GREEN TEA (CAMELLIA SINENSIS) EXTRACT DOES NOT ALTER CYTOCHROME P450 3A4 OR 2D6 ACTIVITY IN HEALTHY VOLUNTEERS

ABSTRACT:

Green tea extract is a widely used dietary supplement. The objective of this study was to assess the influence of a decaffeinated green tea (DGT; Camellia sinensis) extract on the activity of the drug-metabolizing enzymes cytochrome P-450 2D6 and 3A4. Probe drugs dextromethorphan (30 mg, CYP2D6 activity) and alprazolam (ALPZ, 2 mg, CYP3A4 activity) were administered orally to healthy volunteers (n = 11) at baseline, and again after treatment with four DGT capsules/day for 14 days. Each DGT capsule contained 211 ± 25 mg of green tea catechins and <1 mg of caffeine. Dextromethorphan metabolic ratios (DMRs) and alprazolam pharmacokinetics were determined at baseline and after DGT treatment. There were no significant differences in ALPZ pharmacokinetics at baseline and after DGT treatment (all P values ≥0.05; maximum concentration in plasma, 33 ± 8 versus 34 ± 13 ng/ml; time to reach maximum concentration in plasma, 1.4 ± 1.2 versus 1.4 ± 1.2 h; area under the plasma concentration versus time curve, 480 ± 119 versus 510 ± 107 h · ng · ml⁻¹; half-life of elimination, 12.3 ± 1.7 versus 13.1 ± 3.4 h). The DMR was 0.053 ± 0.045 at baseline and 0.041 ± 0.032 after DGT supplementation (P > 0.05). The plasma concentration of the green tea flavonoid, (−)-epigallocatechin gallate, reached 1.3 ± 1.8 μM 2 h after DGT treatment. Our results indicate that DGT is unlikely to alter the disposition of medications primarily dependent on the CYP2D6 or CYP3A4 pathways of metabolism.

Materials and Methods

Subjects. The clinical protocol was approved by the Medical University of South Carolina’s Institutional Review Board, and all subjects provided informed consent. All subjects were determined to be healthy by history, physical examination, basic laboratory indices, and ECG. Subjects were nonsmokers, taking no medications, and abstained from caffeine, ethanol, and grapefruit juice during the study. All were phenotyped as normal metabolizers of CYP2D6 using DM before enrollment in the study, as previously described (Schmid et al., 1985).

Study Design and Drug Administration. The clinical study design has been used extensively by our laboratory to determine the in vivo effects of dietary supplements on P450 activity and has been previously described in detail (Donovan et al., 2003; Markowitz et al., 2003a,b). After an overnight fast and urinary void, each subject was given 30 mg of DM (Robitussin cough syrup; Wyeth-Ayerst, Princeton, NJ), and 2 mg of ALPZ (Mylan Pharmaceuticals, Morgantown, WV) orally with 30 to 60 ml of water. Subjects were fed a standard breakfast 30 to 45 min after drug administration. An 8-h urine collection commenced immediately after drug administration. Blood samples were obtained in heparinized tubes before (i.e., 0 h) drug administration, and again at 0.5-, 1-, 1.5-, 2-, 3-, 4-, 6-, 8-, 12-, 24-, 36-, 48-, and 60-h time points. This phase served as the baseline measurement of CYP2D6 and CYP3A4 activity.

After a minimum 7-day wash-out period, subjects were provided a 14-day supply of DGT extract (Decaffeinated Super Green Tea Extract; Life Exten-
tion; Fort Lauderdale, FL). The capsules were dispensed in preloaded medication organizers embossed with the days of the week (Ezy-Dose; Apothecary Products, Burnsville, MN) to enhance subject compliance with dosing schedule and duration (Park et al., 1991). The subjects took two capsules two times each day at 8:00 AM and 8:00 PM. The dosage was chosen to represent an amount of green tea and its catechins that could normally be consumed by the general public.

After 14 days of treatment with DGT, subjects were dosed with 30 mg of DM and 2 mg of ALPZ as in the baseline phase with identical specimen collection times and identical meals. Additionally, two DGT capsules were given concomitantly with the ALPZ and DM and continued at 8:00 AM and 8:00 PM for 1 day thereafter.

**Analytical Methods.** DM and dextrophan, and ALPZ were determined using previously described HPLC methods (Miller and DeVane, 1988; Hoskins et al., 1997). The pharmacokinetic program WinNonlin was used to estimate ALPZ pharmacokinetics (Pharsight, Mountain View, CA). Mean pharmacokinetic parameters and the dextromethorphan to dextrophan metabolic ratio (DMR) at baseline and after DGT administration were analyzed using the paired t test. The level of significance was set at P = 0.05.

