

Note

Inhibitory Effects of Wasabi Isothiocyanates on Chemical Mediator Release in RBL-2H3 Rat Basophilic Leukemia Cells

Tomoe YAMADA-KATO¹, Masashi NAGAI¹, Motoko OHNISHI² and Kazutoshi YOSHIDA³

¹ Kinjirushi Co., Ltd., 2–61 Yahata-hontori, Nakagawa-ku, Nagoya, Aichi 454–8526, Japan

² Department of Biological Chemistry, College of Bioscience and Biotechnology, Chubu University, 1200 Matsumoto-cho, Kasugai, Aichi 487–8501, Japan

³ Hyogo Prefectural Institute of Technology, 3–1–12 Yukihira-cho, Suma-ku, Kobe, Hyogo 654–0037, Japan

(Received February 22, 2012)

Summary Wasabi is a plant of Japanese origin. It belongs to the family Brassicaceae and produces various isothiocyanates (ITCs). To clarify the type I allergies inhibited by wasabi ITCs, we investigated the inhibitory effect on chemical mediator release from dinitrophenylated bovine serum albumin (DNP-BSA)-stimulated RBL-2H3 rat basophilic leukemia cells. Allyl ITC (AITC), *sec*-butyl ITC (*s*-BuITC), and 3-butenyl ITC (3-BuITC), which have 3 or 4 carbon chains, inhibited histamine release but did not inhibit the release of leukotriene B₄ (LTB₄) or cysteinyl LTs (CysLTs). 4-Pentenyl ITC (4-PeITC) and 5-hexenyl ITC (5-HeITC), which have 5 or 6 carbon chains and an unsaturated bond at the end, inhibited LTB₄ release but did not inhibit the release of histamine or CysLTs. 6-Methylthiohexyl ITC (6-MTITC), 6-methylsulfinylhexyl ITC (6-MSITC), and 6-methylsulfonylhexyl ITC (6-MSFITC), which have a sulfur atom inserted at the end of a 6-carbon chain, inhibited the release of histamine, LTB₄, and CysLTs and the elevation in intracellular Ca²⁺. These results suggest that wasabi ITCs inhibited type I allergies by inhibiting chemical mediator release and that the inhibitory effects on each chemical mediator were due to differences in the side chain structure of the wasabi ITCs.

Key Words antiallergic, wasabi isothiocyanate, RBL-2H3 rat basophilic leukemia cells, chemical mediator

Type I allergy is a hypersensitivity reaction that occurs as a result of antigen stimulation. Allergic disorders such as asthma, rhinitis, atopic dermatitis, and anaphylactic shock are classified as type I allergies. The number of patients with these allergies has been increasing in most parts of the world (1, 2). Present allergic medicines suppress symptoms but do not cure the illness completely (3, 4). Consequently, patients have to continue the medication as long as symptoms exist. Furthermore, these medications have side effects such as drowsiness (5). Thus, suppressing allergic symptoms by consuming normal, safe, and effective functional food is important to maintain a patient's quality of life.

An allergic reaction starts when an antigen invades the body. The antigen induces the production of anti-specific IgE antibodies, and the IgE interacts with receptors on the mast cell membrane surface (6–8). Once the mast cells are activated by the bridging of IgE and the antigen, induced phospholipase C (PLC) produces inositol 1,4,5-trisphosphate (IP₃) and 1,2-diacylglycerol (DG) from phosphatidylinositol 4,5-bisphosphate (9, 10). IP₃ releases Ca²⁺ from internal stores (11), while DG activates protein kinase C (PKC) (12). Histamine, which is a chemical mediator, is stored in mast cell

