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Original article

Agarwood Inhibits Histamine Release from Rat Mast Cells and Reduces Scratching Behavior in Mice

-Effect of Agarwood on Histamine Release and Scratching Behavior-

Eiji Inoue*, Yasuharu Shimizu, Ryo Masui, Tomoe Tsubonoya, Tomomi Hayakawa, Keiichi Sudoh

Tokyo Research Center, Kyushin Pharmaceutical Co, Ltd, Tokyo, Japan

Key Words

agarwood, histamine release, mast cells, scratching behavior

Abstract

Objectives: This study was conducted to clarify the effects of agarwood on histamine release from mast cells in rats and on the scratching behaviors in mice.

Methods: Histamine release from rat mast cells induced by compound 48/80 or concanavalin A (Con A) and compound 48/80-induced scratching behavior in mice were examined to investigate the effects of agarwood. The hyaluronidase activity and the 3',5'-cyclic adenosine monophosphate (cAMP) levels in mast cells were examined to investigate the mechanisms for the inhibition of histamine release. The correlation between the inhibitory effects of agarwood on histamine release and the content of its typical ingredients, a 2-(2-phenylethyl)chromone derivatives, was analyzed using thin-layer chromatography.

Results: Agarwood showed an inhibitory effect on mast-cell histamine release induced by compound 48/80 or Con A without any effect on hyaluronidase activity; this effect involves an increase in the cAMP levels in mast cells. Oral administration of agarwood showed an inhibitory effect on compound 48/80-induced scratching behavior in mice. The inhibitory effects of agarwood on histamine release were quite different, depending on the area where the agarwood was produced, its quality, and its market price. No correlation was found between the inhibitory effects of agarwood on histamine release and the typical ingredients of agarwood, which are 2-(2-phenylethyl)chromone derivatives.

Conclusion: These results show that agarwood inhibits its histamine release from mast cells partially through an increase in the cAMP levels in cells. We suggest that some active ingredients of agarwood must be effective on oral intake and that agarwood can be used to treat patients with a number of conditions, including urticaria, atopic dermatitis, and bronchial asthma, in which an increase in histamine release occurs. Differences in the pharmacological effects of this crude drug among markets may provide important information for the quality control of this herbal medicine.

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1. Introduction

Mast cells participate in allergies and inflammation by releasing a variety of chemical mediators, such as histamines, leukotrienes, and platelet-activating factors. Activation of mast cells occurs in immunoglobulin E (IgE)–mediated (called Type I allergy reactions or anaphylaxis) reactions and in non-IgE-mediated (called anaphylactoid) reactions [1]. Histamine is the principal mediator and causes smooth muscle con-

***Corresponding Author**
Eiji Inoue. Tokyo Research Center, Kyushin Pharmaceutical Co, Ltd. 1-22-10, Wada, Suginami-ku, Tokyo 166-0012, Japan.
Tel: +81-3-5385-3211 Fax: +81-3-5385-3219
E-mail: e-inoue@kyushin.co.jp

tractions, increased vascular permeability, and increased airway mucus secretion [2]. As a result, increased levels of histamine have been observed in patients with a number of conditions/diseases, including bronchial asthma, hay fever, allergic rhinitis, allergic conjunctivitis, urticaria, atopic dermatitis, and anaphylactic shock [2].

Agarwood is fragrant wood and has been used in Oriental medicine as a sedative and an analgesic, as well as for its antiasthma effect. We have previously reported that agarwood shows bronchodilating effects in the trachea of guinea pigs, and this effect is thought to be involved in the antiasthma effects of this drug [3]. Because asthma is strongly linked to histamine, we examined the effect of agarwood on histamine release from rat mast cells. The ingredients in agarwood are well known to be quite different between markets, particularly between different producing areas [4]. Therefore, we examined the correlation between the inhibitory effects of agarwood on histamine release and the content of its typical ingredients, a 2-(2-phenylethyl) chromone derivatives.

