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Relationship between antimutagenic activity and major components of various teas

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The objectives of this study were to determine the major components in tea leaves and tea extracts and to study the relationship between chemical content and antimutagenic activity of various tea extracts. The amount of catechins in various tea extracts was in the order: green tea (26.7%) > oolong tea (23.2%) > pouchong tea (15.8%) > black tea (4.3%). The amounts of caffeine and phenolic compounds in oolong tea extracts were 8.3 and 32.4%, respectively; these amounts were greater than those in the other three tea extracts. The ascorbic acid in green tea extracts was a little higher than in oolong and pouchong tea extracts. The amount of catechins in tea leaves also showed the order: nonfermented (green tea) > semifermented (pouchong tea and oolong tea) > fermented tea (black tea). The amounts of caffeine and phenolic compounds in oolong tea leaves are also higher than in other tea leaves. Besides water soluble components, tea leaves also contain several lipid soluble chemicals such as β-carotene and tocopherols. The tea extracts, especially oolong and pouchong teas, markedly inhibited the mutagenicity of 2-amino-3-methylimidazo(4,5-*f*)quinoline (IQ), 3-amino-1,4-dimethyl-5*H*-pyrido(4,3-*b*)indole (Trp-P-1), 2-amino-6-methyl-dipyrido(1,2-*a*:3′,2′-*d*)imidazole (Glu-P-1), benzo[*a*]pyrene (B[*a*]P) and aflatoxin B_1 (AFB_1). The inhibitory effect of tea extracts against the mutagenicity of IQ and Glu-P-1 in *Salmonella typhimurium* TA100 showed a significant ($P < 0.05$) correlation to the contents of catechins and ascorbic acid. The antimutagenic activity of tea extracts to Trp-P-1 in TA98 or TA100 was well correlated ($P < 0.05$) to the caffeine contents. No significant ($P > 0.05$) correlation was found between the antimutagenicity of tea extracts to B[*a*]P and AFB_1 in TA100 and the content of major components in tea extracts.

Introduction

Tea has been used as a daily beverage and crude medicine in China for thousands of years. Various processing methods have been developed for diverse types of tea, each with its unique composition and characteristic flavor. Green tea is widely used as a beverage in Japan, China and other Asian countries, while black tea is more popular in Western countries. Oolong and pouchong tea production are confined to China and Taiwan (Graham, 1992).

In recent years, the pharmacological effects of tea have been reviewed for antioxidative activity (Matsuzaki and Hara, 1985), antimutagenic (Kada *et al.*, 1985; Yen and Chen, 1994, 1995) and anti-carcinogenic effects (Katiyar *et al.*, 1992). Kada *et al.* (1985) reported that green tea possessed antimutagenic activity; the active component was identified as epigallocatechin gallate (EGCG). Katiyar *et al.* (1992) showed that the polyphenolic fraction isolated from green tea inhibited 12-*O*-tetradecanoylphorbol-13-acetate-induced skin tumor promotion. Polyphenols are the most abundant groups of compounds in tea leaves. Among these, the catechins constitute the major component and seem to be responsible for most of the antimutagenic activity. Moreover, other components in tea, such as ascorbic acid, tocopherol, tannic acid, chlorophyll and gallic acid were also found to be antimutagenic against some mutagens (Okuda *et al.*, 1984; O'Connor *et al.*, 1985). The amounts of these components in tea may vary with the variety, harvesting season and processing method, which may also cause the variable antimutagenic activity of various tea products. Kojima *et al.* (1989) reported that the antimutagenic effect of oolong tea against benzo[*a*]pyrene (B[*a*]P) is greater than that of green tea. Our previous study also showed that the antimutagenic activity of semifermented tea (pouchong and oolong tea) was greater than those of nonfermented tea (green tea) and fermented tea (black tea) (Yen and Chen, 1994). Nevertheless, the amount of catechins in various teas is in the decreasing order: nonfermented tea > semifermented tea > fermented tea (Graham, 1992). Therefore, the variability of antimutagenic effects in various teas may not be completely attributable to the variable contents of these components.

The objectives of this study were to analyze the major chemical components of tea leaves and tea extracts and to study the relationship between components and antimutagenic activity of tea extracts.

