JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

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Epicatechin-3-*O*-(3″-*O*-methyl)-gallate Content in Various Tea Cultivars (*Camellia sinensis* L.) and Its in Vitro Inhibitory Effect on Histamine Release

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ABSTRACT: It has been reported that epigallocatechin-3-*O*-(3″-*O*-methyl)-gallate (EGCG3″Me) and the EGCG3″Me-rich green tea (*Camellia sinensis* L.) cultivar 'Benifuuki' exhibit antiallergic effects. The objective of this study was to investigate the effect of various tea leaf catechins on histamine release from murine bone marrow mast cells (BMMC). At a dose of 50 μg/mL, the rank order of histamine release inhibition was observed to be epicatechin-3-*O*-(3″-*O*-methyl)-gallate (ECG3″Me) > gallocatechin-3-*O*-(3″-*O*-methyl)-gallate (GCG3″Me) > EGCG3″Me > gallocatechin-gallate (GCG) > catechin-gallate (CG) > EGCG > epicatechin-gallate (ECG) > epigallocatechin (EGC) > gallocatechin (GC). Of the various tea cultivars analyzed by HPLC, the greatest content of ECG3″Me was found in the third crop of 'Benifuuki' (1.05% dry weight). Moreover, ECG3″Me content was positively correlated with EGCG3″Me content in 'Benifuuki' tea leaves. In an assay of mixtures of ECG3″Me and EGCG3″Me, inhibitory activity (50 μg/mL in total) was increased as the content of ECG3″Me increased. This suggests that ECG3″Me might link to the antiallergic effect of 'Benifuuki' tea, as has been reported for EGCG3″Me.

KEYWORDS: *epicatechin-3-O-(3″-O-methyl)-gallate (ECG3″Me), 'Benifuuki' tea, histamine release inhibitory effect, HPLC analysis*

INTRODUCTION

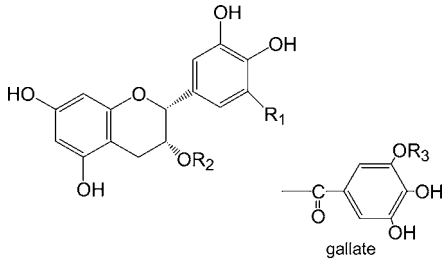
Allergy is recognized as a disease of excessive immune activity, with allergic rhinitis (AR) being one of the most prevalent allergic disorders affecting productivity and quality of life. The prevalence of AR varies among populations, but it is estimated to affect up to 30% of the worldwide population and its incidence is increasing.[1] Because medical costs for treating this disease are high and the possible adverse effects of available medication are not negligible, there is great demand for the development of physiologically functional foods for allergy prevention. Mast cells play a critical role in the effector phase of IgE-dependent immediate hypersensitivity and allergic diseases.[2] Cross-linking of high-affinity IgE receptors (FcεRI) with IgE and allergen initiates the activation process, leading to the release of preformed and de novo synthesized vasoactive amines, proteases, leukotrienes, cytokines, and chemokines.[3−5] These chemical and polypeptide agents elicit various allergy-associated pathophysiological changes locally and systemically; for instance, amines, such as histamine and serotonin, enhance vascular permeability, and cytokines, such as TNF-α, recruit inflammatory cells to the site of allergen exposure.

It has been reported that *O*-methylated EGCGs (epigallocatechin-3-*O*-(3″-*O*-methyl)-gallate (EGCG3″Me; Figure 1), epigallocatechin-3-*O*-(4″-*O*-methyl)-gallate (EGCG4″Me),[6−8] and strictinin[9] have antiallergic functions and that the Japanese tea (*Camellia sinensis* L.) cultivar 'Benifuuki' is rich in EGCG3″Me, which is absent in black tea.[10,11] Oral administration of these *O*-methylated catechins significantly and dose-dependently (5−50 mg/kg) inhibited type I allergic (anaphylactic) reactions in mice sensitized with ovalbumin and Freund's incomplete adjuvant. Both EGCG3″Me and EGCG4″Me inhibited histamine release (as a surrogate marker of degranulation of mast cells) with higher potency than their nonmethylated form of catechins. These catechins also strongly inhibit mast cell activation through the suppression of tyrosine phosphorylation mediated by the cellular protein kinase Lyn,[12] suppression of myosin light chain phosphorylation and high-affinity IgE receptor expression via binding to the 67 kDa laminin receptor,[13] and suppression of histamine and leukotriene release, as well as interleukin-2 secretion after FcεRI cross-linking.

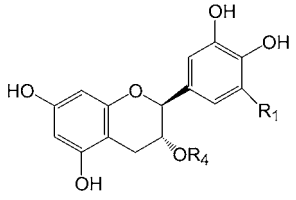
Furthermore, in vitro and in vivo effects of 'Benifuuki' green tea containing EGCG3″Me (in vitro, 50 μg/mL addition; in vivo, 34 mg/day administration to adult human) have been reported, such as suppression of inflammatory cytokine (TNF-α) and chemokine (MIP1-α) production from mast cells after antigen stimulation and significant symptom relief of subjects with Japanese cedar pollinosis compared to 'Yabukita' green tea without EGCG3″Me (in a double-blinded clinical trial).[14] We also reported that the blood level of EGCG3″Me was higher than that of EGCG after administering 'Benifuuki' green tea to humans.[14] Briefly, after the consumption of 'Benifuuki' green tea containing 43.5 mg of EGCG and 8.5 mg of EGCG3″Me, the AUC (area under the drug concentration time curve; min·μg/mL) of EGCG was 6.72 ± 2.87 and that of EGCG3″Me was 8.48 ± 2.54 in healthy human volunteers. Although the dose of

Received: November 3, 2011
Revised: February 6, 2012
Accepted: February 16, 2012
Published: February 16, 2012

dx.doi.org/10.1021/jf204497b | J. Agric. Food Chem. 2012, 60, 2165−2170



	R1	R2	R3
EGC	OH	H	
ECG	H	gallate	H
EGCG	OH	gallate	H
ECG3″Me	H	gallate	CH_3
EGCG3″Me	OH	gallate	CH_3



	R1	R4	R3
CG	H	gallate	H
GCG	OH	gallate	H
GCG3″Me	OH	gallate	CH_3

Figure 1. Structural formulas of catechins used in this study.

