Restorative Effect of Repetitive Administration of Shaoyao-Gancao-tang on Bioavailability of Paeoniflorin Reduced by Antibacterial Synthetic Drugs Treatment in Rats

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Paeoniflorin (PF) is an active glucoside in Shaoyao (peony root), and is transformed into an antispasmodic metabolite, paeonimetabolin-I (PM-I), by intestinal bacteria in the gut after oral administration of Shaoyao or Shaoyao-Gancao-tang (SGT, Shakuyaku-Kanzo-To in Japanese). SGT is a pain-relieving traditional Chinese formulation (Kampo-medicine in Japanese) and is often used together with antibacterial synthetic drugs, such as amoxicillin and metronidazole (AMPC-MET), in peptic ulcer therapy. Since the bioavailability of PF in SGT has been reported to be significantly reduced by co-administered antibacterial drugs, we investigated how to minimize this reducing effect of antibacterial treatment in the present study. We found that repetitive administration of SGT starting 24 h after AMPC-MET treatment rapidly restored the plasma PM-I concentration from SGT reduced by AMPC-MET, due to its restorative effect on the decreased PF-metabolizing activity of intestinal bacteria. Since direct determination of the PM-I formed is hard to achieve by HPLC due to the lack of an intense chromophore in PM-I, the PF-metabolizing activity of rat feces was determined by measuring the rate of formation of PM-I possessing a chromophore from PF, which is equivalent to that of PM-I from PF.10) The formation of PM-I from PF is equivalent to that of PM-I from PF.10) The formation of PM-I from PF is equivalent to that of PM-I from PF.10) The formation of PM-I from PF is equivalent to that of PM-I from PF.10) The formation of PM-I from PF is equivalent to that of PM-I from PF.10) The formation of PM-I from PF is equivalent to that of PM-I from PF.10) The formation of PM-I from PF is equivalent to that of PM-I from PF.10) The formation of PM-I from PF is equivalent to that of PM-I from PF.10) The formation of PM-I from PF is equivalent to that of PM-I from PF.10) The formation of PM-I from PF is equivalent to that of PM-I from PF.10) The formation of PM-I from PF is equivalent to that of PM-I from PF.10) The formation of PM-I from PF is equivalent to that of PM-I from PF.10) The formation of PM-I from PF is equivalent to that of PM-I from PF.10) The formation of PM-I from PF is equivalent to that of PM-I from PF.10) The formation of PM-I from PF is equivalent to that of PM-I from PF.10) The formation of PM-I from PF is equivalent to that of PM-I from PF.10) The formation of PM-I from PF is equivalent to that of PM-I from PF.10) The formation of PM-I from PF is equivalent to that of PM-I from PF.10) The formation of PM-I from PF is equivalent to that of PM-I from PF.10) The formation of PM-I from PF is equivalent to that of PM-I from PF.10) The formation of PM-I from PF is equivalent to that of PM-I from PF.10) The formation of PM-I from PF is equivalent to that of PM-I from PF.10) The formation of PM-I from PF is equivalent to that of PM-I from PF.10) The formation of PM-I from PF is equivalent to that of PM-I from PF.10) The formation of PM-I from PF is equivalent to that of PM-I from PF.10) The formation of PM-I from PF is equivalent to that of PM-I from PF.10) The formation of PM-I from PF is equivalent to that of PM-I from PF.10) The formation of PM-I from PF is equivalent to that of PM-I from PF.10) The formation of PM-I from PF is equivalent to that of PM-I from PF.10) The formation of PM-I from PF is equivalent to that of PM-I from PF.10) The formation of PM-I from PF is equivalent to that of PM-I from PF.10) The formation of PM-I from PF is equivalent to that of PM-I from PF.10) The formation of PM-I from PF is equivalent to that of PM-I from PF.10) The formation of PM-I from PF is equivalent to that of PM-I from PF.10) The formation of PM-I from PF is equivalent to that of PM-I from PF.10) The formation of PM-I from PM-I was achieved by incubating PF (2.0 mM) with a suspension prepared from fresh feces at 0.2 g/ml in 50 mM Na-phosphate buffer (pH 7.2) at 37 °C for 20 min. The MeOH-soluble portion of the reaction mixture was analyzed by HPLC at 255 nm to determine the PT-PM-I produced (7σH and 7SR) are biotransformed from Paeoniflorin (PF) by Intestinal Bacteria in Rat Feces with or without Phenylmercaptan.