Materials and methods

Materials

Teas, including green tea, pouchong tea, oolong tea and black tea, were purchased at a local market in Taichung, Taiwan. B[*a*]P, aflatoxin B_1 (AFB_1), ascorbic acid and β-carotene were purchased from Sigma Chemical Co. (St Louis, MO, USA). 2-Amino-3-methylimidazo(4,5-*f*)quinoline (IQ), 2-amino-6-methyl-dipyrido(1,2-*a*:3′,2′-*d*)imidazole (Glu-P-1), 3-amino-1,4-dimethyl-5*H*-pyrido(4,3-*b*)indole (Trp-P-1) and pyrogallol were from Wako Pure Chemical Co. (Tokyo, Japan); 2,6-dichloroindophenol (DIP), trifluoroacetic acid, tocopherol isomers and caffeine were from E.Merck (Darmstadt, Germany).

Preparation of tea extracts and tea powder

Tea extracts were prepared by the method previously described by Yen and Chen (1994). In brief, each tea (20 g) was extracted with boiled water (400 ml) for 5 min, the filtrate was freeze-dried. Each sample was prepared in triplicate from different batches, and the results were averaged. The yields of tea extracts for green, pouchong, oolong and black tea were 4.09, 4.11, 5.27 and 3.86 g, respectively. Tea leaves were directly ground into a fine powder in a mill, sealed in a plastic bottle, and stored at 4°C until used.

HPLC analysis of catechins and caffeine

The contents of four main tea catechins [epicatechin (EC), epigallocatechin (EGC), epicatechin gallate (ECG) and epigallocatechin gallate (EGCG)] and caffeine of tea leaves and tea extracts were determined by HPLC. Tea extracts (1 mg) in 1 ml deionized water were filtered through an 0.45 μm filter before use. The filtrate was analysed by HPLC (Hitachi, Japan) using the LiChrosphere RP-18 column (250 × 4 mm, 5 μm) and UV detector (measured at 280 nm). Trifluoroacetic acid–methanol (1%, 75:25, v/v) was used as the mobile phase

with a flow rate of 0.7 ml/min. Catechins and caffeine were identified by comparison of their retention time with those of known standards and determined by peak areas from the chromatograms. Tea powder (0.5 g) was extracted by 10 ml methanol and was analyzed as described above. All analyses were run in three replicates and averaged.

Spectrophotometric estimations of ascorbic acid and phenolic compounds

Tea extracts (1 mg) in 1 ml distilled water were mixed with 9 ml DIP (50 μm). Absorbance of the mixture at 515 nm was determined 15 s later against a blank solution containing tea extracts and distilled water. Ascorbic acid content was calculated directly from the absorbance relative to standard curve. Tea powder (0.5 g) was extracted by 1% metaphosphoric acid (10 ml) and the filtrate was determined as described above.

The concentration of phenolic compounds was measured by the method of Taga *et al.* (1984) and calculated using catechin as the standard. Tea powder (0.1 g) or tea extracts (5 mg) were extracted by 5 ml acidified methanol/water (60:40, 0.3% HCl). The filtrate (100 μl) was added to 2 ml of 2% Na_2CO_3. After 2 min, 50% Folin–Ciocalteau reagent (100 μl) was added to the mixture which was then left to stand for 30 min. Absorbance was measured at 750 nm on a spectrophotometer. All analyses were run in three replicates and averaged.

HPLC analyses of tocopherol isomers and β-carotene

Tocopherol isomers were extracted by the method of Carpenter (1979). Tea powder (1 g) or tea extracts (50 mg) were mixed with pyrogallol (6 ml, 6%) and potassium hydroxide (4 ml, 60%), and the mixtures were incubated at 70°C for 20 min. A portion (15 ml) of distilled water was added to the mixture which was then extracted with *n*-hexane (15 ml). The hexane layer was dried over Na_2SO_4 and then evaporated to dryness. The resultant residue was redissolved in 5 ml *n*-hexane and separated on an HPLC system. Tocopherols were determined in the LiChrosorb Si-60 column with a UV detector (295 nm), and with a mobile phase of *n*-hexane–isopropanol–ethanol (100:3:2, v/v/v) at a flow rate of 1.0 ml/min.

