The Influence of Commonly Prescribed Synthetic Drugs for Peptic Ulcer on the Pharmacokinetic Fate of Glycyrrhizin from Shaoyao-Gancao-tang

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The influence of synthetic drugs prescribed for peptic ulcer on the pharmacokinetic fate of glycyrrhizin (GL) from Shaoyao-Gancao-tang (SGT, a traditional Chinese formulation, Shakuyaku-Kanzo-to in Japanese) was investigated in rats. Co-administration of histamine H2-receptor antagonist (cimetidine) and anticholinergic drug (scopolamine butyl bromide, SBB) with SGT didn’t influence the area under the plasma concentration–time curves (AUC) of glycyrrhetic acid (GA), an active metabolite derived from GL in SGT. The AUC of GA from SGT were significantly reduced by co-administration of synthetic drugs commonly used for peptic ulcer in a triple therapy (OAM), a combination of a proton pump inhibitor (omeprazole) and two antibiotics (amoxicillin and metronidazole). We found that the reduction of AUC in OAM treatment was due to the antibacterial effect of amoxicillin and metronidazole on intestinal bacteria in rat which lead to the decrease of GL-hydrolysis activity. The present study suggests that it may not be a proper way to use triple therapy containing antibiotics simultaneously with SGT for healing of chronic ulcers.

Key words pharmacokinetic; peptic ulcer; traditional Chinese formulation; intestinal bacteria; glycyrrhizin; Shaoyao-Gancao-tang

Recently, combination of synthetic drugs and traditional Chinese formulations is used clinically in Japan. Therefore, it is necessary to examine the interactions between synthetic drugs and traditional Chinese formulations to assure the safety and effective clinical use of such combination therapy. Shaoyao-Gancao-tang (SGT), Shakuyaku-Kanzo-to in Japanese, composed of Shaoyao (Paeoniae Radix) and Gancao (Glycyrrhizae Radix), has been used to relieve pains in abdomen and uterus in traditional Chinese medicine. In Japan, SGT is one of the predominant traditional Chinese formulations clinically used and is widely applied for the treatment of abdominal pain, sometimes together with analgesics and antispasmodics such as anticholinergic drug (scopolamine butyl bromide, SBB). As SGT is also used to promote the healing of peptic ulcer, it may also combine with synthetic antisecretory drugs as a histamine H2-receptor antagonist (cimetidine) and triple therapy using OAM, a combination of a proton pump inhibitor (omeprazole, OPZ) to reduce gastric acid secretion and two antibiotics (amoxicillin, AMPC and metronidazole, MET) to remove Helicobacter pylori.

In this study, the influence of such synthetic drugs on the pharmacokinetic fate of glycyrrhizin (GL) from SGT was investigated. GL is the main and active constituent of Gancao in SGT and also SGT with various pharmacological effects. It is well known that orally taken GL is hydrolyzed into glycyrrhetic acid (GA) by intestinal bacteria, and the active metabolite GA is absorbed into blood stream. Therefore, the pharmacokinetic parameters for SGT were evaluated mainly from the area under the concentration–time curve (AUC) of GA, since GA is effective metabolite for peptic ulcer. Furthermore, to clarify the changes of GL-hydrolysis activities in rats administered with synthetic drugs, transforming rates of GL to GA were examined by incubating GL with the rats feces. Although some pharmacokinetic studies on SGT have been reported, no evidence about the influence of the current administration of synthetic drugs on the pharmacokinetic fate of GL from SGT is available yet. As compared to those papers concerning the influences of some traditional Chinese formulations on the bioavailability of synthetic drugs, this study is designed to examine the influences of synthetic drugs on the pharmacokinetic fate of an active constituent in traditional Chinese formulation. The purpose of this biopharmaceutical study is to examine whether it is proper or not to use synthetic drugs prescribed for peptic ulcer together with SGT.

