Journal of Chromatography B, 1015 (2016) 62–73

Contents lists available at ScienceDirect

Journal of Chromatography B

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

Determination of American ginseng saponins and their metabolites in human plasma, urine and feces samples by liquid chromatography coupled with quadrupole time-of-flight mass spectrometry

Jin-Yi Wan [a,b,c], Chong-Zhi Wang [c], Zhi Liu [c], Qi-Hui Zhang [c], Mark W. Musch [d], Marc Bissonnette [d], Eugene B. Chang [d], Ping Li [b], Lian-Wen Qi [b,c,*], Chun-Su Yuan [c,**]

[a] School of Pharmacy, Jiangsu University, 301 Xuefu Road, Zhenjiang 212013, China
[b] State Key Laboratory of Natural Medicines, China Pharmaceutical University, 24 Tongjia Lane, Nanjing 210009, China
[c] Tang Center for Herbal Medicine Research and Department of Anesthesia and Critical Care, University of Chicago, 5841 South Maryland Avenue, MC 4028, Chicago, IL 60637, USA
[d] Department of Medicine, Knapp Center for Biomedical Discovery, University of Chicago, IL 60637, USA

ARTICLE INFO

Article history:
Received 2 October 2015
Received in revised form 3 February 2016
Accepted 4 February 2016
Available online 6 February 2016

Keywords:
American ginseng
Ginsenosides
Enteric
Microbiota
Ginsenoside Rb_1
Compound K
LC-Q-TOF-MS

ABSTRACT

American ginseng is a commonly consumed herbal medicine in the United States and other countries. Ginseng saponins are considered to be its active constituents. We have previously demonstrated in an *in vitro* experiment that human enteric microbiota metabolize ginseng parent compounds into their metabolites. In this study, we analyzed American ginseng saponins and their metabolites in human plasma, urine and feces samples by liquid chromatography coupled with quadrupole time-of-flight mass spectrometry (LC-Q-TOF-MS). Six healthy male volunteers ingested 1 g of American ginseng twice a day for 7 days. On day 7, biological samples were obtained and pretreated with solid phase extraction. The ginseng constituents and their metabolites were characterized, including 5 ginseng metabolites in plasma, 10 in urine, and 26 in feces. For the plasma, urine and feces samples, the levels of ginsenoside Rb_1 (a major parent compound) were 8.6, 56.8 and 57.7 ng/mL, respectively, and the levels of compound K (a major metabolite) were 58.4 ng/mL, 109.8 ng/mL and 10.06 μg/mL, respectively. It suggested that compound K had a remarkably high level in all three samples. Moreover, in human feces, ginsenoside Rk_1 and Rg_5, Rk_3 and Rh_4, Rg_6 and F_4 were detected as the products of dehydration. Further studies are needed to evaluate the pharmacological activities of the identified ginseng metabolites.

1. Introduction

American ginseng or *Panax quinquefolius* is a commonly consumed herbal medicine in the United States and other countries. Ginseng saponins are considered to be active constituents of this botanical [1]. Until now, nearly a hundred ginsenosides have been isolated and characterized from American ginseng, and many of them have shown pharmacological activities [2,3]. Yet, compared with studies of other ginseng species, such as *Panax ginseng* and *Panax notoginseng*, analytical studies of American ginseng and its components are relatively limited [4,5].

The chemical and pharmacological diversity of different ginseng constituents has been investigated. In studies of many parent compounds of ginseng, attention also has been given to their potential structure-activity relationship [6–8]. Reductionist methodology in ginseng research during *in vitro* screening has been applied primarily to the bioactivity of parent compounds [9,10]. However, the bioavailability of ginseng compounds, an important consideration for their effects *in vivo*, has been overlooked.

