Effects of Co-Administration of Tea Epigallocatechin-3-gallate (EGCG) and Caffeine on Absorption and Metabolism of EGCG in Humans

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Based on the ratios of (−)-epigallocatechin-3-gallate (EGCG) and caffeine (CAF) levels found in commercial tea drinks, EGCG and CAF were co-administered to human volunteers at various EGCG/CAF ratios, and plasma EGCG was determined by high performance liquid chromatography with chemiluminescence detection. As for the results, in plasma taken after ingestion of a beverage containing 95 mg of EGCG alone, the area under the plasma EGCG concentration-time curve (AUC) was 857 ng·h/ml. A higher AUC (1,370 ng·h/ml) was observed when subjects ingested a beverage containing EGCG (95 mg) and a low amount of CAF (40 mg). In the case of ingestion of a beverage containing EGCG (95 mg) and a high amount of CAF (180 mg), the AUC tended to be somewhat higher (1,165 ng·h/ml), but not significantly so, compared with the beverage with EGCG alone. These findings (modulation of plasma AUC) tended to be somewhat higher (1,165 ng·h/ml) when subjects ingested a beverage containing EGCG (95 mg) and a low amount of CAF (40 mg). In the case of ingestion of a beverage containing EGCG (95 mg) and a high amount of CAF (180 mg), the AUC tended to be somewhat higher (1,165 ng·h/ml), but not significantly so, compared with the beverage with EGCG alone. These findings (modulation of plasma AUC) tended to be somewhat higher (1,165 ng·h/ml), but not significantly so, compared with the beverage with EGCG alone.

Key words: tea catechins; caffeine; human plasma

Green tea is consumed as a popular beverage in Japan and many countries throughout the world. Recent biological studies have revealed that green tea has beneficial effects on health that can be ascribed to characteristic constituents in tea leaves.¹,²

Tea leaves contain many polyphenols, catechins being particularly numerous. Among tea catechins, (−)-epigallocatechin-3-gallate (EGCG) is the major component, and it is believed to be the most bioactive agent. Other important constituents of tea leaves are xanthines, such as caffeine (CAF).³

Since the effects of tea catechins have become known,¹,² products (especially drinks) containing the polyphenol compounds have begun to appear on the market. In Japan, there are several tea drinks which have approved by Japan’s Ministry of Health, Labor, and Welfare for labeling as a Food for Specified Health Use (FOSHU). For instance, Ito En (Tokyo) has marketed a FOSHU tea drink (called Catechin Ryokucha) for people of age 1 to 2 months.

Abbreviations: AUC, area under the plasma concentration-time curve; C, (−)-catechin; CAF, caffeine; CL, chemiluminescence; EC, (−)-epicatechin; ECG, (−)-epicatechin-3-gallate; EG, (−)-epigallocatechin; EGCG, (−)-epigallocatechin-3-gallate; FOSHU, Food for Specified Health Use; HPLC, high performance liquid chromatography
Table 1. Catechins and CAF Concentrations in Fifteen Commercial Tea Drinks (mg/100 ml)

