Gut microbiota in the pharmacokinetics and colonic deglycosylation metabolism of ginsenoside Rb1 in rats: Contrary effects of antimicrobials treatment and restraint stress

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Abstract

Ginsenoside Rb1, 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 Rb1, a comprehensive and comparative study of colonic deglycosylation metabolism and systemic exposure of ginsenoside Rb1 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 Rb1 and its deglycosylation metabolites (Rd, F2, and C-K) were studied. Moreover, the in vitro metabolism of Rb1 in fecal suspension and the fecal b-D-glucosidase activity were probed. Systemic exposure of the deglycosylation metabolites of ginsenoside Rb1 (F2, C-K) were significantly higher in restraint stressed rats, but ATMs treated rats exhibited a decreased plasma levels of F2 and C-K, compared with normal rats. Further studies illustrated that altered systemic Rb1 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 b-D-glucosidase, in vitro elimination of Rb1, and formation of these deglycosylation metabolites afforded further evidence for the in vivo data. In conclusion, the dysregulated fecal b-D-glucosidase activity and deglycosylation metabolism may contribute to the altered pharmacokinetic of ginsenoside Rb1 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 stature.

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 Rb1, 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 Rb1 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 Rb1 is relatively low (around 0.1%) due to poor intestinal absorption and extensive
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 Rb1 in vitro and in vivo [13]. Notably, considerable colonic deglycosylation of the ginsenoside Rb1 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-xilosidase [15]. Through cleavage of sugar moieties by β-D-glucosidase, ginsenoside Rb1 underwent stepwise hydrolysis to secondary ginsenosides or aglycone [14]. Specifically, ginsenoside Rb1 was rapidly hydrolyzed to ginsenoside Rf, which was then largely deglycosylated to ginsenoside F2 rather than ginsenoside Rg3 [16]. Ginsenoside F2 formation from Rb1 is the rate-limiting step, and this further hydrolyzed Compound K (C-K), a major deglycosylated metabolite readily absorbed into the systemic circulation following consummation 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 Rb1 and Rd with a sugar number of 4 and 3 exhibit no significantly cytotoxicity against cancer cells. In contrast, ginsenoside Rg3 (two sugar), Rb2 (one sugar at C-3), C-K (one sugar at C-20) and PPD (no sugar) showed more potent anticancer activity, and now ginsenoside Rg3 and Rb2 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 Rb1 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 Rb1, ultimately changes in the biological activity from ginsenoside Rb1 [21]. Nevertheless, the actual metabolism and pharmacokinetics changes under different gut microbial dysbiosis statuses 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 Rb1. 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 Rb1 were conducted in normal, ATMs treated and restraint stressed rats. Moreover, the in vitro deglycosylation metabolism of ginsenoside Rb1 and β-D-glucosidase activity in different group were also studied.

2. Materials and Methods

2.1. Chemicals and reagents

Ginsenoside Rb1, Rd, F2, Rg3, C-K and Rh2 (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 8th 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 7th 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 Rb1 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/
kg) of ginsenoside Rb1 in 0.9% saline. The dosage was selected as previously described with minor modifications [25,26], and this dose could give more comprehensive plasma concentration time profiles of ginsenoside Rb1 and its deglycosylation metabolites [14]. Blood samples (120 µL) were collected under light ether anesthesia via the orbital sinus with heparinized tubes at the time points of 0, 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 6, 8, 12, 24, 36, 48 and 72 h. All blood samples were immediately centrifuged at 5000 g for 10 min and 50 µL supernatants were transferred and stored at −80 °C until analysis. Non-compartmental analysis using DAS 3.2.6 software (Chinese Pharmacological Association, China) was employed and the pharmacokinetic parameters were calculated from the plasma concentration-time data of each rat.

2.5. Excretion study

To collect urinary and fecal samples, rats were housed individually in metabolic cages during the period of 0–72 h after oral administration of ginsenoside Rb1 (80 mg/kg). Urine and fecal samples were collected at intervals of 0–3 h, 3–6 h, 6–12 h, 12–24 h, 24–48 h, and 48–72 h after dosing. Aliquots of urine and fecal samples were stored at −80 °C until analysis.