β-Carotene was extracted by the method of Kitada *et al.* (1989). Tea powder (0.2 g) or tea extracts (50 mg) were mixed with 10 ml 1% pyrogallol in methanol–dichloromethane (1:1, v/v). The mixtures were filtered through an 0.45 μm filter and were injected into the HPLC. The analysis of β-carotene was conducted by HPLC with a UV–VIS detector (470 nm) and employing a LiChrosphere RP-18 column (250 × 4 mm, 5 μm). The column was equilibrated with acetone/methanol/acetonitrile (1:2:2, v/v) at a flow rate of 0.7 ml/min. All analyses were run in three replicates and averaged.

Antimutagenicity assays

The antimutagenic effect of tea extracts was assayed according to the Ames test using *Salmonella typhimurium* strains TA98 and TA100 (Maron and Ames, 1983). The mutagens used were IQ (0.1 μg/plate for TA98 and 0.5 μg/plate for TA100), B[*a*]P (10 μg/plate for TA98 and 5 μg/plate for TA100), AFB_1 (0.5 μg/plate for TA98 and TA100), Trp-P-1 (0.25 μg/plate for TA98 and 2 μg/plate for TA100), and Glu-P-1 (0.5 μg/plate for TA98 and 2 μg/plate for TA100). Tea extracts (0.35 mg/plate) were added to overnight cultures of *S.typhimurium* TA98 or TA100 (0.1 ml), mutagen (0.1 ml) and S9 mix (0.5 ml). The entire mixture was preincubated at 37°C for 20 min before molten top agar (2 ml) was added; the mixture was poured onto a minimal medium agar plate. The His^+ revertant colonies were counted after incubating at 37°C for 48 h. Each sample was assayed using triplicate plates per run, and the data presented were the means of three experiments using tea extracts from different batches. The calculation of percent inhibition follows that described by Ong *et al.* (1986):

$$\text{Inhibition (\%)} = \left[1 - \frac{\text{number of His}^+ \text{ revertants in the presence of tea extracts}}{\text{number of His}^+ \text{ revertants in the absence of tea extracts}}\right] \times 100\%$$

The number of spontaneous revertants was subtracted from the numerator and denominator.

Statistical analysis

The correlations between the antimutagenicity and the major components of tea extracts were calculated by Duncan's multiple range test (Duncan, 1955).

Results and discussion

Major components analysis

The amounts of major components in tea extracts and tea leaves were evaluated. Table I shows the catechin contents of various tea extracts and tea leaves. Among four teas, green tea contained the highest amount of total catechins in either tea extracts or tea leaves. The amount of individual catechin in all tea extracts showed the order: EGC > EGCG > EC > ECG. This is in contrast to earlier findings (Millen

Table I. Catechins content of various tea extracts and tea leaves[a]

Tea samples	EC		ECG		EGC		EGCG		Total catechins	
	Tea extracts[b] (mg/g)	Tea leaf[c] (mg/g)	Tea extracts (mg/g)	Tea leaf (mg/g)	Tea extracts (mg/g)	Tea leaf (mg/g)	Tea extracts (mg/g)	Tea leaf (mg/g)	Tea extracts (mg/g)	Tea leaf (mg/g)
Green	$23.50^{A,d}$	19.23^{A}	12.58^{A}	14.19^{A}	153.62^{A}	97.79^{A}	77.18^{A}	90.74^{A}	266.88^{A}	221.94^{A}
Pouchong	16.58^{B}	9.50^{B}	8.68^{B}	13.64^{A}	77.37^{B}	77.97^{B}	55.31^{B}	97.77^{B}	157.94^{B}	198.95^{B}
Oolong	17.30^{B}	14.07^{C}	10.63^{C}	13.02^{A}	137.89^{C}	78.82^{B}	66.53^{C}	81.93^{C}	232.35^{C}	187.84^{C}
Black	4.54^{C}	0.77^{D}	3.47^{D}	2.09^{B}	59.94^{D}	19.94^{C}	16.53^{D}	5.66^{D}	43.48^{D}	16.64^{D}

[a]Values are means of three replicate analyses.
[b]Tea (20 g) was extracted with boiled water (400 ml) for 5 min and the filtrate was freeze-dried. The yields of tea extracts for green tea, pouchong tea, oolong tea and black tea were 4.09, 4.11, 5.27 and 3.86 g, respectively.
[c]Catechin content in tea leaf on a wet-weight basis.
[d]Data bearing different superscript letters in the same column were significantly different ($P < 0.05$).

