Anti-allergic Activity of Naringenin Chalcone from a Tomato Skin Extract

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The anti-allergic activity of a tomato extract was studied by using an in vitro histamine-release assay. The tomato skin extract exerted the strongest inhibition of histamine release. Chlorogenic acid, rutin and naringenin were identified in the 60% ethanol extract of tomato skin. However, the extract contained an unknown compound which strongly inhibited histamine release. This active compound in tomato skin was identified as naringenin chalcone (trans-2′,4′,6′-tetrahydroxychalcone). Naringenin chalcone inhibited histamine release with an IC50 value of 68 μg/ml. The anti-allergic activity of the tomato skin extract was next investigated by the in vivo mouse ear-swelling response. We found that naringenin chalcone showed the strongest inhibitory effect of the polyphenols of the tomato skin extract. These results indicate that a tomato skin extract could inhibit allergic reactions.

Key words: naringenin chalcone; tomato skin extract; histamine

There has recently been an increasing incidence of allergic disorders from food and airborne allergens. According to Coombs and Gell, allergic reactions can be classified into four types, and the immediate hypersensitive reaction is generally classified as a type I allergy. This allergic reaction induces a series of events and the inhibition of any steps may attenuate the allergic symptoms.

Histamine, an inflammatory substance released from mast cells, is regarded as one of the chemical mediators which triggers an allergic reaction, and is generally known as an autacoid. The inhibition of histamine release could therefore be a matter of attenuating the allergic symptoms. Foods contain many physiological factors which may prevent the incidence of diseases and may promote human health, in addition to their functions as nutrients. Polyphenols, which are widely distributed in fruits and vegetables, can act as antioxidants. It has been reported that some polyphenols could exert anti-allergic activities. However, relatively little is known about the anti-allergic activities of tomato polyphenols.

We examined in this study the anti-allergic effects of a tomato skin extract on the histamine release from rat peritoneal mast cells and the ear-swelling response by using the mouse model for type I allergy.

Material and Methods

Chemicals. Compound 48/80 and cyproheptadine hydrochloride were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Chlorogenic acid, rutin, carboxymethyl cellulose sodium salt (CMC), acetone and olive oil were purchased from Wako Pure Chemicals Co. (Osaka, Japan). Naringenin was purchased from Funakoshi (Tokyo, Japan). Picryl chloride (PCI) and caffeic acid were purchased from Nakalai Tesque (Kyoto, Japan). Bovine serum albumin (BSA) was purchased from Intergen Co. (Toronto, Ontario, Canada) and anti-trinitrophenyl (TNP) IgE was purchased from BD PharMingen (San Diego, CA, USA).

Animals. Female BALB/c mice (6-week-old) and male Sprague-Dawley rats (13-week-old) were purchased from Charles River Japan (Tsukuba, Japan). These animals were acclimatized in an environmentally controlled room (temperature: 23 ± 1°C; humidity: 55 ± 5%; illumination time: 7:00 to 19:00), with an MF diet (Oriental Yeast. Co., Tsukuba, Japan) and water available ad libitum for at least 1 week before the experiment. Experiments were performed according to the Guidelines for the Care and Use of Experimental Animals of the Japanese Association for Laboratory Animals.

Sample preparation of the tomato extract. Crude tomato polyphenols were obtained from the skin and seed of tomatoes (Lycopersicon esculentum Miller). Tomato extracts containing polyphenols were prepared from seeds and skin with 60% (v/v) ethanol at 60°C for 2 hours with subsequent lyophilization.

Abbreviation: NGC, naringenin chalcone
Assay of the inhibitory effect on histamine release from rat peritoneal mast cells. Preparation of the mast cells and the assay of the inhibitory effect on histamine release from rat peritoneal mast cells were based on the methods of Inoshiri et al.\textsuperscript{12} Rat peritoneal mast cells were collected from the peritoneal cavity after injecting a buffer solution (0.15 M NaCl, 3.7 mM KCl, 3.0 mM Na\textsubscript{2}HPO\textsubscript{4}, 3.5 mM KH\textsubscript{2}PO\textsubscript{4}, 0.9 mM CaCl\textsubscript{2}, 5.6 mM d-glucose and 0.1% (w/v) gelatin) and then partially purified by centrifugation. Test samples were added to the cell suspension (2.5 × 10\textsuperscript{5} cells/ml in 80 μl) with subsequent incubation at 37°C for 10 min with a buffer solution, and the cells were then stimulated with compound 48/80 for 10 min. HCl (0.1 N, 80 μl) was added in order to stop the histamine release from the cells. After centrifugation of the cell suspension at 1500 × g for 5 min at 4°C, the supernatant was collected and the amount of histamine in the solution was analyzed by HPLC according to the on-column conversion method by using o-phthalaldehyde.\textsuperscript{13} Ketotifen, an anti-allergic drug, was used as a positive control in the assay.

