

Note

Preventive Effects of C-2 Epimeric Isomers of Tea Catechins on Mouse Type I Allergy

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Summary The preventive effects of C-2 epimeric isomers of (–)-epigallocatechin-3-O-gallate (EGCG) and the O-methylated derivative, (–)-epigallocatechin-3-O-(3-O-methyl)gallate (EGCG3"Me), against ovalbumin-induced type I allergy in male mice were investigated. EGCG and EGCG3"Me exhibited strong antiallergic effects by oral administration at doses of 25 and 50 mg/kg body weight. The antiallergic effects of their C-2 epimers, (–)-gallocatechin-3-O-gallate and (–)-gallocatechin-3-O-(3-O-methyl)gallate (GCG3"Me), on mouse type I allergy were almost equivalent to and/or as strong as those of the corresponding original catechins, respectively. Oral administration of these compounds at a dose of 50 mg/kg body weight tended to suppress the increases in interleukin-4 levels in the abdominal walls of allergic mice and immunoglobulin E levels in the serum of allergic mice. In particular, the administration of GCG3"Me exhibited significant effects on the production and/or release of these parameters stimulating type 2 T helper cells and mast cells in the type I allergic process. These results indicated that C-2 epimerization of tea catechins, which are produced during heat processing at high temperatures, would not be disadvantageous for preventive effects on type I allergy.

Key Words tea, catechin, O-methylated catechin, type I allergy, epimerization

Recently, the increases in incidence rates of various allergic diseases due to lifestyle changes, including dietary habits, have become serious social problems. Allergies are generally divided into 4 types based on their mechanisms (1). The most frequent allergic diseases are type I allergy, such as asthma and pollinosis. Type I allergy is known as immediate hypersensitivity and occurs via a humoral immune response. It was reported that the water extracts of green tea, oolong tea, and black tea produced from tea leaves (*Camellia sinensis* L.) and their catechin components inhibited passive cutaneous anaphylaxis reaction in rats after oral administration (2). This efficiency of (–)-epigallocatechin-3-O-gallate (EGCG), a major catechin in tea leaves, was due to the suppressive effects on activation of mast cells and the enhancement of vascular permeability (3). We reported previously that (–)-epigallocatechin-3-O-(3-O-methyl)gallate (EGCG3"Me), an O-methylated derivative of EGCG, was present in certain tea leaves, such as Tong ting oolong tea and some cultivars of "Benihomare" and "Benifuuki" (4, 5). EGCG3"Me also prevented mouse type I allergy as well as EGCG, and the effects of EGCG3"Me exceeded those of EGCG (6). Therefore, daily intake of tea as a beverage could be beneficial in the prevention of allergic disorders.

Heat-processing at high temperature is carried out to extract the tea components from leaves and for sterilizing the spores of thermophilic anaerobes in the production of packaged tea beverages (7). In green tea infusions with hot water or in heated catechin solutions, some C-2 epimers (2S, 3R type) of the original tea catechins (2R, 3R type) were detected (8, 9). The heating of EGCG and EGCG3"Me dissolved in buffer solution at pH 6.0 for 30 min at 90°C resulted in approximately 30–40% epimerization (9), and (–)-gallocatechin-3-O-gallate (GCG) and (–)-gallocatechin-3-O-(3-O-methyl)gallate (GCG3"Me) were produced, respectively. We demonstrated previously that GCG and GCG3"Me prevented mouse type IV allergy, a delayed-type hypersensitivity, as well as EGCG and EGCG3"Me (10). However, there have been few reports regarding the antiallergic effects of GCG and GCG3"Me on type I allergy. In an in vitro experiment, the inhibitory activity of GCG on histamine release from rat peritoneal mast cells passively sensitized with anti-egg albumin immunoglobulin E (IgE) antibody was greater than that of EGCG (11). In the present study, we investigated the ability of GCG and GCG3"Me to prevent mouse type I allergy.

