Inhibitory Effects of Tea Catechins and *O*-Methylated Derivatives of (−)-Epigallocatechin-3-*O*-gallate on Mouse Type IV Allergy

Masazumi Suzuki,[†] Kyoji Yoshino,[‡] Mari Maeda-Yamamoto,[§] Toshio Miyase,[†] and Mitsuaki Sano*[,†]

Laboratory of Health Science, School of Pharmaceutical Sciences, University of Shizuoka, 52-1 Yada, Shizuoka, Shizuoka 422-8526, Japan; Department of Chemistry and Biochemistry, Numazu College of Technology, 3600 Ooka, Numazu, Shizuoka 410-8501, Japan; and National Research Institute of Vegetables, Ornamental Plants and Tea, Ministry of Agriculture, Forestry and Fisheries, 2769 Kanaya, Shizuoka 428-8501, Japan

The inhibitory effects of tea catechins, the *O*-methylated derivatives of (−)-epigallocatechin-3-*O*-gallate (EGCG), and the polyphenol extracts from tea leaves (*Camellia sinensis* L.) on oxazolone-induced type IV allergy in male ICR mice were investigated. Four major tea catechins and two *O*-methylated derivatives, (−)-epigallocatechin-3-*O*-(3-*O*-methyl)gallate (EGCG3″Me) and (−)-epigallocatechin-3-*O*-(4-*O*-methyl)gallate (EGCG4″Me), showed significant inhibitory effects on mouse type IV allergy after a percutaneous administration at a dose of 0.13 mg/ear. Among tea catechins, the compounds including galloyl moieties, such as EGCG and (−)-epicatechin-3-*O*-gallate (ECG), showed the strongest inhibitory activities on mouse type IV allergy. The inhibitory activities of EGCG3″Me and EGCG4″Me were higher than that of EGCG at a dose of 0.05 mg/ear. Polyphenol extract from tea leaves of Benihomare cultivar, which includes EGCG3″Me, strongly inhibited mouse type IV allergy after percutaneous administration in comparison with that from Yabukita cultivar, which does not include EGCG3″Me, at doses of 0.05 and 0.13 mg/ear. EGCG3″Me is thought to contribute, at least in part, to the inhibitory ability of Benihomare tea leaves on mouse type IV allergy. EGCG and the polyphenol extracts from Benihomare and Yabukita tea leaves also inhibited mouse type IV allergy by oral administration at 1 h before the sensitization and at 1 h before the challenge with oxazolone. Therefore, daily intake of tea drinks could have potential to prevent type IV allergy.

Keywords: *Tea; catechin; mouse; type IV allergy; EGCG; O-methylated EGCG*

INTRODUCTION

Extracts from some kinds of teas (*Camellia sinensis* L.), green, oolong, and black teas, are known to exhibit antiallergic effects in rats, mice, and guinea pigs. The antiallergic effects were thought to be due to some components, catechins (Matsuo et al., 1997), flavonoids (Toyoda et al., 1997), and saponins (Akagi et al., 1997), in tea leaves and stems. The major catechins in tea leaves are (−)-epigallocatechin-3-*O*-gallate (EGCG), (−)-epicatechin-3-*O*-gallate (ECG), (−)-epigallocatechin (EGC), and (−)-epicatechin (EC). These catechins were reported to inhibit rat and mouse type I allergy (Shiozaki et al., 1994; Ohmori et al., 1995; Fukui et al., 1998). We also reported the inhibitory effect of the *O*-methylated derivatives of EGCG, (−)-epigallocatechin-3-*O*-(3-*O*-methyl)gallate (EGCG3″Me) and (−)-epigallocatechin-3-*O*-(4-*O*-methyl)gallate (EGCG4″Me), on mouse type I allergy (Sano et al., 1999). EGCG3″Me and EGCG4″Me were separated from Tong ting oolong tea, a Taiwanese oolong tea product, and Benihomare cultivar, one of the cultivars used for Japanese black tea. EGCG3″Me and EGCG4″Me inhibited type I allergy more effectively compared with EGCG. Chemical structures of tea catechins, EGCG3″Me, and EGCG4″Me are summarized in Figure 1. The inhibitory effect of tea catechins on type I allergy could be partly due to an inhibition of the histamine release from animal mast cells (Yamamoto et al., 1998; Ohmori et al., 1995) and an inhibition of hyaluronidase activity in animal tissues (Yamamoto et al., 1990). In contrast with the case of type I allergy, only EGCG was reported as an available catechin for the inhibition of mouse type IV allergy (Abe et al., 1995).

