Combinational enhancing effects of formulation and encapsulation on digestive stability and intestinal transport of green tea catechins

Yu-Ra Son, Jae-Hwan Chung, Sanghoon Ko*, and Soon-Mi Shim*

Department of Food Science & Technology, Sejong University, Seoul, Republic of Korea

ABSTRACT

The hypothesis was that green tea catechins (GTCs) formulated with vitamin C and xylitol followed by enteric coating with hydroxypropyl methyl cellulose phthalate (HPMCP) or encapsulated into γ-cyclodextrin (γ-CD) could enhance intestinal absorption of GTCs. Surface morphology and size obtained by SEM were different. Digestive stability of GTCs encapsulated into γ-CD or coated with HPMCP was enhanced up to 65.56% or 57.63%, respectively. When GTCs were formulated, the digestive stability was greater than the one not formulated. Formulated GTCs followed by encapsulation into γ-CD significantly increased intestinal transport. Absorption of GTCs was 2.8%, 9.64%, 11.97%, 8.41% and 14.36% for only GTCs, GTCs encapsulated into γ-CD, formulated GTCs encapsulated into γ-CD, GTCs coated with HPMCP and formulated GTCs coated with HPMCP, respectively. This study suggests that GTCs, formulated with vitamin C and xylitol followed by γ-CD encapsulation or HPMCP enteric coating, provide combinational effect to increase bioavailability of GTCs.

Introduction

Green tea catechins (GTCs) are well-known to provide many biological activities, including anti-carcinogenesis, anti-oxidation and anti-platelet aggregation (Han et al., 1997; Duffy et al., 2001; Young et al., 2002). However, the bioavailability of tea catechins has been suggested to be low in animals as well as humans (Yang et al., 1998; Zhu et al., 2000; Baba et al., 2001; Chow et al., 2005). A previous study discovered that GTCs were highly degradable and unstable in alkaline solution, while they were stable in acidic solution (Zhu et al., 1997). In vitro experiments have also shown that catechins are stable in acid but are extensively degraded in degradates of near-neutral or greater pH, such as intestinal juice, plasma, bile, cell culture media or simulated digestive conditions (Neilson et al., 2007). Hence, many studies have been conducted to improve catechin stability by adding an acid such as vitamin C, and several studies have disclosed that the addition of sucrose as well as vitamin C can advance catechin stability, thereby enhancing the bioaccessibility of catechins (Peters et al., 2010; Shim et al., 2012). In addition, xylitol was used instead of sucrose due to its higher hydrophilic properties than sucrose, resulting from replacing ketone or aldehyde groups with alcohol and transforming a ring structure into a linear structure for formulating GTCs (Koo and Lee, 2000; Shim et al., 2012). Xylitol in combination with vitamin C increased digestive stability of catechins, showing higher viscosity than sucrose and glucose (Shim et al., 2012).

As another means of improving bioavailability, GTCs were encapsulated into cyclodextrin (CD). Some previous studies have reported that enhancement in the solubility of catechin through phase solubility experiments was observed with CD (Ishizu et al., 1999; Jullian et al., 2007). Polyphenols such as catechins (molecular size: approximately 100 μm) are suitable in γ-CD when considering its size (molecular size: 150 μm; Reid et al., 2000). Among CD, γ-CD was degraded to glucose quickly and entirely in the upper intestinal tract by intestinal enzymes (Del Valle, 2004). Therefore, γ-CD protected catechins during digestion and delivered the catechins to the small intestine safely. Enteric-coating material such as hydroxypropyl methyl cellulose phthalate (HPMCP) has been used to protect drugs from degradation by gastric acid or to prevent them from causing side effects in the stomach (Sanders et al., 1997). For instance, HPMC, the non-toxic semi-synthetic ether derivative of cellulose, is frequently employed as the matrix of oral dosage forms to enhance the bioavailability of flavonoids because it was found to form a coating layer (Lee and Ha, 2007).

It is plausible that the formulation with acid and xylitol, as well as encapsulation or enteric coating, could provide combinational effects on enhancing catechin bioavailability. Therefore, the objectives of current study were to improve the digestive stability and intestinal transport of catechins by (1) coating with HPMCP, (2) encapsulating into γ-CD, (3) formulating with vitamin C and xylitol followed by an HPMCP coating and (4) formulating with vitamin C and xylitol followed by γ-CD encapsulation.

