Antiallergic Effect of Apple Polyphenols on the Allergic Model Mouse

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We studied here the antiallergic effect of apple condensed tannins (ACT) administered orally to a type I allergy model mouse transplanted with an IGEL a2 hybridoma secreting anti-2,4,6-trinitrophenyl (TNP) immunoglobulin E (IgE). The oral administration of ACT significantly inhibited the ear swelling responses at 1 h after antigen-stimulation with picryl chloride. The response was dose dependent within 0.1 to 10 mg/mouse. The inhibition of the ear swelling response reached the maximal level (90% inhibition) when ACT was administered 2 h before the antigen challenge. These findings suggest that ACT has an antiallergic effect on type I allergic symptoms.

Key words antiallergic activity; procyanidin; apple

The apple contains several phenolic substances, i.e., chlorogenic acid, catechin, epicatechin, phlorizin, rutin, flavonoids, and condensed tannins. Unripe apples contain condensed tannins at a level ten times that of a ripe one, and the condensed tannins are mainly composed of various polymerized catechins. Ohnishi-Kameyama et al. reported that the condensed tannins obtained from unripe apples were composed of various polymerized catechins.

Recently, several authors have published that polyphenols showed antiallergic activities in addition to antioxidant anti-tumor, and anti-human immunodeficiency virus activities. Matsuo et al. have reported that tea polyphenols inhibited histamine release from rat peritoneal exudate cells and basophilic leukemia cells. Sano et al. showed that two catechin derivatives in Oolong tea inhibited type I allergic reactions in mice. In a previous paper, we described that condensed tannins prepared from unripe apples (ACT) had an inhibitory effect on histamine release from both rat basophilic leukemia (RBL-2H3) cells by antigen stimulation and rat peritoneal mast cells stimulated by compound 48/80. However, it is not clear that ACT has antiallergic activity in vivo. In the present study, we studied the effects of ACT administered orally on ear swelling responses using the mouse model for type I allergy. Furthermore, we examined the effects of the interval between the antigen challenge and the oral administration of ACT on the inhibition of the ear swelling responses.

MATERIALS AND METHODS

Animals Female BALB/c mice, 6 weeks of age, were purchased from SLC (Shizuoka, Japan). These animals were housed in an air-conditioned room (24°C) and given a diet and water ad libitum. The animals were kept for at least 7 d before being used.

Culture of Hybridoma The mouse hybridomas, IGEL a2 cells (American Type Culture collection; ATCC, TIB 142), which secreted anti-2,4,6-trinitrophenyl (TNP) immunoglobulin E (IgE), were maintained in RPMI 1640 medium (Gibco BRL, Life Technologies, Inc., Rockville, MD, U.S.A.) supplemented with 10% fetal bovine serum (FBS, Sanko Junyaku Co., Ltd., Tokyo, Japan), 10 μM N-2-hydroxyethylpiperazine-N'-2-ethane sulfonic acid (HEPES, Gibco BRL, Life Technologies, Inc., Rockville, MD, U.S.A.), 2 mM L-glutamine, 50 U/ml of penicillin and 50 μg/ml of streptomycin (Gibco BRL, Life Technologies, Inc., Rockville, MD, U.S.A.).

Induction of Ear Swelling Response Using the Mouse Model of Type I Allergy Ear swelling responses using the mouse model for type I allergy were carried out according to the method reported by Naito et al. with some modifications. The IGEL a2 cells were washed four times with RPMI-1640 medium, and 200 μl of the cell suspension containing 1×10⁶ cells with RPMI 1640 medium without FBS was then injected subcutaneously into the back of the neck of the mice. Ten days after the transplantation of the hybridomas, the ear thickness (time 0) was measured with an upright dial thickness gauge (Ozaki MFG Co., Ltd., Tokyo, Japan). Ear swelling responses were elicited by applying 10 μl of antigen (1% picryl chloride in acetone) to the ventral sides of the left ears of the mice. Ear thickness measurements were carried out at 1, 2, 4 and 24 h after the picryl chloride challenge, and the increase in the ear thickness from that time (time 0) was evaluated. To examine the effect of ACT on ear swelling, 0.1—10 mg (equivalent to 5—500 mg/kg body weight) of ACT was orally administered to the mice before the antigen challenge, and the ear thickness was measured as previously described. The percent inhibition of ear swelling responses was calculated using the following formula: Inhibition (%) = 100−[(test-blank)/(control-blank)]×100. The values of the test, control and blank are the ear thickness at 1 h after antigen challenge of the hybridoma-transplanted and ACT-pretreated mice, of the hybridoma-transplanted mice, and of normal mice, respectively.