2. Materials and Methods

Eight-week-old male Wistar rats and 6-week-old male ddY mice were obtained from Japan Laboratory Animals (Tokyo) and Japan SLC (Shizuoka), respectively. The experimental protocol complied with the laws and notifications of the Japanese government. Prior to commencement of the study, the design was approved by the Animal Care and Use Committee of Kyushin Pharmaceutical Co, Ltd. In this study, significant differences in data were calculated using a one-way analysis of variance (ANOVA), followed by Dunnett's multiple range test. The inhibitory concentration $(IC)_{50}$ value was calculated using a probit analysis.

Agarwood was obtained from anonymous suppliers (M, K, W) (Table 1) and was pulverized using a tablet grinder (Konishi Seisakusho, Nagoya, Japan). For *in vitro* studies, this drug was suspended in Tyrode's solution (137.9-mM NaCl, 2.7-mM KCl, 1.8-mM $CaCl_2$, 0.5-mM $MgCl_2$, 1.1-mM Na_2HPO_4, 5.6-mM D-glucose, and 11.9-mM $NaHCO_3$) or distilled water and was then extracted using an ultrasonic generator (Model 2510, Branson, CT, USA) for 30 minutes. The extract was centrifuged (3,000 rpm, 5 minutes), and the supernatant was used for tests. For *in vivo* studies, the drug was suspended in distilled water and administered orally using an esophageal tube at a volume of 10 mL/kg. Agarotetrol was kindly provided by Prof. Dr. F Kiuchi and

Table 1 Sample list of agarwood

Sample No	Supplier	Producing area	Lot No.	Market price (yen/g)
1	M	Indonesia	87199	70
2	M	Indonesia	87203	36
3	M	Indonesia	W04603	112
4	M	Indonesia	87253	135
5	M	Indonesia	W04602	154
6	M	Indonesia	87252	174
7	M	Indonesia	W04601	269
8	M	Indonesia	87251	283
9	M	Indonesia	A237	—
10	K	Vietnam	S09092991	—
11	K	Indonesia (Sumatra)	09091481①	—
12	K	Indonesia (Kalimantan)	—	185
13	K	Indonesia	3092609	185
14	K	Indonesia (Ambon)	—	295
15	K	Indonesia	3092616	295
16	K	Indonesia (Sumatra [Lampung])	—	—
17	K	Indonesia	04120723	—
18	W	Vietnam	—	117

M, K, and W refer to three different randomly chosen suppliers.

Asst. T Sugiyama (Division of Natural Medicines, Faculty of Pharmacy, Keio University, Japan).

Compound 48/80, concanavalin A (Con A), hyaluronidase, and *o*-phthalaldehyde were purchased from Sigma-Aldrich (St. Louis, MO, USA). Sodium cromoglycate, diphenhydramine hydrochloride, *p*-dimethylaminobenzaldehyde (*p*-DAB), potassium hyaluronate, and *L*-α-phosphatidyl-*L*-serine were purchased from Wako Pure Chemical Industries (Osaka, Japan). The 3′,5′-cyclic adenosine monophosphate (cAMP) CLIA kit was purchased from Arbor Assays (Ann Arbor, MI, USA).

The histamine release from rat mast cells induced by compound 48/80 or Con A was studied using the method described by Saito *et al* [5], with slight modifications. Rats were euthanized by using cervical dislocation. The peritoneal cavity was opened and injected with 25 mL of Tyrode's solution. The abdomen was gently massaged, and the fluid was then collected. The cavity was injected with 5 mL of Tyrode's solution 3 times, and the fluid was collected. The fluid was centrifuged (1,500 rpm, 3 minutes, 4°C), and cell pellet (rat peritoneal mast cells) was re-suspended at 1×10^6 cells/mL in Tyrode's solution.

Rat mast cell suspensions (0.9 mL, 1×10^6 cells/mL) were pre-incubated with 50 μL of test drug for 10 minutes at 37°C and then incubated with 50 μL of 1-μg/mL compound 48/80 or 25 μL of 4-mg/mL Con A and 25 μL of 400-μg/mL *L*-α-phosphatidyl-*L*-serine for 10 minutes at 37°C. The reaction was stopped by cooling the reacting mixture on ice. Part of the mixture (0.25 mL) was moved to another tube, the remainder was centrifuged (1,500 rpm, 3 minutes, 4°C), and 0.25 mL of the supernatant was placed in another tube. The histamine contents in these tubes were measured by using the fluorometric method described by Shore *et al* [6]. The percent histamine release and the percent inhibition of histamine release were calculated using the following formulae:

- % histamine release = 100 × (histamine content of the supernatant/histamine content of the reaction mixture),
- % inhibition of histamine release = 100 × [1 – (% histamine release with drug/% histamine release without drug)].

