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Gut microbiota in the pharmacokinetics and colonic deglycosylation metabolism of ginsenoside Rb_1 in rats: Contrary effects of antimicrobials treatment and restraint stress

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ABSTRACT

Ginsenoside Rb_1, an active ingredient in *Panax ginseng*, was widely used for its various biological activities. To clarify the role of the gut microbiota in pharmacokinetics and metabolism of Rb_1, a comprehensive and comparative study of colonic deglycosylation metabolism and systemic exposure of ginsenoside Rb_1 in normal rats, antimicrobials (ATMs) treated rats, and restraint stressed rats was conducted. ATMs treated rats received oral administration of non-absorbable antimicrobial mixtures for 7 consecutive days. Restraint stressed rats were subjected to repeated restraint stress for a period of 2 h once daily for 7 days. Plasma concentration dynamics, urine and fecal excretion of Rb_1 and its deglycosylation metabolites (Rd, F_2, and C-K) were studied. Moreover, the *in vitro* metabolism of Rb_1 in fecal suspension and the fecal β-D-glucosidase activity were profiled. Systemic exposure of the deglycosylation metabolites of ginsenoside Rb_1 (F_2, C-K) were significantly higher in restraint stressed rats, but ATMs treated rats exhibited a decreased plasma levels of F_2 and C-K, compared with normal rats. Further studies illustrated that altered systemic Rb_1 and its deglycosylation metabolites exposure in restraint-stressed rats and ATMs treated rats may be partially attributed to alternations in cumulative fecal excretion. The distinguishing fecal β-D-glucosidase, *in vitro* elimination of Rb_1, and formation of these deglycosylation metabolites afforded further evidence for the *in vivo* data. In conclusion, the dys-regulated fecal β-D-glucosidase activity and deglycosylation metabolism may contribute to the altered pharmacokinetic of ginsenoside Rb_1 and its hydrolysis metabolites after ATMs treatment or restraint stress exposure. Our results may offer valuable insights into the pharmacological changes of bioactive ginsenosides in dys-regulated gut microbiota statue.

1. Introduction

Ginseng, the root of *Panax ginseng C.A. Meyer*, is one of the best known remedy and dietary supplement as it exerts a broad range of pharmacological actions [1]. Ginseng saponins, namely ginsenosides, are considered to be the primary constituents responsible for ginseng's pharmacological actions [2], including immune enhancement [3], anti-tumor [4], anti-diabetic properties [5], and cognitive improvement [6]. Ginsenoside Rb_1, a 20(*S*)-protopanaxadiol (PPD) type ginsenoside featuring sugar moieties at the C-3 and/or C-20 positions, is the most abundant component in ginseng total saponins and possesses various biological effects [7]. Accumulating evidence has demonstrated that ginsenoside Rb_1 has anxiolytic-like [8], antidepressant [9], anti-inflammatory [10], learning and memory improving [7], hypoglycemic [11] and neuroprotective effects [7].

The oral bioavailability of ginsenoside Rb_1 is relatively low (around 0.1%) due to poor intestinal absorption and extensive

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metabolism [12]. Gastric acid-mediated hydrolysis or hydration, gut microbiota-mediated deglycosylation and cytochrome P450 enzymes-mediated oxygenation were involved in the metabolism in ginsenoside Rb_1 *in vitro* and *in vivo* [13]. Notably, considerable colonic deglycosylation of the ginsenoside Rb_1 occurred following oral administration (see Fig. 1) [14]. Gut microbiota possess different types of glycosidases, such as β-D-glucosidase, α-L-rhamnosidase and β-D-xylosidase [15]. Through cleavage of sugar moieties by β-D-glucosidase, ginsenoside Rb_1 underwent stepwise hydrolysis to secondary ginsenosides or aglycone [14]. Specifically, ginsenoside Rb_1 was rapidly hydrolyzed to ginsenoside Rd, which was then largely deglycosylated to ginsenoside F_2 rather than ginsenoside Rg_3 [16]. Ginsenoside F_2 formation from Rd is the rate-limiting step, and this further hydrolyzed Compound K (C-K), a major deglycosylated metabolite readily absorbed into the systemic circulation following consumption of ginseng related products [17].

Quantitative structure-activity relationship between the number and the position of sugar moieties and inhibitory capacity against tumor growth demonstrated that anticancer activity significantly increased with a decreased sugar number [18]. Ginsenoside Rb_1 and Rd with a sugar number of 4 and 3 exhibit no significantly cytotoxicity against cancer cells. In contrast, ginsenoside Rg_3 (two sugar), Rh_2 (one sugar at C-3), C-K (one sugar at C-20) and PPD (no sugar) showed more potent anticancer activity, and now ginsenoside Rg_3 and Rh_2 have been widely used for the adjunct therapy of cancer [19]. Decreased number of sugar can not only affect the anticancer activity [12], but also impact their intestinal absorption due to enhanced membrane permeability but poorer solubility [20]. Colonic β-D-glucosidase mediated hydrolysis of ginsenoside Rb_1 plays a crucial role in its pharmacokinetic and metabolism [13]. Not surprisingly, a dysregulated gut microbiota may affect the β-D-glucosidase activity, leading to altered metabolism and pharmacokinetic of ginsenoside Rb_1, ultimately changes in the biological activity from ginsenoside Rb_1 [21]. Nevertheless, the actual metabolism and pharmacokinetic changes under different gut microbial dysbiosis statues need to be well clarified.