EGCG was 5.1 times the dose of EGCG3″Me, the AUC of EGCG3″Me was higher than that of EGCG.

HPLC analysis of *O*-methylated catechins in green tea detected the presence of not only EGCG3″Me but also epicatechin-3-*O*-(3″-*O*-methyl)-gallate (ECG3″Me). However, the antiallergic activity of ECG3″Me has not yet been investigated. In this study, we attempt to clarify the ECG3″Me content in various cultivars of tea, and its inhibitory effect on histamine release using bone marrow derived mast cells (BMMC), which is used as an in vitro assay.[15]

MATERIALS AND METHODS

Tea Samples. (1) Various cultivars of fresh tea leaves (*C. sinensis* L.) were hand plucked from April 29 (first crop) to August 9 (third crop), 2007, at the plantation of the National Institute of Vegetable and Tea Science (NITVS) in Kanaya, Shizuoka, Japan. (2) Fresh 'Benifuuki' tea leaves were hand plucked from March 17 (first crop) to October 27 (fourth crop), 2006, in Shizuoka, Okinawa, Kagoshima, and Ooita prefectures. The freshly plucked tea leaves were immediately dried in a microwave oven and finely powdered using a sample cyclone mill (1 mm mesh; Shizuoka Seiki, Shizuoka, Japan) and stored in a refrigerator until analysis.

Chemicals. Ethyl gallate, (−)-epicatechin (EC), (+)-catechin (C), (−)-epigallocatechin-3-*O*-gallate (EGCG), epicatechin-3-*O*-gallate (ECG), epigallocatechin (EGC), gallocatechin-3-*O*-gallate (GCG), and catechin-3-*O*-gallate (CG) were purchased from Wako Pure Chemical (Osaka, Japan). EGCG3″Me,[6] ECG3″Me, and GCG3″Me (>98% purity) were kindly provided by Prof. Toshio Miyase of University of Shizuoka. The chemical structures of the catechins used in this study are shown in Figure 1.

Acetonitrile (MeCN), *N*-acetylcysteine (NAC), calcium chloride ($CaCl_2$), ethanol (EtOH), ethylenediaminetetraacetic acid (EDTA), ferrous sulfate ($FeSO_4$), gelatin, glucose, glutamine, HEPES, hydroxy chloride (HCl), magnesium chloride ($MgCl_2 \cdot 6H_2O$), 2-mercaptoethanol, methanol (MeOH), penicillin, *o*-phthalaldehyde (OPA), phosphoric acid (H_3PO_4), potassium chloride (KCl), potassium dihydrogen phosphate (KH_2PO_4), potassium sodium tartrate, sodium azide, sodium borate, sodium chloride (NaCl), sodium dihydrogen phosphate (NaH_2PO_4), and streptomycin were purchased from Wako Pure Chemical. Anti-2,4,6-trinitrophenyl (TNP) mouse monoclonal IgE antibody (IgE) was purchased from BD Pharmingen (Franklin Lakes, NJ, USA). TNP−bovine serum albumin (TNP-BSA) was purchased from LSL Lifescience (Cosmo Bio Co. Ltd., Tokyo, Japan). Murine recombinant IL-3 (IL-3) was purchased from PeproTech Inc. (Rocky Hill, NJ, USA). RPMI 1640 medium and fetal bovine serum albumin (FBS) were purchased from Invitrogen Japan K.K. (Tokyo, Japan).

Preparation of Tea Infusion. Powdered tea was extracted at 100 °C for 30 min in a 100-fold dilution with distilled water (w/b). After filtration, the polyphenol content in the infusion was measured, as described in the following section, to standardize the polyphenol levels in each sample.

Analysis of Polyphenol Content. The polyphenol (tannin) content in the infusion was measured by colorimetry using the ferrous tartrate method.[16] Briefly, solution A (0.1% $FeSO_4$ and 0.5% potassium sodium tartrate), Sorensen buffer (67 mM Na_2HPO_4/67 mM KH_2PO_4, 84:16), and an ethyl gallate standard solution were prepared. A 5 mL aliquot of infusion sample (standard solution or distilled water as blank) was placed in a 25 mL flask, 5 mL of solution A was added and mixed completely, and Sorensen buffer was added to 25 mL. Absorbance of the reaction was measured by spectrophotometry at 540 nm (Benchmark Plus; Nippon Bio-Rad Laboratories K.K., Tokyo, Japan). Ethyl gallate was used as the polyphenol standard. Standard solutions were prepared at 0, 20, 40, 60, 80, and 100 mg/100 mL to generate a linear calibration curve. Polyphenol content (mg/100 mL) was calculated by multiplying ethyl gallate content by a coefficient of 1.5.

Analysis of Catechins. Tea leaves were pulverized into powder for analysis. Catechins were extracted from 250 mg of tea leaf powder using 20 mL of 2% H_3PO_4/EtOH (1:1) at 30 °C for 60 min in a water bath.[17] The extracts were added to 25 mL of distilled water and centrifuged at 1200*g* for 5 min at 4 °C, and the supernatant was diluted 10-fold with distilled water. Twenty microliters of the sample, filtered through a membrane filter (DISMIC-13HP, PTFE; pore size = 0.45 μm; Advantec, Osaka, Japan), was injected into the HPLC apparatus. HPLC was performed with a Shimadzu LC-10A pump coupled with a UV−vis detector (SPD-M10Avp; Shimadzu Corp., Kyoto, Japan) using a reverse-phase Wakopak Navi C18-5 column (4.6 mm i.d. × 150 mm; granule diameter, 5 μm; Wako Pure Chemical) with Wakopak Navi C18-5 (4.6 mm i.d. × 10 mm; granule diameter, 5 μm; Wako Pure Chemical) as a guard column and eluted with an eluent (as described below) at a flow rate of 1 mL/min at 40 °C. Catechins were measured at 272 nm.

