Journal of Functional Foods 46 (2018) 268–277

Contents lists available at ScienceDirect

Journal of Functional Foods

journal homepage: www.elsevier.com/locate/jff

Metagenomics analysis of gut microbiota modulatory effect of green tea polyphenols by high fat diet-induced obesity mice model

Xin Zhang[a], Man Zhang[b], Chi-Tang Ho[b], Xiaojing Guo[a], Zufang Wu[a], Peifang Weng[a], Meidi Yan[c], Jinxuan Cao[a,*]

[a] Department of Food Science and Engineering, School of Marine Sciences, Ningbo University, Ningbo 315211, PR China
[b] Department of Food Science, Rutgers University, New Brunswick, NJ 08901, United States
[c] Ningbo No.7 Hospital, Ningbo 315202, PR China

ARTICLE INFO

Keywords:
Green tea polyphenols
Obesity
Modulatory effect
Intestinal microbiota
Metagenomics

ABSTRACT

This study focused on the modulatory effect of green tea polyphenols (GTP) on human intestinal microbiota, and its underlying anti-obesity mechanisms. GTP was prepared from Chinese green tea by column chromatography, and then the influence of GTP on intestinal microbiome was analyzed with a human flora-associated (HFA) high fat diet-induced obesity mice model by metagenomics. GTP ameliorated the obesity-induced gut dysbiosis; in addition, a significant decrease was observed in *Firmicutes/Bacteroidetes* after GTP treatment. Moreover, KEGG pathways of ATP-binding cassette (ABC) transporters, two-component system and biosynthesis of amino acids enriched the most differentially expressed genes after GTP intervention for 8 weeks. Our results may have important implications for the use of GTP as a functional food component with potential therapeutic utility against high fat diet induced obesity, and prebiotic-like activity by modulating intestinal microbiota and affecting certain metabolic pathways, contributing to the improvement of host health.

1. Introduction

Obesity has become a global concern recently, which is usually associated with chronic diseases, including hypertension, diabetes, etc. (Franks & McCarthy, 2016). Although many proposed genetic and environmental factors have predisposed individuals to weight gain, the fundamental cause of obesity is an imbalance between dietary intake and energy expenditure (Hill, Wyatt, Reed, & Peters, 2003). Therefore, more attentions have been paid to the effective therapy, especially natural products as potential functional ingredients with anti-obesity activity. More recently, the relationship between intestinal flora and obesity has increasingly aroused general concerns.

In human gastrointestinal tract, the vast majority of microbial residents play a critical role for the development and lifelong maintenance of the health (Gill et al., 2006). They form a stable microbial ecosystem which can not only digest food, but also regulate the immune function, and the disturbance of gut microbiota is associated with obesity epidemic and metabolic syndromes (Liu et al., 2017). Disrupted composition of gut microbiota in high fat diet (HFD) induced obesity mice model led to metabolic endotoxemia, inflammation and associated disorders by a mechanism that could increase intestinal permeability (Cani et al., 2008). Some research indicated that there are significant differences in intestinal flora between lean and obese individuals, and the goal of weight-loss could be achieved by manipulating microbiota (Wang et al., 2014). Recent studies have indicated that dietary polyphenol-rich sources may modulate the intestinal microbiota *in vivo*, promoting the proliferation of beneficial bacteria and increasing the biodiversity degree in the gut (Viveros et al., 2011; Zhang et al., 2013; Zhang, Yang, Wu, & Weng, 2016). Thus, the development of an effective dietary regimen has great significance for the prevention of obesity.

In China, tea has been used as an anti-obesity therapy for more than a millennium, and considerable accumulated evidences have demonstrated the effects of tea on obesity prevention (Yang, Qiao, Zhang, Wu, & Weng, 2015). Green tea is traditionally considered to have weight-reducing effect, and prolonged consumption of it could maintain a lower body fat content (Kao, Chang, Lee, & Chen, 2006).

