Arch Pharm Res Vol 23, No 4, 401-406, 2000

Inhibitory Effect of Mast Cell-dependent Anaphylaxis by *Gleditsia sinensis*

Tae Yong Shin and Dae Keun Kim

College of Pharmacy, Woosuk University, Chonju, Chonbuk, 565-701, Korea

(Received May 25, 2000)

We investigated the effect of aqueous extract of *Gleditsia sinensis* thorns (Leguminosae) (GSAE) on the mast cell-dependent anaphylaxis. GSAE (0.005 to 1 g/kg) dose-dependently inhibited systemic anaphylaxis induced by compound 48/80 in rats. GSAE (0.1 and 1 g/kg) also significantly inhibited local anaphylaxis activated by anti-DNP IgE. When GSAE was pretreated at the same concentrations with systemic anaphylaxis, the plasma histamine levels were reduced in a dose-dependent manner. GSAE (0.001 to 1 mg/ml) dose-dependently inhibited the histamine release from rat peritoneal mast cells (RPMC) activated by compound 48/80 or anti-DNP IgE. The level of cyclic AMP in RPMC, When GSAE (1 mg/ml) was added, transiently and significantly increased about fourfold compared with that of basal cells. Moreover, GSAE (0.01 and 0.1 mg/ml) had a significant inhibitory effect on anti-DNP IgE-induced tumor necrosis factor-α production from RPMC. These results suggest a possible use of GSAE in managing mast cell-dependent anaphylaxis.

Key words: *Gleditsia sinensis*, Anaphylaxis, Compound 48/80, anti-DNP IgE, Tumor necrosis factor-α, Cyclic AMP

INTRODUCTION

The thorns of *Gleditsia sinensis* Lam. (Leguminosae), well known as "Jo-gak-ja" in Korea, has been used for centuries as traditional medicine. This crude drug is successfully used for the management of swelling, suppuration, carbuncle and skin diseases (But *et al.*, 1997). It is now well established that the mast cell triggers anaphylaxis in response to allergens by releasing chemical mediators (Wasserman and Marquardt, 1988). Among the preformed and newly synthesized inflammatory substances released on degranulation of mast cells, histamine is the best characterized and most potent vasoactive mediator implicated in the acute phase of immediated-type allergic reactions (Petersen *et al.*, 1996). Mast cell degranulation can be elicited by a number of positively charged substances, collectively known as the basic secretagogues of mast cells (Lagunoff *et al.*, 1983). Compound 48/80 and polymers of basic amino acids, such as substance P, are some of the most potent secretagogues of mast cells (Ennis *et al.*, 1980). Compared with the natural process, a high concentration of compound 48/80 induces almost a 90% release of histamine from mast cells. Thus, an appropriate amount of compound 48/80 has been used as a direct and convenient reagent to study the mechanism of anaphylaxis (Allansmith *et al.*, 1989). The secretory response of mast cells can also be induced by aggregation of their cell surface-specific receptors for immunoglobulin E (IgE) by the corresponding antigen (Segal *et al.*, 1977; Metzger *et al.*, 1986; Alber *et al.*, 1991). It has been established that the anti-IgE antibody induces passive cutaneous anaphylaxis (PCA) reactions as a typical model for the immediate hypersensitivity. Given the recent evidence that upon antigen stimulation mast cells are a potential source of various cytokines, including tumour necrosis factor-α (TNF-α), it is likely that they play a crucial role in allergic inflammation ((Plut *et al.*, 1989; Wodnar-Filipowicz *et al.*, 1989; Burd *et al.*, 1989; Gurish *et al.*, 1991; Galli *et al.*, 1991). Therefore, modulation of TNF-α production by mast cells should provide us with a useful therapeutic strategy for allergic disease.

In this study, we showed that GSAE inhibited compound 48/80-induced systemic anaphylaxis, anti-dinitrophenyl (DNP) IgE antibody-induced PCA, and histamine and TNF-α production from rat peritoneal mast cells (RPMC). We also investigated the cyclic AMP content to clarify the mechanism by which the GSAE inhibited histamine release from RPMC.