granules (6, 8) and is released by degranulation induced by elevation in intracellular Ca²⁺ and PKC activation (6, 13). PKC is involved in Ca²⁺ influx from the extracellular environment (14). The elevation in intracellular Ca²⁺ level translocates cytosolic phospholipase A₂ (cPLA₂) to the margin of the nuclear membrane (7). In addition, the activated mast cells induce Ras, which then activates mitogen-activated protein (MAP) kinase (15). MAP kinase activates cPLA₂ (7, 16), and cPLA₂ induces release of leukotrienes (LTs) through an arachidonic acid cascade (7, 17). LTA₄, which is synthesized from arachidonic acid, is converted to LTB₄ and LTC₄ by LTA₄ hydrolase and LTC₄ synthase, respectively (7, 18). LTC₄ is converted to LTD₄ and LTE₄ (7, 18). LTC₄, LTD₄, and LTE₄ are together referred to as cysteinyl LTs (CysLTs) (7, 18). Chemical mediators (histamine, LTs, etc.) dilate blood vessels, accelerate vascular permeability, and promote immunocyte migration, leading to allergic symptoms (4, 7, 18). If the release of these chemical mediators from mast cells can be inhibited, allergic symptoms may be suppressed.

The rat basophilic leukemia cell line (RBL-2H3) is a model for mucosal-type mast cells (8). These cells have been used to investigate the antiallergic effects of medicines and foods (19, 20). After antigen stimulation, these cells release chemical mediators such as histamine

E-mail: t-yamada@kinjirushi.co.jp

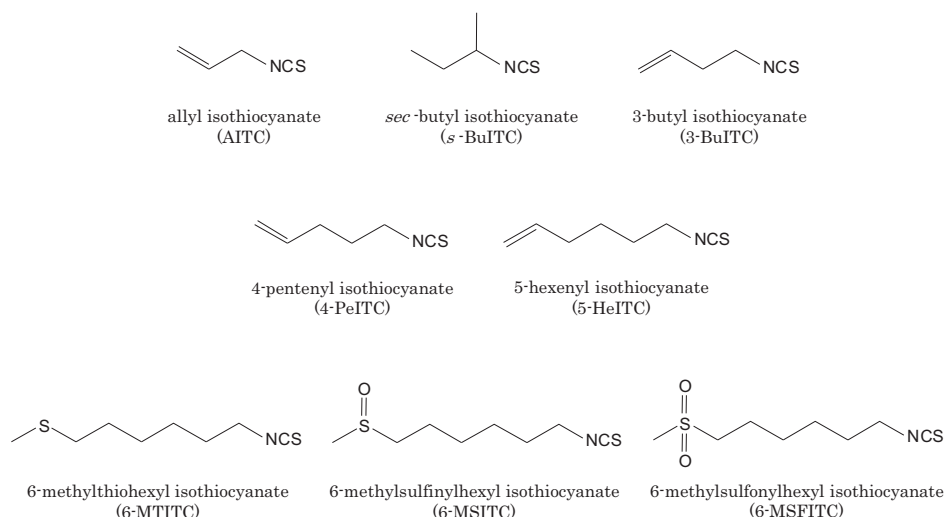


Fig. 1. Chemical structures of wasabi isothiocyanates.

(19), LTB₄, LTC₄, and LTE₄ (20).

Wasabi [*Wasabia japonica* (Miq) Matsumura] is a plant of Japanese origin and belongs to the family Brassicaceae. Grated wasabi rhizome is a popular condiment in Japan. The grated rhizome contains various isothiocyanates (wasabi ITCs) such as allyl ITC (AITC), *sec*-butyl ITC (*s*-BuITC), 3-butenyl ITC (3-BuITC), 4-pentenyl ITC (4-PeITC), 5-hexenyl ITC (5-HeITC), 6-methylthiohexyl ITC (6-MTITC), and 6-methylsulfinylhexyl ITC (6-MSITC) (21, 22). Wasabi ITCs, particularly AITC, 6-MTITC, and 6-MSITC, have physiological functions. AITC is well known for its antimicrobial activity (23). 6-MTITC has anticancer effects (24), while 6-MSITC has inhibitory activity against platelet aggregation (25), inducing activity of phase II enzymes (26), as well as antiinflammatory activity (27).

