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Pharmacological properties of α-mangostin, a novel histamine H_1 receptor antagonist

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Abstract

In the isolated rabbit thoracic aorta and guinea-pig trachea, α-mangostin inhibited histamine-induced contractions in a concentration-dependent manner in the presence or absence of cimetidine, a histamine H_2 receptor antagonist. But KCl-, phenylephrine- or carbachol-induced contractions were not affected by α-mangostin. The concentration-contractile response curve for histamine was shifted to the right in a parallel manner by α-mangostin. In the presence of chlorpheniramine, a histamine H_1 receptor antagonist, α-mangostin did not affect the relaxation of the rabbit aorta induced by histamine. In the guinea-pig trachea, α-mangostin had no effect on the relaxation induced by dimaprit, a histamine H_2 receptor agonist. α-Mangostin caused a concentration-dependent inhibition of the binding of [^{3}H]mepyramine, a specific histamine H_1 receptor antagonist to rat aortic smooth muscle cells. Kinetic analysis of [^{3}H]mepyramine binding indicated the competitive inhibition by α-mangostin. These results suggest that α-mangostin is a novel competitive histamine H_1 receptor antagonist in smooth muscle cells.

Keywords: α-Mangostin; Trachea, guinea-pig; Histamine; Histamine H_1 receptor antagonist; Aorta, rabbit

1. Introduction

Histamine is a naturally occurring mediator of inflammation and is abundantly present in the secretory granules of mast cells and basophils which reside in the respiratory tract. The tissue effects of histamine are presumably mediated by cell membrane receptors. Three histamine receptor subtypes (H_1, H_2 and H_3) are known. The histamine H_1 receptor was identified by Ash and Schild (1966) and histamine H_1 receptor antagonists have been developed and used in the therapy of many allergic diseases including urticaria, allergic rhinitis, pollenosis, and bronchial asthma. In peripheral tissues, the histamine H_1 receptor mediates the contraction of smooth muscles, increase in capillary permeability due to contraction of terminal venules, and catecholamine release from adrenal medulla (Douglas, 1990), as well as mediating neurotransmission in the central nervous system (Schwartz et al., 1991). It has been reported the molecular cloning and sequencing of a cDNA encoding histamine H_1 receptor which provide insight into the molecular biology of histamine action (Yamashita et al., 1991; Fujimoto et al., 1993 and Fukui et al., 1994). Recently, site-directed mutagenesis of the histamine H_1 receptor has been studied and revealed that different histamine H_1 receptor agonists interact in different ways with the receptor proteins (Leurs et al., 1994).

The fruit hull of *Garcinia mangostana* Linn. has been widely used as an anti-inflammatory agent and in the treatment of diarrhea in South East Asia. In the course of our survey of antihistamines from natural sources, α-mangostin (Fig. 1) was isolated as a histamine H_1 receptor antagonist from the fruit hull of this plant. α-Mangostin (1,3,6-trihydroxy-7-methoxy-2,8-bis(3-methyl-2-butenyl)-9*H*-xanthen-9-one), a tetraoxygenated diprenylated xanthone, has been reported to exhibit anti-inflammatory and weak anti-oxidant activity (Shankaranayan et al., 1979 and Yoshikawa et al., 1994). However, the detailed pharmacological properties of α-mangostin have not yet been stud-

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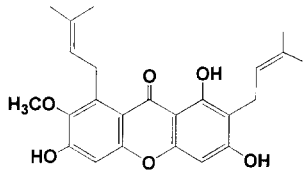

Fig. 1. The structure of α-mangostin.

ied. The present paper is the first report on antihistamine action of α-mangostin. α-Mangostin inhibited the contraction mediated by the histamine H_1 receptor without affecting the relaxation mediated by the histamine H_2 receptor. Furthermore, α-mangostin competitively inhibits [^{3}H]mepyramine binding to rat aortic smooth muscle cells.

