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Integrative Medicine Research

journal homepage: www.imr-journal.com

Original Article

Effect of hydroxypropyl methyl cellulose phthalate coating on digestive stability and intestinal transport of green tea catechins

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ARTICLE INFO

Article history:
Received 3 November 2013
Received in revised form
22 November 2013
Accepted 26 November 2013
Available online 9 December 2013

Keywords:
digestive stability
green tea catechin
hydroxypropyl methyl cellulose phthalate
intestinal transport

ABSTRACT

Background: The purpose of this study was to investigate the effect of hydroxypropyl methyl cellulose phthalate (HPMCP) coating on the digestive stability and intestinal transport of green tea catechins (GTCs).

Methods: Two types of HPMCP coating were prepared: one type with size smaller than 500 μm (S-HPMCP) and the other with size larger than 500 μm (L-HPMCP). An *in vitro* gastrointestinal model system coupled with Caco-2 cells was used for estimating the bioavailability of GTCs. Ultraperformance liquid chromatography with a photodiode array detector was performed to analyze GTCs.

Results: The digestive stability of GTCs was enhanced up to 33.73% and 35.28% for S-HPMCP and L-HPMCP, respectively. Intestinal transport of the GTCs was increased to 22.98% and 23.23% for S-HPMCP and L-HPMCP, respectively. Overall, the bioavailability of GTCs increased by 4.08 and 11.71 times for S-HPMCP and L-HPMCP, respectively.

Conclusion: The results of this study confirm that coating with HPMCP could be a way to improve the digestive stability and intestinal transport of GTCs.

1. Introduction

Green tea is one of most popular beverages worldwide, and it contains a series of polyphenols known as catechins.[1] Although green tea catechins (GTCs) provide a wide range of beneficial health effects, the oral bioavailability of catechins has been suggested to be low in humans.[1–4] Differences in pH levels and varying oxygen conditions during digestion are major factors that degrade GTCs.[5]

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http://dx.doi.org/10.1016/j.imr.2013.11.001

An enteric coating material [i.e., hydroxypropyl methyl cellulose phthalate (HPMCP)] has been used to protect drugs or flavonoids from degradation by gastric acid or to prevent them from causing side effects in the stomach.[6,7] For example, HPMCP, frequently used as a matrix for oral dosage forms, was expected to enhance the bioavailability of flavonoids, because it has the ability to protect it from various levels of pH in the gastrointestinal tract of humans.[8]

Therefore, we aimed to examine the digestive stability and intestinal transport of catechins by coating them with HPMCP.

2. Methods

2.1. *Chemicals and standards*

Standards of epigallocatechin, epigallocatechin gallate, epicatechin (EC), and epicatechin gallate were purchased from Wako (Osaka, Japan). Digestive enzymes (α-amylase from human saliva, pepsin from porcine gastric mucosa, porcine lipase, pancreatin from porcine pancreas, and bile extract porcine) were obtained from Sigma-Aldrich (St. Louis, MO, USA). High-performance liquid chromatography–grade solvent of acetic acids, water, and methanol were obtained from Sigma-Aldrich and J.T.Baker (Phillipsburg, NJ, USA).

2.2. *Sample preparation*

Two types of HPMCP coating were prepared: one with a size smaller than 500 μm (S-HPMCP) and another type with a size larger than 500 μm (L-HPMCP). The ratio of the catechin to HPMCP coating for L-HPMCP was 7:3 and for S-HPMCP it was 2:8. The origin of catechin for the coating of S-HPMCP was China and that for the coating of L-HPMCP was Korea.

