We found that the epigallocatechin gallate (EGCG)/epigallocatechin (EGC) ratio in a green tea (Camellia sinensis L.) extract was affected by the extraction temperature. The EGCG/EGC ratio in the 4°C extract was around 1:3-4, whereas in the 100°C extract, it was around 1:0.7. Oral administration of the mixture with a high EGC ratio (1:2-3 = EGCG/EGC) resulted in greater IgA production by murine Peyer’s patch cells.

Key words: epigallocatechin (EGC); epigallocatechin gallate (EGCG); Peyer’s patch cell; green tea (Camellia sinensis L.) extract

Tea from the Camellia sinensis L. plant is one of the most popular beverages consumed worldwide in its green, black, or oolong form. It contains such compounds as polyphenols, polysaccharides, amino acids and vitamins, and reduces the risk of a variety of diseases.1) Catechins, one of the main components of this tea extract, have antioxidative activity1) and immunomodulating activities,2–4) and play an important role in reducing the risk of disease. The most abundant catechins in a green tea extract are epigallocatechin gallate (EGCG), epicatechin gallate (ECG), epicatechin (EC) and epigallocatechin (EGC), and there are many reports on each catechin and green tea extract; however, no study focusing on the catechin ratio in a green tea has been reported. We analyzed in this study the EGCG/EGC ratio in green tea extracts from different mixture ratios of EGCG and EGC, and investigated the immunomodulating activitys, 2–4) and play an important role in reducing the risk of disease. The most abundant catechins in a green tea extract are epigallocatechin gallate (EGCG), epicatechin gallate (ECG), epicatechin (EC) and epigallocatechin (EGC), and there are many reports on each catechin and green tea extract; however, no study focusing on the catechin ratio in a green tea extract has been reported. We analyzed in this study the catechin ratio in green tea extracts at various temperatures, and investigated the immunomodulating activity of different mixture ratios of EGCG and EGC, the main catechins in the green tea extract.

BALB/c female mice, 7 weeks old, were purchased from Charles River (Kanagawa, Japan) and were housed three per cage in a room at 24°C and 60% relative humidity. A time-controlled system provided a 12-h light/dark cycle (lights on 7:00–19:00 h). Food (CRF-1; Oriental Yeast, Tokyo) and water were supplied ad libitum. The three mice per group were administered with 1 ml of either a test solution or distilled water (DW) once daily for 2 weeks via an orogastric tube, and were sacrificed by cervical dislocation 24 h after the last administration. The small intestines (jejunum and ileum) were isolated, and Peyer’s patches (PPs) were excised from the small intestine. PPs were mashed with a stainless steel mesh and a syringe plunger in ice-cold PBS and, after washing twice, the cells were resuspended at a concentration of 2.0 × 10⁶ cells/ml in an RPMI 1640 medium supplemented with 10% FBS. Two hundred microliters of the PP cell suspension was seeded into five wells each of 96-well plates, and incubated for 5 d at 37°C with 5% CO₂. The animal experiment was performed according to Guidelines for Animal Experimentation no. 105 and Notification no. 6 implemented by the government. A total IgA analysis was then performed. IgA in the PP cell culture supernatant was measured by ELISA. A MaxiSorp ELISA plate (Nunc, Rochester, NY, USA) was coated with 100μl of goat anti-mouse IgA (Zymed Laboratories, San Francisco, CA, USA), and diluted 1:1000 in a 50mM Na-carbonate buffer (pH 9.6) overnight at 4°C. After washing with 0.05%Tween 20-PBS (PBS-T), the plate was blocked with 1% (w/v) bovine serum albumin in PBS, and then 100μl of each test sample and standard mouse IgA (Bethyl Laboratories, Montgomery, TX, USA) were added. The plate was incubated at 37°C for 60 min, washed three times with PBS-T, and then 100μl of a goat anti-mouse IgA horseradish peroxidase conjugate (Zymed Laboratories), diluted 1:2000 in 1% BSA in PBS, was added. The plate was further incubated at 37°C for 60 min, washed three times with PBS-T, and then 100μl of 0.8 M 2,2’-azinobis (3-ethylbenzthiazoline) 6-sulfonic acid in a 150 mM citrate buffer (pH 4.0) with 0.0045% hydrogen peroxide was added. The plate was finally incubated at room temperature for 5 min, and 100μl of 1.5% (w/v) oxalic acid was added. The absorbance was measured at 415 nm by a microplate reader.