In the present study, we investigated the inhibitory effect of catechins, EGCG3″Me, EGCG4″Me, and tea polyphenol extracts on mouse type IV allergy and discuss the important chemical structures in the polyphenols for the antiallergic effect. For an animal model of type IV allergy, previously acquired delayed type reaction, mouse contact hypersensitivity to oxazolone (4-ethoxymethylene-2-phenyl-2-oxazolin-5-one) is often used (Nakano, 1977; Nakamura et al., 1988). The formation of ear edema with oxazolone as a simple hapten is observed in this response, and the immunosuppressive activity of various chemicals can be estimated using this model. Therefore, we used oxazolone-induced mouse type IV allergy for the evaluation of the antiallergic effects of the samples.

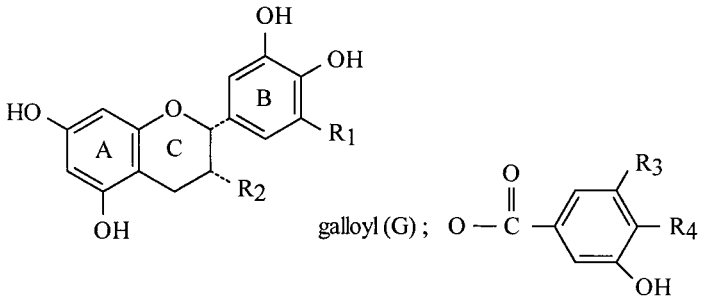
* Author to whom correspondence should be addressed (telephone/fax +81-542-64-5680; e-mail sano@ys2.u-shizuoka-ken.ac.jp.

[†] University of Shizuoka.

[‡] Numazu College of Technology.

[§] Ministry of Agriculture, Forestry and Fisheries.

10.1021/jf000313d CCC: $19.00 © 2000 American Chemical Society
Published on Web 10/24/2000



Compounds	R_1	R_2	R_3	R_4
(-)-Epigallocatechin-3-*O*-(3-*O*-methyl)gallate (EGCG3"Me)	OH	G	OCH_3	OH
(-)-Epigallocatechin-3-*O*-(4-*O*-methyl)gallate (EGCG4"Me)	OH	G	OH	OCH_3
(-)-Epigallocatechin-3-*O*-gallate (EGCG)	OH	G	OH	OH
(-)-Epicatechin-3-*O*-gallate (ECG)	H	G	OH	OH
(-)-Epigallocatechin (EGC)	OH	OH	-	-
(-)-Epicatechin (EC)	H	OH	-	-

Figure 1. Chemical structures of tea catechins and the *O*-methylated derivatives of EGCG.

MATERIALS AND METHODS

Materials and Animals. Oxazolone was purchased from Aldrich Chemical Co., Milwaukee, WI, and was used as a sensitizer for type IV allergy. EGCG, ECG, EGC, and EC were purchased from Kurita Co., Tokyo, Japan. Gallic acid was obtained from Wako Pure Chemicals Industry, Ltd., Tokyo, Japan. EGCG3″Me and EGCG4″Me were prepared from Benihomare and Tong ting tea leaves according to the method described previously (Sano et al., 1999). Polyphenol extracts from Yabukita and Benihomare cultivars were prepared as follows. Tea was extracted with hot water for 30 min, and the extract was passed through a porous polymer gel Mitsubishi Diaion HP-20 column. The adsorbed material was eluted with MeOH/H_2O (1:1) after a washing with water. The MeOH/H_2O (1:1) eluate was passed through an Amberlyst 15 column to remove caffeine. The eluate was concentrated under reduced pressure to give a polyphenol fraction. The catechin contents in polyphenols were determined using an HPLC system equipped with an electrochemical detector as described previously (Sano et al., 1999). Other chemicals used were of a reagent grade. Male ICR 4-week-old mice were purchased from Japan SLC, Inc., Shizuoka, Japan.