MATERIALS AND METHODS

Shaoyao, Gancao, SGT and Chemicals Each crude drug in SGT, Shaoyao (Paeoniae Radix produced in Japan: Yamato-Shakuyaku in Japanese) and Gancao (Glycyrrhizae Radix: Dongbei-Gancao imported from China, Touhoku-Kanzo in Japanese), a Japanese Pharmacopoeia XIII standard, were purchased from Tsumura & Co. (Tokyo Japan). A voucher specimen is deposited in the Department of Pharmacognosy, Toyama Medical and Pharmaceutical University. The extract of SGT (6 g each of Shaoyao and Gancao) were prepared by boiling in water (600 ml) for 40 min, filtered and freeze-dried into powder (yield: 4.00±0.06 g which is human common daily dose).

GL, GA, liquiritin and OPZ purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan), SBB and AMPC purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.), clarithromycin (CAM) purchased from Taisho Pharmaceutical Co., Ltd. (Tokyo), cimetidine and MET purchased from Nacalai Tesque, Inc. (Kyoto, Japan) and Aldrich Chem. Co. (Milw., WI, U.S.A.) were used. All of the other chemicals and solvents used were of analytical and/or HPLC grade.

Pharmacokinetic Investigation 1) Animals: Wistar male rats (8 weeks old, weighing about 250 g) were purchased from Japan SLC Inc., Hamamatsu, Japan and maintained in

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the Laboratory for Animal Experiments, Toyama Medical and Pharmaceutical University. They were given free access to water and standard laboratory chow before experiments.

2) Single Regimen (Single Simultaneous Administration with SGT): Rats were fasted overnight (17 h) prior to SGT oral administration (665 mg/10 ml/kg, equivalent to GL 41 mg/kg, at 10 times common daily single dose of adult human). Synthetic drugs at 10 times common single dose of adult human as cimetidine (50 mg/kg), SBB (3.33 mg/kg), OAM (OPZ: 1.7 mg/kg, AMPC: 83.3 mg/kg, MET: 41.7 mg/kg), AMPC-MET (AMPC: 83.3 mg/kg, MET: 41.7 mg/kg) and CAM-MET (CAM: 41.7 mg/kg, MET: 41.7 mg/kg) were orally administered simultaneously with SGT, respectively.

3) Multiple Regimen (3 d Pre-administration Prior to SGT): Synthetic drugs were given twice a day for three days prior to SGT administration, at same dose as single regimen. On the fourth day, the drugs were singly administered with SGT simultaneously, respectively.

4) Collection of Blood Samples: Blood samples (about 0.3 ml) were collected from the tail vein through heparinized microcapillaries at 1, 2, 4, 6, 9, 12, 18, 24 and 32 h after the last administration, and centrifuged at 3000 rpm for 10 min to obtain the plasma which were stored at −20 °C until analysis.

**Determination of GA in Plasma**

Plasma GA concentration was determined as previously reported. Briefly, the mixture of plasma samples (100 μl) and MeOH (200 μl) was centrifuged at 10000 rpm for 10 min and the resulting supernatant was filtered through a 0.45 μm membrane filter. The filtrate (100 μl) was applied to HPLC shown in the legend of Fig. 1. The analytical conditions were as follows: Mobile phase: CH3CN : 2% AcOH (pH 2.5) from 90 : 10 to 0 : 100 in 60 min). Flow-rate: 1.0 ml/min. Column temperature: 40 °C; UV absorption to display the peak area of GA: 251 nm. The standard curve for determination of plasma GA concentration: Y = 1613.23X, r = 0.999. Detect limit: 16 ng/ml. Recovery: 99.6%.

**Determination of GL Hydrolysis Activity in Rat Feces**

The feces of rat pre-administered with synthetic drugs for three days were collected just before SGT oral administration. GL-hydrolysis enzyme activity of rats feces was measured as described previously. Briefly, the suspension (200 μl) prepared from feces (0.40 g) in 4.0 ml of 0.1 M phosphate buffer (pH 6.5) was added with 2.5 mM GL (50 μl) and incubated at 37 °C for 1 h. The reaction mixture was extracted by 500 μl MeOH and the filtrate was analyzed using the same conditions as GA determination in plasma.

**Data and Statistical Analysis**

The peak plasma concentration \( (C_{\text{max}}) \) and the time to reach \( C_{\text{max}} \) \( (T_{\text{max}}) \) of GA were obtained from the actual data. The \( AUC_{0-32h} \) for GA was calculated for each rat from zero to 32 h by the trapezoidal rule. Student’s \( t \)-test was performed for a comparison of the means.

