CONTRIBUTION OF PRESYSTEMIC HEPATIC EXTRACTION TO THE LOW ORAL BIOAVAILABILITY OF GREEN TEA CATECHINS IN RATS

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(Received May 20, 2002; accepted August 5, 2002)

This article is available online at http://dmd.aspetjournals.org

ABSTRACT:
Green tea and green tea catechins have been shown to possess potent cancer-preventive activities in rodent cancer models. At present, epidemiological evidence of the protective effect of green tea consumption against the development of human cancers is not conclusive. Oral bioavailability of green tea catechins has been shown to be low in animals and possibly in humans. This study is designed to determine the contribution of first-pass hepatic elimination to the low oral bioavailability of green tea catechins. Green tea catechin mixture was dosed to rats by intravenous or intraportal infusion. Blood samples were collected after dosing and analyzed using high-performance liquid chromatography with the coulometric electrode array detection system. The systemic clearance of epigallocatechin gallate (EGCG), epigallocatechin (EGC), and epicatechin (EC) was 8.9, 6.3, and 9.4 ml/min, respectively. The steady state volume of distribution (Vss) of EGCG, EGC, and EC was 432, 220, and 187 ml, respectively. We found that high percentage of green tea catechins escaped first-pass hepatic elimination, with 87.0, 108.3, and 94.9% of EGCG, EGC, and EC, respectively, available in the systemic blood following intraportal infusion. Our results suggest that factors within the gastrointestinal tract such as limited membrane permeability, transporter mediated intestinal absorption, and phase II detoxification enzymes (Khan et al., 1992; Katiyar et al., 1993). Despite of the compelling laboratory evidence, the epidemiological evidence on the protective effect of green tea consumption against the development of human cancer is not conclusive.

At clinically relevant doses, the oral bioavailability (F) of tea catechins was found to be low in animals and possibly in humans. Chen et al. (1997) reported that less than 2% EGCG was available in the systemic blood after oral administration in rats. Recently, we have determined the pharmacokinetics of green tea catechins in humans following oral administration of EGCG or a green tea catechin mixture (Chow et al., 2001). The oral clearance (CL/F) and the apparent volume of distribution (V/F) of EGCG were found to be around 6 to 14.6 l/min and 1000 to 4800 liters, respectively. The large oral clearance and apparent volume of distribution observed in humans are also likely to be attributed to low oral bioavailability.

This study is designed to determine the contribution of hepatic first-pass elimination on the low oral bioavailability of green tea catechins. Information generated from this study contributes to the understanding of mechanism(s) responsible for low oral systemic availability of green tea catechins and could help identify potential factors affecting the systemic exposure of these important phytochemicals.

Materials and Methods
Chemicals and Reagents. EGCG, EGC, and EC were supplied by the Food Research Laboratories, Mitsui Norin Co. (Fujieda City, Japan) through the National Cancer Institute (Bethesda, MD). All other reagents were of HPLC grade or of the highest grade commercially available.

Animals. Male Sprague-Dawley rats (370–400 g) were obtained from Harlan Laboratories (Indianapolis, IN). Animals were allowed to acclimate to
a standard environment for 2 weeks before the experiment and were fasted overnight before the study day.

**Dosing Solution.** EGCG, EGC, and EC were dissolved in normal saline in a ratio similar to that in one of the green tea catechin formulations used in our clinical study (Chow et al., 2001). The dosing solution was prepared fresh immediately prior to the initiation of infusion. For intravenous administration, each animal received 6000, 1101, and 930 µg of EGCG, EGC, and EC, respectively. For intraportal infusion, each animal received 6041, 1285, and 1048 µg of EGCG, EGC, and EC, respectively.

**Animal Experiments.** Rats were randomly assigned to receive tea catechin dosing solution via intravenous or intraportal infusion (5 rats/group). All rats were weighed on the study day and anesthetized with an intraperitoneal injection of pentobarbital (10 mg/ml) at a dose of 50 mg/kg body weight and were maintained under anesthesia throughout the blood collection period. Right femoral venous or pyloric vein was cannulated for intravenous or intraportal infusion, respectively, and right external jugular vein was cannulated for sample collection. The dosing solution was delivered via a syringe infusion pump (model PHD 2000; Harvard Apparatus, Inc., Holliston, MA) at a rate of 0.05 ml/min for 30 min. Blood samples were collected from the jugular vein catheter at 5, 15, 30, 60, 90, 120, 180, 240, 300, and 360 min after the initiation of infusion. Samples were centrifuged at 2,000 g at 4°C for 10 min. Plasma was transferred into microcentrifuge tubes containing 10 µl of acetic acid/EDTA solution [0.4M NaH₂PO₄ buffer containing 20% ascorbic acid and 0.1% EDTA (pH 3.6)] and stored at −80°C until analysis.

