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Screening of Korean Marine Plants for Their Inhibitory Effect on Histamine Release from RPMC *in vitro*

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Abstract Allergy, meaning 'heightened reactivity' of a host on being exposed to an antigen, is an immediate reaction which included anaphylaxis following contact with an antigen. An anaphylatic reaction is caused by the release of pharmacological mediators, like histamine, from mast cells. The potential anti-allergic activities of 27 seaweed and 19 salt marsh extracts collected from the coast of Korea were tested against the inhibition of histamine release in rat peritoneal mast cells (RPMCs). Among them, three salt marsh plants (*Persicaria lapathifolia, Ixeris tamagawaensis,* and *Salsola komarovii*) significantly showed more than 75% of inhibition of the histamine release at a concentration of 100 μg/mL, and also three salt marsh (*Messerschmidia sibirica, Rosa rugosa,* and *Portulaca oleraceae*) and three seaweed (*Colpomenia bullosa, Derbesia marina,* and *Sargassum thunbergii*) extracts exhibited moderately inhibition effects when compared to the control.

Keywords: histamine release, marine plant, anti-allergic activity

Allergic diseases, both systemic and site-specific, affect approximately 20~30% of the general population and constitute a major source of suffering, disability, and loss of productivity throughout the world [1]. The pathological features of allergic diseases and asthma include the production of allergen-specific IgE, which results in immediate phase responses, with mast cell and basophil degranulation, with the release of mediators, including histamine, proteases, cytokines, and arachidonic acid products [2,3]. Of the preformed and newly synthesized inflammatory substancs released on degranulation of mast cells, histamine remains the best characterized and most potent vasoactive mediator implicated in the acute phase of type I-allergic reactions [4].

Histamine is a primary amine which is synthesized and stored within the secretary granules of human mast cells (~3 pg/cell) and basophils (~1 pg/cell) [5]. It has been shown that histamine can induce and or modulate cytokine synthesis in allergic inflammation. Two types of effects have been described: (1) Direct effects of histamine on cytokine and (2) modulation of cytokine synthesis induced by immunologic stimuli [6].

The mast cell has also long been thought to play a crucial role in the development of many physiologic changes uring anaphylactic and allergic responses [7]. Mast cell degranulation can be elicited by a number of positively charged substances, collectively known as the basic secretagogues of mast cells [8]. The most potent secretagogues include the synthetic compound 48/80, which contains polymers of basic amino acids [9]. The compound is a mixture of polymers synthesized by condensing *N*-methyl-*p*-methoxyphenylethylamine with formaldehyde [10, 11]. Compared with the natural process, a high concentration of compound 48/80 induces almost a 90% release of histamine from mast cells. Thus, it has been used as a good tool for studying the histamine release activity mechanism of anaphylactic reactions [12].

Seaweeds contain high amounts of essential minerals and are recommended as food to ameliorate skin atopic symptoms. Nevertheless, no scientific data for allergy therapy have been reported concerning the biological activities of marine plants including seaweeds [13-15].

In this study, we have investigated the inhibitory effects of salt-resisted halophytes and seaweeds on compound 48/80 induced-histamine release in the rat peritoneal mast cells *in vitro*.

The seaweeds were collected along the shores of Cheju Island and Busan, South Sea, Korea. All the seaweeds were identified by Dr. J. S. Yu, at the Research Institute of Marine Science and Technology, Korea Maritime University, Korea. The salt marsh plants were collected at Daebudo, Yangpori, Pohang, and Geojedo, Korea. The taxonomic identification of salt marsh plants was confirmed by a botanist, S. G. Moon, at the Kyungsung University, Korea.

The shade-dried salt marsh plants were chopped into

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small pieces and sequentially extracted with CH_2Cl_2 and MeOH, for 2 days each. Both extracts were combined, evaporated and subjected to the test for inhibition activity of histamine release from mast cells. The samples obtained were stored in a refrigerator at −5°C until further experiments.

Compound 48/80 was purchased from Sigma Chemical Co. (St. Louis, MO, USA). The original stock of Wistar rats (Wistar Charles River, Wilmington, MA, USA) was purchased from the Damul Experimental Animal Center (Daejeon, Korea), and the animals were maintained in the College of Pharmacy, Wonkwang University. The rats were housed five to ten per cage in a laminar air-flow room maintained at a temperature of 22 ± 1°C and with a relative humidity of 55 ± 10% throughout the study. The rats were sacrificed in accordance with National Institutes of Health animal care and use guidelines.

RPMCs were isolated as previously described [16]. In brief, rats were anesthetized by ether, and injected with 20 mL of Tyrode buffer B (NaCl, glucose, $NaHCO_3$, KCl, and NaH_2PO_4) containing 0.1% gelatin (Sigma) into the peritoneal cavity; the abdomen was gently massaged for about 90 sec. The peritoneal cavity was carefully opened, and the fluid containing peritoneal cells was aspirated using a Pasteur pipette. The peritoneal cells were then sedimented at 150 g for 10 min at room temperature and resuspended in Tyrode buffer B. RPMCs were separated from the major components of the rat peritoneal cells (*i.e.*, macrophages and small lymphocytes) according to the method described by Yurt *et al.* [17]. In brief, peritoneal cells suspended in 1 mL of Tyrode buffer B were layered onto 2 mL of 0.225 g/mL metrizamide (density 1.120 g/mL, Sigma) and centrifuged at 400 g and room temperature for 15 min. The cells remaining at the buffer-metrizamide interface were aspirated and discarded; the cells in the pellet were washed and resuspended in 1 mL of Tyrode buffer A (10 mM HEPES, 130 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 5.6 mM glucose, 0.1% bovine serum albumin) containing calcium (1.4 mM $CaCl_2$). RPMCs preparations were about 95% pure as assessed by toluidine blue staining. More than 97% of the cells were viable as adjudged from the trypan blue uptake.

