Identification of a Methylated Tea Catechin as an Inhibitor of Degranulation in Human Basophilic KU812 Cells

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We examined the constituents of tea that had an inhibitory effect on the degranulation process in the human basophilic cell line, KU812. Among the constituents purified from a extract of ‘Benihomare’ oolong tea by column chromatography, a methylated (−)-epigallocatechin gallate ((−)-epigallocatechin 3-O-(3-O-methyl) gallate) was found to inhibit the degranulation of KU812 cells that had been stimulated with calcium ionophore A23187. The inhibitory effect of this methylated (−)-epigallocatechin gallate on degranulation was greater than that of (−)-epigallocatechin gallate. This result indicates that methylated (−)-epigallocatechin gallate may be an effective modification for the catechin to inhibit degranulation from human basophils.

Key words: tea catechin; basophil; degranulation; methylation; (−)-epigallocatechin gallate

Basophils appear to play an important role in allergic reactions as effector cells in IgE-dependent immune responses. Basophils possess cytoplasmic granules containing various potent inflammatory mediators such as histamine, proteases and chemotactic factors. When a specific allergen associates with receptor-bound IgE, which induces an elevation of the intracellular Ca2+ concentration, basophils can release preformed mediators and arachidonic acid metabolites. However, the mechanism that regulates degranulation, especially suppression, in human basophils has remained largely unknown.

Green tea contains polyphenols, which include flavanols, flavonol glycosides, and phenolic acids. Most of the green tea polyphenols are flavanols, and are commonly known as catechins. Tea catechins have been reported to inhibit degranulation from rat mast cells and rat basophilic leukemia RBL-2H3 cells. Some sensitivity differences between human and rat mast cells for stimulating degranulation have been reported. Therefore, we investigated tea constituents that have an inhibitory effect on the degranulation of human basophils. Since the polyphenol fraction of the ‘Benihomare’ oolong tea cultivar (semifermented) has been shown to include some anti-allergic factors, the polyphenol fraction of ‘Benihomare’ (Camellia sinensis L. cv. benihomare) oolong was used as a source of anti-degranulating agents.

‘Benihomare’ oolong was prepared as described previously. The tea cultivar was extracted by adding six volumes of boiling water to the tea, standing it for 30 min at 100°C, and filtering through four sheets of gauze. The extract was passed through a porous polymer gel (Mitsubishi Diaion HP-20) column, and the adsorbed materials were eluted with water/methanol (1:1). The eluate was further applied to Amberlyst 15 (ORGANO, Tokyo) column chromatography, and then eluted with methanol. The methanol eluate was used as the polyphenol fraction. This polyphenol fraction was fractionated in a reversed-phase chromatographic column (ODS, Nomura Chemicals, Seto), using a linear gradient of CH3CN–H2O (8:92–20:80, by volume) as the eluting solvent, the elution being monitored at 280 nm. Each fraction was lyophilized to give powdery material for later use at a concentration of 1 mg/ml when dissolved in water.

We have recently demonstrated that IL-4 induced differentiation in KU812 cells to morphologically and functionally mature into human basophilic cells, so these IL-4-treated KU812 cells were used as human mature basophils in this study. KU812 cells were obtained from the Japanese Cancer

