Antiallergic Constituents from Oolong Tea Stem

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The antiallergic constituents of oolong tea stem were examined. The stem extracts inhibited the 48 h homologous passive cutaneous anaphylaxis (PCA) reactions of rats in a dose-dependent manner and showed the same extent of inhibitory activity as ketotifen. All antiallergic constituents from the stem were concentrated into chloroform and ethyl acetate fractions, when extracted by various solvents. These fractions were treated with polyvinylpolypyrrolidone (PVPP), which resulted in the elimination of antiallergic activity in the ethyl acetate fraction, suggesting that one of the antiallergic constituents may be tea catechins. Then, six kinds of catechins, (−)-epigallocatechin gallate (EGCG), (−)-epigallocatechin (EGC), (−)-epicatechin gallate (ECG), (−)-epicatechin (EC), (+)-catechin (C) and (−)-gallocatechin gallate (GCG), were isolated from the ethyl acetate fraction, and the inhibitory activity of these catechins on histamine release from rat peritoneal mast cells passively sensitized with anti-egg albumin (EA) IgE antibody was investigated. Among these catechins, significant inhibitory activity was observed in all the catechins except for EC. In addition, the inhibitory activity of GCG was greater than that of EGCG, which is well known to be an antiallergic constituent in tea. These results suggest that GCG may be a novel antiallergic constituent among tea catechins, and also the most potent.

Key words catechin; histamine release; antiallergic activity; oolong tea stem; antiallergic constituent

It has been well established that tea extracts exhibit physiological and pharmacological actions. Several studies have indicated that green tea acts as inhibitor of the growth of Streptococcus mutans,1) and may play a role in the prevention of cancer formation.2) Furthermore, considerable information has been accumulated on the regulatory effect of tea on plasma glucose level,3) lipid metabolism4,5) and blood pressure.6) Recently, antiallergic and desmutagenic effects of tea extracts have been also reported.7,8) It seems that these effects may due to several tea catechins, especially (−)-epigallocatechin gallate (EGCG), (−)-epicatechin gallate (ECG) and (−)-epicatechin (EC).

Despite recent progress on the elucidation of constituent properties, little is known about the active constituents of tea other than EGCG and ECG. In our present study, therefore, six kinds of tea catechins were isolated from the oolong tea stem, and the antiallergic activity of these catechins was examined.

MATERIALS AND METHODS

Materials Egg albumin (EA) crystallized five times was purchased from Seikagaku Kogyo Co., Ltd. Bordetella pertussis was from Chiba Serum Institute. Oolong tea stem was purchased commercially. All other chemicals were of special grade.

Animals Wistar rats weighing 200–250 g were used throughout.

Preparation of Rat Anti-EA IgE Antibody Rat anti-EA IgE antibody was prepared by the method of Mota.9) Briefly, rats were injected with 1 mg of EA intramuscularly and with 1 ml of Bordetella pertussis containing 2 × 10^10 organisms intraperitoneally. The rats were then bled by cardiac puncture 12 d after the injection, the blood was allowed to clot, and the serum was separated. The pooled serum was then estimated by the passive cutaneous anaphylaxis (PCA) titer as 1:64.

Production of PCA Reactions PCA reactions were produced by the method of Mota and Wong.10) Briefly, intradermal injections of 0.1 ml of 32-fold-diluted antiserum were performed on each side from the midline of the rat skin. After 48 h, the rats were challenged by an intravenous injection of 5 mg of EA and 1 ml of 0.25% Evans blue. Thirty minutes after the challenge, the rats were killed, the skin was inverted, and the amount of dye was determined by the method of Katayama et al.11) Tea extracts were orally administered 1 h before the challenge.

Extraction and Assay of Histamine Mast cells were extracted and partially purified from rats by the method of Sullivan et al.12) A 5 ml volume of the peritoneal cell suspension was layered on top of 2 ml of 28% bovine serum albumin (BSA) in saline and centrifuged at 300 × g for 10 min at 4°C. Mast cells were recovered from the BSA layer, washed, and resuspended in the medium. A direct count of the cell suspension with toluidine blue showed that about 95% of the total cells were mast cells. Then, the mast cell suspension was incubated with anti-EA IgE antibody at 37°C for 30 min. Tea catechins were added, and it was gently incubated at 37°C for 10 min. The suspension was then mixed with EA (10 μg/ml) and phosphatidyl-L-serine (PS) (10 μg/ml) and the mixture was incubated at 37°C for 20 min. Histamine was assayed using the o-phthalaldehyde spectrofluorometric procedure of May et al.13)

Isolation of Tea Catechins from Oolong Tea Stem Tea catechins were isolated from oolong tea stem by the method of Wilkins et al.14) Briefly, hot water extracts were successively partitioned with chloroform, ethyl acetate and n-butanol. The ethyl acetate fraction was further applied to silica gel column chromatography, then washed with methanol-chloroform (20:1). The crude catechins were then eluted with methanol-chloroform (10:1).