Purified RPMCs were resuspended in Ca-added Tyrode buffer A for the treatment with compound 48/80. RPMCs suspensions (2×10^5 cells/mL) were preincubated for stabilization, for 10 min at 37°C, before the addition of compound 48/80. Each sample with cells was preincubated for 30 min, and then incubated for 15 min with compound 48/80 (6 g/mL). The reaction was stopped by cooling the tubes in ice. The cells were separated from the released histamine by centrifuging at 400 g and 4°C for 5 min. Residual histamine in the cells was released by disrupting the cells with 60% perchloric acid and centrifuging at 400 g and 4°C for 5 min. The histamine content was measured by the *o*-phthalaldehyde spectrofluorometric procedure of Shore *et al.* [18]. The fluorescent intensity was measured at 440 nm (excitation at 360 nm) in a spectrofluorometer. The percentage inhibition of histamine release was calculated using the following equation: % inhibition = (A − B) × 100/A, where A is the histamine release without a sample and B is the histamine release with a sample.

Table 1. Inhibitory effects of seaweed extracts on histamine release

Seaweeds	Inhibition rate (%)
Carpopeltis affinis	22.50
Chondria crassicaulis	–
Cladophora sakaii	–
Codium contractum	–
Colpomenia bullosa	43.14
Colpomenia sinuosa	23.27
Corallina pilulifera	18.20
Dasya sessilis	11.08
Derbesia marina	42.07
Gelidium amansii	12.30
Gracilaria textori	–
Grateloupia elliptica	–
Hypnea japonica	–
Laruencia okamurae-I	12.89
Laruencia okamurae-II	28.98
Lomentaria catenata	2.14
Pachydictyon coriaceum	29.56
Pterocladia capillacea	2.46
Sagassum thunbergii	49.80
Sargassum hemiphyllun	25.13
Sargassum horneri-I	5.34
Sargassum horneri-II	10.63
Sargassum muticum	14.72
Sargassum patens	4.47
Sargassum serratifolium	11.19
Sargassum siliquastrum	–
Sargassum sp.	26.82
Scytosiphon lomentaria	–

Isolated mast cells (1×10^6 cells/mL) were preincubated with 100 μg/mL concentrations of each sample and then incubated for 10 min with compound 48/80. The reaction was stopped by cooling tubes on ice. The cells were centrifuged at 400 × g for 5 min at 4°C after disrupting the cells with perchloric acid. Then, the inhibition percentage of histamine release was then calculated.

A total number of 48 species of marine plants (27 seaweeds; 19 salt marsh plants) were tested on compound 48/80 – induced histamine release from rat peritoneal mast cells (RPMCs). The results for histamine release inhibition from RPMCs was summarized in Table 1, and 2. Three crude extracts of salt marsh plants were found to have high inhibitory effects on histamine release from RPMCs showing more than 75% inhibition compared to the control which was not given any plants extract. They were as follows: *Persicaria lapathifolia* (82.30%), *Sasola komarovii* (76.90%), and *Ixeris tamagawaensis* (75.33%). Some other plants, three salt marsh (*Rosa rugosa, Messerschmidia sibirica,* and *Portulaca oleraceae*) and three seaweeds (*Colpomenia bullosa, Derbesia marina,* and *Sargassum thunbergii*) showed a moderate inhibition, about 40% at crude extracts concentration of 100 μg/mL. Other plant extracts showed very low inhibitory activity.

Table 2. Inhibitory effect of salt marsh plants on histamine release

Salt marsh plants	Inhibition rate (%)
Artemisia capillaris	35.23
Aster spathulifolius	0.01
Calystegia soldanella	22.80
Carex scabrifolia	30.93
Erigeron annus	24.24
Glehnia littoralis	27.56
Imperata cylindrical	3.87
Ixeris tamagawaensis	75.33
Lactuca indica Linne	21.41
Lathyrus japonicus Willdenow	12.06
Limonium tetragonum	12.53
Messerschmidia sibirica	47.12
Persicaria lapathifolia	82.30
Portulaca oleraceae	43.00
Rosa rugosa	43.18
Salsola komarovii	76.90
Suaeda asparagoides	14.72
Suaeda japonica	14.19
Tetragonia tetragonoides	9.40

Isolated mast cells (1×10^6 cells/mL) were preincubated with 100 μg/mL concentrations of each sample and then incubated for 10 min with compound 48/80. The reaction was stopped by cooling tubes on ice. The cells were centrifuged at 400 × g for 5 min at 4 °C after disrupting the cells with perchlolic acid. The inhibition percentage of histamine release was then calculated.

The some marine plants with histamine inhibitory effect have also been reported significant antioxidative activities on stable free radical DPPH and peroxynitrite by previous our results [19-23]. It has been reported that mast cells generate intracellular reactive oxygen species (ROS) in responses to antigen challenge, and the ROS is involved in histamine release [24]. Moreover, it has also been reported that naturally occurring polyphenolic antioxidants reduce ROS levels in antigen-Ig E activated mast cells and concomitantly inhibit histamine release from the activated mast cells [25]. Thus, although it is not clear what the inhibitory mechanism on compound 48/80 induced histamine release by these marine plants is, it may be supposed to be due to the antioxidant activity of the marine plant extracts.

The search for antihistamines began in the 1930s, and a variety of compounds based on the ethylenediamine structure were found to partially block the effects of histamine [26]. Further study for the isolation and purification of active components in these potent extracts should be followed.

In summary, some of the Korean marine plants had significant effects on mast cell degranulation due to the inhibiting of histamine release in RPMCs and are expected to provide an insight for the discovery of new drugs for treating allergic disease involving mast cells.

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