**HPLC analysis of the polyphenolic composition of the tomato skin extract.** Polyphenols in the tomato skin extract were determined by HPLC, using a CapcellPak C18 column (shiseido, 4.6 mmΦ × 150 mm) at a flow rate of 1.0 ml/min. The sample (10 μl) was injected, and elution was performed with a system composed of solvent A (0.1% TFA in water) and solvent B (0.1% TFA in acetonitrile) mixed in a linear gradient starting from 0% solvent B and increasing to 100% solvent B in 30 min. The elution was monitored by photodiode array detection at 310 nm. The compounds corresponding to each peak were identified by a comparison with the retention time and absorption spectrum of reference standards under identical conditions.

**LC/MS analysis.** The JMS-BU30 (Jeol) HPLC system for LC/MS was used with a CapcellPak C18 column (4.6 mmΦ × 150 mm, Shiseido) for calculating the molecular weight. The LC/MS conditions were as follows: electrospray ionization (ESI) in the positive ion mode, selected ion monitoring (SIM; 305.3, 579.2, 731.3, 867.3), desolving plate, 220°C; orifice I, 70°C; and lens ring voltage, 50 V. The column was isocratically eluted with 30% acetonitrile containing 0.1% TFA at a flow rate of 1 ml/min. Each peak was identified from UV and MS data which provided information on the flavonoid skeleton patterns.\textsuperscript{14}

**NMR analysis.** To determine the structure of the active polyphenol compound in tomato skin, \textsuperscript{1}H-, \textsuperscript{13}C-NMR, \textsuperscript{1}H–\textsuperscript{13}C COSY, HOHAGO, HMOC, and HMBC spectra were recorded in dimethyl-d\textsubscript{6} sulfoxide at 300 k with a Bruker avance 500 spectrometer operated at 500.1 MHz for \textsuperscript{1}H and at 125.7 MHz for \textsuperscript{13}C.

**Determination of total polyphenol.** Freeze-dried tomato juice and the tomato extract were dissolved in water for a total polyphenol determination. The total polyphenol content was determined by the Folin–Ciocalteu method. The Folin–Ciocalteu reagent (0.25 ml, Fluka) and an aqueous solution of 20% sodium carbonate (0.75 ml) were added to 4 ml of the aqueous extract, and the mixture was incubated at 40°C for 20 min. After cooling to room temperature, the absorbance was measured at 755 nm by a spectrophotometer. An aqueous solution of caffeic acid was used as a standard.

**Assay for anti-allergic activity using the mouse type I allergy.** The ear-swelling response according to the mouse model for type I allergy was evaluated by the method reported by Lavaud et al.\textsuperscript{15} with some modifications. Mice were intravenously sensitized by 2 μg of anti-TNP IgE in a 0.1% BSA-phosphate-buffered saline (PBS) solution. After 30 minutes, the ear thickness (before the challenge) was measured with an upright dial thickness gauge (Ozaki Mfg. Co., Tokyo, Japan). The ear-swelling response was elicited by applying 10 μl of the antigen (0.8% PCI in acetone:olive oil (1:1)) to the ventral side of the ears of the mice. After two hours from the PCI challenge, the mice were killed, and the ear thickness was measured (after the challenge). The ear-swelling response was determined as the difference in ear thickness before and after the challenge. To examine the effect of the samples on the ear-swelling response, the tomato skin extract, polyphenols, cyproheptadine hydrochloride and their vehicle, 0.5% CMC, as the control were orally administered for 5 days before the antigen challenge.

The following values were then calculated:

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edema A = \frac{\text{edema control}}{\text{edema sample}}\times 100
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**Statistical Analysis.** A statistical analysis was performed by using Student’s t-test with the SPSS software system for Windows Ver. 11.0J (SPSS Japan, Tokyo, Japan). In all cases, probability (p) values below 0.05 were considered significant.

