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Analytical Methods

Simulated gastrointestinal digestion, intestinal permeation and plasma protein interaction of white, green, and black tea polyphenols

Gian Carlo Tenore [a,*], Pietro Campiglia [b], Daniela Giannetti [a], Ettore Novellino [a]

[a] Department of Pharmacy, University of Naples Federico II, Via D. Montesano 49, 80131 Napoli, Italy
[b] Department of Pharmaceutical and Biomedical Sciences, University of Salerno, Via Ponte Don Melillo, 1, 84084 Salerno, Italy

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ABSTRACT

The gastrointestinal digestion, intestinal permeation, and plasma protein interaction of polyphenols from a single tea cultivar at different stages of processing (white, green, and black teas) were simulated. The salivary phase contained 74.8–99.5% of native polyphenols, suggesting potential bioavailability of significant amounts of antioxidants through the oral mucosal epithelium that might be gastric sensitive and/or poorly absorbed in the intestine. White tea had the highest content and provided the best intestinal bioaccessibility and bioavailability for catechins. Since most of native catechins were not absorbed, they were expected to accumulate in the intestinal lumen where a potential inhibition capacity of cellular glucose and cholesterol uptake was assumed. The permeated catechins (approximately, 2–15% of intestinal levels) significantly bound (about 37%) to plasma HDLs, suggesting a major role in cholesterol metabolism. White tea and its potential nutraceuticals could be effective in the regulation of plasma glucose and cholesterol levels.

1. Introduction

In spite of numerous data about the effects of green and black teas on human health, very little is known about white tea, which is the rarest and the least processed. White tea is, generally, composed only of the first white hairy leaves while green and black teas are the products of postharvest treatments that, along with genotype and growing conditions, influence the chemical content of the tea and its flavour, taste, and health characteristics (van der Hooft et al., 2012).

Flavan-3-ols, also known as catechins, constitute up to 30% of tea leaves dry weight. (−)-Epigallocatechin-3-gallate (EGCG) has been identified as the major polyphenol in both white and green teas, but (−)-epigallocatechin (EGC) and (−)-epicatechin-3-gallate (ECG), along with gallic acid, caffeine, and theobromine, are present at higher concentrations in white tea (Santana-Rios et al., 2001). EGCG is thought to be responsible primarily for many of the health benefits associated with tea consumption, including reduced oxidation (Mildner-Szkudlarz, Zawirska-Wojtasiak, Obuchowski, & Golinski, 2009) and inflammatory (Cao et al., 2007) processes, glucose/insulin regulation (Nishiumi et al., 2010) and lipid metabolism (Ikeda, Yamahira, Kato, & Ishikawa, 2010). The higher concentrations of several white tea constituents compared with black and green teas may be responsible for the apparent increase in biological activity (Santana-Rios et al., 2001). White tea has been reported to alleviate inflammation and rheumatoid arthritis more effectively than some green teas (Thring, Hili, & Naughton, 2009). Sõhle et al. (2009) showed white tea lipolytic activity in human subcutaneous (pre)-adipocytes and inhibition of adipogenesis.

To achieve a specific effect, polyphenols must be bioavailable, i.e. extracted from the food matrix and absorbed from the gut. In this sense, the term bioaccessibility has been defined as the fraction of a compound that is released from the food matrix in the gastrointestinal tract and, thus, becomes available for intestinal absorption (Tagliazucchi, Verzelloni, Bertolini, & Conte, 2010). Bioavailability is used to describe the proportion of the ingested compound that reaches the systemic circulation (Manach, Scalbert, Morand, Rémésy, & Jiménez, 2004). Digestion is a physiological process that allows the release of both nutrients and non-nutrients (e.g., polyphenols) from the food matrix (Hinsberger & Sandhu, 2004; Pedersen, Bardow, Jensen, & Nauntofte, 2002). In humans, the digestive process starts in the mouth where the initial degradation of polysaccharides and triglycerides takes place due to mastication and the action of salivary enzymes (α-amylase and lipase) (Hinsberger & Sandhu, 2004; Pedersen et al., 2002). Then, the food bolus is subjected to gastrointestinal (GI) digestion, where both

* Corresponding author. Tel./fax: +39 081 678610.
E-mail address: giancarlo.tenore@unina.it (G.C. Tenore).