For the analysis of 2-(2-phenylethyl)chromone derivatives by using thin-layer chromatography, agarwood (0.1 g/20 mL) was suspended in Tyrode's solution and extracted using an ultrasonic generator for 30 minutes. The extract was centrifuged (3,000 rpm, 5 minutes), and the supernatant was evaporated under reduced pressure. The residue was re-suspended in 0.5 mL of methanol, and the fluid was centrifuged (3,000 rpm, 5 minutes). The supernatant was used as a sample solution. Agarotetrol (0.1 mg/mL) was dissolved in methanol as a standard solution. In accordance with the method described by the Japanese Pharmacopoeia Sixteenth Edition [7], 20 μL each of the sample and the standard solutions were spotted on a silica gel plate for thin-layer chromatography (TLC) (Silicagel 60 F254, Merck, NJ, USA). After the plate had been dried, it was developed with a mixture of chloroform/methanol/water (40:10:1) to a distance of approximately 10 cm from the original spot. Detection was conducted by using irradiation with ultraviolet light (main wavelength: 254 nm).

The effect on hyaluronidase activity was studied using the method described by Kubo *et al* [8] and by Maeda *et al* [9], with some modifications. Hyaluronidase (0.05 mL, 2,200 unit/mL) in a 0.1-M acetate buffer (pH 4.0) was incubated with 0.1 mL of test drug for 20 minutes at 37°C; then, 0.1 mL of 0.5-mg/mL compound 48/80 was added, and the mixture was incubated for 20 minutes at 37°C. Potassium hyaluronate (0.25 mL, 0.8 mg/mL) was then added to the mixture and incubated for 40 minutes at 37°C. The reaction was stopped by adding 0.1 mL of 0.4-M NaOH and cooling on ice. A boric acid solution (0.1 mL, 0.8-M boric acid adjusted to pH 9.1 with 1-M NaOH) was added to the reaction mixture, boiled for 3 minutes in a water bath, and then cooled on ice. *p*-DAB reagent (3 mL, 0.67-M *p*-DAB in a mixture of 10-M HCl/CH_3COOH (1:4) diluted 10 times with CH_3COOH) was added to the reaction mixture and incubated for 20 minutes at 37°C. The mixture (0.25 mL) was added to a 96-well microplate, and the absorbance of the mixture was measured at 585 nm by using a microplate reader (Synergy H1, Biotek, VT, USA). The percent inhibition of hyaluronidase activity was calculated using the following formula:

- % inhibition of hyaluronidase activity = 100 × [1 – (C – D)/(A – B)], where A is the absorbance without the drug, but with the enzyme; B is the absorbance with neither the drug nor the enzyme; C is the absorbance with both the drug and the enzyme; D is the absorbance with the drug, but without the enzyme.

The effect on the cAMP levels of rat mast cells was studied by using the method described by Dai *et al* [10], with some modifications. The rat mast-cell suspensions (50 μL, 4×10^6 cells/mL) were pre-incubated for 5 minutes at 37°C and then incubated with 50 μL of test drug at 37°C. After a fixed time interval, the reaction was stopped by adding ice-cold acid ethanol (0.9 mL, 86% ethanol in 0.01-M HCl). The sample was then snap-frozen in liquid nitrogen and thawed repeatedly, four times, to destroy the cell membrane. The debris was centrifuged (400 × g, 5 minutes, 4°C), and 0.9 mL of the supernatant was transferred and evaporated to dryness under a gentle stream of nitrogen. The dried sample was reconstituted in 150 μL of the assay buffer. The cAMP levels were measured by using the cAMP CLIA kit to perform an enzyme immunoassay.

The effect on compound 48/80-induced scratching behavior in mice was studied using the method described by Kubo *et al* [11] and by Jeon *et al* [12], with some modifications. Test drugs were orally administered to mice that had been deprived of food for 18 hours. One hour later, compound 48/80 (100 μg/0.1 mL saline) was intradermally injected into the back of the mice. Immediately after the injection, the number of scratches around the injection site with the hind paw in ten minutes was determined.