Determination of Mouse Type IV Allergic Response. The determination of the type IV allergic response was identified by the following procedures (Nakano, 1977; Nakamura et al., 1988). Briefly, the hair of the abdominal region of the mice was carefully cut off, and 0.1 mL of a 0.5% oxazolone solution in ethanol was applied to the skin. This operation was called sensitization. Five days after the sensitization, 20 μL of a 0.5% oxazolone solution in acetone was applied to both sides of the mouse's right ear. This operation was called challenge. Twenty-four hours after the challenge, mice were killed under anesthesia with diethyl ether. The circular parts (5.0 mm in diameter) of both ears of mice were removed using a punching apparatus. The weights of the right ear (WR) and left ear (WL) were measured.

In cases of percutaneous administration of the samples to mice, catechins, EGCG3″Me, EGCG4″Me, gallic acid, and tea polyphenol extracts were dissolved in an oxazolone/acetone solution at the challenge, and the dose was 0.05 or 0.13 mg/ear. Hydrocortisone, a well-known steroid-type anti-inflammatory agent, was also assayed. Control experiments were performed with the solvents alone used for the dissolution of the samples.

In cases of oral administration of the samples to mice, tea polyphenol extracts were dissolved in 0.5% tragacanth gum solution and administered orally to mice at 1 h prior to the sensitization or challenge. The dose of tea polyphenol extracts was 10, 50, or 100 mg/kg of body weight, respectively. Control experiments were performed using 0.5% tragacanth gum solution.

Table 1. Effect of the Percutaneous Administration of Tea Catechins, Gallic Acid, and the *O*-Methylated Derivatives of EGCG on Oxazolone-Induced Type IV Allergy in 5-Week-Old Male ICR Mice

sample	dose (mg/ear)	ear swelling ratio[a] (%)
control (oxazolone only)		100.0 ± 3.7
steroid-type anti-inflammatory agent as positive control		
hydrocortisone	0.05	26.8 ± 9.5** (73.2)
	0.13	16.2 ± 1.9** (83.8)
polyphenolic compounds		
EGCG	0.05	103.4 ± 13.6 (−3.4)
	0.13	12.0 ± 1.5** (88.0)
ECG	0.05	101.1 ± 11.7 (−1.1)
	0.13	37.8 ± 7.4** (62.2)
EGC	0.05	116.6 ± 14.9 (−16.6)
	0.13	53.7 ± 11.1* (46.3)
EC	0.05	94.9 ± 16.4 (5.1)
	0.13	52.3 ± 11.2* (47.7)
gallic acid	0.05	91.7 ± 7.9 (8.3)
	0.13	70.5 ± 5.7** (29.5)
EGCG3″Me	0.05	63.8 ± 10.0** (36.2)
	0.13	26.8 ± 6.4** (73.2)
EGCG4″Me	0.05	58.4 ± 4.7** (41.6)
	0.13	22.4 ± 6.9** (77.6)

[a] Values are the mean ± SE, $n = 5$. Statistically significant difference from the control value: *, $P < 0.05$; **, $P < 0.01$. Numbers in parentheses represent percentage inhibition of type IV allergy, which was calculated using the following equation: percentage inhibition = 100 − ear swelling ratio.

The ear swelling ratio was calculated using the following equation:

$$\text{ear swelling ratio (\%)} = [(WR_{sample} - WL_{sample})/WL_{sample}]/[(WR_{control} - WL_{control})/WL_{control}] \times 100.$$

Statistical Analysis. Statistical analysis in this study was tested using the nonparametric Mann−Whitney U test.

RESULTS AND DISCUSSION

Inhibitory effects of tea catechins, gallic acid, and the *O*-methylated derivatives of EGCG, EGCG3″Me, and EGCG4″Me on type IV allergy in ICR mice after percutaneous administration are shown in Table 1. All

Table 2. Catechin Contents of Tea Leaves and the Polyphenol Extracts[a]

sample	EGCG	GCG	ECG	EGC	EC	EGCG3″Me	total catechins
			% of Dry Weight of Tea Leaves				
Yabukita	9.22	0.36	1.78	3.45	0.97	ND[b]	15.78
Benihomare	10.09	0.41	2.36	4.43	1.08	0.50	18.87
			% of Dry Weight of Polyphenol Extracts				
Yabukita	34.66	10.10	10.02	5.96	10.59	tr[c]	71.33
Benihomare	38.75	13.77	15.53	6.88	7.83	3.90	86.66

