Mechanism of Gastrointestinal Absorption of Glycyrrhizin in Rats

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The mechanism of gastrointestinal absorption of glycyrrhizin (GZ) was examined in rats. Using an in situ loop technique, it was found that the intestinal absorption of glycyrrhetic acid (GA), a major metabolite of GZ, was larger than that of GZ and that the absorption of GA was larger in the small intestine than in the large intestine. Although GZ was poorly absorbable, both GZ and GA were detected in rat plasma after oral administration of GZ, suggesting that GZ can be absorbed in both parent and metabolite forms, although their bioavailabilities were low. GZ was hydrolyzed to GA by rat gastric and large-intestinal contents, but not by the small-intestinal contents. This hydrolysis was diminished after boiling of the gastrointestinal contents and was not observed in the gastrointestinal contents of kanamycin-treated rats. These results suggest that GZ is hydrolyzed by bacteria in the stomach and large-intestinal contents and that most of the GA formed is absorbed from the large intestine. Since GZ was extensively excreted in bile after intravenous administration, the first-pass elimination might be the reason for its low bioavailability, in addition to the poor mucosal permeability.

Keywords: glycyrrhizin; glycyrrhetic acid; intestinal absorption; biliary excretion; pseudo-germ-free rat; first-pass elimination effect

Glycyrrhizin (GZ), a glycoside of glycyrrhetic acid (GA), is a natural compound with an intensely sweet taste, and one of the most commonly used herbal drugs in traditional Chinese prescriptions. It is an active ingredient extracted from the root of Glycyrrhiza glabra L. (licorice, Chinese name: Gancao), which contains 2.4–24% of GZ. In addition to its use as a sweetening agent in many food and luxury products such as chocolates, beer, liquor and chewing tobacco, GZ has been used as a medicine in the treatment of chronic hepatitis, allergic disorder, inflammation and gastric ulcers. It has been reported to have an inhibitory effect on the in vitro infective and cytopathic activity of human immunodeficiency virus, and has been responsible for some improvement in immune function and an objective clinical improvement in patients with immunodeficiency virus infection. However, GZ has been reported to produce an adverse effect of aldosteronism when given in massive doses. Several studies on the disposition of GZ and GA in human and animals have been reported, although few papers have covered the mechanism of GZ gastrointestinal absorption.

The purpose of the present study was to clarify the time course of plasma concentrations of GZ and the active metabolite GA after oral administration of GZ and to elucidate the mechanism of the gastrointestinal absorption in rats.

MATERIALS AND METHODS

Materials GZ and GA were purchased from Tokyo Kasei Kogyo Co., Ltd. (Tokyo, Japan). Kanamycin sulfate was the gift of Meiji Seika Co., Ltd. (Tokyo, Japan). All other reagents were the best available commercial products of analytical grade.

Preparation of Drug Solutions GZ was dissolved in saline for oral administration. For intravenous administration, GZ and GA were dissolved in saline and in saline containing 2% polysorbate 20, respectively. For in situ absorption experiments (loop methods), GZ was dissolved in 0.1 N HCl–NaCl, pH 6.5 isotonic sodium phosphate buffer and pH 7.4 isotonic sodium phosphate buffer, each containing 10% methanol, for the stomach, small and large intestines, respectively, while GA was dissolved in mixed solutions of pH 6.5 and 7.4 isotonic sodium phosphate buffer: propylene glycol (1:1, v/v) for the small and large intestines, respectively.

Animals Male Wistar rats weighing 240–280 g were fasted overnight (15 to 18 h) prior to the experiments of oral administration of GZ, but water was allowed ad libitum. The animals were lightly anesthetized with ethyl ether for surgical procedures of all cannulations, or anesthetized with pentobarbital sodium for surgical procedures of in situ experiments. The right femoral artery was cannulated with a polyethylene tube (PE-50, medical grade, flushed with heparinized saline) for blood sampling in the i.v. administration experiments. In the experiments of oral administration, the blood sample was taken from the tail vein. The dosed rats were kept in restraining cages for in vivo experiments, with free access to water, or on restraining plates for in situ experiments. The body temperature was kept at 37°C throughout the experiments using heat lamps.

