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FULL PAPER

Effect of components of green tea extracts, caffeine and catechins on hepatic drug metabolizing enzyme activities and mutagenic transformation of carcinogens

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Abstract

Green tea contains catechins and caffeine as major constituents. Treatment of rats with green tea (2.5% w/v) significantly increased 7-ethoxycoumarin O-deethylase (7-ECOD), caffeine N-1 demethylase (CN1D) and UDP-glucuronyltransferase (UGT) activities. Treatment with caffeine similarly activated CYP1A2 and related monooxygenases as well as UGT, while treatment with catechins induced UGT activity but not 7-ECOD or CN1D activity. Numbers of benzo[a]pyrene (BP)-induced revertant colonies in an Ames test (mutation assay) with *S. typhimurium* TA98 as the test strain were markedly larger when BP was preincubated with the liver S-9 (9000 ×g supernatant of liver homogenate) from green tea-treated rats than when preincubated with that from control rats. In a modified Ames assay system in which UGT was activated by the addition of UDP-glucuronic acid to the preincubation mixture, the numbers of revertant colonies in the assay using liver S-9 from green tea-treated rats decreased to a similar level to that in the assay using S-9 from controls. The acceleration of two enzymatic reactions may contribute to the rapid elimination of BP; the first step, the formation of a metabolic intermediate (which is mutagenic) by CYP1A2 and the second, the conjugation of active metabolic intermediates by UGT. We speculated that green tea can reduce the amount of time carcinogens reside in the body and the chance that body tis-

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sues will be exposed to active metabolites of carcinogens thorough rapid elimination due to the simultaneous induction of CYP1A2 and UGT activities.

Key words：Cytochrome P450, Green tea, Mutagens, UDP-glucuronyltransferase

Introduction

Diet and beverages play an important role in determining the metabolism of xenobiotics and incidence of carcinogenesis[20]. Green tea is one of the most popular beverages in Asian countries. It contains catechins, caffeine, vitamins and amino acids and is reported to possess anticarcinogenic and antimutagenic properties against changes induced by polycyclic aromatic hydrocarbons (PAH)[13].

PAH carcinogens such as benzo[a]pyrene (BP) in the environment are metabolically activated by cytochrome P450 (CYP) to DNA-reactive and mutagenic forms[10]. In contrast, one of the phase II enzymes, UDP-glucuronyl transferase (UGT), catalyzes glucuronidation and contributes to the elimination of metabolically activated toxicants. Because of their strong antioxidative activity and inhibitory effects on the CYP-mediated activation of carcinogens, catechins have been believed to be the most important factor in cancer prevention due to ingestion of green tea[18].

In contrast to a report that CYP-mediated reactions were inhibited by green tea in vitro[22], a number of studied have found that treatment of rats with green tea increased CYP1A and UGT activities in vivo[7,8,17,21]. The cytosolic aromatic hydrocarbon (Ah) receptor contributes to the induction of expression of the above enzymes, and Williams et al. have showed that tea extracts weakly activate Ah receptor-dependent transcription[23]. Moreover, another study demonstrated that treatment of rats with caffeine significantly increased CYP1A2 activity in a Ah receptor-independent manner[4]. Decaffeinated green tea extract was unlikely to alter CYP2D or CYP3A-dependent activity in drug-metabolism[9]. These observations raise doubt over, or even contradict, the hypothesis that the prevention of chemical carcinogenesis by green tea may be due, at least partly, to the inhibitory effect of catechins on the CYP-mediated activation of carcinogens.

A reduction in the activation of pre-mutagens by green tea extracts has been reported; however, the contribution of UGT is still unclear. In this study, we tested the effect of green tea, catechins and caffeine on hepatic enzyme activities and the mutagenic transformation of BP. The Ames test is a sensitive biological method for measuring the mutagenic potency of chemicals. We carried out the Ames test to see if the induction of CYP1A expression and the phase II conjugating enzyme UGT play any role in the prevention of the mutagenic transformation of carcinogens and elucidate the possible mechanism of cancer prevention by green tea.

