JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Inhibitory Effects of the C-2 Epimeric Isomers of Tea Catechins on Mouse Type IV Allergy

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The inhibitory effects of C-2 epimeric isomers of (−)-epigallocatechin-3-*O*-gallate (EGCG) and two O-methylated EGCG derivatives, (−)-epigallocatechin-3-*O*-(3-*O*-methyl)gallate (EGCG3″Me) and (−)-epigallocatechin-3-*O*-(4-*O*-methyl)gallate (EGCG4″Me), against oxazolone-induced type IV allergy in male mice were investigated. These compounds exhibited strong antiallergic effects by percutaneous administration at a dose of 0.13 mg/ear. The inhibition rates of (−)-gallocatechin-3-*O*-gallate (GCG), (−)-gallocatechin-3-*O*-(3-*O*-methyl)gallate (GCG3″Me), and (−)-gallocatechin-3-*O*-(4-*O*-methyl)gallate (GCG4″Me) on mouse type IV allergy were 52.1, 53.3, and 54.8%, respectively. However, the antiallergic effects were weaker than those of their corresponding original tea catechins (2*R*,3*R* type). The inhibition rates of those were 88.0, 73.2, and 77.6%, respectively. For all of the catechins tested, oral administration at a dose of 50 mg/kg body weight significantly suppressed the allergic symptoms. The inhibitory rates varied from 24.0 to 60.6%. No significant differences were observed between the effects of the epimers (2*S*,3*R* type) and their corresponding original catechins (2*R*,3*R* type). The antiallergic effects of tea catechins and their C-2 epimers observed in this study were dose-dependent. These results suggest that C-2 epimers of tea catechins, which are produced during heat processing at high temperatures, could be disadvantageous for the antiallergic effects on type IV allergy.

KEYWORDS: Tea; catechin; O-methylated catechin; mouse; type IV allergy; epimerization

INTRODUCTION

EGCG, a specific and major flavan-3-ol derivative in tea leaves (*Camellia sinensis* L.), is known to have inhibitory effects on allergic reactions in animal models (*1*). The antiallergic effects of the *O*-methylated derivatives of EGCG, EGCG3″Me, and EGCG4″Me on mouse type I and type IV allergy were also reported (*2*, *3*). These *O*-methylated catechins are present in certain tea leaves, such as Tong ting oolong tea and the cultivars of Benihomare, Benifuuki, and Benifuji, which are classified as Assam hybrids (*4*, *5*). These studies demonstrated that daily intake of tea as a beverage could be beneficial in the prevention of allergic disorders.

Some C-2 epimers (2*S*,3*R* type) of the original tea catechins (2*R*,3*R* type) were detected in green tea infusions with hot water or in heated catechin solutions (*6*, *7*). Heat processing at a high temperature is required to extract the tea components from leaves and to sterilize the spores of thermophilic anaerobes in the production of packaged tea beverages (*8*). It has been reported that the C-2 epimers could be more effective to produce the precipitate with cholesterol in the micelles than the original tea catechins (*9*). While the antioxidant activities of the C-2 epimers of tea catechins have also been reported (*10*, *11*), little is known about the antiallergic effects of the C-2 epimers. In this study, we investigated the ability of the C-2 epimers of EGCG and its *O*-methylated derivatives (**Figure 1**) to prevent mouse type IV allergy induced by oxazolone.

MATERIALS AND METHODS

Materials and Animals. Oxazolone (4-ethoxymethylene-2-phenyl-2-oxazolin-5-one) was purchased from Sigma-Aldrich Chemical Co. (Milwaukee, WI) and used as a sensitizer for type IV allergy. EGCG was purchased from Kurita Co. (Tokyo, Japan). Other tea catechins and their C-2 epimers (**Figure 1**) were prepared from Benihomare and Tong ting tea leaves by the method described in our previous paper (*2*). Male ICR mice, 4 weeks old, were purchased from Japan SLC Inc. (Shizuoka, Japan).

Determination of Mouse Type IV Allergic Response. The type IV allergic response was evaluated using mouse contact hypersensitivity to oxazolone. The formation of ear edema by oxazolone as a simple hapten was observed in this response. The type IV allergic response was determined according to the previously reported procedures (*3*, *12*). The hair of the abdominal region of the mice was carefully removed, and 0.1 mL of 0.5% oxazolone solution in ethanol was applied to the skin (sensitization procedure). Five days after sensitization, 20 μL of 0.5% oxazolone solution in acetone was applied to both sides of each animal's right ear (challenge procedure). Twenty-four hours after the challenge, the mice were sacrificed under anesthesia with diethyl ether. Circular parts (5.0 mm in diameter) of both ears were removed

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10.1021/jf035476r CCC: $27.50
Published on Web 06/24/2004

