Effects of Repeated Green Tea Catechin Administration on Human Cytochrome P450 Activity

H-H. Sherry Chow,[1] Iman A. Hakim,[1] Donna R. Vining,[1] James A. Crowell,[2] Catherine A. Cordova,[1] Wade M. Chew,[1] Min-Jian Xu,[1] Chiu-Hsieh Hsu,[1] James Ranger-Moore,[1] and David S. Alberts[1]

[1]Arizona Cancer Center, The University of Arizona, Tucson, Arizona and [2]Division of Cancer Prevention, National Cancer Institute, Bethesda, Maryland

Abstract

Purpose: **Preclinical studies suggested that green tea or green tea catechins can modulate the activities of drug-metabolizing enzymes. We conducted this clinical study to determine the effect of repeated green tea catechin administration on human cytochrome P450 (CYP) enzyme activities.**
Methods: **Forty-two healthy volunteers underwent a 4-week washout period by refraining from tea or tea-related products. At the end of the washout period, study participants received a cocktail of CYP metabolic probe drugs, including caffeine, dextromethorphan, losartan, and buspirone for assessing the activity of CYP1A2, CYP2D6, CYP2C9, and CYP3A4, respectively. Blood and urine samples before and 8 h after probe drug administration were collected to determine parent drug and metabolite concentrations for measurements of baseline CYP enzyme activities. Following the baseline evaluation, study participants underwent 4 weeks of green tea catechin intervention at a dose that contains 800 mg epigallocatechin gallate (EGCG) daily. The green tea catechin product was taken on an empty stomach to optimize the p.o. bioavailability of EGCG. The EGCG dose given in this study exceeded the amounts provided by average green tea consumption. Upon completion of the green tea catechin intervention, the postintervention CYP enzyme activities were evaluated as described above.**
Results: **There are large between-subject variations in CYP enzyme activities in healthy individuals. Four weeks of green tea catechin intervention did not alter the phenotypic indices of CYP1A2, CYP12D6, and CYP12C9, but resulted in a 20% increase (P = 0.01) in the area under the plasma buspirone concentration-time profile, suggesting a small reduction in CYP3A4 activity.**
Conclusions: **We conclude that repeated green tea catechin administration is not likely to result in clinically significant effects on the disposition of drugs metabolized by CYP enzymes.** (Cancer Epidemiol Biomarkers Prev 2006; 15(12):2473–6)

Introduction

Tea (*Camellia sinesis*) is one of the most consumed beverages in the world. Tea consumption may be linked to low incidences of various chronic pathologic conditions, including cancer. The principal active constituent in green tea is believed to be catechins. Green tea catechin–enriched extracts prepared in p.o. formulations are available over the counter as dietary supplements. Defined and enriched green tea catechin formulations, such as Polyphenon E, have been made available for the conduct of controlled clinical trials. There have been no reports of clinical adverse events when green tea is consumed as a beverage. However, cases of liver toxicity have recently been reported following the use of over-the-counter products that contain green tea extracts (1, 2). The cause of these liver toxicity cases has not been determined.

Green tea or green tea catechins have been shown to modulate various xenobiotic metabolizing enzymes in animal studies and in *in vitro* systems (3-5). Modulation of carcinogen activation and detoxification enzymes has been suggested as one of the biochemical mechanisms responsible for the cancer-preventive effect of green tea. Such changes may also affect the efficacy and toxicity of clinically used drugs and may augment drug-related toxicity such as liver toxicity. Because green tea consumption and usage of green tea extracts have increased in the general public, and green tea extracts at doses higher than that consumed from tea are used in multiple ongoing clinical trials, there is a potential for clinically relevant metabolic drug interactions. We conducted a clinical study to determine whether repeated green tea catechin administration could result in metabolic drug interactions by affecting the cytochrome P450 (CYP) enzyme activity. The activities of CYP1A2, CYP2D6, CYP2C9, and CYP3A4 were assessed by isozyme-specific metabolic probe drugs. These four isozymes were selected for study because they account for the metabolism of the majority of medications currently on the market (6) and clinically significant metabolic interactions have been observed with drugs metabolized by these isozymes (7).

