Experimental Gerontology 47 (2012) 672–679

Contents lists available at SciVerse ScienceDirect

Experimental Gerontology

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

Influence of large molecular polymeric pigments isolated from fermented Zijuan tea on the activity of key enzymes involved in lipid metabolism in rat

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ARTICLE INFO

Article history:
Received 21 March 2012
Received in revised form 1 June 2012
Accepted 5 June 2012
Available online 13 June 2012

Section Editor: Andrzej Bartke

Keywords:
Zijuan tea
Pigments
Hyperlipidemia
PCR analysis
Hormone-sensitive lipase
HMG-CoA reductase

ABSTRACT

Influence of large molecular polymeric pigments (LMPP) isolated from fermented Zijuan tea on the activity and mRNA expression of key enzymes involved in lipid metabolism in rat was explored. The results show that intragastric infusion of high-dose LMPP (1.215 g/kg body weight) effectively suppressed the elevation in TC and LDL-C ($p<0.05$), and prevented the reduction in HDL-C ($p<0.05$), compared with the hyperlipidemia model group. LMPP significantly enhanced the activity of HL and HSL, and increased the HSL mRNA expression in the liver tissue and adipose tissue. High-LMPP treatment significantly reduced the HMG-CoA reductase expression by 56.5% in the liver compared with hyperlipidemia model group. In contrast, LDL-R expression was increased by 120% in the presence of high-LMPP treatment. These results suggest that LMPP have the hypolipidemic effect to some extent and significantly enhance HSL mRNA expression in the liver and adipose tissue, thereby increasing HSL activity in rat.

1. Introduction

Zijuan tea plant is a cultivar developed from an individual plant of *Camellia sinensis var. kitamura* obtained at Menghai county of China (Fig. 1a). It has purple stems, buds, and leaves, a light purple calyx and pedicle, and pale purple fruit skin. Its leaves are processed into Zijuan sun-dried green tea (Fig. 1b) by multiple procedures, including fixation, rolling, and sun drying. The liquor of the resulting Zijuan green tea appears purple and tastes bitter, with indigo leaves residing at the bottom (Bao et al., 2008; Liang and Xia, 2003; Yang et al., 2009). Zijuan green tea can be further manufactured into the more valuable fermented Zijuan tea (Fig.1c) by appropriate wetting and solid-state fermentation. The fermented Zijuan tea is brown, and its liquor appears thick and brightly red, with a rich taste without bitterness, and it has a unique fragrance. Because of these enjoyable characteristics, the fermented Zijuan tea is well accepted by consumers despite its high price.

Our study showed that during the fermentation of Zijuan tea by solid-state fermentation, the composition of the tea leaves changes dramatically. The concentration of tea polyphenols decreased from 21.83 to 9.02%, total catechins decreased from 7.25 to 3.18%, thearubigin decreased from 6.31 to 0.39%, and theaflavin decreased from 6.31 to 0.39%. The concentration of large molecular polymeric pigments **(LMPP)** in fermented Zijuan tea increased from 2.38 to 12.43% (Wang, 2011). The substantial decrease in phenols and increase in **LMPP** suggest that **LMPP** may be a unique component in fermented Zijuan tea. **LMPP** (Fig.1d) is a family of polymeric substances, and are soluble in water but insoluble in organic solvents such as methanol, anhydrous ethanol, ethyl acetate, n-butanol, and trichloromethane. In light of these unique characteristics of fermented Zijuan tea, we studied the biological activity of **LMPP** fractionated in fermented Zijuan tea, including its ability to lower blood lipid concentrations and action mechanism of **LMPP** in hyperlipidemic rats. Our objectives were to determine the influence of **LMPP** on the activity of key enzymes involved in lipid metabolism and mRNA expression of hormone-sensitive lipase and HMG-CoA reductase in rat liver, epididymis and mesenteric adipose tissue, and provide a basis for its application in functional foods, health care, cosmetics, and natural pigments. For example, a better understanding of its biological functions may guide a rational development of this natural substance into a new dietary supplement for lowering blood lipids.

Abbreviations: LMPP, large molecular polymeric pigments; FC, free cholesterol; TC, total cholesterol; TG, triacylglycerol; HDL-C, high density lipoprotein-cholesterol; LDL-C, low density lipoprotein-cholesterol; HL, hepatic lipase; HSL, hormone-sensitive lipase; LCAT, lecithin cholesterol acyltransferase; LPL, lipoprotein lipase; PCR, polymerase chain reaction; HMGR, 3-hydroxy-3-methyl-glutaryl-CoA reductase; LDLr, low-density lipoprotein receptor; RT-PCR, reverse transcription polymerase chain reaction.

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0531-5565/$ – see front matter.
doi:10.1016/j.exger.2012.06.002

Fig. 1. Zijuan tea plant (a), Zijuan sun-dried green tea (b), Fermented Zijuan tea (c), large molecular polymeric pigments (**LMPP**) extracted from fermented Zijuan tea (d).

2. Materials and methods

2.1. Materials

Zijuan sun-dried green tea was purchased from the Tea Research Institute of Yunnan Academy of Agricultural Sciences (Menghai, Yunnan, China). Assay kits for total cholesterol (TC), triacylglycerol (TG), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) were obtained from Shanghai Kehua Bioengineering Co., Ltd. (Shanghai, China). Rat HMG-CoA reductase (HMGR) enzyme-linked immunosorbent assay (ELISA) kits were obtained from Rapidbio (RB) company (USA). Lovastatin capsules were obtained from Jiangsu D&Y Pharmaceutical Co., Ltd. (Suqian, China). Kits for total RNA extraction, RT-PCR, reverse transcriptase M-MLV, TaqDNA polymerase and DNA Marker-1 were obtained from Beijing Quanshijin Biotechnology Co., Ltd. β-actin gene primers, HSL and LDLr gene primers were obtained from Beijing Bomaide Biotechnology Co., Ltd. Heparin sodium was obtained from Shanghai Chemical Regent Company. The total esterase detection kit was obtained from Nanjing Jiancheng Bioengineering Institute. Freshly redistilled water was prepared in our laboratory. All of the other reagents used are of analytical grade.