2.6. Metabolism of ginsenoside Rb1 in fecal suspension

The fresh feces of each rat were separately collected from the three groups at the 7th day. Feces were carefully vortexed and

Fig. 1. Stepwise deglycosylation of ginsenoside Rb1 by gut microbiota. Glc, β-D-glucose.
suspended with 9 vol (v/m) ice-cold phosphate buffer solution (PBS). After centrifugation at 500 g for 5 min at 4 °C, the supernatant (100 µL) was collected and transferred to a new test tube. Ginsenoside Rb1 was added and the final concentration of ginsenoside Rb1 was 20 mM by adding ice-cold 0.1 mM PBS to a final volume of 200 µL. The mixture was incubated in shaking water bath at 37 °C for 0, 5, 10, 20, 30, 45 min, 1, 1.5, 2, 4, 8, and 12 h. The mixture was immediately applied to sample preparation and the concentration of ginsenoside Rb1 and its metabolites were determined using a validated UPLC-MS/MS method.

2.7. UPLC-MS/MS analyses of ginsenosides in biological samples

Validated bioanalytical methods developed in our group were used to measure ginsenoside Rb1 and related metabolites in biomatrices using a UPLC-MS/MS method [14]. Briefly, ginsenoside Rb1 and its deglycosylation metabolites were extracted with water-saturated n-butanol: ethyl acetate (3:1, v/v) from various biological samples, including plasma, urine, fecal stool, and in vitro incubation mixture. Analytes were separated using an Acquity™ UPLC BEH C18 analytical column (50 mm × 2.1 mm, 1.7 µm; Waters, Milford, MA, USA) with water containing 1 mM ammonium formate (A) and acetonitrile (B) in a gradient elution mode in a flow rate of 0.25 mL/min. The elution program was set as follows: 20–35% B at 0–1 min, 35–50% B at 1–4 min, 50–20% B at 4–4.5 min, and hold for 1 min. An Acquity Xevo TQD mass spectrometer with electrospray ionization (ESI) source in negative ion mode and multiple reaction monitoring was used. The transitions from molecular ion to dominant product ion were at m/z 1107.44 → 179.01 for ginsenoside Rb1, m/z 945.33 → 783.22 for ginsenoside Rd, m/z 783.34 → 621.24 for ginsenoside F2 and Rg3, m/z 621.22 → 160.87 for ginsenoside Rh2 and C-K, and m/z 779.18 → 649.11 for digoxin (IS). The Masslynx 4.1. Software (Waters Corporation, Milford, MA) was applied to process data.

2.8. Assay of β-glucosidase activity

Fresh feces (approximately 2 g) of each rat from the three groups after the 0, 1, 3, 5, 7 day treated with and without antibiotic, feces were carefully mixed with a spatula and suspended with 3.8 mL of ice-cold phosphate buffer solution. After centrifuged in 500 g for 5 min at 4 °C, the supernatant were collected and assayed for the β-glucosidase activity as previously described with some modifications [26]. Briefly, 1 mL reaction system consisted of 0.4 mL of 2 mM p-nitrophenyl-β-D-glucopyranoside, 0.4 mL of 0.1 M phosphate buffer, and 0.2 mL of the enzyme solution. The reaction was incubated for 20 min at 37 °C and then stopped by adding 200 µL of 0.5 N NaOH. The reaction mixture was then centrifuged at 3000 g for 10 min, and measured the absorbance at 405 nm.

2.9. Statistical analysis

Pharmacokinetic parameters, including the area under the concentration-time curve (AUC), terminal elimination half-life (T1/2), mean retention time (MRT), apparent volume of distribution (Vz/F) and clearance (CL) were calculated by non-compartmental...
3. Results

3.1. Validation of the gut microbiota dysbiosis in rat models

After oral administration with antibiotic cocktails for 7 days, the body weight was not obviously changed, while the fecal moisture was significantly increased in the antibiotic treated rats compared with that of normal rats at the 3rd and 7th days. On the other hand, rats suffering from restraint stress for 7 consecutive days exhibited much lower body weight gain, while the fecal moisture was not significantly changed.

The SCFAs are beneficial metabolic products of dietary fiber fermented by probiotics and may be changed in the context of dysbiosis. Our study of fecal SCFAs revealed that major SCFAs, including acetic acid, propionic acid, i-butyric acid, n-butyric acid, i-valeric acid, and n-valeric acid, were significantly reduced in these ATMs treated rats (p < 0.01). Also, the propionic acid and n-valeric acid in restraint stressed rats were obviously decreased as compared with the normal rats (p < 0.05 or p < 0.01). These data demonstrated that both two groups exhibit a dysregulated gut microbiota in different extent.