The antiallergic effects of wasabi ITCs have been studied. AITC has an asthma-protective effect when administered orally to guinea pigs (28). A wasabi rhizome extract diet (including 6-MSITC without other ITCs) improves atopic dermatitis-like symptoms in HR-1 hairless mice (29). Morimitsu et al. (26) reported that 6-MSITC is absorbed into the body following its oral administration and enters the circulatory system. These reports suggest that wasabi ITCs could be absorbed into the body and inhibit type I allergies.

Therefore, we investigated the inhibitory effects of chemical mediators released from RBL-2H3 cells. In addition, the structure-activity relationships of wasabi ITCs were investigated. 6-Methylsulfonylhexyl ITC (6-MSFITC), which has not been reported in wasabi, was also investigated, because the structure of 6-MSFITC resembles that of 6-MTITC and 6-MSITC.

Materials and Methods

Reagents. Mustard oil (AITC) was purchased from T. Hasegawa Co., Ltd. (Tokyo, Japan). *s*-BuITC, 3-BuITC, 4-PeITC, and 5-HeITC were kind gifts from Ogawa & Co., Ltd. (Tokyo, Japan). 6-MTITC was purchased from Ogawa & Co., Ltd. 6-MSITC was synthesized by 6-MTITC

oxidation in our laboratory (30), and 6-MSFITC was a kind gift from Dr. Morimitsu (Ochanomizu University, Tokyo, Japan). These chemical structures are shown in Fig. 1.

Dinitrophenylated bovine serum albumin (DNP-BSA) was purchased from Cosmo Bio Co., Ltd. (Tokyo, Japan). Fetal bovine serum (FBS), penicillin-streptomycin, and trypan blue were purchased from Invitrogen (Carlsbad, CA, USA). Anti-DNP-IgE, ketotifen fumarate, Eagle's minimum essential medium (MEM), BSA, and 4-nitrophenyl *N*-acetyl- β -D-glucosaminide were purchased from Sigma (St. Louis, MO, USA).

Cell culture. The RBL-2H3 rat basophilic leukemia cell line (JCRB0023) was purchased from the Health Science Research Resources Bank (Tokyo, Japan). Cells were cultured in MEM with 10% heat-inactivated FBS, 100 U/mL penicillin, and 100 μ g/mL streptomycin at 37°C in a 5% CO₂ incubator.

Chemical mediator release assay. RBL-2H3 cells were stimulated according to the method described by Kobayashi et al. (19), with some modifications. The cells were plated at 5.0×10^5 cells/mL in 24-well culture plates (0.4 mL/well), and the plated cells were then sensitized with 0.05 μ g/mL anti-DNP-IgE at 37°C for 24 h. They were then washed twice with PBS (–), and 260 μ L of a releasing mixture (116.9 mM NaCl, 5.4 mM KCl, 0.8 mM MgSO₄ · 7H₂O, 5.6 mM glucose, 25 mM HEPES, 2.0 mM CaCl₂, and 1.0 mg/mL BSA at pH 7.7) was added. Test samples (ITCs and ketotifen fumarate), which were dissolved in dimethylsulfoxide (DMSO) and diluted with the releasing mixture, were added 20 μ L to cells and incubated at 37°C for 10 min. After adding 20 μ L of DNP-BSA (4 μ g/mL), the stimulated cells were further incubated at 37°C for 30 min. The final concentration of the test samples was 50 μ M, and the DMSO concentration was 0.1%. The histamine concentration in the supernatant was measured using the A05890 enzyme-linked immunosorbent assay (ELISA) kit (SPI-Bio, Massey Cedex, France), and the LTB₄ and CysLTs concentrations were measured with the RPN223 and