2. Materials and methods

2.1. Tissue preparation

The animals used in this study were treated in accordance with the principles and guidelines of Tohoku University Council on Animal Care. The procedure of preparing the tissues and the technique for measurement of contractions were performed as described by Furukawa et al. (1996). Male albino rabbits weighing 2–3 kg were used for this study. The thoracic aorta was dissected and connective tissues were carefully removed. The endothelium was removed from the tissues by gently rubbing the endothelial surface with cotton pellets. The aorta was cut into helical strips, approximately 4 mm in width and 20 mm in length. One end of the strip was secured to the glass tissue holder by a silk ligature and the other end was connected to a force-displacement transducer. The strips were suspended in a 20 ml organ bath containing modified Krebs-Ringer-bicarbonate solution of the following composition (mM): NaCl, 120; KCl, 6.0; $CaCl_2$, 1.2; $MgSO_4$, 1.3; $NaHCO_3$, 25.2; glucose 5.8; pH 7.4. The solution was gassed with 95% O_2 and 5% CO_2 and temperature was maintained at 37°C.

Male, Dunkin-Hartley, guinea-pigs (250–300 g) were used for this study. A section of the trachea between larynx and sternum was isolated, cleaned of all connective tissue and cut open longitudinally along the anterior side. The opened trachea was cut transversely along ring cartilages into 8–9 strips, which were tied up to about 3 cm long. The strips were suspended in a 20 ml organ bath containing modified Krebs-Ringer-bicarbonate solution. The solution was gassed with 95% O_2 and 5% CO_2 and temperature was maintained at 37°C.

The strips of aorta and trachea were mounted under 1 and 0.5 g tension, respectively. The strips were allowed to equilibrate for 60–90 min. Isometric contractions were measured by the transducer and recorded on a polygraph (Nihon Kohden, Tokyo, Japan). After equilibration, the strips were precontracted with 60 mM KCl (10 min) and two or three contractile responses to 60 mM KCl were obtained until the response remained constant.

2.2. Experimental protocols

The experiments were started after the strips had been allowed to equilibrate for 1 h. The control concentration-contractile response curves for the agonists (histamine, KCl, phenylephrine or carbachol) were obtained cumulatively and the contractions were expressed as a percentage of the maximal contraction produced by each agonist [histamine (7×10^{-4} M), KCl (120 mM), phenylephrine (10^{-5} M) or carbachol (3×10^{-6} M)]. To examine the antagonism, α-mangostin (10^{-6}–1.5×10^{-5} M) was added to the bath 10 min before the addition of agonist in the concentration previously used as control. The concentration-response curves for the agonist were then obtained in the presence of α-mangostin. The time interval between two consecutive curves was usually set at 60 min.

To investigate the mechanism of action of α-mangostin on the histamine receptor, the experiments were done on both histamine H_1 and H_2 receptors. In the first set of experiments, the effect of α-mangostin on the histamine H_1 receptor was determined. The strips of aorta and trachea were incubated with cimetidine (10^{-4} M) for 10 min to block the histamine H_2 receptor. The control concentration-contractile response curves of histamine (10^{-7}–7×10^{-4} M) were obtained cumulatively followed by a recovery and equilibration period. The strips were incubated with α-mangostin (10^{-6}–1.5×10^{-5} M) 10 min prior to the addition of cimetidine, followed by a second contraction to histamine in the concentration previously used as control. The contractions induced by histamine were expressed as a percentage of maximal contraction induced by histamine (7×10^{-4} M).

In the second set of experiments, the effect of α-mangostin on the histamine H_2 receptor was determined. The rabbit aortic strips were treated with chlorpheniramine (10^{-5} M) for 10 min to block the histamine H_1 receptor followed by precontraction with phenylephrine (10^{-5} M). After the contraction had stabilized, control histamine-induced concentration-dependent relaxations were obtained. The relaxation response to histamine of the aortic strips are expressed as percentage of the contraction induced by phenylephrine (10^{-5} M). To test the antagonism, α-mangostin (1.5×10^{-5} M) was added to the organ bath 10 min before the addition of chlorpheniramine. In the guinea-pig trachea, the strips were precontracted with carbachol (10^{-7} M) to 65–80% of its maximal response. After the precontraction had stabilized, the relaxation response to dimaprit, a histamine H_2 agonist, was observed. Two cumulative concentration-effect curves for dimaprit were determined in the absence or presence of α-mangostin (1.5×10^{-5} M). α-Mangostin was added 10 min before the precontraction with carbachol. The relaxation response

to dimaprit of the trachea strips are expressed as a percentage of the contraction induced by carbachol (10^{-7} M).