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Abbreviations: EGCG, (−)-epigallocatechin gallate
Research Resources Bank (Tokyo). The cells were maintained in an RPMI-1640 medium (Nissui, Tokyo) supplemented with 10% fetal bovine serum (Intergen, NY), 100 U/ml of penicillin G, 100 mg/ml of streptomycin and a 10 mm HEPES buffer. To prepare mature basophils, the KU812 cells were stimulated with 1 ng/ml of IL-4 for 1 week. Degranulation of the KU812 cells was monitored by measuring the released histamine. KU812 cells (1.0 x 10^6 cells) were washed and resuspended in 2 ml of a Tyrode buffer consisting of 137 mm NaCl, 2.7 mm KCl, 1.8 mm CaCl_2, 1.1 mm MgCl_2, 11.9 mm NaHCO_3, 0.4 mm Na_2HPO_4, and 5.6 mm glucose at pH 7.2. Five μM of calcium ionophore A23187 (Wako, Osaka) was incubated with various tea constituents (1 μg/ml) and was then added to the cell suspension. After adding the mixture to the cells, the mixture was incubated at 37°C for 20 min, and the reaction was terminated by cooling at 4°C for 15 min. The cell suspension was then centrifuged at 300 x g for 10 min, and the amount of histamine in the supernatant was measured by a fluorometric assay. After mixing 2 ml of the supernatant with 0.75 g of NaCl and 0.5 ml of 1 N NaOH, 5 ml of a 3:2 (v/v) mixture of n-butanol and chloroform was added and mixed for 5 min. After centrifugation at 270 x g for 5 min, 4 ml of the organic solvent layer was recovered, mixed with 0.15 ml of 1 N NaOH and 0.1 ml of 0.2% o-phenthaldehyde (Wako), and stood for 5 min at room temperature. The reaction was terminated by adding 0.14 ml of 0.5 N H_2SO_4, and then the fluorescence intensity was measured by a spectrofluorophotometer (RF 500, Shimazu, Kyoto) with excitation at 360 nm and emission at 450 nm. The percentage histamine release was calculated as follows: histamine release (%) = (test - negative control)/(positive control - negative control) x 100. Supernatant from unstimulated cells was used as the negative control, while the supernatant from cells stimulated only with A23187 was used as the positive control. Each result is expressed as the mean ± SE. To establish the exact nature of the differences between the groups, a one-way analysis of variance was followed by Duncan’s new multiple-range test.

The tea constituents obtained by ODS column chromatography from the polyphenol fraction of ‘Benihomare’ oolong tea on histamine release from human basophilic KU812 Cells. KU812 cells were stimulated with A23187 (5 μM) in the presence of each constituent (1 μg/ml), and the amount of histamine released into the supernatants was determined by a fluorometric assay (a). The effects of fractions Q, R, S, U, V and W obtained by second ODS column chromatography, which correspond to fractions 1 and m from the first column chromatography (a) were examined for histamine release from KU812 cells (b).

W) were obtained and assessed for their degranulation inhibitory activity. Only fraction U diminished the histamine release from KU812 cells (Fig. 1(b)). To clarify the constituent in the fraction U, we determined the structure of U by an NMR analysis. The ^1H-NMR spectrum of U is in agreement with the data for epigallocatechin 3-O-(3-O-methyl)-gallate reported previously. This compound was found to be a methylated form of (−)-epigallocatechin gallate (EGCg) (Fig. 2). Therefore, we compared methylated EGCg with EGCg (Kurita Water Industries, Tokyo) for the ability to inhibit the degranulation of KU812 cells. As shown in Fig. 3, both EGCg and (−)-epigallocatechin 3-O-(3-O-methyl)-gallate dose-dependently inhibited histamine release from the KU812 cells. At 50 μM, the inhibitory effect of epigallocatechin 3-O-(3-O-methyl)-gallate on the histamine release was higher (28% histamine release)
Fig. 2. Structure of (−)-Epigallocatechin 3-O-(3-O-methyl) Gallate.

Fig. 3. Inhibitory Effect of EGCg and Methylated EGCg on the Degranulation of KU812 Cells.

KU812 cells were stimulated with A23187 in the presence of various concentrations of either EGCg or (−)-epigallocatechin 3-O-(3-O-methyl) gallate (1, 10 and 50 μM). The concentration of histamine in the supernatant was measured by a fluorometric assay. Each data value is the mean ± SE (n = 3). Values not sharing a common letter (a, ab, b or c) are significantly different between groups (p < 0.05).

than non-methylated EGCg (42% histamine release). This result suggests that methylation of the galloyl moiety is an effective modification for EGCg to inhibit degranulation from human basophils. Methylated EGCg has been shown to inhibit type I allergic (anaphylactic) reactions in mice sensitized with ovalbumin and Freund’s incomplete adjuvant. In rat basophilic leukemia RBL-2H3 cells and rat peritoneal exudate cells, the triphenol structure of catechins has been shown to play a role determining the degree of inhibition of degranulation. Clarifying the relationship between methylation and the inhibitory effect on degranulation may provide an approach to suppress allergic and/or inflammatory reactions.

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