The green tea extract was prepared from a tea cultivar harvested from a plantation of the National Institute of Vegetable and Tea Science in Kanaya, Shizuoka, Japan. These tea leaves were dried in a microwave oven, pulverized, and stored at 4°C before being subjected to analysis. The tea leaves (2.5 g) in DW (100 ml) were left to stand at various temperatures for 2 or 60 min. The catechin assay was performed as previously described.5) Briefly, the tea extract was diluted with DW, and 20μl of the extract was injected into HPLC apparatus with a reverse-phase C18 column and UV detector. The solution was then eluted as next described at a flow rate of 1 ml/min and 40°C. EC, catechin (+C), EGCG, ECG, and catechin gallate (CG) were measured at 272 nm, and EGC was measured at 242 nm. The HPLC analysis was performed by using a linear gradient composed of

Note
Effect on the Epigallocatechin Gallate/Epigallocatechin Ratio in a Green Tea (Camellia sinensis L.) Extract of Different Extraction Temperatures and Its Effect on IgA Production in Mice

Manami MONOBE,1 Kaori EMA, Yoshiko TOKUDA, and Mari MAEDA-YAMAMOTO

National Institute of Vegetable and Tea Science, NARO, 2769 Kanaya, Shimada, Shizuoka 428-8501, Japan

Received July 6, 2010; Accepted September 4, 2010; Online Publication, December 7, 2010 [doi:10.1271/bbb.100498]
mobile phase A (H$_2$O-acetonitrile-H$_3$PO$_4$, 400:10:1) and mobile phase B (methanol-mobile phase A, 1:2). The eluates were quantified by using the external standard method.

We first analyzed the catechin ratio in green tea extracted with water at various temperatures. As shown in Fig. 1A, EGC in the 4°C extract made up 60% of the total catechins, its ratio being decreased with increasing extraction temperature. On the other hand, EGCG in the 4°C extract made up around 15%, its ratio being increased with increasing extraction temperature. The respective proportions of EGCG and EGC in the boiled extract were around 30% and 40% (Fig. 1A).

As shown in Fig. 1B, the ratio of EGCG and EGC was affected by the change in extraction efficiency of EGCG. The inefficient extraction of EGCG may have been due to its high membrane interaction, and increased EGCG extraction may have resulted from physical damage to the membrane due to the increasing extraction temperature.

We next investigated the immunomodulating activity of the mixture of EGCG and EGC, the main catechins in the green tea extract (Fig. 1). Secretory immunoglobulin A (sIgA) plays an important role as the first line of defense against pathogens in a mucosal membrane, and its increase is considered to help to prevent an infectious disease such as influenza. Such dietary components as nucleotides, fructooligosaccharides, and vitamin A have been found to increase the mucosal IgA secretion. We hence investigated the total IgA produced by ex vivo PP cells after orally administering the mixture of EGCG and EGC to mice. The respective EGCG and EGC contents of the 4°C extract were around 0.6 and 0.15 mg/ml, and around 0.7 and 0.9 mg/ml in the 100°C extract. Hence, we orally administered 1.0 mg each of mixtures of EGCG and EGC in different ratios, as well as 0.5 mg of EGC or EGCG to mice for 2 weeks, and then examined total IgA produced by PP cells cultured for 5 d. As shown in Fig. 2A, the 0.5-mg EGC administration group resulted in significantly
We thus infer that the lower response from the 100°C extract may have been due to offsetting the effect of EGC by the increased EGCG with a negative effect.

The mucosal immune system is regulated by the interaction of various immune cells. An increase in the mucosal IgA secretion by dietary components is due to an enhanced antigen response caused by antigen-presenting cells (APC) and lymphocyte activation. EGCG has a phagocytosis-enhancing effect on macrophage-like cells via caspase activation, and we confirmed that the administration of EGCG for two weeks enhanced the phagocytic activity of ex vivo PP cells, but that EGC did not (data not shown). The enhanced IgA production by EGCG administration may have been due to enhancement of the antigen response by APC activation. Most previously reported immunomodulation of catechins has been due to such immunosuppressive effects as anti-allergy and anti-inflammatory, and most of these effects were brought about by EGCG. The 67-kDa laminin receptor (67LR) has recently been identified as a cell surface receptor for EGCG and it has been suggested that 67LR participated in the immunosuppressive activity of EGCG. EGCG and EGC can have different effects on immune cells. Hence, the different content ratio in green tea extracts may have the potential to induce different effects on the mucosal immune system. Further studies are needed to clarify the molecular mechanism concerning modulation of the immune system by catechins; however, the findings in this study may provide a fundamental clue to the immunomodulatory effect of an oral intake of green tea.

Fig. 3. Effect of an Oral Administration of the Green Tea Extract on IgA Produced in a PP Cell Culture.

The effect is shown of orally administering the 4°C extract (around 1 mg of total catechins; EGCG:EGC = 1:3–4) or the 100°C extract (around 2.5 mg of total catechins; EGCG:EGC = 1:0.7) on IgA produced in the PP cell culture. The tea leaves (2.5 g) in DW (100 ml) were respectively left to stand at 4°C or 100°C for 60 min or 2 min. The mice were administered with 1 ml of the 100°C extract, 4°C extract or DW once daily for 14 d via an orogastric tube. Thirteen independent experiments were each performed with the 4°C and 100°C green tea extracts. Each point represents the value obtained by using three mice. IgA production in DW was normalized to 100%. The bar is a median value. a p < 0.05 (Wilcoxon signed-rank test for non-normal distribution).

References