2.3. Cell cultures

Vascular smooth muscle cells were isolated from the aorta of Wistar rats by enzymatic dispersion as described by Chamley et al. (1977). The resulting cells were seeded in 18-mm culture dishes for measurements of ligand binding. Cells were cultured for 5–6 days in Dulbecco's modified Eagle's media (DMEM) supplemented with 10% heat-inactivated fetal calf serum, 10 U/ml penicillin and 100 μg/ml streptomycin. After reaching confluency, cells were cultured in serum-free medium (Cosmedium 001) for an additional 24 h to enhance redifferentiation (Schwartz et al., 1986).

2.4. Measurement of [^{3}H]mepyramine binding

Confluent vascular smooth muscle cells (2×10^5 cells/ml) in culture dishes were incubated for 30 min at 37°C with [^{3}H]mepyramine in 250 μl balanced salt solution (BSS) containing (in mM): NaCl 146; KCl 4; $MgCl_2$ 2; $CaCl_2$ 0.5; glucose 10; bovine serum alblumin 0.1% and Hepes 10 (adjusted to pH 7.4 with Tris base) in the absence or presence of α-mangostin. Incubation was terminated by the addition of 1 ml of ice-cold BSS. Cells were then washed five times with cold BSS, and then cell-bound radioactivity was determined. Non-specific binding of [^{3}H]mepyramine was determined in the presence of 10^{-6} M chlorpheniramine. Specific binding of ligand to cells was estimated by subtracting the non-specific component from total binding.

2.5. Protein determination

Protein concentration was determined by the protein-dye method of Bradford (1976), using bovine serum albumin as a standard.

2.6. Drugs and chemicals

The following drugs were used: carbamylcholine chloride (carbachol), cimetidine, histamine hydrochloride and *S*-(3-dimethylaminopropyl)isothiourea · 2HCl (dimaprit) (Sigma, St. Louis, MO, USA), DL-chlorpheniramine maleate (chlorpheniramine), phenylephrine and KCl (Wako Pure Chemical Industries, Osaka, Japan), Dulbecco's modified Eagle's medium (Nissui Pharmaceutical, Tokyo, Japan), streptomycin sulfate (Meiji Seika, Tokyo, Japan), penicillin (Banyu Pharmaceutical, Tokyo, Japan) and fetal calf serum (JRH Biosciences Lenexa, KS, USA). [^{3}H]Mepyramine (20 Ci/mmol) was purchased from Du Pont New England Nuclear (Boston, MA, USA). α-Mangostin was obtained from the fruit hull of *G. mangostana* L. as previously reported. The fruit hull of *G. mangostana* L. was crushed and soaked in methanol. The methanol extract was purified by silica gel chromatography to give α-mangostin. α-Mangostin was dissolved in dimethyl sulfoxide of which the final concentration was kept less than 1% (v/v) in all experiments. In the control experiments, dimethylsulfoxide was added instead of the solution of α-mangostin to minimize the effect of the vehicle solvent. Other chemicals or drugs were of reagent grade of the highest quality available.

2.7. Data analysis

Three or four preparations were used for each experiment. Data are presented as means ± S.E.M. Statistical analyses were done by means of Student's *t*-test. A P value of less than 0.05 was considered a significant difference. Dissociation constant (K_d) and maximum binding site (B_{max}) of the tritium ligands were obtained by Scatchard analysis (Nakahata et al., 1989).

