



ARTICLE

Pretreatment with broad-spectrum antibiotics alters the pharmacokinetics of major constituents of Shaoyao-Gancao decoction in rats after oral administration

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The influence of broad-spectrum antibiotics on the pharmacokinetics and biotransformation of major constituents of Shaoyao-Gancao decoction (SGD) in rats was investigated. The pharmacokinetic behaviors of paeoniflorin (PF), albiflorin (AF), liquiritin (LT), isoliquiritin (ILT), liquiritin apioside (LA), isoliquiritin apioside (ILA), and glycyrrhizic acid (GL), seven major constituents of SGD, as well as glycyrrhetic acid (GA), a major metabolite of GL, were analyzed. A 1-week pretreatment with broad-spectrum antibiotics (ampicillin, metronidazole, neomycin, 1 g L⁻¹; and vancomycin, 0.5 g L⁻¹) via drinking water reduced plasma exposure of the major constituents. The AUC_{0-24 h} of PF and LT was significantly decreased by 28.7% and 33.8% ($P < 0.05$ and $P < 0.005$), respectively. Although the differences were not statistically significant, the AUC_{0-24 h} of AF, ILT, LA, ILA, and GL was decreased by 31.4%, 50.9%, 16.9%, 44.1%, and 37.0%, respectively, compared with the control group. In addition, the plasma GA exposure in the antibiotic-pretreated group was significantly lower ($P < 0.005$) than the control group. The in vitro stability of the major constituents of SGD in the rat intestinal contents with or without broad-spectrum antibiotics was also investigated. The major constituents were comparatively stable in the rat duodenum contents, and the biotransformation of GL mainly occurred in the rat colon contents. In summary, broad-spectrum antibiotics suppressed the absorption of the major constituents of SGD and significantly inhibited the biotransformation of GL to GA by suppressing the colon microbiota. The results indicated a potential clinical drug–drug interaction (DDI) when SGD was administered with broad-spectrum antibiotics.

Keywords: Shaoyao-Gancao decoction; broad-spectrum antibiotics; pharmacokinetics; drug–drug interaction; intestinal microbiota.

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INTRODUCTION

Traditional Chinese medicine (TCM) is a crucial part of medical science worldwide, particularly in Asian countries. Shaoyao-Gancao decoction (SGD) is a well-known TCM prescription composed of *Radix Paeoniae Alba* and licorice in a 1:1 ratio; it is widely used in clinical settings to treat pain, including headaches, inflammatory pain [1], and abdominal spasmodic pain [2]. SGD also has remarkable curative effects in patients with muscle cramps [3] and peptic ulcers [4]. In addition, SGD exerts significant anti-inflammatory and analgesic effects on an arthritis rat model [5] and shows potential suppressive effects on the inflammatory response following cerebral ischemia-reperfusion injury [6]. The main constituents of SGD include paeoniflorin (PF), albiflorin (AF), liquiritin (LT), isoliquiritin (ILT), liquiritin apioside (LA), isoliquiritin apioside (ILA), and glycyrrhizic acid (GL) [7–9] (Fig. 1). These constituents are also regarded as the dominant phytochemicals in the quality control of SGD.

The intestine is considered a major organ for the absorption of orally administered TCM [10–12]. Meanwhile, the intestinal

microbiota plays an important role in the metabolism of chemicals, particularly glycosides [13–15]. In general, all major constituents of SGD possess glucose units, which are susceptible to hydrolysis by the β -glucosaccharase expressed by intestinal microbiota [16, 17]. Moreover, due to the wide applications of SGD in treating inflammatory responses and infectious diseases, it is likely to be co-administered with antibiotics as an adjuvant therapy. SGD may be combined with amoxicillin and metronidazole for the clinical treatment of peptic ulcers and excessive *Helicobacter pylori* [18–20]. Therefore, the drug–drug interactions (DDIs) of SGD and antibiotics during combination therapy should be clarified.

However, no systematic study examining the influence of broad-spectrum antibiotics on the pharmacokinetics of major constituents of SGD after oral administration has yet been published. In the present study, we analyzed the pharmacokinetic fates and biotransformation processes of the major constituents of SGD in rats pretreated with broad-spectrum antibiotics or not, to investigate the compatibility of broad-spectrum antibiotics with SGD.

