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Influence of acute ingestion and regular intake of green tea catechins on resting oxidative stress biomarkers assays in a paralleled randomized controlled crossover supplementation study in healthy men

Mahendra P. Kapoor[a,*], Masaaki Sugita[b], Akinobu Nishimura[c], Akihiro Sudo[c], Tsutomu Okubo[a]

[a] Taiyo Kagaku Co. Ltd., Nutrition Division, 1-3 Takaramachi, Yokkaichi, Mie 510 0844, Japan
[b] Faculty of Sport Science, Nippon Sport Science University, 7-1-1 Fukusawa, Setagaya-Ku, Tokyo 158 8508, Japan
[c] Graduate School of Medicine, Faculty of Medicine, University of Mie, 2-174 Edobashi, Tsu, Mie 514 8507, Japan

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ABSTRACT

In this paralleled randomized controlled crossover study, we examined the hypothesis whether acute or regular green tea catechins (GTC) supplements have the potential to stimulate resting oxidative stress metabolites in healthy individuals. Sixteen subjects were randomly divided equally and assigned into regular intake (GTC-RI) or control group (GTC-CG) after the screening. GTC-RI group consumed 780 mg/day EGCG-enriched GTC (506 mg EGCG). While GTC-CG received water under the identical diet regime and lifestyle conditions. Then the GTC-CG crossed over to acute intake (GTC-AI) group and consumed a single dose of 780 mg ECGC-enriched GTC. Blood aliquots were collected at baseline and after GTC ingestion according to the prescribed study protocol. We measured oxidative stress blood biomarkers using the diacron reactive oxygen metabolite (d-ROMs) and biological antioxidant potential (BAP) tests. Urinary 8-hydroxydeoxyguanosine (8-OHdG) and 8-OHdG/creatinine were also analyzed. Analysis of variance (ANOVA) was used to determine statistical significance ($P < 0.05$) within the group as well as between and among the groups. The relative ratio of BAP and 8-OHdG concentrations to baseline showed statistical significance ($P < 0.05$) between the GTC-AI and GTC-RI groups and among all groups as determined by two-way repeated measures factorial ANOVA. The present results support the importance of appropriate GTC intake as antioxidant supplements for reducing oxidative stress that might lead to a sedentary behavior under resting conditions. Regular intake of GTC offers protection against oxidation-induced DNA damage in healthy humans and decreased oxidative stress. When GTC is taken in safe dosages under acceptable limits they reduce oxidative stress via associated and/or unassociated oxidant effect without any relevant pro-oxidant activity related to redox-sensitive adaptations.

1. Background

Dietary polyphenols are widely consumed antioxidants and have been shown to inhibit oxidative damages (Jówko, Długołęcka, Makaruk, & Cieśliński, 2015; Prior & Wu, 2013; Zhang & Tsao, 2016). Green tea is rich in polyphenols, especially catechins, which are equivalent to 20 to 35% of the dry weight of green tea leaves (Graham, 1992; Kumar & Pandey, 2013). Green tea extracts are comprised primarily of flavanols and their gallic acid derivatives, namely, catechin (C), epicatechin (EC), gallocatechin (GC), epigallocatechin (EGC), epicatechin 3-gallate (ECG), and epigallocatechin 3-gallate (EGCG) (Chaturvedula & Prakash, 2011; Graham, 1992; Kapoor et al., 2013).

The bioactivity of the GTC is well established and attributed to, antibacterial (Stapleton et al., 2004), antimicrobial (Marín, Miguélez, Villar, & Lombó, 2015), anti-inflammatory (Donà et al., 2003), anti-aging (Esposito et al., 2002), anti-carcinogenesis (Yang, Maliakal, & Meng, 2002), anti-angiogenic (Cao & Cao, 1999), anti-mutagenic (Han, Tian, & Chen, 1997; Wang et al., 1989), anti-HIV (Nance & Shearer, 2003), hypocholesterolemic (Yang & Koo, 2000), and anti-atherosclerotic plaque-forming activity (Chyu et al., 2004). Metabolism of GTC via conjugation reactions such as glucuronidation, acetylation, and sulfation, convert GTC to polar inactive metabolites, but results in poor gastrointestinal absorption combined with urinary excretion of conjugated catechins, severely limits their bioavailability and bioactivity (Del Rio et al., 2010; Manach, Williamson, Morand, Scalbert, & Rémésy, 2005; Stalmach et al., 2010). The effect of GTC on the oxidative stress is not predictable, especially if there is no reliable measure of activity.

