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Metabonomics study of the protective effects of green tea polyphenols on aging rats induced by D-galactose

Chunwang Fu[a], Tiejie Wang[b], Yang Wang[c], Xiaohui Chen[c], Jian Jiao[c], Feng Ma[a], Min Zhong[b], Kaishun Bi[c,*]

[a] School of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University, Shenyang 110016, PR China
[b] Shenzhen Institute for Drug Control, Shenzhen 518057, PR China
[c] School of Pharmacy, Shenyang Pharmaceutical University, Wenhua Road 103, Shenyang 110016, PR China

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ABSTRACT

This article was designed to study metabonomic characters of aging induced by D-galactose (D-gal) and the protective effects of green tea polyphenols (GTP). Plasma samples from control, D-gal and GTP treated rats were analysed by ultra-performance liquid chromatography coupled with mass spectrometry (UPLC/MS) in positive mode. Coupled with biochemistry and histopathology results, the significant difference in metabolic profiling between D-gal treated group and the GTP treated group by using the principal components analysis (PCA) indicated that changes in global plasma metabolites were occurred. Some significantly changed metabolites like lysophosphatidylcholines, tryptophan, dihydrosphingosine and phytosphingosine have been found and identified. These changes in plasma metabolites are related to the perturbations of lecithin metabolism, amino acid metabolism and phospholipids metabolism, which may be helpful to further understand the action mechanisms of GTP.

1. Introduction

Aging, an aspect of almost all living organism, is a multifactorial process of enormous complexity and is characterized by impairment of various cellular modulation and functions, affecting various systems such as nervous system and immune system [1]. It is an inevitable biological process that eventually causes many chronic age-associated diseases, including cancer, cardiovascular diseases and neurodegenerative diseases. Anti-aging has already become a major public issue as the elderly population increases in the world.

Accumulated evidence has shown that the generation of free radical or reactive oxygen species (ROS) can lead to cell and tissue damage, resulting in aging and ultimately cell death [2]. Indeed, with the aging increasing, the antioxidative defense system include enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), glutathione peroxidase (GPx) and non-enzymatic antioxidants such as ascorbate, a-tocopherol and glutathione decline during the aging process [3], accordingly, oxidative stress induced by an overproduction of ROS leads to a disruption of cellular macromolecules functions, such as DNA, proteins and lipids of cell membranes [4]. Oxidative stress plays an important role during the pathogenesis of aging and age-associated diseases [5]. Antioxidant supplementation has been proved to be an ameliorator of oxidative stress [6]. Dietary antioxidants, in particular, green tea polyphenols (GTP) has been found beneficial in protecting against the generation of ROS. GTP is a group of polyphenolic compounds extracted from green tea (*Camellia sinensis*), a widely consumed beverage in many countries around the world, especially in China and Japan. It has been reported that the main active components of GTP are flavanols, commonly known as tea catechins including (−)-epigallocatechin-3-gallate (EGCG), (−)-epicatechin-3-gallate (ECG), (−)-epigallocatechin (EGC), (−)-epicatechin (EC), (+)-gallocatechin (GC) and (+)-catechin (C). Numerous studies have revealed that GTP possesses the potent radical scavenging, iron-chelating and also indirectly through modulation of transcription factors or enzymes [7,8] and has wide health promoting properties, such as anti-aging, anti-artherosclerosis and the cancer chemopreventive effects [9–11]. Until now, many works on the pharmacological mechanism of GTP mainly focus on gene expression, cell morphology, as well as biochemical and pathological changes studies. While, few are known about the change of the whole metabolites in an organism treated with GTP. Neither has it been experimentally investigated how GTP affects the metabolism of whole body.

Metabonomics is an important platform of systems biology, defined as the quantitative measurement of the dynamic multiparametric metabolic response of living systems to patho-

* Corresponding author. Tel.: +86 24 23986296; fax: +86 24 23986259.
E-mail address: bikaishun@yahoo.com (K. Bi).

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physiological stimuli or genetic modifications [12]. The ability to measure global alteration in metabolism in biological fluids and tissues is well coincident with the integrity and systemic feature of traditional Chinese medicine, which precedes conventional biochemical and pathological changes, has contributed to the emergence of metabonomics as a promising scientific platform for understanding the biochemical basis of diseases, drug toxicity, the diagnosis and treatment of diseases [13,14]. However, there is no report on the molecular biomarkers for anti-aging effects of GTP with a metabonomic approach.

Recent metabonomic technologies based on mass spectrometry (MS) and nuclear magnetic resonance (NMR) are all powerful analytical tools to measure low molecular weight metabolites in biological samples. Compared with NMR, MS is inherently considerable more sensitive than NMR spectrometry. When MS is coupled with ultra performance liquid chromatography (UPLC), high sensitivity, high resolution, wide dynamic range, coverage of a wide chemical diversity, robustness and feasibility to elucidate the molecular weight and structure of unknown compounds can be achieved for the low abundance metabolites. UPLC/MS has been used for observing the subtle metabolic changes under some diseases or treatment of diseases and provided informative data for elucidating biochemical basis of diseases and addressing the therapeutic effect of medicines [15,16].