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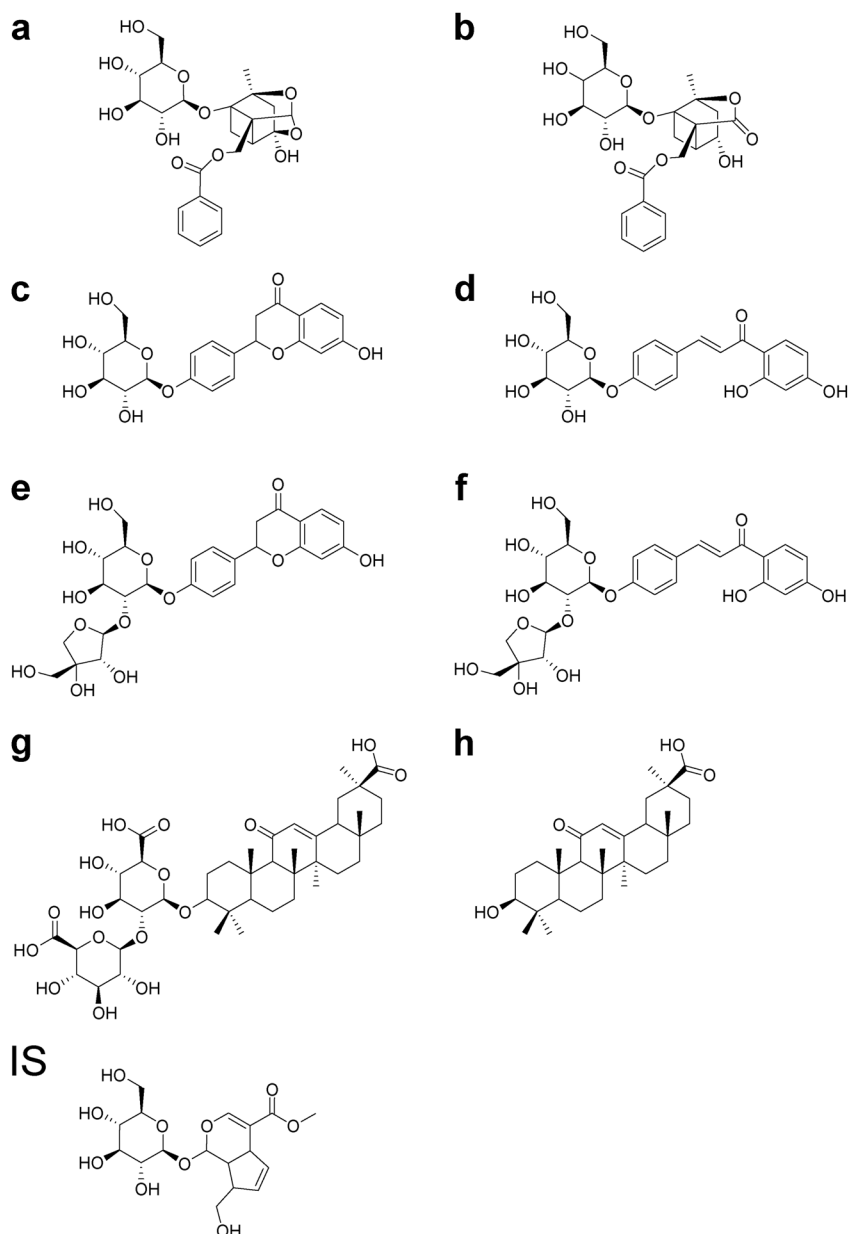


Fig. 1 Chemical structures of the analytes and IS. **a:** Paeoniflorin (PF); **b:** albiflorin (AF); **c:** liquiritin (LT); **d:** isoliquiritin (ILT); **e:** liquiritin apioside (LA); **f:** isoliquiritin apioside (ILA); **g:** glycyrrhizic acid (GL); **h:** glycyrrhetic acid (GA); IS: geniposide

MATERIALS AND METHODS

Chemicals and reagents

Radix Paeoniae Alba (the roots of *Paeonia lactiflora*, RP, 161227) and licorice (the roots of *Glycyrrhiza uralensis*, GU, 161031) were purchased from Tongren Tang Chinese Medicine Co. Ltd. (Shanghai, China). PF (purity $\geq 99.1\%$, ST00700120MG), AF (purity $\geq 98.0\%$, ST03830120MG), LT (purity $\geq 98.0\%$, ST07010120MG), ILT (purity $\geq 98.0\%$, ST04480120MG), LA (purity $\geq 98.0\%$, ST04390120MG), ILA (purity $\geq 98.0\%$, ST18930105MG), GL (purity $\geq 98.0\%$, ST00660120MG), glycyrrhetic acid (GA) (purity $\geq 96.9\%$, ST00710120MG), and geniposide (purity $\geq 99.1\%$, ST03330120MG, internal standard, IS) were purchased from Shanghai Standard Technology Co. Ltd. (Shanghai, China). Methanol (HPLC grade) and formic acid (HPLC grade) were purchased from Merck KGaA (Darmstadt, GER). Phosphate-buffer saline (PBS) was purchased from Corning Cellgro (Manassas, USA). Ultrapure water for HPLC analysis was prepared using a Milli-Q water purification system (Millipore, Milford, USA). Ampicillin and vancomycin were

purchased from J&K (Beijing, China). Metronidazole and neomycin were purchased from Sigma Aldrich (Louis, USA), and all other reagents were of analytical grade.

SGD preparation

Forty grams of RP and 40 g of GU were mixed together and extracted twice by refluxing with 800 mL water for 1 h each time to prepare SGD. After cooling, both extracts were evaporated to dryness at 40 °C under reduced pressure.

The dried SGD was dissolved in 0.5% sodium carboxymethylcellulose (700 mg mL^{-1}) for the pharmacokinetic experiment. Concentrations of the seven constituents in the SGD were quantitatively analyzed using LC-MS/MS. The SGD was stored at 4 °C until further use.

Animals

Male Sprague-Dawley rats ($200 \pm 20 \text{ g}$) were purchased from B&K Universal Group Co. Ltd. (Shanghai, China), with certificate number

2008001682547. All animal use procedures were conducted in accordance with the guidelines of the National Research Council. The experimental protocol was approved by the Institutional Animal Care and Use Committee of the Shanghai Institute of Materia Medica, with permit number 2017-02-YY-05. Animals were maintained in different cages (six animals per cage) and acclimated to the laboratory for at least 1 week prior to the experiment. Animals were housed in a well-lit air-conditioned room at 21 ± 2 °C with 50–60% relative humidity under standard environmental conditions (12 h light–dark cycle) and had free access to standard rodent chow and water prior to the experiment. The animals used in the pharmacokinetic study were fasted for 12 h with free access to water prior to drug administration. For experiments in which intestinal contents were collected, the animals had free access to standard rodent chow and water.

Antibiotic pretreatment

Pharmacokinetic experiments were performed on 12 rats that were randomized into two groups of six animals per group: the control group and the broad-spectrum antibiotic-pretreated group. Animals in the broad-spectrum antibiotic-pretreated group were administered broad-spectrum antibiotics (ampicillin, metronidazole, neomycin, 1 g L^{-1} ; and vancomycin, 0.5 g L^{-1}) via drinking water supplemented with 1% (wt/vol) glucose, as previously reported [21, 22], for 1 week. Control drinking water was prepared in the same manner but lacked broad-spectrum antibiotics. Drinking water was replaced three times a week.