A number of studies have been previously reported that

* Corresponding author.
E-mail address: mkapoor@taiyokagaku.co.jp (M.P. Kapoor).

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consumption of GTC results in an increase in plasma catechins and decrease oxidative stress (Renouf et al., 2013; Takechi et al., 2016). An acute dose of GTC intake can increase in plasma catechins that peak 1–2 h post-ingestion (Chow et al., 2003; Müller et al., 2010). Fung, Ho, Choi, Chung, and Benzie (2013) reported a comparison of catechin profiles in plasma and urine after a single dose as well as chronic green tea consumption (150 mg GTC containing 75 mg EGCG). In an acute study, the EGCG and ECG were more rapidly absorbed into the plasma than other catechins and escaped rapid conjugation and renal loss. However, with regular intake of GTC for 7 consecutive days, the EGCG and ECG were predominately accumulated in fasting plasma in their conjugated forms. Despite a clear difference, they did not investigate changes in oxidative stress biomarkers, which could be effectively influenced by acute or regular GTC intake. While, Muller et al. revealed that despite increased concentrations of several flavan-3-ols in plasma (600 mL green tea), the oxidative stress biomarker (8-iso-prostaglandin-F2α) were not affected in healthy, non-smoking subjects (Müller et al., 2010).

Kimura, Umegaki, Kasuya, Sugisawa, and Higuchi (2002) studied the relationship between single/double or repeated green tea consumption (164 mg catechins of which 100 mg was EGCG) on antioxidant activity in humans. This study reported no change in antioxidant activity after 7 days GTC supplementation; however, antioxidant activity evaluated by the ferric reducing ability of plasma (FRAP) assay was significantly lowered after withdrawal of GTC intake (Kimura et al., 2002). Takahashi et al. (2014) studied both acute and regular intake of catechin-rich green tea (615 mg catechins of which 125.9 mg was EGCG) for 4 weeks and reported no clear influence on fasting oxidative stress or antioxidant capacity in postmenopausal women (Takahashi et al., 2014). The possible reason for inconsistent results from aforementioned studies could be a relatively low content of most pharmacologically active catechin (EGCG) in the green tea used in their studies. However, some investigators have reported that daily consumption of green tea catechins (379 mg catechins of which 208 mg was EGCG) for 3 consecutive months positively attenuated the resting oxidative stress markers in obese individuals (Bogdanski et al., 2012; Suliburska et al., 2012).

Suffice to say that there are handful of studies, which assessed green tea supplements with adequate EGCG content, either as acute or chronic intake in humans to influence oxidative stress metabolites during resting conditions. Regular intake of GTC lowers DNA damage in humans via direct antioxidant effect plausibly related to redox-sensitive adaptations. In order to strengthen the limited and/or missing pieces of evidence, in the present study, we investigated the hypothesis whether acute or regular green tea catechins consumption (780 mg of which 506 mg was EGCG) can strengthen or influence the oxidative stress metabolite assays under resting conditions in healthy men. We performed the diacron reactive oxygen metabolites (d-ROMs) and biological antioxidant potential (BAP) tests to evaluate an oxidative and/or reducing the potential of GTC supplementation. In addition to biomarkers for oxidative stress, we measured 8-hydroxydeoxyguanosine (8-OHdG), which directly reflects the consequences of oxidative stress including the generation of mutagenic damages on DNA, responsible for secondary chronic morbidity.