Materials and methods

Chemicals and standards

Standards of epigallocatechin (EGC), epigallocatechin gallate (EGCG), epicatechin (EC) and epicatechin gallate (ECG) were purchased from Wako (Osaka, Japan). HPMC with a particle size larger than 500-μm coating was kindly provided and encapsulation was prepared by a Biogenics Company (Daejeon, South Korea). An aqueous solution of γ-cyclodextrin (γ-CD, W8) was purchased from Wacker Chemical Corporation (Adrian, MI). The powdered forms of
vitamin C and xylitol were obtained from AmorePacific R&D Center (Gyeonggi-do, South Korea). Digestive enzymes (α-amylase from human saliva, pepsin from porcine gastric mucosa, porcine lipase, pancreatic from porcine pancreas and bile extract porcine) were obtained from Sigma-Aldrich (St. Louis, MO). High-performance liquid chromatography (HPLC) grade solvents of acetic acids and water were purchased from Sigma-Aldrich, and methanol was obtained from J.T. Baker (Phillipsburg, NJ).

Sample preparation

Green tea (GT) extract was prepared by previous study (Shim et al., 2012) and contained 2542.25 μg of total flavanols including major GTCs (EC, ECG, EGCG and EGC). The formulation ratio and components concentration of GT with vitamin C and xylitol for the current study were chosen on the basis of the results from our previous study (Chung et al., 2013). Formulated GTCs consisted of GT, vitamin C (20 ppm) and xylitol (49.5 ppm) with 37%, 0.6% and 62.2% of total volume, respectively. For coating with HPMC, GTCs or formulated GTCs were melted into HPMC solution and then dried at room temperature for producing a coating. The confluence of Caco-2 cell monolayers was checked before each experiment by using a transepithelial electrical resistance (TEER) test with Millicell ERS-2 system (Millipore, New Bedford, MA). When the cells had a TEER value higher than 350 Ω, they were used for transport study. Each treatment mixed with basal cell culture medium was dispensed to apical Caco-2 human intestinal cells and then incubated at 37 °C for 2 h. Transport (%) was calculated by assessing transport from apical to basolateral cell media, and the ratio was expressed as a fraction of 100.

Surface characterisation of GTCs, γ-CD encapsulation and HPMC coating

The surface microscopic structure of catechin, γ-CD encapsulation and HPMC coating was observed with a scanning electron microscope (SEM; model S-4700; Hitachi, Japan). The particles were coated with platinum by sputter. The scales used in magnification were 5 K, 3 K and 500 K for catechin, γ-CD encapsulation and HPMC coating, respectively.

In vitro digestion model system simulating the human gastrointestinal tract

The in vitro digestion model system simulating the human gastrointestinal tract including salivary, gastric and small intestinal phases was adopted from a previous study (Shim et al., 2012; Kim, 2014). GTC treatment (5 mg) was suspended in aliquots (5 mL) of 20-mM phosphate buffer (pH 7). For the salivary phase, 60 μL of α-amylase (0.2 mg/mL in 20 mM phosphate buffer, pH 7) was added and the initial pH was adjusted to pH 6.9 by addition of 20-mM phosphate buffer (pH 7). Samples were allowed to incubate in a shaking water bath at 37 °C with 150 rpm for 5 min. The gastric phase was initiated with 120 μL of porcine pepsin (3 mg/mL in 100 mM of sodium bicarbonate solution), and the pH was kept at pH 2.0 with 0.1-M HCl. Solutions were required to incubate in a shaking water bath (37 °C, 150 rpm) for 30 min. After that, 0.1 M of sodium bicarbonate solution was added to neutralise the pH to 5.3. For the small intestinal phase, samples were kept at pH 7.0 by using 0.1 M of NaOH solution following the addition of 60-μL pancreatic enzyme mixture (bile, lipase and pancreatin extract) and then incubated in a shaking water bath at 37 °C with 150 rpm for 1 h. All samples were brought to 5 mL of final volume using 20 mM of phosphate buffer (pH 7) and handled with a gentle stream of nitrogen gas at each step. Finally, the supernatants from the digesta were obtained by centrifugation at 4 °C and 3000 rpm for 30 min and they were then prepared for UPLC-MS/MSn analysis.