Pharmacokinetic study

Animals in the two groups were orally administered 10 mL kg^{-1} of the SGD, equivalent to 7 g kg^{-1} of the dried SGD. Blood ($40 \mu\text{L}$) from the femoral vein was collected into a heparinized tube 0, 0.083, 0.167, 0.333, 0.5, 1, 2, 4, 8, and 24 h after drug administration. Blood samples were immediately centrifuged at $12000 \times g$ for 5 min at 4 °C to obtain plasma, which was then stored at -20 °C until analysis.

Sample preparation

The plasma sample ($20 \mu\text{L}$) was precipitated with $200 \mu\text{L}$ of methanol containing the IS (20 ng mL^{-1}) and vigorously vortex-mixed for 1 min. After centrifugation at $12000 \times g$ for 5 min at 4 °C, the supernatants from each sample were analyzed using LC-MS/MS.

LC-MS/MS analysis

The assay was performed on an Acquity ultra performance liquid chromatography (UPLC) system (I-class, Waters, Milford, USA) coupled to a triple quadrupole mass spectrometer (Xevo TQ-S, Waters, Milford, USA).

Chromatographic separation was performed using a C18 column ($1.7 \mu\text{m}$, $2.1 \times 50 \text{ mm}$) supplied by Waters at a flow rate of 0.5 mL min^{-1} . Sample or calibration standards ($5 \mu\text{L}$) were injected into the system, and the column temperature was maintained at 45 °C. The autosampler was maintained at 10 °C.

The mobile phase was composed of 0.1% formic acid aqueous (A) and 0.1% formic acid in methanol (B). The following gradient program was used: 0.0–2.0 min, 5–55% (v/v) B; 2.0–3.0 min, 55–95% B; 3.0–3.5 min, 95% B; 3.5–3.8 min, 95–98% B; 3.8–4.3 min, 98% B; and 4.3–4.8 min, 98–5% B.

The mass spectrometer was equipped with an electrospray ionization probe and was operated in negative ion mode. The following operating parameters were used: the capillary voltage was 3.00 kV at a temperature of 500 °C, and the desolvation gas flow was 1000 L h^{-1} . Quantitative analysis was performed by monitoring $[\text{M} - \text{H}]^-$ or $[\text{M} + \text{HCOO}]^-$ for the analytes and IS in multiple reaction monitoring (MRM) mode. The m/z values of precursor and product ions and parameters for voltages are listed in Table 1. All data were acquired in centroid mode and processed using Masslynx 4.1 software (Waters, Milford, USA).

Data analysis

All data from the pharmacokinetic experiments were processed using the pharmacokinetic data analysis software WinNolin 5.2 (Certara, Princeton, USA). The major pharmacokinetic parameters were calculated using a non-compartmental model. Plasma concentrations of the constituents at different time points were expressed as the mean values + the standard deviations (SD), and the mean concentration–time curves were plotted. Statistical analyses were performed using the unpaired *t*-test, as calculated using SPSS 19.0, and the differences with *P* values < 0.05 were considered statistically significant.

In vitro stability study

The intestinal contents were removed from the duodenum and colon of three rats using sterile techniques and placed into separate sterilized tubes. PBS was added to an inoculation ratio of 0.2 g mL^{-1} . Samples were mixed with a vortex mixer, and the inoculant was filtered to remove the large insoluble material before placement in the anaerobic chamber (Don Whitley Scientific, Shipley West Yorkshire, UK).

The rat duodenum and colon contents were each divided into a control group and a broad-spectrum antibiotics group, with triplicate samples for each group. The total incubation volume was $500 \mu\text{L}$, including 0.16 g mL^{-1} intestinal contents and $10 \mu\text{g mL}^{-1}$ analytes. The broad-spectrum antibiotics group was also incubated with 10 mg mL^{-1} ampicillin, neomycin, and vancomycin and 1 mg mL^{-1} metronidazole.

Samples were incubated at 37 °C at 500 rpm under anaerobic conditions. Aliquots were sampled 0, 0.083, 0.167, 0.333, 0.5, 0.75, 1, 2, and 4 h after 10 min of pre-incubation. The reaction was stopped by adding 9 volumes of methanol followed by sonication. After vortexing and centrifugation, the supernatants from each sample were analyzed using LC-MS/MS. The relative levels of the constituents remaining at different time points were expressed as the means + SD, and the mean remaining levels–time curves were plotted.

Table 1. The monitoring ions, m/z values, and voltages of the analytes and IS

Analyte	Monitoring ion	Precursor ion (m/z)	Product ion (m/z)	Cone voltage (V)	Collision voltage (V)
Paeoniflorin (PF)	$[\text{M} + \text{HCOO}]^-$	525.2	121.0	18	22
Albiflorin (AF)	$[\text{M} + \text{HCOO}]^-$	525.2	121.0	18	22
Liquiritin (LT)	$[\text{M} - \text{H}]^-$	417.2	255.1	40	20
Isoliquiritin (ILT)	$[\text{M} - \text{H}]^-$	417.2	255.1	40	20
Liquiritin apioside (LA)	$[\text{M} - \text{H}]^-$	549.2	255.1	70	32
Isoliquiritin apioside (ILA)	$[\text{M} - \text{H}]^-$	549.2	255.1	70	32
Glycyrrhizic acid (GL)	$[\text{M} - \text{H}]^-$	821.4	351.1	80	40
Glycyrrhetic acid (GA)	$[\text{M} - \text{H}]^-$	469.4	425.4	22	40
Geniposide (IS)	$[\text{M} + \text{HCOO}]^-$	433.2	225.1	12	12

RESULTS

Bioanalysis of the major constituents of SGD

The mass spectra of the analytes and IS are shown in Fig. 2. All monitored analytes were separated well, and no interfering peaks

from the endogenous substance were observed within the period in each MRM channel (Fig. 3).