3. Results

Agarwood (Tyrode's solution extract, Sample No. 1) dose-dependently inhibited compound 48/80- or Con A-induced histamine release in the mast cells, with an IC_{50} value of 1.13 or 8.82 μg/mL, respectively. On the other hand, the inhibition of compound 48/80-induced histamine release caused by ethanol-extracted agarwood was

quite weak compared to that of the Tyrode's-solution-extracted agarwood. Agarotetrol (100 μM; 31.9 μg/mL), which is a 2-(2-phenylethyl)chromone derivative and a typical ingredient of agarwood, did not show a significant inhibitory effect on histamine release (Fig. 1). The inhibitory effects (IC_{50} values) of 18 kinds of agarwood were found to be vastly different (Table 2).

To clarify the active ingredients of agarwood, we examined the TLC patterns of 2-(2-phenylethyl)chromone derivatives of several kinds of agarwood that inhibited histamine release. Agarotetrol was detected in most agarwood preparations extracted with Tyrode's solution, but in sample No. 10, it was observed as a thin spot, and in sample No. 11, no spot was detected. The TLC patterns of samples No. 10 and No. 11 were quite different from those of the other samples, and no correlations between the TLC patterns of the 2-(2-phenylethyl)chromone derivatives and the efficacy of inhibition of histamine release were found (Fig. 2).

In other experiments, the hyaluronidase activity of several kinds of agarwood that inhibited histamine release were examined. Agarwood (distilled water extract) did not show more than a 50% inhibitory effect on hyaluronidase activity. The IC_{50} value of sodium cromoglycate was found to be 157 μM (Fig. 3). Furthermore, agarwood (Tyrode's solution extract, Sample No. 1) significantly increased cAMP levels in mast cells 10 and 60 s after incubation (Fig. 4). Moreover, agarwood (oral administration, Sample No. 1) dose-dependently inhibited compound 48/80-induced scratching behavior in mice, and the effect was significant at 100 mg/kg; diphenhydramine (oral administration) at 50 mg/kg also significantly inhibited compound 48/80-induced scratching behavior in mice (Fig. 5).

Table 2 Effect of agarwood on compound 48/80-induced histamine release from rat mast cells

Sample No	IC_{50} (μg/mL)
1	1.13
2	> 100
3	3.28
4	36.1
5	1.26
6	> 100
7	11.3
8	44.4
9	18.3
10	8.36
11	68.9
12	132
13	28.1
14	20.8
15	24.8
16	36.4
17	36.7
18	4.74

IC_{50}, 50% inhibitory concentration.

4. Discussion

This study was conducted to clarify the effects of agarwood on histamine release from rat mast cells. Agarwood (Tyrode's solution extract) showed inhibitory effects on compound 48/80- and Con A-induced histamine release in rat mast cells. Kim *et al* [13] also reported that the water extract of agarwood showed inhibitory effects on compound 48/80-induced histamine release in such cells. Compound 48/80 is known to induce non-IgE-mediated histamine release [14]. Furthermore, as Con A is known to induce IgE-mediated histamine release by cross linkage of the IgE receptor, this reaction is thought to be based on an antigen-antibody reaction [15]. These results indicate that agarwood inhibits both non-IgE and IgE-mediated histamine release from rat mast cells.

Because agarwood is known to have quite different ingredients depending on characteristics such as the producing area [4], the inhibitory effects of 18 kinds of agarwood on compound 48/80-induced histamine release were examined. No correlations were found between the inhibitory effects of agarwood on histamine release and the producing area, its quality, or its market price. Furthermore, agarotetrol hardly inhibited histamine release, and no correlations between the TLC patterns of 2-(2-phenylethyl) chromone derivatives and the efficacy of histamine release inhibition were found. These findings suggest that no single ingredient of agarwood is involved in the inhibition of histamine release. On the other hand, the inhibitory effect of ethanol-extracted agarwood on histamine release was quite weaker compared to that of Tyrode's-solution-extracted agarwood. These findings suggest that highly polar molecules of agarwood are involved in the inhibition of histamine release.