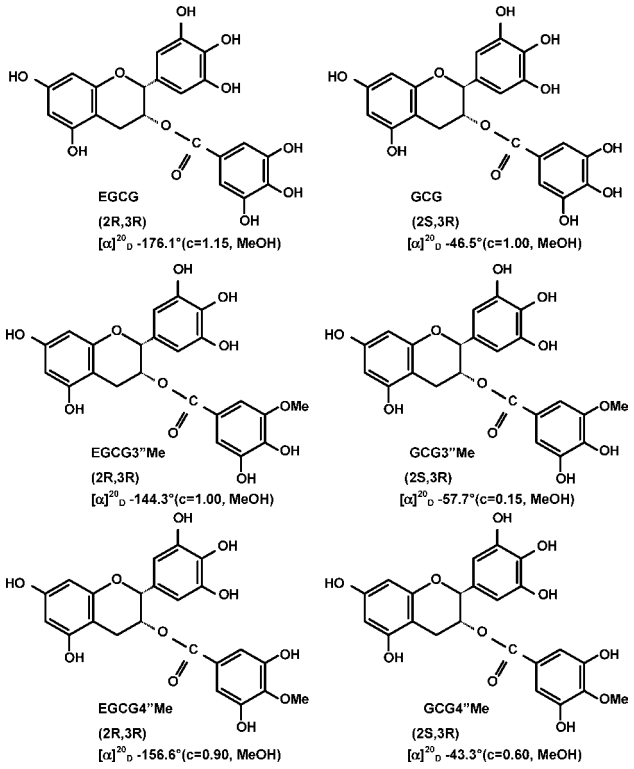

Figure 1. Chemical structures of original tea catechins (2*R*,3*R* type) and their epimers (2*S*,3*R* type). EGCG, GCG, EGCG3″Me, GCG3″Me, EGCG4″Me, and GCG4″Me.

using a punching apparatus. The weights of the right (WR) and left (WL) ears were measured. In the case of percutaneous administration of samples to the mice, the tea catechins and their C-2 epimers were dissolved in an oxazolone/acetone solution at challenge. The mixture was prepared in chilled water just before use. The tea catechins were administered at doses of 0.025, 0.05, 0.10, and 0.13 mg/ear to study the dose dependency of the antiallergic effects, and the C-2 epimers of the tea catechins were administered at doses of 0.05 and 0.13 mg/ear to compare their antiallergic effects with those of the original tea catechins. Hydrocortisone, a well-known steroid type antiinflammatory agent, was assayed in the same manner. A control was performed with only the solvents used for the dissolution of samples. In the case of oral administration, the catechin samples were dissolved in 0.5% tragacanth gum solution and administered orally to mice 1 h prior to the challenge. EGCG was administered at doses of 5, 10, 50, and 100 mg/kg body weight to study the dose dependency of the antiallergic effect, and the tea catechins and their C-2 epimers were administered at doses of 10 and 50 mg/kg body weight to compare their antiallergic effects. Hydrocortisone was assayed at doses of 10, 50, and 100 mg/kg body weight. The leaf extract of Benihomare tea was prepared by extraction for 40 min at 30 °C, followed by filtration with a 0.45 μm membrane filter. This extract was heated for a further 40 min at 100 °C. When a 200 μL aliquot of the tea extract with or without the heat treatment was percutaneously administered, the antiallergic effect was determined by the same procedure described above. The ear swelling ratio was calculated using the following equation:

$$\text{ear swelling ratio (\%)} = \{(WR_{sample} - WL_{sample})/WL_{sample}\}/\{(WR_{control} - WL_{control})/WL_{control}\} \times 100$$

Statistical Analysis. Statistical analyses were performed with the nonparametric Kruskal−Wallis test to determine the significance of differences within experimental groups, followed by the nonparametric Mann−Whitney U test for differences between the appropriate groups. A p value less than or equal to 0.05 was considered statistically significant.

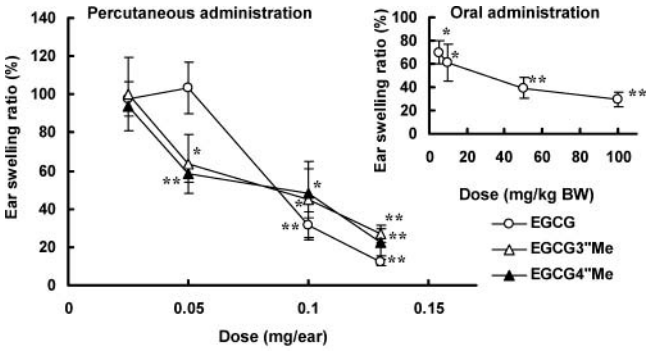

Figure 2. Dose-dependent preventive effect of percutaneous and oral administrations of tea catechins on oxazolone-induced type IV allergy in 5 week old male ICR mice. Values are the means ± SE; $n = 5$. Significant difference from the control value: *$p < 0.05$, **$p < 0.01$.