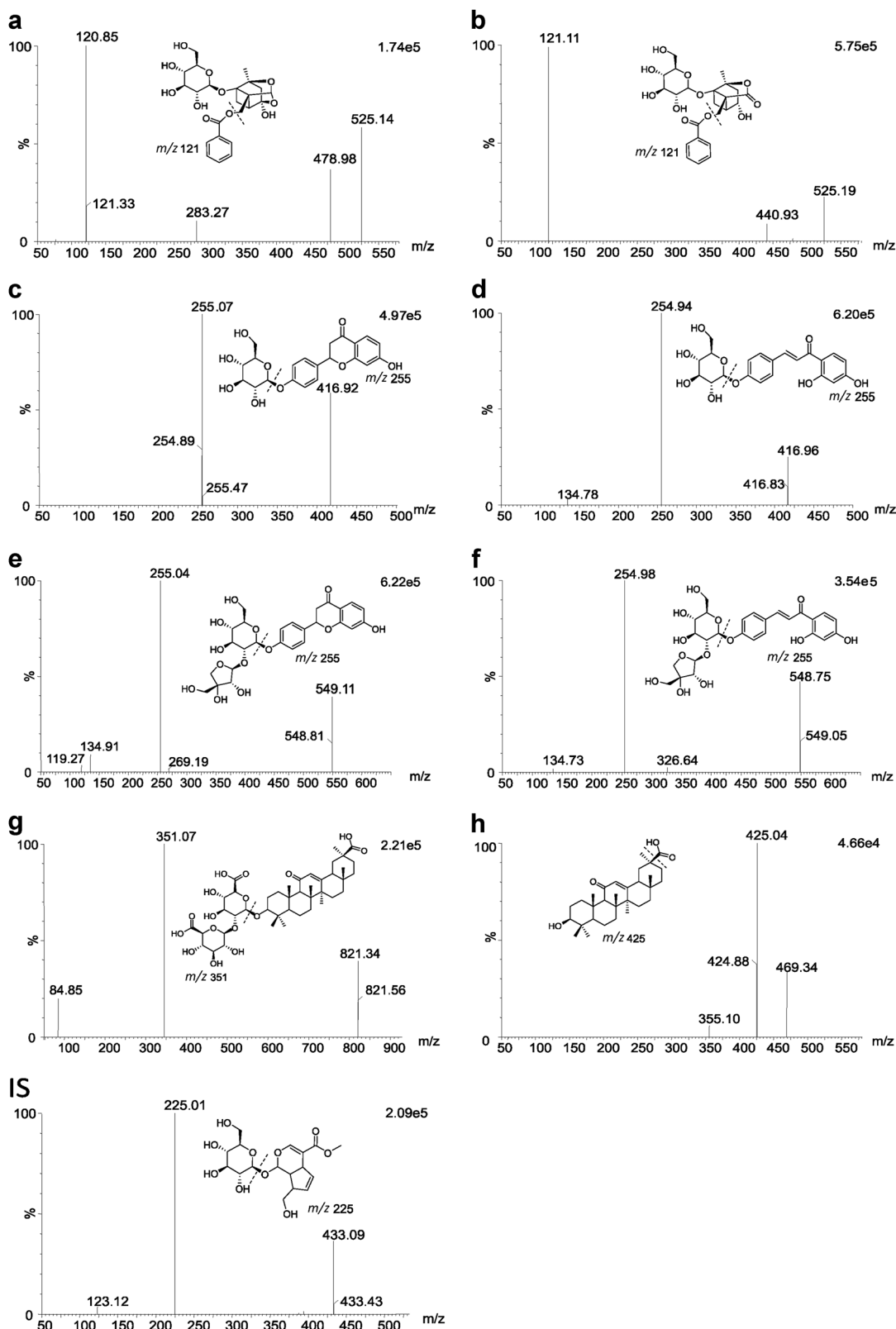


Fig. 2 Mass spectra of the analytes and IS. **a:** PF; **b:** AF; **c:** LT; **d:** ILT; **e:** LA; **f:** ILT; **g:** GL; **h:** GA; IS: geniposide