3. Results

3.1. Mechanical response of the rabbit thoracic aorta

Histamine (10^{-7}–7×10^{-4} M) caused a concentration-dependent contraction in the aorta with a pD_2 value of 5.21 ($n = 4$) (Fig. 2A). The contractile response to histamine was antagonized in a concentration-dependent manner by α-mangostin (10^{-6}–1.5×10^{-5} M) without depression of the maximal response. As shown in Fig. 2B, the Schild plot of the data revealed the pA_2 value to be 5.78 with a slope of the regression line (1.16) not being significantly different from unity. Conversely, the concentration-response curves for KCl (7.5–120 mM, pD_2

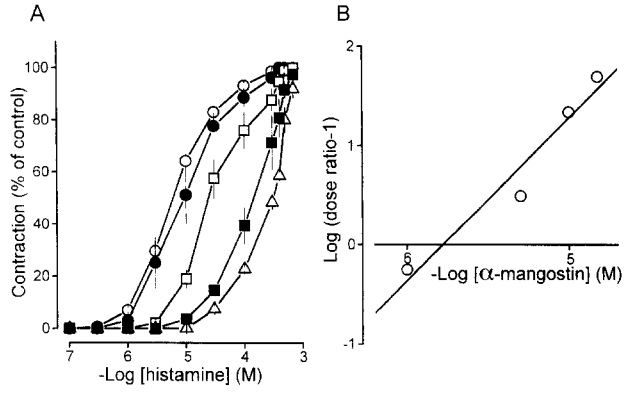

Fig. 2. Rabbit thoracic aorta. (A) Concentration-response curves to histamine-induced contraction in the absence (○) and presence of four increasing concentrations of α-mangostin (● 10^{-6} M, □ 5×10^{-6} M, ■ 10^{-5} M, △ 1.5×10^{-5} M). Tissues were preincubated with α-mangostin 10 min before the addition of agonists. Each point is the mean of at least four experiments and vertical bars are S.E.M. (B) Schild plot for antagonism of histamine by α-mangostin. The data are taken from experiments shown in (A).

1.71) and phenylephrine (3×10^{-8} to 10^{-5} M, pD_2 6.28) were unaffected by α-mangostin (1.5×10^{-5} M).

To investigate the histamine receptor type involved in the inhibitory effect of α-mangostin, the experiments were done in both histamine H_1 and H_2 receptors. In the presence of cimetidine (10^{-4} M), histamine (10^{-7}–7×10^{-4} M) induced a contraction of the aorta via activation of the histamine H_1 receptor with a pD_2 value of 4.96. Pretreatment of the aortic strips with α-mangostin (10^{-6}–1.5×10^{-5} M) for 10 min caused a parallel rightward shift of the histamine-induced contractile response curves. Analysis of this displacement, by using the Schild plot yields a pA_2 value of 5.74 with a slope of 0.91. The value of the slope is not significantly different from unity. After blocking the histamine H_1 receptor by using chlorpheniramine (10^{-5} M), histamine (3×10^{-7}–5×10^{-4} M) induced a relaxation of the aorta mediated by the histamine H_2 receptor with a pD_2 value of 4.32. α-Mangostin (1.5×10^{-5} M) did not affect the concentration-response curve of histamine-induced relaxations in the rabbit aorta.

3.2. Mechanical response of the guinea-pig trachea

As shown in Fig. 3A, histamine induced contractions of the guinea-pig trachea in a concentration-dependent manner (10^{-7}–7×10^{-4} M) with a pD_2 value of 5.47. Pretreatment of the trachea for 10 min with α-mangostin (10^{-6}–1.5×10^{-5} M) resulted in a concentration-related rightward shift of the histamine-induced contractile-response curve. No significant changes in the maximal response were observed. The pA_2 value for α-mangostin was calculated to be 5.80 and the slope of the Schild plot is 0.95 (Fig. 3B). In contrast, α-mangostin (1.5×10^{-5} M) had no effect on the contractile responses to KCl (7.5–120 mM, pD_2 1.65) or carbachol (10^{-8}–3×10^{-6} M, pD_2 6.87).