3.2. Pharmacokinetic profiles of ginsenoside Rb1 and its deglycosylation metabolites following oral administration

After oral administration of the ginsenoside Rb1, ginsenoside Rb1, Rd, F2 and C-K could be detected at all or some of the time points. Plasma concentrations-time profiles of ginsenoside Rb1, Rd, F2 and C-K in different groups following an oral dosage of ginsenoside Rb1 (80 mg/kg) were illustrated in Fig. 3 and the main pharmacokinetic parameters were listed in Table 1.

The results revealed that restraint stressed rats exhibit a lower systemic exposure of ginsenoside Rb1 (p < 0.05), as evidenced by significantly decreased Cmax and AUC0-∞, and a higher CLz (p < 0.05) compared with normal rats. However, there was no significant difference in main pharmacokinetic parameters between normal rats and ATMs treated rats. It was also found that restrain stress showed a trend to increase systemic ginsenoside Rd exposure (Table 2), while the ATMs treatment resulted in a significantly decreased systemic exposure of ginsenoside Rd as seen from decreased Cmax and AUC0-1 (p < 0.01 vs. normal rats).

For other deglycosylation metabolites, ginsenoside F2 and C-K could not be detected in the plasma collected from the ATMs treated rats based on our UPLC-MS/MS analysis, which indicated that the plasma concentrations of ginsenoside F2 and C-K were less than 1.61 and 1.03 ng/mL, respectively. However, there was a trend of increase of ginsenoside F2 at 8 and 12 h after oral administration of the ginsenoside Rb1 (with no statistical difference) in restraint stressed rats. However C-K could be detected and the concentration of C-K was higher in restraint stressed rats than that found in normal rats at 8, 12 and 24 h after an oral dosage of ginsenoside Rb1 (p < 0.05 or p < 0.01 vs. normal rats).
3.3. Urine excretion of ginsenoside Rb1 and Rd

UPLC-MS/MS analysis of the urine sample collected at different time points revealed that only ginsenoside Rb1 and Rd could be detected. As shown in Fig. 4A and B, the cumulative excretions of ginsenoside Rb1 in ATMs treated and restraint stressed group were significantly decreased as compared with the normal rats. Concerning urine cumulative excretion of ginsenoside Rd, rats in ATMs treated and restraint stressed group exhibit different changes: there is a trend in the increase of urine excretion in restraint stressed rats, while the urine excretion of ginsenoside Rd was slightly decreased in ATMs treated rats.

3.4. Fecal excretion of ginsenoside Rb1 and its deglycosylation metabolites

The fecal excretion study showed that a large amount of ginsenoside Rb1, Rd, F2, Rg3 and C-K could be detected in stool samples.
ginsenoside Rb1 to C-K was significantly inhibited in ATMs treated rats as evidenced from a lower fecal excretion in contrast to that of the normal rats ($p < 0.05$). In addition, the 0–12 h cumulative fecal excretion of C-K was significantly increased in restraint stressed rats ($p < 0.05$) and the 0–72 h fecal excretion was slightly increased in contrast to normal rats.

3.5. In vitro metabolism of ginsenoside Rb1 in fecal suspension

To further clarify whether $\beta$-o-glucosidase in gut microbiota contribute to the altered pharmacokinetic and metabolism of ginsenoside Rb1 and its deglycosylation metabolites after microbial manipulation, time-dependent ginsenoside Rb1 depletion and its metabolites formation in different fecal suspensions were investigated.

When ginsenoside Rb1 was incubated with the fecal suspension from control and model rats, the clearance of ginsenoside Rb1 in feces from rats treated with ATMs for 7 days was much slower than that from normal rats. In contrast, similar elimination profiles of ginsenoside Rb1 could be seen in normal and restraint-stressed rats (Fig. 6A). Formation of Rd in the incubation systems varied at different time points (Fig. 6B). Rd generation reached the peak at 2 h and then eliminated gradually in normal rats. However, Rd formation in fecal suspension of ATMs treated rats were inhibited.