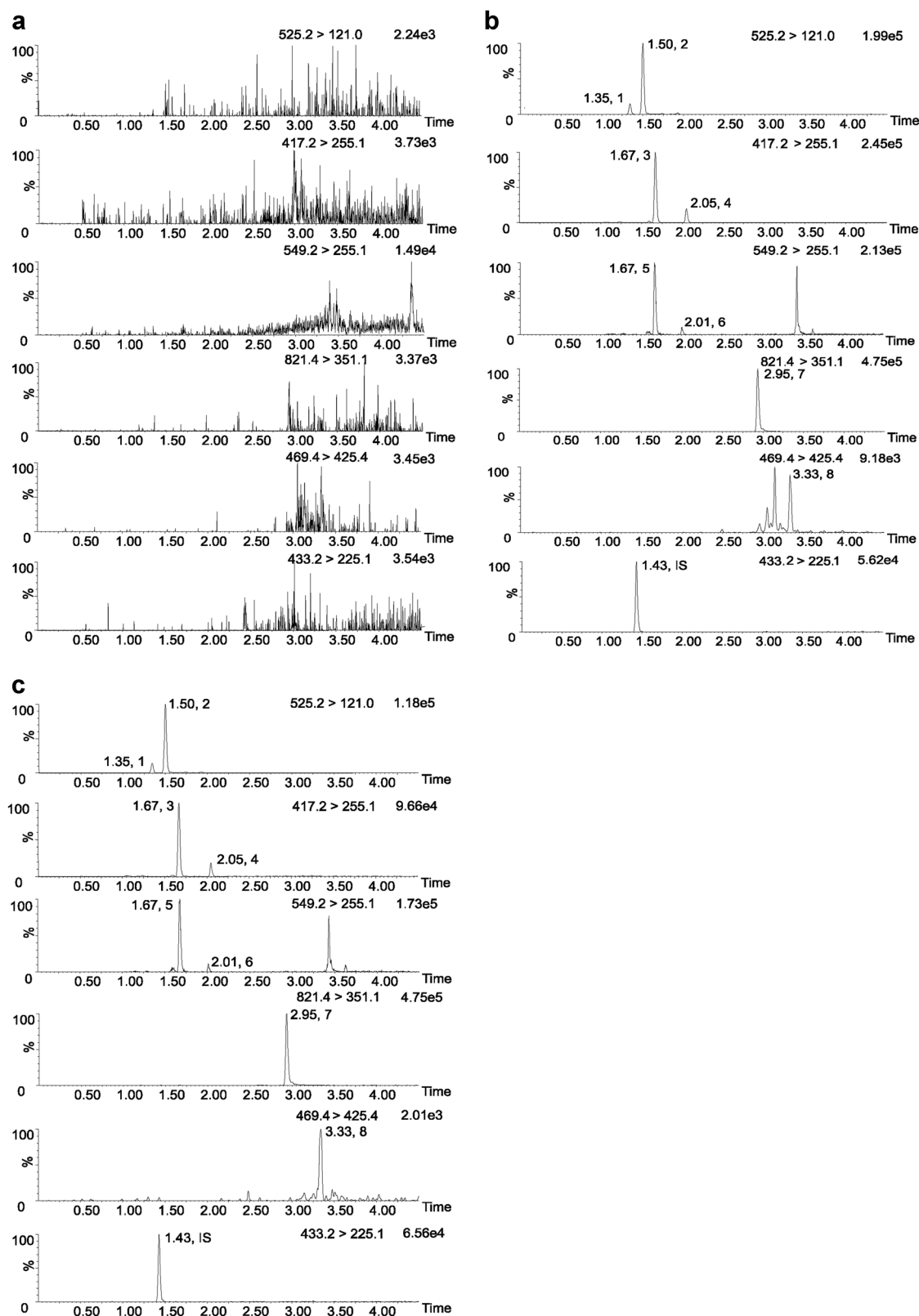


Fig. 3 Representative multiple reaction monitoring chromatograms of **a** blank rat plasma, **b** plasma sample collected from the control group 0.5 h after oral administration of SGD, and **c** the broad-spectrum antibiotic-pretreated group. 1: AF; 2: PF; 3: LT; 4: ILT; 5: LA; 6: ILA; 7: GL; 8: GA; IS: geniposide

Doses of the major constituents in the SGD

In the SGD (700 mg mL⁻¹) used in the pharmacokinetic study, PF was present in the highest percentage at a concentration of 26.9 mg mL⁻¹. GL was present at the second highest percentage at a

concentration of 9.82 mg mL⁻¹. The concentrations of AF, LT, and LA were approximately the same, whereas ILT and ILA concentrations were lower. The doses of the major constituents used in the pharmacokinetic study are shown in Table 2.