When SGT is administered orally to rats, PF in SGT is metabolized into PM-I by intestinal bacteria in the gut. The PF-metabolizing rate into PM-I by intestinal bacteria in the rat gut is analogized with that by intestinal bacteria in rat feces. Since direct determination of the PM-I formed is hard to achieve by HPLC due to the lack of an intense chromophore in PM-I, the PF-metabolizing rate into PM-I by intestinal bacteria in rat feces was determined by measuring the rate of formation of PT-PM-I possessing a chromophore from PF, which is equivalent to that of PM-I from PF.10) The formation of PT-PM-I from PF is achieved by incubating PF (2.0 mM) with a suspension prepared from fresh feces at 0.2 g/ml in 50 mM Na-phosphate buffer (pH 7.2) in the presence of phenylmercaptan (5.0 mM), which is equivalent to that of PM-I from PF.
MATERIALS AND METHODS

Materials The Shaoyao and Gancao used were described previously. Their voucher specimens are deposited in the Department of Pharmacognosy, Institute of Natural Medicine, Toyama Medical and Pharmaceutical University. Freeze-dried extracts were prepared from SGT (made of 6 g each of Shaoyao and Gancao), Shaoyao (6 g) and Gancao (6 g) by boiling in water (600 ml) for 40 min as previously described. The yield of the freeze-dried extract of SGT was 4.02 ± 0.05 g (a common human daily dose) containing PF at 39.4 ± 0.5 mg/(g extract) and glycyrrhizin (GL, another bioactive ingredient in SGT) at 63.6 ± 0.7 mg/(g extract). The HPLC fingerprint of SGT was examined to ensure homogeneity, and to prepare batches of formulations as previously described. The yield of the freeze-dried extract of Shaoyao was 2.12 ± 0.03 g containing PF at 86.0 ± 2.6 mg/(g extract) and that of Gancao was 1.95 ± 0.02 g containing GL at 85.4 ± 0.3 mg/(g extract).

The reagents used (AMPC, MET, OFLX, phenylmercaptan, 4-methylumbelliferyl β-D-galactoside, β-galactosidase, goat anti-rabbit IgG and so on) were described previously.

Animals Male Wistar rats (8 weeks old, approx. 250 g) were purchased from Japan SLC Inc., (Hamamatsu, Japan) and maintained in the Laboratory for Animal Experiments, Toyama Medical and Pharmaceutical University. The rats were fed standard laboratory chow with freely available water throughout the study. Animal experiments were carried out in accordance with the guidelines after obtaining permission from the Animal Care and Use Committee of Toyama Medical and Pharmaceutical University approved by the Japanese Association of Laboratory Animal Care.

Administration of Antibacterial Synthetic Drugs and Extracts Antibacterial synthetic drugs were orally administered to rats in a single dose on day 0 at 10-fold the common human daily dose (AMPC, 83.3 mg/kg; MET, 41.7 mg/kg; OFLX, 50 mg/kg). The control group was given tap water instead of antibacterial synthetic drugs. The three extracts were each orally administered twice a day (morning and evening) at total daily doses of 10-fold the common human daily doses (SGT extract, 645 mg/kg equivalent to 25 mg/kg PF and 41 mg/kg G; Shaoyao extract, 290 mg/kg equivalent to 25 mg/kg PF; Gancao extract, 480 mg/kg equivalent to 41 mg/kg GL) according to the specific schedule shown in Figs. 2, 3 and 4.

Determination of Plasma PM-I Concentration Blood samples (approx. 0.24 ml each on days 0 and 14, and 0.12 ml each over days 1 to 13) were collected from the tail vein using heparinized micro capillaries according to the specific schedule shown in Fig. 2. The samples were immediately centrifuged at 3000 rpm for 10 min and the plasma samples thus obtained were stored at −20 °C until analysis. The plasma PM-I concentration was determined as previously reported using an enzyme immunoassay method.

