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 antigen-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 (PtdIns(4,5)P2). 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...
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 (6-MTITC, and 6-MSITC, have physiological functions. AITC has inhibitory activity against platelet aggregation (6-MSITC) (21). 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 μg/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 × 10⁴ cells/mL in 24-well culture plates (0.4 mL/well), and the plated cells were then sensitized with 0.05 μg/mL anti-DNP-IgE at 37°C for 24 h. They were then washed twice with PBS (−), and 260 μL 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 μL to cells and incubated at 37°C for 10 min. After adding 20 μL of DNP-BSA (4 μg/mL), the stimulated cells were further incubated at 37°C for 30 min. The final concentration of the test samples was 50 μM, 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. 1. Chemical structures of wasabi isothiocyanates.](image-url)
Wasabi Isothiocyanates Inhibit Chemical Mediator Release

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-MTITC and 6-MSFITC: p<0.05, 6-MTITC: p<0.005) (Fig. 4). AITC, 6-MTITC, 6-MSITC, and 6-MSFITC significantly inhibited intracellular Ca2+ elevation (6-MSFITC: p<0.05, AITC, 6-MTITC, and 6-MSFITC: 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 μM) of ketotifen fumarate significantly inhibits histamine release. In this study, ketotifen fumarate (50 μM), 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

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**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±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±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 Ca^{2+} (Fig. 5), which originates from the extracellular environment or internal stores (11, 14). Histamine is released by degranulation induced by elevation in intracellular Ca^{2+} and activation of PKC (6, 13). PKC is involved in the Ca^{2+} influx from the extracellular environment (14). LTs are produced by activation of cPLA2 induced by Ca^{2+} influx from the extracellular environment and by MAP kinase phosphorylation (7, 16, 17). These results suggest that these ITCs may not activate cPLA2 activation caused by MAP kinase but may inactivate the elevation in intracellular Ca^{2+} 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 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 Ca^{2+} 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 Ca^{2+} 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.

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