Inhibitory effects of O-methylated catechin derivatives on mouse type-I allergy.

Mitsuaki Sano¹, Masazumi Suzuki¹ Toshio Miyase¹, Yoshiyuki Nakamura¹, Kyoji Yoshino¹ Hirofumi Tachibana⁶ and Mari Maeda-Yamamoto⁷

¹: University of Shizuoka, 52-1 Yada, Shizuoka, 422-8526, Japan
²: Numazu College of Technology, 3600 Ooka, Numazu, 410-8501, Japan
³: Kyushu University, 6-10-1 Hakoizaki Higashiku, Fukuoka, 812-8581, Japan
⁴: Nat. Res.Inst. Vegetable and Tea Science, NARO, 2769 Kanaya-cho, Shizuoka, 428-8501, Japan

Summary

Oral administration of the O-methylated catechin derivatives, (-)-epigallocatechin-3-O-(3'-O-methyl)gallate (EGCG 3'-Me) and (-)-epigallocatechin-3-O-(4'-O-methyl)gallate (EGCG 4'-Me), significantly and dose-dependently (5–50 mg/kg) inhibited anaphylactic reactions in mice sensitized with ovalbumin (OVA) and Freund's incomplete adjuvant (FIA). These inhibitory effects exceeded that of the major tea catechin, (-)-epigallocatechin-3-O-gallate (EGCG), which had known potent anti-allergic properties. The O-methylated catechins significantly inhibited histamine release from mouse mast cells and human basophilic cells. The EGCG 3'-Me content in the cultivars "Benifuuki", "Benifuji" and "Benihomare" was higher than those in the other tea cultivars tested. EGCG 4'-Me was localized exclusively in "Tong ting" oolong tea manufactured in Taiwan. The results suggest that the intake of tea extracts containing the O-methylated catechins may be effective in the treatment of allergic disorders.

Key words: tea, methylated catechin, type I anti-allergic effect

Introduction

Several studies have demonstrated that EGCG strongly inhibited type I allergic reactions.¹,² Yamamoto et al.³ have reported that infusions of oolong tea manufactured in Taiwan significantly inhibited the release of histamine from mouse mast cells, and that the inhibitory activity exceeded that of green tea infusions prepared from the cultivar "Yabukita", which accounts for most of the green tea in Japan. In this paper, we report that two O-methylated catechin derivatives isolated from Taiwanese tea (Tong ting oolong tea) are excellent type-I anti-allergic catechins.

Materials and Methods

Tea. Samples of Tong ting oolong tea were obtained from markets in Japan and Taiwan. All other teas were cultivated at the plantation of National Research Institute Vegetable and Tea Science in Kanaya, Shizuoka.

Analysis of catechins. The major catechins (EGCG, ECG, EC, EGC, GC) and O-methylated catechin derivatives in tea leaves were determined by HPLC with
electrochemical detector (ECD). \(^4,5\)

**Assay of anti-allergic activity.** The *in vivo* type I anti-allergic activity of tea catechins was determined using mouse abdominal wall (AW method) according to Kataoka *et al.*\(^6\) with slight modification. Briefly, male *ddY* mice at 5 weeks of age were sensitized intraperitoneally with a 1:1 mixture of OVA (2 mg/mL N-saline) and FITC. The catechin sample (or distilled water as control) was administered orally to mice 9 days after initial exposure to OVA. Sixty minutes after the administration of the sample, 0.1 mL solution of Evans blue dye (10 mg/mL N-saline) was injected intravenously. Within 5 minutes after the injection of the dye, the abdominal skin of the mice was detached under ether anesthesia, without injury to the abdominal wall. Five minutes after injection of the dye, 50 µL of OVA solution (5 µg/site) was injected in the exposed abdominal wall. The mouse was killed by cervical dislocation 7 minutes after the challenge, then the abdominal wall was removed. The area of the abdominal wall permeated by blue dye was measured using a densitograph with spot image processing software. Anti-allergic activity was expressed as the inhibition ratio compared with control.

### Results and Discussion

**Anti-allergic activities of catechin mixtures.** Catechin mixtures were fractionated from hot water extracts of tea leaves. The effects of the catechin mixture on the anaphylactic reaction are shown in Figure 1. The catechin mixture "Tong ting" oolong tea had the most inhibitory effect, then "Benihomare" green tea and then "Yabukita" green tea.

**Isolation of EGCG3'Me and EGCG4'Me.** Three hundred grams of "Tong ting" oolong tea was extracted with 500 mL boiling water for 30 min. Two O-methylated catechin derivatives were obtained from the extracts by column chromatography techniques\(^7\) as shown in Fig. 2.

**Anti-allergic activities of EGCG and the methylated catechin derivatives.** Oral administration of EGCG inhibited the anaphylactic reaction induced in mouse abdominal wall by 12.6%, 26.9% and 22.1% after a single dose of 10, 50, 100 mg/kg, respectively (Figure 3). The potency of EGCG is highest in major tea catechins. EGCG3"Me and EGCG4"Me were significantly more inhibitory than corresponding
The strongly inhibitory effects of the O-methylated catechins were dose-dependent. The two methylated catechins strongly inhibited mast cell activation through the prevention of tyrosine phosphorylation of cellular protein and histamine release after FceRI cross-linking. The potency is higher than that of EGCG. These catechins inhibited significantly the degranulation of human basophils stimulated with calcium ionophore A23187.8)

Catechin content of tea leaves. The concentrations of EGCG3"Me and EGCG4"Me in fresh green tea leaves manufactured from 42 tea cultivars were examined by HPLC-ECD analysis. The cultivars "Benifuuki", "Benifuji" and "Benihomare" classified as Assam hybrids, contained more EGCG3"Me than the other tea cultivars as shown in Fig. 4. Under the analytical conditions, neither catechin derivative was detected in 16 of the 42 green teas. EGCG4"Me was localized
exclusively in "Tong ting" oolong tea and in a few cultivars such as Seishin-taipan. The concentration was 0.02-0.2 % of dry weight in 14 "Tong ting" oolong tea tested.

It is well known that catechol-O-methyl transferase catalyzes O-methylation of various plant polyphenols, and EGCG3'Me and EGCG4'Me were produced when EGCG was incubated with mouse liver homogenate or authentic catechol-O-methyltransferase in the presence of S-adenosylmethionine. Interestingly, more EGCG4'Me than EGCG3'Me was produced. Therefore, EGCG in vivo may also be partly converted to EGCG4'Me and EGCG3'Me by the methyltransferase, and these catechin complexes may have anti-allergic properties.

In conclusion, we demonstrated that two O-methylated galloyl epigallocatechins isolated from tea leaves have potent anti-allergic effects and that their concentrations differ markedly among tea cultivars. Tea extracts that contain these constituents is thought to have potential to prevent an immediate hypersensitive (type-I) allergy.

References