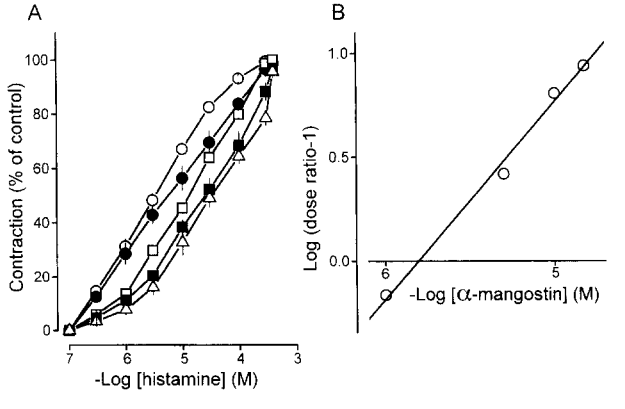

Fig. 3. Guinea-pig trachea. (A) Concentration-response curves to histamine-induced contraction in the absence (○) and presence of α-mangostin (● 10^{-6} M, □ 5×10^{-6} M, ■ 10^{-5} M, △ 1.5×10^{-5} M). Tissues were preincubated with α-mangostin 10 min before the addition of agonists. Each point is the mean of at least four experiments and vertical bars are S.E.M. (B) Schild plot for antagonism of histamine by α-mangostin. The data are taken from experiments shown in (A).

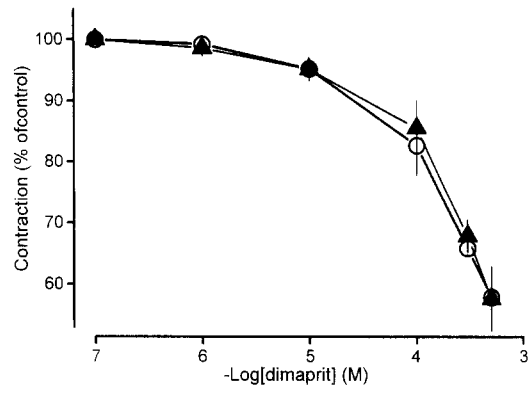

Fig. 4. Guinea-pig trachea. Concentration-response curves to dimaprit-induced relaxation, precontraction with carbachol (10^{-7} M). Control (○), α-mangostin (▲ 1.5×10^{-5} M). Tissues are pretreated with α-mangostin 10 min before the addition of carbachol. The response to dimaprit is expressed as a percentage of the contraction induced by 10^{-7} M carbachol. Each point is the mean of at least three experiments and vertical bars are S.E.M.

In the presence of cimetidine (10^{-4} M), histamine (10^{-7}–4×10^{-4} M) produced contractions of the trachea (pD_2 5.79) in a concentration-dependent manner. The addition of α-mangostin (10^{-6}–1.5×10^{-5} M) caused a parallel rightward shift of the histamine-induced contractile response curves with the same maximal response as previously. The Schild plot was linear with a slope of 1.14 and a pA_2 value of 5.53. As shown in Fig. 4, dimaprit, a specific histamine H_2 agonist caused a concentration-dependent relaxation of the trachea (pD_2 3.92) which was completely inhibited by cimetidine. α-Mangostin (1.5×10^{-5} M) did not inhibit the concentration-relaxation curve of the trachea induced by dimaprit.

3.3. [^{3}H]Mepyramine binding

α-Mangostin competitively inhibited histamine-induced contractions of both aorta and trachea. Then, receptor