Materials and methods

Chemicals.

Caffeine monohydrate, 7-ethoxycoumarin and *p*-nitrophenol (*p*-NP) were purchased from Wako Pure Chemical Co. (Tokyo, Japan). Glucose 6-phosphate, glucose 6-phosphate dehydrogenase and NADPH were obtained from Oriental Yeast Co. (Tokyo, Japan). UDP-glucuronic acid (UDPGA) was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Goat anti-rat CYP1A antiserum was from Daiichi Pure Chemical Co. (Tokyo, Japan). Green tea was bought locally (a prod-

uct of Shizuoka, Japan). Catechin mixtures (catechins), (-)-epigallocatechin, (-)-epicatechin, (-)-epigallocatechin gallate and epicatechin gallate, were provided by the Food Research Laboratories, Mitsui Norin Co. (Shizuoka, Japan). The other reagents were of analytical grade.

Animals.

Male Wistar rats (4 and 7 weeks old) were purchased from Japan SLC Inc. (Shizuoka, Japan) and used for experiments after 1 week of acclimatization. Animals were housed at 24±1℃ with a 12 h light and 12 h dark cycle, and given laboratory feed and water *ad libitum*. Rats (5 weeks old) were treated with freshly prepared green tea (2.5% w/v) in drinking water for 4 weeks. All experiments using animals were performed under the supervision and with the permission of the University's Institutional Animal Care and Use Committee. Control animals were given water ad lib. Rats (8 weeks old) were injected intraperitoneally with caffeine (150 mg /kg/day) or catechins (800 mg/kg/day) in saline solution for three days in separate experiments. Animals were killed by decapitation 24 h after the last treatment.

Preparation of hepatic S-9, cytosol and microsomes.

Hepatic S-9, cytosol and microsomes were prepared as described by Omura and Sato[19]. All samples were frozen in liquid nitrogen and stored at -80℃ until assayed. The protein concentration of hepatic samples was determined as described by Lowry *et al*.[16]. Microsomal cytochrome P450 contents were determined as described by Omura and Sato[19].

Preparation and analysis of tea components.

Aqueous extracts of green tea (2.5% w/v) were prepared as described by Sohn *et al*.[21]. The quantitation of tea components was performed using high-pressure liquid chromatography (HPLC). Catechins or green tea extracts (2.5% w/v) dissolved in the mobile phase were subjected to HPLC. The HPLC was performed by the Food Research Laboratories, Mitsui Norin Co. (Shizuoka, Japan), using a Shimadzu LC-6A liquid chromatograph equipped with a data processor (Chromatopac C-R6A) and a SPD-6AV spectrophotometric detector. The column used was Inertsil ODS (4.6 mm i.d.×250 mm) (GL Science Inc., Tokyo, Japan). The mobile phase was a mixture of acetonitrile : ethyl acetate : 0.05% phosphoric acid (12:6:90, v/v), and the flow rate was 2.0 ml/min. The wavelength for absorbance was 280 nm.

Enzyme assays.

The assay of 7-ethoxycoumarin O-deethylase (7-ECOD) activity was described by Greenlee *et al*.[11]. Caffeine N-1 demethylase (CN1D) activity was measured as described by Agundez et al.[1]. UGT activity was measured as described by Bock *et al*.[6].

CYP1A immunoblot analysis.

Immunoblot analysis for the determination of CYP1A protein was performed as described by Laemmli[14] using antibody to rat CYP1A. The spectral configuration of the immunoblot was analyzed using NIH Image as described by Lennard[15].

Ames assay.

