Inhibitory Effects of Aloe Extracts on Antigen- and Compound 48/80-Induced Histamine Release from Rat Peritoneal Mast Cells

MASATOSHI YAMAMOTO,^a KIYOSHI SUGIYAMA,^a MASAMI YOKOTA,^a YUMIE MAEDA,^b
KAZUYA NAKAGOMI^c and HIROYUKI NAKAZAWA^d

School of Pharmaceutical Sciences, University of Shizuoka,^a 52-1 Yada, Shizuoka 422,
Japan, Shizuoka Prefectural Institute of Public Health and Environmental Science,^b

4-27-2 Kita-ando, Shizuoka 420, Japan, Fermentation Research

Institute,^c 1-1-3 Higashi, Tsukuba, Ibaraki 305, Japan and

The National Institute of Public Health,^d

4-6-1 Shirokanedai, Minato-ku,

Tokyo, 108, Japan

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The inhibitory effects of water extracts of five genera of aloe (*Aloe arborescens* MILLER, *A. ferox* M., *A. barbadensis* M., *A. africana* M., *A. saponaria* HAWAII) and aloe powder of Japanese Pharmacopoeia (JP Aloe powder) on histamine release from rat peritoneal mast cells induced by antigen and compound 48/80 were investigated *in vitro*.

All the aloe extracts tested inhibited histamine release from mast cells induced by antigen and compound 48/80 in a concentration-dependent manner. The extract of *A. ferox* M. and JP Aloe powder particularly strongly inhibited release by either antigen or compound 48/80 at medium inhibitory concentration (IC_{50}) of 0.16 and 0.07 mg/ml, respectively, for antigen and of 0.41 and 0.29 mg/ml for compound 48/80. These results suggest that aloes may be useful in the treatment of type I immediate allergic disorders.

Barbaloin, a major constituent of aloes, also exhibited concentration-dependent inhibitory effects on histamine release induced by these two substances, with IC_{50} being 0.02 and 0.06 mg/ml, respectively.

From its yield and inhibitory effects, barbaloin is considered to account for the inhibitory action of the water extracts of *A. arborescens* M., *A. africana* M. and JP Aloe powder. Although tranilast, a current antiallergic agent, strongly inhibited antigen-induced histamine release ($IC_{50}=0.07$ mg/ml), it showed a weak inhibitory effect on compound 48/80-induced histamine release ($IC_{50}=0.84$ mg/ml). These results suggest that the mechanism of inhibitory action of barbaloin on histamine release differs from that of tranilast.

Keywords — aloe ; barbaloin ; histamine release from mast cell ; IgE ; compound 48/80 ; antiallergic action

Introduction

Plants of the aloe species have been utilized for constipation, common cold, asthma, burns and insect bites, and more recently also in cosmetics and health foods.¹⁻³⁾ Extensive study has demonstrated that aloes have vari-

ous biological activities: antitumor activity, antimicrobial activity, anti-inflammatory activity and inhibitory activity of gastric juice secretion, in addition to being a cathartic in animal experiments.⁴⁾

We previously showed that *Aloe arborescens* MILLER (Kidachi-aroe in Japanese)

strongly inhibits carrageenan-induced rat-paw inflammatory edema, and that one of the effective constituents of the aloe is barbaloin, a major component.⁵⁾ We also found that barbaloin had an inhibitory effect on histamine release from rat peritoneal mast cells induced by compound 48/80.⁶⁾

Among plants of the aloe species, *A. arborescens* M. has most commonly been utilized as a folk remedy in Japan, however, many species have been used throughout the world as folk remedies for purposes similar to that for which *A. arborescens* M. is used. In this study, we investigated the inhibitory effects of five kinds of aloe (*A. arborescens* M., *A. ferox* M., *A. barbadensis* M., *A. africana* M., *A. saponaria* HAWAII) and aloe powder of Japanese Pharmacopoeia (JP Aloe powder) on histamine release from rat peritoneal mast cells. The role of barbaloin in these inhibitory actions was also examined.