2.3. *Digestive stability of catechins using the in vitro digestion model system*

The method used in this study was designed by Lee et al.[9] The *in vitro* digestion model system, including the human gastrointestinal tract, including salivary, gastric, and small intestinal phases, was simulated as described previously.[5] Approximately 5 mg of GTC and 5 mg of HPMCP-coated GTCs were suspended in 5-mL aliquots of 20 mM phosphate buffer. For the salivary phase, 60 μL of α-amylase (0.2 mg/mL in 20 mM phosphate buffer) was added, and the initial pH was controlled to 6.9 by adding 20 mM phosphate buffer. Samples were shaken at a constant speed of 150 rpm in a shaking water bath at a temperature of 37 °C for 5 minutes. The gastric phase was initiated with 120 μL of porcine pepsin (3 mg/mL in 100 mM sodium bicarbonate solution), and the pH was maintained at 2.0 by adding 0.1 M hydrogen chloride. Solutions were then incubated in a shaking water bath for 30 minutes, following which a 0.1 M solution of sodium bicarbonate was added to neutralize the pH to 5.3. For the small intestinal phase, the pH was regulated to 7.0 by adding 0.1 M sodium hydroxide solution, followed by the addition of 60 μL pancreatic enzyme mixture. The solution mixture was then incubated in a shaking water bath at 37 °C and 150 rpm for 1 hour. All samples were brought to the final volume of 5 mL using 20 mM phosphate buffer and a gentle stream of nitrogen gas was passed in each step. Finally, the supernatant from the digesta was obtained after centrifuging the solution mixture at 4 °C and 3000 rpm for 30 minutes for LCQ-Fleet ultraperformance liquid chromatography using a photodiode array detector (UPLC–PDA; Thermo Fisher Scientific, Waltham, MA, USA).

2.4. *Observation of microscopy*

The residues after continuous digestion were observed with an optical microscope (CKX41, Olympus, Japan). The magnification scale was 40×.

2.5. *Study of intestinal transport of catechins using Caco-2 human intestinal cell culture*

Passage numbers 32–36 of the Caco-2 cell cultures were obtained from the Korean Cell Line Bank (Seoul, South Korea) for this study. A 12-transwell plate (Corning, NY, USA) was used for Caco-2 cell cultures seeded in a growth medium consisting of Dulbecco's modified Eagle's medium (DMEM; Gibco Rockville, MD, USA) with 10% fetal bovine serum (FBS; Gibco, NE, USA), 1% nonessential amino acid (Sigma), 1% penicillin (Gibco), and 0.1% gentamicin (Gibco). The cells were maintained at 37 °C in an incubator with 5% CO_2 and 95% air. A 10% FBS-supplemented DMEM was used to change the medium of the apical and basal compartments each day. Cellular transport of catechins was assessed when the cells grew between 2 and 3 weeks after being confluent. The value of transepithelial electric resistance (TEER) was measured by the Millicell ERS-2 system (Millipore, New Bedford, MA, USA) to ensure rigidity. When the cells had a TEER value higher than 350 Ω, they were used for the transport study. Each treatment mixture with a basal cell culture medium was dispensed to the apical Caco-2 human intestinal cells, which were then incubated at 37 °C for 2 hours. The basal medium was then collected for the liquid chromatography–mass spectrometry analysis.

2.6. *UPLC–PDA analysis*

The method used for the UPLC–PDA analysis was designed by Chung et al.[5] The amount of catechins in the supernatant after *in vitro* digestion and the transported amount of catechin from the apical to basal compartment was quantified using a UPLC–PDA/electrospray ionization/multistage mass spectrometry. Methanol was added into the collected supernatant and basal media, and it was sonicated for 3 minutes followed by vortexing and centrifugation. The supernatant was filtered through a 0.45-μm polyvinyl difluoride syringe filter (Millipore, MA, USA) before the analysis. Chromatographic separation was performed on a Hypersil GOLD C18 (2.1 × 50 mm, 1.9 μm) column with mobile phases of solvents A and B (vol:vol, 0.1% acetic acid in water:methanol). A gradient elution was performed by varying the proportion of solvents A and B with a flow rate of 200 μL/minute and with an initial phase of 5% solvent B. The gradient increased linearly to 20% of solvent B for 15 minutes, increased linearly to 50% for next 5 minutes, and remained at 50% for 5 minutes until injection of the next sample for 10 minutes. The injection volume was 2 μL. The wavelength of the ultraviolet spectrum was set at 280 nm.