*Correspondence to:*Tae Yong Shin, College of Pharmacy, Woosuk University, Chonju, Chonbuk, 565-701, Korea
E-mail: tyshin@core.woosuk.ac.kr

MATERIALS AND METHODS

Reagents

Compound 48/80, anti-DNP IgE, DNP-human serum albumin (HSA), α-minimal essential medium (α-MEM), o-phthaldialdehyde (OPA) and metrizamide were purchased from Sigma Chemical Co. (St Louis, MO). Cyclic AMP was purchased from Amerham Pharmacia Biotec (UK), and Murine TNF-α was obtained from RD Systems Inc. (USA).

Animals

The original stock of male Sprague-Dawley rats (200~300 g) were purchased from Dae-Han Experimental Animal Center (Taejeon, Korea), and the animals were maintained in the College of Pharmacy, Woosuk University. The animals were housed five to ten per cage in a laminar air flow room maintained under a temperature of 22 ± 2 and relative humidity of 55 ± 5% throughout the study.

Preparation of GSAE

The *Gleditsia sinensis* thorns were purchased from the oriental drug store, Bohwa Dang (Chonju, Korea). A voucher specimen (number WSP-99-35) was deposited at the Herbarium of the College of Pharmacy, Woosuk University. The plant sample was extracted with distilled water at 70°C for 5 h (two times). The extract was filtered through a 0.45 μm filter, and the filtrate was lyophilized, and kept at -4°C. The yield of dried extract from starting crude materials was about 9.8%. 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 anaphylactic reaction was examined as previously described (Shin *et al.*, 1999). Rats were given an intraperitoneal injection of 8 mg/kg body weigh (BW) of the mast cell degranulator, compound 48/80. Compound 48/80 and GSAE were dissolved in saline and administered by intraperitoneal injection from 0.001 to 1 g/kg 1 h before the injection of compound 48/80 (n=10/group). In time dependent experiment, GSAE (1 g/kg BW) was injected intraperitoneally at 5 and 10 min after compound 48/80 injection (n=10/group). Mortality was monitored for 1 h after induction of anaphylactic shock. After the mortality test, blood was obtained from the heart of each rat.

PCA reaction

An IgE-dependent cutaneous reaction was generated by sensitizing the skin with an intradermal injection of anti-DNP IgE followed 48 h later with an injection of DNP-HSA into the rat's tail vein. The anti-DNP IgE and DNP-HSA were diluted in PBS. The rats were injected intradermally with 0.5 μg of anti-DNP IgE into each of four dorsal skin sites that had been shaved 48 h earlier. The sites were outlined with a water-insoluble red marker. Each rat, 48 h later, received an injection of 1 μg of DNP-HSA in PBS containing 4% Evans blue (1:4) via the tail vein. GSAE (0.0001 to 1 g/kg BW) was orally administered 1 h before the challenge. Then 30 min after the challenge, the rats were sacrificed and the dorsal skin was removed for measurement of pigment area. The amount of dye was then determined colorimetrically after extraction with 1 ml of 1.0 M KOH and 9 ml of mixture of acetone and phosphoric acid (5:13) based on the method of Katayama *et al.* (1978). The absorbent intensity of the extraction was measured at 620 nm in a spectrophotometer (Shimadzu, UV-1201, Japan).

Preparation of plasma and histamine determination

The blood was centrifuged at 400× g for 10 min. The plasma was withdrawn and histamine content was measured by the OPA spectroflurometric procedure of Shore *et al.* (1959). The fluorescent intensity was measured at 438 nm (excitation at 353 nm) in a spectrofluorometer (Shimadzu, RF-5301 PC, Japan).