**Tea Catechin Concentration Measurements.** Plasma samples were extracted according to the procedure published previously (Chen et al., 1997). Briefly, plasma samples were extracted with methylene chloride to remove lipid soluble components in the presence of acetic acid/EDTA solution. The aqueous supernatant was then extracted with ethyl acetate. The ethyl acetate fraction was collected, mixed with a small volume of 10% acetic acid, and dried by vacuum centrifugation. The dried ethyl acetate fraction was reconstituted in 100 µl of 15% acetonitrile and centrifugation at 16,000g for 10 min before injecting onto HPLC.

The HPLC system used in this study consisted of an ESA model 540 refrigerated autosampler, an ESA 582 two-pump solvent delivery system, an ESA 5600 Coulochemistry electrode array system (ESA Inc., Chelmsford, MA), and a Supelcosil C₁₈ reversed-phase column (150 x 4.6 mm; particle size, 5 µm; Supelco Inc., Bellefonte, PA). The autosampler and column temperatures were maintained at 6°C and 35°C, respectively. Gradient mobile phase was used to separate tea catechins. Buffer A consisted of 30 mM NaH₂PO₄ buffer, acetonitrile, and tetrahydrofuran in a volume ratio of 98:1.3:11.7:0.12 (pH 3.35). Buffer B consisted of 15 mM NaH₂PO₄ buffer, acetonitrile, and tetrahydrofuran in a volume ratio of 4:1:58:5:12.5 (pH 3.45). The flow rate was maintained at 1 ml/min. The column was eluted at 96% buffer A and 4% buffer B from 0 to 7 min, and then the gradient was changed linearly to 17% buffer B at 25 min, 28% buffer B at 31 min, 33% buffer B at 37 min, and 98% buffer B at 38 min. It was maintained at 98% buffer B from 38 to 43 min and finally changed back to 4% buffer B at 44 min for the analysis of the next sample. The effluent was monitored by the Coulochemistry electrode array system with potential settings at −90, −10, 70, and 150 mV, and four chromatograms were obtained simultaneously.

**Data Analysis.** The following pharmacokinetic parameters of unchanged EGCG, EGC, and EC were estimated using the WinNonlin program (Pharsight, Mountain View, CA) with a noncompartmental approach (Gibaldi and Perrier, 1982); terminal elimination rate constant (kₑ); terminal elimination half-life (t₁/₂ₑ); area under the plasma concentration-time curve (AUC); systemic clearance (CL); and the steady state volume of distribution (Vss). Dose corrected AUC from intraportal administration was compared with that from intravenous administration to determine the contribution of the liver to the presystemic loss of tea catechins. Pharmacokinetic parameters of each green tea catechin were compared using one way analysis of variance. Bonferroni’s t test was used for the pairwise multiple comparisons. A p value <0.05 was considered statistically significant.

**Results and Discussion**

Table 1 summarizes the pharmacokinetic parameters of green tea catechins following intravenous infusion. Terminal elimination rate constant of EGCG (0.0052 ± 0.0011 min⁻¹) was smaller than that of EGC (0.0131 ± 0.0022 min⁻¹) and EC (0.0117 ± 0.0013 min⁻¹), which corresponded to the observed differences in the elimination half-life of the three tea catechins (139.5, 54.2, and 59.7 min for EGCG, EGC, and EC, respectively). The volume of distribution of EGCG was significantly larger than that of EGC and EC (432 ± 70 ml versus 220 ± 114 versus 187 ± 64 ml, respectively). This is consistent with the differences observed in the octanol/water partition coefficient (log KWP) values of the three catechins (Hashimoto et al., 1999). The systemic clearance of EGCG was significantly smaller than that of EC and EGCG (6.3 ± 1.0 versus 9.4 ± 1.5 versus 8.9 ± 1.4 ml/min).