Preparation of Pseudo-Germ-Free Rats In accordance with the method reported by Gingell et al., kanamycin sulfate dissolved in saline (200 mg/rat × 5) was given orally twice daily for 2 d and once more 4 h before the experiment.

Procedure of Absorption Experiments in Situ The absorption from the stomach, the small intestine (the portion from the proximal end of the duodenum to the ileocecal junction) and the large intestine (from the proximal end of the colon to the anus) was examined by the in situ loop method. Briefly, under pentobarbital anesthesia, the segment to be tested was exposed by a midline abdominal incision and a loop was formed. For the stomach, the cardia was ligated and the pylorus ligated around a short silicone cannula. For the intestines, each end was cannulated with silicone tubing. Care was taken

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to exclude major blood vessels from the ligatures. The bile duct was ligated in most experiments, but in some experiments, it was cannulated with polyethylene tubing to examine the biliary excretion. The contents of the lumen were washed out with saline warmed to 37°C, then an isotonic drug solution was injected into the loop and each cannula was clamped with forceps. The organs were returned to the abdomen, and the incision was closed. The volume of the solution injected was 4 ml for the stomach, 5 ml for the small intestine, and 2 ml for the large intestine. One h after the injection, the solution in the segment was withdrawn and the lumen was washed with the isotonic buffer. The solution and the washings were combined and the volume was adjusted to 50 ml by addition of the buffer. The amount of drug absorbed was calculated as the difference in amount between the initial and final solutions.

**Oral Administration Study** GZ dissolved in saline was administered orally (200 mg/kg) to normal rats and bile-fistula rats using a gastric sonde. Blood samples (0.3 ml) were taken from the tail vein at 0.5, 1, 2, 4, 6, 9, 12, and 24 h after the administration and then immediately centrifuged to obtain the plasma. GZ and GA concentrations were determined by HPLC.

**Biliary Excretion of GZ after Oral Administration of GZ** In bile-fistula rats, bile samples were collected 0—1, 1—2, 2—4, 4—6, 6—9, 9—12, 12—24, and 24—30 h after oral administration of GZ (200 mg/kg). The volumes of collected bile were estimated from their weight. GZ concentration was determined by HPLC.

**Biotransformation of GZ by Gastrointestinal Contents in Vitro** Non-treated and kanamycin-treated rats were sacrificed under pentobarbital anesthesia and an abdominal operation performed. After the excision, the gastrointestinal tract was divided into five segments (stomach, proximal half of small intestine, distal half of small intestine, cecum and colon with rectum). Each luminal content specimen was collected as quickly as possible. A saline solution of GZ (1 mmol) warmed to 37°C was added to each gastrointestinal content specimen (5 ml/g wet weight) and the resulting mixture was incubated at 37°C under anaerobic condition using nitrogen gas. After 3 h, an aliquot was removed and the reaction was immediately stopped by the addition of methanol (3 vol.). The concentrations of GZ and the metabolite GA in the supernatant fraction were determined by HPLC.

**Fate of GZ in Gastrointestinal Tract in Vivo** GZ (200 mg/kg) saline solution was administered to rats through a gastric sonde. One or four h thereafter, the gastrointestinal tract was removed and segmented as in the in vitro experiment described above. The content of each segment was separated from the tissue by washing with cold saline. The tissues were homogenized with saline on ice. The individual luminal content and the tissue homogenate were adjusted to appropriate volume, and the remaining amount of GZ and the amount of GA formed were determined by HPLC.

**Intravenous Administration Study** To estimate the bioavailability, intravenous administration studies for GZ and GA were carried out. GZ (100 mg/kg) or GA (20 mg/kg) was administered as the solution, which was prepared as described above, by bolus injection into the left femoral vein. Blood samples of about 300 μl were taken through the right femoral artery cannula at 5, 15, 30, 60, 90, 120, 180, and 240 min after the administration and were immediately centrifuged to obtain the plasma. GZ and GA concentrations were determined by HPLC.