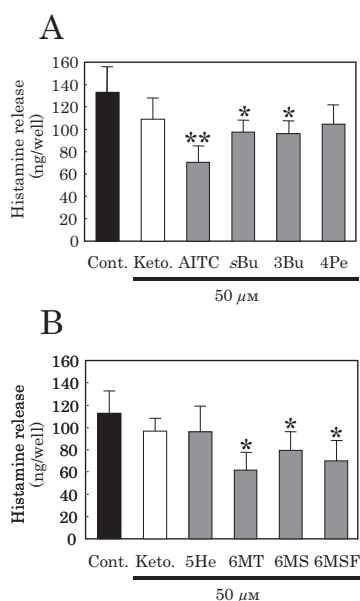


Fig. 2. Inhibitory effect of wasabi isothiocyanates on histamine release from RBL-2H3 cells. Sensitized cells were preincubated with test samples at 37°C for 10 min before 30 min dinitrophenylated bovine serum albumin stimulation. Each data value is expressed as mean $\pm$ SD ($n=4$). Statistically significant differences (Student's *t*-test) are indicated as * $p<0.05$ or ** $p<0.005$ when compared with the control value. Cont.: control, Keto.: ketotifen fumarate, sBu: s-BuITC, 3Bu: 3-BuITC, 4Pe: 4-PeITC, 5He: 5-HeITC, 6MT: 6-MTITC, 6MS: 6-MSITC, 6MSF: 6-MSFITC.

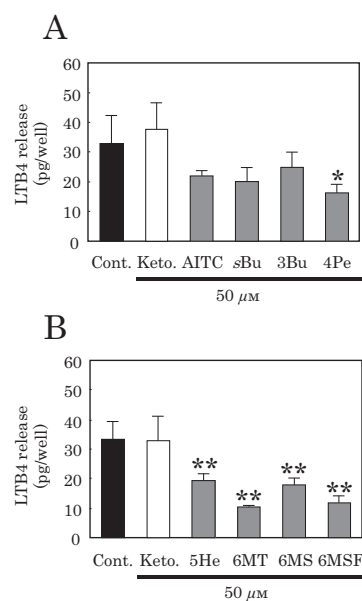


Fig. 3. Inhibitory effect of wasabi isothiocyanates on leukotriene B4 release in RBL-2H3 cells. Sensitized cells were preincubated with test samples at 37°C for 10 min before 30 min dinitrophenylated bovine serum albumin stimulation. Each data value is expressed as mean $\pm$ SD ($n=4$). Statistically significant differences (Student's *t*-test) are indicated as * $p<0.05$ or ** $p<0.005$ when compared with the control value. Cont.: control, Keto.: ketotifen fumarate, sBu: s-BuITC, 3Bu: 3-BuITC, 4Pe: 4-PeITC, 5He: 5-HeITC, 6MT: 6-MTITC, 6MS: 6-MSITC, 6MSF: 6-MSFITC.

RPN224 ELISA kits, respectively (GE Healthcare UK Ltd., Little Chalfont, Buckinghamshire, England), according to the manufacturer's instructions. The viability of the stimulated cells at 37°C for 30 min was checked by trypan blue exclusion.

Measurement of intracellular Ca^{2+} level. The intracellular Ca^{2+} level was measured with Calcium Kit-Fluo3 (Dojindo Laboratories, Kumamoto, Japan). The cells were plated at 3.0×10^5 cells/mL in 96-black-walled bottom-clear well plates (0.1 mL/well). They were sensitized at 37°C for 24 h with 0.05 μ g/mL anti-DNP-IgE, washed twice with PBS (–), and then incubated with 100 μ L of a loading buffer containing Fluo 3-AM (Calcium Kit-Fluo3) at 37°C for 1 h. The cells were then washed twice with PBS (–) and incubated at 37°C for 10 min with 100 μ L of a recording medium (Calcium Kit-Fluo3) including a test sample (50 μ M) and 0.1% DMSO. After adding 7 μ L of DNP-BSA (20 μ g/mL), the stimulated cells were further incubated for 50 s. Fluorescence intensity was measured at an excitation wavelength of 485 nm and an emission wavelength of 530 nm using a fluorometric plate reader (Wallac 1420 ARVO SX multilabel counter; PerkinElmer, Inc., Waltham, MA, USA).