To exert systemic activities, drugs/chemicals administered orally need to be absorbed into the systemic circulation and then distributed to different target organs. In animal studies, the oral bioavailability of green tea catechins was found to be less than 2% (Chen et al., 1997). Small changes in the presystemic elimination of green tea catechins could have significant biological consequences because the systemic exposure dose would vary considerably. The oral bioavailability of green tea catechins has not been determined in humans because of the lack of an intravenous formulation. We have determined the plasma pharmacokinetics of green tea catechins in humans after oral administration of EGCG and a green tea catechin mixture (Chow et al., 2001) and found that EGCG had high CL/F and large oral volume of distribution (V/F). In the current animal study, the CL of green tea catechins was found to be 15 to 25 ml/min/kg following intravenous dosing. Since small fractions of unchanged green tea catechins were excreted in the urine (Chen et al., 1997), the hepatic clearance is likely to contribute significantly to the total systemic clearance. Comparing to an average hepatic blood flow of 50 ml/min/kg in rats (Lin, 1990), the tea catechins can be considered to have moderate clearances. The steady state volumes of distribution (Vss) of these catechins in rats are in the range observed for other polar drugs (Fabre et al., 1977; Maza et al., 1996; Burstein et al., 1999) and are considered to have small distribution volumes. The discrepancies observed between the animal pharmacokinetic data and human situations could be because the oral bioavailability (F) of green tea catechins is also low in humans. Since F values range between 0–1, small F values would result in high oral clearance and large oral apparent volume of distribution.

<table>
<thead>
<tr>
<th>PK Parameter</th>
<th>EGCG</th>
<th>EC</th>
<th>EGCG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aₚ (min⁻¹)</td>
<td>0.0131 ± 0.0022ᵃ</td>
<td>0.0117 ± 0.0013</td>
<td>0.0052 ± 0.0011ᵇ</td>
</tr>
<tr>
<td>t₁/₂ₑ (min)</td>
<td>54.2 ± 10.1</td>
<td>59.7 ± 7.4</td>
<td>139.5 ± 35.0ᵇ</td>
</tr>
<tr>
<td>Vₐ (ml)</td>
<td>220 ± 114</td>
<td>187 ± 64</td>
<td>432 ± 70ᵇ</td>
</tr>
<tr>
<td>CL (ml/min)</td>
<td>6.3 ± 1.0ᵃ</td>
<td>9.4 ± 1.5</td>
<td>8.9 ± 1.4</td>
</tr>
</tbody>
</table>

ᵃ Mean ± S.D. ᵇ Significantly different from that of EGCG and EC; p < 0.05 ᶜ Significantly different from that of EGCG and EC; p < 0.05

The data in Table 1 show that the systemic clearance of EGCG is significantly smaller than that of EGC and EC, indicating that only a small fraction of the dose is absorbed into the systemic circulation. The high systemic clearance of EGC and EC suggests that they are rapidly cleared from the circulation, which could have significant biological consequences because the systemic exposure dose would vary considerably. The oral bioavailability of green tea catechins has not been determined in humans because of the lack of an intravenous formulation. We have determined the plasma pharmacokinetics of green tea catechins in humans after oral administration of EGCG and a green tea catechin mixture (Chow et al., 2001) and found that EGCG had high CL/F and large oral volume of distribution (V/F). In the current animal study, the CL of green tea catechins was found to be 15 to 25 ml/min/kg following intravenous dosing. Since small fractions of unchanged green tea catechins were excreted in the urine (Chen et al., 1997), the hepatic clearance is likely to contribute significantly to the total systemic clearance. Comparing to a hepatic blood flow of 50 ml/min/kg in rats (Lin, 1990), the tea catechins can be considered to have moderate clearances. The steady state volumes of distribution (Vss) of these catechins in rats are in the range observed for other polar drugs (Fabre et al., 1977; Maza et al., 1996; Burstein et al., 1999) and are considered to have small distribution volumes. The discrepancies observed between the animal pharmacokinetic data and human situations could be because the oral bioavailability (F) of green tea catechins is also low in humans. Since F values range between 0–1, small F values would result in high oral clearance and large oral apparent volume of distribution.
green tea catechins undergo minimal presystemic hepatic elimination. Most of EGC (108.3%) and EC (94.9%) infused into portal vein entered into the systemic circulation without undergoing significant first-pass hepatic elimination. Similarly, high percentage of EGCG (87.0%) entered into the systemic blood following intraportal infusion. Figure 1 illustrates that the average plasma concentration-time profiles of each green tea catechin were similar after i.v. and intraportal infusion. The data suggest that first-pass hepatic elimination does not play an important role in the presystemic loss of orally administered green tea catechins.