Analyte	Dose (mg kg ⁻¹)
PF	269
AF	38.9
LT	45.8
ILT	4.95
LA	47.7
ILA	6.67
GL	98.2

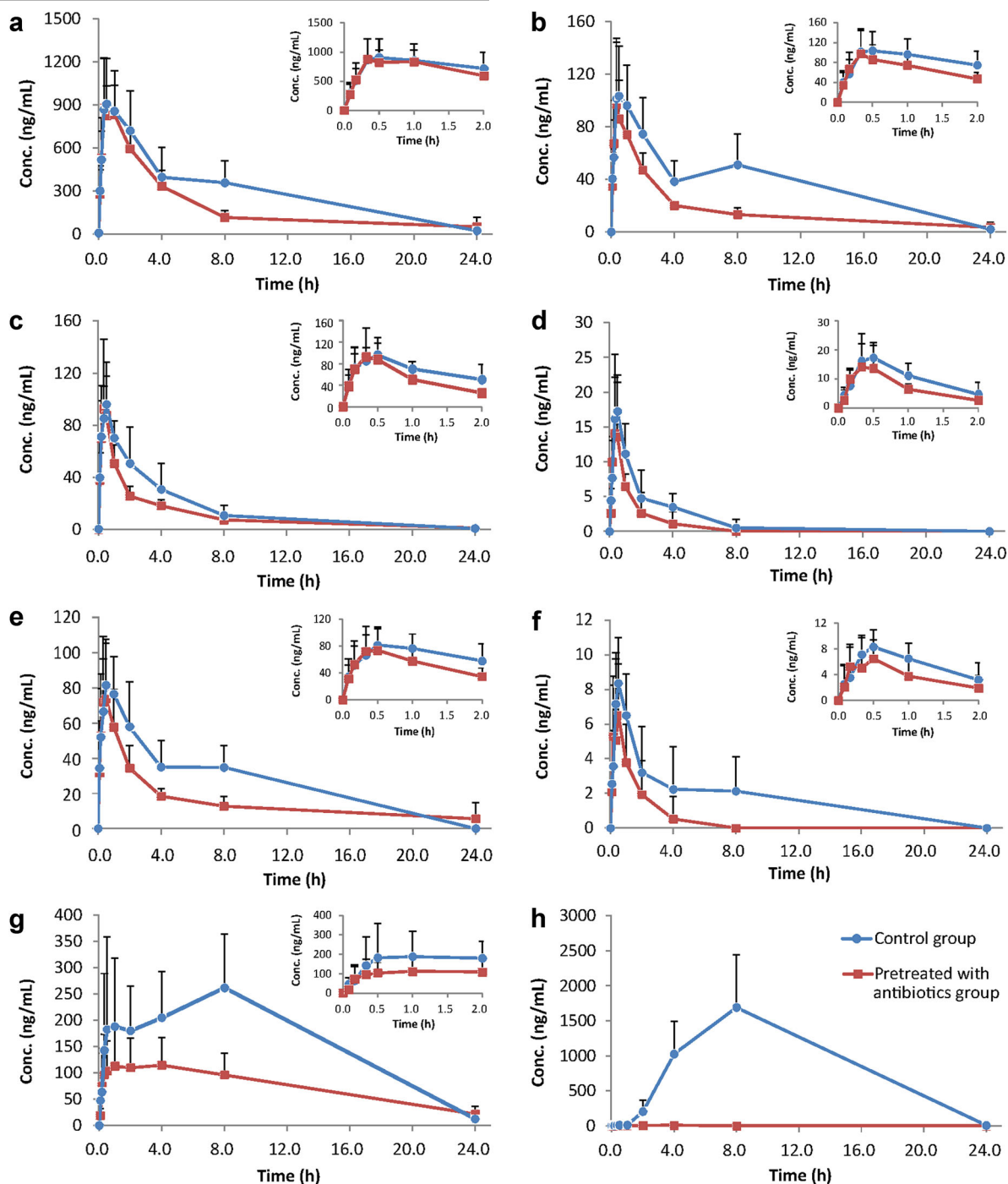


Fig. 4 Mean plasma concentration-time profiles of the analytes in two groups rat plasma after oral administration of SGD. **a:** PF; **b:** AF; **c:** LT; **d:** ILT; **e:** LA; **f:** ILA; **g:** GL; **h:** GA. Each point and bar represent a mean + SD ($n = 6$)

Pharmacokinetic study

The plasma concentration-time curves of the major constituents of SGD, as well as the GL metabolite GA, are plotted in Fig. 4, and the major pharmacokinetic parameters are listed in Table 3. The respective half-lives ($T_{1/2}$) and mean retention times (MRT) were not substantially affected by broad-spectrum antibiotics. However, the plasma exposure of the major constituents was lower in the antibiotic-pretreated group than in the control group. The $AUC_{0-24\text{ h}}$ of PF and LT was significantly decreased by 28.7% and 33.8% ($P < 0.05$ and $P < 0.005$), respectively, compared with that of the control group. Although the differences were not statistically significant, the plasma exposure levels ($AUC_{0-24\text{ h}}$) of AF, ILT, LA,

Table 3. Pharmacokinetic parameters of the analytes in the two groups rat plasma after oral administration of SGD (mean \pm SD, $n = 6$)

Analyte	Pharmacokinetic parameter	Control group	Pretreated with antibiotics group
PF	C_{max} (ng mL ⁻¹)	1411 \pm 1064	1011 \pm 342
	T_{max} (h)	1.03 \pm 1.46	0.767 \pm 0.325
	$T_{1/2}$ (h)	5.63 \pm 1.69	5.67 \pm 2.30
	AUC _{0-24 h} (ng h mL ⁻¹)	5585 \pm 1372	3981 \pm 549*
	MRT (h)	8.20 \pm 2.28	6.57 \pm 2.67
AF	C_{max} (ng mL ⁻¹)	191 \pm 194	106 \pm 43.0
	T_{max} (h)	0.694 \pm 0.645	0.306 \pm 0.125
	$T_{1/2}$ (h)	5.54 \pm 1.25	7.51 \pm 5.66
	AUC _{0-24 h} (ng h mL ⁻¹)	593 \pm 238	407 \pm 181
	MRT (h)	8.54 \pm 1.93	10.8 \pm 6.94
LT	C_{max} (ng mL ⁻¹)	103 \pm 22.3	109 \pm 50.6
	T_{max} (h)	0.611 \pm 0.696	0.300 \pm 0.139
	$T_{1/2}$ (h)	3.49 \pm 1.72	2.48 \pm 0.637
	AUC _{0-24 h} (ng h mL ⁻¹)	296 \pm 50.9	196 \pm 31.6***
	MRT (h)	5.50 \pm 2.83	3.44 \pm 0.963
ILT	C_{max} (ng mL ⁻¹)	17.8 \pm 5.45	16.5 \pm 10.1
	T_{max} (h)	0.444 \pm 0.0862	0.361 \pm 0.125
	$T_{1/2}$ (h)	2.07 \pm 1.35	1.96 \pm 1.59
	AUC _{0-24 h} (ng h mL ⁻¹)	30.3 \pm 15.6	14.9 \pm 6.11
	MRT (h)	3.27 \pm 2.00	3.23 \pm 2.75
LA	C_{max} (ng mL ⁻¹)	88.5 \pm 22.4	81.5 \pm 32.7
	T_{max} (h)	0.917 \pm 0.585	0.472 \pm 0.287
	$T_{1/2}$ (h)	6.07 \pm 2.36	6.10 \pm 4.12
	AUC _{0-24 h} (ng h mL ⁻¹)	379 \pm 136	314 \pm 138
	MRT (h)	9.33 \pm 3.53	9.67 \pm 5.97
ILA	C_{max} (ng mL ⁻¹)	9.28 \pm 2.80	8.75 \pm 2.58
	T_{max} (h)	0.433 \pm 0.149	0.375 \pm 0.159
	$T_{1/2}$ (h)	3.83 \pm 3.90	1.31 \pm 0.258
	AUC _{0-24 h} (ng h mL ⁻¹)	16.6 \pm 14.2	9.28 \pm 3.73
	MRT (h)	5.74 \pm 5.75	2.02 \pm 0.379
GL	C_{max} (ng mL ⁻¹)	278 \pm 146	157 \pm 77.6
	T_{max} (h)	3.67 \pm 3.60	2.33 \pm 3.24
	$T_{1/2}$ (h)	5.32 \pm 1.07	7.00 \pm 1.60
	AUC _{0-24 h} (ng h mL ⁻¹)	2564 \pm 1020	1616 \pm 629
	MRT (h)	8.64 \pm 1.38	10.8 \pm 1.88
GA	C_{max} (ng mL ⁻¹)	1690 \pm 751	11.9 \pm 4.73***
	AUC _{0-24 h} (ng h mL ⁻¹)	11374 \pm 5577	22.4 \pm 11.0***

* $P < 0.05$, *** $P < 0.005$

ILA, and GL were decreased by 31.4%, 50.9%, 16.9%, 44.1%, and 37.0%, respectively, compared with that of the control group. Moreover, the C_{max} of PF, AF, and GL in the antibiotic-pretreated group was reduced by 28.3%, 44.4%, and 43.5%, respectively, compared with that of the control group.