Statistical analysis. Results are expressed as mean $\pm$ standard deviation. Statistical comparisons were performed with Student's *t*-test for paired values. Results with $p<0.05$ were considered statistically significant.

Results and Discussion

As shown in Fig. 2, AITC, s-BuITC, 3-BuITC, 6-MTITC, 6-MSITC, and 6-MSFITC significantly inhibited histamine release compared with the control (s-BuITC, 3-BuITC, 6-MTITC, 6-MSITC, and 6-MSFITC: $p<0.05$, AITC: $p<0.005$). 4-PeITC, 5-HeITC, 6-MTITC, 6-MSITC, and 6-MSFITC significantly inhibited LTB4 release compared with the control (4-PeITC: $p<0.05$, 5-HeITC, 6-MTITC, 6-MSITC, and 6-MSFITC: $p<0.005$) (Fig. 3). Furthermore, 6-MTITC, 6-MSITC, and 6-MSFITC significantly inhibited CysLTs release compared with the control (6-MSITC and 6-MSFITC: $p<0.05$, 6-MTITC: $p<0.005$) (Fig. 4). AITC, 6-MTITC, 6-MSITC, and 6-MSFITC significantly inhibited intracellular Ca^{2+} elevation (6-MSFITC: $p<0.05$, AITC, 6-MTITC, and 6-MSITC: $p<0.005$) (Fig. 5). Cell viability checked by trypan blue exclusion was $>90\%$ (data not shown).

Ketotifen fumarate, an anti-allergic drug, is a stabilizer of mast cell chemical mediator release and an antagonist of these mediator receptors (4). Kobayashi et al. (19) reported that in comparison to the control, 0.1 mg/mL (235 μ M) of ketotifen fumarate significantly inhibits histamine release. In this study, ketotifen fumarate (50 μ M), which was at the same concentration as wasabi ITCs, did not inhibit the release of histamine, LTB4, or CysLTs.

Wasabi ITCs showed various inhibitory effects on the 3 chemical mediators. They were roughly divided into 3

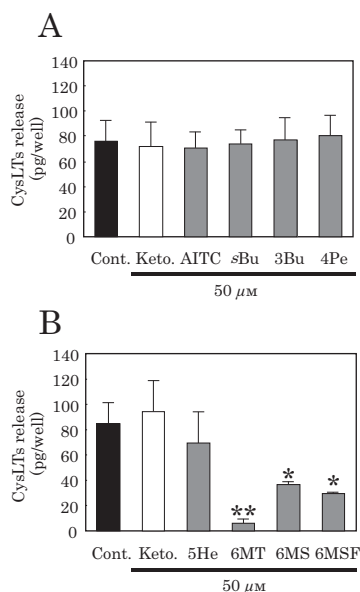


Fig. 4. Inhibitory effect of wasabi isothiocyanates on cysteinyl leukotriene release in RBL-2H3 cells. Sensitized cells were preincubated with test samples at 37°C for 10 min before 30 min dinitrophenylated bovine serum albumin stimulation. Each data value is expressed as mean $\pm$ SD ($n=4$). Statistically significant differences (Student's *t*-test) are indicated as * $p<0.05$ or ** $p<0.005$ when compared with the control value. Cont.: control, Keto.: ketotifen fumarate, sBu: *s*-BuITC, 3Bu: 3-BuITC, 4Pe: 4-PeITC, 5He: 5-HeITC, 6MT: 6-MTITC, 6MS: 6-MSITC, 6MSF: 6-MSFITC.

types based on differences in their inhibitory effect and molecular structure.

AITC, *s*-BuITC, and 3-BuITC have 3 or 4 carbon chains (Fig. 1). These ITCs inhibited histamine release (Fig. 2) but did not inhibit LTB₄ (Fig. 3) or CysLTs (Fig. 4) release. In addition, AITC inhibited the elevation in intracellular Ca²⁺ (Fig. 5), which originates from the extracellular environment or internal stores (11, 14). Histamine is released by degranulation induced by elevation in intracellular Ca²⁺ and activation of PKC (6, 13). PKC is involved in the Ca²⁺ influx from the extracellular environment (14). LTs are produced by activation of cPLA2 induced by Ca²⁺ influx from the extracellular environment and by MAP kinase phosphorylation (7, 16, 17). These results suggest that these ITCs may not inactivate cPLA2 activation caused by MAP kinase but may inactivate the elevation in intracellular Ca²⁺ from the extracellular environment and/or internal stores.