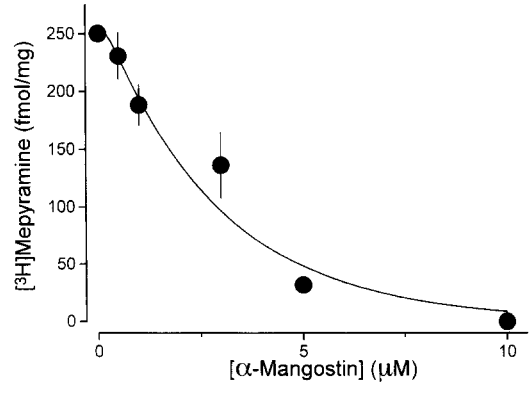

Fig. 5. Concentration-inhibition curve for α-mangostin in [^{3}H]mepyramine binding to rat aortic smooth muscle cells. Rat aortic smooth muscle cells were incubated with 10^{-8} M [^{3}H]mepyramine for 30 min at 37°C. Non specific binding in the presence of 10^{-5} M chlorpheniramine has been subtracted from the results. Each point is the mean of at least three experiments and vertical bars are S.E.M.

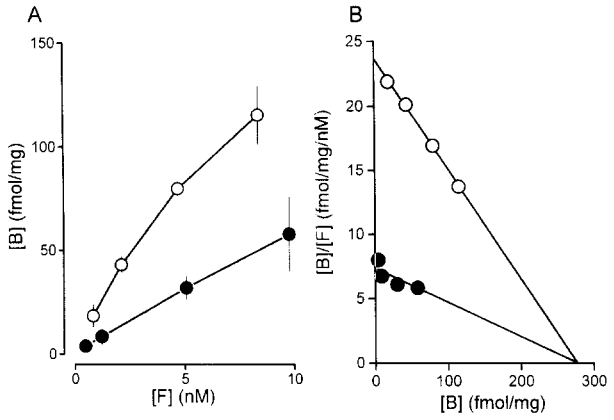

Fig. 6. Binding of [^{3}H]mepyramine to rat aortic smooth muscle cells in the presence or absence of α-mangostin. Rat aortic smooth muscle cells were incubated with [^{3}H]mepyramine (1.25×10^{-9}–2×10^{-8} M) for 30 min at 37°C. Non specific binding in the presence of 10^{-5} M chlorpheniramine has been subtracted from the results. Each point is the mean of at least three experiments and vertical bars are S.E.M. (A) [^{3}H]Mepyramine binding [B] was measured in the presence (●) or absence (○) of 5×10^{-6} M α-mangostin and are expressed versus the free [^{3}H]mepyramine concentration [F]. (B) [^{3}H]Mepyramine binding in the presence (●) or absence (○) of 5×10^{-6} M α-mangostin is presented as a Scatchard plot.

binding analysis was carried out to clarify whether α-mangostin is a histamine H_1 receptor antagonist. Fig. 5 illustrates the inhibition of specific [^{3}H]mepyramine to rat aortic smooth muscle cells by α-mangostin. α-Mangostin inhibited the [^{3}H]mepyramine binding with an IC_{50} value of 2.27 μM. The dependence of [^{3}H]mepyramine binding on free [^{3}H]mepyramine concentration in the presence and absence of α-mangostin (5×10^{-6} M) is illustrated in Fig. 6A. Specific binding of [^{3}H]mepyramine to rat aortic smooth muscle cells was saturable. Scatchard analysis showed that [^{3}H]mepyramine bound to a high affinity receptor site with $K_d = 11.72$ nM and $B_{max} = 275.95$ fmol/mg (Fig. 6B). α-Mangostin increased the K_d value to 38.02 nM without affecting the B_{max} value. These results clearly indicate that α-mangostin is a specific histamine H_1 receptor antagonist.

4. Discussion

As far as we know, the present study is the first one to describe the pharmacological studies of α-mangostin on histamine-induced contractions and relaxations of isolated rabbit thoracic aorta and guinea-pig trachea. In both tissues, α-mangostin produced concentration-dependent antagonism of contractile responses to histamine, causing parallel dextral shifts of histamine concentration-response curves with no or little effect on the maximal response (Fig. 2A and Fig. 3A). The slope of the Schild plot in each case was not significantly different from unity (Fig. 2B and Fig. 3B). Thus, α-mangostin proved to be a competitive antagonist of histamine receptors of rabbit aorta and guinea-pig trachea. Furthermore, α-mangostin had no effect on the contractile-response curves for KCl or phenylephrine of the aorta and for KCl or carbachol of the trachea. These results suggest that α-mangostin is a selective and competitive histamine antagonist.