Table II. The contents of caffeine, ascorbic acid and phenolic compounds of various tea extracts and tea leaves[a]

Tea samples	Caffeine		Ascorbic acid		Phenolic compounds	
	Tea extracts[b] (mg/g)	Tea leaf[c] (mg/g)	Tea extracts (mg/g)	Tea leaf (mg/g)	Tea extracts (mg/g)	Tea leaf (mg/g)
Green	$54.63^{A,d}$	32.88^{A}	3.05^{A}	2.57^{A}	285.46^{A}	208.80^{A}
Pouchong	56.53^{A}	46.35^{B}	2.70^{B}	2.22^{A}	281.28^{A}	219.95^{B}
Oolong	83.10^{B}	51.51^{C}	2.93^{A}	2.39^{A}	323.94^{B}	221.75^{B}
Black	50.38^{C}	40.84^{D}	–	–	88.97^{C}	87.70^{C}

[a]Values are means of three replicate analyses.
[b]Tea (20 g) was extracted with boiled water (400 ml) for 5 min and the filtrate was freeze-dried. The yields of tea extracts for green tea, pouchong tea, oolong tea and black tea were 4.09, 4.11, 5.27 and 3.86 g, respectively.
[c]Content in tea leaf on a wet-weight basis.
[d]Data bearing different superscript letters in the same column were significantly different ($P < 0.05$).

et al., 1969) which suggested that EGCG is the most common flavonol present. Wang *et al.* (1992) also reported that EGCG is the most abundant catechin in green tea extracts by weight and on a molar basis. Other recent data indicate that EGC is the more prevalent flavonol in tea extracts (Price and Spitzer, 1993). In our results, the amount of EGC is greater than EGCG, especially in tea extracts. This could be due to the different content of catechins in different varieties and to the cultivated conditions of teas. Catechins are the most abundant group of compounds in fresh tea leaves and are oxidized by polyphenol oxidase during the fermentation process of black tea manufacture. Therefore, the catechins are found in green and black teas at 30–42 and 3–10% of the total dry matter, respectively (Graham, 1992). Pouchong and oolong teas are considered to be about one-third and one-half fermented, respectively, compared to black tea (Yamanishi, 1981). In this study, the total catechins in oolong tea extracts (23.2%) are higher than pouchong tea (15.8%).

Table II shows the analytical data including caffeine, ascorbic acid and phenolic compounds of various tea extracts and tea leaves. The amounts of caffeine in oolong tea extracts and tea leaves were 8.3 and 5.1%, respectively, and these amounts were greater than those in the other three teas. Terada *et al.* (1987) indicated that the caffeine contents in tea infusions prepared from the same variety with various degrees of fermentation were not different. Therefore, the effects of a different variety or cultivation conditions on the caffeine contents of tea leaves are more remarkable than the actual manufacturing process. The ascorbic acid in green tea extracts was a little higher than in oolong and pouchong tea extracts, but none was detected in black tea extracts. Kitada *et al.* (1989) reported that the highest ascorbic acid content of green tea was 367 mg/100 g and 64.2% of this amount was extracted by a normal infusion process. The results also indicated that the amounts of phenolic compounds in oolong tea leaves and extracts were greater than in the other three teas.

Besides water-soluble components, tea leaves possess several lipid-soluble chemicals such as carotenoids and tocopherols. As shown in Table III, the β-carotene content in oolong tea leaves was greater than in green and pouchong tea leaves, but it was not detected in black tea leaves. Sanderson *et al.* (1971) reported that several carotenoids including β-carotene, lutein, neoxanthin and violaxanthin were found in fresh tea leaves. All these carotenoids are oxidatively degraded to several volatile and non-volatile products during the fermentation stage of black tea manufacture (Hazarika and Mahanta, 1983). As shown in the results, β-carotene was not detected in black tea, and this could be due to the β-carotene being destroyed in the manufacturing process. The amounts of tocopherols in tea leaves decreased in the order: oolong tea > pouchong tea > green tea > black tea. α-Tocopherol was the highest among four tocopherol isomers in all teas; however, δ-tocopherol was not detected except in oolong tea. Hiroshi and Kenji (1990) reported that the content of tocopherol in green tea was the highest of all tested foods, and almost all of the tocopherol was α-tocopherol. In this study, carotenoids and tocopherols