2.2. Methods

2.2.1. Preparation of LMPP isolated from fermented Zijuan tea

The Zijuan sun-dried green tea leaves were fermented by the following procedures. Ten kilograms of leaves (moisture content: 8.5%) was wetted with 9000 ml of distilled water; they were covered with gas-permeable food-grade polyethylene films, and kept in a fermentation oven (45 °C, relatively humidity: 70%) for 40 d with constant rotation. The leaves were then dried at 60 °C and Zijuan pu-erh tea leaves were obtained.

Extraction of LMPP from fermented Zijuan tea was as follows. Fermented Zijuan tea (3000 g) was extracted 3 times in advance with anhydrous ethanol (1:4, w/v) for 12 h at 35 °C, after which the ethanol extraction was discarded. The tea residues were dissolved in distilled water (1:5, w/v) for 3 h at 85 °C and filtered 3 times. The resulting aqueous extract was extracted with chloroform (1:1, w/v), ethyl acetate (1:1, w/v), and n-butanol (1:1, w/v) at room temperature, and the extract was discarded. The residual tea aqueous extract was precipitated by anhydrous ethanol (1:4, v/v) for 12 h at room temperature and the precipitation was collected by centrifuge method. The water solution of the precipitation (10%, w/v) was vacuum freeze-dried (FD-1PF vacuum lyophilizator; Detanyou Scientific, Beijing, China) into powder. The powder (ethanol precipitate) consists of large molecular polymeric pigments (LMPP) 74.0 ± 0.85%, protein residual 13.11 ± 1.48%, and carbohydrate residual 10.14 ± 1.78%.

2.2.2. Curie-point pyrolysis-gas chromatography-mass spectroscopy (Py-GC/MS) analysis

A small amount (0.05–0.1 mg) of sample (LMPP) was wrapped in a ferromagnetic foil and pyrolyzed in a JPH-5 Curie-point pyrolyzer (Japan Analytical Industry, Tokyo, Japan) at 485 °C for 5 s. The injector temperature was maintained at 280 °C. The evolved gas products were separated and analyzed with a coupled GC–MS system (6890N/5973N, Agilent Technologies, Santa Clara, CA, USA) equipped with an HP-5 column. The GC conditions were: GC analysis using a program temperature, stays at 45 °C for 4 min, heating up to 280 °C at 3 °C/min to maintain 15 min, inlet temperature, 250 °C; detector temperature, 280 °C; carrier gas, helium; flow rate, 1.0 mL/min; and split ratio, 100:1. The MS conditions were: electron bombardment ionization source, DB-1701MS, 70 eV and 230 °C; and scan range, 15–500 U.

MS spectra were assigned by computerized searches against the NIST02 spectral library assisted by manual interpretation with the help of the Spectral database for Organic Compounds (SDBS, National Institute of Advanced Industrial Science and Technology of Japan, Tokyo, Japan). For quantitative analyses, the areas of ion current peaks were determined and normalized into relative peak areas.

2.2.3. Animals and groups

Healthy adult male Sprague–Dawley (SD) rats (weighing 150 to 200 g and 4 months old) were obtained from the Animal Experimental Center of Kunming Medical College (license nr: SCXK [Dian 2005-0008]). All animal procedures were approved by the National Natural Science Fund of China, in accordance with guidelines from the China Council for Healthy Food.

2.2.4. Feeding method and management

Basal feed diet was obtained from the Animal Experimental Center of Kunming Medical College. The formulation was as follows: corn, 350 g/kg; wheat bran, 250 g/kg; bean pulp, 250 g/kg; fish meal, 80 g/kg; yeast, 20 g/kg; bone meal, 20 g/kg; whey powder, 10 g/kg; salt sodium, 5 g/kg; rape oil, 5 g/kg; mineral mix, 1 g/kg; vitamin mix, 0.3 g/kg; methionine, 1.3 g/kg; lysine, 0.7 g/kg; and cod liver oil, 0.5 g/kg. The formulation of the high lipid diet (basal: feed, 78.8%; lard oil, 10.0%; yolk powder, 10.0%; cholesterol, 0.1%; and bile salt, 0.2%) was based on the *Technical Standards for Determination and Assessment of Health Foods* issued by the China Ministry of Health in February 2003 (Wei, 2008).

2.2.5. Groups of animals

Sixty healthy male SD rats (150–200 g) were separately maintained in cages (20 ± 2 °C, relative humidity: 45–60%) with free access to water and food. Their body weights and lengths were measured weekly. After adaptive feeding with basal feed diet and distilled water for 1 week, a blood sample was collected from the orbit of each rat for hematological analysis. The rats were divided into six groups (10 rats/group) according to their TC concentrations: normal control group (Group I, basal feed diet and distilled water), hyperlipidemia model group (Group II, high lipid diet and distilled water), low-dose **LMPP** treatment group (Group III, high lipid diet and **LMPP** (0.135 g/kg.BW)), medium-dose **LMPP** treatment group (Group IV, high lipid diet and **LMPP** (0.405 g/kg.BW)), high-dose **LMPP** treatment