Transport study using Caco-2 human intestinal cell culture

Passage Nos. 32–36 of Caco-2 cell cultures were obtained from Korean Cell Line Bank (KCLB, Seoul, South Korea) for this study. A 12-transwell plate (Corning, NY) was used for Caco-2 cell cultures seeded in a growth medium consisting of Dullbeco’s modified eagle media (DMEM; Gibco, Rockville, MD) with 10% foetal bovine serum (Gibco), 1% non-essential amino acid (Sigma), 1% penicillin (Gibco) and 0.1% gentamycin (Gibco). The cells were maintained at 37 °C in an incubator with 5% CO2 and 95% air (Yang et al., 2014). A 10% foetal bovine serum (FBS) supplemented with DMEM was used to change the medium of the apical and basal cell media every other day. Cellular transport of catechins was assessed when the cells grew between 2 and 3 weeks after being confluent.

UPLC-PDA/ESI-MS/MSn analysis for GTCs

The amount of catechins recovered from digesta and transported from apical to basal was quantified by using an UPLC with a PDA detector and an LCQ-Fleet ESI mass spectrometer (Thermo Fisher Scientific, Whatman, MA). Methanol was added into collected basal media, sonicated for 3 min followed by vortexing and then centrifuged. The supernatant was filtered through a 0.45-μm PVDF syringe filter (Whatman Ltd, Piscataway, NJ) prior to analysis. Chromatographic separation was performed on a Thermo scientific hypersil gold C18 (50 × 2.1 mm, 1.9 μm) column with mobile phases of solvents A and B (V/V, 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/min with an initial phase of 5% Solvent B. The gradient increased linearly to 20% of Solvent B for 15 min, increased linearly to 50% for the next 5 min and held at 50% for 5 min. The gradient sharply decreased to 5% of Solvent B for 25.1 min, and was held at 5% to 30 min until the injection of the next sample. The injection volume was 2 μL. The wavelength of the UV spectrum was set at 280 nm.

For the validation of the metabolite analysis, the mass conditions were as follows: capillary temperature and heater temperature were 275 °C and 50 °C, respectively. Sheath gas and auxiliary gas were 30 and 5 arb, respectively, and spray voltage was 4.2 kV. Analysis of samples was initially carried out using a full scan mode, data-dependent mass spectrometry (MS) scanning from m/z 80 to 1000. The tuning of the MS was optimised by infusing a standard of each catechin dissolved in the initial mobile phase.

Chromatographic peaks and mass spectra in samples were identified using comparative retention times and molecular weights of pure standards. Quantitative analysis was conducted using a standard curve.
**Statistical analysis**

Values were reported as mean ± standard deviation (SD) from at least three different experiments. A one-way analysis of variance (ANOVA) was performed to measure significant differences among the groups at the significant value $p < 0.05$ by using a Graphpad Prism 3.0 software (Graphpad, CA).

**Results**

**Characterisation of surface macroscopic structure**

Surface microscopic structures for GTCs, GTCs encapsulated into $\gamma$-CD and GTCs coated with HPMCP were obtained by SEM images (Figure 1). Each surface morphology and size was apparently different. The catechin without encapsulation or coating appeared to be smooth and of perfect sphere shape (Figure 1A), whereas several hollows were found in the surface of GTCs encapsulated into $\gamma$-CD (Figure 1B). Size of GTCs with $\gamma$-CD encapsulation was two times bigger than that of GTCs only. Many spherical structures having bumps with an average size of 25 $\mu$m were assembled, and the largest area was observed in the HPMCP coating compared with others (Figure 1C). Results from surface macroscopic structures suggest that GTCs are successfully encapsulated in $\gamma$-CD and HPMCP coating and plausible to change digestive properties or intestinal mechanism in human gastrointestinal tract.