Preparation of RPMC

RPMC were isolated as previously described (Kanemoto *et al.*, 1993). In brief, rats were anesthetized by ether and injected with 20 ml of Tyrode buffer B (137 mM NaCl, 5.6 mM glucose, 12 mM $NaHCO_3$, 2.7 mM KCl, 0.3 mM NaH_2PO_4 and 0.1% gelatin) into the peritoneal cavity and the abdomen was gently massaged for about 90 seconds. The peritoneal cavity was carefully opened and the fluid containing peritoneal cells was aspirated by a Pasteur pipette. Thereafter, 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, according to the method described by Yurt *et al.* (1977). In brief, peritoneal cells suspended in 1 ml of Tyrode buffer B were layered on 2 ml of metrizamide (22.5 W/V%) and centrifuged at room temperature for 15 min at 400× g. The cells remaining at the buffer-metrizamide interface were aspirated and discarded; the cells in the pellet were washed and resuspended in 1 ml 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 trypan blue uptake.

Inhibition of histamine release

Purified RPMC were resuspended in Tyrode buffer A

for the treatment of compound 48/80. RPMC suspensions (2×10^5 cells/ml) were preincubated for 10 min at 37°C before the addition of compound 48/80 (5 μg/ml). The cells were preincubated with the GSAE preparations, and then incubated (10 min) with the compound 48/80. RPMC suspensions (2×10^5 cells/ml) were also sensitized with anti-DNP IgE (10 μg/ml) for 6 h. The cells were preincubated with the GSAE at 37°C for 10 min prior to the challenge with DNP-HAS (1 μg/ml). The cells were separated from the released histamine by centrifugation at 400× g for 5 min at 4°C. Residual histamine in cells was released by disrupting the cells with perchloric acid and centrifugation at 400× g for 5 min at 4°C.

Assay of histamine release

The inhibition percentage of histamine release was calculated using the following equation:

$$\% \text{ Inhibition} = \frac{A-B}{A} \times 100$$

A: Histamine release without GSAE
B: Histamine release with GSAE

Assay of TNF-α production

TNF-α production was measured with the quantitative sandwich enzyme immunoassay technique, using a commercial kit (R D Systems, U.S.A.). RPMC (3×10^5 cells/ml) were sensitized with anti-DNP IgE (1 μg/ml) and incubated for 18 h in the absence or presence of GSAE (0.01 to 0.1 mg/ml) before the challenge DNP-HAS (0.1 μg/ml). TNF-α production was measured by ELISA. The ELISA was performed by coating 4-well plates with murine polyclonal antibody with specificity for murine TNF-α Standard, controls, and samples are pipetted into the wells and any mouse TNF-α present is bound by the immunobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for mouse TNF-α is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution (100 μl) is added to the wells. The enzyme reaction yields a blue product that turns yellow when the Stop solution (100 μl) is added. The intensity of the color measured is in proportion to the amount of mouse TNF-α bound in the initial step. Optical density readings were made on a Titertek Multiscan (Flow Laboratories) with a 405 nm filter. The sample values are then read off the standard curves.

Measurement of cyclic AMP level

The cyclic AMP level was measured according to the method of Peachell *et al.* (1988). In brief, purified mast cells were resuspended in prewarmed (37°C) Tyrode buffer A. Typically, an aliquot of cells (2×10^5 cells) were added to an equivalent volume (50 μl) of prewarmed buffer containing the drug in an Eppendorf tube. The reaction was allowed to proceed for discrete time intervals, terminated by the addition of ice-cold acidified ethanol (0.9 ml of 86% ethanol/1 M HCl, 99:1) with brief vigorous vortexing and then snap frozen in liquid nitrogen. The sample was later thawed and vortexed, then the debris was sedimented in a centrifuge (400× g at 4, for 5 min), and an aliquot (0.9 ml) of the supernatant was removed and evaporated to dryness under reduced pressure. The dried sample was reconstituted in assay buffer (150-200 μl) and stored frozen. The cyclic AMP level was determined by enzyme immunoassay, using a commercial kit (Amersham Pharmacia Biotech).

Statistical analysis

The results obtained were expressed as mean ± SEM. The Student's t-test was used to make a statistical comparison between the groups. Results with $p<0.05$ were considered statistically significant.