The Ames assay was performed as described by Ames *et al*.[3], with some minor modifications, using *S. typhimurium* TA98. Preincubation mixtures (0.7ml) consisted of an overnight culture of *S. typhimurium* TA98 and a hepatic S-9 and S-9 cofactor mix (Oriental East Co., Tokyo) containing a complete NADPH generating system and NADH. In ex-

periments to investigate the effect of the conjugation reaction (phase II) on the metabolic activation of BP by CYP (phase I), the substrate (5 μg BP/plate) was added to preincubation mixtures containing 15 mM UDP-glucuronic acid (UDPGA). Preincubation was initiated by addition of the substrate, and mixtures were preincubated at 37℃ for 20 min. Immediately after the preincubation, 2 ml of soft agar containing 0.5 mM L-histidine and 0.5 mM biotin was added. The mixtures were poured onto a glucose agar plate. The numbers of revertant colonies were counted after 48 h of incubation at 37℃.

Analysis of results.

All results are expressed as the mean ± SD. Statistical significance was determined with an unpaired t-test and Turkey-test. .

Results

The results of the quantitative analyses of components in the green tea and the catechin preparations used in the experiment are shown in Table 1. When lyophilized, 1 ml of green tea (2.5% w/v) gaveo 7.18 mg of tea solids. As shown in Table 1, the green tea preparation used contained 30% catechins and 7% caffeine.

Treatment of rats with green tea or caffeine produced a moderate, but significant, increase in 7-ECOD, CN1D and UGT activities. CN1D activity has been reported to be specific for CYP1A2-catalyzed enzyme activity 5). Treatment of rats with crude catechins also significantly increased UGT activity. 7-ECOD and CN1D activities were not affected by catechin treatment (Table 2).

Immunoblot analysis of hepatic micro-

Table 1. Quantitative analysis of components in tea preparations by HPLC.

Components	Green tea (2.5% w/v)		Crude catechins	
	mg/ml (percentage of total solids)			
Total solids	7.18	(100.0)	2.50	(100.0)
Total catechins	2.14	(29.8)	1.64	(65.6)
EGC	0.75	(10.4)	0.48	(19.2)
EC	0.41	(5.8)	0.25	(10.0)
EGCG	0.78	(10.9)	0.65	(26.0)
ECG	0.20	(2.8)	0.26	(10.4)
Caffeine	0.49	(6.9)	0.02	(0.8)

EGC : (-)-epigallocatechin, EC : (-)-epicatechin, EGCG : (-)-epigallocatechin gallate, ECG : (-)-epicatechin gallate.

Table 2. Comparison of P450 levels and phase I and phase II enzyme activities after pretreatment of rats with green tea, caffeine or catechins.

Pretreatment	P450.	CN1D	7-ECOD	UGT
	nmol/mg protein	pmol/min/mg protein	nmol/min/mg protein	
Control	0.60±0.13	9.07±1.97	0.63±0.02	7.56±0.93
Green tea	0.58±0.08	16.30±2.30**	0.95±0.04**	9.73±0.76**
Control	0.73±0.05	10.60±0.74	0.65±0.15	6.87±1.16
Caffeine	0.86±0.13	17.67±3.52*	0.97±0.18*	9.57±0.56*
Control	0.79±0.02	6.48±0.63	0.54±0.10	9.03±0.05
Catechins	0.83±0.01	6.23±0.92	0.45±0.01	11.59±0.66*

Rats were treated with green tea (2.5% w/v, 4 weeks) in drinking water, or injected with caffeine (150 mg/kg/day, 3 days) or catechins (800 mg/kg/day, 3 days) in saline in a separate experiment. Other experimental details are discussed in the text. Each value is expressed as the mean ± SD from 4 rats. Significantly different from control; * : $P<0.05$. ** : $P<0.01$. 7-ECOD: 7-ethoxycoumarin O-deethylase activity, CN1D : caffeine N-1 demethylase activity, UGT : UDP-glucuronyltransferase activity