Materials and Methods

Study Drugs. Polyphenon E is a green tea catechin extract produced by Mitsui Norin, Ltd (Shizuoka, Japan). It contains 80% to 98% total catechins by weight with epigallocatechin gallate (EGCG) as the main component accounting for 50% to 75% of the material. Other catechins, including epicatechin, epigallocatechin, epicatechin gallate, and gallocatechin gallate, are present in levels ranging from ~2% to 12% each. Polyphenon E contains small quantities of caffeine (around 0.5% w/w) and can be considered a decaffeinated product. Polyphenon E, formulated as p.o. capsules (200 mg of EGCG per capsule), was supplied by the Chemoprevention Agent Development Research Group, National Cancer Institute (Bethesda, MD).

Study Participants. Forty-two nonsmoking healthy individuals participated in the study. There were 10 male and 32 female participants. Average age was 38 years (range, 19-73 years) and average body mass index was 24 kg/m^2 (range, 19-37 kg/m^2). The participants had normal liver and renal function. Participants were excluded if they were pregnant

Received 5/10/06; revised 9/21/06; accepted 10/10/06.
Grant support: National Cancer Institute contract no. N01-CN-25119.
The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
Requests for reprints: H-H. Sherry Chow, Arizona Cancer Center, The University of Arizona, Tucson, AZ 85724. Phone: 520-626-3358; Fax: 520-626-5348. E-mail: schow@azcc.arizona.edu

doi:10.1158/1055-9965.EPI-06-0365

Table 1. CYP phenotypic indices before and after 4 weeks of daily Polyphenon E administration

CYP isozyme	Phenotypic index	Index values*		*P*
		Baseline	Posttreatment	
CYP1A2	Paraxanthine/caffeine ratio in 4-h postdose plasma (n = 41)	0.28 ± 0.13 (0.05-0.6)	0.29 ± 0.16 (0.03-0.84)	0.75
CYP3A4	Buspirone AUC min·ng/ml (n = 41)	136.7 ± 115.7 (15.2-626.7)	166.0 ± 137.7 (10.5-545.3)	0.01
CYP2D6	Dextromethorphan/dextrorphan molar ratio in 0-8 h postdose urine (n = 32)	0.22 ± 0.69 (0.00058-3.33)	0.21 ± 0.52 (0.00027-2.23)	0.47
CYP2C9	Losartan/E3174 ratio in 0-8 h postdose urine (n = 42)	1.53 ± 1.07 (0.38-4.75)	1.52 ± 1.07 (0.24-5.86)	0.84

*Mean ± SD (range).

or breast feeding, had invasive cancers within the past 5 years, had uncontrolled severe metabolic disorders or other serious acute or chronic diseases, consumed more than three drinks of alcohol per week on average, consumed tea regularly, had known hypersensitivity to green tea or metabolic probe drugs (caffeine, dextromethorphan, losartan, or buspirone), were taking medications/supplements that are known CYP enzyme inducers or inhibitors, or had participated in other clinical research studies within the past 3 months. The study was approved by the University of Arizona Human Subjects Committee. Written informed consent was obtained from all participants.

Study Design. Eligible subjects underwent a 4-week washout period in which they were required to refrain from tea or its related products and herbal/botanical supplement, and to minimize the consumption of cruciferous vegetables. At the end of the washout period, study subjects underwent baseline CYP enzyme activity assessment. For enzyme activity determination, low doses of four CYP metabolic probe drugs (100 mg caffeine, 30 mg dextromethorphan, 25 mg losartan, and 10 mg buspirone) were coadministered p.o. Subjects were required to abstain from caffeine-containing products and food items that have been reported to affect drug-metabolizing enzymes (e.g., grapefruit juice, cruciferous vegetables, and food cooked over charcoal), and over-the-counter medications beginning 72 h before and until 8 h after the probe drug administration. Study subjects were instructed to fast overnight for 8 h before and until 4 h after the administration of the probes. A standardized lunch was provided to the subjects at 4 h after probe drug administration. Blood samples were collected before and at 0.5, 1, 2, 4, 6, and 8 h after dosing. Plasma was isolated and stored at −70°C until analysis. Total voided urine was collected for 8 h after probe drug administration. Total urine volume was determined and an aliquot was stored at −70°C until analysis. Probe drug and metabolite levels in plasma and urine samples from selected time points were determined.