Experimental

Animals — Seven-week-old male Wistar rats (mean weight, 150 g) were obtained from Japan SLC, Inc., Hamamatsu, Japan, and kept in rooms with a controlled temperature ($23 \pm 1^\circ\text{C}$), humidity ($55 \pm 15\%$), and 12 h dark-light cycle. They were fed on commercial mouse chow (MF; Oriental Yeast Co., Ltd., Tokyo) for one week, and were then used in the experiments.

Materials — Dried leaves of the five kinds of aloes and the JP Aloe powder were obtained from Aroe Seiyaku Co., Ltd., Shizuoka. Barbaloin, shown in Fig. 1, was isolated from *A. arborescens* M., and identified by comparing the physicochemical data (mass spectra, nuclear magnetic resonance and infrared spectra) with those of a standard substance (obtained from Wako Pure Chemical Industries, Ltd., Tokyo).

Aloe Extract Preparation — Dried leaves of aloes and JP Aloe powder were powdered finely and extracted with water at room temperature for 24 h. After centrifuging the extracts at $1500 \times g$ for 10 min, aloe extracts were obtained by lyophilizing the supernatants.

$\text{C}_{21}\text{H}_{22}\text{O}_9$ MW : 418.39

Barbaloin

Fig. 1. Chemical Structure of Barbaloin

Assay of Histamine Release — The preparation of mast cells and the assay of histamine release from mast cells were modifications of the method of Hirai *et al.*⁷⁾ The mast cells were collected from the rat peritoneal cavity after injection of 30 ml of phosphate buffered saline (PBS), and centrifuged at $150 \times g$ for 10 min. The cells were purified by centrifugation at $100 \times g$ for 15 min on a solution of bovine serum albumin (BSA, $d = 1.068$). The purity of the mast cells was 80–90% as observed by a phase contrast microscope.

Twenty microliters of the cell suspension (10^6 cells/ml in 0.1% BSA-PBS) was added to 20 μl of aloe extract or barbaloin solution, which was incubated at 37°C for 10 min, followed by 10 min incubation with an inducer of histamine release. Then, 0.08 $\mu\text{l}/\text{ml}$ of dinitrophenyl (DNP)-BSA + 10 $\mu\text{g}/\text{ml}$ of phosphatidylserine (PS, Sigma Chemical Co., St. Louis, MO, U.S.A.), or 1 $\mu\text{g}/\text{ml}$ of compound 48/80 (Sigma) was used as the inducer of histamine release. When the antigen was used, mast cells were prepared by incubation with anti-DNP mouse monoclonal IgE (Seikagaku Kogyo Co., Ltd., Tokyo) at 37°C for 1 h before the assay. After the incubation, the cells were cooled on ice and centrifuged at $1500 \times g$ for 5 min. Histamine in the supernatant was determined by high performance liquid chromatography. The amounts of histamine released were expressed as values relative to that released from 0.05% Triton X-100-treated cells, which corresponds to 16.6 ± 0.8 nmol of released histamine per 2×10^4 cells in

a tube (mean $\pm$ S.E., $n = 6$).

The inhibitory effect of the test samples was calculated by the following equation: Inhibition (%) = $\{1 - (SR - C)/(R - C)\} \times 100$, where C is the value of the histamine released of the control without sample and inducer, and SR and R are the value of the histamine released by the inducer with and without sample, respectively.

Quantitative Analysis of Barbaloin — The content of barbaloin in aloe extracts was determined according to the previously outlined procedure.^{2,8)}

Results

1. Inhibitory Effects of Aloes on Histamine Release

The inhibitory effects of various aloes on histamine release from mast cells sensitized by IgE antibody are shown in Fig. 2. All the species used inhibited release in a concentration-dependent manner. Inhibition by JP Aloe powder, *A. ferox* M. and *A. barbadensis* M. was, respectively, 7.6, 3.3 and 3.8 times as strong as that by *A. arborescens* M. (medium inhibitory concentration, $IC_{50} = 0.53$ mg/ml). JP Aloe powder showed the strongest inhibitory effect on histamine release, and its IC_{50} value was the same as that of

Fig. 2. Inhibitory Effects of Aloe Extracts on Histamine Release from Rat Mast Cells Induced by Antigen

● *A. arborescens* M., ◆ *A. ferox* M.
▲ *A. barbadensis* M., × *A. africana* M.
▼ *A. saponaria* H., ■ JP Aloe powder.