**RESULTS AND DISCUSSION**

**HPLC-Profile and GL Contents of SGT (Fig. 1)** In order to assure the homogeneity of the formulation and prepare batches of constant formulation, HPLC-profile of SGT was analyzed. The components of Gancao, GL, liquiritin, liquiritin apioside, and of Shaoyao, paoniflorin are demonstrated (Fig. 1). The content of GL in SGT extract was 62.7 ± 1.5 mg/g of extract.

**Combination of Cimetidine or SBB with SGT (Table 1, Fig. 2)** No significant differences in the pharmacokinetic parameters of GA from SGT were observed between the control and cimetidine-treated groups (Table 1). Although in the multiple regimen of cimetidine, the \( AUC_{0-32h} \) was reduced in appearance (Fig. 2), but no significant difference was observed.

However, it is well known that cimetidine is a powerful inhibitor of cytochrome P450, decreasing the metabolism of many drugs and potentially causing toxic plasma concentrations of therapeutic agents such as some oral anticoagulants, beta-blockers, benzodiazepines and so on. Even if cimetidine didn’t show significant influence on the pharmacokinetic fate of GL in the present study, whether it affects some
other components of SGT or not during oral co-administration, still need to be further clarified.

No significant differences in the pharmacokinetic parameters were observed between the control and SBB-treated groups (Table 1). Although orally administered SBB delays gastric emptying and reduces contraction amplitude to influence the metabolism or absorption of other drugs, it didn’t clearly influence the pharmacokinetic fate of GL from the co-administered SGT in this study.

**Combination of OAM, OPZ, AMPC-MET and CAM-MET with SGT (Table 2, Figs. 3—5)** In the single regimen studies, significant decrease in the bioavailability of GA from SGT was observed between the control and OAM-treated groups. The $AUC_{0-32h}$ of OAM-treated group reduced to approximately 16% of the control value (Table 2).

In the multiple regimen studies of OAM, the $AUC_{0-32h}$ of GA was also markedly reduced to that of the control (Fig. 3, Table 2). In order to elucidate the reason for the reducing effects of OAM on the $AUC$ of GA, the same multiple regimen was carried out on OPZ and AMPC-MET, respectively. The results for $AUC$ for OPZ were almost same to those for the control (Fig. 4), while $AUC$ for AMPC-MET significantly reduced (Fig. 5). Therefore, it is clear that the significant reducing effects of OAM on the $AUC$ of GA were caused by the antibiotics, AMPC-MET, not by the proton pump inhibitor, OPZ.

Furthermore, another antibiotics combination, CAM-MET, which is also frequently prescribed together with the proton

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**Table 1. Pharmacokinetic Parameters of GA after Oral Administration of SGT together with Cimetidine and SBB**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Single regimen</th>
<th></th>
<th>Multiple regimen</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (n=40)</td>
<td>With cimetidine (n=12)</td>
<td>With SBB (n=15)</td>
<td></td>
</tr>
<tr>
<td>$C_{\text{max}}$ (µg/ml)</td>
<td>1.26±0.88</td>
<td>1.25±0.74</td>
<td>1.27±1.04</td>
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<tr>
<td>$T_{\text{max}}$ (h)</td>
<td>8.0±1.5</td>
<td>7.8±2.0</td>
<td>8.8±0.8</td>
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</tr>
<tr>
<td>$AUC_{0-32h}$ (µg·h/ml)</td>
<td>11.01±5.84</td>
<td>12.03±5.41</td>
<td>11.21±5.41</td>
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</tr>
</tbody>
</table>

Each value represents the mean±S.D.