RESULTS

In vivo effect of GSAE on compound 48/80-induced systemic anaphylaxis

To assess the contribution of GSAE in anaphylaxis, we first used the *in vivo* model of systemic anaphylaxis. We used compound 48/80 (8 mg/kg BW) as a systemic fatal anaphylaxis inducer. After the intraperitoneal injection of compound 48/80, the rats were monitored for 1 h, after which the mortality rate was determined. As shown in Table I, an intraperitoneal injection of 200 μl saline as a control induced a fatal shock in 100% of rats. When rats were pretreated with GSAE at concentrations ranging from 0.001 to 1 g/kg BW for 1 h, the mortality with com-

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

GSAE treatment (g/kg BW)	Compound 48/80 (8 mg/kg BW)	Mortality (%)
None (saline)		100
0.001		100
0.005		90
0.01		80
0.05		40
0.1		0
0.5		0
1		0
1		0

Groups of rats ($n=10$/group) were intraperitoneally pretreated with 200 μl saline or GSAE. GSAE was given at various doses 1 h before the compound 48/80 injection. The compound 48/80 solution was intraperitoneally given to the group of rats. Mortality (%) within 1 h following compound 48/80 injection was represented as the number of dead rats × 100/total number of experimental rats.

pound 48/80 was reduced dose-dependently. The mortality of rats injected intraperitoneally with GSAE (1 g/kg) 5 min after compound 48/80 injection was 0%. However, the mortality of rats injected with GSAE (1 g/kg) 10 min after compound 48/80 injection was 40% (n=10/group).

Effect of GSAE on compound 48/80-induced plasma histamine release

The ability of GSAE to influence compound 48/80-induced plasma histamine release was investigated. GSAE was given from 0.001 to 1 g/kg BW 1 h before (n=10/group) compound 48/80 injection. The correlation results with those of the mortality test were shown when their plasma histamine contents were measured (Fig. 1). The inhibition rate of histamine by GSAE was significant at doses of 0.5 to 1 g/kg.

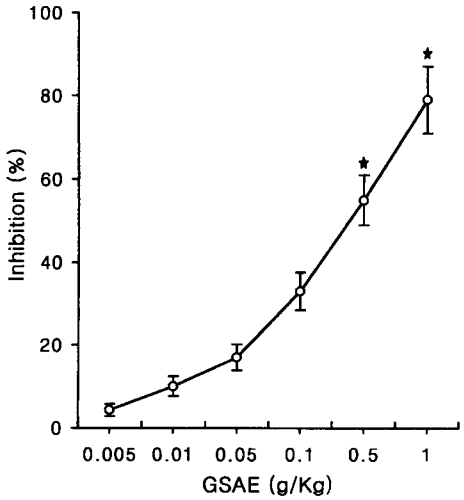

Fig. 1. *In vivo* effect of GSAE on compound 48/80-induced plasma histamine release. Groups of rats were intraperitoneally pretreated with 200 μl saline or GSAE. GSAE was given with various doses 1 h before the compound 48/80 injection. The data represents the mean ± SEM of three independent experiments. *p<0.05; significantly different from the saline value.

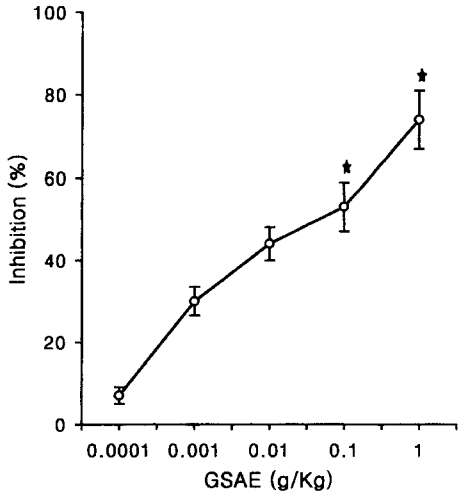

Fig. 2. Effect of GSAE on 48 h PCA. GSAE was administered orally 1 h prior to the challenge with antigen. The data represents the mean ± SEM of three independent experiments. *p<0.05; significantly different from the saline value.

Effect of GSAE on anti-DNP IgE-induced PCA

Another way to test anaphylactic reactions is to induce PCA (Wershil *et al.*, 1987). As described in this experimental procedures, local extravasation was induced by a local injection of anti-DNP IgE followed by an antigenic challenge. Oral administration of GSAE (0.1 and 1 g/kg) showed a marked inhibition rate in PCA reaction (Fig. 2).