The maximum plasma concentration (Cmax) and the time required to reach Cmax (Tmax) were determined directly from the actual drug levels in the plasma. The elimination rate constant (K) was estimated by linear regression analysis on the terminal portion of the semilogarithmic plot of plasma concentration versus time. The half-life of elimination (t1/2K) was calculated from ln 2/t1/2K. The area under the mean concentration versus time curve from zero to 24 h (AUC0−24h) was calculated using the trapezoidal rule.

Determination of PF-Metabolizing Activity of Intestinal Bacteria in Rat Feces Fecal samples (approx. 0.5 g each) were collected according to the specific schedule shown in Figs. 3 and 4. The PF-metabolizing activity of intestinal bacteria in rat feces was estimated by measuring the rate of formation of 8-phenylthio-paeonimetabolin-I (PT-PM-I, Fig. 1) from PF, which was determined using our previously developed HPLC method. This method is based on the finding that the rate of metabolizing PF into PM-I by intestinal bacteria is equivalent to that of metabolizing PF into PT-PM-I, which is produced by incubating PF with a fecal suspension in the presence of phenylmercaptan (thiophenol).

The recovery time (days) of PF-metabolizing activity was defined as the average number of days from the time when the activity was reduced to 70% or less of the initial level to the time when it recovered to 90% or more. The high-level maintenance time was defined as the average number of days when the activity was maintained at 140% or more of the initial value. The criterion of 140% was chosen based on the percentage of the mean value of the rebound on day 3 (or day 2) relative to the mean initial value, in groups administered

![Fig. 2. Restoration Effect of Repetitive Administration of SGT on Plasma PM-I Concentration Reduced by a Single Administration of AMPC-MET](image-url)
AMPC-MET (or OFLX) alone.

Statistical Analysis Statistical analysis was performed using paired or unpaired two-tailed Student's t-test. Differences were considered statistically significant at $p < 0.05$.

RESULTS

Changes in Plasma PM-I Concentration with SGT and AMPC-MET Administration Figure 2A shows the plasma PM-I concentration–time curve after the first administration of SGT 24 h after the pretreatment with or without (control) AMPC-MET on day 0. Significant reductions in the plasma PM-I concentration were observed in the AMPC-MET-pretreated group.

Figure 2B shows the daily plasma PM-I concentration over days 2 to 13 examined at 4 h after each morning administration of SGT with or without (control) AMPC-MET pretreatment on day 0. In response to the repetitive administration of SGT, the plasma PM-I concentration in the control group on day 2 was similar to the day 1 level (0.80 ± 0.07 μg/ml) and remained constant during the study period. However, the plasma PM-I concentration in the AMPC-MET-pretreated group rapidly increased on day 3 (2.77 ± 0.25 μg/ml, $p < 0.01$), remained significantly high for the subsequent 6 d, and thereafter fell back to the initial level on day 10.

Figure 2C shows the plasma PM-I concentration–time curve after the final administration of SGT on day 14, two weeks after the pretreatment with or without (control) AMPC-MET on day 0. No clear difference in the plasma PM-I concentration was observed between the AMPC-MET-pretreated group and the control group.

Table 1 shows that in the AMPC-MET-pretreated group, the $C_{\text{max}}$ and $AUC_{0–24\text{h}}$ for PM-I from the first dose of SGT were extremely small compared with those of control-1, while those from the final dose were equal to those of control-1. There was no significant difference in the parameters between control-1 and control-14.

Recovery of PF-Metabolizing Activity of Intestinal Bacteria in Rat Feces Reduced by AMPC-MET Figure 3A shows the changes in PF-metabolizing activity of feces in response to the three different treatments. In rats receiving AMPC-MET alone, the activity rapidly decreased to 0.01% of the initial level (0.01 ± 0.01 nmol PT-PM-I/min/g feces, $p < 0.01$) 24 h after the treatment with AMPC-MET. The reduced activity drastically rebounded to 1.4-fold the initial level (32.10 ± 1.81 nmol PT-PM-I/min/g feces, $p < 0.01$) on day 3, but thereafter fell back to a level lower than the initial one from days 4 to 7 before returning to its original level on day 8. In rats receiving repetitive administration of SGT starting 24 h after the AMPC-MET treatment, a similar, but much greater rebound (56.25 ± 3.30 nmol PT-PM-I/min/g feces, 2.8-fold the initial level, $p < 0.01$) was observed. The PF-metabolizing activity stayed at a significantly high level over days 4 to 9 and then returned to its original level on day 10. In rats receiving SGT alone (control), the activity remained constant.

