliponsky, A.M., Williams, C.M., and Tsai, M., Mast cells as "tunable" effector and immunoregulatory cells: recent advances. *Annu. Rev. Immunol*. **23**, 749-786 (2005b).

Ha, H.H., Park, S.Y., Ko, W.S., and Kim, Y.J., Gleditsia sinensis thorns inhibit the production of NO through NF-κB suppression in LPS-stimulated macrophage. *J. Ethnopharmacol.* **118**(3), 429-434 (2008).

Kemp, S.F. and Lockey, R.F., Anaphylaxis: a review of causes and mechanisms. *J. Allergy Clin. Immunol.* **110**, 341-348 (2002).

Kim, S.H., Choi, C.H., Kim, S.Y., Eun, J.S., and Shin, T.Y., Anti-allergic effects of *Artemisia iwayomogi* on mast cell-mediated allergy model. *Exp. Biol. Med*. **230**, 82-88 (2005).

Kim, S.H. and Sharma, R.P., Mercury-induced apoptosis and necrosis in murine macrophages: role of calcium-induced reactive oxygen species and p38 mitogen-activated protein kinase signaling. *Toxicol. Appl. Pharmacol*. **196**, 47-57 (2004).

Kim, S.H. and Shin, T.Y., *Amomum xanthiodes* inhibits mast cell-mediated allergic reactions through the inhibition of histamine release and inflammatory cytokine production. *Exp. Biol. Med*. **230**, 681-687 (2005).

Kim, S.H. and Shin, T.Y., Anti-inflammatory effect of leaves of *Eriobotrya japonica* correlating with attenuation of p38 MAPK, ERK, and NF-κB activation in mast cells. *Toxicol. in Vitro* **23**, 1215-1219 (2009).

Kim, S.H., Jun, C.D., Suk, K., Choi, B.J., Lim, H., Park, S., Lee, S.H., Shin, H.Y., Kim, D.K., and Shin, T.Y., Gallic acid inhibits histamine release and proinflammatory cytokine production in mast cells. *Toxicol. Sci*. **91**, 123-131 (2006).

Kim, S.H. and Shin, T.Y., Effect of *Dracocephalum argunense* on mast cell-mediated hypersensitivity. *Int. Arch. Allergy Immunol*. **139**, 87-95 (2006).

Lagunoff, D., Martin, T.W., and Read, G., Agents that release histamine from mast cells. *Annu. Rev. Pharmacol. Toxicol*. **23**, 331-351 (1983).

Metcalfe, D.D., Kaliner, M., and Donlon, M.A., The mast cell. *Crit. Rev. Immunol*. **3**, 23-27 (1981).

Metzger, H., Alcaraz, G., Hohman, R., Kinet, J.P., Pribluda, V., and Quarto, R., The receptor with high affinity for immunoglobulin E. *Annu. Rev. Immunol*. **4**, 419-470(1986).

Mican, J.A., Arora, N., Burd, P.R., and Metcalfe, D.D., Passive cutaneous anaphylaxis in mouse skin is associated with local accumulation of interleukin-6 mRNA and immunoreactive interleukin-6 protein. *J. Allergy Clin. Immunol*. **90**, 815-824 (1992).

Mousli, M.C., Bronner, C., Bockaert, J., Rouot, B., and Landry, Y.,

Interaction of substance P, compound 48/80 and mastoparan with α-subunit C-terminal of G protein. *Immunol. Lett*. **25**, 355-358 (1990).

Shin, T.Y., Kim, S.H., Choi, C.H., Shin, H.Y., and Kim, H.M., *Isodon japonicus* decreases immediate-type allergic reaction and tumor necrosis factor-alpha production. *Int Arch Allergy Immunol*. **135**, 17-23 (2004).

Shin, T.Y., Kim, S.H., Suk, K., Ha, J.H., Kim, I., Lee, M.G., Jun, C.D., Kim, S.Y., Lim, J.P., Eun, J.S., Shin, H.Y., and Kim, H.M., Anti-allergic effects of *Lycopus lucidus* on mast cell-mediated allergy model. *Toxicol. Appl. Pharmacol*. **209**, 255-262 (2005).

Shin, T.Y. and Kim, D.K., Inhibitory effect of mast cell-dependent anphylaxis by *Gledithia sinensis*. *Arch. Pharm. Res*. **23**(4), 401-406 (2000).

Shin, T.Y., Oh, J.M., Choi, B.J., Park, W.H., Kim, C.H., Jun, C.D., and Kim, S.H., Anti-inflammatory effect of *Poncirus trifoliata* fruit through inhibition of NF-κB activation in mast cells. *Toxicol. in Vivo* **20**, 1071-1076 (2006).

Sillaber, C., Bevec, D., Butterfield, J.H., Heppner, C., Valenta, R., Scheiner, O., Kraft, D., Lechner, K., Bettelheim, P., and Valent, P., Tumor necrosis factor alpha and interleukin-1 beta mRNA expression in HMC-1 cells: differential regulation of gene product expression by recombinant interleukin-4. *Exp. Hematol*. **21**, 1271-1275 (1993).

Tasaka, K., Mio, M., and Okamoto, M., Intracellular calcium release induced by histamine releasers and its inhibition by some antiallergic drugs. *Ann. Allergy* **56**, 464-469 (1986).

Walsh, L.J., Trinchieri, G., Waldorf, H.A., Whitaker, D., and Murphy, G.F., Human dermal mast cells contain and release tumor necrosis factor alpha, which induces endothelial leukocyte adhesion molecule 1. *Proc. Natl. Acad. Sci*. **88**, 4220-4224 (1991).

Wershil, B.K., Mekori, Y.A., Murakami, T., and Galli, S.J., ^{125}I-fibrin deposition in IgE-dependent immediate hypersensitivity reactions in mouse skin. Demonstration of the role of mast cells using genetically mast cell-deficient mice locally reconstituted with cultured mast cells. *J. Immunol*. **139**, 2605-2614 (1987).

Received July 26, 2010
Revised August 12, 2010
Accepted August 17, 2010