<table>
<thead>
<tr>
<th>Tea drink</th>
<th>EGCG</th>
<th>EGC</th>
<th>EC</th>
<th>ECG</th>
<th>Total catechins</th>
<th>CAF</th>
<th>EGCG/CAF ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>37.0 ± 0.6</td>
<td>18.6 ± 1.1</td>
<td>10.5 ± 0.1</td>
<td>10.5 ± 0.4</td>
<td>5.9 ± 0.1</td>
<td>82.4</td>
<td>20.9 ± 0.5</td>
</tr>
<tr>
<td>B</td>
<td>28.7 ± 3.2</td>
<td>25.3 ± 0.6</td>
<td>12.2 ± 0.5</td>
<td>5.6 ± 0.8</td>
<td>5.5 ± 1.1</td>
<td>77.3</td>
<td>23.1 ± 0.6</td>
</tr>
<tr>
<td>C</td>
<td>15.5 ± 1.7</td>
<td>8.8 ± 0.1</td>
<td>3.7 ± 0.5</td>
<td>2.8 ± 0.4</td>
<td>3.5 ± 0.1</td>
<td>34.4</td>
<td>19.5 ± 0.1</td>
</tr>
<tr>
<td>D</td>
<td>15.2 ± 0.3</td>
<td>7.4 ± 0.2</td>
<td>4.1 ± 0.1</td>
<td>3.8 ± 0.2</td>
<td>2.7 ± 0.0</td>
<td>33.2</td>
<td>19.1 ± 0.1</td>
</tr>
<tr>
<td>E</td>
<td>17.1 ± 0.4</td>
<td>2.5 ± 0.1</td>
<td>1.5 ± 0.1</td>
<td>6.9 ± 0.1</td>
<td>1.6 ± 0.3</td>
<td>29.6</td>
<td>7.9 ± 0.1</td>
</tr>
<tr>
<td>F</td>
<td>15.3 ± 1.4</td>
<td>3.6 ± 0.1</td>
<td>1.5 ± 0.5</td>
<td>5.9 ± 0.3</td>
<td>1.5 ± 0.0</td>
<td>27.8</td>
<td>6.9 ± 0.1</td>
</tr>
<tr>
<td>G</td>
<td>14.0 ± 0.0</td>
<td>4.8 ± 0.1</td>
<td>3.2 ± 0.1</td>
<td>2.9 ± 0.1</td>
<td>3.1 ± 0.0</td>
<td>27.9</td>
<td>11.8 ± 0.1</td>
</tr>
<tr>
<td>H</td>
<td>9.5 ± 1.3</td>
<td>13.2 ± 0.4</td>
<td>7.5 ± 0.6</td>
<td>2.4 ± 0.1</td>
<td>4.8 ± 0.2</td>
<td>37.3</td>
<td>20.6 ± 0.4</td>
</tr>
<tr>
<td>I</td>
<td>8.1 ± 0.2</td>
<td>4.5 ± 0.0</td>
<td>2.4 ± 1.1</td>
<td>1.2 ± 0.2</td>
<td>1.7 ± 0.0</td>
<td>17.9</td>
<td>11.5 ± 0.1</td>
</tr>
<tr>
<td>J</td>
<td>7.7 ± 0.1</td>
<td>4.4 ± 0.1</td>
<td>2.2 ± 0.0</td>
<td>1.2 ± 0.2</td>
<td>1.4 ± 0.0</td>
<td>17.0</td>
<td>11.5 ± 0.0</td>
</tr>
<tr>
<td>K</td>
<td>6.3 ± 0.3</td>
<td>4.5 ± 0.0</td>
<td>2.3 ± 0.3</td>
<td>0.7 ± 0.1</td>
<td>1.3 ± 0.0</td>
<td>15.2</td>
<td>9.2 ± 0.1</td>
</tr>
<tr>
<td>L</td>
<td>6.6 ± 0.1</td>
<td>4.2 ± 0.2</td>
<td>2.2 ± 0.0</td>
<td>0.6 ± 0.1</td>
<td>1.9 ± 0.1</td>
<td>15.5</td>
<td>10.6 ± 0.3</td>
</tr>
<tr>
<td>M</td>
<td>6.3 ± 0.4</td>
<td>4.8 ± 0.1</td>
<td>2.0 ± 0.1</td>
<td>0.8 ± 0.2</td>
<td>2.4 ± 0.1</td>
<td>16.3</td>
<td>13.5 ± 0.1</td>
</tr>
<tr>
<td>N</td>
<td>5.9 ± 0.6</td>
<td>6.0 ± 0.0</td>
<td>3.0 ± 0.1</td>
<td>0.6 ± 0.1</td>
<td>1.8 ± 0.1</td>
<td>17.2</td>
<td>10.7 ± 0.1</td>
</tr>
<tr>
<td>O</td>
<td>5.5 ± 0.2</td>
<td>1.6 ± 0.1</td>
<td>2.5 ± 0.9</td>
<td>0.7 ± 0.1</td>
<td>1.9 ± 0.2</td>
<td>12.1</td>
<td>12.2 ± 0.6</td>
</tr>
</tbody>
</table>

Values represent means with SD (n = 3). Statistical analysis. The data were expressed as means with SD. Statistical analysis was performed by ANOVA, followed by the Tukey test. Differences were considered significant at p < 0.05. The data on the time course of the plasma EGCG concentration were analyzed by two-way repeated-measures ANOVA.

Results

The catechins and CAF concentrations in 15 different tea drinks are summarized in Table 1. The amounts of the compounds were roughly in the following order: EGCG > (−)-epigallocatechin (EGC) > CAF > (−)-epicatechin (EC) > (−)-epicatechin-3-gallate (EGCG) > (+)-catechin (C). Total catechins concentrations were from 12.1 to 82.4 mg/100 ml, and CAF concentrations were from 6.9 to 23.1 mg/100 ml. Therefore, as we expected, the catechins/CAF ratios were different for the various drinks. This may influence the bioavailability of tea catechins (e.g., pharmacokinetics). This hypothesis was investigated in the next series of human trials.

