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Determination of green tea catechins in human plasma using liquid chromatography–electrospray ionization mass spectrometry

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Abstract

A method for the sensitive and specific determination of eight green tea catechins, consisting of catechin (C), epicatechin (EC), gallocatechin (GC), epigallocatechin (EGC), catechin-3-gallate (CG), epicatechin-3-gallate (ECG), gallocatechin-3-gallate (GCG) and epigallocatechin-3-gallate (EGCG), in human plasma was established. For optimization of conditions for LC–ESIMS, the separation of the eight catechins was achieved chromatographically using Inertsil ODS-2 column combined with a gradient elution system of 0.1 M aqueous acetic acid and 0.1 M acetic acid in acetonitrile. Detection using a mass spectrometer was performed with selected ion monitoring at $m/z = 289$ for E and EC, 305 for GC and EGC, 441 for CG and ECG, and 457 for GCG and EGCG under negative ESI. A preparative procedure, consisting of the addition of perchloric acid and acetonitrile to the plasma for deproteinizing and the subsequent addition of potassium carbonate solution to remove excess acid, was developed. In six different plasma with the eight catechins spiked at two different concentrations, the average recoveries were in the range between 72.7 and 84.1%, which resulted from the matrix effect and preparative loss, with coefficients of variance being 8.2–19.8% among individuals. The levels of the catechins in prepared plasma solutions that were kept at 5 °C within 24 h were stable, which allows us to simply analyze many prepared plasma solutions using an autosampler overnight. When using this method to analyze the eight catechins in human plasma after oral ingestion of a commercial green tea beverage, we detected all the catechins absorbed into human blood for the first time. This also suggested that extremely small amounts of the eight catechins orally ingested may be absorbed based on each absorptive property for the catechins. The method should enable pharmacokinetic studies of green tea catechins in humans.

Keywords: LC–ESIMS; Green tea; Catechins; Deproteinizing; Human plasma

1. Introduction

Green tea catechins, which can be easily infused by hot water, have been extensively reported to possess various biological and pharmacological effects, such as anti-carcinogenic activities [1], anti-oxidant activities [2], lowering of plasma lipid [3] and glucose levels [4], and reducing obesity [5,6]. Among these, the anti-obesity effect in humans following oral ingestion of greater amounts of green tea catechins [6] has been recently reported, while obesity associated with many health risks (so-called lifestyle-related diseases) is a growing problem in many countries worldwide. Naturally occurring green tea catechins in an infusion of green tea leaves with hot water, which are widely consumed in Asian countries, consist of epigallocatechin-3-gallate (EGCG), epicatechin-3-gallate (ECG), epigallocatechin (EGC) and epicatechin (EC). On the other hand, Japanese have recently begun to prefer drinking a commercial canned or bottled green tea beverage, which has a different composition of catechins from the hot water infusion. Thus, owing to the epimerization of the four epi-catechins by heat treatment used for retort pasteurization in the manufacturing, four non-epi-forms, gallocatechin-3-gallate (GCG), catehin-3-gallate (CG), gallocatechin (GC) and catechin (C), are produced in the beverage [7,8]. Therefore, attention has been recently paid to the effects of these non-epi-forms on lipid metabolism [9].

To understand the pharmacokinetics of green tea catechins after oral ingestion of a green tea beverage, all eight green tea catechins must be determined in plasma. Among many methods

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for the analysis of green tea catechins [10,11], LC connected to an ultraviolet detector (UVD), a fluorescence detector (FLD), a chemiluminescence detector (CLD) and an electrochemical detector (ECD) have been used to determine the catechins in plasma [12–22]. However, these methods using LC did not determine all eight catechins in the plasma, due to their problems in sensitivity and specificity. While the use of sensitive and specific LC–MS for the analysis of the catechins has been recently increasing [23–25], LC–MS has not been applied to such analyses of actual plasma after ingestion, although it has been used for the analyses in model plasma with a few spiked catechins [26,27]. In addition, there are no preparative procedures that can be used for high-throughput analyses with simplicity and practicability, although high throughput performance for the analyses of green tea catechins in many plasma samples obtained from different volunteers at several periods after the ingestion is required for understanding the behaviors of the eight catechins absorbed into human blood among individuals and among populations. Thus, complex procedures including liquid–liquid extraction [12,17,21,22,26] and solid phase extraction [13,20] are troublesome and time-consuming. Other simple procedures, such as the addition of either of an acid or an organic solvent [14,15,18,19] and on-line extraction [27], do not permit us to allow the analysis of many plasma samples (intact plasma or prepared plasma solutions) with an autosampler, even at low temperature, due to the instability of the catechins in such non-prepared or prepared plasma samples.

