Suppressive effect of ethanol extract from passion fruit seeds on IgE production

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Abstract
An array of foodstuffs was screened for the suppressive effect on production of IgE, a key molecule in the type I allergic reaction, using U266 cells. An ethanol extract from passion fruit seeds (PFS) has emerged as a food material with the IgE production-suppressive effect as the result of screening. Real-time RT-PCR analysis revealed that the IgE production-suppressive effect was caused by downregulated IgE gene expression. In addition, PFS selectively suppressed IgE production and did not affect the production of IgG, IgA, or IgM by mouse splenocytes. Resveratrol and piceatannol were identified as bioactive ingredients contained in passion fruit seeds. The effect of oral administration of PFS, resveratrol, and piceatannol was evaluated using a mouse model of allergic contact dermatitis. Both stilbenoids significantly suppressed IgE production in orally administered mice. Overall results indicated that passion fruit seeds are a promising candidate with the antiallergy effect for development of functional foods.

1. Introduction
The number of allergic patients goes on increasing rapidly year after year all over the world. Investigation by Ministry of Health, Labor, and Welfare of Japan has revealed that about one in two people has one or more allergic symptoms such as pollinosis, allergic asthma, and atopic dermatitis in Japan. However, treating allergic symptoms with medications sometimes produce a side effect. Hence, intake of foods with an antiallergy effect instead of drugs recently attracts much interest because of very few side effects. The need for such foods with scientific evidence is increasing, and the food industry gives increased attention to the achievements in the research area of functional foods.

Passion fruit (Passiflora edulis) belongs to the Passifloraceae family and consists of 12 genera and over 500 species (Rodriguez-Amaya, 2012). The fruit of P. edulis is an ellipse-rounded form and contains many seeds wrapped with jelly-like pulp. P. edulis is very popular among Passifloraceae family, not only because of its pulp, but also because of its leaves, which have been largely used in American and European countries as medicines such as sedative, tranquilizer, and antinflammatory drug (Montanher, Zucolotto, Schenkel, & Fröde, 2007). In fact, many studies have revealed that passion fruit exhibits many biological functions. Passion fruit seeds contain biologically functional
compounds such as resveratrol, piceatannol, and scirpusin B (Sano, Sugiyama, Ito, Katano, & Ishihata, 2011). Resveratrol exerts anti-inflammatory (de la Lastra & Villegas, 2005), antioxidant (de la Lastra & Villegas, 2007), anticancer, antihyperglycemic, and beneficial cardiovascular activities (Han et al., 2013). Piceatannol has strong antioxidant (Ovesná et al., 2006), anticancer (Kukreja, Wadhwa, & Tiwari, 2014), melanogenesis-suppressive, and collagen synthesis-stimulatory activities (Matsui et al., 2010). Scirpusin B, a dimer of piceatannol, has stronger antioxidant and vasorelaxant activities than piceatannol (Sano et al., 2011).

Although there are many papers reporting biological functions in passion fruit, its antiallergy effect has not reported yet. After screening of a bunch of foodstuffs for antiallergy effects, we have found a suppressive effect on IgE production in passion fruit seeds. IgE plays a major role in allergic reactions and is often called “allergic antibody”. In allergy, the immune system recognizes harmless substances like pollens and foods as hazardous antigens and then starts producing IgE antibodies. Serum IgE levels in patients with allergic diseases are much higher than those in healthy individuals. Allergic reactions are provoked when an allergen encounters IgE in our body. Thus, suppressing IgE production is of great importance to prevent and alleviate allergic symptoms. We herein report that the ethanol extract from passion fruit seeds has an effect to suppress IgE production and suggest that resveratrol and piceatannol would be the bioactive ingredients contained in passion fruit seeds.

