Inhibitory Effects of Guarana Seed Extract on Passive Cutaneous Anaphylaxis and Mast Cell Degranulation

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This study investigated the effects of guarana seed extract (GSE) on an anti-allergic mechanism. GSE orally administered inhibited the anti-dinitrophenol IgE-induced passive cutaneous anaphylaxis reaction in mice. Furthermore, it inhibited the degranulation of rat basophilic leukemia RBL-2H3 cells. It had no cytotoxicity on RBL-2H3 cells. These results show that GSE is a candidate for effective therapeutic material for allergic diseases.

Key words: mast cell; degranulation; passive cutaneous anaphylaxis; anti-allergic effect; guarana

Allergic diseases, such as allergic rhinitis, atopic dermatitis, asthma, and food allergy, have increased in most countries.1,1 Mast cells and basophils play critical roles in various biological processes of allergic diseases.2,3 These cells express the high-affinity receptor for IgE on their surface. The interaction of multivalent antigens with surface-bound IgE secretes granule-stored mediators, and leads to de novo synthesis of cytokines.4,5 These mediators and cytokines activate migration of neutrophils and macrophages, and reaction by these cells causes tissue inflammation.6,7

Guarana (Paullinia cupana Kunth.) seed is used as a traditional remedy in the treatment of ulcers, carcinogenesis, and oxidation.5,6,7,8 However, the effects of guarana seed on allergy have not been reported to date. In this study, we investigated the inhibitory effects of oral administration of an aqueous ethanolic extract of P. cupana seed (guarana seed extract, GSE) on the increase in vascular permeability induced by stimulation with IgE of ICR mice. We also investigated the effects of GSE on the degranulation of mast cells induced by IgE.

Seeds of P. cupana (100 g), purchased from Tochimoto Tenkaido (Osaka, Japan), were extracted with 30% aqueous ethanol (0.9 liter) under reflux for 2 h. After cooling and filtration, the filtrate was concentrated in vacuo and lyophilized to give an extract (GSE; yield, 80%). The dried extract was dissolved in 10% dimethylsulfoxide (DMSO) and diluted with saline or Tyrode's buffer9 before use.

Female 6-week-old ICR mice (25–27 g) were purchased from Japan SLC (Hamamatsu, Japan). All animal experiments were performed according to the “Guidelines Relating to the Care and Use of Experimental Animals” of the Japanese Association for Laboratory Animal Science.

We examined the effects of GSE on the passive cutaneous anaphylaxis (PCA) reaction, one of the most important in vivo models of anaphylaxis in local allergic reactions.10,11 The effect of GSE on an IgE-dependent PCA was measured as follows. The mice were injected intradermally with 0.1 μg of anti-dinitrophenol (DNP) IgG (Sigma-Aldrich Japan, Tokyo) into the ear. Four h after sensitization with IgE, the mice were challenged intravenously with 0.2 ml (1 mg/ml) of DNP-human serum albumin (HSA) (Sigma-Aldrich) containing 2% Evans blue dye (Wako Pure Chemical Industries, Osaka). In some experiments, GSE (0.1, 0.3, or 1.0 g/kg) or saline (control) was administered orally 2 h before antigen challenge. Since we confirmed that 10% DMSO did not affect the value of the PCA reaction, we used saline as the control. Ketotifen fumarate (Sigma-Aldrich, 0.02 g/kg), a histamine H1 receptor antagonist and chemical mediator release suppressor, was administered intraperitoneally 30 min before antigen challenge. Thirty min after antigen challenge, the mice were sacrificed, and the ears were removed and weighed. To measure the amount of Evans blue dye in the exudates, the ears were dissolved in 400 μl of KOH and incubated at 37°C overnight. Dissolved tissue solution was then added to 800 μl of a mixture of acetone and phosphoric acid (5:13 v/v), and the optical density was measured at 620 nm. The amount of exudate dye was calculated from the standard curve of a known concentration of Evans blue.

The results were expressed as percentage of the mean amount of exudate dye in the mice administered each sample to the control mice administered saline.

When a low dose of GSE (0.1 or 0.3 g/kg) was orally administered to the ICR mice, there was no change in the amount of exudate dye. In contrast, when a high dose of GSE (1.0 g/kg) was orally administered, the amount of exudate dye was significantly reduced (Fig. 1). This means that GSE inhibited the PCA reaction in a dose-
The effects of GSE (0.1, 0.3 and 1.0 g/kg) and of Ketotifen fumarate (0.02 g/kg) on an IgE-dependent PCA reaction were investigated. Mean ± SE of 12 to 16 mice are shown. *p < 0.01 by t-test as compared to the value of the control.

Ketotifen fumarate (0.02 g/kg), an anti-allergic drug often used as a positive control for anti-allergic reactions targeting mast cell degranulation, also inhibited the PCA reaction.