Agarwood increased the cAMP levels in rat mast cells. An increase in the cAMP levels in such cells is believed to precede the inhibition of histamine release by suppressing the influx of Ca^{2+} from extracellular Ca^{2+} and endoplasmic reticulum Ca^{2+} stores [16]. We suggest that this increase in the cAMP levels is partly involved in the inhibition of histamine release. In addition, agarwood showed no inhibitory effect on hyaluronidase activity. The inhibition of hyaluronidase activity has often been regarded as one of the mechanisms for the inhibition of histamine release and has been observed in anti-allergic agents such as sodium cromoglycate and tranilast [17]. Our results and these re-

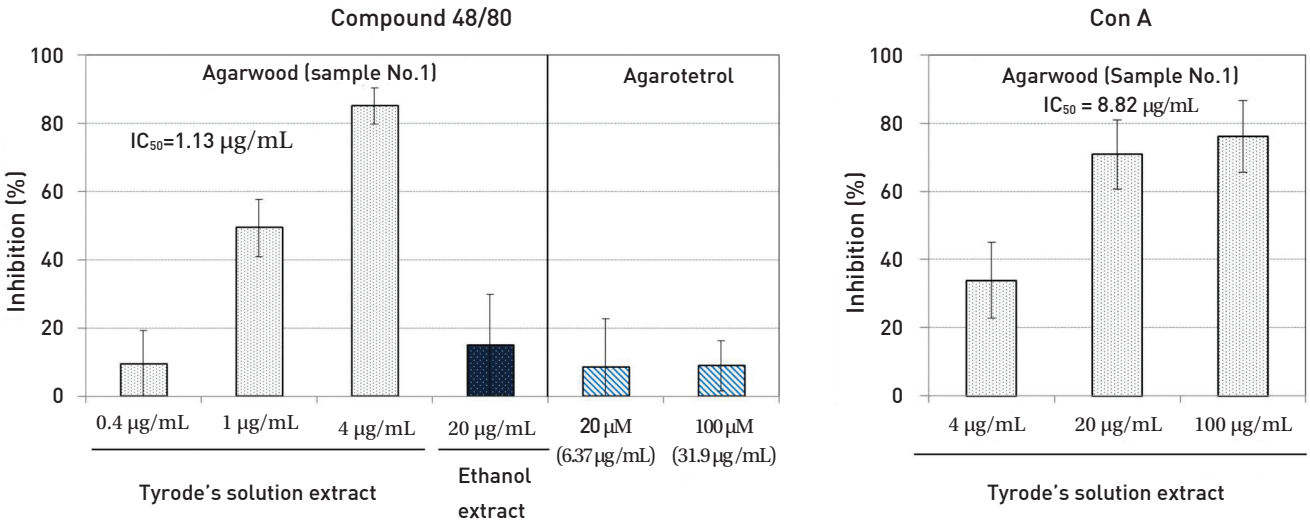

Figure 1 Effects of agarwood (Sample No. 1) and agarotetrol on compound 48/80- or Con A- induced histamine release from rat mast cells. The data are expressed as means ± SEs. (n = 3 − 6).

Con A, concanavalin A; SEs, standard errors.