In vitro effect of GSAE on compound 48/80-induced or anti-DNP IgE-induced histamine release from RPMC

The inhibitory effect of GSAE on compound 48/80-induced or anti-DNP IgE-induced histamine release from RPMC are shown in Fig. 3. GSAE dose-dependently inhibited compound 48/80-induced or anti-DNP IgE-induced histamine release at concentrations of 0.001 to 1 mg/ml. Especially, GSAE significantly inhibited the compound 48/80-induced or IgE-mediated histamine release at the concentrations of 0.1 and 1 mg/ml.

Effect of GSAE on anti-DNP IgE-induced TNF-α production from RPMC

We next examined whether GSAE could also regulate

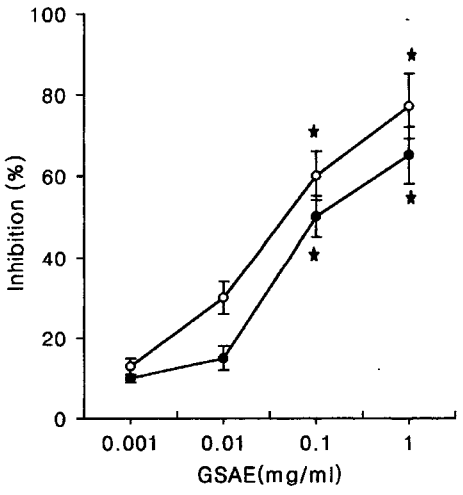

Fig. 3. *In vitro* effect of GSAE on compound 48/80-induced or IgE-mediated histamine release from RPMC. The cells (2×10^5 cells/ml) were preincubated with GSAE at 37°C for 10 min prior to incubation with compound 48/80 (○) or challenge with DNP-HAS (■). The data represents the mean ± SEM of three independent experiments. *p<0.05; significantly different from the saline value.

TNF-α production by RPMC. GSAE significantly inhibited TNF-α production at concentration of 0.01 and 0.1 mg/ml (Table II). No significant cytotoxicity of GSAE on the culture was observed in the concentrations used in the experiments, as assessed by trypan blue uptake.

Effect of GSAE on cyclic AMP level of RPMC

Finally, We investigated the cyclic AMP content to clarify the mechanism by which GSAE inhibits histamine release from RPMC. When RPMC were incubated with GSAE at a concentration of 1 mg/ml, the cyclic AMP content significantly increased. It peaked at 1 min after GSAE was added, then decreased to basal value about four min later (Fig. 4).

DISCUSSION

The present study showed that GSAE treatment profoundly affected compound 48/80-induced systemic anaphylaxis and anti-DNP IgE-induced PCA. There is no doubt that stimulation of mast cells with compound 48/80 or anti-DNP IgE initiates the activation of signal-transduction pathway, which leads to histamine release. Some recent studies have shown that compound 48/80 and other polybasic compounds are able, apparently directly, to activate G-proteins (Mousli *et al.*, 1990a; Mousli *et al.*, 1990b). The evidence indicates that the protein is G inhibitory-like and that the activation is inhibited by benzalkonium chloride (Bueb *et al.*, 1990). Tasaka *et al.* (1986) reported that compound 48/80 increased the permeability of the lipid bilayer membrane by causing a perturbation of the membrane. This result indicates that the permeability increase of the cell membrane 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. GSAE might act on the lipid bilayer membrane affecting the prevention of the perturbation being induced by compound 48/80. The GSAE-administered rats are protected from IgE-mediated allergic reaction. It is conceivable that GSAE inhibits the initial phase of immediate-type allergic reactions, probably through interference with the mast cell/histamine system.

Table II. Effect of GSAE on anti-DNP IgE-induced TNF-α production in RPMC

GSAE treatment (mg/ml)	Anti-DNP IgE plus DNP-HSA	TNF-α production (pg/ml)
None(saline)	-	75.3 ± 4.2
None(saline)	+	221.3 ± 12.9
0.01	+	102.8 ± 11.2*
0.1	+	94.6 ± 9.9*

RPMC (3×10^5 cells/ml) were sensitized with anti-DNP IgE (1 μg/ml) and incubated for 18 h in the absence or presence of GSAE before the challenge with DNP-HAS (0.1 μg/ml). The data represents the mean ± SEM of three independent experiments. *$p<0.05$: significantly different from the saline value.