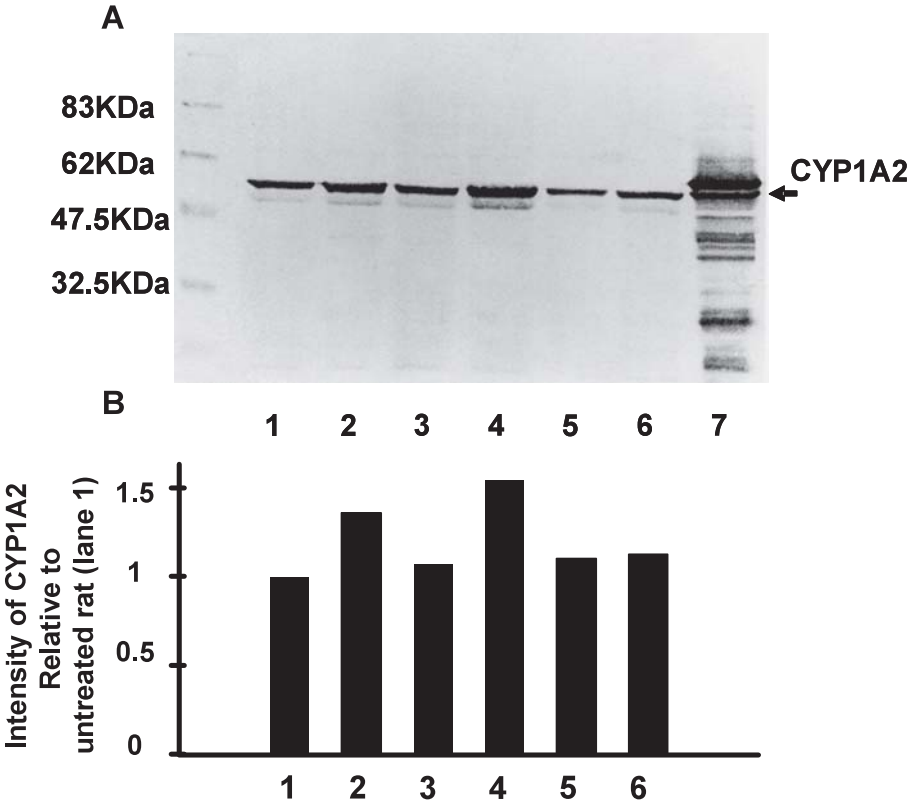

Figure 1. Immunoblot analysis and intensity of CYP1A2 expression in hepatic microsomes from rats using anti-rat CYP1A antiserum. The amount of microsomal protein applied was 15 μg. (A) The arrow indicates the position of CYP1A2 protein. (B) Relative expression levels of CYP1A2. Microsomes used were from control (untreated, 4weeks) rats (lane1), green tea (2.5%w/v, 4weeks)-treated rats (lane 2), control (saline, 3 days) rats (lane 3), caffeine (150mg/kg/day, 3 days)-treated rats (lane 4), control (saline, 3 days) rats (lane 5), crude catechins (800 mg/kg /day, 3 days)-treated rats (lane 6), 3-methylcholanthrene (40 mg/kg/ day, 3 days)-treated rats (lane 7).

somes revealed a moderate increase in the level of CYP1A2 in hepatic microsomes from green tea-and caffeine-treated rats, but not in crude catechin-treated rats (Figure 1). The anti-CYP1A antibody that we used might cross-react with both isoforms of CYP1A1 and CYP1A2. Since CYP1A2 was the major CYP1 A isoform in liver, we detected the constitutive expression of CYP1A2 in treated or untreated rat livers. In the normal condition, the expression level of CYP1A1 might be very low in the liver. Western blot experiments were repeated at least three times to confirm the results.

In the Ames assay using S. *typhimurium* TA98, preincubation of BP with hepatic S-9 from green tea-treated rats significantly increased the number of revertant colonies. However, the addition of UDPGA, a co-substrate of UGT, to preincubation mixtures decreased the numbers of revertant colonies to a level similar to that of the control (Figure 2).