2. Materials and methods

2.1. Study design, ethics, and participants

In this study, sixteen healthy male Japanese subjects age between 20 and 23 years were recruited after an initial screening (Fig. 1). The study was designed as randomized, parallel, controlled intervention study, and protocols including procedure were approved by the research ethics committee of the Mie University, Japan. The study was conducted according to the guidelines laid out in the declaration of Helsinki. All subjects gave written informed consent for the study and were well informed about the purpose of the study and related experimental procedures. Exclusion criteria were smokers, previous history of serious illness, liver or thyroid disorders, gastrointestinal or renal diseases, etc. Also, the subjects were excluded from participation if they were consumers of excessive green tea or any kind of food rich in polyphenols, took antibiotics and having a high habitual intake of alcohol (> 20 g/day) or caffeine (> 100 mg/day).

Subjects were assigned to order of treatment groups according to a computer-generated randomization plan of equal block size (n = 8) as of two groups by an independent researcher. Regular intake group (GTC-RI) received 780 mg GTC per day as the regular intake twice daily (390 mg per dose) at breakfast and dinner for 7 consecutive days. There were also pre and post-intervention measurements of several parameters as indicated in Fig. 2. One subject of the GTC-RI group refused to participate in the study after the recruitment, thus the data of remaining seven subjects are evaluated and presented for GTC-RI group (n = 7, age 21.8 ± 1.2 y; BMI 23.3 ± 2.5 kg/m^2;). The control group (GTC-CG) (n = 8, age 22.4 ± 1.0 y; BMI 22.8 ± 2.4 kg/m^2) was kept under observation period for 7 days with identical lifestyle as of the subjects of GTC-RI group. After 7 days, the GTC-CG group crossover to an acute intake group (GTC-AI) and received a single dose (780 mg) of GTC supplementation with water according to acute intake test trial protocol.

2.2. Diet and lifestyle observations

Subjects were asked to maintain their daily lifestyle during the study period. They were instructed not to change their prescribed food intake pattern ≥2wk before the study and during the trial period. To ensure that all subjects were roughly in energy balance, they were asked to abstain from any intense or structured physical activity such as fitness training. They were also asked to remain inactive on the day before of each trial and throughout the main trials. Additionally, subjects were asked not to consume alcohol or caffeine-containing beverages including coffee, tea, or soda because caffeine is reported to have a synergic effect with GTC on the ability to modulate oxidative stress metabolites (Dean, Braakhuis, & Paton, 2009; Dulloo et al., 1999; Sugita, Kapoor, Nishimura, & Okubo, 2016). In particular, subjects were told not to excessively consume any dairy product with before the study trials as intake of milk protein inhibit the effect of GTC on diet-induced thermogenesis (Hursel, & Westerterp-Plantenga, 2009; Sugita, et al., 2016).

2.3. Supplements

Subjects assigned to both GTC-RI and GTC-AI groups received the identical daily dose of GTC supplement in a tablet form (Sunphenon™; a proprietary green tea extract of Taiyo Kagaku Co. Ltd., Japan), which provide a total catechins content of 780 mg of which 506 mg was EGCG. The product is decaffeinated green tea catechins with caffeine content less than 0.7% (Table 1).

2.4. Protocol procedure and sample collection

All subjects of the present study were asked to finish their breakfast at least two hours prior to the clinical trial test. The trial protocol with pre-/post- intervention design for the GTC-RI group is illustrated in Fig. 2a. Subjects were asked to visit a testing laboratory on the day before the trial (d0) for the baseline anthropometry. Initial blood and urine samples were collected and subjects were instructed to begin their GTC supplementation (half dose; 390 mg) at dinner on the day. On day one (d1), the subjects arrived at the testing laboratory at 10:00 a.m., 2 h after consumption of ascribed GTC dose (half dose; 390 mg) at breakfast. Blood, as well as urine samples were collected accordingly. Subjects were asked to consume their GTC supplementation at breakfast and dinner for 7 consecutive days, and maintain their regular lifestyle