4-PeITC and 5-HeITC have 5 or 6 carbon chains with an unsaturated bond at the end (Fig. 1). Although these ITCs inhibited LTB₄ release (Fig. 3), they did not inhibit the release of histamine (Fig. 2) or CysLTs (Fig. 4). LTA₄ is produced in the arachidonic acid cascade and is converted to LTB₄ and LTC₄ by LTA₄ hydrolase and LTC₄ synthase, respectively (7, 18). ITCs are known as highly reactive electrophiles (30). These results suggest that these ITCs may inactivate LTA₄ hydrolase and/or directly attack LTB₄.

6-MTITC, 6-MSITC, and 6-MSFITC have a sulfur

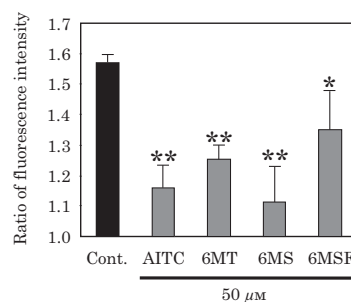


Fig. 5. Inhibitory effect of allyl isothiocyanate (AITC), 6-methylthiohexyl isothiocyanate (6-MTITC), 6-methylsulfonylethyl isothiocyanate (6-MSITC), and 6-methylsulfonylhexyl isothiocyanate (6-MSFITC) on intracellular Ca²⁺ elevation in RBL-2H3 cells. Sensitized and Fluo 3-loaded cells were preincubated with test samples at 37°C for 10 min before dinitrophenylated bovine serum albumin (DNP-BSA) stimulation. Intracellular Ca²⁺ levels were determined to be in the 50 s after DNP-BSA stimulation by spectrofluorometry. Each data value is expressed as mean $\pm$ SD ($n=3$). Statistically significant differences (Student's *t*-test) are indicated as * $p<0.05$ or ** $p<0.005$ when compared with the control value. Cont.: control, 6MT: 6-MTITC, 6MS: 6-MSITC, 6MSF: 6-MSFITC.

atom inserted into the end of a 6-carbon chain (Fig. 1). These ITCs inhibited the release of histamine, LTB₄, and CysLTs (Figs. 2–4) and the intracellular Ca²⁺ elevation (Fig. 5). These results suggest that the inhibitory effects on histamine, LTB₄, and CysLTs by these ITCs might be caused by inhibition of the intracellular Ca²⁺ elevation.

Furthermore, AITC, 3-BuITC, 4-PeITC, and 5-HeITC are characterized by carbon chains with an unsaturated bond at the end. Short carbon chain ITCs (AITC and 3-BuITC) inhibited histamine release, whereas long carbon chain ITCs (4-PeITC and 5-HeITC) did not (Fig. 2). In contrast, LTB₄ release was more strongly inhibited by long carbon chain ITCs (4-PeITC and 5-HeITC) than short carbon chain ITCs (AITC and 3-BuITC) (Fig. 3). CysLT release was not inhibited by the 4 ITCs (AITC, 3-BuITC, 4-PeITC, and 5-HeITC) (Fig. 4). These results revealed that the inhibitory effects on chemical mediators differed according to the length of the carbon chain.

We found that wasabi ITCs inhibit chemical mediator release from RBL-2H3 cells. Furthermore, the inhibitory effects on each chemical mediator differed based on side chain structure. The wasabi rhizome contains various ITCs (21, 22). When patients with allergies consume wasabi, inhibition of chemical mediator release by ITCs may contribute to suppressing symptoms. Future research and development of wasabi is required.