Following the completion of baseline enzyme activity determination, study participants were provided with a 4-week supply of Polyphenon E. They were instructed to take four Polyphenon E capsules every day in the morning, on an empty stomach. Breakfast could be consumed 1 h after Polyphenon E dosing. During the Polyphenon E treatment period, participants continued to refrain from tea or its related products and herbal/botanical supplement, and to minimize the consumption of cruciferous vegetables. Study participants underwent posttreatment CYP enzyme activity assessment the day after completing the Polyphenon E treatment period. Study procedures for posttreatment CYP enzyme activity assessment were the same as those described for baseline activity assessment.

Analytic Methods for Metabolic Probe Drugs. For each assay, baseline and posttreatment samples of the same individual were paired in the analysis (i.e., analyzed in the same batch). Caffeine and paraxanthine in 4-h postdose plasma samples were determined using reversed-phase high-performance liquid chromatography with UV detection (8). Dextromethorphan and dextrorphan in urine were analyzed using reversed-phase high-performance liquid chromatography with fluorescence detection (9, 10). Losartan and its metabolite, E3174, in urine were analyzed using reversed-phase high-performance liquid chromatography with fluorescence detection (11). Plasma buspirone levels were determined using a high-performance liquid chromatography/tandem mass spectrometry method developed and validated in our laboratory (12).

Data Analysis. CYP1A2 phenotypic index was assessed by the paraxanthine to caffeine plasma concentration ratio in plasma samples collected 4 h after probe cocktail dosing (8). CYP3A4 phenotypic index was determined by the area under the plasma buspirone concentration-time profile (AUC; ref. 13) obtained after probe drug administration with the AUC estimated using the WINNONLIN program (version 5.0). CYP2D6 phenotypic index was assessed by the urinary recovery of dextromethorphan to dextrorphan molar ratio in urine collected up to 8 h after probe drug administration (14). CYP2C9 phenotypic index was determined by the urinary recovery of losartan to E3174 ratio in urine collected up to 8 h after probe drug administration (8). The distribution of these phenotypic indices was normalized by logarithmic transformation before tests of significance. The indices determined after repeated green tea catechin administration were compared with those determined at baseline using the paired t test. A $P < 0.05$ was considered statistically significant. Ninety percent confidence intervals (90% CI) for the geometric mean ratio of posttreatment over baseline values were also used to evaluate the equivalence of the indices before and after green tea catechin treatment. A lack of clinically significant green tea catechin effect can be concluded if these 90% CI values fall within the range of 0.8 to 1.25. All statistical analyses were done with SAS version 9.0.

Results

Table 1 summarizes the CYP phenotypic indices determined before and after 4 weeks of daily green tea catechin administration. For CYP1A2 phenotypic measurement, data from one participant was not included for analysis because the presence of high caffeine and paraxanthine levels in the predosing samples precluded the assessment for this participant. Similar to those reported previously (8), there were large between-subject variations in CYP1A2 activity. The mean baseline paraxanthine to caffeine ratio from 41 participants was 0.28, with values ranging from 0.05 to 0.6. After 4 weeks of daily Polyphenon administration, the paraxanthine to caffeine ratio did not change with a mean of 0.29 and values ranging from 0.03 to 0.84. The geometric mean ratio of posttreatment over baseline CYP1A2 index was 1.04 (90% CI, 0.94-1.10).

CYP3A4 activity was assessed in 41 subjects because serial blood samples were not successfully obtained from one participant. Large between-subject variations were also observed for CYP3A4 enzyme activity. The mean baseline buspirone AUC from 41 participants was 136.7 min·ng/ml with values ranging from 15.2 to 626.7 min·ng/ml. Four weeks of green tea catechin administration resulted in a statistically significant increase in buspirone AUC ($P = 0.01$), suggesting a reduction in CYP3A4 activity. The geometric mean ratio of posttreatment over baseline buspirone AUC was 1.21 (90% CI, 1.07-1.30).