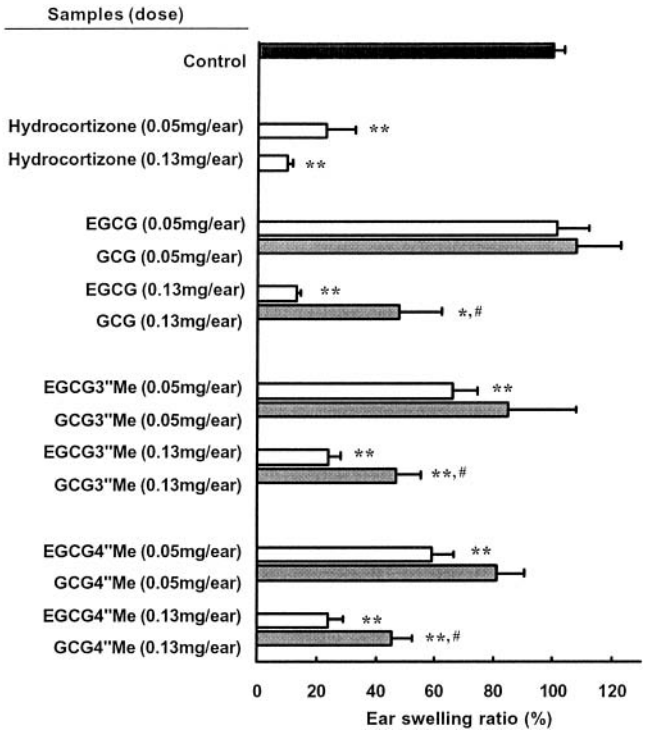

Figure 3. Effect of percutaneous administration of tea catechin epimers on oxazolone-induced type IV allergy in 5 week old male ICR mice. Values are the means ± SE; $n = 5$. Significant difference from the control value: *$p < 0.05$, **$p < 0.01$. Significant difference from the value of the corresponding original tea catechin at the same dose: #$p < 0.05$.

RESULTS AND DISCUSSION

The dose dependencies of the antiallergic effects of the tea catechins on type IV allergy in ICR mice after percutaneous administration are shown in **Figure 2**. The antiallergic activities of *O*-methylated derivatives of EGCG were dose-dependent between 0.025 and 0.13 mg/ear, although that of EGCG was dose-dependent between 0.05 and 0.13 mg/ear. The inhibitory effects of the tea catechins and their C-2 epimers on type IV allergy in ICR mice after percutaneous administration are shown in **Figure 3**. C-2 epimers GCG, GCG3″Me, and GCG4″Me showed significant antiallergic effects only at a dose of 0.13 mg/ear. The effects were, however, weaker than those of the corresponding original tea catechins and hydrocortisone at the same dose. The dose dependency of the antiallergic effect of EGCG on type IV allergy in ICR mice after oral administration was dose-dependent between 5 and 100 mg/kg body weight.

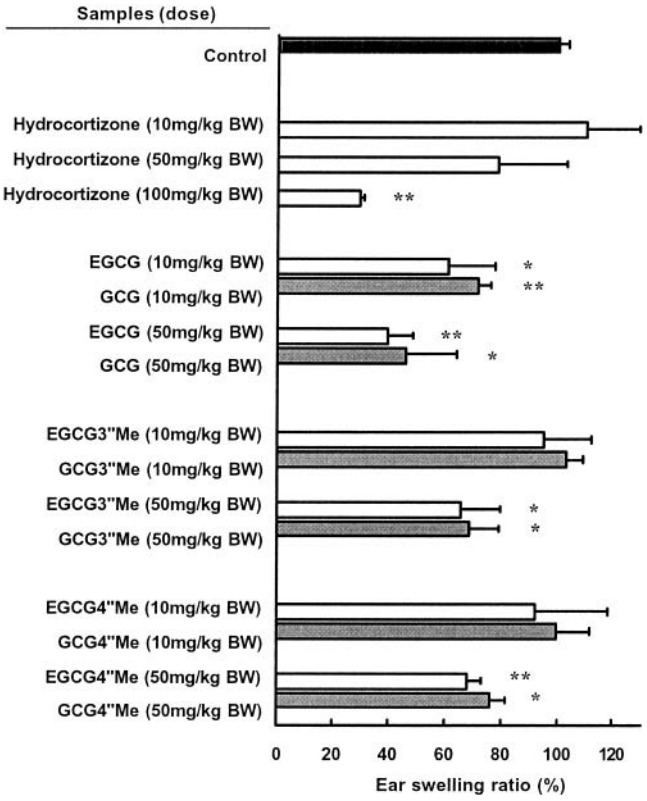

Figure 4. Effect of oral administration of tea catechin epimers on oxazolone-induced type IV allergy in 5 week old male ICR mice. Values are the means ± SE; $n = 5$. Significant difference from the control value: $^{*}p < 0.05$, $^{**}p < 0.01$.