Table III. Contents of β-carotene and tocopherols in various tea leaves[a]

Tea samples	β-Carotene (mg/g)[b]	Tocopherol (mg/g)			
		α	β	γ	δ
Green	$1.25^{A,c}$	0.91^{A}	0.11^{A}	0.08^{A}	–
Pouchong	0.80^{B}	1.04^{A}	0.10^{A}	0.10^{B}	–
Oolong	1.38^{C}	1.18^{B}	0.15^{B}	0.11^{B}	0.07
Black	–	0.58^{C}	0.11^{A}	0.07^{A}	–

[a]Values are means of three replicate analyses.
[b]Content in tea leaf on a wet-weight basis.
[c]Data bearing different superscript letters in the same column were significantly different ($P < 0.05$).

Table IV. Inhibitory effect of tea extracts on the activity of mutagens[a] to *S.typhimurium* TA98 and TA100

Tea extracts	His^+ revertants/plate[b] (% of inhibition)[c]				
	IQ	B[*a*]P	AFB_1	Trp-P-1	Glu-P-1
TA98					
Control[d]	$1739 \pm 116^{A,f}$	380 ± 31^{A}	448 ± 27^{A}	1737 ± 72^{A}	911 ± 81^{A}
Green tea	1287 ± 80^{B} (26.7)	$230 \pm 22^{B,C}$ (45.3)	$203 \pm 13^{B,C}$ (61.4)	1319 ± 88^{B} (21.8)	369 ± 44^{B} (62.9)
Pouchong tea	1564 ± 40^{A} (10.4)	215 ± 13^{B} (49.8)	170 ± 29^{B} (69.7)	1278 ± 32^{B} (27.2)	406 ± 13^{B} (58.6)
Oolong tea	1568 ± 114^{A} (10.1)	209 ± 14^{B} (51.7)	243 ± 20^{C} (51.4)	1144 ± 60^{C} (35.1)	690 ± 53^{C} (25.6)
Black tea	1642 ± 105^{A} (5.7)	255 ± 12^{C} (37.8)	335 ± 37^{D} (28.3)	1341 ± 91^{B} (23.5)	742 ± 52^{C} (19.6)
Spontaneous revertants[e]	49 ± 6				
TA100					
Control	911 ± 109^{A}	797 ± 42^{A}	388 ± 40^{A}	534 ± 43^{A}	596 ± 18^{A}
Green tea	378 ± 27^{B} (69.8)	443 ± 24^{B} (54.5)	275 ± 7^{B} (46.9)	487 ± 22^{B} (12.1)	274 ± 10^{B} (71.7)
Pouchong tea	505 ± 61^{C} (53.1)	$332 \pm 20^{C,D}$ (71.5)	186 ± 8^{C} (83.8)	$495 \pm 12^{A,B}$ (10.1)	282 ± 25^{B} (69.9)
Oolong tea	$442 \pm 20^{B,C}$ (61.4)	295 ± 9^{C} (77.2)	230 ± 6^{D} (65.6)	419 ± 11^{C} (29.7)	263 ± 36^{B} (74.2)
Black tea	815 ± 72^{A} (12.6)	346 ± 16^{D} (69.4)	$253 \pm 13^{B,D}$ (56.0)	$505 \pm 8^{A,B}$ (7.5)	455 ± 34^{C} (31.4)
Spontaneous revertants	147 ± 10				

[a]Mutagen was preincubated with tea extracts (0.35 mg/plate) at 37°C for 20 min before antimutagenicity assay.
[b]Data presented are the means of three experiments.

[c]$$\text{Inhibition (\%)} = \left[1 - \frac{\text{number of His}^+ \text{ revertants in the presence of tea extracts}}{\text{number of His}^+ \text{ revertants in the absence of tea extracts}} \right] \times 100\%.$$

The number of spontaneous revertants was subtracted from the numerator and denominator.
[d]The number of controls was determined without tea extract.
[e]The number of spontaneous revertants was determined without tea extract and mutagen.
[f]Data bearing different superscripts in the same column were significantly different ($P < 0.05$).