Determination of Mouse Serum Anti-TNP IgE Titer by Indirect ELISA For determination of the serum anti-TNP IgE titer, the serum was obtained 7 d after injection of IGEL a2 hybridomas. The mouse serum titer of anti-TNP IgE was determined by the method of Teshima et al. with some modifications. Fifty microliters of dinitrophenylated bovine
serum albumin (DNP-BSA, Cosmobio Co., Ltd., Tokyo, Japan, 20 μg/ml) in 50 μl sodium carbonate buffer, pH 9.5, was added to each well of a 96-well microtiter plate and incubated overnight at 4°C. The solution was discarded, and each well was washed 4 times with 200 μl phosphate-buffered saline (PBS) containing 0.05% polyoxyethylene sorbitan monolaurate (Tween 20, Sigma, St Louis, MO, U.S.A.). To minimize nonspecific binding by blocking unoccupied solid-phase sites. Two hundred microliters of 0.1% casein in PBS (PBS/casein) was added, and the plates were incubated for 1 h at room temperature. The casein solution was removed, and each well was washed as above. Fifty microliters of serum sample diluted with PBS/casein was added to each well, and the plates were incubated for 1 d at 4°C. The solution was removed, and each well was washed as before. Fifty microliters of rabbit anti-mouse IgE (10⁻³ dilution in PBS/casein, Yamasa Shoyu Co., Ltd., Chiba, Japan) was added to each well, and the plates were incubated for 1 h at room temperature. The solution was removed, and each well was washed as before. Fifty microliters of sheep anti-rabbit-IgG-β-galactosidase conjugate (10⁻³ dilution in PBS/casein, Amersham, U.K.) was added to each well, and the plates were incubated for 1 h at room temperature. The antibody-enzyme conjugate solution was removed, and each well was washed. The wells were incubated for 1 h at 37°C with 100 μl PBS containing 0.1 mg 4-methylumbellifereone-β-galactoside (Sigma, St Louis, MO, U.S.A.). Finally, 25 μl of anhydrous sodium carbonate was added to each well. The fluorescence intensity of the liberated 4-methylumbellifereone was monitored by a TiterTek Fluoroskan reader (Flow Laboratories, Inc., Costa Mesa, CA, U.S.A.).

Sample Preparations  ACT from unripe apples was obtained according to the method reported by Ohnishi-Kameyama et al. ⁸

Statistical Analysis  All data were expressed as mean±S.D. Statistical analysis was performed using the Student’s t-test. In all cases, probability (p) values below 0.05 were considered significant.

RESULTS

Effects of ACT on Ear Swelling Responses of the Mice Bearing the IGEL a2 Hybridoma Induced by Epicutaneous Challenge with Picryl Chloride  The titers of serum anti-TNP IgE were quantified by ELISA to confirm the increase in the serum anti-TNP IgE level in the IGEL a2-bearing mice. In all the IGEL a2-bearing mice, the titers increased to more than 1000, of which the value corresponds to 0.5 mg/ml antibody amount, 7 d after the transplantation of the hybridoma (Data not shown).

The administration of 500 mg/kg ACT significantly inhibited ear swelling responses at 1 h after antigen challenge, and the extent of the inhibition was nearly the same as that of the antiallergic drug, 10 mg/kg body weight of ketotifen (Fig. 1). The time course of the ear swelling response of the ACT-administered mice is shown in Fig. 2. The ear swelling response was evoked within 1 h and kept its maximal level 2 h after antigen (picryl chloride) challenge. The swelling was slightly decreased at 4 h after antigen challenge and continued up to 24 h. Also, 500 mg/kg body weight of ACT pretreatment 1 h before antigen challenge inhibited the ear swelling responses.

Fig. 1. Effect of ACT on Ear Swelling Responses of the Mice Bearing the IGEL a2 Hybridoma 1 h after Epicutaneous Challenge

ACT (10 mg/mouse) or ketotifen (0.2 mg/mouse) was administered 1 h before antigen challenge. Ear swelling was measured at 1 h after the antigen challenge. The data for the blank were obtained from the mice that were not transplanted with the hybridoma. p<0.05, p<0.01 was assessed by a significant difference from the control. Bar shows the mean±S.D.

Fig. 2. Time Course of Inhibitory Effect of ACT on the Ear Swelling Responses by Epicutaneous Challenge with Picryl Chloride on the Hybridoma-Transplanted Mice

The hybridoma was not transplanted into the blank mice. ACT (10 mg/mouse) was administered orally 1 h before the antigen challenge. Ear swelling was measured at 1, 2, 4 and 24 h after the antigen challenge. Bar shows the mean±S.D.

