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Transport of gallocatechin gallate and catechin gallate in high-temperature-processed green tea extract from gastrointestinal tract to brain by an *in vitro* bio-mimic model system coupled with sequential cell cultures

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ABSTRACT

The aim of this study was to evaluate the transport of gallocatechin gallate (GCG), catechin gallate (CG) and their metabolites in high-temperature-processed green tea extract (HTP_GTE) from the gastrointestinal tract to blood-brain barrier (BBB) with an *in vitro* bio-mimic model system with sequential cell cultures including Caco-2, HepG2 and HBMECs. Transports from the GI to BBB, the final concentrations of GCC and CG were 18.95 ± 3.24 μM and 5.11 ± 0.83 μM, respectively. Metabolites detected in BBB after systemic circulation were identified as glucuronide or sulfate conjugated form. Results suggest that GCG, CG and their metabolites in HTP_GTE are capable of reaching the brain by oral intake of HTP_GTE, implying that HTP_GTE could be utilized as a natural functional material for the prevention of degenerative brain diseases.

1. Introduction

Neurodegenerative diseases including amyotrophic lateral sclerosis, Alzheimer's and Parkinson's involve the progressive loss of specific neuronal cell population (Barnham, Masters, & Bush, 2004). These diseases occur in consequence of oxidative damage, which might be responsible for the malfunction or death of nerve cells (Floyd & Hensley, 2002). In order to alleviate and delay the progression of these symptoms, acetylcholinesterase inhibitors (AChEI) and L-dihydroxyphenylalanine (L-Dopa) which are drug increasing the amounts of neurotransmitters have generally been used in a clinic (Kihara & Shimohama, 2004; Pardridge, 2003). To attain this function, these compounds should firstly cross the blood-brain barrier (BBB), which finally regulates the composition of extracellular fluid in the central nervous system (CNS) by tightly controlling molecular traffic and buffering against changes in the systemic circulation (Faria et al., 2014; Palmer, 2010; Rossi, Mazzitelli, Arciello, Capo, & Rotilio, 2008). The BBB is composed of the endothelium of brain microvessels, can transport substances of small molecular size, lipophilicity and non-ionization by passive diffusion and regulate drug delivery into CNS by expression of transport carriers (Faria et al., 2011; Löscher & Potschka, 2005).

Polyphenols widely present in plants are well known to provide protective potential for brain function (Vauzour, 2012). Their mechanisms on neuroprotection have been suggested that they act as a various way; an inducer of antioxidant sufficient defenses, a modulator of signaling cascades, apoptotic processes, or synthesis/degradation of the amyloid-β peptide (Stevenson & Hurst, 2007). For instance, flavonoids which are the subcategory of polyphenols exhibited the potential of anti-oxidant capacity for preventing neurodegenerative diseases (Kumar & Pandey, 2013). Studies with resveratrol showed that this compound eliminated O_2^-, $OH^·$ and lipid hydroperoxyl free radicals *in vitro* and this antioxidant activity was related to the beneficial effect for dementia (Pandey & Rizvi, 2009). Anthocyanins abundantly present in berry fruits and grapes are considered as neuroprotective compounds through inhibition of reactive oxygen species (ROS) formulation and apoptosis of mitochondria by H_2O_2 (Kwon et al., 2016; Tarozzi et al., 2007). In particular, green tea catechins are largely composed of (−)-epigallocatechin gallate (EGCG), (−)-epicatechin gallate (ECG), (−)-epigallocatechin (EGC) and (−)-epicatechin (EC). These can be converted to their epimers that are (−)-gallocatechin gallate (GCG),

Abbreviations: BBB, blood-brain barrier; CG, catechin gallate; GCG, gallocatechin gallate; GI, gastrointestinal; HBMECs, human brain microvascular endothelial cells; HTP_GTE, high-temperature-processed green tea extract

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(−)-catechin gallate (CG), (−)-gallocatechin (GC) and (±)-catechin (C) (Ananingsih, Sharma, & Zhou, 2013). They have various and beneficial effects in human health owing to their antioxidant activities, including free radical scavenging, metal chelating, and inhibiting lipid peroxidation (Cho et al., 2013; Mandel, Weinreb, Reznichenko, Kalfon, & Amit, 2006). EGCG and ECG which have a gallate ester in green tea catechins have been studied about their neuroprotective effect on the brain and some studies showed that these compounds are transported into BBB, suggesting that sufficient concentrations of EGCG and ECG which are transported BBB could prevent neurodegenerative diseases (Bastianetto, Yao, Papadopoulos, & Quirion, 2006; Pervin et al., 2017). For example, Pervin et al., (2017) found that the learning ability of mice and human neuroblastoma cell proliferation significantly increased by a low concentration of EGCG, perhaps affecting the precaution of cognitive dysfunction by increasing brain plasticity. Meanwhile, experiments on the neuroprotective effect of the epimers of these catechins, GCG and CG, was rarely done. Our group previously investigated that inhibitory effects high-temperature-processed green tea extract (HTP_GTE) containing higher amount of GCG and CG compared to green tea extracts on $A\beta^{1-42}$ fibril formation and $A\beta^{1-42}$ fibril destabilization (Lee, Jeong, Kim, Kim, & Shim, 2018). The study suggested that HTP_GTE could significantly inhibit and destabilize amyloid oligomer formation associated with Alzheimer's disease due to catechins in HTP_GTE. It is plausible that GCG and CG could transport BBB, resulting in preventing brain aging. It is very important to estimate concentration of GCG and CG that can transport BBB after oral exposure and then provide biological activity on brain. Thus, the objective of this study was to evaluate the transport of GCG and CG in green tea extracts from the gastrointestinal tract to BBB using an *in vitro* bio-mimic model system coupled with human intestinal epithelial cells (Caco-2), human liver hepatocellular carcinoma (HepG2) and human human brain microvascular endothelial cells (HBMECs) and to identify their metabolites in BBB.