[a] Values are the means of duplicate determinations. [b] ND, not detected (<0.01%). [c] Trace.

tea catechins used significantly inhibited the mouse type IV allergy at a dose of 0.13 mg/ear. The inhibitory effects of ECG, EGCG3″Me, and EGCG4″Me on mouse type IV allergy were slightly weaker than that of hydrocortisone used as a positive control at this dose. The inhibitory effect of EC on mouse type IV allergy was similar to that of EGC; therefore, the 3′,4′,5′-triol structure in the B ring of EGC could not be important for the antiallergic effect. However, inhibitory effects of EGCG and ECG on mouse type IV allergy were higher than those of catechins without the galloyl moieties, EGC and EC. This indicates that the galloyl moieties of EGCG and ECG contribute to the inhibitory effect on type IV allergy. Gallic acid significantly inhibited the mouse type IV allergy, although more weakly than EGCG and ECG at a dose of 0.13 mg/ear. Therefore, not only the galloyl moieties in catechins but the flavanol or flavane structures would be important for the inhibitory effects on mouse type IV allergy. One of the hydroxyl groups in the galloyl moieties of EGCG is methylated in EGCG3″Me and EGCG4″Me. However, inhibitory effects of EGCG3″Me and EGCG4″Me were not lowered compared with their parent compound, EGCG, at a dose of 0.13 mg/ear. Inhibitory effects of EGCG3″Me and EGCG4″Me on type IV allergy were stronger than that of EGCG at a dose of 0.05 mg/ear. It was reported that the metabolites of (+)-catechin were more stable compared with (+)-catechin in human plasma (Hackett and Griffiths, 1983). We have previously demonstrated that the inhibitory effects of EGCG3″Me and EGCG4″Me on mouse type I allergy were stronger than that of EGCG (Sano et al., 1999). EGCG is known to be unstable and is degraded easily in animal bodies. However, EGCG3″Me and EGCG4″Me were more stable than EGCG in mouse plasma in an in vitro study, and the flavanol structures could have remained in the animal bodies. Therefore, the high inhibitory activities of EGCG3″Me and EGCG4″Me on mouse type I and type IV allergies are thought to be associated with the stability of the *O*-methylated derivatives of EGCG in animal bodies.

EGCG3″Me and EGCG4″Me, which were stronger antiallergic agents than EGCG, are known to be included in Tong ting oolong tea, and EGCG3″Me was included in the Benihomare cultivar, classified as Assam hybrids, whereas they are not detected in the Yabukita cultivar, which accounts for >90% of the green tea manufactured in Japan, as shown in Table 2. The content of EGCG in the polyphenol extract from Benihomare tea leaves was slightly higher than that from Yabukita tea leaves, as shown in Table 2. Table 3 shows the percutaneous administration of tea polyphenol extracts from Yabukita and Benihomare tea leaves inhibitied the mouse type IV allergy. The extract from Benihomare tea leaves, which included EGCG3″Me, showed a strong inhibitory effect on mouse type IV allergy compared with that from Yabukita tea leaves, which did not include EGCG3″Me, at both doses, 0.05 and 0.13 mg/ear. Because of the strong inhibitory effect of EGCG3″Me on mouse type IV allergy, this compound is thought to contribute, at least in part, to the ability of Benihomare tea leaves.

Table 3. Effect of the Percutaneous Administration of Tea Polyphenolic Extracts on Oxazolone-Induced Type IV Allergy in 5-Week-Old Male ICR Mice

sample	dose (mg/ear)	ear swelling ratio[a] (%)
control (oxazolone only)		100.0 ± 3.7
tea polyphenolic extracts		
Yabukita	0.05	104.6 ± 16.8 (−4.6)
	0.13	31.7 ± 5.3** (68.3)
Benihomare	0.05	55.5 ± 11.9* (44.5)
	0.13	12.4 ± 2.0** (87.6)

[a] Values are the mean ± SE, $n = 5$. Statistically significant difference from the control value: *, $P < 0.05$; **, $P < 0.01$. Numbers in parentheses represent percentage inhibition of type IV allergy, which was calculated using the following equation: percentage inhibition = 100 − ear swelling ratio.