Figure 3B shows the changes in PF-metabolizing activity in response to repetitive administration of Shaoyao or Gancao. The results obtained from administrations of either Shaoyao or Gancao were similar to those from SGT administration. The reduced PF-metabolizing activity was enhanced to peak levels of 46.50 ± 2.29 and 42.37 ± 2.18 nmol PT-PM-I/min/g feces on day 3 by Shaoyao and Gancao treatments, respectively, which were smaller than the rebound level induced by SGT ($p < 0.01$).

Table 2 summarizes the recovery time and high-level maintenance time of the PF-metabolizing activity reduced by AMPC-MET. The recovery time of the activity reduced by AMPC-MET (or OFLX) alone.
AMPC-MET treatment was 8.17 ± 0.48 d, which was significantly shortened by repetitive administrations of SGT (to 2.83 ± 0.17 d), Shaoyao (to 3.00 ± 0.26 d) or Gancao (to 3.17 ± 0.17 d) after the AMPC-MET treatment. The high-level maintenance time (1.33 ± 0.21 d) was also markedly prolonged by the three kinds of repetitive treatments (to 7.33 ± 0.18 d by SGT, 5.67 ± 0.22 d by Shaoyao, and 3.33 ± 0.20 d by Gancao).

**DISCUSSION**

To assure the safety and efficacy of the synthetic drugs and

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**Table 1.** Pharmacokinetic Parameters of Paeonimetabolin-I (PM-I) after the First and Final Oral Doses of Shaoyao-Gancao-tang (SGT) in Rats Pretreated with Amoxicillin and Metronidazole (AMPC-MET)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>After the first dose (on day 1)</th>
<th>After the final dose (on day 14)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SGT with AMPC-MET pretreated&lt;sup&gt;a&lt;/sup&gt;</td>
<td>SGT alone (control-1)</td>
</tr>
<tr>
<td>K (h&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>0.24 ± 0.02</td>
<td>0.25 ± 0.04</td>
</tr>
<tr>
<td>t_{1/2,b} (h)</td>
<td>2.89 ± 0.24</td>
<td>2.72 ± 0.46</td>
</tr>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt; (µg/ml)</td>
<td>5.33 ± 0.47*</td>
<td>3.33 ± 0.40</td>
</tr>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt;%</td>
<td>0.03 ± 0.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.80 ± 0.14</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;C&lt;sub&gt;0&lt;/sub&gt;−24h&lt;/sub&gt; (µg h/ml)</td>
<td>(3.8 ± 0.5)</td>
<td>100%</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;C&lt;sub&gt;0&lt;/sub&gt;−24h&lt;/sub&gt;(%)</td>
<td>0.15 ± 0.02*</td>
<td>4.91 ± 0.69</td>
</tr>
<tr>
<td>Parameters</td>
<td>SGT alone (control-1)</td>
<td>SGT with AMPC-MET pretreated&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>AMPC-MET alone</td>
<td>1.3 ± 0.2</td>
<td>7.3 ± 0.2&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td>AMPC-MET with Shaoyao&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.0 ± 0.3&lt;sup&gt;*&lt;/sup&gt;</td>
<td>5.7 ± 0.2&lt;sup&gt;***&lt;/sup&gt;</td>
</tr>
<tr>
<td>AMPC-MET with Gancao&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
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</tbody>
</table>

Each value represents the mean ± S.E. (<i>n</i> = 6). *<i>p</i> < 0.01 vs. AMPC-MET alone, **<i>p</i> < 0.01 vs. AMPC-MET with SGT; †<i>p</i> < 0.01 vs. AMPC-MET with Shaoyao. a) SGT, Shaoyao and Gancao were each orally administered twice a day (morning and evening) at total daily doses of 645, 290 and 480 mg/kg, respectively, 24 h after AMPC-MET treatment. b) Recovery time for individual rats was the length of the interval from the first day on which the activity was maintained at 90% or more of the initial level to the day the activity fell to 70% or less of the initial level. c) High-level maintenance time for individual rats was the average number of days when the activity was maintained at 140% or more of the initial value. The criterion of 140% was chosen based on the percentage of the mean value of the rebound on day 3 relative to the mean initial value in rats receiving AMPC-MET alone.