Table V. Correlation between antimutagenicity toward *S.typhimurium* TA98 and TA100 and ascorbic acid, caffeine, phenolic compounds and catechins contents of tea extracts

	IQ	B[*a*]P	AFB_1	Trp-P-1	Glu-P-1
TA98					
Ascorbic acid	0.604[a] (0.113)[b]	0.873 (0.005)	0.879 (0.006)	0.360 (0.381)	0.653 (0.079)
Caffeine	−0.152 (0.720)	0.728 (0.041)	0.124 (0.770)	0.943 (0.002)	−0.334 (0.417)
Phenolic compounds	0.466 (0.244)	0.939 (0.003)	0.825 (0.012)	0.523 (0.183)	0.520 (0.187)
EC	0.827 (0.011)	0.671 (0.068)	0.829 (0.011)	0.082 (0.847)	0.759 (0.029)
ECG	0.789 (0.020)	0.702 (0.052)	0.753 (0.031)	0.199 (0.637)	0.637 (0.089)
EGC	0.768 (0.026)	0.494 (0.214)	0.413 (0.309)	0.206 (0.624)	0.339 (0.411)
EGCG	0.752 (0.031)	0.749 (0.032)	0.789 (0.020)	0.239 (0.568)	0.646 (0.084)
Total catechins	0.765 (0.027)	0.696 (0.055)	0.686 (0.060)	0.253 (0.545)	0.553 (0.156)
TA100					
Ascorbic acid	0.985 (0.008)	−0.144 (0.734)	0.204 (0.629)	0.507 (0.199)	0.996 (0.000)
Caffeine	0.451 (0.246)	0.600 (0.116)	0.201 (0.633)	0.991 (0.001)	0.549 (0.159)
Phenolic compounds	0.951 (0.015)	0.030 (0.944)	0.284 (0.496)	0.634 (0.091)	0.995 (0.000)
EC	0.982 (0.009)	−0.447 (0.266)	−0.066 (0.876)	0.334 (0.418)	0.917 (0.006)
ECG	0.989 (0.006)	−0.370 (0.367)	−0.115 (0.787)	0.477 (0.232)	0.925 (0.005)
EGC	0.850 (0.029)	−0.389 (0.340)	−0.444 (0.271)	0.594 (0.121)	0.732 (0.042)
EGCG	0.997 (0.000)	−0.320 (0.440)	−0.041 (0.924)	0.489 (0.218)	0.950 (0.001)
Total catechins	0.976 (0.012)	−0.333 (0.420)	−0.163 (0.699)	0.551 (0.157)	0.908 (0.011)

[a]Correlation coefficients.
[b]Probability: statistical correlations at $P < 0.05$ were considered to be significant.

which were found mainly in the lipid fraction of infused tea, were not detected in all tea extracts. Carotenoids (Olson, 1989) and tocopherols (Packer, 1991) showed antioxidative and anticarcinogenic activities, therefore they may play a role in the physiological effects of the lipid fraction of the tea leaves.

Antimutagenic effects

In our preliminary study, no mutagenicity or toxicity was observed for any tea extracts in a dosage <5 mg/plate towards *S.typhimurium* TA98 and TA100 (data not shown). The antimutagenic activities of tea extracts on five indirect mutagens, including IQ, B[*a*]P, AFB_1, Trp-P-1 and Glu-P-1, were evaluated (Table IV). The tea extracts from green tea exhibited the strongest inhibitory effect against IQ in TA98 and TA100. The antimutagenic effect of four tea extracts on B[*a*]P, AFB_1 and Trp-P-1 decreased in the order: semifermented fermented tea > fermented tea. The tea extracts from pouchong tea showed greater inhibitory effect against AFB_1 in both TA98 and TA100. In addition, oolong tea showed the strongest inhibitory effect against Trp-P-1. For strain TA98, the inhibitory effect to Glu-P-1 of four tea extracts was in the decreasing order: green tea > pouchong tea > oolong tea > black tea. No significant difference ($P > 0.05$) was found for the antimutagenic activity among green, pouchong and oolong teas to Glu-P-1 in strain TA100. Kojima *et al.* (1989) also reported that the antimutagenic effect of oolong tea against B[*a*]P is greater than that of green tea. For strains TA98 and TA100, black tea exhibited the weakest inhibitory activity on the five mutagens studied. The antimutagenic activity of tea extracts varied with the extent of fermentation of tea during the manufacturing process. However, the antimutagenic activity of semifermented tea was greater than other teas for some mutagens.