The stability of green tea catechins in aqueous solutions has been shown to be dependent on a variety of factors, including pH, oxygen concentration, temperature, and ionic strength (Yoshino et al., 1999). Green tea catechins are generally stable in acidic solutions at pH ranging from 1.8 to 6.4. EGC and EGCG are rapidly degraded at pH levels above 7.4, which is the pH of most body fluids. EC is found to be stable between pH 1.8 and 11.2. Since the pH of the intestinal tract ranges from 5 to 8, degradation of EGCG and EGC may occur in the intestinal lumen and may contribute their presystemic loss.

Transporter-mediated intestinal efflux may also play a role in the presystemic loss of green tea catechins. The intestinal epithelial membrane transport of EC was studied recently using the human Caco-2 cell line (Vaidyanathan and Walle, 2001). EC was not absorbed from apical to basolateral side, whereas efflux from basolateral to apical side with a high apparent permeability was reported. The efflux was inhibited by MK-571, a competitive inhibitor of the MRP2 transporter expressed in the apical membrane of Caco-2 cells. A P-glycoprotein inhibitor, verapamil, did not inhibit the efflux of EC from basolateral to apical side at a concentration of 50 μM. Apical to basolateral absorption of EC could be observed, although rather low, in the presence of MK-571. This study suggests that intestinal efflux of

![Graph](image1)

**FIG. 1.** Average plasma green tea catechin concentration-time profiles after i.v. and intraportal (IP) administration. Each point represents the average of five rats, and the cross-vertical bars represent one SD of the mean.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>EGC</th>
<th>EC</th>
<th>EGCG</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC(μg·min/ml)</td>
<td>i.v.</td>
<td>i.p.</td>
<td>i.v.</td>
</tr>
<tr>
<td>177.7±24.5</td>
<td>241.5±43.7</td>
<td>100.2±13.5</td>
<td>123.2±40.2</td>
</tr>
<tr>
<td>Dose (μg)</td>
<td>1101</td>
<td>1285</td>
<td>930</td>
</tr>
<tr>
<td>AUC/dose (min/ml)</td>
<td>0.161±0.022</td>
<td>0.175±0.032</td>
<td>0.108±0.014</td>
</tr>
<tr>
<td>F&lt;sub&gt;iv, p&lt;/sub&gt;</td>
<td>108.3%</td>
<td>94.9%</td>
<td>87.0%</td>
</tr>
</tbody>
</table>

i.p., intraportal.

a Mean ± S.D.

b Calculated by (AUC/dose)_i.p. / (AUC/dose)_i.v.
green tea catechins may contribute to the low oral bioavailability of these phytochemicals.

Catechins have also been shown to be metabolized by intestinal flora and enzymes located in the enterocytes. Meselhy et al. (1997) found that EC, EGC, and EGCG are extensively metabolized by a human fecal suspension. Novel metabolites of EGC and EC have been identified in human plasma and urine and appeared to be produced by intestinal microorganisms (Li et al., 2000). Sulfate and glucuronide conjugates of green tea catechins have been identified in preclinical and clinical samples (Okushio et al., 1999; Yang et al., 1999; Lee et al., 2000; Chow et al., 2001; Kohri et al., 2001). UDP-glucuronosyltransferase and phenolsulfotransferase located in the intestinal mucosa could be responsible for the presystemic gut wall metabolism of green tea catechins.

We conclude that first-pass hepatic elimination of green tea catechins does not play a significant role in the presystemic elimination of orally administered catechins. Studies are needed to delineate the contribution of intestinal efflux and intestinal metabolism to the low oral bioavailability of green tea catechins to better understand factors affecting the oral bioavailability of this important class of potential cancer chemopreventive agents.

References