**Analytical Methods** One hundred μl of luminal solution, plasma or bile sample was deproteinized by the addition of 300 μl of methanol. Then the mixture was vortexed and centrifuged for 5 min at 10000 rpm and the supernantant layer was filtered through a 0.45 μm membrane filter (Nihon Millipore Kogyo, Yonezawa, Japan). The filtrate was used for the HPLC analysis. A high performance liquid chromatograph (LC-5A, Shimadzu Co., Kyoto Japan) equipped with a Shimadzu SPD-2A ultraviolet detector was used. Separation was achieved on an Inertsil ODS-2 packed column (4.6 x 150 mm, 5 μm particle size, GL Sciences Inc., Tokyo, Japan) which was used in reversed-phase mode. The mobile phase was methanol: pH 4.2 phosphate buffer (68.5 mM NaH₂PO₄—38.2 mM H₃PO₄) (5:2, v/v) for the test of GZ, or acetoni-trile: 10 mM ammonium acetate (1:1, v/v) for the test of GA. The flow rate was maintained at 1.0 ml/min. The drugs were monitored at a wavelength of 245 nm throughout all the experiments. The concentrations of GZ and GA were calculated by the peak height measurements.

**Data Analysis** Pharmacokinetic evaluations were carried out by non-compartmental analysis of the plasma concentration-time data based on the statistical moment theory. The moments, the area under the plasma concentration–time curve (AUC) and the mean residence time (MRT), were calculated by the trapezoidal method with a monoexponential extrapolation of the terminal phase. The respective bioavailability (F) of GZ and GA after oral administration of GZ was calculated using AUC values after intravenous administration of each of them.

**RESULTS**

**Pharmacokinetics of GZ and GA Following Oral Administration** Plasma concentrations of both GZ and GA after their intravenous bolus administration at 100 and 20 mg/kg, respectively, declined biexponentially. In the case of GZ, only the parent drug, but not GA, was detected in the plasma. The AUC values for GZ and GA were 2903.6 ± 583.5 μg·h/ml (n = 3) and 59.9 ± 13.2 μg·h/ml (n = 3), respectively.

Figure 1 shows the plasma concentration–time curves of GZ and GA after oral administration of GZ (200 mg/kg). Both GZ and its metabolite GA were detected in rat plasma after oral administration of GZ. The plasma appearance of GA was as soon as 1 h after oral administration of GZ, but the time of maximum concentration was 12 h. The pharmacokinetic parameters are summarized in Table I. The oral bioavailability of GZ was 4.0%, while the bioavailability as GA was 14.2%.

**Gastrointestinal Absorptions in Situ** The absorptions of GZ and GA from the stomach, the small intestine, and the large intestine were examined by the in situ loop method, and the results are summarized in Table II. GZ
was poorly absorbable from any site of the gastrointestinal tract. The absorption of GA was larger than that of GZ in both small and large intestines and was larger in the small intestine than in the large intestine.

**Biotransformation of GZ by Gastrointestinal Contents in Vitro** To investigate its biotransformation in the gastrointestinal tract, GZ was incubated anaerobically with the luminal contents of various gastrointestinal segments of non-treated and kanamycin-treated rats, and the results are shown in Table III. No degradation of GZ was observed in the small intestinal contents within 3 h. On the other hand, GZ was partly degraded to GA by the gastric, cecal and colonic contents within this same period. To investigate whether or not the intra-luminal biotransformation is mediated by the gastrointestinal microflora, the effects of oral pretreatment of the animal with kanamycin sulfate and of boiling of the normal gastrointestinal contents on the biotransformation of GZ were also examined. As is evident from Table III, the degradation of GZ by boiled gastric and large-intestinal contents and the contents of kanamycin-treated rats was significantly reduced compared to those by normal contents.

**Fate of GZ in Vivo** In order to evaluate the time courses of the gastrointestinal distribution and the biodegradation of GZ in vivo, the recovery of GZ and GA in the luminal contents and the tissues was determined after oral administration of GZ (200 mg/kg) (Fig. 2). One hour after