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digestive enzymes of the stomach and the small intestine, and colonic fermentative bacteria in the large intestine, are decisive for making nutrients and bioactive compounds available for the absorption through the intestinal wall (Hinsberger & Sandhu, 2004; Pedersen et al., 2002). These physiological conditions may result in structural changes that affect the stability, bioavailability and bioactivity of food constituents (Cilla, González-Sarrías, Tomás-Barberán, Espín, & Barberá, 2009). It has been demonstrated that digestion decreases the phenolic content by at least 47% in fruit beverages compared with pre-digestion (Cilla et al., 2009). Tagliazucchi et al. (2010), found only 62% of polyphenols originally present in grapes were bioaccessible after GI digestion, and radical-scavenging activities of polyphenols may be pH-dependent, suggesting a greater scavenging capacity in the intestine than in the stomach.

Polyphenols have a high affinity for proteins and bind to them by hydrophobic interactions, and hydrogen and covalent bonds (Brunet, Bladé, Salvadó, & Arola, 2002). However, the formation of protein–phenol complexes depends considerably on individual structures. EGCG binds to several human plasma proteins when serum is incubated *in vitro* with tea catechins (Brunet et al., 2002). Evidently, the processes of absorption and transport affect distribution of polyphenols to tissues, metabolism, and excretion and, despite considerable interest in polyphenols, little is known about these *in vivo*.

Thus, the aim of this work was to evaluate the bioaccessibility, bioavailability and plasma protein interaction of white tea polyphenols *in vitro* using a GI digestion model. GI digestion was categorised into salivary, gastric and duodenal digestion; Caco2 cell lines were used to explore intestinal transit; the interaction of white tea polyphenolic compounds with plasma albumins and lipoproteins after absorption was evaluated *in vitro*.

2. Materials and methods

2.1. Reagents and standards

All chemicals and reagents used were either analytical-reagent or HPLC grade. The water was treated in a Milli-Q water purification system (Millipore, Bedford, MA) before use. Standards used for the identification and quantification of phenolics were: C, (+)-catechin; EC, (−)-epicatechin; ECG, (−)-epicatechingallate; EGC, (−)-epigallocatechin; EGCG, (−)-epigallocatechingallate; GC, (−)-gallocatechin; CG, (−)-catechingallate; TTF, total theaflavins from black tea (Sigma–Aldrich Co., St. Louis, USA). Chemicals and reagents used to simulate the GI digestion, and interaction with plasma proteins, were: potassium chloride (KCl), potassium thiocyanate (KSCN), monosodium phosphate (NaH_2PO_4), sodium sulphate (Na_2SO_4), sodium chloride (NaCl), sodium bicarbonate ($NaHCO_3$), urea, α-amylase, hydrochloric acid (HCl), pepsin, pancreatin, bile salts, human serum albumin (HSA, ⩾97.0%), high density lipoprotein (HDL, ⩾95.0%), low density lipoprotein (LDL, ⩾95.0%) and very low density lipoprotein (VLDL, ⩾95.0%) (Sigma–Aldrich Co.). Acetonitrile (HPLC grade), methyl alcohol (HPLC grade) and formic acid were purchased from Carlo Erba Reagents (Milan, Italy).