**Results**

**Effect of the extracts from tomato juice, tomato seed and tomato skin on the histamine release from rat peritoneal mast cells**

Tomato extracts containing polyphenols were prepared from the seed and skin with 60% ethanol at 60°C for 2 hours, before lyophilization. Total polyphenol was determined as approximately 2%, 1% and 4% in the tomato juice extract, tomato seed extract and tomato

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edema A = \frac{\text{edema control}}{\text{edema sample}}\times 100
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skin extracts, respectively. The inhibitory effects of the tomato juice, seed and skin extracts on the histamine release from rat peritoneal mast cells was examined. As shown in Fig. 1(a), the tomato skin extract in 60% ethanol (60 °C, 2 hours) strongly suppressed the histamine release from rat peritoneal mast cells, while the tomato juice extract and tomato seed extract showed weak suppression. Ketotifen, an anti-allergic drug, was used as a positive control in the assay. As shown in Fig. 1(b), the tomato skin extract inhibited the histamine release in a dose-dependent manner. These results suggested that a specific compound present in tomato skin could inhibit the histamine release from rat peritoneal mast cells.

Purification and identification of the active compound in tomato skin

It is well known that tomato skin contains several polyphenols. Some polyphenols in plants are known to inhibit histamine release. To identify the active compounds involved in inhibiting the histamine release from mast cells, the tomato skin extract was fractionated by reversed-phase HPLC with a CapcellPak ODS column. The eluate was collected into fractions A to K (Fig. 2(a)). In order to identify the fractions containing the active compounds, the inhibitory effect of each fraction was measured. As shown in Fig. 2(b), fraction K showed the strongest inhibition of histamine release. When fraction K underwent HPLC separation in the ODS column with isocratic elution (33% acetonitrile containing 0.1% TFA), two peaks appeared, and the solutions corresponding to each of these peaks were collected as fractions K-1 and K-2 (Fig. 2(c)). Fraction K-2 was identified as naringenin from a comparison with an authentic sample (Funakoshi) and did not strongly inhibit the histamine release from mast cells (data not shown). Spectral analyses by LC-MS and NMR were carried out to identify the structure of fraction K-1. The
LC-MS analysis showed the molecular weight of fraction K-1 to be 272, this being identical to that of C_{15}H_{12}O_{5}. To elucidate the structure in detail, fraction K-1 was analyzed by ^1H- and ^13C-NMR. Based on information from the ^1H-, ^13C-NMR, ^1H–^1H COSY, HOHAHA, HMQC, and HMBC spectra, the structure of the active compound was identified as naringenin chalcone as shown in Fig. 3 (UV \_\text{max} (\text{MeOH}): 365 nm. APCI-MS \text{m/z}: 273 [MH]^+ (100%). NMR δH (DMSO-\text{d}_6, 500 MHz): 12.54 (s, OH-2/6), 10.40 (s, OH-4), 10.06 (s, OH-4), 7.96 (d, J = 15.6 Hz, H-\text{C}11), 7.56 (d, J = 15.6 Hz, H-\beta), 7.51 (d, J = 8.2 Hz, H-2/6), 6.83 (d, J = 8.2 Hz, H-3/5), 5.84 (s, H-\text{C}3/5). The results of these experiments determined the active compound of fraction K-1 to be naringenin chalcone.

Effect of naringenin chalcone and various polyphenols contained in tomato skin on the histamine release from rat peritoneal mast cells

Naringenin chalcone was purified from the tomato skin extract by HPLC fractionation, and its purity was confirmed at as more than 99% by an HPLC analysis (data not shown). The inhibitory effect of the purified naringenin chalcone on the histamine release was examined. As shown in Fig. 4, naringenin chalcone strongly inhibited the histamine release from rat peritoneal mast cells in a dose-dependent manner. The IC_{50} value for naringenin chalcone was determined to be 68 \mu g/ml.

When the peaks from the HPLC analysis were compared with those from the authentic standards, naringenin, rutin and chlorogenic acid were also identified in the tomato skin extract. Among these polyphenols, naringenin chalcone was the most efficient inhibitor of histamine release. Naringenin and caffeic acid showing weak inhibitory effects, while rutin and chlorogenic acid did not show any inhibitory effect (Table 1). These results show that the most active compound contained in the tomato skin extract was naringenin chalcone.