Mast cells sensitized with IgE were first incubated with test samples at 37°C for 5 min, and then incubated with antigen (0.08 μ g/ml) + PS (10 μ g/ml) for 10 min. Anti-DNP mouse monoclonal IgE as antibody and DNP-BSA as antigen were used. Each point indicates the mean of 3 determinations. The values of histamine release from none- and only antigen-treated cells were, respectively, $2.8 \pm 0.4\%$ and $39.4 \pm 2.3\%$ of the value of histamine released from 0.05% Triton X-100-treated cells.

tranilast, a current antiallergic agent (Table I).

The inhibitory effects of various aloes on compound 48/80-induced histamine release showed almost the same concentration-

TABLE I. Inhibitory Effects of Aloe Extracts on Antigen- and Compound 48/80-Induced Histamine Release

Sample	Barbaloin content (%) ¹⁾	Inhibitory effect			
		Antigen		Compound 48/80	
		IC_{50} ²⁾ (mg/ml)	Contribution ³⁾ (%)	IC_{50} ²⁾ (mg/ml)	Contribution ³⁾ (%)
<i>A. arborescens</i> M.	2.3	0.53	59.8	1.52	54.7
<i>A. ferox</i> M.	0.4	0.16	3.2	0.41	2.7
<i>A. africana</i> M.	1.3	1.00	68.1	2.42	49.7
<i>A. saponaria</i> Haw.	N.D. ⁴⁾	>1.00	—	>3.00	—
<i>A. barbadensis</i> M.	1.7	0.14	12.0	0.92	24.9
JP Aloe powder	17.6	0.07	61.5	0.29	80.9
Barbaloin		0.02		0.06	
Tranilast		0.07		0.84	

1) Barbaloin in water extracts of Aloes was measured by HPLC.

2) Calculated from concentration-response curves.

3) Contribution of barbaloin to inhibitory effect of each sample.

4) N. D.: Not detected.

Fig. 3. Inhibitory Effects of Aloe Extracts on Histamine Release from Rat Mast Cells Induced by Compound 48/80

- *A. arborescens* M., ◆ *A. ferox* M.
- ▲ *A. barbadensis* M., × *A. africana* M.
- ▼ *A. saponaria* H., ■ JP Aloe powder.

Mast cells were first incubated with test samples at 37°C for 5 min, and then incubated with compound 48/80 (1 μ g/ml) for 10 min. Each point indicates the mean of 3 determinations. The values of histamine release from none- and only compound 48/80-treated cells were, respectively, $3.5 \pm 0.3\%$ and $62.8 \pm 3.1\%$ of the value of histamine released from 0.05% Triton X-100-treated cells.

dependent tendency as their effects on that induced by antigen (Fig. 3). The effects of JP Aloe powder (IC_{50} ; 0.29 mg/ml), *A. ferox* M. (IC_{50} ; 0.41 mg/ml) and *A. barbadensis* M. (IC_{50} ; 0.92 mg/ml) on histamine release induced by compound 48/80 were stronger than that of *A. arborescens* M. (IC_{50} ; 1.52 mg/ml) as well with antigen-induced histamine release. Specifically, the inhibitory effects with respect to IC_{50} values of JP Aloe powder and *A. ferox* M. were strong, being 2.9 and 2.0 times as great as that of tranilast, respectively.

2. The Role of Barbaloin in Inhibitory Action of Aloes

The concentration-response curves of barbaloin on histamine release induced by antigen and compound 48/80 are shown in Fig. 4. Barbaloin exhibited a concentration-dependent inhibitory effect on the release by both inducers, the IC_{50} value being 0.02 and 0.06 mg/ml, respectively, 3.5 and 14 times stronger than that of tranilast (Table I).

