Pharmacokinetics of Glycyrrhizin in Rats with D-Galactosamine-Induced Hepatic Disease

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The pharmacokinetic behavior of glycyrrhizin (GZ) was examined in D-galactosamine-intoxicated (GAL) rats. When GZ was administered intravenously, the apparent volume of distribution (Vd) and the total body clearance (CLtotal) were more significantly decreased in GAL rats than those in normal rats. When GZ was administered orally, the area under the plasma concentration–time curve (AUC), the mean residence time (MRT) and the time to reach the maximum plasma concentration (Tmax) for GZ were higher, but the maximum plasma concentration (Cmax) in GAL rats was lower than that in normal rats. The bioavailability of GZ, however, was not significantly changed. On the other hand, the AUC for glycyrrhetic acid (GA), a main metabolite of GZ, after oral administration of GZ was higher in GAL rats than in normal rats, although there was no significant difference in MRT or Tmax, Cmax, or the bioavailability for GA between GAL and normal rats. The reasons for these differences in GAL rats would be changes in the absorption rate (reduced gastric emptying rate) and reduction of the hepatic elimination rates (biliary excretion of GZ and hepatic metabolism of GA).

Key words glycyrrhizin; hepatic disease; pharmacokinetics; glycyrrhetic acid; intestinal absorption; rat

The hepatobiliary transport of drugs is affected by factors such as hepatic blood flow, binding to plasma proteins, influx and efflux across the sinusoidal plasma membrane, intracellular transport, interaction with cytoplasmic binding proteins and organelles, metabolism, transport across the bile canalicular membrane, bile flow and so on. A hepatic diseased state might influence these factors. Glycyrrhizin (GZ) is widely used for various types of hepatitis, but has been reported to produce an adverse effect of aldosteronism when given in massive doses. Therefore, investigation of the pharmacokinetic characteristics of GZ in the hepatic diseased state is important and useful for optimal clinical use of this drug.

Tanaka et al. reported the pharmacokinetic profiles of GZ in patients with chronic hepatitis after intravenous administration (120 mg/d), and longer biological half-life (t1/2) and remarkably smaller total body clearance (CLtotal) than in healthy volunteers were observed. Yamamura et al. also reported the relationship between pharmacokinetic behavior of GZ and hepatic function in patients with acute hepatitis and liver cirrhosis, and the t1/2 for GZ in the hepatitis and cirrhosis groups were about twice and eight times longer, and the CLtotal values were smaller, about 0.7 and 0.23 times, than those in normal subjects, respectively. Ishida et al. reported the pharmacokinetic behavior of GZ in chronically CCl4-intoxicated rats. There is no report, however, on gastrointestinal absorption behaviors of orally administered GZ in rats with experimentally induced hepatic disease.

We have reported the mechanism of gastrointestinal absorption of GZ and some differences between pure GZ and GZ in glycyrrhiza extract (GE) in gastrointestinal absorption behaviors, which are caused by some components in GE. In this study, we examined the pharmacokinetics of GZ in rats with D-galactosamine-induced hepatic disease (GAL rats), a rat model which has attracted attention since the liver tissue is very similar to that in viral hepatitis of human.

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MATERIALS AND METHODS

Materials GZ, glycyrrhetic acid (GA) and D(-)-galactosamine hydrochloride were purchased from Tokyo Kasei Kogyo Co. (Tokyo, Japan). All other reagents were the best available commercial products of analytical grade.

Preparation of Diseased Rats Male Wistar rats weighing 230–280 g were used. GAL rats were prepared with a single intraperitoneal administration of 400 mg/kg of D-galactosamine 24 h after each treatment. The animals were lightly anesthetized with ethyl ether for surgical procedures of all cannulations and kept in restraining cages for in vivo oral and intravenous administration experiments, or anesthetized with pentobarbital sodium (32.4 mg/kg, i.p.) and kept on restraining plates for surgical procedures of in situ absorption experiments. The body temperature was kept at 37°C using heat lamps throughout the experiments. The right femoral artery was cannulated with a polyethylene tube (PE-50, medical grade, flushed with heparinized saline) for blood sampling in the intravenous administration experiments. In the experiments of oral administration, the blood sample was taken from the tail vein.