A significant difference ($P < 0.005$) in the plasma exposure of the GL metabolite GA between the two groups was also observed. In the antibiotic-pretreated group, GA concentrations were close to the lower limit of quantitation, whereas the AUC_{0-24 h} of GA was 11374 \pm 5577 ng h mL⁻¹ in the control group. In rats in the control group, GL was largely biotransformed into GA and further absorbed into the systemic circulation. The AUC_{0-24 h} and C_{max} of GA were approximately 4.4-fold and 6-fold greater than GL,

respectively. GA is more lipophilic and more easily absorbed through intestinal epithelial cells, which might result in higher plasma concentrations. However, in antibiotic-pretreated rats, the major constituent in the plasma was the parent drug (GL).

In vitro stability study

Seven major constituents of SGD were incubated with rat intestinal contents to investigate the decomposition and biotransformation of these components by the microbiota of the rat duodenum and colon as well as the effects of broad-spectrum antibiotics. These constituents were comparatively stable in the rat duodenum contents, regardless of the presence of antibiotics. PF, AF, LA, and GL did not exhibit substantial decomposition in the rat duodenum contents within 4 h. In contrast, all of the seven constituents were degraded rapidly and completely in the rat colon contents. The addition of broad-spectrum antibiotics obviously reduced the degradation rates of PF, AF, LA, ILA, and GL in the rat colon contents.

GL exhibited little biotransformation to GA in the rat duodenum contents, regardless of the presence of antibiotics. Nevertheless, obvious biotransformation was observed in the rat colon contents. The amount of GA increased with time and plateaued at approximately 1 h, consistent with the degradation of GL in the rat colon contents. Broad-spectrum antibiotics decreased the biotransformation rate of GL to GA in the rat colon contents (Fig. 5).

DISCUSSION

The absorption, distribution, metabolism, and excretion (ADME) process of drugs is a highly complex process that depends on the physicochemical properties and formulations of the drug, physiological conditions in the body and drug combinations, etc. Antibiotics are some of the most commonly used drugs in combination therapies. Broad-spectrum antibiotics might induce DDI by changing the physiology of the body, such as the physiological function of the intestine, thus affecting the gut inflammatory response [23–25], modulating drug metabolizing enzymes and drug transporters [26, 27], and causing dysbiosis of the intestinal microbiota. In the current study, we focused on the influence of broad-spectrum antibiotics on the glycoside constituents of SGD as well as one of the major metabolites.

One of the most important effects of antibiotics is the alteration of the intestinal microbiota. The microbiota is a crucial component contributing to the intestinal environment [28] and is closely related to glycoside metabolism. It was reported that co-administration of amoxicillin and metronidazole with SGD significantly increased the AUC of PF due to the reduced hydrolysis by the intestinal microbiota [29]. However, in our study, pretreatment with broad-spectrum antibiotics decreased the plasma exposure of the major constituents of SGD in rats, indicating the presence of more complex mechanisms by which antibiotics affect the ADME of SGD.

In our study, the plasma concentration-time curves of PF, AF, LA, ILA, and GL in the control group exhibited double peaks (Fig. 4), which may be attributed to the presence of enterohepatic circulation. However, this phenomenon was not observed in the antibiotic-pretreated group. PF and AF had glucuronic acid-conjugated metabolites in rats [30]. When these constituents are absorbed by the intestine and transported to the liver, they might be converted into glucuronic acid-conjugates and excreted via the bile to intestine. The conjugates would be hydrolyzed by β -glucuronidase produced by the intestinal microbiota in the intestinal tract, and the regenerated parent drugs might be reabsorbed in the intestine. The amount and diversity of the intestinal microbiota would be subsequently suppressed by broad-spectrum antibiotics and further inhibit the β -glucuronidase expression. Therefore, in the antibiotic-pretreated