The percent contribution of barbaloin to

Fig. 4. Dose-dependent Inhibitory Effects of Barbaloin on Antigen- and Compound 48/80-induced Histamine Release from Rat Mast Cells

- compound 48/80, —○— antigen.

Mast cells sensitized with IgE or normal mast cells were first incubated with barbaloin at 37°C for 5 min, and then incubated with antigen (0.08 μ g/ml) + PS (10 μ g/ml) or compound 48/80 (1 μ g/ml) for 10 min. Each point indicates the mean $\pm$ S.E. of 6 determinations. The values of histamine release from none-, only antigen- and only compound 48/80-treated cells were, respectively, $2.9 \pm 0.2\%$, 37.8 ± 2.2 and $59.2 \pm 2.6\%$ of the value of histamine released from 0.05% Triton X-100-treated cells.

the inhibitory action was calculated based on its content in the various aloes and their inhibition of histamine release (Table I); the contribution varied with the aloe genus. Barbaloin did, however, show a similar percent contribution to both stimulants with each of the aloes: contribution was a high 60–70% in the extracts of *A. arborescens* M., *A. africana* M. and JP Aloe powder, whereas the contribution was low in *A. ferox* M. and *A. barbadensis* M.

Discussion

Aloes have traditionally been used as folk remedies for various diseases throughout the world.¹⁾ However, there is little data explaining the mechanism of their anti-inflammatory and antiallergic effects. In this study, we demonstrated that aloes have an inhibitory effect on histamine release induced by antigen and compound 48/80 from rat peritoneal mast cells. We also found that barbaloin, a major component of aloes, is important in this inhibi-

tion by degranulating the mast cells.

Mast cells are well known to trigger inflammation in immediate hypersensitivity reaction.⁹⁾ The current antiallergic agents, disodium cromoglycate and tranilast, are known to have inhibitory effects on degranulation of rat mast cells and release of chemical mediators from these cells.¹⁰⁾ Thus, our results showing the inhibitory activity of aloe extracts on histamine release from mast cells suggest that aloes may be a useful plant for the treatment of type I immediate allergic disorders. Inhibition of the release of chemical mediators from mast cells seems to be an important mechanism of the anti-inflammatory and antiallergic effects of aloe.

Barbaloin, a major component of aloes, showed stronger inhibitory effects than did tranilast on histamine release induced by antigen and compound 48/80, in a concentration-dependent manner. Barbaloin was found to be important in the effect demonstrated by *A. arborescens* M., *A. africana* M. and JP Aloe powder. However, its contribution to effect of *A. ferox* M. and *A. barbadensis* M. was low, suggesting that these aloes may contain other constituents which account for their action.

An increase of the intracellular Ca^{2+} concentration is an essential trigger for the histamine release from mast cells.¹¹⁾ The de-

granulation of mast cells by antigen requires extracellular Ca^{2+} however, the degranulation by compound 48/80 does not always require this.¹²⁾ In this study, tranilast strongly inhibited the histamine release induced by antigen with a potency 14 times greater than that induced by compound 48/80. This observation was similar to results reported by Koda *et al.*,^{10b)} who found that tranilast inhibited the release of histamine by inhibiting the influx of extracellular Ca^{2+} . However, barbaloin showed almost the same inhibitory effect on both inducers, suggesting that barbaloin has a different inhibitory mechanism than tranilast. We previously demonstrated that barbaloin has no inhibitory effect on the release of histamine induced by calcium ionophore A23187.⁶⁾ Therefore, barbaloin may inhibit both the influx of extracellular Ca^{2+} and the mobilization of Ca^{2+} from the intracellular Ca store through the inhibition of IP_3 (inositol-1,4,5-trisphosphate)-relative reactions.¹³⁾

Although further studies must be undertaken, barbaloin seems to be a valuable and important compound. It helps explain the complicated intracellular conducting systems in mast cells and is also a new type of antiallergic agent having an anti-degranulation effect.

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