In bile fistula rats, the abdominal midline was opened and the common bile duct was cannulated with a polyethylene tube (0.5 mm i.d., 0.8 mm o.d.; Dural Plastics & Engineering, Dural, Australia) as deeply as possible. The abdominal incision was closed and the end of the cannula was extruded from the skin.

Preparation of Drug Solution GZ was dissolved in saline for oral administration. For intravenous administration and in vitro degradation experiments, GZ and GA were dissolved in saline alone and in saline containing 2% polysorbate 20, respectively. For in situ absorption experiments of GZ (small intestine loop method), GZ was dissolved in pH 6.5 isotonic sodium phosphate buffer containing 10% methanol.

Administration of Drugs The animals were fasted overnight (15 to 18 h) prior to the oral administration experi-
ments, but water was allowed ad libitum. The saline solution of GZ (200 mg/8 ml/kg) was orally administered to normal or GAL rats with or without bile fistula, using a gastric sonde. The dosed rats were kept in restraining cages with access to water under normal housing conditions.

To examine gastric emptying rate (GER), GZ saline solution was administered to normal or GAL rats through a gastric sonde. One or 4 h after the administration, the stomach was removed, the contents washed out and the stomach tissue was homogenized. Then the remaining amount of GZ in the stomach contents and tissue was estimated as described.\textsuperscript{10}

The absorption of GZ from the small intestine (the portion from the proximal end of the duodenum to the ileo-cecal junction) in normal and GAL rats was examined by the in situ loop method as previously described.\textsuperscript{10,11} Five milliliters of 1 mm GZ solution (pH 6.5 isotonic phosphate buffer) was administered to each rat.

**Collection of Blood and Bile Samples** Blood samples of about 300 μl were taken from the tail vein at 0.5, 1, 2, 4, 6, 9, 12 and 24 h after the oral administration, as described above, and were immediately centrifuged to obtain the plasma. In the case of intravenous administration, GZ in saline (100 mg/4 ml/kg) was administered by bolus injection into the left femoral vein. The dosed rats were kept in restraining plates under normal housing conditions. 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.\textsuperscript{10,11}

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 or intravenous administration of a drug. The volume of collected bile was estimated from its weight. GZ and GA concentrations were determined by HPLC.

**Study on Hepatic Metabolism of GZ and GA** Normal or GAL rats were lightly anesthetized with ethyl ether and an abdominal operation performed. The livers were perfused with cold saline until washed free of blood, then removed and homogenized with twice the volume of saline for 15 min in ice. For the recovery study, six ml of methanol was added to 1800 μl of the homogenate and mixed thoroughly. Two hundred μl of a drug solution (0.5 mm of GZ in saline, or GA in saline containing 2% polysorbate 20) was added to the mixture and mixed again. For the degradation study, 200 μl of a drug solution (0.5 mm of GZ in saline, or GA in saline containing 2% polysorbate 20) was added to the homogenate, and the mixture was incubated at 37°C. Two hundred μl of the incubation mixture was sampled out at 1 h and added to 600 μl of methanol. The degradation rate was calculated from the recovery.

**Analytical Methods** GZ and GA in the plasma and bile samples were determined by HPLC as described previously.\textsuperscript{10,11} Briefly, samples deproteinized by the addition of methanol were injected into HPLC, which was operated in the reversed-phase mode. The column was an Inertsil ODS-2 packed column (4.6 × 150 mm, 5 μm particle size, GL Sciences Inc., Tokyo), and the mobile phase was methanol: pH 4.2 phosphate buffer (68.5 mm NaH₂PO₄–38.2 mm H₂PO₄) (5:2, v/v) for the test of GZ, or acetonitrile : 10 mm ammonium acetate (1:1, v/v) for the test of GA. The flow rate was maintained at 1.0 ml/min and the drugs were monitored at 245 nm. The concentrations of GZ and GA were calculated by peak height measurements.

**Pharmacokinetic Analysis** Pharmacokinetic evaluations were carried out by non-compartment analysis of the plasma concentration–time data based on the statistical moment theory.\textsuperscript{15} The moments, the area under the plasma concentration–time curve (AUC) and the mean residence time (MRT) were calculated by trapezoidal method with a monoexponential extrapolation of the terminal phase. Student's t-test was utilized to determine the significance of differences.