Of the commercially available American ginseng products, nearly all are ingested orally. After oral intake, trillions of gut microbiota in the gastrointestinal tract may affect ginseng biotransformation [11]. Metabolic profiles of ginseng by enteric microbiota have been reported in animals [12,13], and the actions of selected biotransformed metabolites have been elucidated [14]. For example, compound K, a major metabolite in the protopanaxadiols

* Corresponding author.
** Corresponding author at: Tang Center for Herbal Medicine Research and Department of Anesthesia and Critical Care, University of Chicago, 5841 South Maryland Avenue, MC 4028, Chicago, IL 60637, USA.
E-mail addresses: fleude@126.com (L.-W. Qi), CYuan@dacc.uchicago.edu (C.-S. Yuan).

http://dx.doi.org/10.1016/j.jchromb.2016.02.008
1570-0232/

group, has a better bioactivity than its parent compound, ginsenoside Rb_1 [15]. In an *in vitro* study we observed that human enteric microbiota metabolized ginseng parent compounds into 25 metabolites [16]. However, to date, the determination of ginseng metabolites in human biological samples has largely not been carried out. To link the health benefits of ginseng compounds to their effects, we sought to determine the profiles of ginseng and its metabolites after oral administration.

Based on our previous studies, liquid chromatography coupled with quadrupole time-of-flight mass spectrometry (LC-Q-TOF-MS) can be effectively used to characterize ginseng saponins and their metabolites [16]. This technique provides advanced structural information with high sensitivity, specificity, and versatility in characterizing complex metabolite profiles in matrix-based samples. With this method, all ginseng constituents, even at low concentrations, can be successfully detected.

In a previous study in humans, we analyzed a ginsenoside and its metabolism in plasma after a single oral dose of American ginseng using UPLC-TOF-MS [17]. It was indicated that the metabolite profile might not have been adequately revealed after a single dose regimen in the qualitative study. For the study reported here, we recruited six healthy volunteers to ingest American ginseng for 7 successive days. The 2 g daily ginseng used in this study was within the commonly used therapeutic dose range. Plasma, urine and feces samples were collected at the end of the 7 days and analyzed by LC-Q-TOF-MS. Special attention was paid to the determination of a major ginseng parent compound, ginsenoside Rb_1, and its predominant metabolite, compound K.

2. Experimental

2.1. Chemicals and reagents

Reference ginsenosides, including ginsenoside Rb_1, Rb_2, Rb_3, Rc, Rd, Re, Rg_1, F_1, F_2, pseudoginsenoside F_{11}, gypenoside XVII, 20*S*-Rg_2, 20*R*-Rg_2, 20*S*-Rg_3, 20*R*-Rg_3, 20*S*-Rh_1, 20*R*-Rh_1, 20*S*-Rh_2, 20*R*-Rh_2, compound K, and protopanaxatriol were purchased from Jilin University (Changchun, China). Ginsenoside Rk_3, Rh_4, Rk_1 and Rg_5 were prepared in our laboratory by steaming transformation. The structures are shown in Fig. 1C, and were elucidated by ^{1}H NMR, ^{13}C NMR and MS. The internal standard (IS) digoxin was obtained from Sigma-Aldrich (St. Louis, USA). The purities of the reference compounds were more than 95%, as determined by HPLC-DAD. Acetonitrile (ACN) and formic acid of HPLC grade were obtained from Merck (Darmstadt, Germany). Deionized water (18 MΩ cm^{-1}) was supplied with a Milli-Q water system (Millipore, Milford, MA, USA). Other reagents were of analytical grade.

2.2. Plant materials

American ginseng roots (*P. quinquefolius* L.) were obtained from Roland Ginseng, LLC (Wausau, WI, USA). The voucher samples were authenticated by Dr. Chun-Su Yuan and deposited at the Tang Center for Herbal Medicine Research at the University of Chicago (Chicago, IL, USA). Air-dried American ginseng was pulverized to powder and passed through a 40 mesh screen to prepare the sample.