Table 4. Effect of Oral Administration of Polyphenol Extracts of Yabukita and Benihomare on Oxazolone-Induced Type IV Allergy in 5-Week-Old Male ICR Mice

sample	dose (mg/kg of BW)	ear swelling ratio[a] (%)
control (oxazolone only)		100.0 ± 3.7
single application at 1 h before the sensitization		
Yabukita	10	97.6 ± 30.1 (2.4)
	50	87.8 ± 16.5 (12.2)
	100	67.5 ± 10.8* (32.5)
Benihomare	10	39.4 ± 8.2** (60.6)
	50	80.6 ± 17.4 (19.4)
	100	90.3 ± 18.8 (9.7)
single application at 1 h before the challenge		
Yabukita	10	92.4 ± 14.8 (7.6)
	50	45.5 ± 9.8** (54.5)
	100	41.8 ± 8.4** (58.2)
Benihomare	10	42.2 ± 8.7** (57.8)
	50	52.6 ± 14.2* (47.4)
	100	72.6 ± 9.0* (27.4)

[a] Values are the mean ± SE, $n = 5$. Statistically significant difference from the control value: *, $P < 0.05$; **, $P < 0.01$. Numbers in parentheses represent percentage inhibition of type IV allergy, which was calculated using the following equation: percentage inhibition = 100 − ear swelling ratio.

The inhibitory effects of the oral administration of tea polyphenol extracts prepared from Yabukita and Benihomare tea leaves on mouse type IV allergy were investigated, and the findings are presented in Table 4. In the oral administration at 1 h before the challenge, both tea extracts showed significant antiallergic effects at a dose of 50 mg/kg of body weight, and Benihomare extract at a dose of 10 mg/kg of body weight showed the most significant antiallergic effect. However, a dose-dependent manner was not observed in the antiallergic effect of Benihomare extract. Oral administration of tea polyphenol extracts at 1 h before the sensitization also caused significant inhibition of mouse type IV allergy at 100 mg/kg of body weight of Yabukita and at 10 mg/kg of body weight of Benihomare, as shown in Table 4.

The finding suggests that tea polyphenol extracts could also suppress the early immune process in type IV allergy induction. The reasons for the non-dose dependency for Benihomare polyphenols administered orally to mice are unknown, and further investigations are necessary. We have previously found that the polyphenol extracts from Benihomare exhibited strong inhibitory effects on mouse type I allergy at a lower dosage (5 mg/kg, p.o.) than did Yabukita (Sano et al., 1999). It is expected that the optimal dose for the inhibitory effect on type IV allergy would exist in oral administration of tea polyphenols. The optimal dose of Benihomare polyphenols may be around 10 mg/kg.

Very few studies on the mechanisms for the inhibitory effect of tea catechins on type IV allergy have been reported. The difficulty of such an investigation would be due to the complexity of the type IV allergic response (Florentin et al., 1982). In a typical type IV allergic response mediated by appropriately sensitized lymphocytes, early and predominant mononuclear cell infiltration probably occurs (Dietrich and Hess, 1970; Evans et al., 1971). Furthermore, the inflammation by oxazolone sensitization could be mediated by lymphokine, cytokine, and histamine, the metabolites of arachidonic acid with 5-lipoxygenase and cyclo-oxygenase (Nakamura et al., 1988). The inhibitory effects of tea polyphenol extract on the activities of lipoxygenase and cyclooxygenase in the mouse ear (Katiyar et al., 1993) and on the release of histamine from the mast cells (Ohmori et al., 1995; Yamamoto et al., 1998) would partly contribute to the anti-inflammatory effect. However, some active oxygen species, superoxide anion radical, hydrogen peroxide, and hydroxyl radical produced from these free radicals and iron in the Fenton reaction or the Harber−Weiss reaction, play an important role in the process of inflammation (Britigan et al., 1994; Ramos et al., 1995). Tea catechins are well-known as natural antioxidants, especially as an iron chelators and free radical scavengers (Salah et al., 1995; Rice-Evans et al., 1996; Kondo et al., 1999; Yoshino et al., 1999). They could show antioxidative activity in vivo (Yoshino et al., 1994; Zhang et al., 1997; Silva et al., 1998; Nakagawa et al., 1999) and inhibit inflammation (Wei et al., 1993; Katiyar et al., 1995). Part of their antiallergic effects may be due to their anti-inflammatory effects. However, the antiallergic effects of tea catechins and the *O*-methylated derivatives of EGCG could not be explained from their anti-inflammatory effects alone, because they inhibited mouse type IV allergy by oral administration before sensitization. Further investigations are necessary to clarify the mechanisms of the inhibitory effects of tea catechins and the *O*-methylated derivatives of EGCG on mouse type IV allergy. Tea is one of the most popular beverages in the world; therefore, the findings of the present study indicate that daily intake of tea drinks, especially Benihomare tea infusion, is thought to have the potential to prevent type IV allergy.