**Table 2. Pharmacokinetic Parameters of GA after Oral Administration of SGT together with OAM, OPZ, AMPC-MET and CAM-MET**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Single regimen</th>
<th></th>
<th>Multiple regimen</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (n=40)</td>
<td>With OAM (n=6)</td>
<td>With OPZ (n=6)</td>
<td>With AMPC-MET (n=6)</td>
</tr>
<tr>
<td>$C_{\text{max}}$ (µg/ml)</td>
<td>1.26±0.88</td>
<td>0.27±0.17*</td>
<td>0.03±0.01*</td>
<td>0.05±0.01*</td>
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<tr>
<td>$T_{\text{max}}$ (h)</td>
<td>8.0±1.5</td>
<td>5.8±1.8*</td>
<td>3.3±1.0*</td>
<td>4.7±6.6*</td>
</tr>
<tr>
<td>$AUC_{0-32h}$ (µg·h/ml)</td>
<td>11.01±5.84</td>
<td>1.72±0.94*</td>
<td>0.13±0.08*</td>
<td>0.62±0.14*</td>
</tr>
</tbody>
</table>

Each value represents the mean±S.D. *: p<0.01, significantly different from the control.
pump inhibitor OPZ,\textsuperscript{14} was also examined in the multiple regimen studies. The AUC of GA in the CAM-MET-treated groups seriously decreased when compared to the control (Table 2). Moreover, triple therapy is usually used for one or more weeks,\textsuperscript{15} so it is obvious that its reducing effects on the AUC of GA from the orally administered SGT may become much more intense in real practice.

By the way, weak inhibitory activities on H. pylori of Gancao\textsuperscript{16} and Shaoyao\textsuperscript{16} have been reported. However, antibacterial activities of these crude drugs seem to be not potent enough to substitute for antibiotics, therefore, such traditional Chinese drugs may be co-administered with triple therapy to decrease the emergence of resistant colonies induced by antibiotics and to promote gastric mucosal atrophy.\textsuperscript{17}

Correlation between GL-Hydrolysis Activity in Rat Feces and AUC of GA (Fig. 6) To clarify the mechanism of the effects of the orally co-administered synthetic drugs on the AUC of GA from SGT, GL-hydrolysis activities in rats feces were examined. GL-hydrolysis activities in the OAM and the AMPC-MET-treated rats were 0.01±0.01 and 0.03±0.01 (nmol GA/min/g feces), respectively. On the other hand, that in the OPZ-treated rats was 0.31±0.12, which is similar to that of control rats (0.33±0.11). This evidence can be considered that the intestinal bacteria capable of producing GL-hydrolyzing enzyme had been killed by the co-administered antibiotics. As shown in Fig. 6, a good direct correlation (r=0.80) between the AUC of GA and GL-hydrolysis activity was observed. That is, bigger AUC values of GA corresponded to higher GL-hydrolysis activity in feces.

The results obtained after examining the pharmacokinetic fate of GL from SGT co-administered with commonly prescribed synthetic drugs for peptic ulcer would confirm the following facts.

1. Cimetidine, a histamine H\textsubscript{2}-receptor antagonist and SBB, an anticholinergic drug, didn’t significantly influence the AUC of an active metabolite GA from SGT, in both single (without pretreatment) and multiple (with 3 d pretreatment) regimens (Table 1).

2. Triple therapy using OAM, a combination of OPZ, AMPC and MET, significantly reduced the AUC of GA from SGT, in both single and multiple regimens. The reducing effects of OAM were caused by AMPC-MET, not by OPZ. The similar reducing effects on the AUC of GA were also noticed in the co-administration of CAM-MET, another combination of two antibiotics (Table 2).

3. It is clear that such reducing effects were caused because of co-administered antibiotics, which might have killed the intestinal bacteria capable of producing GL-hydrolyzing enzyme.

4. A good correlation between the AUC values of GA and the GL-hydrolysis activities in rats was observed (Fig. 6).

From the results of the present investigation, it is clear that the production and absorption of GA from GL in SGT was decreased markedly by co-administered antibiotics, but was not clearly influenced by cimetidine or SBB. Therefore, it may not be a proper way to use antibiotics of AMPC-MET or CAM-MET simultaneously with SGT to improve gastric defensive mechanisms in peptic ulcer.

Acknowledgments This work was supported in part by a Grant from Toyama Prefecture. Authors thank to Ms. T. Katsuda (Institute of Natural Medicine) for her technical assistance and Prof. P. Basnet (present address: The School of Pharmaceutical Sciences, Pokhara University, Nepal) for his helpful discussion.

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