Correlation between antimutagenicity and major components of tea extracts

Calculated coefficients of correlation between antimutagenic activity and the major components are shown in Table V. For *S.typhimurium* TA98, the antimutagenic effect of tea extracts against the mutagenicity of IQ showed a significant ($P < 0.05$) correlation to their catechin contents. The same trends were observed in the correlation of inhibition by tea extracts against B[*a*]P and AFB_1 and the amounts of ascorbic acid, phenolic compounds and EGCG. However, there was no significant correlation between the antimutagenic activity of tea extracts against Glu-P-1 and the amount of major components except EC. For TA100, significant positive correlations were observed between the inhibitory effect of tea extracts against IQ and Glu-P-1 and the amounts of catechins, ascorbic acid and phenolic compounds. No significant ($P > 0.05$) correlation was found between the antimutagenicity of tea extracts to B[*a*]P and AFB_1 and the content of major components in tea extracts. The inhibitory effect of tea extracts against Trp-P-1 in either TA98 or TA100 was well correlated ($P < 0.05$) to the content of caffeine.

The mechanisms of action of five indirect mutagens used in this study are different. IQ, Trp-P-1 and Glu-P-1 are food mutagens isolated from heated meat and fish. The first step in the activation of these heterocyclic amines is *N*-hydroxylation by cytochrome P-448, and the *N*-hydroxy amine and the *N*-acyl derivative react with DNA to induce mutations (Hashimoto *et al.*, 1980). As shown above, the inhibitory effects of tea extracts against IQ and Glu-P-1 were correlated to their contents of catechins, ascorbic acid and phenolic compounds for TA100, a strain which primarily detects base-pair substitution mutagens. However, the correlation did not exist in TA98, a strain which detects frame-shift mutagens. B[*a*]P is a typical polycyclic aromatic hydrocarbon and is widely distributed through the environment. It is activated to a dihydrodiol epoxide metabolite (Sims and Grover, 1974). AFB_1 is one of the most common aflatoxins. It requires metabolic activation by cytochrome P-450 to produce biologically active epoxide metabolites (Gurtoo *et al.*, 1978). Wood *et al.* (1982) indicated that several phenolic acids, especially ellagic acid, bind to B[*a*]P and reduce its mutagenicity by opening the epoxide ring. San and Chan (1987) reported that phenolic compounds, including gallic acid and caffeic acid, inhibited the activation of AFB_1. The inhibitory effect of phenolic compounds against AFB_1-induced mutagenesis may

be due to the inhibition of the activation enzyme. In this study, the inhibitory effects of tea extracts against B[*a*]P and AFB_1 in TA98 were correlated ($P < 0.05$) to the contents of ascorbic acid and the phenolic compounds. Therefore, the mechanism of antimutagenic effects of components in tea extracts varied with the mutagens and Salmonella strains.

Our results indicate that the antimutagenic activity and major components of tea extracts vary with the extent of fermentation of the tea during manufacture. The amounts of catechins and ascorbic acid in green tea extracts are greater than in the other three tea extracts, whereas the caffeine and phenolic compounds in oolong tea extracts are slightly higher than in green tea extract. The antimutagenic activity of semifermented tea was greater than other teas for some mutagens.

It has been reported in the literature (Taniguchi *et al.*, 1992) that catechins, especially EGCG, in the polyphenolic fraction isolated from green tea afford protection against some mutagen-induced tumors. In this study, the antimutagenic activity of tea extracts against some mutagens appears to be related to the content of catechins, caffeine, ascorbic acid or polyphenols. However, the reason that compounds afford stronger antimutagenic effects in semifermented tea extracts is not clear. Since the manufacture of oolong tea allows for a short period of oxidation, the composition of oolong tea would be expected to be intermediate between green and black teas. A large number of new flavanoids, such as theasinensins, oolongtheanin, oolonghomobisflavins and the ester of EGCG with ascorbic acid, have been isolated from oolong tea (Hashimoto *et al.*, 1988, 1989). These transitory products of the oxidation of catechins have not been found in nonfermented and fermented teas. Further studies on the isolation of the antimutagenic component from semifermented teas and their antimutagenic mechanisms are in progress.

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