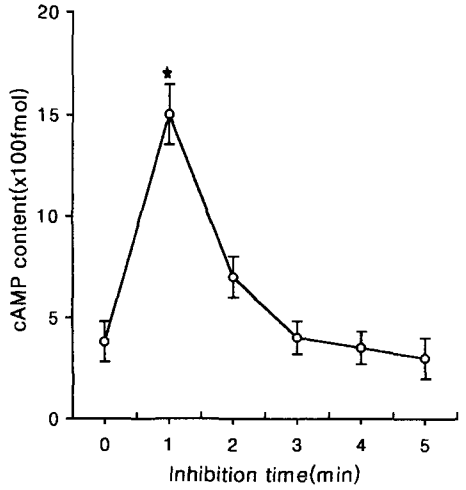

Fig. 4. Time course of increase in the cyclic AMP level of RPMC caused by GSAE. RPMC (2×10^5 cells/ml) were pretreated with GSAE at 37°C. The data represents the mean ± SEM of three independent experiments. *$p<0.05$; significantly different from the saline value

In this study, the compound 48/80-induced or anti-DNP IgE-induced histamine release from RPMC was significantly inhibited by GSAE at the concentrations of 0.1 and 1 mg/ml. TNF-α is a multifunctional cytokine that has a pro-inflammatory role. Our data showed that GSAE inhibited anti-DNP IgE-induced TNF-α production from mast cells. The effect of GSAE on mast cell cytokine production *in vivo* and the relative importance of mast cells as a source of TNF-α during inflammatory and immune responses are important areas for future studies. The release of histamine is known to be depressed by an increase in the intracellular cyclic AMP content due to the activation of adenylate cyclase or inhibition of cyclic AMP phosphodiesterase (Makino *et al.*, 1987). The intracellular cyclic AMP content of the mast cells, when incubated with GSAE (1mg/ml), increased about four-fold in comparison with that of basal cells. Our results demonstrated that GSAE inhibited the mast cell-dependent anaphylaxis *in vivo* and in an *in vitro* murine model. The studies on the isolation and characterization of the active chemical constituents are in progress.

ACKNOWLEDGEMENTS

This work was supported by Woosuk University

REFERENCES

Alber, G., Miller, L., Jelsema, C., Varin-Blank, N. and Metzger, H., Structure/function relationships in the mast cell high-affinity receptor for IgE (Fc ε RI): Role of cytoplasmic