**Digestive stability of GTCs by formulation and encapsulation**

The digestive stability of the four major catechins (EC, ECG, EGC and EGCG) was assessed in accordance with formulation and encapsulation by using an in vitro biomimic system (Figure 2). The stability of EC in digestive fluid was 13.42%, 56.92%, 72.36%, 57.63% and 51.49% for GTCs, GTCs encapsulated into $\gamma$-CD, formulated GTCs encapsulated into $\gamma$-CD, GTCs coated with HPMCP and formulated GTCs coated with HPMCP, respectively (Figure 2A). There was no significant difference in the EC stability in GTCs between $\gamma$-CD and HPMCP encapsulation. However, the digestive stability of EC in formulated GTCs was greater in $\gamma$-CD compared with that of HPMCP coating. In HPMCP coating, there was no significant difference between GTCs and formulated GTCs. Figure 2B presents the digestive stability of ECG as 65.56, 66.85, 56.41 and 60.82% for GTCs encapsulation into $\gamma$-CD, formulated GTCs encapsulated into $\gamma$-CD, GTCs coated with HPMCP and formulated GTCs coated with HPMCP, respectively. Digestive stability of ECG in GTCs coated with HPMCP was higher than GTCs only, but it was least effective in enhancing digestive stability compared with other treatments. For the digestive stability of EGC, formulated GTCs encapsulated into $\gamma$-CD were remarkably more effective than the others, indicating 44.12% digestive stability, which was from 1.01 to 4.32 times higher than the others (Figure 2C). As shown in Figure 2D, GTCs encapsulated into $\gamma$-CD showed the highest digestive stability (53.09%) of EGCG among the treatments. Neither $\gamma$-CD encapsulation nor HPMCP coating significantly affected EGCG stability on
GTCs formulated with vitamin C and xylitol. Overall, the digestive stability of GTCs, including EC, ECG, EGC and EGCG, was enhanced by formulating both with vitamin C and xylitol compared with GTCs only. GTCs formulated with vitamin C and xylitol, followed by γ-CD encapsulation or HPMCP coating, remarkably increased the bioaccessibility of most catechins tested.

Intestinal transport of GTCs by formulation and encapsulation

Intestinal transport of GTCs was measured by Caco-2 cells (Figure 3). Intestinal transport (%) of EC was 8.01 for GTCs, 16.61 for GTCs encapsulated into γ-CD, 28.12 for formulated GTCs encapsulated into γ-CD, 10.13 for GTCs coated with HPMCP and 17.15 for formulated GTCs coated with HPMCP (Figure 3A). The intestinal transport of GTCs encapsulated into γ-CD was observed to be greater than that of GTCs coated with HPMCP. In formulated GTCs, γ-CD encapsulation indicated higher intestinal transport than that of HPMCP coating. Both formulation and encapsulation of GTCs were observed in the enhancement of the intestinal transport. Among treatments, formulated GTCs encapsulated into γ-CD showed the highest level of intestinal transport.

Figure 3B presents the intestinal transport of ECG which was 16.16%, 24.43%, 38.82%, 25.04% and 28.01% for GTCs, GTCs encapsulated into γ-CD, formulated GTCs encapsulated into γ-CD, GTCs coated with HPMCP and formulated GTCs coated with HPMCP, respectively. The GTCs encapsulated into γ-CD showed no significant difference from GTCs coated with HPMCP. The intestinal transport of formulated GTCs coated with HPMCP was higher than GTCs coated with HPMCP. Among the treatments, the intestinal transport of formulated GTCs encapsulated into γ-CD was 2.4 times higher than that of GTCs only.

When it comes to EGC, intestinal transport was 16.98%, 26.39%, 27.15%, 25.21% and 28.51% for GTCs, GTCs encapsulated into γ-CD, formulated GTCs encapsulated into γ-CD, GTCs coated with HPMCP and formulated GTCs coated with HPMCP, respectively (Figure 3C).
The intestinal transport of EGC in both formulation and encapsulation was higher than that of GTCs, but there was no significant difference among treatments. In terms of EGCG, the intestinal transport of GTCs was not significantly different in encapsulations. Formulated GTCs followed by γ-CD encapsulation was 1.18 times higher in intestinal transport than that of HPMCP coating (Figure 3D). However, encapsulated GTCs into γ-CD and GTCs coated with HPMCP showed no significant difference. Among the treatments, formulated GTCs encapsulated into γ-CD showed the highest level of intestinal transport, 2.2 times greater than GTCs. Overall, formulated GTCs which were encapsulated into γ-CD had higher intestinal transport of the catechin than formulated GTCs which were coated with HPMCP. Our results suggest that these encapsulations of catechins formulated with vitamin C and a sugar substitute mixture such as xylitol could provide a combinational effect on increasing catechin bioavailability.