Materials and Methods

The allergen ovalbumin (OVA) was purchased from Sigma-Aldrich Co. (St. Louis, MO, USA) and Freund's

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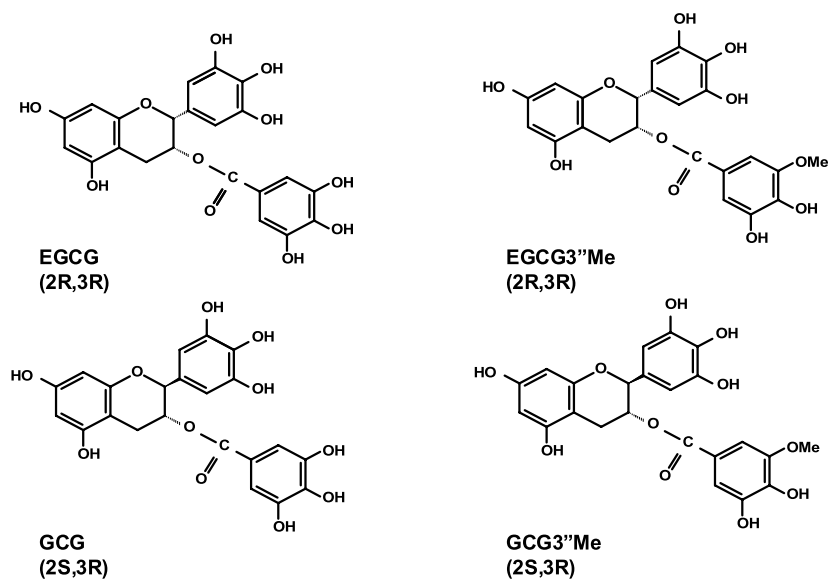


Fig. 1. Chemical structures of original tea catechins (2R,3R type) and their epimers (2S,3R type). EGCG, (–)-epigallocatechin-3-O-gallate; GCG, (–)-gallocatechin-3-O-gallate; EGCG3''Me, (–)-epigallocatechin-3-O-(3-O-methyl)gallate; GCG3''Me, (–)-gallocatechin-3-O-(3-O-methyl)gallate.

incomplete adjuvant (FIA) and diphenhydramine hydrochloride were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). GCG and EGCG were purchased from Sigma-Aldrich Co. and Kurita Co. (Tokyo, Japan), respectively. EGCG3''Me and GCG3''Me were prepared from Benihomare and Tong ting tea leaves by the method described previously (6). The chemical structures of these catechin samples are summarized in Fig. 1. Other chemicals used were of reagent grade. Four-week-old male ddY mice were purchased from Japan SLC, Inc. (Shizuoka, Japan).

The antiallergic activities of EGCG, EGCG3''Me, GCG, and GCG3''Me in mouse type I allergy were examined using the mouse abdominal wall method reported previously (12), with slight modifications. Briefly, 4-wk-old male ddY mice were sensitized intraperitoneally with a 1 : 1 mixture of OVA (2 mg/mL N-saline) and FIA. The catechin samples were administered orally to mice 9 d after initial exposure to OVA. In the Allergy group, distilled water was administered instead of the catechin samples. The doses of catechins and their C-2 epimers were 25 or 50 mg/kg body weight. Diphenhydramine hydrochloride, a known antihistamine, was used as a positive control. Its dose was 1 mg/kg body weight. Sixty minutes after administration of the sample, 0.1 mL of Evans blue dye solution (10 mg/mL N-saline) was administered intravenously. Within 5 min after injection of the dye, the abdominal skin of the mice was detached under ether anesthesia, without injury to the abdominal wall. Five minutes after injection of the dye, 50 μ L of OVA solution (5 μ g/site) was injected into the exposed abdominal wall. The mice were killed by cervical dislocation 7 min after challenge, and the abdominal wall was removed. The area of the abdominal wall permeated by blue dye was measured by densitography with spot image processing software (AE-6920; Atto, Tokyo, Japan).