The histamine receptors in various types of smooth muscle are divided in two broad classes: histamine H_1 and H_2 receptors. The histamine-induced contraction is primarily mediated through activation of the histamine H_1 receptor on smooth muscle cells including rabbit aorta and guinea-pig trachea, while the histamine-induced relaxation results from stimulation of the histamine H_1 and/or H_2 receptors (Leusen and Van de Voorde, 1988; Levi et al., 1991). In the present experiment, the relaxing influence of histamine in both aorta and trachea were mediated by the histamine H_2 receptor. The inhibitory effects of α-mangostin on histamine H_1 and H_2 receptors were examined in both tissues. α-Mangostin inhibited histamine-induced contractions of both aorta and trachea at the histamine H_1 receptor (in the presence of the histamine H_2 antagonist) with a pA_2 value of 5.74 (rabbit aorta) and 5.53 (guinea-pig trachea). α-Mangostin appears to be slightly potent as an antagonist of the histamine-induced contraction of the rabbit aorta than as an antagonist of the histamine-induced contraction of the guinea-pig trachea. However, the histamine H_2 receptor-mediated relaxation was not affected by α-mangostin. Furthermore, kinetic analysis of the [^{3}H]mepyramine binding to rat aortic smooth muscle cells revealed that α-mangostin increased the K_d value without affecting the B_{max} value, indicating the mode of competitive inhibition by α-mangostin. On the basis of these results, it is suggested that α-mangostin acts as a competitive histamine H_1 receptor antagonist.

It is well known that histamine is one of the major mediators of inflammation. In human skin, the immediate inflammatory reaction is of the flush wheal-flare type, which involves the axon reflex (Douglas, 1990). At this stage, the reaction is reduced by antihistamines. The main use of the histamine H_1 receptor antagonist is the relief of histamine-mediated effects in allergic and anaphylactic reactions. Xanthones and their derivatives were shown to be effective as an allergy inhibitor and bronchodialator in treatment of asthma (Jones et al., 1977). It has been reported that α-mangostin, a tetraoxygenated diprenylated xanthone, exhibits anti-inflammatory activity by intraperitoneal and oral administration in normal and bilaterally adrenalectomized rats (Gopalakrishnan et al., 1980). In the present study, α-mangostin competitively antagonized histamine on the histamine H_1 receptor. Therefore, the anti-inflammatory activity of α-mangostin may be due to its ability to antagonize the histamine H_1 receptor.

Generally, the common structure of antihistamines possess at least one tertiary nitrogen group and have an aromatic portion consisting generally of two phenyl rings. Almost all antihistamines are synthetic nitrogen compounds such as pyrilamine, phenothiazine and chlorpheni-

ramine (Douglas, 1990). It has been reported that 4,4′-diacetyl curcumin (non-nitrogenous compounds) inhibited the effect of histamine on smooth muscle, but these compounds do not act on the cellular surface receptor sites for histamine, as do the synthetic antihistamine drugs (Douglas, 1993). α-Mangostin showed lower affinity for the histamine H_1 receptor than chlorpheniramine, a classical histamine H_1 receptor antagonist (pA_2 8.3) (Bökesoy and Onaran, 1991). It is of interest that the structure of α-mangostin does not even resemble only a type of antihistamines; however, α-mangostin possesses the histamine receptor blocking activity and blocks directly to the histamine H_1 receptor sites. α-Mangostin may be the first natural product which specifically antagonizes the histamine H_1 receptor.

In conclusion, these pharmacological and biochemical studies revealed that α-mangostin competitively inhibited not only the histamine H_1 receptor-mediated smooth muscle contraction but also the [^{3}H]mepyramine binding to histamine H_1 receptor sites on intact smooth muscle cells. α-Mangostin is a novel type of histamine H_1 receptor antagonist and may become a valuable leading compound for the development of antihistamines.

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