HPLC analysis was performed using a linear gradient system with mobile phase A (DW/MeCN/H_3PO_4, 400:10:1) and mobile phase B (MeOH/mobile phase A, 1:2). Linear gradient elution was performed as follows: 100% mobile phase A for 2 min; 20% mobile phase A for 27 min; maintain 20% mobile phase A for 10 min; and return to 100% mobile phase A for 7 min.[18]

Quantification was carried out using the external standard method. Quantification of catechins was performed after data acquisition using an LC workstation (Class VP system; Shimadzu).

Cells and Culture. Pathogen-free 5-week-old female NC/Nga mice were purchased from Charles River Japan, Kanagawa, Japan. The animals were handled and sacrificed in accordance with the procedures outlined in the Guidelines for Animal Experimentation of National Institute of Vegetable and Tea Science, NARO. Mice were sacrificed by cervical dislocation prior to evisceration of their femurs. Ten milliliters of 4 ng/mL of IL-3-containing RPMI 1640 medium, supplemented with 10% heat-inactivated FBS, 100 μg/mL of streptomycin, and 100 U/ml of penicillin, 2 mM glutamine, and 50 μM 2-mercaptoethanol, was injected into each femur, which was cut at the top and bottom, and with a syringe attached to a 26G needle (Terumo Corp., Tokyo, Japan); bone marrow cells were ejected into 25 cm^2 falcon cell culture flasks (Japan Becton Dickinson, Tokyo, Japan). The obtained bone marrow cells were subsequently cultured in humidified 95% air/5% CO_2 at 37 °C. After 4 weeks of culture, the obtained BMMC were found to be >95% pure mast cells.

Degranulation by FcεRI Cross-Linking and Histamine Analysis. BMMC were passively sensitized at a density of 2×10^6 cells/mL

with 0.5 μg/mL of IgE at 37 °C overnight. After washing in Tyrode solution (Ca^{2+}-free; 10 mM HEPES, pH 7.4; containing 0.8% NaCl, 0.02% KCl, 0.056% NaH_2PO_4, 0.1% glucose, 0.05% gelatin, and 1 μM $MgCl_2 \cdot 6H_2O$), the cells were resuspended in Tyrode buffer at a density of 1×10^7 cells/mL, incubated for 20 min with samples at 37 °C, and then stimulated by 300 ng/mL of TNP-BSA with 300 μM $CaCl_2$ for 10 min at 37 °C. Cell suspensions were briefly centrifuged at 15000*g* for 5 min at 4 °C to separate Tyrode solution from cell pellets. The supernatant was added to 4 mM EDTA/Tyrode solution and cooled on ice to stop the reaction.

For histamine determination, an equivalent volume of 0.1 N HCl was added to the supernatant, and the released histamine was measured by on-column HPLC.[19] HPLC was performed using a Shimadzu LC-10A pump coupled with a fluorescent photometric detector (ex 330 nm, em 430 nm; RF-10AxL; Shimadzu) with a reverse-phase Asahipak-ODP-50-4E column (4.6 mm i.d. × 250 mm; particle size, 5 μm; Showa Denko, Tokyo, Japan). The solution was eluted with 50 mM sodium borate/MeCN (80:20) buffer containing 1 mM OPA and 1 mM NAC at a flow rate of 0.5 mL/min at 37 °C. Quantification was carried out using the external standard method.

Moisture Analysis. The moisture content of tea samples was determined using a moisture analyzer (MX-50; A&D Co., Ltd., Tokyo, Japan).

Statistical Analysis. The data are expressed as the mean ± SD of triplicate experiments. Differences in histamine release between each group were assessed using the Tukey–Kramer multiple-comparison test, and the correlation between ECG3″Me and EGCG3″Me was assessed using the Pearson correlation coefficient test, assuming a significance level of 5 or 1%, using Statcel software (ver. 2).

RESULTS

Inhibitory Effect of Various Catechins on Histamine Release. To reveal the catechin with the highest activity in tea leaves, we investigated the inhibitory effects of EGC, GC, ECG, CG, EGCG, GCG, EGCG3″Me, GCG3″Me, and ECG3″Me on histamine release using BMMC. Figure 2 shows the histamine release inhibitory effects of nine catechins (50 μg/mL) in BMMC after antigen (TNP–BSA) stimulation. The rank order of histamine release inhibition was as follows: ECG3″Me > GCG3″Me > EGCG3″Me > GCG > CG > EGCG > ECG > EGC > GC, with 42.0 > 32.6 > 29.2 > 22.0 > 19.0 > 15.5 > 6.8 > 5.1% inhibition, respectively.

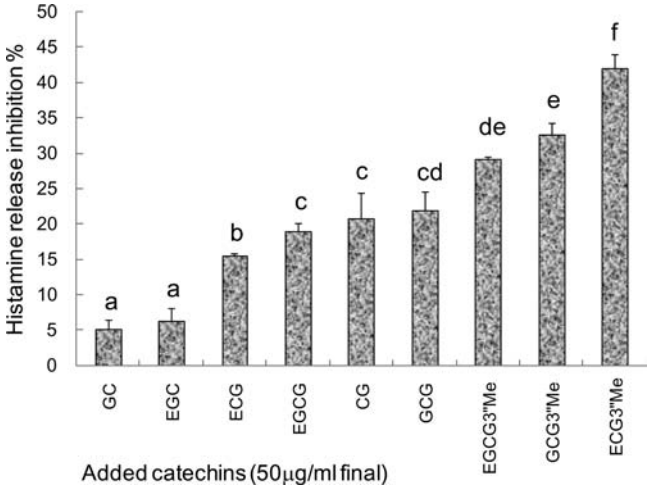


Figure 2. Inhibitory effects of various catechins on histamine release in BMMC. Results are expressed as the percentage of histamine release compared to vehicle after antigen stimulation (%) (n = 3, mean ± SD). Mean values in a column not sharing letters are significantly different by the Tukey–Kramer test ($P < 0.01$).