**RESULTS AND DISCUSSION**

**Pathophysiological Changes in D-Galactosamine-Treated Rats** As mentioned in our previous paper, the pathophysiological parameters of d-galactosamine-treated rats were markedly changed.\textsuperscript{14} After the intraperitoneal administration of d-galactosamine (400 mg/kg), plasma transaminases (GOT, GPT), which are indices of hepatic injury, reached a maximum level in 24 h; we therefore used rats 24 h after administration as GAL rats. Plasma GPT and GPT activities as well as free fatty acid (FFA) were significantly increased in GAL rats, but the direct bilirubin concentration was not. The total plasma protein concentration and plasma albumin concentration were significantly decreased in these rats. These indices suggest that the state of GAL rats is very similar to the acute virus hepatitis of human.\textsuperscript{12,13} The hepatic plasma and blood flow were also significantly decreased in GAL rats (64.6% and 68.7% of normal rats, respectively).\textsuperscript{14}

**Pharmacokinetics of GZ after Intravenous Administration** The plasma profiles after intravenous administration of GZ to normal or GAL rats are shown in Fig. 1, and the pharmacokinetic parameters based on these data are listed in Table 1. The plasma level of GZ was significantly
higher in GAL rats than that in normal rats. The apparent volume of distribution (Vdss) and CLtotal were significantly decreased while AUC and MRT were significantly increased in GAL rats compared with normal rats. It was reported that Vdss and CLtotal values for GZ in chronically CCl4-intoxicated rats were similar to those in normal rats.9) However, Yamamura et al. showed that the Vdss for GZ in a patient with acute hepatitis became larger with improvement of liver function and that the CLtotal for GZ decreased in patients with acute hepatitis and liver cirrhosis.9) Our results are consistent with those in patients with liver disease. The mechanism of the change in Vdss

value in the disease state remains unsolved.

The biliary excretion of GZ after the intravenous administration to normal or GAL rats is shown in Fig. 2. GZ was almost completely recovered in bile within 24 h in normal rats, but only 50% in GAL rats; the difference was statistically significant (p < 0.001). This is consistent with the result of Ishida et al. in chronically CCl4-intoxicated rats.9) We have reported that the biliary excretion of cefpirome, cefoperazone, bromphenol blue and phenol red were markedly diminished in rats with obstructive jaundice, while the urinary excretion of these drugs was increased.16) Similar results were observed for cefpiramide in GAL and acutely CCl4-intoxicated rats (unpublished data). On the other hand, the biliary excretion rate of indocyanine green in GAL rats was significantly lower than that in normal rats, but no complement-ary excretion was observed in this drug.14) The urinary excretion of GZ in GAL rats has not yet been examined.

Pharmacokinetics after Oral Administration of GZ

The plasma profiles after oral administration of GZ to normal or GAL rats are shown in Fig. 3, and the pharmacokinetic parameters based on these data are listed in Table 2. As is evident from the table, in the case of plasma GZ, the AUC, MRT and time to reach the maximum plasma concentration (Tmax), but not bioavailability (F), were increased with the decrease of maximum plasma concentration (Cpmax) compared with those in normal rats. The low F value of GZ after the oral administration would be due to the poor absorption from the small intestine.10,17) The first-pass biliary elimination would also be one reason for the low F of GZ.10) The recovery of GZ in bile within 24 h after oral administration was lower in GAL rats (1.2 ± 0.6%) than in normal rats (1.8 ± 0.3%), though the difference was not statistically significant.

Unabsorbed GZ is hydrolyzed by bacteria in the large-intestinal contents and most of the GA, an active metabolite, formed is absorbed from the large intestine.10) The plasma concentration of GA after oral administration of GZ in GAL rats is also shown in Fig. 3. The AUC was increased similarly to the case of GZ, although the deviations were large (Table 2). However, since the AUC for GA after intravenous administration (20 mg/kg) in GAL rats increased in comparison with that in normal rats,
Table 2. Pharmacokinetic Parameters of GZ and GA after Oral Administration in Normal and GAL Rats