Discussion

It has been reported that treatment of rats with green tea significantly increased

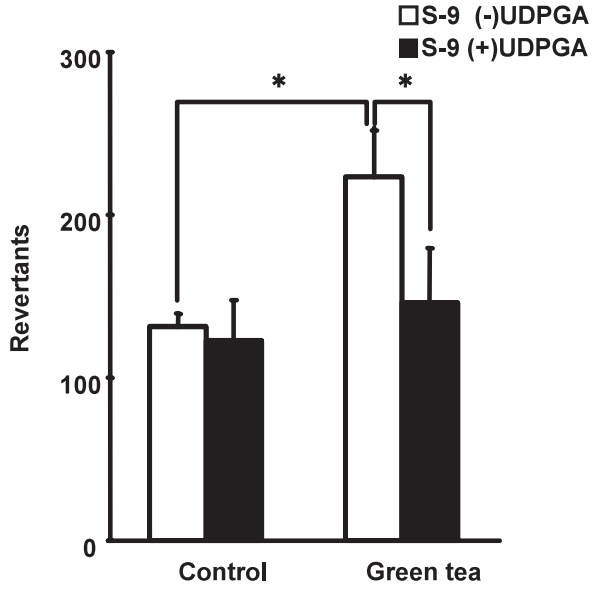

Figure 2. Effect of the addition of UDP-glucuronic acid on ability to transform BP into a mutagen using S. *typhimurium* TA98. The ames assay was performed using hepatic S-9 from rats treated with green tea (2.5% w/v, 4 weeks) in drinking water. Control rats received water ad lib. Each value is expressed as the mean±SD from 3 or 4 rats. Significantly different from control; * :P<0.05.

CYP1A and UGT activities[7,8,17,21]. In this study, we confirmed this finding and demonstrated that treatment with caffeine also produced a similar increase in CYP1A2 and UGT activities. On the other hand, treatment with catechins did not significantly affect CYP1A2. However, UGT activity was significantly increased in catechin-treated rats. Catechin preparations contained 0.8% caffeine as a contaminant, the effect of which we thought would be negligible in this study. The amount of caffeine-impurity was less than the level which could affect phase I and phase II enzymes. It has been reported that treatment with the ellagic acid of natural polyphenols such as catechins significantly increased UGT activity but not CYP1A-catalyzed enzyme activity[2]. Williams et al. reported that treatment with a high concentration of green tea extracts activated the promoter region of CYP1A1 in HepG2 cells[23]. However, they also showed inhibiting effects of several catechins on the activation of the Ah receptor by TCDD. They suggested that green tea extracts contain both agonists and antagonists of the Ah receptor. Notably, (-)-epigallocatechin gallate was revealed to be a potent inhibitor of Ah receptor function, and did not induce CYP1A1 transcription. Taken together, these results indicate that caffeine in green tea is an inducer of CYP1A2 and UGT activities, while the catechins in green tea selectively induce UGT activity.

Cytochrome P450 enzymes have been shown to play an important role in the oxidative metabolic activation and detoxification of many mutagens and carcinogens[12]. The metabolic activation of polycyclic aromatic hydrocarbon pro-carcinogens to DNA-reactive and mutagenic forms is often catalyzed by the CYP1A subfamily[10]. Treatment with green tea induced expression of CYP1A and, hence, an increase in active metabolites of BP in the Ames assay. However, it also induced UGT activity, which catalyzes the detoxification of such active metabolites. Since the conjugation of BP with UDPGA by UGT might be the detoxification route, the addition of UDPGA to the Ames test is associated with a reduction in BP mutagenicity. This reaction system is closer to the situation in vivo because UGT is operative. These results suggest that the elimination of BP from rats is accelerated by green tea, while the levels of active metabolic intermediates do not exceed the control levels. The acceleration of two enzymatic reactions may contribute to the rapid elimination of BP ; the first step, the formation of a metabolic intermediate (which is mutagenic) by CYP1A2 and the second, a conjugation reaction of active metabolic intermediates involving UGT.

We showed the possible role of green tea in the prevention of chemical carcinogenesis through the induction of CYP1A and UGT activities using a modified Ames assay system in which UGT is operative. The induction of CYP1A2 and UGT activities by caffeine and the induction of UGT activity by catechins may contribute to the inactivation and accelerate the elimination of active metabolic intermediates of pro-carcinogens.

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