**Table 2.** Recovery Time and High-Level Maintenance Time of Paeoniflorin (PF)-Metabolizing Activity in Feces of Rats Pretreated with Amoxicillin and Metronidazole (AMPC-MET)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recovery time (d)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>AMPC-MET alone</td>
</tr>
<tr>
<td>High-level maintenance time (d)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>8.2 ± 0.5</td>
</tr>
<tr>
<td>Groups</td>
<td>AMPC-MET alone</td>
</tr>
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<td></td>
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</table>

Each value represents the mean ± S.E. (<i>n</i> = 6). *<i>p</i> < 0.01 vs. AMPC-MET alone, **<i>p</i> < 0.01 vs. AMPC-MET with SGT; †<i>p</i> < 0.01 vs. AMPC-MET with Shaoyao. a) SGT, Shaoyao and Gancao were each orally administered twice a day (morning and evening) at total daily doses of 645, 290 and 480 mg/kg, respectively, 24 h after AMPC-MET treatment. b) Recovery time for individual rats was the length of the interval from the first day on which the activity was maintained at 90% or more of the initial level to the day the activity fell to 70% or less of the initial level. c) High-level maintenance time for individual rats was the average number of days when the activity was maintained at 140% or more of the initial value. The criterion of 140% was chosen based on the percentage of the mean value of the rebound on day 3 relative to the mean initial value in rats receiving AMPC-MET alone.
traditional Chinese formulations widely used in combination therapies in Japan, pharmacokinetic studies on the drug–drug interactions are of great importance. Although some reports concerning the influence of traditional Chinese formulations on the pharmacokinetics of co-administered synthetic drugs are available, little is known about the effect of the synthetic drugs on the bioavailability of the active ingredients in the traditional Chinese formulations.

Intestinal bacteria play a key role in the bioavailability of some bioactive glycosides in orally administered traditional Chinese formulations, since these glycosides are first metabolized into bioactive metabolites by intestinal bacteria in the gut prior to absorption into the blood. In view of this, synthetic drugs, such as some antibacterial synthetic drugs, which are capable of interfering with the intestinal bacteria, may influence the bioavailability of glycosides in the co-administered traditional Chinese formulations. Therefore, during combination therapies of antibacterial synthetic drugs and traditional Chinese formulations, it is important to examine the influence of the former on the bioavailability of the bioactive glycosides in the latter.

SGT is a famous pain-relieving traditional Chinese formulation, and is often administered together with antibacterial synthetic drugs such as AMPC-MET in clinical situations. In a previous study, we demonstrated that simultaneous oral administration of AMPC-MET with SGT significantly reduced the \( AUC \) of PM-I. PM-I is a bioactive metabolite, derived from PF in SGT by intestinal bacteria and absorbed into the blood. The aim of the present study was to seek an appropriate schedule for the administration of the combination therapy that would minimize the reducing effect of co-administered AMPC-MET on the bioavailability of PF from SGT as much as possible.

As shown in Figs. 2A and B, the reducing effect of AMPC-MET on the plasma PM-I concentration from SGT was still severe even at 2 d after the AMPC-MET treatment. The reductions in the plasma PM-I concentration were comparable with those observed in the previous report, which also demonstrated that the \( AUC \) of the original PF from SGT was inversely increased by co-administration of antibacterial synthetic drugs.

As shown in Fig. 2B, in response to repetitive administration of SGT, the plasma PM-I concentration reduced by AMPC-MET rapidly recovered to a dramatically high level on day 3, remained significantly high over the subsequent 6 d, and thereafter returned to the initial level on day 10. Since PM-I possesses anticonvulsant and muscle relaxant activities, the increase in the plasma PM-I concentration may be beneficial for the analgesic and antispasmodic therapeutic efficacies of SGT in pain-relieving treatment. Furthermore, this observation also provides pharmacokinetic evidence that may, in part, account for the popular view that traditional Chinese formulations usually take a relatively long time to exert their effects.