Because the EGCG/CAF ratios in commercial tea drinks were from 0.4 to 2.2 (Table 1), beverage low CAF (95 mg of EGCG and 40 mg of CAF; EGCG/CAF ratio = 2.4, equivalent to 1.6 mg of EGCG and 0.7 mg of CAF/kg subject), beverage high CAF (95 mg of EGCG and 180 mg of CAF; EGCG/CAF ratio = 0.5, equivalent to 1.6 mg of EGCG and 3.0 mg of CAF/kg subject), and beverage Cont (95 mg of EGCG alone; equivalent to 1.6 mg of EGCG/kg subject) were prepared for human study. When plasma taken 1.5 h after ingestion, beverage Cont was analyzed by HPLC-CL, and a clear EGCG peak was observed (Fig. 1). A more intense EGCG peak was documented when the same volunteer ingested beverage low CAF. In the case of ingestion of beverage high CAF, a somewhat large EGCG peak was found as compared with beverage with EGCG alone. The time course of the plasma EGCG concentration is shown in Fig. 2A, and two-way repeated measures ANOVA revealed that CAF influenced the plasma EGCG concentration (p = 0.007) when EGCG and CAF were co-administered to humans. The AUC of plasma EGCG was significantly different between the Cont group (857 ng·h/ml) and the low CAF group (1,370 ng·h/ml), but was not significantly different between the Cont group (857 ng·h/ml) and the high CAF group (1,165 ng·h/ml) (Fig. 2B). This was also con-

was approved by the local research ethics committee, and it followed the Declaration of Helsinki. The subjects gave written informed consent to the protocol. The subjects were asked to abstain from consuming tea, coffee, tea-related products, CAF, vitamins, minerals, and dietary and herbal supplements throughout the study period. Each subject fasted for 12h, following ingestion of one of three different beverages in random order, with a 5-d washout period for each ingestion. In a preliminary experiment, we confirmed that the 5-d washout period was adequate, with no carry-over effect. Blood was collected in heparinized tubes at 0–8h after ingestion, and was subjected to centrifugation at 1,000 × g for 15 min at 4 °C to separate out the plasma.

Determination of EGCG in plasma. For extraction of plasma EGCG, plasma (250 μl) was mixed with 250 μl of 0.4 mol/l phosphate buffer (pH 3.9, containing 2% ascorbic acid and 0.1% EDTA). To this mixture, acetonitrile (500 μl) and ethyl acetate (3 ml) were added, and subjected to centrifugation at 1,000 × g for 15 min at 4 °C, the supernatant was collected. Extraction was repeated 3 times. The supernatants obtained were combined, evaporated, and dissolved in 900 μl of a mixture of methanol and water (8:1 v/v). The solution was filtered through a chromatodisc (0.45 μm pore size, GL science) with 4 ml of methanol as eluent. The filtrate was evaporated, and the residue was redisolved in 100 μl of 10% acetonitrile aqueous solution. An aliquot portion (50 μl) was injected into HPLC with chemiluminescence (CL) detection for determination of plasma EGCG.5,6 An ODS column (Capcell Pak C18 UG120, 4.6 × 250 mm, Shiseido) was used with a mixture of methanol and water (2:8 v/v, containing 0.1% phosphoric acid) at flow rate of 1.0 ml/min, and this was maintained at 40 °C. CL reagent A was prepared by dissolving 8.2 mol acetaldehyde in 50 mmol/l of phosphate buffer (pH 7.4, containing 108 mg of horseradish peroxidase); CL reagent B was 8.8 mol/l of hydrogen peroxide aqueous solution. At post column, CL reagents A and B were mixed with a column eluent, and CL generation from EGCG was measured with a CLD-100 chemiluminescence detector (Tohoku Electronic Industries, Sendai). The plasma EGCG concentration was calculated with a calibration curve of standard EGCG. The area under the plasma EGCG concentration-time (0–8h) curve (AUC) was estimated by the linear trapezoidal rule.

Analysis of EGCG metabolites in the plasma. For determination of plasma EGCG metabolites (glucuronide and sulfate conjugates), plasma (250 μl) was mixed with 50 μl of 0.4 mol/l phosphate buffer (pH 6.8, containing 600 U of β-glucuronidase (G-7896, Sigma, St. Louis, MO) and 2 U of sulfatase (S-9754, Sigma)). The mixture was incubated at 37 °C for 45 min. The sample was then extracted and analyzed by HPLC-CL. Plasma EGCG metabolites (the sum of glucuronide and sulfate conjugates) were calculated from the difference between the concentrations derived with and without treatment of the plasma with β-glucuronidase/sulfatase.

Determination of CAF in the plasma. The plasma CAF was determined by HPLC-UV (272 nm)7.
firmed by the results of plasma EGCG determined using HPLC coupled to a hybrid quadrupole/linear ion trap tandem mass spectrometer (HPLC-MS/MS), instead of HPLC-CL (data not shown). On plasma CAF, a dose-response increase was observed (data not shown). Overall, these findings (Figs. 1 and 2) suggest that when EGCG and CAF are co-administrated to humans, CAF affects the absorption and metabolism of EGCG.