In this study, we aimed to systemically investigate the effect of gut microbial dysbiosis on the pharmacokinetics and colonic metabolism of ginsenoside Rb_1. An obvious characteristic of stress-related psychiatric disorders is a dysregulated gut microbiota. Animal study revealed that chronic restraint stress obviously affect the microbiota composition with a reduction of the relative abundance of *Allobaculum*, *Bifidobacterium*, *Turicibacter*, *Clostridium* and the family *S24-7*, and *Lachnospiraceae* increased significantly when comparing with normal animal [22]. Oral administration of non-absorbable antimicrobials mixture (neomycin, bacitracin, and pimaricin) may also affect the microbial composition, and have become a validated gut microbiota dysbiosis animal model [23]. Thus, in this study, comprehensive and comparative colonic deglycosylation metabolism and pharmacokinetics of ginsenoside Rb_1 were conducted in normal, ATMs treated and restraint stressed rats. Moreover, the *in vitro* deglycosylation metabolism of ginsenoside Rb_1 and β-D-glucosidase activity in different group were also studied.

2. Materials and methods

2.1. Chemicals and reagents

Ginsenoside Rb_1, Rd, F_2, Rg_3, C-K and Rh_2 (purity > 98.0%) were obtained from the College of Chemistry, Jilin University (Changchun, China). Digoxin (internal standard, IS) (purity 99.0%) was purchased from the National Institute for the Control of Pharmaceuticals and Biological Products (Beijing, China). Non-absorbable antimicrobials mixtures (ATMs) including Bacitracin (60 Units/mg), pimaricin (purity 95%) and neomycin (purity 98%) were purchased from Aladin Ltd. (Shanghai, China). P-nitrophenyl-β-D-glucopyranoside was purchased from Sigma Chemicals (St.Louis, MO, USA). HPLC-grade methanol and acetonitrile were purchased from Merck (New Jersey, USA). Other chemicals were all of analytical grade.

2.2. Animal model

Male Sprague-Dawley (SD) rats (8 weeks, 180–220 g) were purchased from the Laboratory Animal Center of Nantong University (Certificate No. SCXK-2009-0002) and acclimated to the laboratory environment for 1 week. The rats were kept in controlled environment of temperature (23 ± 1 °C) and relative humidity (50 ± 5%) with 12 h light/darkness cycle. All animals were provided free access to food and water and the animal studies were conducted in accordance with protocols approved by the Animal Ethic Committee of Nanjing University of Chinese Medicine. The SD rats were randomly divided into three groups: normal control, ATMs treated, and restraint stress group with 12 SD rats in each group. 6 rats in each group were assigned for pharmacokinetic study, the other rats were used for urine and fecal excretion study.

For ATMs treated animal model, SD rats orally received a mixture of non-absorbable ATMs, including neomycin (500 mg/kg), bacitracin (500 mg/kg), and pimaricin (0.125 mg/kg) once daily for 7 days. The following experiments were performed 1 day after the final administration.

Stressed rat were subjected to repeated episodes of restraint stress under a bright light for a period of 2 h for 7 consecutive days using a transparent cylinder (7.0 cm diameter, 18 cm long). Rats were under a full rodent immobilization and deprivation of food and water during the 2 h stress period. The following experiments were performed on the 8^{th} day.

2.3. Validation of the animal model

Body weight of each rat in different groups was recorded and fresh stool samples were collected for the determination of fecal moisture. Stool samples were placed in a 105 ± 5 °C oven until constant weight. Then the moisture content of the sample was calculated as the ratio of sample weight loss to initial weight.

The stool samples collected at the 7^{th} were also subjected to analysis the short chain fatty acids (SCFAs) using a validated gas chromatography (GC) method with minor modifications [24]. Briefly, 0.1 g fresh stool sample was suspended and vortexed in 0.5% phosphoric acid for 5 min, then centrifuged at 18000 *g*. An aqueous of 1 mL ethyl acetate was added to fecal suspensions to yield high extraction efficient. After vortex for 2 min, the mixture was centrifuged for 10 min at 18000 *g*. The organic phase was transferred and 4-methyl valeric acid was added at a final concentration of 40 μg/mL. Supernatant was analyzed using a chromatographic system Agilent 6890 GC system equipped with a flame ionization detector (FID). Separation was achieved using a capillary column fitted with FFAP (30 m, 0.25 mm id, 0. 5 μm film thickness, Nanjing Jianuo Technologies Inc.). Initial oven temperature was 90 °C and was kept there for 2 min and then raised to 150 °C by 15 °C/min, then raised to 230 °C by 20 °C/min and kept for 1 min. The injected sample volume for GC analysis was 0.2 μL, and the running time for each analysis was 12 min.

2.4. Pharmacokinetics of ginsenoside Rb_1 following oral administration

For oral administration, rats were fasted for 12 h before the experiment. The rats were administrated with an oral dose (80 mg/