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RESULTS

Effect of the Stem Extracts on PCA Reactions Oral administration of the stem extracts dose-dependently inhibited rat 48 h homologous PCA reactions and showed almost the same extent of inhibitory activity as that of ketotifen. The results are given in Fig. 1.

Among various fractions obtained from the stem extracts, chloroform and ethyl acetate fractions inhibited PCA reactions, while no significant effect was observed from either n-butanol or residual fractions, as shown in Fig. 2.

When the chloroform and ethyl acetate fractions were treated with polyvinylpolymeridone (PVPP), which is known to specifically adsorb the phenolic compounds, the inhibitory activity of ethyl acetate fraction was decreased, as shown in Fig. 3.

Effect of Several Constituents from Oolong Tea Stem on Histamine Release To clarify the antiagrillergic constituents contained in oolong tea stem, the effect of several constituents from the stem on histamine release from rat peritoneal mast cells passively sensitized with anti-EA IgE antibody was examined. The results are summarized in Fig. 4 and Table 1. In this experiment, the ethyl acetate fraction inhibited histamine release in a dose-dependent manner, and purified GCG, one of the tea catechins, exhibited stronger inhibition than ethyl acetate fraction. EGCG, which is known to be a strong antiagrillergic constituent in teas, inhibited the histamine release induced by anti-EA IgE antibody and showed more than 80% inhibition at 1 mg/ml. (-)-Epigallocatechin (EGC) and ECG also significantly inhibited the histamine release, but the maximum inhibition by each of them was less than that by EGCG. In contrast, (+)-catechin (C) showed a weak inhibitory effect and EC failed to substantially inhibit the histamine release at concentrations up to 1 mg/ml. GCG, however, exhibited a remarkable inhibitory effect on histamine release and showed more than 80% inhibition, even at 0.3 mg/ml. Caffeine also strongly inhibited the histamine release. The IC_{50} values (×10^{-4}M) of EGCG, EGC, ECG, C, GCG and caffeine were 5.7, 7.3, 6.7, 31, 3.1 and 2.9, respectively.
Fig. 3. Effect of PVPP Treatment on the Antiallergic Activity of Chloroform (A) and Ethyl Acetate (B) Fractions

Each extract (1g) was dissolved in water, and then mixed with 1g of PVPP. The mixture was incubated at room temperature for 1 h. The total amount of adsorption to PVPP in chloroform and the ethyl acetate fractions were 0.09 g (9.1%) and 0.52 g (52.3%), respectively. The total amount of dye (control value) in A (none), A (PVPP), B (none) and B (PVPP) was 11.8 ± 1.1, 13.5 ± 2.1, 13.5 ± 2.3 and 13.7 ± 2.1, respectively. Each value represents the mean ± S.E. of 4 determinations. * p < 0.05; ** p < 0.01; significantly different from control.

Fig. 4. Inhibitory Effect of Ethyl Acetate Fraction and GCG on Histamine Release from Rat Peritoneal Mast Cells Passively Sensitized with Anti-EA IgE Antibody

Each value represents the mean ± S.E. of 4 determinations. The total amount of histamine (control value) was 0.312 ± 0.006 µg/ml, and the basal value obtained in the absence of EA and PS was 0.095 ± 0.005 µg/ml. ○, ethyl acetate fraction; ●, GCG.

DISCUSSION

Coombs and Gell have classified allergies as type I, II or III, corresponding to immediate-type hypersensitivity, and type IV, which corresponds to a delayed-type sensitivity. Among these types, the type I allergy is known to be an atopic or anaphylaxis reaction, and symptoms such as bronchial asthma, hay fever and atopic dermatitis are included in type I. It has been found that the histamine release from mast cells passively sensitized with antigen-IgE antibody is an essential step in the pathological process of a type I allergy. In addition, accumulated findings have suggested that the antigen-IgE antibody induces PCA reactions as a typical model of the type I allergy. In this study, therefore, antiallergic constituents of oolong tea stem were examined using these methods.

The stem extracts significantly inhibited rat 48h PCA reactions and showed the same extent of inhibitory activity as ketotifen, suggesting that the pharmacological characteristics of oolong tea stem may be partially clarified. Remarkable inhibitory activity was observed in both chloroform and ethyl acetate fractions. Thus, the almost antiallergic constituents may be concentrated into these fractions by separating the stem extracts. Treatment of each fraction with PVPP decreased the inhibitory activity, not in chloroform, but in the ethyl acetate fraction. The remaining inhibitory effect in the ethyl acetate fraction may be due to some catechins not removed by PVPP, since the total amount of catechins adsorbed to PVPP was 52.3% of the ethyl acetate fraction. Accordingly, these findings suggested that the antiallergic constituents in an ethyl acetate fraction are quite different from those in the chloroform fraction. Furthermore, about 85% of the chloroform fraction is considered to be caffeine according to analysis of the constituent contents. Indeed, the dose-response curve of the chloroform fraction for PCA reactions was completely similar to that of caffeine (data not shown).