ECG3″Me Content in Various Tea Cultivars. To identify the tea cultivars with high ECG3″Me content, we determined the ECG3″Me content in various tea cultivars. Figure 3 shows the HPLC chromatograms for catechin standards (Figure 3A) and 'Benifuuki' green tea infusion from the second crop of the 2007 season (Figure 3B). Table 1 shows ECG3″Me and EGCG3″Me contents in various tea cultivars from the first to third crops of the 2007 season. Among the tea cultivars tested, ECG3″Me was most abundantly found in 'Benifuuki', and ECG3″Me content in the third crop was 1.05% (dry weight). Moderate amounts of ECG3″Me were also found in the cultivars 'Asahi', 'Benihomare', 'Ujihikari', 'Tamamidori', and 'Yamatomidori', and all other cultivars contained minimal amounts.

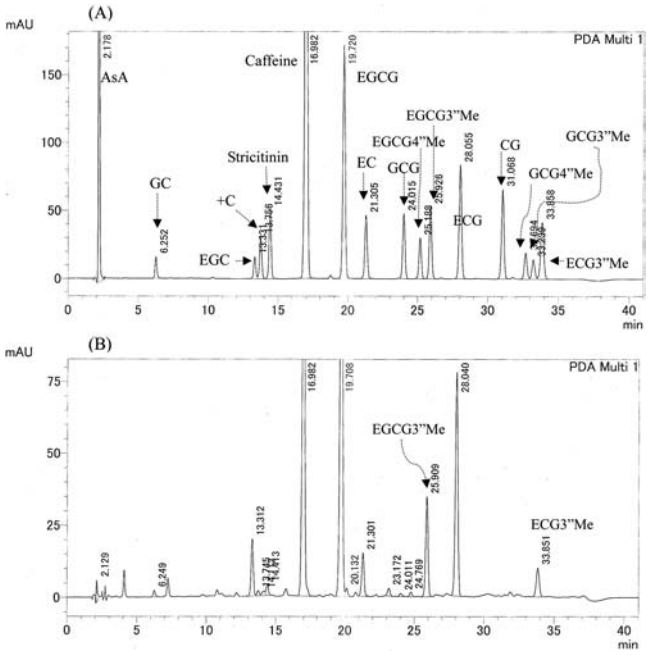


Figure 3. HPLC chromatograms of catechin standards (A) and 'Benifuuki' green tea infusion (B). The 'Benifuuki' green tea used was plucked from the second crop of the 2007 season.

Figure 4 shows the correlation between EGCG3″Me and ECG3″Me contents in 'Benifuuki' tea leaves. ECG3″Me content was positively correlated with EGCG3″Me content in 'Benifuuki' tea leaves (r = 0.803, t = 21.50, $P = 4.10 \times 10^{-59}$).

Inhibitory Effect of Mixtures of ECG3″Me and EGCG3″Me on Histamine Release. To obtain further information on the inhibitory effect of ECG3″Me, we examined the effect of mixtures of EGCG3″Me and ECG3″Me on histamine release inhibition. Mixtures 1 (EGCG3″Me 50 μg/mL + ECG3″Me 0 μg/mL), 2 (EGCG3″Me 37.5 μg/mL + ECG3″Me 12.5 μg/mL), 3 (EGCG3″Me 25 μg/mL + ECG3″Me 25 μg/mL), 4 (EGCG3″Me 12.5 μg/mL + ECG3″Me 37.5 μg/mL), and 5 (EGCG3″Me 0 μg/mL + ECG3″Me 50 μg/mL) and a 'Benifuuki' infusion (50 μg/mL as tannin content) were examined with respect to histamine release inhibition. As shown in Figure 5, the inhibitory activity was increased in proportion to the ECG3″Me dose, up to 50 μg/mL addition; however, the activity of the 'Benifuuki' infusion was the greatest.

DISCUSSION

In our previous study, we demonstrated that EGCG3″Me exhibited greater inhibition of histamine release than EGCG,[8,12]

dx.doi.org/10.1021/jf204497b | J. Agric. Food Chem. 2012, 60, 2165–2170

Table 1. Contents of ECG3″Me and EGCG3″Me in Various Cultivars of Green Tea from the First to Third Crops of the 2007 Season in Kanaya (Mean ± SD)