<table>
<thead>
<tr>
<th>Parameter (Unit)</th>
<th>Normal rat</th>
<th>GAL rat</th>
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<tbody>
<tr>
<td>For GZ:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUC (μg h/ml)</td>
<td>233.5 ± 26.3</td>
<td>515.8 ± 259.6</td>
</tr>
<tr>
<td>MRT (h)</td>
<td>3.5 ± 0.6</td>
<td>13.9 ± 4.6a</td>
</tr>
<tr>
<td>T_{max} (h)</td>
<td>0.5 ± 0.0</td>
<td>8.0 ± 2.0a</td>
</tr>
<tr>
<td>C_{max} (μg/ml)</td>
<td>120.4 ± 37.7</td>
<td>22.1 ± 5.8</td>
</tr>
<tr>
<td>F (%)</td>
<td>4.1 ± 0.5</td>
<td>3.0 ± 1.5</td>
</tr>
<tr>
<td>For GA:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUC (μg h/ml)</td>
<td>85.2 ± 17.8</td>
<td>531.1 ± 361.3a</td>
</tr>
<tr>
<td>MRT (h)</td>
<td>16.8 ± 2.1</td>
<td>19.5 ± 1.2</td>
</tr>
<tr>
<td>T_{max} (h)</td>
<td>13.4 ± 1.9</td>
<td>16.0 ± 4.0</td>
</tr>
<tr>
<td>C_{max} (μg/ml)</td>
<td>5.0 ± 1.4</td>
<td>25.0 ± 17.7</td>
</tr>
<tr>
<td>F (%)</td>
<td>14.2 ± 3.0</td>
<td>36.9 ± 25.1</td>
</tr>
</tbody>
</table>

Dose of GZ was 200 mg/kg. Results are expressed as the mean ± S.E. (n = 3—10). a) p < 0.05; b) p < 0.01, compared with the corresponding parameter of normal rats.

Table 3. Hepatic Metabolism of GZ and GA in Normal and GAL Rats

<table>
<thead>
<tr>
<th>Substrate</th>
<th>% remaining after 1 h incubation</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Normal rat</td>
</tr>
<tr>
<td>GZ</td>
<td>102.6 ± 3.6</td>
</tr>
<tr>
<td>GA</td>
<td>62.5 ± 2.7</td>
</tr>
</tbody>
</table>

Initial concentration of substrate was 0.5 mM. Results are expressed as the mean ± S.E. (n = 3—4). a) p < 0.001, compared with normal rats.

144.1 ± 23.4 μg h/ml (n = 5) and 59.9 ± 13.2 μg h/ml (n = 3), respectively, no significant change was observed in F of GA after oral administration of GZ in this disease state. Neither the MRT nor T_{max} was significantly different from those in normal rats.

Gastric Emptying Rate and Small-Intestinal Absorption of GZ. After oral administration of GZ (200 mg/kg) to GAL rats, the remaining amounts of GZ in the stomach at 1 and 4 h were 95.4 ± 6.1 and 66.5 ± 12.2% of dose, respectively, which are remarkably smaller than those in normal rats (29.9 ± 3.7 and 25.2 ± 11.1%, respectively). These results indicate that the GER was reduced in GAL rats. Reduced GER in patients with liver disease has not been confirmed.

The absorption of GZ from the small intestine was examined by an in situ loop method. The absorption in 1 h was slightly higher in GAL rats (16.2 ± 3.1%) than in normal rats (11.8 ± 2.8%), but the difference was not statistically significant. The reduction of GER and CL_{tota}, but not the change in the small-intestinal permeability, of GZ in GAL rats is believed to result in the change in the plasma profile after oral administration of GZ (Fig. 3).

Hepatic Metabolism of GZ and GA. Table 3 shows the degradation of GZ and GA in the homogenate of rat liver. There was no significant difference in metabolic degradation of GZ between normal and GAL rats, while metabolic degradation of GA was significantly decreased in GAL rats. Since GA was not detected in plasma after intravenous administration of GZ, the metabolism of GZ to GA in the liver might not be significant, and GA in plasma after oral administration of GZ is the result of the large-intestinal absorption of GA produced from GZ. On the other hand, GA might be exclusively metabolized in the liver; the metabolites are sulfate, monoglucuronide and diglucuronide conjugates. The increased AUC of GA in GAL rats after the intravenous administration mentioned above, i.e., the decreased CL_{tota}, is mainly the result of the decrease in the hepatic metabolism. This would be the reason for the higher plasma concentration of GA after oral administration of GZ in GAL rats.

In conclusion, GZ and GA plasma kinetics after oral or intravenous administration of GZ differed in normal and GAL rats; this might be caused by the changes in GER, the biliary elimination of GZ and hepatic metabolism of GA due to the experimental liver damage with D-galactosamine.

REFERENCES