Finally, we evaluated the mechanism by which CAF influences the pharmacokinetics of EGCG. Because part of EGCG orally administered to humans is known to be metabolized into glucuronide and sulfate conjugates, these conjugates in the plasma were determined by HPLC-CL. Based on the results of the plasma conjugate concentrations (Fig. 3), it is likely that when volunteers ingest a beverage containing CAF, CAF suppresses conjugation reactions (glucuronidation and sulfation) of EGCG, thereby increasing the plasma EGCG level. However, high amounts of CAF might interact with intestinal absorption of EGCG, since AUC tended to be lower (but not significantly) in the high CAF group than in the low CAF group (Fig. 2B).

Discussion

About 10 years ago, we developed a combined system of HPLC and CL detection for determination of tea catechins in biological samples. The system consists of separation of catechins with HPLC and detection of catechin-specific CL, which is generated from the reaction of catechins with hydrogen peroxide and acetaldehyde in the presence of horseradish peroxidase as catalyst. For instance, the method enables the determination of tea catechins (e.g., EGCG) in human plasma with high sensitivity and selectivity. Therefore, the method should be a useful tool in studying the metabolic fate of tea catechins as well as their bioavailability.

In this study, we focused on differences in catechin/CAF ratios among commercial tea drinks (Table 1). EGCG and CAF were co-administrated to humans at various EGCG/CAF ratios, and the pharmacokinetic profiles of EGCG were evaluated by HPLC-CL as well as HPLC-MS/MS. In our results, we found a modulating effect of CAF on the plasma EGCG concentration (Figs. 1 and 2).

To explain our present findings (Figs. 1 and 2), we evaluated the possibility that CAF influences EGCG metabolism, because many studies have reported that tea catechins undergo glucuronidation and sulfation in cultured cells, animals, and humans. Considering the results of plasma EGCG conjugates (Fig. 3), the mod-
ululating effect of CAF on the plasma EGCG concentration is probably related in part to the ability of CAF to reduce conjugation reactions of EGCG. However, besides glucuronidation and sulfation, tea catechins are known to undergo methylidyne.\(^9\) In addition, efflux transporters such as Pgp, MRP1, and MRP2 have been suggested to play roles in the absorption and excretion of tea catechins.\(^13\) Therefore, the effects of CAF on EGCG methylation as well as efflux transporters are a subject of ongoing investigations. On the other hand, AUC tended to be lower (but not significantly) in the high CAF group than in the low CAF group (Fig. 2B). This suggests that a relatively high amount of CAF causes suppression of both conjugation and intestinal absorption of EGCG, but further studies (e.g., evaluation of the effects of high doses of CAF on EGCG excretion into the urine and feces) are needed.

Tea catechins have attracted a great deal of attention for their antioxidative, anti-inflammatory, and anti-cancer properties,\(^1,2\) but the oral bioavailability of tea catechins in humans is not so profound,\(^9\) because several processes, including intestinal, microbial, and hepatic metabolisms as well as chemical degradation, are all involved in the metabolic fate of tea catechins. There are several reports on the effects of tea catechin metabolites. For instance, Spencer \textit{et al.} reported that glucuronidation of epicatechin considerably impairs the beneficial properties of native epicatechin (e.g., protective activity against oxidative stress-induced cell death).\(^14\) Hence we believe that minimizing metabolites might enhance the bioavailability of tea catechins. Based on this, some researchers focus on food materials having effects on the absorption and metabolism of tea catechins. Lambert \textit{et al.} reported that black pepper alkaloid (piperine) has inhibitory effects on the glucuronidation of EGCG, thereby enhancing the bioavailability of EGCG in mice.\(^15\) Another study showed that certain types of emulsifying agents improve the intestinal absorption of flavonoids similarly to catechins.\(^16\) However, application of black pepper alkaloid and emulsifying agents is limited by certain of their properties, such as taste and flavor. In contrast, because tea leaves contain CAF in nature, it appears easy to apply CAF to a wide range of tea-related foods and drinks in order to enhance the bioavailability of tea catechins. Considering the contradictory results between AUC found after ingestion of beverage low CAF and beverage high CAF (Fig. 2B), it is desirable to elucidate the optimal ratio and doses of tea catechins and CAF to maximize bioavailability of tea catechins.

Recently, green tea is manufactured as container-packed beverages, which are therefore easier to drink than in conventional tea-making. As the beneficial effects of tea catechins on health becomes better known, drinks with extra tea catechins have been increasingly provided in the marketplace. Under these circumstances, we suggest that the present finding (the modulation of plasma EGCG levels by CAF) should provide ideas how to improve the bioavailability of tea catechins, which can be applied to tea-related drinks and foods. Hence, cell, animal, and human studies are required to prove definitely the usability as well as the safety of catechin/CAF ingestion for a realistic prospect of their use in human therapy. Also, further studies are needed to evaluate the effects of EGCG on the absorption and metabolism of CAF.

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**References**