	first crop (% dry weight)		second crop (% dry weight)		third crop (% dry weight)	
cultivar	ECG3″Me	EGCG3″Me	ECG3″Me	EGCG3″Me	ECG3″Me	EGCG3″Me
Benifuuki	**0.38 ± 0.00**	**0.74 ± 0.01**	**0.65 ± 0.01**	**1.90 ± 0.01**	**1.05 ± 0.01**	**3.16 ± 0.03**
Hatsumomiji	0.38 ± 0.02	0.08 ± 0.01	0.19 ± 0.01	0.00	0.09 ± 0.00	0.00
Benihomare	0.27 ± 0.01	0.62 ± 0.01	0.44 ± 0.01	1.13 ± 0.03	0.35 ± 0.01	1.04 ± 0.03
Benihikari	0.24 ± 0.01	0.30 ± 0.01	0.26 ± 0.01	0.66 ± 0.06	0.31 ± 0.01	1.20 ± 0.03
Ujihikari	0.22 ± 0.00	0.25 ± 0.01	0.26 ± 0.01	0.34 ± 0.05	0.22 ± 0.01	0.49 ± 0.02
Asahi	0.20 ± 0.00	0.00	0.09 ± 0.00	0.00	0.04 ± 0.00	0.00
Yamatomidori	0.20 ± 0.01	0.32 ± 0.01	0.25 ± 0.02	0.53 ± 0.03	0.23 ± 0.00	0.75 ± 0.01
Benifuji	0.20 ± 0.00	0.83 ± 0.01	0.36 ± 0.01	1.89 ± 0.04	0.68 ± 0.03	3.07 ± 0.15
Okumusashi	0.17 ± 0.01	0.26 ± 0.00	0.17 ± 0.03	0.26 ± 0.00	0.13 ± 0.00	0.45 ± 0.01
Ryofu	0.16 ± 0.00	0.25 ± 0.01	0.15 ± 0.01	0.32 ± 0.01	0.19 ± 0.00	0.80 ± 0.02
Yutakamidori	0.16 ± 0.01	0.12 ± 0.00	0.15 ± 0.00	0.24 ± 0.01	0.13 ± 0.01	0.44 ± 0.02
Inzatsu131	0.15 ± 0.00	0.19 ± 0.00	0.15 ± 0.01	0.44 ± 0.01	0.16 ± 0.00	0.43 ± 0.01
Minamisayaka	0.15 ± 0.01	0.26 ± 0.01	0.17 ± 0.00	0.42 ± 0.00	0.24 ± 0.01	0.85 ± 0.02
Hokumei	0.14 ± 0.01	0.00	0.10 ± 0.00	0.04 ± 0.00	0.05 ± 0.00	0.00
Sayamakaori	0.14 ± 0.01	0.00	0.09 ± 0.00	0.00	0.06 ± 0.01	0.00
Kanayamidori	0.12 ± 0.01	0.25 ± 0.01	0.09 ± 0.01	0.42 ± 0.01	0.15 ± 0.01	0.73 ± 0.09
Sayamamidori	0.12 ± 0.00	0.05 ± 0.01	0.09 ± 0.00	0.00	0.02 ± 0.00	0.00
Izumi	0.12 ± 0.00	0.00	0.06 ± 0.00	0.00	0.05 ± 0.00	0.00
Minekaori	0.11 ± 0.00	0.00	0.07 ± 0.00	0.00	0.07 ± 0.00	0.00
Yamakai	0.11 ± 0.00	0.24 ± 0.01	0.13 ± 0.00	0.38 ± 0.02	0.17 ± 0.00	0.79 ± 0.01
Ooiwase	0.11 ± 0.01	0.05 ± 0.00	0.10 ± 0.00	0.14 ± 0.01	0.07 ± 0.00	0.00
Tamamidori	0.11 ± 0.00	0.00	0.09 ± 0.01	0.00	0.07 ± 0.01	0.00
Gokou	0.11 ± 0.01	0.00	0.05 ± 0.00	0.00	0.06 ± 0.00	0.00
Okumidori	0.11 ± 0.01	0.26 ± 0.01	0.12 ± 0.00	0.41 ± 0.00	0.19 ± 0.00	0.83 ± 0.01
Asatsuyu	0.10 ± 0.01	0.00	0.06 ± 0.00	0.00	0.05 ± 0.01	0.00
Saemidori	0.10 ± 0.00	0.00	0.06 ± 0.01	0.00	0.04 ± 0.00	0.00
Sofu	0.10 ± 0.00	0.12 ± 0.00	0.09 ± 0.01	0.15 ± 0.00	0.15 ± 0.01	0.54 ± 0.01
Kuritawase	0.09 ± 0.00	0.13 ± 0.01	0.11 ± 0.00	0.20 ± 0.01	0.06 ± 0.00	0.18 ± 0.00
Samidori	0.09 ± 0.01	0.00	0.11 ± 0.01	0.00	0.03 ± 0.00	0.00
Fushun	0.09 ± 0.01	0.00	0.05 ± 0.00	0.00	0.04 ± 0.00	0.00
Asagiri	0.08 ± 0.01	0.10 ± 0.00	0.10 ± 0.00	0.17 ± 0.00	0.11 ± 0.00	0.55 ± 0.02
Fukumidori	0.08 ± 0.00	0.00	0.03 ± 0.00	0.00	0.03 ± 0.00	0.00
Toyoka	0.08 ± 0.00	0.00	0.05 ± 0.00	0.00	0.03 ± 0.00	0.00
Surugawase	0.07 ± 0.00	0.18 ± 0.01	0.18 ± 0.01	0.34 ± 0.01	0.20 ± 0.00	0.66 ± 0.01
Seishin-oolong	0.07 ± 0.01	0.00	0.06 ± 0.00	0.00	0.03 ± 0.00	0.00
Komakage	0.07 ± 0.00	0.00	0.04 ± 0.00	0.00	0.05 ± 0.01	0.04 ± 0.00
Meiryoku	0.07 ± 0.00	0.00	0.04 ± 0.00	0.00	0.06 ± 0.01	0.00
Minamikaori	0.07 ± 0.00	0.00	0.06 ± 0.00	0.00	0.04 ± 0.00	0.00
Shunmei	0.06 ± 0.00	0.15 ± 0.00	0.15 ± 0.00	0.24 ± 0.01	0.14 ± 0.01	0.52 ± 0.02
Okuyutaka	0.06 ± 0.00	0.00	0.04 ± 0.00	0.00	0.03 ± 0.00	0.00
Sakimidori	0.05 ± 0.00	0.14 ± 0.01	0.11 ± 0.00	0.44 ± 0.00	0.10 ± 0.01	0.38 ± 0.01
Yabukita	0.05 ± 0.00	0.00	0.05 ± 0.00	0.00	0.06 ± 0.00	0.00
Seishin-taipan	0.05 ± 0.00	0.26 ± 0.01	0.15 ± 0.01	0.56 ± 0.00	0.22 ± 0.01	1.30 ± 0.07
Harumidori	0.05 ± 0.00	0.00	0.05 ± 0.00	0.00	0.01 ± 0.00	0.00
Yaeho	0.17 ± 0.00	0.22 ± 0.01	0.14 ± 0.01	0.48 ± 0.04		
Ohba-oolong	0.09 ± 0.03	0.31 ± 0.00	0.23 ± 0.00	0.95 ± 0.01		

in which an antiallergic action had been observed, using an in vivo type-I allergic PCA test.[6] Furthermore, when the in vitro antiallergic effects of EGCG3″Me and EGCG and their C-2 epimers GCG3″Me and GCG were compared, it was found that GCG3″Me had the greatest activity, with the order of potency as follows: GCG3″Me > EGCG3″Me > GCG > EGCG.[20] GCG3″Me and GCG, EGCG3″Me, and EGCG C-2 isomers were not present in the fresh tea leaves, but are generated by the heating process (>80 °C) in infusions.[21,22] It is known that tea leaves include various catechins,[23] and the presence of *O*-methylated catechin, with the exception of EGCG3″Me, is suggested in 'Benifuuki' tea leaves. Therefore, to reveal the catechin with the greatest activity in 'Benifuuki' tea leaves, we investigated the histamine release inhibitory effects of EGC, GC (EGC isomer), ECG, CG (ECG isomer), EGCG, GCG, EGCG3″Me, GCG3″Me, and ECG3″Me.