### Table I. Pharmacokinetic Parameters of GZ and GA after Oral Administration of GZ in Rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>(Unit)</th>
<th>GZ</th>
<th>GA</th>
</tr>
</thead>
<tbody>
<tr>
<td>T&lt;sub&gt;max&lt;/sub&gt;</td>
<td>(h)</td>
<td>0.5 ± 0.0</td>
<td>12.3 ± 2.2</td>
</tr>
<tr>
<td>C&lt;sub&gt;Pmax&lt;/sub&gt;</td>
<td>(µg/ml)</td>
<td>121.4 ± 35.0</td>
<td>3.6 ± 0.6</td>
</tr>
<tr>
<td>AUC</td>
<td>(µg h/ml)</td>
<td>233.5 ± 26.3</td>
<td>85.2 ± 17.8</td>
</tr>
<tr>
<td>MRT</td>
<td>(h)</td>
<td>3.5 ± 0.6</td>
<td>16.8 ± 2.1</td>
</tr>
<tr>
<td>F</td>
<td></td>
<td>0.040 ± 0.005</td>
<td>0.142 ± 0.030</td>
</tr>
</tbody>
</table>

*Dose of GZ was 200 mg/kg. Results are expressed as the mean ± S.E. (n = 3–6).*

### Table II. Gastrointestinal Absorption of GZ and GA Examined by in Situ Loop Method

<table>
<thead>
<tr>
<th>Site</th>
<th>% absorbed in 1 h</th>
<th>GZ</th>
<th>GA</th>
</tr>
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<tbody>
<tr>
<td>Stomach</td>
<td>8.3 ± 3.3&lt;sup&gt;a&lt;/sup&gt; (3)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Small intestine</td>
<td>9.0 ± 3.0 (6)</td>
<td>77.1 ± 1.8 (6)</td>
<td></td>
</tr>
<tr>
<td>Large intestine</td>
<td>8.3 ± 2.2 (6)</td>
<td>43.7 ± 3.6 (6)</td>
<td></td>
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</table>

*Initial concentration of each drug was 1.0 mg/ml.<sup>a</sup> In the luminal solution of the stomach, a small amount (2.2 ± 0.3%) of GA was detected, and thus the value of absorption was corrected considering this as remaining GZ. <sup>b</sup> The experiment was not done because of the solubility problem at the low pH. Results are expressed as the mean ± S.E. with the number of experiments in parentheses.*

### Table III. Biotransformation of GZ by Rat Gastrointestinal Contents in Vitro

<table>
<thead>
<tr>
<th>Site</th>
<th>GA formation (%)</th>
<th>Normal</th>
<th>Boiled&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Antibiotic-treated&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stomach</td>
<td>28.6 ± 6.2</td>
<td>0.3 ± 0.2</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Proximal small intestine</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Distal small intestine</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Cecum</td>
<td>21.1 ± 6.5</td>
<td>2.2 ± 1.9</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Colon</td>
<td>16.1 ± 2.3</td>
<td>1.5 ± 1.0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

*GZ solution (1 mg/ml) was added to the luminal contents (5 ml/g wet weight) and the mixture was incubated anaerobically at 37°C for 3h. <sup>a</sup> The luminal contents from normal rats were boiled for 3 h. <sup>b</sup> Kanamycin sulfate (5 × 200 mg/animal) was given orally twice daily for 2 d and also 4 h before the experiment. Results are expressed as the mean ± S.E. of 3 experiments.*

![Fig. 2. Recovery of GZ and GA from Luminal Contents and Tissues of Different Sites after Oral Administration of GZ](image-url)

(a) 1 h after administration; (b) 4 h after administration. Key: dotted column, GZ; closed column, GA. Dose of GZ was 200 mg/kg. Results are expressed as the mean ± S.E. (n = 6). PSI and DSI mean proximal small intestine and distal small intestine, respectively. ND, not detected.
the administration, a considerable amount of unchanged GZ was recovered from the stomach and the small intestine; a small but significant amount of GA was found only in the stomach. Even 4 h after GZ administration, the formation of GA was not recognized in the small intestine. On the other hand, in the lower intestine, i.e. the cecum and the colon, the formation of GA was recognized.

**Biliary Excretion of GZ after Oral Administration**

Figure 3 shows the biliary excretion of GZ after its oral administration at 200 mg/kg. As is evident, a small but significant amount of GZ was excreted in bile. In 24 h the amount of GZ excreted in bile was 1.7 ± 0.3% of the administered dose. The metabolite GA was not detected in bile.