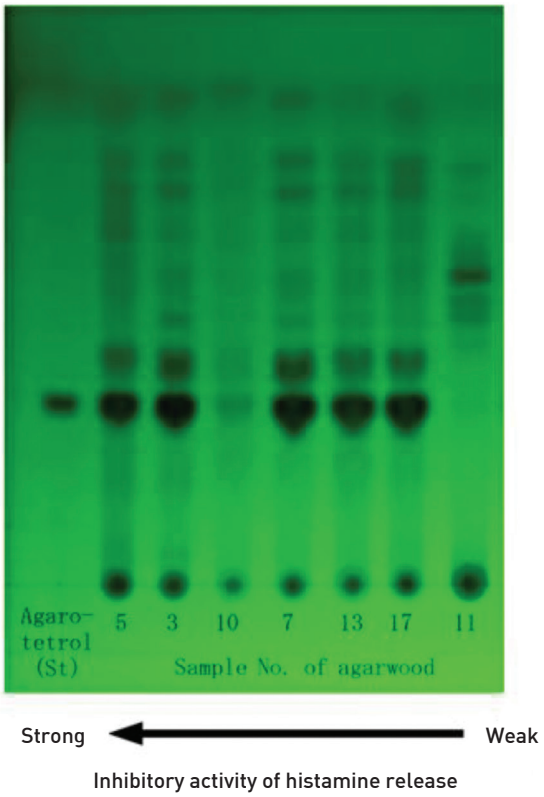

Figure 2 TLC comparison of 2-(2-phenylethyl)chromone derivatives for several kinds of agarwood.
(Solvent system: chloroform/methanol/water (40 : 10 : 1); detection: UV 254-nm inhibitory effect on histamine release (IC_{50}, μg/mL); Samples: No. 5 (1.26), No. 3 (3.28), No. 10 (8.36), No. 7 (11.3), No. 13 (28.1), No. 17 (36.7), and No. 11 (68.9).

TLC, thin-layer chromatography; UV, ultraviolet; IC, inhibitory concentration.

ports suggest that the inhibitory mechanism of histamine release due to agarwood is different from those of sodium cromoglycate and tranilast.

Oral administration of agarwood showed inhibitory effects on compound 48/80-induced scratching behavior in mice. Compound 48/80 is well known to cause scratching behavior due to histamine release [11]. Although the histamine levels in the mice were not measured in this study, the results of this study suggest that oral intake of agarwood probably lessens the scratching behavior in mice by inhibiting histamine release.

In summary, agarwood showed an inhibitory effect on histamine release in rat mast cells, and data suggest that an increase in the cAMP levels is partly involved in this inhibitory effect. Because oral administration of agarwood showed an inhibitory effect on compound 48/80-induced scratching behavior in mice, some active ingredients of this drug must be effective on oral intake. These results suggest that agarwood can be used to treat patients with a number of conditions/diseases, including urticaria, atopic dermatitis, and bronchial asthma, in which an increased release of histamine occurs. Finally, no correlations were found between the inhibitory effects of histamine release due to agarwood and its producing area, quality, or market price; therefore, more detailed studies on the ingredients of agarwood are needed if its quality as a herbal medicine is to be controlled.

5. Conclusion

This study was conducted to clarify the effects of agarwood on histamine release from rat mast cells. Agarwood was found to inhibit histamine release from rat mast cells partially through an increase in the cAMP levels in those cells. We suggest that some active ingredients of agarwood must be effective on oral intake and that agarwood can