For CYP2D6 phenotypic assessment, the urine dextromethorphan levels from 10 participants were not detectable or below the limit of quantification, which preclude the dextromethorphan to dextrorphan molar ratio calculation. The mean baseline dextromethorphan to dextrorphan molar ratio from 32 participants was 0.22, with values ranging from 0.00058 to 3.33. The mean posttreatment dextromethorphan to dextrorphan molar ratio was 0.21, with values ranging from 0.00027 to 2.23. Based on the cutoff defined previously for poor metabolizers (15, 16), five participants can be considered CYP2D6 poor metabolizers. Green tea catechin intervention did not result in statistically significant changes in CYP2D6 phenotypic index when all data were used for the analysis or when data from poor metabolizers were excluded from the analysis. The geometric mean ratio of posttreatment over baseline CYP2D6 index was 0.95 (90% CI, 0.83-1.58) when all data were used and 1.19 (90% CI, 0.75-1.34) when excluded poor metabolizers.

For CYP2C9 phenotypic assessment, the mean baseline losartan to E3174 ratio was 1.53 with values ranging from 0.38 to 4.78. Four weeks of green tea catechin intervention did not result in significant changes in CYP2C9 phenotypic index. The geometric mean ratio of posttreatment over baseline CYP2C9 index was 1.07 (90% CI, 0.91-1.08).

Table 2 lists the reported adverse events deemed possibly or probably related to study agent because of temporal proximity. All reported adverse events were Common Toxicity Criteria grade 1 or 2, and many were very mild and transient. Nineteen subjects reported at least one episode of nausea that was deemed possibly or probably related to Polyphenon E administration. Nausea was generally very mild (grade 1) and resolved when the morning meal was taken an hour after dosing. It is not known whether nausea reported was related to Polyphenon E or to taking gelatin capsules on an empty stomach. Four weeks of Polyphenon E dosing on an empty stomach did not result in clinically significant changes in most blood chemistry and hematology measurements (data not shown).

Discussion

This clinical study showed that repeated green tea catechin administration at a daily dose of 800 mg EGCG for 4 weeks had no effects on CYP1A2, CYP2D6, and CYP2C9 phenotypic indices but resulted in 20% increase in buspirone AUC, suggesting a small reduction in CYP3A4 activity. The data suggests that green tea catechin administration is not likely to result in clinically relevant metabolic drug interactions for drugs metabolized by these CYP isozymes.

Table 2. Summary of adverse events possibly or probably related to Polyphenon E

Adverse event	No. subjects reporting the adverse event
Nausea	19
Vomiting	1
Diarrhea	5
Abdominal pain	4
Dyspepsia	2
Dizziness	2
Facial flushing	1
Flatulence	1

Donovan et al. (17) recently reported that administration of decaffeinated green tea extract at a daily dose of 504 mg EGCG (252 mg EGCG, bid) for 14 days did not alter CYP2D6 or 3A4 activity in healthy individuals. This study used probe drugs dextromethorphan and alprazolam for CYP2D6 and 3A4 activity determination, respectively. With a sample size of 11, this study was not powered to detect small changes in CYP enzyme activities. The lack of effect on CYP3A4 activity could also be related to disparity in the sensitivity of different probe drugs to enzyme modulations because the same CYP3A4 inhibitors led to greater changes in buspirone AUC than that of midazolam AUC (18).

The magnitude of metabolic drug interactions is dependent on the dose size of the interacting drug. It is recommended that the maximum planned dose should be used to maximize the possibility of finding an interaction (18). The Polyphenon E dose used in this study contains 800 mg EGCG. This is equivalent to the EGCG content in 8 to 16 cups of green tea given that one cup of green tea contains 40 to 110 mg EGCG (19). In addition to the high catechin dose, we have instructed our participants to take the study product on an empty stomach to further enhance the p.o. bioavailability of EGCG (20). Because no clinically relevant changes in CYP phenotypic indices were observed at the selected dose and dosing condition, minimum metabolic drug interactions are expected with regular green tea consumption at a lower catechin exposure.