2.3. Human subjects and study protocol

With approval from the Institutional Review Board, six healthy male volunteers (ages 18–45 years) were enrolled in the clinical trial. The subjects were screened for drug abuse disorders or medical contraindications for participation in the study. The volunteers underwent a physical examination and completed a health history questionnaire. On day 1, each subject was asked to fast overnight, and their biological samples (blood, urine and feces) were collected as the control. Then all volunteers were instructed to ingest 1 g of American ginseng powder with water twice a day for 7 consecutive days. On day 7, biological samples from these subjects were again obtained for analysis. The blood samples in the heparin tubes were centrifuged at 4000 rpm for 5 min and the plasma was transferred to Eppendorf tubes. All samples were then immediately chilled on ice and stored at −80 °C until analysis.

2.4. Sample preparation

To prevent matrix effects and concentrate the analytes, all the biological samples were pretreated with solid phase extraction (SPE) before LC-Q-TOF-MS. Oasis HLB columns (1 mL, 30 mg, Waters, Milford, MA, USA) were preconditioned with 2 mL of methanol and equilibrated with 2 mL of deionized water.

The plasma samples (200 μL) were mixed with 20 μL of 2% acetic acid and vortexed for 30 s at room temperature. Acetic acid was used to release the protein binding ginsenosides and metabolites to increase the recovery of the ingredients for the measurement. The samples were subsequently diluted with 1 mL of physiological saline. The homogenates were loaded onto the preconditioned SPE columns, washed with 2 mL of deionized water and slowly eluted using 1 mL of methanol. The elution was evaporated to dryness under nitrogen at 20 °C, and the residue was dissolved in 100 μL of methanol and centrifuged at 13,000 rpm for 10 min before further analysis.

The urine samples were centrifuged at 4000 rpm for 10 min at room temperature and the supernatants (200 μL) were purified by the preconditioned SPE columns. The cartridge was cleaned with 2 mL of deionized water, and slowly eluted using 1 mL of methanol. The elution was evaporated to dryness under nitrogen at 20 °C, and the residue was dissolved in 100 μL of methanol and centrifuged at 13,000 rpm for 10 min.

The feces samples (0.5 g) were extracted with 3 mL of 80% methanol by ultrasound (30 °C, 100 Hz) for 15 min. They were then centrifuged at 4000 rpm for 10 min at room temperature, and the supernatants (200 μL) were purified by the preconditioned SPE columns. The cartridge was cleaned with 2 mL of deionized water and slowly eluted using 1 mL of methanol. The elution was evaporated to dryness under nitrogen at 20 °C, and the residue was dissolved in 100 μL of methanol and centrifuged at 13,000 rpm for 10 min.

2.5. LC–MS/MS instrument and conditions

Chromatographic analysis was performed on an Agilent 1290 Series LC system (Agilent Technologies, Santa Clara, CA, USA) equipped with a binary pump, an online degasser, an auto plate-sampler, and a thermostatically controlled column compartment. Sample separation was carried out at 25 °C on an Agilent Zorbax Extend-C_{18} column (4.6 mm × 250 mm, 5 μm) with a C_{18} guard column (4.6 mm, 0.2 μm). The mobile phase consisted of water (0.1% formic acid, Solvent A) and acetonitrile (0.1% formic acid, Solvent B), using a gradient elution of 21% B at 0–15 min, 21–30% B at 15–18 min, 30–33% B at 18–30 min, 33% B at 30–34 min, 33–45% B at 34–40 min, 45–60% B at 40–50 min, 60–80% B at 50–55 min, 80–100% B at 55–60 min, and 100% B at 60–65 min. The flow rate was kept at 1 mL/min, and the injection volume of plasma, urine, and feces samples was set at 10 μL, 5 μL, and 10 μL, respectively.

Detection was performed by a 6530 Q-TOF mass spectrometer (Agilent) with a Dual electrospray ionization (ESI) source. The operating parameters were optimized as follows: drying gas (N_2) flow rate, 10.0 L/min; drying gas temperature, 320 °C; nebulizer, 35 psig; capillary, 3500 V; OCT RFV, 750 V; and fragmentor voltage, 120 V. Mass spectra were recorded across the range *m*/*z* 100–3000 in both