Throughout the experiment, the animals were handled in accordance with The Guide for the Animal Experiments in Numazu National College of Technology, which is based on Fundamental Guidelines for Proper Conduct of Animal Experiment and Related Activities in Academic Research Institutions under the Jurisdiction of the Ministry of Education, Culture, Sports, Science and Technology.

Another experiment was performed according to the method described above with some alterations. The dose of the catechin samples was 50 mg/kg body weight. The solution of Evans blue dye was not injected because it would inhibit determination of cytokines and antibodies in the samples. After the experiment, the abdominal wall was taken and the supernatant of 0.5% mouse ear homogenate in 0.04 M phosphate buffer (pH 7.4) was prepared. Mouse blood was also taken and centrifuged at 1,500 $\times g$ for 10 min, and the supernatant was used as the serum fraction. The levels of interleukin-4 (IL-4) and IL-10 in the mouse abdominal wall were determined by enzyme-linked immunosorbent assay (ELISA) with Mouse IL-4 and IL-10 ELISA Kits, respectively (BioSource, San Jose, CA, USA). The levels of IgE, an antibody related to type I allergic responses, in mouse serum were determined by ELISA with a Mouse IgE ELISA Quantitation Kit (Bethyl Laboratories, Inc., Montgomery, TX, USA).

Statistical analyses were performed with Student's *t* test to determine the significance of differences between the appropriate experimental groups, and $p < 0.05$ was considered statistically significant.

Results and Discussion

As shown in Fig. 2, oral administration of EGCG, GCG, EGCG3''Me, and GCG3''Me at 25 or 50 mg/kg body weight significantly inhibited mouse type I allergy, although their activities were lower than that of

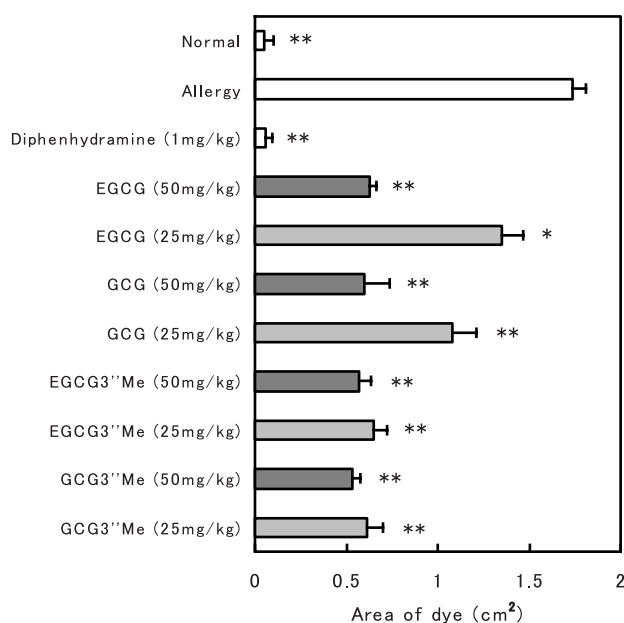


Fig. 2. Preventive effects of oral administration of EGCG, EGCG3''Me, GCG, and GCG3''Me on mouse type I allergy. Values are the means $\pm$ SE. $n=5$. Significant difference from Allergy group: * $p<0.05$, ** $p<0.01$.

diphenhydramine. The activities were dose-dependent at doses of 25 and 50 mg/kg body weight for all catechin samples used. The antiallergic effects of oral administration of GCG and GCG3''Me were almost equivalent to or higher than those of EGCG and EGCG3''Me, respectively. There were no significant differences between the two groups for any catechin tested. These results suggest that C-2 epimerization of tea catechins is not disadvantageous for the preventive effects on mouse type I allergy. Similar to the results of our previous study (6), the antiallergic effects of *O*-methylated derivatives, EGCG3''Me and GCG3''Me, tended to be stronger than those of EGCG and GCG. Significant differences ($p<0.05$) between catechins and their *O*-methylated derivatives were observed at a dose of 25 mg/kg body weight.