Anti-allergic effect of the tomato extracts and polyphenols contained in the tomato extracts on the mouse type I allergic model

In order to obtain \textit{in vivo} information about the anti-allergic effects of the tomato extracts, an assay of the inhibitory effects on a mouse type I allergy model was carried out. To examine the preventive effect of each sample on the ear-swelling response, each sample was orally administered for 5 days before the antigen challenge. In past studies, some active compounds have been given as a pretreatment.\textsuperscript{23,24} Cyproheptadine (10 mg/kg, i.p.), a positive control, inhibited ear-swelling by 72.3% (data not shown). The tomato skin extract had an inhibitory effect on the ear-swelling response in a dose-dependent manner. The inhibitory ratios of a daily administration of 4 mg/kg and 0.8 mg/kg of body weight were 41.0% and 26.9%, respectively (Fig. 5). These results show that the tomato skin extract had an inhibitory effect on the ear-swelling response by using the mouse model for the type I allergy. In this study, we identified chlorogenic acid, rutin, naringenin chalcone and naringenin as polyphenols in the tomato skin extract. We examined the inhibitory effects of these polyphenols contained in the tomato skin extract on the ear-swelling response. Among the tested polyphenols, naringenin chalcone showed the strongest inhibitory effect on the ear-swelling response (Fig. 6). The inhibitory ratio of a daily administration of 0.8 mg/kg naringenin chalcone was 46.7%. On the other hand, the inhibitory ratios of chlorogenic acid, rutin and naringenin were 20.1%, 21.0%, 14.5%, respectively. Naringenin chalcone strongly inhibited the ear-swelling response,
while chlorogenic acid, rutin, naringenin, quercetin and caffeic acid showed a slight inhibitory effect.

**Discussion**

Type I allergy is an immediate hypersensitive reaction to food or environmental allergens. With this type of allergy, mast cells play a crucial role in the pathogenesis of allergic symptoms through the production and release of such chemical mediators as histamine and eicosanoids. These chemical mediators cause various pathophysiologic events in acute allergic reactions, including increased vascular permeability, induction of bronchial smooth muscle contraction, mucus production, and neutrophil chemotaxis. It is important to reduce the mediator release for preventing and alleviating the allergic symptoms. We have shown in this study that the tomato skin extract strongly inhibited the histamine release from rat peritoneal mast cells. It is well known that tomato skin contains several polyphenols. Some polyphenols are known to inhibit histamine release. We found a novel fraction in the tomato skin extract which could exert strong inhibition of histamine release and identified naringenin chalcone as the active compound inhibiting histamine release. Naringenin chalcone has been reported to represent an intermediate in the biosynthesis of flavonols and to be converted into naringenin by the chalcone isomerase. Some chalcones have been shown to have antibacterial and antifungal activities. We have shown in this study that naringenin chalcone inhibited the histamine release from mast cells.

We have shown that the tomato skin extract had inhibitory activity against the ear-swelling response by using the mouse model for type I allergy. The inhibitory activity of naringenin chalcone toward mouse type I allergy was stronger than that of the other polyphenols included in the tomato skin extract. This indicates that naringenin chalcone contributed to the anti-allergic activity of the tomato skin extract. However, other polyphenols included in the tomato skin extract slightly inhibited ear-swelling. It is possible that these compounds also contribute to the anti-allergic activity of the tomato skin extract. In fact, naringenin is known as a histidine decarboxylase inhibitor. Rutin and chlorogenic acid inhibited histamine release by decreasing the reactive oxygen species level in antigen-IgE-activated mast cells. These results suggest that the various compounds contained in the tomato skin extract showed anti-allergic activity through different in vivo mechanisms.

The tomato is a common vegetable that is known to contain many compounds with health benefits such as lycopene. Tomato skin is a rich source of polyphenols, and attention has been focused on the physiological functions of polyphenols. We assessed in this study the capacity of naringenin chalcone to suppress an allergic reaction through the inhibition of histamine release. However, the mechanism for this histamine-release inhibition by naringenin chalcone is not clear. Not only naringenin chalcone, but other compounds contained in the tomato skin extract may have inhibited the degranulation and synthesis of allergic chemical mediators and contributed to the inhibitory activity of the tomato skin extract toward the acute-phase and late-phase allergic responses. Further examination is underway.

The results obtained in this study suggest that the ingestion of a tomato skin extract would be useful for preventing and/or improving allergic symptoms. Further studies for the mechanism of the anti-allergic action are needed.
References