**Absorption of GTCs after formulation and encapsulation**

Absorption value indicates the relative amount of catechins accumulated in the Caco-2 cell after undergoing in vitro digestion.

**Figure 3.** Effect of formulation and encapsulation on intestinal transport (%) of major catechins by Caco-2 cell culture (n = 3). Different letters indicate that each catechin’s intestinal transport (%) is significantly different among treatments (p < 0.05). *GTCs: Green tea catechins, γ-CD_GTCs: Green tea catechins encapsulated into γ-CD, γ-CD_FGTCs: Formulated green tea catechins into γ-CD, HPMCP_GTCs: Green tea catechins coated with HPMCP, HPMCP_FGTCs: Formulated green tea catechins coated with HPMCP.

**Figure 4.** Effect of formulation and encapsulation on absorption of total catechins (n = 3). Different letters indicate a significant difference among treatments (p < 0.05). Absorption indicates that the relative amount of catechins accumulated in the Caco-2 cell after in vitro digestion. *GTCs: Green tea catechins, γ-CD_GTCs: Green tea catechins encapsulated into γ-CD, γ-CD_FGTCs: Formulated green tea catechins into γ-CD, HPMCP_GTCs: Green tea catechins coated with HPMCP, HPMCP_FGTCs: Formulated green tea catechins coated with HPMCP.
The absorption of total catechin (%) was 2.8% for GTCs, 9.64% for GTCs encapsulated into γ-CD, 11.97% for formulated GTCs encapsulated into γ-CD, 8.41% for GTCs coated with HPMCP and 14.36% for formulated GTCs coated with HPMCP (Figure 4). Even though the bioavailability of catechins tends to be very low due to low digestive stability and poor intestinal absorption due to its degradation under simulated human digestive conditions, GTC formulation and encapsulation were revealed to increase the absorption of total catechins up to 3–5.13 times.

Discussion

The small intestinal condition of having slightly alkali pH and the presence of reactive oxygen species made GTCs unstable (Neilson et al., 2007). Catechins have been found to be limitedly absorbed in the human body because they are effluxed by apical intestinal membranes. Several studies have suggested that catechins serve as substrates for MRP2 and P-glycoprotein, which are major efflux transporters in intestinal membranes (Peters et al., 2010). Formulation of GT with ingredients containing sugars and ascorbic acid was observed to increase catechin stability under simulated digestive conditions and to alter accumulation of catechins by human intestinal cells (Caco-2) in culture (Green et al., 2007; Peters et al., 2010; Shim et al., 2012). Our previous study also found that GT formulated with vitamin C increased the intestinal uptake of total catechins up to 6 and 11 times compared with GT alone (Shim et al., 2012). A previous study also reported that vitamin C and xylitol formulation for EC, ECG, EGC and EGCG showed 8, 2, 2.4 and 1.9 times higher intestinal transport than EC, ECG, EGC and EGCG only formulations, respectively (Chung et al., 2013).