The aim of this study was to establish a method for the sensitive and specific determination of eight green tea catechins in human plasma after oral ingestion of a green tea beverage. We have optimized the LC–ESIMS (liquid chromatography coupled to electrospray ionization mass spectrometry) conditions to simultaneously determine all eight catechins and we have developed a simple preparative procedure for deproteinizing plasma, in which the catechins are stable at 5 °C within 24 h after preparation. This method has been applied to analyze the eight catechins in the plasma after the ingestion of a commercial green tea beverage, in which we succeeded for the first time in detecting and determining all eight catechins in human plasma.

2. Experimental

2.1. Chemicals

Authentic green tea catechins such as C, EC, GC, EGC, CG, ECG, GCG and EGCG were purchased from Kurita Industrial (Tokyo, Japan). Ultra-pure water was prepared using a Milli-Q purification system (Millipore, Bedford, MA, USA). All other chemicals were of analytical grade or of HPLC grade. A commercial green tea beverage, which has been approved as a food for specified health use in the Japanese market by the Ministry of Health, Labour and Welfare in Japan, was orally ingested. This beverage contained 1.66 mg/ml total green tea catechins, consisting of 0.12 mg/ml C, 0.08 mg/ml EC, 0.37 mg/ml GC, 0.22 mg/ml EGC, 0.11 mg/ml CG, 0.09 mg/ml ECG, 0.36 mg/ml GCG and 0.31 mg/ml EGCG, as determined by quantitative HPLC with UV detection at 280 nm.

Each stock standard solution (ca. 1 mg/ml) was prepared by dissolving a known amount of each authentic compound in buffer consisting of 0.1 M sodium phosphate, 25 vol% methanol, 0.5 wt% ascorbic acid and 0.025 wt% disodium ethylenendiaminetetraacetate (pH 3.9) and their small aliquots were stored at −80 °C until used. The stock solutions remained stable under −80 °C for at least 6 months. Working authentic mixtures were always obtained just before use by further dilutions of the stock solution aliquots with the buffer.

2.2. Apparatus and analytical conditions

An Agilent 1100 Series LC-MSD SL system (single quadrupole) equipped with ChemStation software, an 1100 well plate autosampler, a diode array detector (Agilent Technologies, Palo Alto, CA, USA) and an Inertsil ODS-2 column 2.1 mm Ø × 250 mm (GL Science, Tokyo, Japan) was used for the analysis of the eight catechins. In this LC-MSD system, an 1100 binary pump connected to eluents A (0.1 M aqueous acetic acid) and B (0.1 M acetic acid in acetonitrile) was used. The mobile phases were consecutively programmed as follows: an isocratic elution of A 94% (B 6%) for 5 min, a linear gradient of A 94–90% between 5 and 10 min, a linear gradient of A 90–80% between 10 and 20 min, a linear gradient of A 80–77% between 20 and 29 min, an isocratic elution of A 77% for 6 min, a linear gradient of A 77–0% between 35 and 40 min, and finally an isocratic elution of A 94% from 40.1 to 65 min (a total run time of 65 min). The injection volume was 10 μl of each sample solution. The column temperature was maintained at 35 °C and was eluted at flow rate of 0.2 ml/min. Electrospray ionization (ESI) in the mass spectrometer was performed with the following parameters: ionization; negative ion mode, V-cap voltage; 3000 V, fragmentor voltage; 140 V, flow of heated dry nitrogen gas; 10 l/min, heater temperature of gas; 350 °C, and nebulizer gas pressure; 50 psi. Negative ions in scan measurements were acquired from m/z = 100–1000 with a scan time of 0.67 s per cycle. The mass spectrometer was also operated in selected ion monitoring (SIM) with m/z = 289 for E and EC at 15–37 min in the run time, 305 for GC and EGC at 15–31 min, 441 for CG and ECG at 37–50 min, and 457 for GCG and EGCG at 31–50 min. When parameters associated with an impact on performance of ESI were examined, intensities of the monitoring ions for the eight catechins were most strongly affected by the fragmentor voltage of all parameters tested. Based on the test of the fragmentor voltages in the range between 40 and 240 V, 140 V providing with higher intensities for the eight catechins was chosen. Each calibration line was generated by plotting the concentrations of an authentic compound against their peak areas.

2.3. Human plasma

The protocol was approved by the Ethical Committee of the Kao Corporation of Japan, based on the Recommendations from the Declaration of Helsinki. All plasma samples were obtained from six healthy nonsmoking male volunteers (26–38 years, 55–70 kg body weight, #1 to #6), who had not consumed tea or tea-related beverages for 3 days prior to this experiment. After