Acknowledgments

I would like to thank Dr. Motoyasu Minami (Chubu University) and collaborators from the Ohnishi laboratory (Chubu University) for their cooperation and kind support, and Enago (www.enago.jp) for the English language review.

REFERENCES

- 1) Sigurkarlsson S, Clausen M, Gislason T, Gislason D. 2011. Prevalence of respiratory symptoms and use of asthma drugs are increasing among young adult Icelanders. *Laeknabladid* **97**: 463–467.
- 2) Thomsen SE, van der Sluis S, Kyvik KO, Skytthe A, Skadhauge LR, Backer V. 2011. Increase in the heritability of asthma from 1994 to 2003 among adolescent twins. *Respir Med* **105**: 1147–1152.
- 3) Okuda M, Baba H, Konno A, Takenaka H. 2005. Practical Guideline for the Management of Allergic Rhinitis in Japan, p 32–33. Life Science, Tokyo.
- 4) Nakagawa T. 1991. Chemical mediators and anti-mediator drugs. *Kokyu to Junkan* **39**: 533–538 (in Japanese).
- 5) Kamikawa Y. 1999. Current progress in antihistamines and their adverse reactions. Sedative side effects and permeability of blood-brain barrier. *J New Remedies Clinics* **48**: 39–47 (in Japanese).
- 6) Furuno T. 2005. Confocal laser scanning microscopy to study molecular mechanism of mast cell activation. *Yakugaku Zasshi* **125**: 671–683 (in Japanese).
- 7) Taketomi Y, Murakami M. 2011. Lipid networks in mast cell biology. *Yakugaku Zasshi* **131**: 73–84 (in Japanese).
- 8) Teshima R, Nakamura R, Furuno T, Nakanishi M. 1999. Degranulation in mast cells and basophils: confocal and atomic force microscopy. *Bioimages* **7**: 97–103.
- 9) Dainaka J, Ichikawa A, Koibuchi Y, Nakagawa M, Tomita K. 1986. Effect of the tridecamer of compound 48/80, a Ca^{2+} -dependent histamine releaser, on phospholipid metabolism during the early stage of histamine release from rat mast cells. *Biochem Pharmacol* **35**: 3739–3744.
- 10) Okano Y, Takagi H, Tohmatsu T, Nakashima S, Kuroda Y, Saito K, Nozawa Y. 1985. A wasp venom mastoparan-induced polyphosphoinositide breakdown in rat peritoneal mast cells. *FEBS Lett* **188**: 363–366.
- 11) Meyer T, Holowka D, Stryer L. 1988. Highly cooperative opening of calcium channels by inositol 1,4,5-trisphosphate. *Science* **240**: 653–656.
- 12) Chakravarty N. 1990. The role of protein kinase C in histamine secretion from mast cells. *Acta Physiol Scand* **139**: 319–331.
- 13) Kimata M, Shichijo M, Miura T, Serizawa I, Inagaki N, Nagai H. 1999. Ca^{2+} and protein kinase C signaling for histamine and sulfidoleukotrienes released from human cultured mast cells. *Biochem Biophys Res Commun* **257**: 895–900.
- 14) Teshima R, Ikebuchi H, Terao T, Nakanishi M. 1991. The effect of staurosporine on Ca^{2+} signals in rat basophilic leukemia (RBL-2H3) cells. *Chem Pharm Bull (Tokyo)* **39**: 747–751.
- 15) Reischl IG, Coward WR, Church MK. 1999. Molecular consequences of human mast cell activation following immunoglobulin E-high-affinity immunoglobulin E receptor (IgE-FcεRI) interaction. *Biochem Pharmacol* **58**: 1841–1850.
- 16) Hirasawa N, Santini F, Beaven MA. 1995. Activation of the mitogen-activated protein kinase/cytosolic phospholipase A2 pathway in a rat mast cell line. Indications of different pathways for release of arachidonic acid and secretory granules. *J Immunol* **154**: 5391–5402.