Figure 2 shows that the in vitro antiallergic activity of ECG3″Me is greater than that of EGCG3″Me. High levels of ECG3″Me were observed in 'Benifuuki', 'Asahi', 'Benihomare', 'Ujihikari', 'Tamamidori', and 'Yamatomidori', and minimal levels were found in the remaining cultivars (Table 1). However, previous results revealed that EGCG3″Me was not found in any

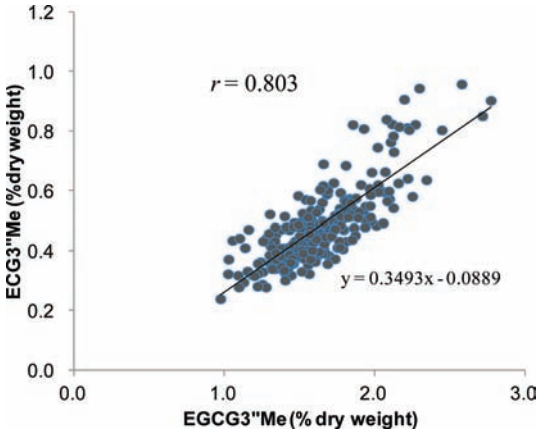


Figure 4. Correlation between EGCG3″Me and ECG3″Me contents in 'Benifuuki' tea leaves. The test samples (256) were plucked in Shizuoka, Kagoshima, Okinawa, and Ooita from the first to fourth crops of the 2006 season.

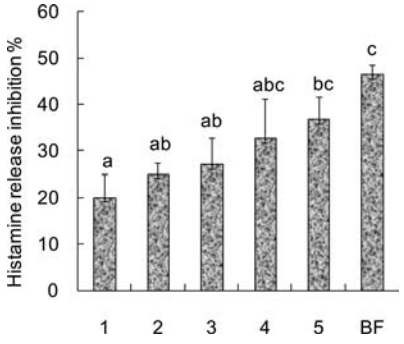


Figure 5. Inhibitory activity of various mixtures of EGCG3″Me and ECG3″Me. Results are expressed as the percentage of histamine release compared to vehicle after antigen stimulation (%) (n = 3, mean ± SD). 1, EGCG3″Me 50 μg/mL + ECG3″Me 0 μg/mL; 2, EGCG3″Me 37.5 μg/mL + ECG3″Me 12.5 μg/mL; 3, EGCG3″Me 25 μg/mL + ECG3″Me 25 μg/mL; 4, EGCG3″Me 12.5 μg/mL + ECG3″Me 37.5 μg/mL; 5, EGCG3″Me 0 μg/mL + ECG3″Me 50 μg/mL; BF, 'Benifuuki' infusion (as tannin content; 50 μg/mL). Mean values in columns not sharing letters are significantly different by the Tukey–Kramer test (P < 0.01).

cultivar.[11] ECG3″Me content in 'Benifuuki' showed the same trend as observed with EGCG3″Me, and the ECG3″Me content increased in the rank order of third > second > first crop season.

Although the EGCG3″Me content of 'Benifuji' was almost the same as that of 'Benifuuki', the ECG3″Me content of 'Benifuji' was about half that of 'Benifuuki'. We demonstrate that ECG3″Me in 'Benifuuki' varies in the same manner as EGCG3″Me content. 'Benifuuki' is rich in not only EGCG3″Me but also ECG3″Me. As shown in Figure 3, the activity of 'Benifuuki' green tea infusion (50 μg tannin) was greater than that of 50 μg of ECG3″Me. Interestingly, 'Benifuuki' infusion included only 3.61 μg of EGCG3″Me and 0.72 μg of ECG3″Me per 50 μg equivalent to tannin. These results suggest that an unknown antiallergic substance or a synergistic effect with other substances might exist in 'Benifuuki' green tea infusion. To clarify this point, further experimentation will be initiated.

Previous studies have demonstrated antiallergic activity, using the histamine-dependent PCA test, of a single oral dose of 50 mg/kg EGCG3″Me in *dd*Y mice,[6] as well as improvements in the nasal symptoms of allergic rhinitis with 'Benifuuki' green tea infusion containing 34 mg of EGCG3″Me in a double-blind placebo-controlled trial.[14] EGCG is the most abundant polyphenol in tea leaves, and the major EGCG metabolites found in the plasma of EGCG-treated mice were monomethylated EGCG, monosulfated methyl EGCG, monoglucuronide EGCG, and monoglucuronide methyl EGCG.[24] Moreover, 4″-*O*-methyl-EGCG (EGCG4″Me) was identified as the major monomethylated EGCG metabolite in mouse plasma,[24] whereas in rats, the ECG metabolites were shown to be (−)-epicatechin-gallate, 3′-*O*-methyl-ECG, 4′-*O*-methyl-ECG, 4″-*O*-methyl-ECG, and 3′,4″-di-*O*-methyl-ECG.[25] Therefore, the EGCG3″Me and ECG3″Me detected in plasma are likely to be primarily derived from ingested material. On the other hand, it has been reported that the maximum drug concentration (C_{max}) for EGCG3″Me after oral administration of 'Benifuuki' green tea containing 8.5 mg of EGCG3″Me and 43.5 mg of EGCG was 12.4 ng/mL at 6 h compared with 16.3 ng/mL for EGCG at <1 h in a human trial.[14] The delay in the methylated EGCG (EGCG3″Me) achieving its C_{max} might suggest that it is formed in part by methylation of EGCG in the liver. We have previously reported that EGCG4″Me inhibited histamine release in BMMCs, with a rank order of inhibition of EGCG3″Me > EGCG4″Me at a dose of 50 μg/mL.[12] ECG3″Me (0.07%) has an absorption rate similar to that of EGCG3″Me (0.06%) in humans (unpublished data). According to HPLC analysis of 'Benifuuki' tea leaves, ECG3″Me content is expected to be approximately 15 mg in a 'Benifuuki' green tea infusion containing 34 mg of EGCG3″Me. From these findings, the C_{max} of ECG3″Me is expected to be 21 ng/mL after oral administration of 'Benifuuki' green tea. In this study, we compared the inhibition of histamine release by tea leaf catechins at 50 μg/mL, a dose that was determined according to the results of our previous in vitro study.[12]