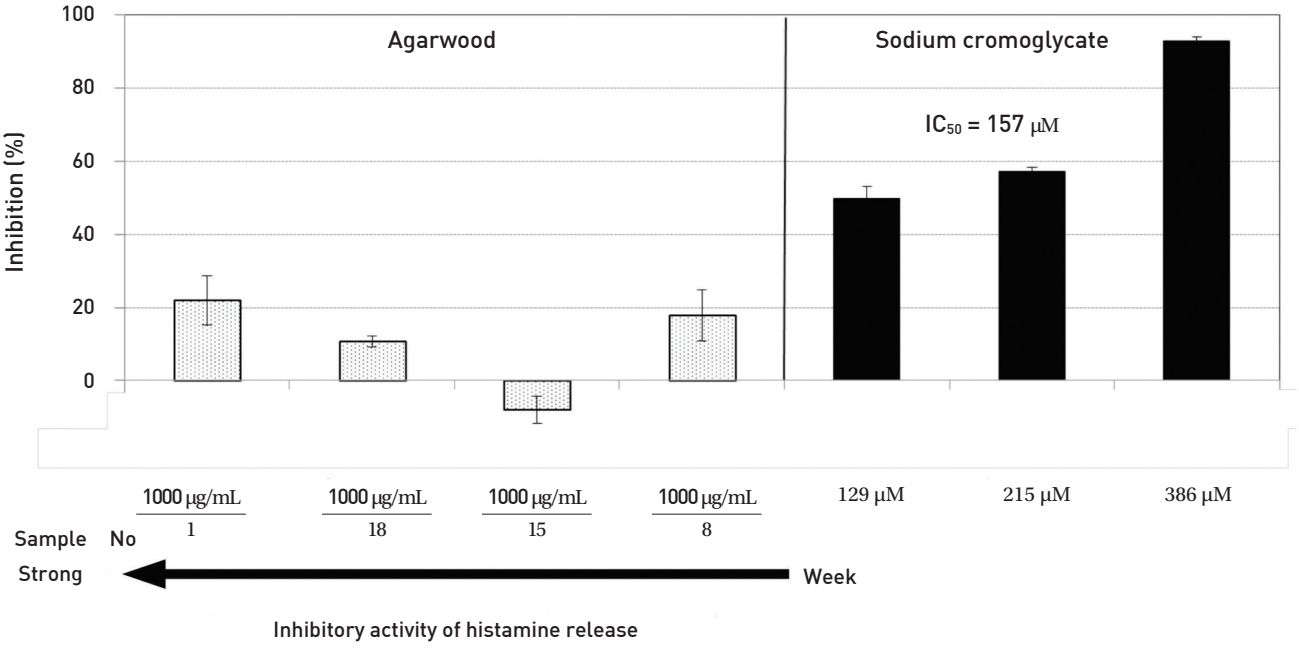

Figure 3 Effects of agarwood and sodium cromoglycate on hyaluronidase activity. The data are expressed as means ± SEs (n = 3 except for sodium cromoglycate 215 μM for which n = 6). The inhibitory effects on histamine release (IC_{50}, μg/mL) are shown for samples No. 1 (1.13), No. 18 (4.74), No. 15 (24.8), and No. 8 (44.4).

SEs, standard errors; IC, inhibitory concentration.

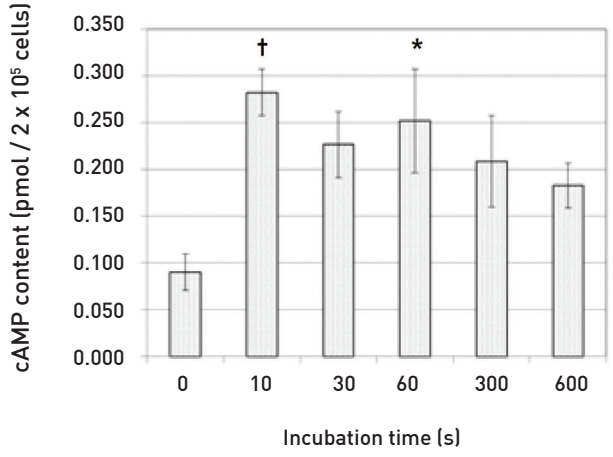

Figure 4 Effect of agarwood (Sample No. 1) on the cAMP levels in rat mast cells. Rat mast cells (2×10^6 cells/mL) were pretreated with agarwood (100 μg/mL). The data are expressed as means ± SEs (n = 5). $^{\dagger}P < 0.01$ and $^{*}P < 0.05$ indicate statistically significant differences from the values at zero incubation time, as determined by using Dunnett's test.

cAMP, 3',5'-cyclic adenosine monophosphate; SEs, standard errors.

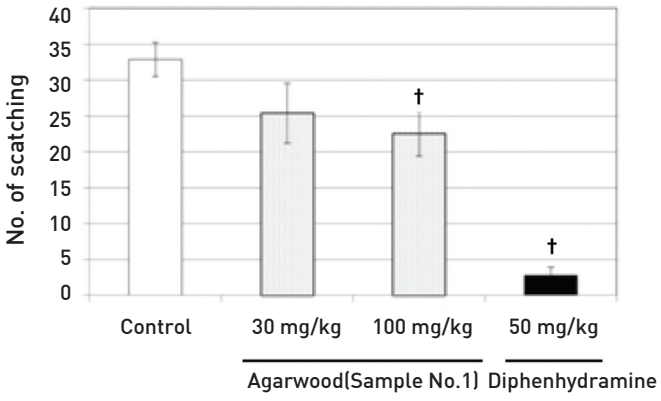

Figure 5 Effects of agarwood (Sample No. 1) and diphenhydramine on compound 48/80-induced scratching behavior in mice. The data are expressed as means ± SEs (n = 10 except for diphenhydramine 50 mg/kg for which n = 4 and agarwood 30 mg/kg for which n = 5). $^{\dagger}P < 0.01$ as compared with the control group by using Dunnett's test.