ABBREVIATIONS USED

EGCG3″Me, (−)-epigallocatechin-3-*O*-(3-*O*-methyl)-gallate; EGCG4″Me, (−)-epigallocatechin-3-*O*-(4-*O*-methyl)gallate; EGCG, (−)-epigallocatechin-3-*O*-gallate; ECG, (−)-epicatechin-3-*O*-gallate; EGC, (−)-epigallocatechin; EC, (−)-epicatechin.

LITERATURE CITED

Abe, Y.; Shiozaki, T.; Sugiyama, K.; Takeo, T. Antiallergic effect of tea II: Effect of tea on type IV allergic reaction. *J. Trad. Med.* **1995**, *12*, 452−453.

Akagi, M.; Fukuishi, N.; Kan, T.; Sagesaka, Y. M.; Akagi, R. Anti-allergic effect of tea-leaf saponin (TLS) from tea leaves (*Camellia sinensis* var. *sinensis*). *Biol. Pharm. Bull.* **1997**, *20*, 565−567.

Britigan, B. E.; Serody, J. S.; Cohen, M. S. The role of lactoferrin as an anti-inflammatory molecule. *Adv. Exp. Med. Biol.* **1994**, *357*, 143−156.

Dietrich, F. M.; Hess, R. Hypersensitivity in mice. I. Induction of contact sensitivity to oxazolone and inhibition by various chemical compounds, *Int. Arch. Allergy* **1970**, *38*, 246−259.

Evans, D. P.; Hossack, M.; Thomson, D. S. Inhibition of contact sensitivity in the mouse by topical application of corticosteroids. *Br. J. Pharmacol.* **1971**, *43*, 403−408.

Florentin, I.; Taylor, E.; Davigny, M.; Mathe, G.; Hadden, J. W. Kinetic studies of the immunopharmacologic effects of NPT 15392 in mice. *Int. J. Immunopharmacol.* **1982**, *4*, 225−234.

Fukui, H.; Kataoka, H.; Semma, M.; Ito, Y.; Takagaki, Y. Development of the mouse passive abdominal wall anaphylaxis (PAA) method and application of the method to search for anti-allergic effect of foods. *J. Food Hyg. Soc. Jpn.* **1998**, *39*, 251−255.

Hackett, A. M.; Griffiths, L. A.; Broillet, A.; Wermaille, M. The metabolism and excretion of (+)-[^{14}C]cyanianol-3 in man following oral administration. *Xenobiotica* **1983**, *13*, 279−286.

Katiyar, S. K.; Agarwal, R.; Ekker, S.; Wood, G. S.; Mukhtar, H. Protection against 12-O-tetradecanoylphorbol-13-acetate-caused inflammation in SENCAR mouse ear skin by polyphenolic fraction isolated from green tea. *Carcinogenesis* **1993**, *14*, 361−365.

Katiyar, S. K.; Elmets, C. A.; Agarwal, R. A.; Mukhtar, H. Protection against ultraviolet-B radiation-induced local and systemic suppression of contact hypersensitivity and edema responses in C3H/HeN mice by green tea polyphenols. *Photochem. Photobiol.* **1995**, *62*, 855−861.

Kondo, K.; Kurihara, M.; Miyata, N.; Suzuki, T.; Toyoda, M. Scavenging mechanisms of (−)-epigallocatechin gallate and (−)epicatechin gallate on peroxyl radicals and formation of superoxide during the inhibitory action. *Free Radical Biol. Med.* **1999**, *27*, 855−863.

Matsuo, N.; Yamada, K.; Shoji, K.; Mori, M.; Sugano, M. Effect of tea polyphenols on histamine release from rat basophilic leukemia(RBL-2H3) cells: the structure-inhibitory activity relationship. *Allergy* **1997**, *52*, 58−64.