Numerous preclinical studies suggested that green tea would modulate CYP activities, with induction of CYP1A1 and1A2 activities consistently observed in rats treated with a variety of green tea preparations (4, 5). However, the modulation effect observed in preclinical studies could be attributed to caffeine present in green tea because caffeine has been shown to be a potent inducer of CYP1A2 (21, 22). This may explain the lack of clinical effect on human CYP1A2 activity observed in our study because Polyphenon E is a decaffeinated green tea catechin product.

This study was designed to determine the effect of repeated green tea catechin administration on CYP enzyme activities with postintervention probe drugs administered 1 day following completion of the green tea catechin intervention. Based on the clinical pharmacokinetics of green tea catechins (20), these compounds should have been mostly cleared from the body before the probe drug administration. Therefore, our study design would not be able to detect the acute metabolic interactions from concomitant administration. This design was selected because acute interactions can be more easily avoided by taking the green tea catechin product hours away from other drugs.

The age range of the volunteers who participated in the study was 19 to 73 years of age. CYP enzyme activities are not changed or reduced moderately in the elderly (>70 years of age; refs. 23, 24). In our study, we did not see a significant correlation between each of the CYP indices with age. Because each subject served as his/her own control, and baseline and posttreatment indices are highly correlated, the wide age range is not likely to obscure the study outcome.

As shown in Table 2, 4 weeks of Polyphenon E intervention at a daily dose of 800 mg EGCG on an empty stomach was generally well tolerated. All reported adverse events were Common Toxicity Criteria grade 1 or 2, and many were very mild and transient. Similar to previous trials (20, 25), the most common adverse events reported during the Polyphenon E intervention were gastrointestinal in nature. Four weeks of Polyphenon E intervention did not result in clinically

significant changes in most blood chemistry and hematology measurements.

We conclude that repeated green tea catechin administration had no effects on CYP1A2, CYP2D6, and CYP2C9 activities but seemed to result in a small reduction in CYP3A4 activity. These results suggest that green tea catechin administration is not likely to result in clinically significant effects on the disposition of drugs metabolized by CYP enzymes.

