Note

Analysis of (−)-Epigallocatechin Gallate in Human Serum Obtained after Ingesting Green Tea

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A method for analyzing the EGCg concentration in human serum was developed by using high-performance liquid chromatography with electrochemical detection. EGCg was detected in human serum after the ingestion of 5 g of green tea powder (matsu-cha) dissolved in 200 ml of hot water. The concentration of EGCg in the serum reached the highest level about 2 h after ingesting the green tea, and then decreased.

Key words: HPLC analysis; EGCg; human serum; HPLC-ECD; absorption

Green tea has recently been receiving strong attention as a preventive agent against cancer and coronary heart disease. Several studies have shown various biological and pharmacological activities that had antioxidative,23 antimutagenic,24 and antiallergic effects.35 Moreover, catechins (the main constituents of green tea) have significantly inhibited tumor promotion and carcinogenesis in animal experiments.4 Similarly, epidemiologic studies have also reported the protective effects of green tea consumption against gastric cancer,5 cardiovascular disease and hepatic disorders.51

Tea catechins comprise (−)-epicatechin (EC), (−)-epigallocatechin (EGC), (−)-epicatechin gallate (ECg), and (−)-epigallocatechin gallate (EGCg). We have previously clarified that EGCg was absorbed into the circulation system of rats after an oral administration.6 Therefore, an analysis of the catechin concentration in human serum would be useful for evaluating the preventive role of drinking green tea against cancer and coronary heart disease as a biomarker for checking the amount of green tea consumed. Lee et al. have also detected catechins, which were mostly in their conjugated forms, in the plasma and urine of humans that were collected after drinking decaffeinated green tea.30 However, details of the metabolic fate such as the concentration change of catechin in the serum after ingesting green tea remain unclear. In this study, the change in EGCg level in human serum collected after drinking green tea was studied by high-performance liquid chromatography with electrochemical detection (HPLC-ECD).

EGCg was purified from green tea as described previously,10 green tea powder (matsu-cha) of a commercial product (type RS-50, Itoen, Tokyo, Japan) being used. Human serum was purchased from Wako Pure Chemical Ind. (Osaka, Japan), the other reagents and solvents being from Nacalai Tesque (Kyoto, Japan). A stock solution of EGCg was prepared by dissolving in 1% acetic acid and was stored at −20°C until needed.

Four healthy volunteers each ingested one cup containing 5 g of green tea powder dissolved in 200 ml of hot water. The green tea powder contained 2.1% of EGCg by dry weight. Blood samples were collected in tubes before (0 h) and at 1, 2, 4, and 6 h after the oral administration of green tea. Serum samples were obtained by centrifuging the blood, and were stored at −20°C until needed.

The thawed serum (200 µl) was mixed with 50 µl of an ethyl gallate solution (100 ng/ml), as an internal standard. 50 µl of 2-mercaptoethanol and 300 µl of 50% (w/v) of a trichloroacetic acid (TCA) solution. The mixture was sonicated at 20 kHz and 50% of the duty cycle for 1 min, and after sonication, was centrifuged at 2000 × g for 10 min at 4°C. The supernatant was transferred to another micro-tube and vortexed with 1 ml of chloroform for 1 min. After removing the organic phase, a second extraction was carried out in a similar manner with 1 ml of chloroform. An aliquot (400 µl) of the aqueous phase was transferred to a test tube and diluted with 4 ml of distilled water. This sample was passed through Bond Elut disposable solid-phase extraction column cartridges (type CH; Varian, Harbor City, CA, U.S.A.) that had been pretreated with 2 ml of methanol, 1 ml of distilled water and 1 ml of 5 mM Na2 EDTA. The unretained solution was rinsed from the cartridge with 1 ml of distilled water. After setting the Bond Elut column over a collection tube, the sample was eluted with 1 ml of methanol containing 0.1% (w/v) TCA and concentrated by blowing with nitrogen. The concentrated sample was then dissolved in 200 µl of the mobile phase for the HPLC analysis, using 20 µl of the resulting sample after being filtered through a 0.45-µm membrane. The HPLC conditions were as follows: apparatus, model 8010 (Tosoh Co., Tokyo, Japan); column, 250 µl/min; absorbance wavelength, 280 nm; mobile phase, 50 mM KH2PO4 aqueous solution (pH 3.0)–acetonitrile (1:9, v/v); flow rate, 1 ml/min; injection volume, 20 µl; run time, 20 min; column temperature, 25°C; voltage, 400 mV; data processing, Shimadzu LC-10ATVP UV detector.

The HPLC-ECD analysis parameters were as follows: detector, microcolumn electrochemical detector, model LC-5A (Shimadzu); sensitivity, 0.05 nA; temperature, 25°C; mobile phase, 50 mM KH2PO4–acetonitrile (1:9, v/v); flow rate, 1 ml/min; injection volume, 20 µl; run time, 20 min; column temperature, 25°C; voltage, 400 mV; data processing, Shimadzu LC-5A UV detector.

Fig. 1. Calibration Curve for the Concentration in Commercial Human Serum versus the Peak Height Ratio of EGCg to the Internal Standard. Details of the extraction from serum after adding different amounts of EGCg are described in the text.

Abbreviations: EC, (−)-epicatechin; EGC, (−)-epigallocatechin; ECg, (−)-epicatechin gallate; EGCg, (−)-epigallocatechin gallate; ECD, electrochemical detection; TCA, trichloroacetic acid.
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WAKOPAK 3C18 (Wako Pure Chemical Ind., Osaka, Japan) reversed-phase type (4.6 mm i.d. x 150 mm); eluent, a 20 mm sodium trichloroacetic acid buffer (pH 2.5) containing 0.1 mm Na2EDTA-methanol (84:16); flow rate, 1.0 ml/min; column temperature, 40°C; detection, ECD model 8011 with the applied voltage set at 600 mV. A standard curve was obtained with commercial human serum spiked with known amounts of EGCg. The EGCg concentration in each assay sample was obtained by comparing the ratio of the peak height of EGCg in the sample to that of an authentic sample plotted against the internal standard.

In a preliminary experiment, the extraction of catechin from the serum was difficult because of interaction with the serum protein. This difficulty was resolved by adding 2-mercaptoethanol as a reducing reagent.

The calibration curve for peak height versus concentration in human serum had good linearity with a satisfactory recovery of 78% of EGCg (correlation coefficient of 0.99) over the 10–250 ng/ml range by adding an authentic sample to 200 μl of commercial serum (Fig. 1).

This method was applied to the analysis of human serum obtained after an oral administration of green tea. Figure 2 shows HPLC chromatograms of extracts from human serum before (A) and at 2 h (B) after the administration. EGCg in the serum samples from volunteers who had ingested green tea was detected at a retention time of 8 min. Time-course plots of the EGCg concentration in the serum samples are individually shown in Fig. 3. The highest level of EGCg in the serum samples ranged from 63 to 142 ng/ml from 4 volunteers. Their maximum level of EGCg in each serum sample was similar, except for that from the No. 2 volunteer. The concentration of EGCg in each serum sample was observed to reach the highest level about 2 h after dosing as an average for the 4 volunteers.

EGCg was detected in the serum of volunteers who had ingested green tea by using the HPLC-ECD system. Although it has been
reported that catechins were metabolized mostly to the conjugated form of glucuronide or sulfite in the human body,9] details of the metabolic pathway are not yet clear. To better evaluate the pharmacological effects of catechins, it is necessary not only to observe after a single ingestion, but also after a long-term administration of dietary catechins. Further studies are required to clarify the metabolism of catechins and the distribution to different organs after absorption from the intestines.

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References