- 17) Murakami M, Nakatani Y, Atsumi G, Inoue K, Kudo I. 1997. Regulatory functions of phospholipase A2. *Crit Rev Immunol* **17**: 225–283.
- 18) Peters-Golden M, Gleason MM, Togias A. 2006. Cysteinyl leukotrienes: multi-functional mediators in allergic rhinitis. *Clin Exp Allergy* **36**: 689–703.
- 19) Kobayashi M, Matsushita H, Yoshida K, Tsukiyama R, Sugimura T, Yamamoto K. 2004. In vitro and in vivo anti-allergic activity of soy sauce. *Int J Mol Med* **14**: 879–884.
- 20) Yamashita M, Kushihara M, Hirasawa N, Takasaki W, Takahagi H, Takayanagi M, Ohuchi K. 2000. Inhibition by troglitazone of the antigen-induced production of leukotrienes in immunoglobulin E-sensitized RBL-2H3 cells. *Br J Pharmacol* **129**: 367–373.
- 21) Etoh H, Nishimura A, Takasawa R, Yagi A, Saito K, Sakuta K, Kishima I, Ina K. 1990. ω -Methylsulfinylalkyl isothiocyanates in wasabi. *Wasabi japonica* Matsum. *Agric Biol Chem* **54**: 1587–1589.
- 22) Kumagai H, Kashima N, Seki T, Sakurai H, Ishii K, Ariga T. 1994. Analysis of volatile components in essential oil of upland wasabi and their inhibitory effects on platelet aggregation. *Biosci Biotechnol Biochem* **58**: 2131–2135.
- 23) Isshiki K, Tokuoka K, Mori R, Chiba S. 1992. Preliminary examination of allyl isothiocyanate vapor for food preservation. *Biosci Biotechnol Biochem* **56**: 1476–1477.
- 24) Yano T, Yajima S, Virgona N, Yano Y, Otani S, Kumagai H, Sakurai H, Kishimoto M, Ichikawa T. 2000. The effect of 6-methylthiohexyl isothiocyanate isolated from *Wasabia japonica* (wasabi) on 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol-induced lung tumorigenesis in mice. *Cancer Lett* **155**: 115–120.
- 25) Morimitsu Y, Hayashi K, Nakagawa Y, Fujii H, Horio F, Uchida K, Osawa T. 2000. Antiplatelet and anticancer isothiocyanates in Japanese domestic horseradish, wasabi. *Mech Ageing Dev* **116**: 125–134.
- 26) Morimitsu Y, Nakagawa Y, Hayashi K, Fujii H, Kumagai T, Nakamura Y, Osawa T, Horio F, Itoh K, Iida K, Yamamoto M, Uchida K. 2002. A sulforaphane analogue that potently activates the nrf2-dependent detoxification pathway. *J Biol Chem* **277**: 3456–3463.
- 27) Uto T, Fujii M, Hou DX. 2005. Inhibition of lipopolysaccharide-induced cyclooxygenase-2 transcription by 6-(methylsulfinyl) hexyl isothiocyanate, a chemopreventive compound from *Wasabia japonica* (Miq.) Matsu-mura, in mouse macrophages. *Biochem Pharmacol* **70**: 1772–1784.
- 28) Dorsch W, Adam O, Weber J, Ziegeltrum T. 1985. Antiasthmatic effects of onion extracts—detection of benzyl- and other isothiocyanates (mustard oils) as antiasthmatic compounds of plant origin. *Eur J Pharmacol* **107**: 17–24.
- 29) Nagai M, Okunishi I. 2009. The effect of wasabi rhizome extract on atopic dermatitis-like symptoms in HR-1 hairless mice. *J Nutr Sci Vitaminol* **55**: 195–200.
- 30) Murata M, Uno M, Nagai Y, Nakagawa K, Okunishi I. 2004. Content of 6-methylsulfinylhexyl isothiocyanate in wasabi and processed wasabi products. *Nippon Shokuhin Kagaku Kogaku Kaishi* **51**: 477–482 (in Japanese).