In a previous study, EGCG, *O*-methylated EGCGs, or procyanidin C1 inhibited histamine release through the prevention of tyrosine phosphorylation by cellular protein kinases.[26−29] We predict that ECG3″Me might also inhibit histamine release through the suppression of tyrosine phosphorylation; therefore, we are currently investigating whether or not ECG3″Me affects signal transduction in mast cells.

These results suggested that ECG3″Me has greater histamine release inhibitory activity than EGCG3″Me in vitro and that 'Benifuuki' green tea, which is rich in ECG3″Me and EGCG3″Me, could be a valuable tea cultivar with antiallergic effects. The present study was undertaken in an attempt to elucidate the differences in in vitro inhibitory activity of ECG3″Me and EGCG3″Me on histamine release, which has been shown to have antiallergic effects in previous in vivo studies.[6,7] Further experimentation on the antiallergic effect of ECG3″Me at lower doses, comparable to levels expected in vivo, should be initiated in the future.

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Funding

This work was supported by a grant from the Program for the Promotion of Basic Research Activities for Innovative Biosciences, Ministry of Agriculture, Fishery and Forestry, Japan.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank Prof. Toshio Miyase (University of Shizuoka, Japan) for kindly donating the ECG3″Me, EGCG3″Me, and GCG3″Me. We also thank Dr. Toshiaki Kawakami and Dr. Yuko Kawakami (La Jolla Institute for Allergy and Immunology, USA) for pertinent advice on experimental methodology.

■ ABBREVIATIONS USED

EC, epicatechin; C, catechin; EGC, epigallocatechin; GC, gallocatechin; ECG, epigallocatechin-3-*O*-gallate; CG, catechin-3-*O*-gallate; 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; ECG3″Me, epicatechin-3-*O*-(3″-*O*-methyl)-gallate; BMMC, bone marrow derived mast cells; PBS, phosphate-buffered saline; BSA, bovine serum albumin.

■ REFERENCES

(1) Jacobs, R. L. Ciclesonide for the treatment of seasonal allergic rhinitis. *Expert Rev. Clin. Immunol.* **2011**, *7*, 735−741.

(2) Galli, S. J.; Maurer, M.; Lantz, C. S. Mast cells as sentinels of innate immunity. *Curr. Opin. Immunol.* **1999**, *11*, 53−59.

(3) Kinet, J. P. The high-affinity IgE receptor (Fc*ε*RI): from physiology to pathology. *Annu. Rev. Immunol.* **1999**, *17*, 931−972.

(4) Beaven, M. A.; Metzger, H. Signal transduction by Fc receptors: the Fc epsilon RI case. *Immunol. Today* **1993**, *14*, 222−226.

(5) Kawakami, T.; Galli, S. J. Regulation of mast-cell and basophil function and survival by IgE. *Nat. Rev. Immunol.* **2002**, *2*, 773−786.

(6) Sano, M.; Suzuki, M.; Miyase, T.; Yoshino, K.; Maeda-Yamamoto, M. Novel antiallergic catechin derivatives isolated from oolong tea. *J. Agric. Food Chem.* **1999**, *47*, 1906−1910.

(7) Suzuki, M.; Yoshino, K.; Maeda-Yamamoto, M.; Miyase, T.; Sano, M. Inhibitory effects of tea catechins and O-methylated derivatives of (−)-epigallocatechin-3-*O*-gallate on mouse type-IV allergy. *J. Agric. Food Chem.* **2000**, *48*, 5649−5653.

(8) Fujimura, Y.; Tachibana, H.; Maeda-Yamamoto, M.; Miyase, T.; Sano, M.; Yamada, K. Antiallergic tea catechin: (−)-epigallocatechin-3-*O*-(3-*O*-methyl)-gallate, suppresses Fc*ε*RI expression in human basophilic KU812 cells. *J. Agric. Food Chem.* **2002**, *50*, 5729−5730.

(9) Tachibana, H.; Kubo, T.; Miyase, T.; Tanino, S.; Yoshimoto, M.; Sano, M.; Maeda-Yamamoto, M.; Yamada, K. Identification of an inhibitor for interleukin 4-induced e germline transcription and antigen-specific IgE production in vivo. *Biochem. Biophys. Res. Commun.* **2001**, *280*, 53−60.

(10) Maeda-Yamamoto, M.; Kawahara, H.; Matsuda, N.; Nesumi, K.; Sano, M.; Tsuji, K.; Kawakami, Y.; Kawakami, T. Effects of tea infusions of various varieties or different manufacturing types on inhibition of mouse mast cell activation. *Biosci., Biotechnol., Biochem.* **1998**, *62*, 2277−2279.

(11) Maeda-Yamamoto, M.; Sano, M.; Matsuda, N.; Miyase, T.; Kawamoto, K.; Suzuki, N.; Yoshimura, M.; Tachibana, H.; Hakamata, K. The change of epigallocatechin-3-*O*-(3-*O*-methyl) gallate contents in tea of different varieties, tea seasons of crop and processing method [in Japanese]. *J. Jpn. Food Sci. Technol.* **2001**, *48*, 64−68.