It was reported that chronic D-galactose (D-gal) exposure could induce memory loss, neurodegeneration, oxidative damage and impair neurogenesis in the dentate gyrus, a process similar to the natural aging in mouse [17,18]. Rodent chronically injected with D-gal has been used as an animal aging model for anti-aging research and health food testing [19].

Based on the reasons above, in this article, aging model rats were established by injecting the male Wistar rats intraperitoneally with D-gal once a day. Corresponding plasma samples were collected from control rats, D-gal treated rats and GTP treated rats for the metabonomic analysis by using UPLC/MS system. Principal component analysis (PCA) was performed for investigating the metabolic changes among the plasma of rats, here, we study the effects of GTP in the aging rats and explore potential molecular biomarks for the anti-aging effects of GTP using metabonomic method.

2. Experimental

2.1. Chemicals and reagents

Acetonitrile and formic acid were HPLC grade (NJ, Fisher Corp., USA), water was purified by redistillation and was filtered through 0.22 μm membrane filter before use. The standard of tryptophan was purchased form Sigma–Aldrich (Sigma Corp., Mo, USA). Green tea polyphenols was purchased from Zhejiang Orient Tea Development Company (Hangzhou, China). The purity of green tea polyphenols was 98%, the content of EGCG was 75.2%, EGC was 2.62%, EC was 1.51%, ECG was 9.7%, GC was 5.95%, C was 3.19%, gallic acid was 1.02% and caffeine was 0.81% in GTP (by HPLC-UV). The total antioxide capacity (T-AOC), total superoxide dismutase (T-SOD), glutathione reductase (GR), glutathione peroxidase (GPx), catalase (CAT) and malondialdehyde (MDA) commercial kits were purchased from Nanjing Jiancheng Institute of Biotechnology (Nanjing, China). D-galactose was purchased from Sigma–Aldrich (Sigma Corp., Mo, USA). The D-galactose was dissolved in physiological saline (0.9% saline and distilled water) at concentration of 20 mg/mL.

2.2. Animals and treatment

35 male Wistar rats of 6 week-old weighing 200–220 g from Shanghai Slack Laboratory Animal Co. Ltd., were used in the study. Animal care was in accordance with the Guidelines for Animal Experimentation of Shenyang Pharmaceutical University (Shenyang, China) and the protocol was approved by the Animal Ethics Committee of the institution. The animals were fed with a certified standard diet and tap water *ad libitum*, and were allowed to acclimate for a period of 1 week before the experiment. During the study, all animals were kept in animal room with temperature: 18–21 °C, humidity: 45–65%, and 12 h dark to light cycle. To reduce contamination, the rats were placed in clean cages each day. After 1 week of acclimatization, all rats were randomly divided into 5 groups (n = 7/group): control group (CG); D-gal group (DG); high dosage group of GTP (HG); middle dosage group of GTP (MG) and low dosage group of GTP (LG). The rats of DG, HG, MG and LG received injection of D-gal intraperitoneally at a dose of 100 mg/(kg/day) for 8 weeks, from the fifth week, all rats of LG, MG and HG received daily GTP at a dose of 200, 400 and 800 mg/(kg/day) in distilled water by oral gavage for another 4 weeks, respectively; and the rats of control group (CG) received injection of physiological saline intraperitoneally and oral gavage with the approximately same volume distilled water as the other groups.

2.3. Sample collection and preparation

After 8 weeks treatment, blood samples of CG, DG, HG, MG and LG rats were collected from the suborbital vein into heparinized tubes and immediately centrifuged at 3000 rpm for 10 min. The plasma was transferred into clean tubes and stored at −20 °C until analysis.

Prior to analysis, the plasma samples were thawed at room temperature, then centrifuged at 15,000 rpm for 10 min. Acetonitrile (400 μL) was added to plasma (200 μL) and vortex-mixed vigorously for 3 min, then centrifuged at 15,000 rpm for 10 min at 4 °C. The supernatant was pipetted out and evaporated to dryness at 30° C under a gentle stream of nitrogen. The dried residue was then reconstituted in 200 μL of acetonitrile-water (15:85, v/v) and vortex-mixed for UPLC/MS analysis.

2.4. Biochemical assay

Rats were deeply anesthetized and then sacrificed by decapitation. Brains were prompted removed and dissected on ice to obtain the hippocampus, frozen in liquid nitrogen and stored at −80 °C until further processing. The activities of T-AOC, T-SOD, GR, GPx, CAT and the level of MDA were determined by using commercially available kits. All procedures completely complied with the manufacture′s instructions.

2.5. Histopathology

For histological evaluation, rats were deeply anesthetized with 10% chloral hydrate, and the brain was infused with physiological saline. After perfusion fixation with paraformaldehyde, the brain was removed and stored in fixative for 24 h, then embedded in paraffin wax and sectioned at 5 μm. Sections of hippocampal CA1 area were stained with hematoxylin and eosin.

2.6. Chromatography

Liquid chromatography was performed on ACQUITY™ UPLC system (Waters Corp., Milford, MA, USA) equipped with cooling autosampler and column oven. Chromatographic separation was