Nakagawa, K.; Ninomiya, M.; Okubo, T.; Aoi, N.; Juneja, L. R.; Kim, M.; Yamanaka, K.; Miyazawa, T. Tea catechin supplementation increases antioxidant capacity and prevents phospholipid hydroperoxidation in plasma of humans. *J. Agric. Food Chem.* **1999**, *47*, 3967−3973.

Nakamura, H.; Motoyoshi, S.; Ishii, K.; Kadokawa, T. Inhibitory effect of AD-1590, a non-steroidal anti-inflammatory drugs, on allergic inflammation in mice and rats. *Folia Pharmacol. Jpn.* **1988**, *92*, 29−38.

Nakano, Y. Antigenic competition in the induction of contact sensitivity in mice. *Immunology* **1977**, *33*, 167−178.

Ohmori, Y.; Ito, M.; Kishi, M.; Mizutani, H.; Katada, T.; Konishi, H. Antiallergic constituents from oolong tea stem. *Biol. Pharm. Bull.* **1995**, *18*, 683−686.

Ramos, C. L.; Pou, S.; Rosen, G. M. Effect of anti-inflammatory drugs on myeloperoxidase-dependent hydroxyl radical generation by human neutrophils. *Biochem. Pharmacol.* **1995**, *49*, 1079−1084.

Rice-Evans, C. A.; Miller N. J.; Paganga, G. Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radical Biol. Med.* **1996**, *20*, 933−956.

Salah, N.; Miller, N. J.; Paganga, G.; Tijburg, L.; Bolwell, G. P.; Rice-Evans, C. A. Polyphenolic flavanols as scavengers of aqueous phase radicals and as chain-breaking antioxidants. *Arch. Biochem. Biophys.* **1995**, *322*, 339−346.

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.

Shiozaki, T.; Sugiyama, K.; Takeo, T. Antiallergic effect of tea. *J. Trad. Med.* **1994**, *11*, 444−445.

Silva, E. L. D.; Piskula, M.; Terao, J. Enhancement of antioxidative ability of rat plasma by oral administration of (−)-epicatechin. *Free Radical Biol. Med.* **1998**, *24*, 1209−1216.

Toyoda, M.; Tanaka, K.; Hoshino, K.; Akiyama, H.; Tanimura, A.; Saito, Y. Profiles of potentially antiallergic flavonoids in 27 kinds of health tea and green tea infusions. *J. Agric. Food Chem.* **1997**, *45*, 2561−2564.

Wei, H.; Frenkel, K. Relationship of oxidative events and DNA oxidation in SENCAR mice to in vivo promoting activity of phorbol ester-type tumor promoters. *Carcinogenesis* **1993**, *14*, 1195−1201.

Yamamoto-Maeda, M.; Masui, T.; Sugiyama, K.; Yokota, M.; Nakagomi, K.; Tanaka, H.; Takahashi, I.; Kobayashi, T.; Kobayashi, E. Inhibitory effect of tea extracts on hyaluronidase (Studies on anti-allergic activity in tea. II). *J. Food Hyg. Soc. Jpn.* **1990**, *31*, 233−237.

Yamamoto-Maeda, 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.

Yoshino, K.; Tomita, I.; Sano, M.; Oguni, I.; Hara, Y.; Nakano, M. Effects of long-term dietary supplement of tea polyphenols on lipid peroxide levels in rats. *Age* **1994**, *17*, 79−85.

Yoshino, K.; Suzuki, M.; Sasaki, K.; Miyase, T.; Sano, M. Formation of antioxidants from (−)-epigallocatechin gallate in mild alkaline fluids, such as authentic intestinal juice and mouse plasma. *J. Nutr. Biochem.* **1999**, *10*, 223−229.

Zhang, A.; Zhu, Q. Y.; Luk, Y. S.; Ho, K. Y.; Fung, K. P.; Chen, Z. Y. Inhibitory effects of jasmine green tea epicatechin isomers on free radical-induced lysis of red blood cells. *Life Sci.* **1997**, *61*, 383−394.

Received for review March 8, 2000. Revised manuscript received August 25, 2000. Accepted August 25, 2000. This work was supported by a grant from the Program for the Promotion of Basic Research Activities for Innovative Biosciences, Japan.

JF000313D