**Fig. 5.** Cumulative fecal excretion of ginsenoside Rb1 (A) and its main hydrolysis metabolites, including, ginsenoside Rd (B), ginsenoside F2 (C), ginsenoside Rg3 (D) and C-K (E) in normal rats, ATMs treated rats and restraint stressed rats following oral administration of 80 mg/kg ginsenoside Rb1. Data are expressed as mean ± SD. ($n = 6$), * $p < 0.05$, ** $p < 0.01$ vs. normal rats.
during the incubation period \( p < 0.05 \) or \( p < 0.01 \), suggesting that the hydrolysis of ginsenoside Rb1 by \( \beta \)-D-glucosidase was suppressed under ATMs treatment for 7 days. Rd elimination in fecal suspension of restraint stressed rats were significantly different from that in control rats, which may partially result from the formation of F2 from Rd, the rate-limiting step in ginsenoside deglycosylation. Similar results could be seen in other secondary decomposition metabolites, like F2 and C-K (Fig. 6C and D). The \textit{in vitro} formation of ginsenoside F2 and C-K were all inhibited in the ATMs treated rats' fecal suspension when compared with the normal rats \( p < 0.05 \) or \( p < 0.01 \). Interestingly, the formation of the C-K in the first 8 h after incubation with the fecal suspension in restraint stressed rats were much higher than that in normal rats \( p < 0.05 \). These results indicated that the deglycosylation of ginsenoside Rb1 may be increased under restraint stress.

3.6. Fecal \( \beta \)-D-glucosidase activity in normal, ATMs treated, and restraint stressed rats

To directly evaluate the metabolic activity of gut microbiota, we measured the activities of \( \beta \)-D-glucosidase in fecal samples from different groups by using p-nitrophynyl-\( \beta \)-D-glucopyranoside as its substrate. As shown in Fig. 7, the fecal \( \beta \)-glucosidase activity in these ATMs treated rats was significantly lower than that from control rats at the 1st, 3rd, 5th, and 7th day after treating ATM mixtures \( p < 0.05 \) or \( p < 0.01 \). In contrast, in rats suffering from restraint stress, the fecal \( \beta \)-glucosidase activity was significantly increased at the 5th and 7th day after modeling \( p < 0.05 \) or \( p < 0.01 \).
4. Discussion

In this study, we selected ginsenoside Rb1 as a model drug and thoroughly investigated the pharmacokinetic and colonic hydrolysis of ginsenoside Rb1 in two animal models featuring gut microbiota dysbiosis. The main finding of this study was that the systemic exposure of the deglycosylation metabolites of ginsenoside Rb1 was significantly decreased under ATMs treatment, while the systemic exposure of hydrolysis metabolites of ginsenoside Rb1 was significantly increased in rats suffering from restraint stress. By modeling microbial changes in conditions of antibiotic treatment and chronic restraint stress, we provided a detailed illustration of how the pharmacokinetics and metabolism of ginsenoside Rb1 were affected by microbiota dysbiosis. These results may offer valuable insights into the pharmacological changes of bioactive ginsenosides in pathological conditions, and provide important reference for further clinical use of ginseng and related products.

It is well known that the ileum and colon of humans and other animals are colonized by more than a thousand microorganisms named gut microbiota [27]. Gut microbiota not only shaped various host function, like innate immune response, energy and vitamin supplying, growth control [28], but also mediated the metabolism of orally administered nature products, such as ginsenoside, bagatellite, and glycyrrhizic acid [29]. The composition of the gut microbiota is susceptible to the change by pathological conditions and the use of antibiotics [23,29]. Thus, it is important to illustrate the pharmacokinetic and metabolism of these nature products in statues of gut microbiota dysbiosis. Ginsenosides show promise in the treatment of various disease [30], including stress-related disease, diabetes, diarrhea and obesity [8,31]. These diseases all share a common characteristic of gut microbiota dysbiosis, which is another reason why we are interested in the pharmacokinetic and metabolic changes of ginsenosides. Indeed, previous work largely focused on the pharmacological effects of ginsenosides [3,8,32] and the pharmacokinetics and/or metabolism of ginsenosides under normal conditions [14,33], which may not offer reliable information under pathological conditions.

SCFAs, the end products of the microbial fermentation of dietary fiber or resistant starch [27], have a critical role in host metabolic functions and maintaining the intestinal homeostasis. In response to chronic psychological stress or antimicrobial challenges [23,34], the normal gut microbiota may be disturbed and the concentration of SCFAs in caecum colon and rectum may change. Previous study showed that ATMs treatment resulted in a notably decrease in probiotic strains, like proteobacteria and Bacteroidetes, and a significantly increase in Firmicutes and Actinobacteria [23,26]. Also, mice exposed to a prolonged restraint stressor exhibit a lower abundance of Porphyromonadaceae, and an overgrowth of aerobi-cally Gram-positive and Gram-negative bacteria, like Citrobacter rodentium, a typically opportunistic pathogenic [34]. These altered gut microbiota composition may lead to the change of the fecal moisture and SCFAs concentration. In consistence with this rationale, the fecal moisture (Fig. 2B) and the SCFAs (Fig. 2C) both in ATMs treated rats and restraint stressed rats were changed in our study, and future studies are warranted to determine the detailed composition changes of gut microbiota in these two animal models.