Cyclodextrin (CD), cyclic oligosaccharides composed of glucopyranose units, provides internal and external surfaces of apolar and polar nature, respectively (Julian et al., 2007). The inclusion properties of catechin into β-CD were carried out in order to confine unfavourable properties of catechins including bitterness and ease of oxidation (Julian et al., 2007). The transport-enhancing properties of the γ-CD, an eight-membered sugar ring molecule, were found to tag along the lipophilicity of the core in their cyclic structure, causing an increase in the permeability of the cytoplasmic membrane (Hovgaard and Brøndsted, 1995). A previous study revealed that various sizes of CDs act as a transporter enhancer in Caco-2 cell with slight disruption of tight junction complexes (Hovgaard and Brøndsted, 1995). Although that study could not elucidate a possible mechanism, CDs could serve as an absorption enhancer for poorly absorbed components. HPMCP, an enteric coating material, has been used for the controlled release of drugs to avoid gastrointestinal side effects (Torres et al., 1995). It is well known that HPMCP will not dissolve in the acidic juices of the stomach (pH about 3), but it will dissolve in the alkaline (pH 7–9) condition of the small intestine. According to a previous study, various amounts (2.5–17% W/W) of HPMC was used to improve drug absorption (Baluom et al., 2000). The study found that intestinal absorption of the enteric-coated ingredient by HPMC increased due to controlling both the erosion rate and the length of intestinal segment. In our preliminary study, HPMCP ranging from 28 to 80% (V/V) affected digestive stability of GTCs, and the lower amount of HPMCP strongly released GTCs by the control erosion rate (data not shown). Therefore, the basic finding of the current study was that formulation and encapsulation can protect GTCs from oxygen exposure or different pH environments in digestive fluids as well as enhanced transport of GTCs by intestinal membranes. The tablet form of product was well solubilised in the water as well as 50% of ethanol.

The current study found good agreement between the experimental and theoretical approaches of the bioavailability of each catechin tested. We confirmed that the combinational effects of formulation and encapsulation on improving GTC stability were found with each catechin tested (Figure 2). As per the hypothesis of
the current study, γ-CD encapsulation and HPMC enteric coating may enhance the digestive solubility of catechins and may protect them from a slightly alkaline pH and oxidation condition in the small intestine. When γ-CD is compared with HPMC, the formulated GTCs encapsulated into the former showed 1.41 times higher digestive stability of GTCs than that of the latter, indicating that γ-CD encapsulation effectively protects gallated catechins (ECG and EGCG) in GTCs (Figure 2). Similar to our results, a previous study indicated that the presence of sucrose may serve to interfere with these associations and enhance catechin solubility in the gastrointestinal tract, thereby enhancing the stability of individual catechins (Peters et al., 2010). Chemical interactions between the aqueous solution and carbohydrates may influence the stabilisation, imparting various viscosities (Shim et al., 2012). Hence, it is plausible that γ-CD is broken down by posing a glucose unit in the upper small intestine, resulting in increased viscosity and decreased water activity (Figure 5). Our results from the current study suggest that these encapsulations of GTCs, formulated with vitamin C and xylitol, could provide a combinational effect on increasing intestinal transport of catechins. In good agreement with our study, the positive effect of GT formulated with vitamin C and xylitol on intestinal cell transport of gallated and non-gallated catechin was previously characterised (Shim et al., 2012; Chung et al., 2013). The study concluded that vitamin C was revealed to be the most effective on the intestinal transport of EC, implying the inhibition of the efflux transport mechanism of EC. A previous study also confirmed that intestinal retention for ECG and EGCG increased with sucrose and ascorbic acid formulation (Green et al., 2007; Peters et al., 2010). In addition, the γ-CD might be disassembled and broken down to release glucose during the digestive process. It is plausible that glucose could be used as an energy source for the active transport of catechins (Figure 5). It could be worthwhile in a further study to find mechanism of the effect of vitamin C on encapsulation via directly or indirectly, which was not observed in the current study. In addition, catechin metabolites mainly including methylated forms were identified after transport across the intestinal membrane for formulated and encapsulated GTCs (Figure 6). Methylated flavonoids do not have free hydroxyl groups, and so these metabolites can be bioavailable because they do not seem to undergo glucuronidation and sulphation for protection from rapid intestinal and hepatic metabolism.

Conclusion

The current study confirmed the combinational enhancing effect of formulation with vitamin C and xylitol followed by γ-CD encapsulation or HPMC enteric coating on digestive stability and intestinal transport of GTCs. Therefore, the bioavailability of poorly absorbed GTCs may be advanced by formulating with vitamin C and xylitol followed by encapsulating into γ-CD or coating with HPMC.

Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the article.

Funding information

This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MEST) (No.2014R1A2A2A01007 627).

References