2.2. Tea samples and preparation

White, green and black tea samples were obtained from the same tea cultivar Chun Mee 41022 (Vicony Teas Company, Huangshan, China). Green leaves had been harvested from the same field in spring, summer and autumn 2011. Fresh leaves were then processed as follows: withering in natural sunlight (12% moisture content, 3 days) and drying (15 min, 90 °C), for white tea; steaming (1 min), withering (75% moisture content, 24 h), and drying (20 min, 90 °C), for green tea; withering (72% moisture content, 12–18 h), crush-tear-curl in Lawrie tea processor (30 s), oxidation (55 min, from 27–30 °C to 22–23 °C) and drying (20 min, 90 °C), for black tea. The teas were ground to obtain a homogeneous fine powder. The infusions were prepared by pouring 20 mL of water at 90 °C on 0.5 g of tea and brewed for 7 min. They were then filtered through Whatman paper filters 43–48 μm and diluted appropriately with water according to each specific assay.

2.3. In vitro gastrointestinal digestion

The assay was performed according to the procedure described by Raiola, Meca, Mañes, and Ritieni (2012) with slight modifications, as follows. GI digestion was split into three categories: salivary, gastric and duodenal digestion. For the salivary digestion, the tea infusions (20 mL) were mixed with 6 mL of artificial saliva composed of: KCl (89.6 g/L), KSCN (20 g/L), NaH_2PO_4 (88.8 g/L), Na_2SO_4 (57.0 g/L), NaCl (175.3 g/L), $NaHCO_3$ (84.7 g/L), urea (25.0 g/L) and 290 mg of α-amylase. The pH of the solution was adjusted to 6.8 with HCl 0.1 mol/L. The mixture was put in a plastic bag containing 40 mL water, and homogenised in a Stomacher 80 Microbiomaster (Seward, Worthing, UK) for 3 min at 37 °C. Pepsin (0.5 g, 14,800 U) dissolved in HCl 0.1 mol/L was added, pH adjusted to 2 with HCl (6 mol/L), and the mixture incubated at 37 °C in a Polymax 1040 orbital shaker (250 rpm) (Heidolph, Schwabach, Germany) for 2 h. After the gastric digestion, the pancreatic digestion was simulated as follows: pH was adjusted to 6.5 with $NaHCO_3$ (0.5 mol/L) and 5 mL of a mixture containing pancreatin (8 mg/mL) and bile salts (50 mg/mL) (1:1; v/v), dissolved in water (20 mL), was added before the mixture was incubated at 37 °C in an orbital shaker (250 rpm) for 2 h. After each step of digestion, 10 mL was removed, centrifuged at 4000 rpm and 4 °C for 1 h, and the supernatant was freeze-dried. To determine the polyphenolic profile, the residues were extracted with an acetonitrile-water (84:16; v/v) mixture, centrifuged at 4000 rpm and 4 °C for 1 h, and then the supernatants were analysed by HPLC.

2.4. Cell culture and in vitro study of polyphenolic transepithelial transport

The human colon carcinoma cell line Caco-2 (HTB-37) was obtained from the American Type Culture Collection (LGC Promochem, Molsheim, France). Cells were cultured routinely in HEPES buffered Dulbecco's modified Eagle's medium (DMEM) with 4.5 g/L glucose and supplemented with 12.5% heat-decomplemented fetal calf serum (FCS), 1% nonessential amino acids, 5 mmol/L L-glutamine, 40 U/mL penicillin, 100 μg/mL gentamycin, and 40 μg/mL streptomycin (DMEMc). Cells were maintained at 37 °C in a humidified atmosphere of CO_2/air (5:95) and passaged every 7 days by trypsinisation. They were seeded in transwells at 6×10^4 cells/cm^2. The medium (15 mL DMEM containing 10% FCS) was changed every 2 days until cells reached confluence (7–8 days). The integrity of the monolayers was evaluated by measurement of the transepithelial electrical resistance (TEER) using a Millicell-ERS device (Millipore, Zug, Switzerland) before and after the treatments. To evaluate transepithelial permeability, medium was removed from the apical and basal sides of the cultures and replaced by 2 mL of the transport solution consisting of Hanks' balanced salt solution (HBSS) and intestinal tea polyphenols, and pH was adjusted to 6 or 7.4. After 4 h of incubation at 37 °C, apical and basal solutions were collected and to determine the polyphenolic profile, aliquots (5 mL) were immediately mixed with 1 mL