2. Materials and methods

2.1. Reagents

Resveratrol (3,4',5-trihydroxy-trans-stilbene; >98% purity, Fig. 1) and piceatannol (3,3',4,5-tetrahydroxy-trans-stilbene; >98% purity, Fig. 1) were purchased from Wako Pure Chemical Industries (Osaka, Japan) and Tokyo Chemical Industry (Tokyo, Japan), respectively, and dissolved in ethanol to make stock solutions. Roswell Park Memorial Institute-1640 (RPMI-1640) medium, penicillin, streptomycin, insulin, transferrin, ethanolamine, sodium selenite, fetal bovine serum (FBS), ovalbumin (OVA), bovine serum albumin (BSA) and mineral oil were products of Sigma-Aldrich (St. Louis, MO, USA). Anti-human IgE antibody and biotin-conjugated anti-human IgE antibody were purchased from Biosource International (Camarillo, CA, USA). Rat anti-mouse IgE and biotin-conjugated rat anti-mouse IgE were from BD Pharmingen (San Diego, CA, USA). Goat anti-mouse IgA antibody, horseradish peroxidase (HRP)-conjugated goat anti-mouse IgA antibody, rabbit anti-mouse IgM antibody, HRP-conjugated goat anti-mouse IgM antibody, and HRP-conjugated streptavidin were purchased from Invitrogen (Carlsbad, CA, USA). Goat anti-mouse IgG antibody was purchased from MP Biomedicals (Santa Ana, CA, USA). HRP-conjugated goat anti-mouse IgG antibody was purchased from Thermo Fisher Scientific (Waltham, MA, USA). Goat anti-actin IgG antibody and HRP-conjugated donkey anti-goat IgG antibody were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). HRP-conjugated anti-rabbit IgG antibody and rabbit antibodies against NF-κB p65 and lamin B1 were purchased from Cell Signaling Technology (Danvers, MA, USA). 1-Fluoro-2,4-dinitrobenzene (dinitrofluorobenzene; DNFB) was obtained from Nacalai Tesque (Kyoto, Japan). All other chemicals were purchased from Wako Pure Chemical Industries or Nacalai Tesque unless otherwise noted.

2.2. Sample preparation

The fruit of Passiflora edulis was provided by Okinawa Miyakolichiba (Okinawa, Japan). Seeds were freeze-dried, milled, and suspended in ethanol at 0.25 g/mL. After stirring for 20 h at 4 °C, the suspension was centrifuged at 10,000g for 20 min at 4 °C. The supernatant was evaporated and dissolved in a small volume of ethanol. The extract was then filtered through a 0.22 μm membrane filter and used as a passion fruit seed ethanol extract (PFS).

2.3. Mice

BALB/c mice were purchased from Japan SLC (Shizuoka, Japan) and kept in an animal room under a 12 h light/dark cycle at a temperature of 24 ± 1 °C. Animals received a pelleted basal diet and water ad libitum. All animal experiments described in this study were carried out in accordance with the protocol approved by the Laboratory Animal Care Committee of Ehime University. Mice were maintained in accordance with the Guidelines for the Care and Use of Laboratory Animals of Ehime University.

2.4. Cells and cell culture

Human myeloma U266 cells, a cell line producing IgE, were obtained from American Type Culture Collection (Rockville, MD, USA). Mouse primary splenocytes were prepared from 6-week-old female BALB/c mice as previously described (Daiifu et al., 2011). U266 cells and primary splenocytes were cultured in RPMI-1640 medium supplemented with 100 U/mL of penicillin, 100 μg/mL of streptomycin, and 5% FBS at 37 °C under humidified 5% CO2.

2.5. Mouse IgE enzyme-linked immunosorbent assay (ELISA)

The concentration of mouse IgE secreted into culture media was measured by an in-house-developed ELISA. Each well of a 96-well microtiter plate (Nunc, Roskilde, Denmark) was coated with anti-mouse IgE diluted at 0.5 μg/mL in 50 mM carbonate buffer (pH 9.6) at 4 °C overnight. After washing with phosphate-buffered saline (PBS) containing 0.05% Tween 20 (PBS-T) 3 times, each well was blocked with PBS containing 5% (w/v) skim milk for 2 h at
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