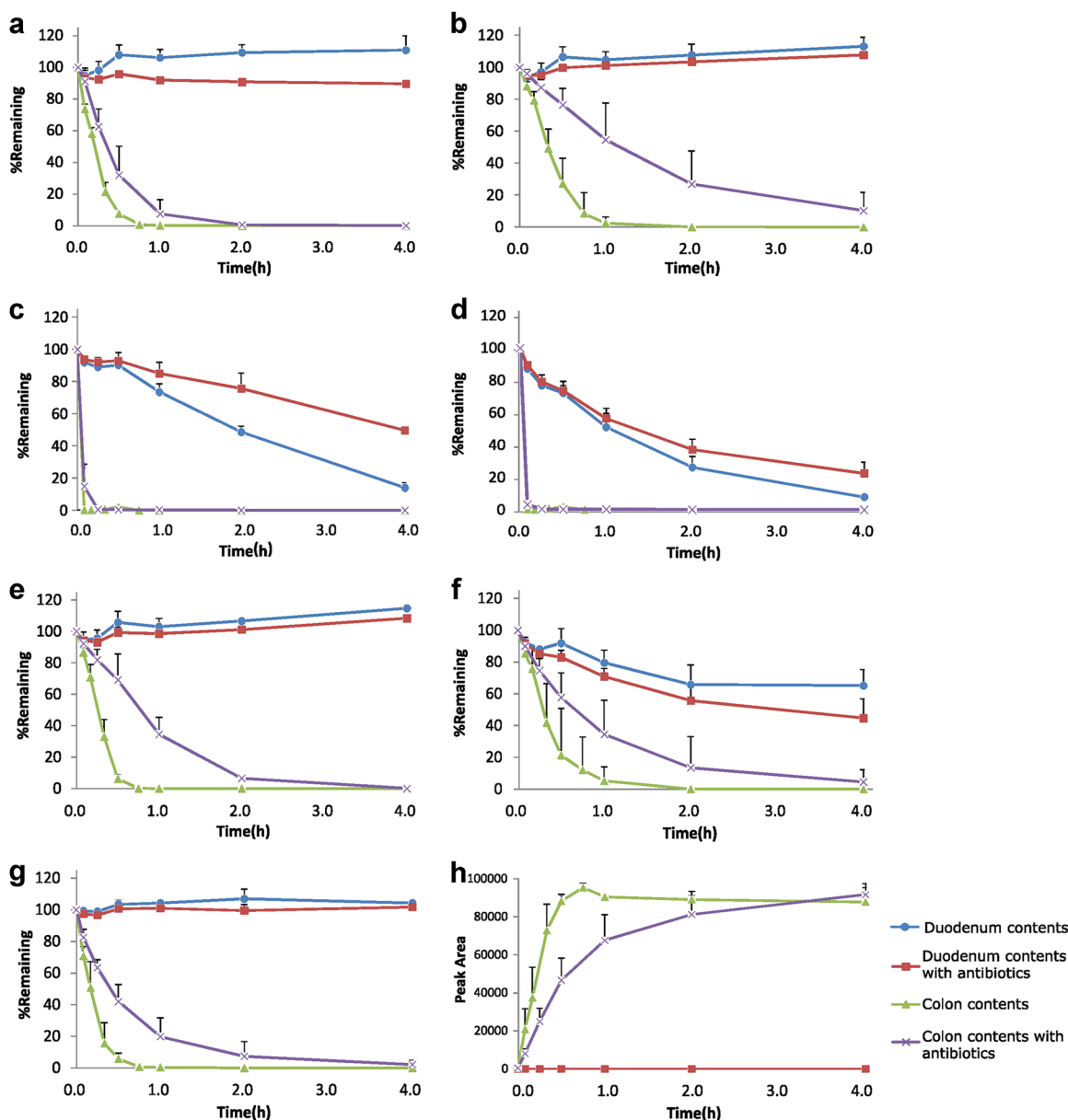


Fig. 5 Mean relative remaining-time profiles of the analytes in rat intestinal contents with or without broad-spectrum antibiotics. **a:** PF; **b:** AF; **c:** LT; **d:** ILT; **e:** LA; **f:** ILA; **g:** GL. **h:** peak area of GA. Each point and bar represent a mean + SD ($n = 3$)

group, enterohepatic circulation was not present, and these animals subsequently showed lower plasma exposure during the elimination phase. This finding might be one of the reasons why plasma exposure of the components was decreased in the antibiotic-pretreated group.

In addition to the effect on the enterohepatic circulation, our in vitro study revealed another possible reason for the decreased plasma exposure in the antibiotic-pretreated group. All seven glycoside constituents showed comparative stability in the rat duodenum contents. The environment of the upper part of the small intestine is more acidic and contains higher levels of oxygen compared with the large intestine, resulting in a lower amount of microbiota. The amount of microbiota in the duodenum is approximately 10 orders of magnitude lower than the amount in the colon [31]. Meanwhile, the small intestine plays the most important role in drug absorption. The duodenum has been reported as the main intestinal segment responsible for the absorption of PF [32]. Therefore, these glycoside constituents were predicted to be affected by the microbiota to a lesser extent

during the major absorption process in the small intestine. Broad-spectrum antibiotics might affect the absorption of these glycoside constituents via other mechanisms that require further investigation.

We also examined the biotransformation of GL. GA, one of the major metabolites of GL, is formed by the intestinal microbiota, including *Eubacterium* and *Streptococcus* [33]. The biotransformation process mainly occurred in the rat colon contents in our in vitro study. Although the broad-spectrum antibiotics were added in a single dose in the in vitro study for only a short time, the rate of GL biotransformation to GA was obviously decreased. This finding correlated with our in vivo result showing that pretreatment with broad-spectrum antibiotics for one week exhibited complete inhibition of the biotransformation of GL to GA. Therefore, broad-spectrum antibiotics reduced the plasma exposure of GA mainly by suppressing the colon microbiota. Although we only assessed the GL metabolite in the current study, this mechanism of antibiotic-induced inhibition may exert similar effects on the other aglycone metabolites of SGD.

Based on the results of the present study, a 1-week pretreatment with broad-spectrum antibiotics via drinking water significantly reduced the plasma exposure of PF and LT and decreased the plasma exposure of the other five constituents of SGD in rats after oral administration. Moreover, the biotransformation of GL to GA was almost completely inhibited by the antibiotic-induced dysbiosis of the colon microbiota. Although some clinical differences may exist because the types of antibiotics and schedules for administering antibiotics differ from humans to rats, the potential DDIs should be interpreted with caution when SGD is co-administered with broad-spectrum antibiotics.

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AUTHOR CONTRIBUTION

J.L., S.Y., and M.L. designed this study; M.L., J.Y., W.-J.H., and C.-Q.K. carried out the experiments; M.L. and W.-J.H. analyzed the data; M.L., J.L., and S.Y. prepared the manuscript; S.Y., J.L., Y.Y., D.-F.Z., Y.-F.Z., and G.-R.Z. critically revised the manuscript. All authors have read and approved the final manuscript.

ADDITIONAL INFORMATION

Competing interests: The authors declare no competing financial interests.

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