**DISCUSSION**

There have been several studies on the disposition of GZ and GA in experimental animals. However, the concentration–time profiles of these substances after intravenous and oral administrations of GZ in human or animals from various laboratories do not agree, perhaps due to the difference in analytical methods. In this study, we compared the pharmacokinetic behaviors of GZ and GA in rats after oral administration. A small amount of GZ was found in plasma after its oral administration at the dose of 200 mg/kg, suggesting that GZ is partly absorbed as the intact form from the gastrointestinal tract. The bioavailability of GZ was only 4.0%. The metabolite GA, on the other hand, was found in the plasma after oral dosing of GZ. Like GZ, this metabolite has anti-inflammatory and interferon-inducing effects. A possible explanation for the appearance of GA in the plasma after oral administration of GZ may be the absorption of the hydrolytic product GA by the gastric juice, intestinal bacteria or other enzymatic processes in the gastrointestinal wall. Hattori et al. reported that GZ is hydrolyzed to GA by an intestinal bacterial mixture obtained from fresh human feces. The in vitro studies on the biotransformation of GZ by rat gastrointestinal contents (Table III) showed GA formation in the stomach and the large intestine, and these biotransformations were almost completely inhibited by either the boiling or the pretreatment with kanamycin sulfate. These results suggest that the gastrointestinal bacteria hydrolyze GZ to GA, and that the bacteria which hydrolyze GZ is not localized in the small intestine of rats. As to the hydrolysis of GZ in the stomach, a hydrolysis experiment in artificial gastric juice was carried out and no degradation of GZ was observed during the incubation at 37 °C for 24 h (results not shown), suggesting that GZ is stable under such an acidic condition. Since the hydrolytic reaction observed in the normal gastric contents was inhibited by the boiling and the antibiotic-treatment (Table III), the reaction might also be mediated by the bacteria, but not by the simple acidic condition. This can be explained by the fact that the rat is an animal with coprophagy and there are some bacteria which can hydrolyze GZ in the stomach. However, most of the GZ dose orally must be transferred through the intestinal tract and reach the lower part of intestine, where the bacterial cleavage occurs. On the other hand, after intravenous administration of GZ, only the parent drug could be detected in the plasma (data not shown) and it is reported that about 80% of the dose is excreted in an unchanged form in the bile, suggesting that the hydrolysis of GZ is relatively small in the systemic circulation. Consequently, GA appearing in the plasma after oral administration of GZ was regarded as the presystemic metabolite mainly in the large intestine.

Although GZ is poorly absorbable from the gastrointestinal tract (Table II), the parent GZ could be detected in the plasma after oral administration (Fig. 1). The peak plasma concentration of GZ was seen in the first sampling time, 30 min. The bioavailability was 4.0%. GZ remaining in the intestinal lumen is transferred to the lower part and is hydrolyzed to GA in the large intestine by the action of intestinal bacteria, and then the metabolite GA is absorbed from the large intestine. While GA absorption in the small intestine is better than in the large intestine (Table II), GA cannot be formed in the small intestine (Table III); the rate-determining step in the absorption of GA is the hydrolytic formation. Thus, the time of peak plasma concentration of GA after oral dosing was late, 12 h (Fig. 1), and its relatively rapid appearance in the plasma might be explained by the small-intestinal absorption of GA formed in the gastric contents (Table III). Furthermore, the plasma concentrations of GA after oral dosing were very low in comparison with those of GZ. However, this is due to the large apparent volume of distribution of GA; GA bioavailability after oral administration of GZ was 14.2%, which is larger than the parent drug.

Several studies on the biliary excretion of GZ after intravenous administration have been reported. Ichikawa et al. reported that about 80% of the dose of GZ is excreted in the bile following intravenous administration (100 mg/kg) to rats. There has been no report on GZ excretion in the bile after oral administration. In this study, we did detect the biliary excretion of GZ after oral administrations of GZ (Fig. 3). Thus, the gastrointestinal absorption of GZ would be around 5.7%
(bioavailability + biliary excretion), in addition to the absorption as GA (14.2%). Besides the poor gastrointestinal absorbability of GZ, the existence of the first-pass elimination effect might also be one of the main reasons for low GZ bioavailability. That is, GZ administered orally was slowly absorbed from the small intestine and transported through the portal vein to the liver, followed by its immediate elimination to the bile, rather than entering the systemic circulation.

REFERENCES