To clarify the mechanism of the alterations in plasma PM-I concentrations from SGT observed above, the changes in PF-metabolizing activity of intestinal bacteria in the feces in response to five different treatments were examined. As shown in Fig. 3A, the PF-metabolizing activity of feces was severely decreased by even a single treatment with AMPC-MET, and this reducing effect lasted 8 d although there was a transient rebound on day 3. By repetitive administration of SGT starting 1 d after the treatment with AMPC-MET, the reduced PF-metabolizing activity was enhanced to a significantly high rebound on day 3, remained markedly high until day 9 and then returned to the initial level on day 10. These results are consistent with those of the previous report showing that the \( AUC \) for PM-I was positively correlated with the corresponding PF-metabolizing activity of rat feces, and clarified that the alterations in the plasma PM-I concentrations from SGT were due to changes in the PF-metabolizing activity of the intestinal bacteria.

As shown in Fig. 3B, the restorative effect of SGT on the PF-metabolizing activity was contributed by both Shaoyao and Gancao, although the effect of each of the latter two drugs separately is much weaker \((p<0.01)\) than that of SGT. Regarding the reason for the enhancing effect of Shaoyao, PF in Shaoyao might have served as such a good nutrition source for the intestinal bacteria capable of transforming PF to PM-I that the populations of these intestinal bacteria proliferated. The reason for the increasing effects of Gancao, however, remains unknown.

Although the precise reason is unclear, the occurrence of the transient rebound in the PF-metabolizing activity on day 3 might result from some predominant colonization of intestinal bacteria capable of metabolizing PF to PM-I in the gut of rats after the AMPC-MET treatment. It has been reported that \( Bacteroides fragilis \), a strain possessing potent PF-metabolizing activity, colonized predominantly in the guts of neonates aged 3—4 d, whose intestines were sterile at birth. This may also be the case in rats treated with strong antibacterial drugs that might have rendered the guts of the rats as sterile as those of newborns. However, in the group pretreated with AMPC-MET and administered repetitively with SGT, the PF-metabolizing activity remained at high level for approximately 7 d after the rebound and then returned to the initial level. This happened probably because that the other intestinal bacteria incapable of metabolizing PF grew following the proliferation of the PF-metabolizing bacteria due to SGT, and finally a bacterial balance similar to the initial case was resulted.

As shown in Fig. 4 and Table 3, the restorative effect of repetitive administration of SGT on the PF-metabolizing activity reduced by antibacterial treatment was confirmed by experiments using another antibacterial synthetic drugs, OFLX, which is predominantly used for the treatment of urinary tract infection and sometimes prescribed together with SGT. Compared with AMPC-MET, OFLX showed a similar, but weaker reducing effect on the PF-metabolizing activity of feces, which was probably due to the lower antibacterial activity of OFLX than AMPC-MET.

Further investigations are necessary to clarify the responses of the plasma PM-I concentration and the PF-metabolizing activity to changes in the doses of antibacterial synthetic drugs and SGT. Since the bioavailability of glycyrrhizin (GL), another bioactive glycoside in SGT, has also been reported to be reduced by co-administered antibacterial synthetic drugs, another study on whether or not repetitive administration of SGT after antibacterial synthetic drugs treatment would minimize the negative effect of those drugs on the bioavailability of GL in SGT is in progress.

In summary, the present study investigated how to restore
the bioavailability of PF in SGT reduced by co-administered antibacterial synthetic drugs such as AMPC-MET. Our findings proved that repetitive administration of SGT after the antibacterial synthetic drugs treatment significantly restored the reduced bioavailability of PF from SGT by accelerating the recovery of the PF-metabolizing activities of intestinal bacteria decreased by the antibacterial treatment. The present results suggest that it may be clinically useful to administer SGT repetitively, starting 1 or 2 d after treatment with AMPC-MET and OFLX during their combination therapy, to ensure the spasmolytic and pain-relieving effects of SGT.

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REFERENCES AND NOTES