Abbreviations: ABC, ATP-binding cassette; BLAST, basic local alignment search tool; C, (−)-catechin; CV, central vein; ECG, (−)-epicatechin gallate; EGC, (−)-epigallocatechin; EGCG, (−)-epigallocatechin gallate; GCG, (−)-gallocatechin-3-gallate; GO, gene ontology; GTP, green tea polyphenols; H&E, hematoxylin-eosin; HFA, human flora-associated; HFD, high fat diet; HFD-GTP, high fat diet with GTP; HPLC-DAD, high performance liquid chromatography-diode array detection; KEGG, Kyoto Encyclopedia of Genes and Genomes; KOs, KEGG orthologues; LEfSe, linear discriminant analysis effect size; LDA, linear discriminant analysis; NR, non-redundant; OTUs, operational taxonomic units; ORFs, open reading frames; PCoA, principal coordinate analysis; QIIME, Quantitative Insights Into Microbial Ecology; SCFA, short chain fatty acid; SD, standard deviation

* Corresponding author.
E-mail address: caojinxuan@nbu.edu.cn (J. Cao).

https://doi.org/10.1016/j.jff.2018.05.003
Received 30 October 2017; Received in revised form 3 May 2018; Accepted 3 May 2018

Epidemiological evidences have supported that the effect of green tea reducing body weight is mainly attributed to its polyphenols (Kao et al., 2006; Lu, Zhu, Shen, & Gao, 2012). The anti-obesity effects of green tea polyphenols (GTP) may be produced by interacting with digestive enzymes as well as by reducing the digestibility of nutrients (Fei et al., 2014; Jiang, Cheng, Zhang, Wu, & Weng, 2017). In addition, our previous studies suggested green tea polyphenols benefits the stability of certain gut microbiota, especially in an environment-triggered microbial imbalance in high-fat diet-induced obesity mice model (Cheng, Zhang, Miao et al., 2017; Guo et al., 2017).

The biotransformation of tea polyphenols by gut microbiota has been investigated extensively (Chen & Sang, 2014), and the metabolic and colonic microbiota transformation may enhance the bioactivities of dietary polyphenols (Chiou et al., 2014). Although it has been shown GTP reduced body weight in rats by modulating certain obesity-related genes (Lu et al., 2012), its potential mechanisms on the prevention of gut dysbiosis and anti-obesity are complicated. Recently, metagenomics based molecular techniques have been widely approached to identify the rare and uncultivated bacterial communities as well as the functional genes enriched by external intervention (Koo et al., 2017; Udayangani et al., 2017). Thus, in the present study, to develop a more applicable model, we transplanted adult human fecal microbiota into germ-free C57BL/6J mice (Turnbaugh, Ridaura, Faith, & Rey, 2009), and the effect of GTP on HFD induced obesity was determined by high-throughput sequencing, in addition, the abundance of genes enriched in various metabolic pathways altered by GTP in the humanized mouse gut microbiome were investigated.

2. Materials and methods

2.1. Chemicals and reagents

Polyamide resin was obtained from Ocean Chemical Co., Ltd. (Qingdao, China). Standards of (−)-epigallocatechin (EGC) (> 98%), (−)-epigallocatechin gallate (EGCG) (> 98%) and (−)-epicatechin gallate (ECG) (> 98%) were purchased from Funakoshi (Tokyo, Japan). Standards of caffeine (> 98%) and theophylline (> 98%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Standards of (−)-catechin (C) and (−)-gallocatechin-3-gallate (GCG) were prepared according to out reported methods (Zhang et al., 2013). Germ-free C57BL/6J mice were obtained from the Experimental Animal Centre of Academy of the Military Medical Sciences (Beijing, China). Research Diets D12450B 10 kcal% Fat and D12492 60 kcal% Fat were purchased from Research Diets, Inc (New Brunswick, NJ, USA). All other chemicals and reagents were analytical grade.

2.2. Preparation of GTP

Green tea was obtained locally from Zhejiang province, Ningbo Beilun District, Baifeng tea plantation, and it was harvested in spring, 2017. The sample was ground into powder using a milling machine, and the material that passed through a 40-mesh sieve was kept in sealed polyethylene bags at −20 °C until use. GTP was prepared from green tea. Briefly, tea powders were extracted with distilled water in a ratio (v/w) of water/tea powder at 16. Upon extraction, the extract was centrifuged at 4500*g* for 15 min. The resulting residue was dissolved, filtered and purified by polyamide column according to our reported method (Guo et al., 2017). The eluted fractions were analyzed by HPLC-DAD (high performance liquid chromatography-diode array detection), and the desired fractions were collected, concentrated, loaded onto the polyamide column, and treated as described above. As results, fractions containing GTP were concentrated and lyophilized.