Here, we noticed antiallergic constituents in ethyl acetate fraction. In this fraction, a possible constituent involved in antiallergic activity may be tea catechins. Several reports have suggested that the antiallergic activity observed in tea extracts is mainly due to catechins such as EGCG, EGC, ECG and EC, and that EGCG may be the most potent antiallergic constituent in tea catechins. Our results from this study showed good agreement with the previous study. Furthermore, we strongly demonstrated that GCG and C are novel antiallergic constituents, and that GCG may show a stronger inhibitory activity than

Table 1. Inhibitory Effect of Tea Catechins on Histamine Release from Rat Peritoneal Mast Cells Passively Sensitized with Anti-EA IgE Antibody

<table>
<thead>
<tr>
<th>Catechins</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>0.3</td>
</tr>
<tr>
<td>EGCG</td>
<td>55.1 ± 4.3</td>
</tr>
<tr>
<td>EGC</td>
<td>44.0 ± 3.9</td>
</tr>
<tr>
<td>ECG</td>
<td>3.6 ± 2.9</td>
</tr>
<tr>
<td>EC</td>
<td>0</td>
</tr>
<tr>
<td>C</td>
<td>0</td>
</tr>
<tr>
<td>GCG</td>
<td>37.5 ± 3.0</td>
</tr>
<tr>
<td>Caffeine</td>
<td>58.2 ± 1.6</td>
</tr>
</tbody>
</table>

a) Each value represents the mean ± S.E. of 4 determinations. b) p < 0.01; significantly different from control (0%). c) p < 0.001; significantly different from control (0%). The total amount of histamine (control value) was 0.524 ± 0.007 µg/ml, and the basal value obtained in the absence of EA and PS was 0.285 ± 0.004 µg/ml.
that of EGCG. These results suggest that GCG may be one of the most useful constituents of teas in the development of novel anti-allergic drugs. The contents of GCG, EGCG, EGC, ECG, EC and C in ethyl acetate fraction were 3.8, 9.5, 6.9, 2.5, 6.0 and 4.3%, respectively, and the total amount of these catechins was 33%. Unknown catechins may also be involved in the inhibitory effect of this fraction.

The dose-response of these catechins was observed in the concentration range of 0.1—0.5 mg/ml. The inhibitory effects of GCG, caffeine, EGC and ECG were found to be saturable around 0.5 mg/ml. A similar finding was observed in an experiment using compound 48/80 as a histamine releaser. These results suggested that the effective concentration range of tea catechins may be 0.1—0.5 or 1 mg/ml.

Although the ethyl acetate fraction inhibited histamine release, the inhibition (%) of 1 mg/ml was significantly decreased as compared with that of 0.3 mg/ml. These results suggested that the high concentration of this fraction may have a moderate toxicity on mast cells. In contrast, no similar findings were observed in the catechins and caffeine used in this study, suggesting that these constituents, at least, may have low toxicity for mast cells. In the ethyl acetate fraction, however, the possibility that other constituents may contribute to the toxicity has been demonstrated. In our laboratory, it has been found that some kinds of catechin polymers exhibit toxicity for mast cells, resulting in the stimulation of histamine release by modulating the membrane fluidity of the mast cell.

Our present study also demonstrated several findings about the relationship between the structures of catechins and their anti-allergic activity. First, the inhibitory activity of GCG and EGCG on histamine release was stronger than that of EGC. ECG was also more active than EC and C. The structure of GCG and EGCG is similar to that of EG except for a galloyl moiety. The structure of EGC is also similar to that of EC except for a galloyl moiety. Thus, these results strongly suggested that the galloyl moiety in their molecules may be involved in the regulation of inhibitory activity. Secondly, a remarkable difference in the inhibitory activity between EGC and EC was observed, although the molecular structure of EGC was completely similar to that of EC except for a hydroxy moiety on the B ring. The same phenomenon was observed between EGCG and ECG. These results demonstrated that the hydroxy moiety on the B ring may also be important in the inhibitory activity on histamine release. Hagiwara et al. have reported that the inhibitory effect of EGC and galloclatechin on 12-O-tetradecanoylpholbol-13-acetate-induced Epstein-Barr virus activation is more active than either EC or C, suggesting that the three hydroxy moieties on the B ring play an important role in their inhibition. Similar findings have also been found regarding the inhibitory effect of tea catechins on the growth of Streptococcus mutans. In general, the hydroxy moieties on the B ring of tea catechins may relate to their pharmacological characteristics. In addition, the inhibitory activity of trans-isomers such as GCG and C were stronger than that of the corresponding cis-isomers such as EGCG and EC, respectively, suggesting that the anti-allergic activity of tea catechins may be partially due to their conformation. Although the mechanism of inhibitory action of tea catechins is not yet clear, it is likely that these catechins regulate the degradation from the mast cells by stabilizing membrane fluidity.

In conclusion, we clearly demonstrated that GCG may be a novel and potent anti-allergic constituent of teas and would therefore be useful for therapeutic application to allergic or inflammatory disease. Further investigation is also necessary to clarify unknown anti-allergic constituents which may be more active than GCG.

REFERENCES

9) Mota I., Immunology, 7, 681 (1964).