Dysregulated gut microbiota may be accompanied by abnormal fecal β-D-glucosidase activity. Based on the critical role of β-D-glucosidase in the colonic metabolism of ginsenoside Rb1, its in vitro incubation of ginsenoside Rb1 with fecal suspension was studied. In our assay of the fecal β-D-glucosidase activity (Fig. 7), the hydrolysis activity of fecal sample suspension was significantly increased in restraint stressed rats but decreased in ATMs treated rats. In terms of the ATMs-treated rat model, long time use of antimicrobials, like lincomycin [26], streptomycin [35] and neomycin [23] could induce a significantly decreased fecal β-D-glucosidase activity. These three non-absorbable ATMs (Bacitracin, pimaricin and neomycin) were selected in our study as these three antimicrobials possess extensive inhibitory effects on most of the gut microbiota existing in the colon [23]. Moreover, since these drugs cannot be absorbed to the systemic circulation [36], potential drug-ginsenosides interactions in hepatic or other organs could be avoided. Fecal suspension in restraint stressed rats caused a significantly increase in the in vitro generation of C-K (Fig. 6D), a main metabolite of ginsenoside Rb1 which could be absorbed to the systemic circulation, also these results are in accordance with the increased fecal β-D-glucosidase activity (Fig. 7) in stool samples in restraint stressed rats. Opposite results were observed in the ATMs treated rats, that is, the in vitro formation of secondary deglycosylation metabolites of ginsenoside Rb1, including ginsenoside Rd, F2, and C-K (Fig. 6), were all inhibited in these rats treated with non-absorbable ATMs. Similar results were reported in rats orally treated with lincomycin at dosages of 0.12 and 4.8 g/kg for 13 days [26].

Decreased fecal β-D-glucosidase activity may lead to a lower generation of the secondary metabolites of ginsenoside Rb1 along the gastrointestinal tract [25,37]. Our results showed that the cumulative fecal excretion of F2, Rg3, and C-K (Fig. 5C, D and 5E) in ATMs treated rats were all decreased, but an increased cumulative fecal excretion of the ginsenoside Rd was observed (Fig. 5B). The decreased intestinal concentration of these deglycosylation metabolites may ultimately result in a decreased intestinal absorption despite that these secondary metabolites were of poor membrane permeability or low solubility. Pharmacokinetic behavior of ginsenoside Rd and other deglycosylation metabolites (Fig. 3B, C and 3D) in ATMs rats provided further evidence that inhibition of the β-D-glucosidase may leading to lower exposure of these deglycosylation metabolites of ginsenoside Rb1, which proved to exhibit more potent pharmacological activity compared with ginsenoside Rb1.

Fecal excretion could partially reflect the gastrointestinal exposure of oral drug or natural products [12]. Fecal excretion of the C-K was increased in restraint stressed rats when compared with that of the normal rats at the period of 0–12 h following oral administration of ginsenoside Rb1. This phenomena may largely result from increased β-D-glucosidase activity (Fig. 7) and generation of C-K (Fig. 6D) in fecal suspensions of the restraint stressed rats. Increased concentrations of these metabolites along the gastrointestinal tract could lead to an increased systemic exposure of ginsenoside Rg3 and Rb1 (Fig. 3C) and C-K (Fig. 3D) in restraint stressed rats. However, the AUC0-t of ginsenoside Rb1 in stressed rats was decreased, but a slight increase was observed in the AUC0-t of ginsenoside Rd. Of note, chronic stress can not only cause a dys-regulated gut microbiota, but also act as a critical player in the up-regulation of the major CYP450s, like CYP3A, CYP2C and CYP2D [38], which may divert the liver metabolism of ginsenoside Rb1 to its oxygenated metabolites as CYP3A4 is the primary isozyme responsible for the metabolism of most of the ginsenosides [39].

5. Conclusions

Our results demonstrated that ATMs treated rats showed a lower systemic exposure of the deglycosylation metabolites of ginsenoside Rb1 following oral administration, while restraint stressed rats showed opposite results. The dys-regulated fecal β-D-glucosidase activity and deglycosylation metabolism may partially explain these contrary phenomena.

Conflict of interest statement

The authors declare no conflict of interest.
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