The mechanisms for the antiallergic effects of the catechin samples were investigated. The effects of diphenhydramine were not examined in this study, because it has been shown to act as an H1 blocker. As shown in Fig. 3, the levels of IL-4 and IL-10 in the abdominal wall and those of IgE in the serum of mice with type I allergy were significantly higher than those of normal mice. Figure 3A shows that oral administration of EGCG, EGCG3''Me, GCG, and GCG3''Me tended to suppress the increase in IL-4 level in the abdominal wall of mice with type I allergy at a dose of 50 mg/kg body weight. A significant difference was observed only between normal mice and GCG3''Me-administered mice. The approximate order of the effects was GCG3''Me>EGCG3''Me>GCG>EGCG as well as that of their antiallergic effects. Similar results were observed in the suppressive effects of these compounds at a dose of 50 mg/kg body weight on IgE levels in mouse sera, as shown in Fig. 3C. With regard to IgE levels in mouse sera, significant differ-

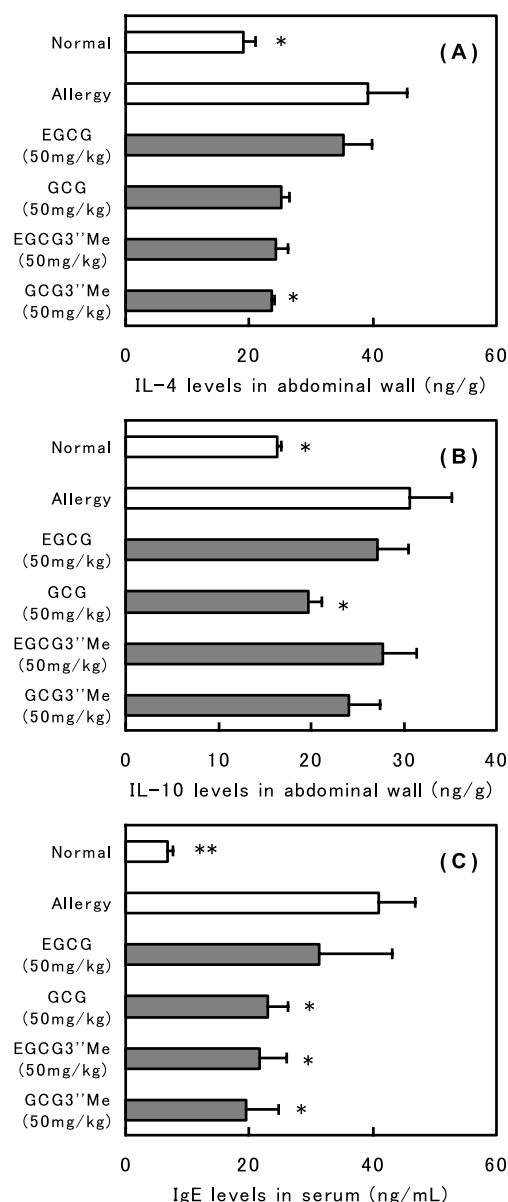


Fig. 3. Suppressive effects of oral administration of C-2 epimers of tea catechins on abdominal wall IL-4 and IL-10 levels and serum IgE levels in mice affected by type I allergy. (A) IL-4 levels in abdominal wall; (B) IL-10 levels in abdominal wall; (C) IgE levels in serum. Values are the means $\pm$ SE. $n=5$. Significant difference from Allergy group: * $p<0.05$, ** $p<0.01$.

ences were observed in all cases except between normal control and EGCG-administered mice. Oral administration of EGCG, EGCG3''Me, GCG, and GCG3''Me at a dose of 50 mg/kg body weight also tended to suppress the increase in IL-10 level in the abdominal wall of mice with type I allergy, as shown in Fig. 3B. In the case of IL-10, however, the approximate order of the effects was different from that of the antiallergic effects, GCG>GCG3''Me>EGCG>EGCG3''Me. The effects of GCG and GCG3''Me tended to be higher than those of EGCG and EGCG3''Me, although the effects of *O*-methylated catechins tended to be lower than those of the catechins.