domains. *J. Biol. Chem.*, 266, 22613-22620 (1991).
Allansmith, M.R., Baird, R.S., Ross, R.N., Barney, N.P. and Bloch, K.J., Ocular anaphylaxis induced in the rat by topical application of compound 48/80. Dose response and time course study. *Acta Ohpthalmol.*, 67, 145-153 (1989).
Bueb, J. L., Mousli, M. C., 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).
Burd, P. R., Rogers, H. W., Gordon, J. R., Martin, C. A., Jayaraman, S., Wilson, S. D., Dvorak, A. M., Galli, S. J. and Dorf, M. E., Interleukin 3-dependent and independent mast cells stimulated with IgE and antigen express multiple cytokines. *J. Exp. Med.*, 170, 245-257 (1989).
But, P. P. H., Kimura, T., Guo, J. X. and Han, B. H. (Eds). *International Collation of Traditional and Folk Medicine*(2), World Scientific, Singapore, pp.74-75, (1997).
Ennis, M., Pearce, F. L., and Weston, P. M., Some studies on the release of histamine from mast cells stimulated with polylysine. *Br. J. Pharmacol.*, 70, 329-334 (1980).
Galli, S, J., Gorden, J. R. and Wershil, B. K., Cytokine production by mast cells and basophils. *Cur. Opin. Immunol.*, 3, 865-872 (1991).
Gurish, M. F., Ghildyal, N., Arm, J., Austen, K. F., Avraham, S., Reynolds, D. S. and Stevens, R. L., Cytokine mRNA are preferentially increased relative to secretory granule protein mRNA in mouse bone marrow-derived mast cells that have undergone IgE-mediated activation and degranulation. *J. Immunol.*, 146, 1527-1533 (1991).
Kanemoto, T. J., Kasugai, T., Yamatodani, A., Ushio, H., Mochizuki, T., Tohya, K., Kimura, M., Nishimura, M. and Kitamura, Y., Supernormal histamine release and normal cytotoxic activity of Biege rat mast cells with giant granules. *Int. Arch. Allergy Immuniol.*, 100, 99-106 (1993).
Katayama, S., Shionoya, H. and Ohtake, S., A new method for extraction of extravasated dye in the skin and the influence of fasting stress on passive cutaneous anaphylaxis in guinea pigs and rats. *Microbiol. Immunol.*, 22, 89-101 (1978).
Lagunoff, D., Martin, T. W. and Read, G., Agents that release histamine from mast cells. *Annu. Rev. Pharmacol. Toxicol.*, 23, 331-351 (1983).
Makino, H., Saijo, T., Ashida, Y. Kuriki, H. H. and Maki, Y., Mechanism of action of an antiallergic agent: Amlexanox(AA-673), in inhibiting histamine release from mast cells. *Int, Arch. Allergy Immunol.*, 82, 66-71 (1987).
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).
Mousli, M. C., Bronner, C., Bockaert, J., Rouot, B. and Landry, Y., Interaction of substance P, compound 48/80 and mastoparan with -subunit C-terminal of G protein. *Immunol. Lett.*, 25, 355-358 (1990a).
Mousli, M.C., Bronner, C., Landry, Y., Bockaert, J. and Rouot, B., Direct activation of GTP-binding regulatory proteins (G proteins) by substance P and compound 48/80. *FEBS Lett.*, 259, 260-262 (1990b).
Peachell, P. T., MacGlashan, D. W., Lichtenstein, L. M. and Schleimer, R. P., Regulation of human basophil and lung mast cell function by cyclic adenosine monophosphate. *J. Immunol.*, 140, 571-579 (1988).
Petersen, L. J., Mosbech, H. and Skov, P., Allergen-induced histamine release in intact human skin *in vivo* assessed by skin microdialysis technique: Characterization of factors influencing histamine releasability. *J. Allergy Clin. Immunol.*, 97, 672-679 (1996).
Plaut, M., Pierce, J. H., Watson, C. J., Hanley-Hyde, J., Nordon, R. P. and Paul, W. E., Mast cell lines produce limphokines in response to cross-linkage of FcεRI or to calcium ionophores. *Nature*, 339, 64-67 (1989).
Segal, D.M., Taurog, J. and Metzger, H., Dimeric immunoglobulin E serves as a unit signal for mast cell degranulation. *Proc. Natl. Acad. Sci.*, USA, 74, 2993-2997 (1977).
Shin, T. Y., Park, J, H. and Kim. H. M., Effect of Cryptotympana atrata extract on compound 48/80-induced anaphylactic reactions. *J. Ethnopharmacol.*, 66, 319-325 (1999).
Shore, P. A., Burkhalter, A. and Cohn, V. H., A method for fluorometric assay of histamine in tissues. *J. Pharmacol. Exp. Ther.*, 127, 182-186 (1959).
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).
Wasserman, S. I. and Marquardt, D. L., Anaphylaxis in Allergy: Principles and Practice. 3rd ed. C. V. Mosby Co., St. Louis, pp.1365, (1988).
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 Immunol., 139, 2605-2614 (1987).
Wodnar-Filipowicz, A., Heusser, C. H. and Moroni, C., Production of the haemopoietic growth factors GM-CSF and interleukin-3 by mast cells in response to IgE receptor-mediated activation. *Nature*, 339, 150-152 (1989).
Yurt, R. W., Leid, R. W. and Austen, K. F., Native heparin from rat peritoneal mast cells. *J. Biol. Chem.*, 252, 518-521 (1997).