To understand the mechanism of inhibition of GSE on the anaphylactic reaction, we investigated the effects of GSE on anti-DNP IgE-mediated mast cell degranulation. Since it has been reported that the release of β-hexosaminidase was well correlated with that of histamine, a major component of mast cell granules,12) degranulation of mast cells was determined by β-hexosaminidase release assay, as previously described.13) Briefly, rat basophilic leukemia RBL-2H3 cells were maintained in Dulbecco’s modified Eagle’s medium (ICN Biochemicals, Costa Mesa, CA) supplemented with 10% heat-inactivated fetal bovine serum. RBL-2H3 (ICN Biochemicals, Costa Mesa, CA) supplemented with 10% heat-inactivated fetal bovine serum. RBL-2H3 were treated with various concentrations of GSE for 1 h. Cell supernatants and total cell lysates solubilized with 1% Triton X-100 (Sigma-Aldrich) were collected, and the β-hexosaminidase in the supernatants and cell lysates was quantified by spectrophotometric measurement of hydrolysis of p-nitrophenyl-N-acetyl-β-D-glucopyranoside (Sigma-Aldrich) in 0.1 M sodium citrate buffer (pH 4.5). The reaction was terminated by the addition of 0.2 M glycine (pH 11.0). The percentage of inhibition of β-hexosaminidase release was calculated using the following formula: percentage of inhibition = [(β-hexosaminidase release without GSE − β-hexosaminidase release with GSE)/β-hexosaminidase release without GSE] × 100.

As Fig. 2 shows, β-hexosaminidase release was drastically decreased by the addition of GSE (12.5–200 µg/ml) in a dose-dependent manner. This indicates the suppressive effects of GSE on the degranulation of RBL-2H3 cells induced by IgE-antigen complex. These findings indicate that GSE exerted an anti-allergic effect by inhibiting the release of chemical mediators from the mast cells.

Next, we examined the effects of GSE on the viability of RBL-2H3 cells. RBL-2H3 cells were treated with various concentrations of GSE for 24 h. Viability was determined by the trypan blue exclusion method. Each value represents the mean ± SE for three experiments.

exclusion method.14) GSE (50–200 µg/ml) had no cytotoxic effect (Fig. 3). This result is consistent with a study that indicated that guarana extract was not toxic in vitro.15) Furthermore, Espinola et al. demonstrated the absence of toxicity of guarana extract in vivo.16) Guarana extract contains caffeine and tea catechins that have proven anti-allergic activity.17–20) We examined to determine whether these substances are the main anti-allergic constituents in the GSE. The contents of caffeine and tea catechins were measured with a LC-10 high-performance liquid chromatography (HPLC) system (Shimadzu, Kyoto, Japan) consisting of pumps, a column oven, a 20-µl injection loop, an auto-injector, a SPD-10A ultraviolet-visible spectrophotometer, and a C-R7A chromatopack equipped with a Cosmosil 5C18-MS-II column (5 μm pore size, 150 mm × 4.6 mm, Nacalai Tesque, Kyoto, Japan). The temperature of the column oven was set at 40 °C. The mobile phase components were 10 mM sodium phosphate buffer (pH 2.6) (solvent A) and acetonitrile (solvent B). The analyses were performed under gradient elution conditions. Elution with a flow rate of 1.2 ml/min was as
follows: 0–25 min, 0–25% B in A; 25–35 min, 25–100% B in A; 35–45 min, 100% in B. The detection wavelength and sample volume were set at 280 nm and 10 μl respectively. The concentrations of the caffeine and catechins in the extracts were calculated from the calibration curves. The content of caffeine in the GSE was 16%. Although 100 μg/ml of GSE showed inhibitory effect of mast cell degranulation, 100 μg/ml of caffeine (Wako) did not have any effect on mast cell degranulation in our experimental system. Since Shin et al. reported that caffeine inhibited histamine release from mast cells at concentrations from 5 to 20 μM,19 there is a possibility that the effectiveness of caffeine depends on the concentration used in each experiment. These results indicate that caffeine was not responsible for the anti-allergic effects observed in the present study.

In the next experiment, we examined the effects of removal of polyphenolic substances such as tea catechins from GSE using polyvinylpolypyrrolidone (PVPP).21 Twenty g of PVPP (Polyclay VT®, ISP Japan, Tokyo) was mixed with 2 g of GSE dissolved in 30% EtOH, and this was stirred at room temperature for 1 h. After centrifugation at 3,000 g for 15 min and filtration, the filtrate was concentrated in vacuo and lyophilized to give a PVPP-treated by GSE (yield, 55%). The total polyphenol content was measured by Folin-Ciocalteu’s method as (+)-catechin (C) (Tokyo Chemical Industries, Tokyo) equivalent. The polyphenol contents of GSE and the PVPP-treated GSE were 38% and 2.7% respectively. Treatment by PVPP significantly reduced the inhibitory effect of mast cell degranulation (data not shown), suggesting that anti-allergic constituents are polyphenolics in the GSE. Although the contents of C and (-)-epicatechin (EC) (Sigma-Aldrich) in the GSE were 5.8% and 4.7% respectively by HPLC analyses, those of (-)-epicatechin gallate (ECg), (-)-epigallocatechin (EGC), (-)-epigallocatechin gallate (EGCG), and (-)-catechin gallate were not detected, or were detected only in trace quantities (<0.1%). Neither C nor EC inhibited mast cell degranulation in our system (data not shown). This is consistent with a report that ECg, EGC, and EGCG showed anti-allergic activity, whereas C and EC did not.23 These results indicate that the anti-allergic effects of GSE are not due to tea catechins. Taking all this together, besides caffeine and tea catechins, GSE contained certain substances that showed the anti-allergic effects observed in the present study. Further investigation is necessary to identify substances that have anti-allergic effects in the GSE.

In conclusion, GSE orally administered inhibited the anti-DNP IgE-induced PCA reaction. It also inhibited the release of β-hexosaminidase from RBL-2H3 cells elicited by the IgE receptor-mediated pathways. These results indicate that GSE had an inhibitory effect on an allergic reaction. Furthermore, the in vivo and in vitro anti-allergic effects of GSE suggest possible therapeutic application of this component in inflammatory allergic diseases.

Acknowledgments

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References