References

1. Gloro R, Hourmand-Ollivier I, Mosquet B, et al. Fulminant hepatitis during self-medication with hydroalcoholic extract of green tea. Eur J Gastroenterol Hepatol 2005;17:1135–7.
2. Bonkovsky H. Hepatotoxicity associated with supplements containing Chinese green tea (*Camellia sinesis*). Ann Intern Med 2006;144:68–9.
3. Maliakal PP, Coville PF, Wanwimolruk S. Tea consumption modulates hepatic drug metabolizing enzymes in Wistar rats. J Pharm Pharmacol 2001; 53:569–77.
4. Bu-Abbas A, Clifford MN, Walker R, Ioannides C. Selective induction of rat hepatic CYP1 and CYP4 proteins and of peroxisomal proliferation by green tea. Carcinogenesis 1994;15:2575–9.
5. Sohn OS, Surace A, Fiala ES, et al. Effects of green and black tea on hepatic xenobiotic metabolizing systems in the male F344 rat. Xenobiotica 1994;24: 119–27.
6. Danielson P. The cytochrome P450 superfamily: biochemistry, evolution and drug metabolism in humans. Curr Drug Metab 2002;3:561–7.
7. Dresser G, Bailey D. A basic conceptual and practical overview of interactions with highly prescribed drugs. Can J Clin Pharmacol 2002;9: 191–8.
8. Christensen M, Andersson K, Dalen P, et al. The Karolinska cocktail for phenotyping of five human cytochrome P450 enzymes. Clin Pharmacol Ther 2003;73:517–28.
9. Park YH, Kullberg MP, Hinsvark ON. Quantitative determination of dextromethorphan and three metabolites in urine by reverse-phase high-performance liquid chromatography. J Pharm Sci 1984;73:24–9.
10. Lam YWF, Rodriguez SY. High-performance liquid chromatography determination of dextromethorphan and dextrorphan for oxidation phenotyping by fluorescence and ultraviolet detection. Ther Drug Monit 1993;15: 300–4.
11. Ritter MA, Furtek CI, Lo MW. An improved method for the simultaneous determination of losartan and its major metabolite, EXP3174, in human plasma and urine by high-performance liquid chromatography with fluorescence detection. J Pharm Biomed Anal 1987;15:1021–9.
12. Chew W, Xu MJ, Cordova C, Chow HHS. Quantification of a cytochrome P450 3A4 substrate, buspirone, in human plasma by liquid chromatography-tandem mass spectrometry. J Chromatogr B. In press.
13. Lilja J, Kivisto K, Backman J, Lamberg T, Neuvonen P. Grapefruit juice substantially increases plasma concentrations of buspirone. Clin Pharmacol Ther 1998;64:655–60.
14. Wang Z, Gorski JC, Hamman MA, Huang SM, Lesko LJ, Hall SD. The effect of St. John's wort (*Hypericum perforatum*) on human cytochrome P450 activity. Clin Pharmacol Ther 2001;70:317–26.
15. Kashuba AD, Nafziger AN, Kearns GL, et al. Effect of fluvoxamine therapy on the activities of CYP1A2, CYP2D6, and CYP3A as determined by phenotyping. Clin Pharmacol Ther 1998;64:257–68.
16. Streetman DS, Bleakley JF, Kim JS, et al. Combined phenotypic assessment of CYP1A2, CYP2C19, CYP2D6, CYP3A, *N*-acetyltransferase-2, and xanthine oxidase with the ''Cooperstown cocktail.'' Clin Pharmacol Ther 2000;68: 375–83.
17. Donovan JL, Chavin KD, Devane CL, et al. Green tea (*Camellia sinesis*) extract does not alter cytochrome p450 3A4 or 2D6 activity in healthy volunteers. Drug Metab Dispos 2004;32:906–8.
18. Bjornsson T, Callaghan J, Einolf H, et al. The conduct of *in vitro* and *in vivo* drug-drug interaction studies: a Pharmaceutical Research and Manufacturers of America (PhRMA) perspective. Drug Metab Dispos 2003;31: 815–32.
19. Graham HN. Green tea composition, consumption, and polyphenol chemistry. Prev Med 1992;21:334–50.
20. Chow HHS, Hakim IA, Vining DR, et al. Effects of dosing condition on the oral bioavailability of green tea catechins after single-dose administration of Polyphenon E in healthy individuals. Clin Cancer Res 2005;11: 4627–33.
21. Ayalogu E, Snelling J, Lewis D, Talwar S, Clifford M, Ioannides C. Induction of hepatic CYP1A2 by the oral administration of caffeine to rats: lack of association with the Ah locus. Biochim Biophys Acta 1995;1272:89–94.
22. Goasduff T, Dreano Y, Guillois B, Mendez J, Berthou F. Induction of liver and kidney CYP1A1/1A2 by caffeine in rat. Biochem Pharmacol 1996;52: 1915–9.
23. Dorne JLCM. Impact of inter-individual differences in drug metabolism and pharmacokinetics on safety evaluation. Fundam Clin Pharmacol 2004;18: 609–20.
24. Durnas C, Loi CM, Cusack BJ. Hepatic drug metabolism and aging. Clin Pharmacokinet 1990;19:359–89.
25. Chow HHS, Cai Y, Hakim IA, et al. Pharmacokinetics and safety of green tea polyphenols after multiple-dose administration of epigallocatechin gallate and polyphenon E in healthy individuals. Clin Cancer Res 2003;9:3312–9.

Effects of Repeated Green Tea Catechin Administration on Human Cytochrome P450 Activity

H-H. Sherry Chow, Iman A. Hakim, Donna R. Vining, et al.

Cancer Epidemiol Biomarkers Prev 2006;15:2473-2476.