SEs, standard errors.

be used to treat patients with a number of conditions, including hives, atopic dermatitis, and bronchial asthma, in which an increase in histamine occurs.

Acknowledgment

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Conflict of interest

The authors declare that there are no conflict of interest.

References

1. Luskin AT, Luskin SS. Anaphylaxis and anaphylactoid reactions: diagnosis and management. Am J Ther. 1996;3(7):515-20.
2. White MV. The role of histamine in allergic diseases. J Allergy Clin Immunol. 1990;86(4):599-605.
3. Noguchi T, Shimizu Y, Sudoh K. [Basic pharmacological studies of Agarwood for its medicinal application for asthma]. The 129th Annual Meeting of the Pharmaceutical Society of Japan. 2009;abstracts papers 2:164. Japanese.
4. Yoneda K, Yamagata E, Sugimoto Y, Nakanishi T. [Pharmacognostical studies on the crude drug of "agarwood" (I): comparison of the constituents of the essential oil from agarwood by means of GLC and GC-MS]. Shoyakugaku Zasshi. 1986;40(3):252-8. Japanese.
5. Saito SY, Tanaka M, Matsunaga K, Li Y, Ohizumi Y. The combination of rat mast cell and rabbit aortic smooth muscle is the simple bioassay for the screening of anti-allergic ingredient from methanolic extract of corydalis tuber. Biol Pharm Bull. 2004;27(8):1270-4.
6. Shore PA, Burkhalter A, Cohn VH Jr. A method for the fluorometric assay of histamine in tissues. J Pharmacol Exp Ther. 1959;127:182-6.
7. The Japanese Pharmacopoeia sixteenth Edition. 2011. Japanese.
8. Kubo Y, Shozen K, Seto Y. [Hyaluronidase inhibitory effect in diatom extracts isolated from deep sea water]. Deep Ocean Water Research. 2002;3(2):71-6. Japanese.
9. Maeda Y, Yamamoto M, Masui T, Sugiyama K, Yokota M, Nakagomi K, *et al*. [Inhibitory effect of tea extracts on hyaluronidase. (studies on anti-allergic activity in tea. 2)]. J Food Hyg Soc Jpn. 1990;31(3):233-7. Japanese.
10. Dai Y, Hou LF, Chan YP, Cheng L, But PP. Inhibition of immediate allergic reactions by ethanol extract from *Plumbago zeylanica* stems. Biol Pharm Bull. 2004;27(3):429-32.
11. Kubo M, Matsuda H, Dai Y, Ido Y, Yoshikawa M. [Studies on kochiae fructus. I. antipruritogenic effect of 70% ethanol extract from kochiae fructus and its active component]. Yakugaku Zasshi. 1997;117 (4):193-201. Japanese.
12. Jeon IH, Kim HS, Kang HJ, Lee HS, Jeong SI, Kim SJ, *et al*. Anti-inflammatory and antipruritic effects of luteolin from Perilla *(P. frutescens L.)* leaves. Molecules. 2014;19(6):6941-51.
13. Kim YC, Lee EH, Lee YM, Kim HK, Song BK, Lee EJ, *et al*. Effect of the aqueous extract of *Aquilaria agallocha* stems on the immediate hypersensitivity reactions. J Ethnopharmacol. 1997;58(1):31-8.
14. Allansmith MR, Baird RS, Ross RS, Barney NP, Bloch KJ. Ocula anaphylaxis induced in the rat by topical applacation of compound 48/80. Acta Ophthalmol Suppl. 1989;192:145-53.
15. Windelborg Nielsen B, Hansen B, Damsgaard TM, Herlin T, Soderberg U, Bjerke T, *et al*. Basophil histamine release induced by anti-IgE and concanavalin A. relation to the total plasma IgE content. Allergy. 1993;48(1):54-61.
16. Weston MC, Peachell PT. Regulation of human mast cell and basophil function by cAMP. Gen Pharmacol. 1998;31(5):715-9.
17. Kakegawa H, Matsumoto H, Satoh T. Activation of hyaluronidase by metallic salts and compound 48/80, and inhibitory effect of anti-allergic agents on hyaluronidase. Chem Pharm Bull. 1985;33(2):642-6.