Analysis of caffeine and catechins in the DGT was performed three times on three DGT capsules from the lot used in the present study. A 0.1 mg/ml solution was prepared in 30% acetonitrile containing 0.5% H₃PO₄. For HPLC analysis, a linear gradient of 12 to 24% acetonitrile in aqueous 0.5% H₃PO₄ was performed over 40 min with a flow rate of 1.0 ml/min with a Luna C18 (2) 250 × 4.6 mm, 5-µm reversed phase column (Phenomenex, Torrance, CA). Detection was performed by absorption at 280 nm. Compounds were identified by their retention times through comparisons with authentic standards (Sigma-Aldrich, St. Louis, MO).

Analysis of plasma EGCG concentrations was performed during the post-treatment P450 activity assessment phase on the plasma obtained 2 h after dosing with the DGT. One-milliliter plasma samples were acidified to pH 5.0 with 70 µl of 1.0 M acetic acid. The internal standard, (+)-taxifolin, was added to achieve a final concentration of 2 µM. To determine the total amount of EGCG, 6000 U of β-glucuronidase and 125 U of aroylsulfatase (Sigma-Aldrich) were added and samples were incubated at 37°C for 45 min. This incubation step was omitted to determine the amount of unconjugated EGCG. Extraction was performed with 3 ml of ethyl acetate. After centrifugation and evaporation of the solvent to dryness, the residue was dissolved in 100 µl of 24% acetonitrile containing 0.5% phosphoric acid. Samples were analyzed by HPLC using the conditions described above for the DGT product analysis.

**Results**

**Human Subjects.** Eleven subjects enrolled in and completed the study (six women and five men; seven white; two Asian, two black), with a mean (±S.D.) age of 35 ± 9 years. No adverse events occurred that were attributable to treatment with the DGT or the study in general. The data for one subject were not used due to suspected use of medication during the study period.

**Decaffeinated Green Tea Product Analysis.** The DGT extract contained 2 ± 0 mg of catechin, 11 ± 2 mg of epicatechin, 18 ± 4 mg of epigallocatechin, 126 ± 16 mg of epigallocatechin gallate, 13 ± 2 mg of catechin gallate, and 41 ± 5 mg of epicatechin gallate. Each DGT capsule contained 0.9 ± 0.1 mg of caffeine; thus, the daily dose of caffeine in this study was <4 mg/day.

**CYP2D6 Activity. Dextromethorphan Metabolic Ratio.** All 10 subjects metabolized DM extensively to its metabolite at baseline and after DGT administration. The DMR values for these subjects are presented in Fig. 1. The (mean ± S.D.) ratio of DM to its metabolite was 0.053 ± 0.045 at baseline and 0.041 ± 0.032 after DGT administration. The magnitude of changes in the DMR values was representative of normal interindividual variation rather than CYP2D6 inhibition (Zhang et al., 1992; Liston et al., 2002). No significant differences in the DMR values were found at baseline and after treatment with DGT.

**Alprazolam Pharmacokinetics.** The mean plasma ALPZ concentra-

![Fig. 1. DMRs for 10 subjects following administration of 30 mg of dextromethorphan at baseline and after supplementation with a DGT extract for 14 days. There were no significant differences in the DMR values after supplementation with DGT (P < 0.05).](image)

**Discussion**

Despite the widespread use of green tea-containing supplements by the general public, there has been no data available on the propensity for green tea to be involved in drug interactions. The present study focused on the activity of two major P450 isoforms, CYP3A4 and CYP2D6, which, together, are involved in the metabolism of approximately 70% of medications (Wrighton and Thummel, 2000; Zanger and Eichelbaum, 2000). Our results indicate that supplementation with a DGT extract providing 844 mg of catechins/day is unlikely to significantly alter the disposition of medications primarily dependent on the CYP2D6 or CYP3A4 pathways of metabolism.

The product used in this study contained all the principal flavonoid components of green tea but was essentially devoid of caffeine. Caffeine consumption does not significantly alter the activity of CYP3A4 or CYP2D6 in humans (Frye et al., 1997). The use of a decaffeinated green tea extract permitted the administration of higher amounts of green tea catechins without the adverse effects of caffeine. We have also confirmed that the main catechin component of the DGT used in this study, EGCG, was bioavailable from this supplement. Therefore, the lack of effect of DGT on CYP3A4 and CYP2D6 activity cannot be due to a lack of bioavailability of the extract used in this study.

This investigation did not assess the effect of DGT on other
important P450 isozymes or drug transporters (Jodoin et al., 2002). In vitro and animal studies indicate that flavonoid components of green tea may inhibit the activity of CYP2B1, steroid 5α-reductase, as well as phase II conjugation enzymes (Hiipakka et al., 2002; Huynh and Teel, 2002; Lu et al., 2003b). Thus, our finding that DGT is unlikely to participate in significant interactions with drugs that are metabolized by CYP3A4 or CYP2D6 must be regarded only as the initial clinical investigation into the drug interaction potential of DGT.

References