Fig. 3. Dose Dependency of ACT on the Inhibition of Ear Swelling Responses of the Hybridoma-Transplanted Mice

ACT (0.1, 1, 10 mg/mouse) was administered orally 1 h before the antigen challenge. Ear swelling was measured at 1 h after the antigen challenge. Bar shows the mean±S.D.
during the first 24 h. Figure 3 shows the dose-dependency of ACT. ACT exerted significant inhibition at the 1 mg administration level (40% inhibition) and at the 10 mg administration level (67% inhibition).

**Effects of the Interval Between the Oral Administration of ACT and the Antigen Challenge on the Inhibition of the Ear Swelling Responses** ACT (500 mg/kg body weight) was orally administered to mice at 1, 2, 4, 6 and 24 h before the antigen challenge to investigate the effects of the interval between the ACT oral administration and the antigen challenge. The ear swelling was measured at 1 h after the antigen challenge. As shown in Fig. 4, the inhibition (%) reached the maximal level (90% inhibition) at the 2 h interval and then decreased. Only a 20% inhibition was observed at the interval time of 24 h.

**DISCUSSION**

The type I allergic model mouse used in our studies was assumed to be a biphasic allergic skin reaction. With priority given to immediate phase response, the oral administration of ACT inhibited an IgE-dependent allergic reaction in a 500 mg/kg body weight oral administration (Fig. 1). Also, the anti-allergic effect of 500 mg/kg body weight ACT was nearly the same as that of 10 mg/kg body weight ketotifen, which has been widely used in allergic disorders, such as bronchial asthma and/or atopic dermatitis. In addition, we showed that the inhibition of the ear swelling reached the maximal level at the 2 h interval between the ACT oral administration and the antigen challenge (Fig. 4). These results suggested that ACT was absorbed through the intestine and reached the connective tissue about 2—3 h after administration to exert the antiallergic effect. We used the in vivo model for type I allergy by transplanting antigen-specific IgE-secreting hybridomas into syngeneic mice and confirmed the increase in the antigen specific IgE level in the serum. Thus it was difficult to explain the antiallergic effect as suppression of the serum IgE level. It is believed that the release of histamine from the intracellular secretory granules is one of the important steps in the allergenic action. In the previous report, we showed that ACT inhibited histamine release from RBL-2H3 cells during antigen-specific stimulation by the antigen-IgE complex with anti-DNP IgE and DNP-BSA. These results might explain that the direct inhibition of histamine release from mast cells is involved in the inhibitory action of ACT.

The ear swelling at 24 h after antigen challenge seemed to be a late phase response (LPR) in the biphasic skin reaction. When ACT (500 mg/kg body weight) was administered orally 1 h before antigen challenge, the swelling derived LPR has a tendency to decrease. Nagai et al. demonstrated that the LPR was independent of mast cells and chemical mediators such as histamine. Moreover it was shown that flavonoids such as luteolin, baicalein, and quercetin inhibited the IgE-mediated biphasic cutaneous reaction mainly by the inhibition of histamine and cytokine release from mast cells, but not through mediator antagonistic effects. The ACT's function concerned with allergy was not enough to identify mechanisms other than inhibition of histamine release. Further study is needed to elucidate the inhibitory effect on LPR and its mechanism.

Many researchers have reported that tannins have biological activities, such as anti-tumor, antioxidant and anti-human immunodeficiency virus activity. In addition, absorption from the intestine and distribution to the tissues of monomeric tannins and their derivatives have been reported by several researchers. However, the biological effects of oligomeric tannins administered orally remain obscure, because it is not so clear that the oligomeric tannins would be absorbed in the intestine. The analysis with matrix-assisted laser desorption/ionization time-of-flight mass spectrometry and fast-atom bombardment mass spectrometry have revealed that ACT is a mixture of oligomeric procoyandins containing the dimer to the pentadecamer of catechin as a unit. Because our in vivo studies clearly showed the efficiency of ACT as an administered mixture on allergic symptoms, the absorption from the intestine, distribution to tissues, and the metabolism of components of ACT become of research interest.

In conclusion, the oral administration of ACT showed an inhibitory effect on the ear swelling response of a type I allergy mouse model. Fractionation of ACT according to the number of polymerized units is now in progress. Further studies on their isolated procoyanidin oligomers are needed to define the structural features involved in the antiallergic effect.

**Acknowledgement** The study was partly supported by a grant from the Japan Health Sciences Foundation.

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