2.3. HPLC analysis

The contents of tea catechins, caffeine and theophylline in green tea were determined according to our reported method using an Agilent 1100 series HPLC (Agilent, CA, USA) (Zhang et al., 2013). The separation was achieved on a TSKgel ODS-100Z column (4.6 × 150 mm, 5 μm, Tosoh, Tokyo, Japan). The mobile phase consisted of formic acid solution (pH 2.5, A) and methanol (B). Elution was performed with a linear gradient as follows: 0–15 min, A from 82% to 40%. The temperature of the column oven was set at 40 °C, the flow rate was set at 1.0 mL/min. The injection volume was 20 μL.

2.4. Animals and experimental design

The anti-obesity effects of GTP were evaluated according to the reported method with proper modifications (Guo et al., 2017). Furthermore, all procedures involving animals were conducted in strict accordance with Chinese legislation on the use and care of laboratory animals during the entire experimental period at Center for Laboratory Animals, Ningbo University (Permission No. SYXK (Zhejiang) 2013-0191). We performed an initial colonization of young adult (6-week old) male C57BL/6J mice using the microbial community present in a freshly voided fecal sample from 6 healthy volunteers (3 females and 3 males, 25–30 years old) who did not have any history of gastrointestinal disorders and had not been treated with antibiotics for the previous 6 months. Humanization was performed under anaerobic conditions by diluting a freshly voided human fecal sample (1 g) in 10 mL reduced phosphate buffered saline (PBS, 0.1 M, pH 7.2), the fecal material was then suspended by vortexing, and 0.2 mL of the suspension introduced by gavage, into each germfree recipient. Mice were housed in separate cages within a gnotobiotic isolator, in an air-conditioned room with the temperature at 22–24 °C under a 12 h light-dark cycle and humidity of 50 ± 10%. The mice adapted to the environment for 7 days with HFD (Research Diets D12492 60 kcal% Fat), then they were randomly divided into 3 groups of 8 each: low fat diet group (LFD, Research Diets D12450B 10 kcal% Fat), high fat diet group (HFD) and high fat diet with GTP group (HFD-GTP). GTP was added to the high fat diet at a final concentration of tea polyphenols at 0.1% (w/w). Sample food and water consumption were measured on a per cage basis 3 times per week and the averages of food and water consumed were calculated weekly. The body mass of each animal was recorded soon after feeding with different diets for the entire period since the start of breeding, and fecal samples were collected from HFD-GTP group after 0 (GTP-0), 2 (GTP-2), 4 (GTP-4) and 8 weeks (GTP-8). All administrations were conducted for 8 consecutive weeks, and then laparotomy was conducted under nembutal anesthesia. After euthanized by excessive nembutal, the internal organs were removed.

2.5. Histopathological evaluation

Livers from mice in different groups were fixed in 10% neutral formalin solution, dehydrated in graded alcohol, and embedded in paraffin. Thin sections of 6 μm thickness of liver tissue were cut and mounted on glass slides and then were counter-stained with hematoxylin-eosin (H&E). Then, thin sections of liver were made into permanent slides and were examined for possible histopathological changes under a high-resolution microscope with photographic facility.

2.6. DNA extraction and intestinal microbiota analysis

DNA extraction and high-throughput sequencing were carried out according to our reported method with some modifications (Cheng, Zhang, Miao et al., 2017). DNA from different samples was extracted using the E.Z.N.A. ®Stool DNA Kit (D4015, Omega, Inc., USA) according to manufacturer's instructions. The total DNA was eluted in 50 μL of elution buffer and stored at −80 °C until measurement in the PCR by LC-Bio Technology (Hang Zhou, China), and the isolation was confirmed by 1.2% agarose gel electrophoresis. Before sequencing, the above 16S rDNA V3-V4 region of each sample was amplified with a set