(12) Maeda-Yamamoto, M.; Inagaki, N.; Kitaura, J.; Chikumoto, T.; Kawahara, H.; Kawakami, Y.; Sano, M.; Miyase, T.; Tachibana, H.; Nagai, H.; Kawakami, T. *O*-methylated catechins from tea leaves, inhibit multiple protein kinases in mast cells. *J. Immunol.* **2004**, *172*, 4486−4492.

(13) Fujimura, Y.; Umeda, D.; Yano, S.; Maeda-Yamamoto, M.; Yamada, K.; Tachibana, H. The 67 kDa laminin receptor as a primary determinant of anti-allergic effects of *O*-methylated EGCG. *Biochem. Biophys. Res. Commun.* **2007**, *364*, 79−85.

(14) Maeda-Yamamoto, M.; Ema, K.; Shibuichi, I. *In vitro* and *in vivo* anti-allergic effects of 'Benifuuki' green tea containing *O*-methylated catechin and ginger extract enhancement. *Cytotechnology* **2007**, *55*, 135−142.

(15) Kimata, M.; Inagaki, N.; Nagai, H. Effects of luteolin and other flavonoids on IgE-mediated allergic reactions. *Planta Med.* **2000**, *66*, 25−29.

(16) Iwasa, K; Torii, H. A colorimetric determination of tea tannin with ferrous tartrate [in Japanese]. *Study Tea* **1962**, *26*, 87−91.

(17) Horie, H.; Maeda-Yamamoto, M.; Ujihara, T.; Kohata, K. Extraction of tea catechins for chemical analysis. *Tea Res. J.* **2002**, *94*, 60−64.

(18) Maeda-Yamamoto, M.; Nagai, H.; Asai, K.; Moriwaki, S.; Horie, H.; Kohata, K.; Tachibana, H.; Miyase, T.; Sano, M. Changes in epigallocatehin-3-*O*-(3-*O*-methyl) gallate and strictinin contents of tea (*Camellia sinensis* L.) cultivar 'Benifuuki' in various degrees of maturity and leaf order. *Food Sci. Technol. Res.* **2004**, *10*, 186−190.

(19) Saito, K.; Horie, M.; Nose, N.; Nakagomi, K.; Nakazawa, H. High-performance liquid chromatography of histamine and 1-methylhistamine with on-column fluorescence derivatization. *J. Chromatogr.* **1992**, *595* (1−2), 163−168.

(20) Nagai, H.; Maeda-Yamamoto, M.; Suzuki, Y.; Sato, K.; Mitsuda, H. The development of suitable manufacturing process for 'Benifuuki' green tea beverage, with anti-allergic effects. *J. Food Sci. Agric.* **2005**, *85*, 1601−1612.

(21) Komatsu, Y.; Suematsu, S.; Hisanomu, Y.; Saigo, H.; Matsuda, R.; Hara, K. Effects of pH and temperature on reaction kinetics of catechin in green tea infusion. *Biosci., Biotechnol., Biochem.* **1993**, *57*, 907−910.

(22) Seto, R.; Nakamura, H.; Nanjo, F.; Hara, Y. Preparation of epimers of tea catechins by heat treatment. *Biosci., Biotechnol., Biochem.* **1997**, *61*, 1434−1439.

(23) Hu, B.; Wang, L.; Zhou, B.; Zhang, X.; Sun, Y.; Ye, H.; Zhao, L.; Hu, Q.; Wang, G.; Zeng, X. Efficient procedure for isolating methylated catechins from green tea and effective simultaneous analysis of ten catechins, three purine alkaloids, and gallic acid in tea by high-performance liquid chromatography with diode array detection. *J. Chromatogr., A* **2009**, *1216*, 3223−3231.

(24) Sang, S.; Yang, I.; Buckley, B.; Ho, C. T.; Yang, C. S. Autoxidative quinone formation in vitro and metabolite formation in vivo from tea polyphenol (−)-epigallocatechin-3-gallate: studied by real-time mass spectrometry combined with tandem mass ion mapping. *Free Radical Biol. Med.* **2007**, *43*, 362−371.

(25) Kohri, T.; Suzuki, M.; Nanjo, F. Identification of metabolites of (−)-epicatechin gallate and their metabolic fate in the rat. *J. Agric. Food Chem.* **2003**, *51*, 5561−5566.

(26) Kanda, T.; Akiyama, H.; Yanagida, A.; Tanabe, M.; Goda, Y.; Toyoda, M.; Teshima, R.; Saito, Y. Inhibitory effects of apple polyphenol on induced histamine release from RBL-2H3 cells and rat mast cells. *Biosci., Biotechnol., Biochem.* **1998**, *62* (7), 1284−1289.

(27) Shimoda, H.; Tanaka, J.; Yamada, E; Morikawa, T.; Kasajima, N.; Yoshikawa, M. Anti type I allergic property of Japanese Butterbur extract and its mast cell degranulation inhibitory ingredients. *J. Agric. Food Chem.* **2006**, *54*, 2915−2920.

(28) Nakano, N.; Nishiyama, C.; Tokura, T.; Nagasako-Akazome, Y.; Ohtake, Y.; Okumura, K. Procyanidin C1 from apple extracts inhibits Fc*ε*RI-mediated mast cell activation. *Int. Arch. Allergy Immunol.* **2008**, *147*, 213−221.

(29) Yamashita, K.; Suzuki, Y.; Matsui, T.; Yoshimura, T.; Yamaki, M.; Suzuki-Karasaki, M.; Hayakawa, S.; Shimizu, K. Epigallocatechin gallate inhibits histamine release from rat basophilic leukemia (RBL-2H3) cells; role of tyrosine phophorylation pathway. *Biochem. Biophys. Res. Commun.* **2000**, *274*, 603−608.