We propose a scheme of the possible preventive effects of EGCG, EGCG3''Me, GCG, and GCG3''Me

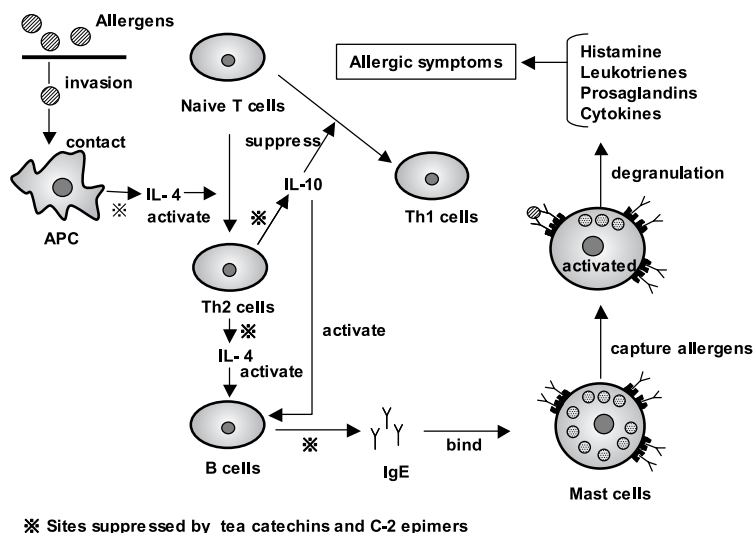


Fig. 4. Prospective preventive roles of C-2 epimers of tea catechins on mouse type I allergy.

against mouse type I allergy in Fig. 4. Antiallergic effects of tea catechins and their C-2 epimers could be mainly due to the following suppressive functions: (i) inhibition of production and/or release of IL-4 from macrophage-like antigen presenting cells (APC) and type 2 T helper (Th2) cells; (ii) inhibition of production and/or release of IgE from B cells. The former action would suppress the differentiation of naive T cells into Th2 cells and decrease the subsequent immune responses. The latter action would suppress the degranulation of mast cells and the decrease in vascular permeability caused by histamine. It is also expected that the latter action occurred because of the former action. It has been reported that the extract of tea and catechins could inhibit histamine release from rat mast cells (2, 13) and we demonstrated that EGCG3"Me inhibited the degranulation process in human basophils (14). The results of this study indicated that GCG and GCG3"Me exerted the same effects as EGCG and EGCG3"Me. EGCG downregulated IL-10 production of macrophages induced by *Legionella pneumophila* (15) and this effect promoted the differentiation of naive T cells into Th1 cells. The decreases in numbers of Th2 cells and levels of γ -interferon, a cytokine produced by macrophages and Th1 cells, would suppress the differentiation of naive T cells into Th2 cells and restrain the activation of B cells. In this study, the suppressive effects of GCG and GCG3"Me on production and/or release of IL-10 from macrophages and/or Th2 cells were demonstrated. The decrease in IL-10 level would partly contribute to the antiallergic effects of GCG and GCG3"Me.

In addition to type IV allergy, some metabolites of arachidonic acid, such as prostaglandins and leukotrienes, are also known as proinflammatory chemical mediators in type I immediate hypersensitivity. Active oxygen species also play an important role in the inflammatory process. Tea catechins are potent natural antioxidants and can inhibit inflammation (16). It was reported that the antioxidant activities of GCG and GCG3"Me were almost equivalent to and/or as high as

those of EGCG and EGCG3"Me (17, 18). The antioxidant activities of these compounds would contribute to their preventive effects on type I allergy. To study the mechanisms of the antiallergic effects of catechins and their C-2 epimers, it is important to examine absorption from the intestinal tract and metabolism of these compounds. However, little information is available regarding these points. Further investigations are necessary to clarify the absorption, distribution, and metabolism of these compounds after oral administration.

The results of the present study suggest that treatment of tea infusions at high temperature for a long period, heat-processing for extraction and sterilization, and warming in vending machines, could enhance the type I allergy preventive ability.

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