The inhibitory effects of the tea catechins and their C-2 epimers on type IV allergy in ICR mice after oral administration are shown in **Figure 4**. All of the catechins tested showed significant inhibition of mouse type IV allergy at 50 mg/kg body weight, while only EGCG and GCG showed significant effects at 10 mg/kg body weight. Hydrocortisone suppressed the allergic reaction at the higher dose of 100 mg/kg body weight. The inhibitory effects in the oral administration also tended to be weaker as compared with the corresponding original catechins, but there was no significant difference between the two groups for all compounds tested. We reported previously that the heating of EGCG, EGCG3″Me, and EGCG4″Me dissolved in pH 6.0 buffer solution for 30 min at 90 °C resulted in ca. 30−40% epimerization (*7*). The inhibitory effects of heated Benihomare tea extracts on type IV allergy in ICR mice after percutaneous administration are shown in **Figure 5**. The contents of the epimers of EGCG and EGCG3″Me in the heated extract were over 45 and 18% of the total contents of them and their epimers, respectively. Percutaneous administration of the tea extract showed a significant preventive effect against mouse type IV allergy. However, when the tea extract was heated, the antiallergic effect of the heated extract was significantly decreased to 25% of that of the original extract.

Delayed type allergic reactions, such as type IV allergy, are often accompanied by serious inflammation. Active oxygen species play an important role in the process of this inflammation. The enzymatic oxidation of arachidonic acid is also important to produce some chemical mediators for the inflammation, such as leukotrienes and prostaglandins. Some antioxidants, such as methionine (*13*), mannitol (*14*), tea catechins (*15*), and superoxide dismutase (*16*), were reported to inhibit inflammation. It has been shown that tea catechins and their *O*-

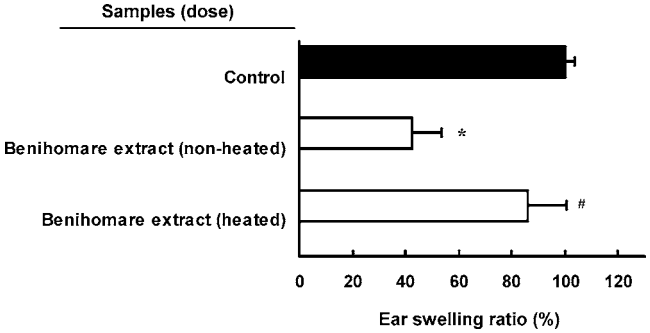

Figure 5. Effect of heating on the preventive activity of percutaneously administered extract of Benihomare tea leaves against oxazolone-induced type IV allergy in 5 week old male ICR mice. Values are the means ± SE; $n = 5$. Significant difference from the control value: $^{*}p < 0.01$. Significant difference from the value of the nonheated extract: $^{\#}p < 0.05$.

methylated derivatives show strong antioxidant activities in vitro. An electron spin resonance study indicated that the antioxidant activities of C-2 epimers are stronger than those of the original catechins (*10*). On the other hand, in another study, there were no differences in the antioxidant activities between the C-2 epimers and the original tea catechins based on the discoloration of β-carotene (*17*). Recently, we reported the peroxyl radical scavenging activities of C-2 epimers of tea catechins, including the *O*-methylated derivatives, but there were no differences in the activity between the C-2 epimers and the original catechins (*11*). The antiallergic effects of these catechins on mouse type IV allergy would be influenced by their absorption through the epidermis of the ears and metabolism. It was reported previously that the amount of C-2 epimer incorporated into the liposomes was less than that of the corresponding original catechin (*18*). The lower antiallergic effects of the C-2 epimers in the percutaneous administration may be explained partly by the presumed lower absorption rate of the C-2 epimers. Further investigations are, however, necessary to clarify the absorption, distribution, and metabolism of these catechins after percutaneous and/or oral administration.

The results of the present study suggest that treatment of tea infusions at high temperatures for a long period, heat processing for extraction and sterilization, and warming in vending machines should be avoided to maintain its type IV allergy preventive ability.

ABBREVIATIONS USED

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; EGCG4″Me, (−)-epigallocatechin-3-*O*-(4-*O*-methyl)-gallate; GCG4″Me, (−)-gallocatechin-3-*O*-(4-*O*-methyl)gallate.

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Received for review December 17, 2003. Revised manuscript received March 22, 2004. Accepted March 31, 2004. This work was supported by a grant from the Program for the Promotion of Basic Research Activities for Innovative Biosiences (PROBRAIN), Japan.

JF035476R