2. Material and methods

2.1. Chemical and standard reagents

Analytical standards of (−)-gallocatechin gallate (GCG), (−)-catechin gallate (CG) and (−)-epigallocatechin gallate (EGCG) were purchased from Wako (Osaka, Japan). A α-amylase from human saliva, pepsin, from porcine gastric mucosa, bile extract from porcine, lipase from porcine pancreas and pancreatin from porcine pancreas were purchased from Sigma Aldrich (St. Louis, MO, U.S.A.). Water, methanol, acetonitrile and acetic acid for using in ultra-performance liquid chromatography (UPLC) were purchased from J.T. Baker (Phillipsburg, NJ, U.S.A.) and Sigma Aldrich (St. Louis, MO, U.S.A.), respectively.

2.2. Preparation of high-temperature-processed green tea extract (HTP_GTE)

The *Camellia sinensis* (CS) leaf used in this study was obtained from the Jeju Island in South Korea. The dried CS leaf (1000 g) was extracted two times with 50% aqueous ethanol (4 L × 3 times) by using a water bath at 60 °C for 3 h to give 50% aqueous ethanol extract (255 g). The 50% aqueous ethanol extract was dissolved with 1L of water and then treated with sterilized at 121 °C for 90 min. The sterilized extract was concentrated with a rotary evaporator (Buchi R200, Flawil, Switzerland) in vacuo to make it 250 g of extract. The components of extracts are shown in Table 1.

2.3. BBB transport study of GCG and CG from HTP_GTE by HBMECs

For the permeability target components by BBB, HBMECs (1.0×10^5/well) were seeded on polytetrafluoroethylene (PETE)/collagen-coated transwell inserts (12 mm diameter, 0.4 μm pore size, Corning Costar®, NY, U.S.A.) and put in 12 well plates. Before the experiment, the trans-epithelial electric resistance (TEER) value was measured to check the integrity of HBMEC and Caco-2 cells with the Millicell ERS-2 system (Millipore Corp., New Bedford, MA, U.S.A.). HBMECs that obtained a TEER > 100 Ω cm^{-2} were used. Samples were dissolved in Dulbecco's phosphate buffered saline (DPBS) and were dispensed to the apical side of cell. Samples were collected from the basolateral side for ultra-performance liquid chromatography-electron spray ionization/mass spectrophotometry (UPLC-ESI/MS) analysis. This experiment was performed in triplicate, and permeability was calculated according to Chung et al. (2013).

Table 1
Components of high-temperature-processed green tea extract (HTP_GTE).

Contents	Amount (%)
Gallocatechin gallate (GCG)	7.59
Catechin gallate (CG)	0.87
Epigallocatechin gallate (EGCG)	10.39
Epigallocatechin (EGC)	4.56
Epicatechin gallate (ECG)	2.79
Epicatechin (EC)	2.27
Caffeine	4.58

2.4. Transport from gastrointestinal tract to brain of GCG and CG from HTP_GTE by in vitro bio-mimic model system coupled with Caco-2 cell, HepG2, and HBMECs

Standards of catechins (GCG and CG) and HTP_GTE were artificially digested in three continuous phases by *in vitro* bio-mimic system from Kim et al. (2016) with modifications. All digestive enzymes were stored in ice with 4 °C. Each sample, dissolved in 20 mM phosphate buffer (PB), was mixed with α-amylase (2 unit) and was incubated for 3 min in a shaking water bath with 200 rpm at 37 °C for salivary phase. In the gastric phase, pepsin (20 mg/mL 0.1 M HCl) was added and the sample was acidified to pH 2.0 ± 0.1 with 1 M HCl. And then the sample was incubated for 1 h with the same conditions above. In the small intestinal phase, pH was adjusted to 5.3 ± 0.1 with 1 M $NaHCO_3$ and added pancreatin (1 mg/mL PB), lipase (0.5 mg/mL PB), and bile acid (6 mg/mL PB). The final pH was neutralized to 7.0 ± 0.1 with 1 M NaOH and incubated for 2 hrs with the same conditions above. The final volume of all samples was equalized with 20 mM PB and centrifuged at 4000 rpm for 30 min at 4 °C. The supernatants were collected for cell treatments and further analysis by UPLC-ESI/MS.

Caco-2 cells and HepG2 cells were obtained from the Korean Cell Line Bank (KCLB, Seoul, Republic of Korea) and American Type Culture Collection (ATCC, Manassas, VA), respectively. Both cells were preserved in DMEM supplemented with 10% FBS and 1% Pen/Strep in a humidified atmosphere containing 5% CO_2, 95% air at 37 °C. Cell media was changed every 2–3 days and sub-cultured at 80% of cell confluence. For the *in vitro* bio-mimic system, Caco-2 cells (seeding density of 1.0×10^5) were seeded on PETE / collagen-coated transwell inserts for 2 weeks. HepG2 cell (seeding density of $1.5–3.0 \times 10^5$) was seeded in basolateral side of 12 well plate for 2–3 days before incubation in co-culture. When TEER value of Caco-2 cells was reached > 200 Ω cm^{-2}, the cell was used. CG, GCG and HTP_GTE digesta were treated to the apical side of Caco-2-HepG2 co-culture system. After 1 hr incubation, basal media collected from the basolateral side was dispensed into the apical side of HBMECs, which is fully grown and TEER value is over 100 Ω cm^{-2}. Basal media collected after 1 hr incubation were used for further analysis by UPLC-ESI/MS. The overall process of this experiment is shown in Fig. 2. All experiments were performed in triplicate.