DOWN-REGULATION OF A HEPATIC TRANSPORTER MULTIDRUG RESISTANCE-ASSOCIATED PROTEIN 2 IS INVOLVED IN ALTERATION OF PHARMACOKINETICS OF GLYCIRRHIZIN AND ITS METABOLITES IN A RAT MODEL OF CHRONIC LIVER INJURY

Toshiaki Makino, Nobuhiro Ohtake, Akito Watanabe, Naoko Tsuchiya, Sachiko Imamura, Seiichi Iizuka, Makoto Inoue, and Hajime Mizukami

Department of Pharmacognosy, Graduate School of Pharmaceutical Science, Nagoya City University, Nagoya, Japan (T.M., A.W., H.M.); Tsumura Research Laboratories, Tsumura Co. Ltd., Ami-machi, Ibaraki, Japan (N.O., N.T., S.I., S.Ii.); and Laboratory of Medicinal Resources, School of Pharmacy, Aichi Gakuin University, Nagoya, Japan (M.I.)

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ABSTRACT:

Glycyrrhizin (GL) has been used to treat chronic hepatitis in Japan and Europe. It is thought to induce pseudoaldosteronism via inhibition of type 2 11β-hydroxysteroid dehydrogenase (11β-HSD2) by glycyrrhetinic acid (GA), a major metabolite of GL. A previous clinical study suggested that 3-monoglucuronyl-glycyrrhetinic acid (3MGA), another metabolite of GL, might play a more important role in the pathogenesis of pseudoaldosteronism. The present study evaluates the pharmacokinetics of GL and its metabolites in rats with chronic liver injury induced by a choline-deficient L-amino acid-defined (CDAA) diet to clarify the relationship between 3MGA and pseudoaldosteronism. In rats fed a CDAA diet, plasma concentrations and urinary eliminations of GL and 3MGA were markedly higher than in the rats fed the control diet; the plasma concentration of GA was unaffected when GL was orally administered. Immunohistochemical analysis revealed the suppression of levels of multidrug resistance-associated protein (Mrp) 2 and its localization in the hepatic tissue of rats fed a CDAA diet. When 3MGA was i.v. injected in rats fed a CDAA diet or injected in Mrp2-dysfunctional Eisai hyperbilirubinemic rats, plasma concentrations of 3MGA were higher, and biliary excretion of 3MGA was lower than in each control group. The results suggested that 3MGA would be excreted to bile via hepatic Mrp2 and that its dysfunction would reduce 3MGA clearance. 3MGA accumulated by liver fibrosis resulted in the increased excretion through renal tubule and might be strongly related to the pathogenesis of pseudoaldosteronism because 11β-HSD2 is expressed in renal tubular epithelial cells.

Glycyrrhizin (GL) is a glycoside of glycyrrhetinic acid (GA) with two molecules of glucuronic acid (Fig. 1). GL is one of the active ingredients of licorice, roots of Glycyrrhiza glabra and Glycyrrhiza ularensis. This was frequently prescribed in the herbal formula of Japanese traditional Kampo medicine to treat various diseases. GL has also been used as a chemical drug to treat chronic hepatitis and allergic diseases for more than 20 years in Japan and Europe (Arase et al., 1997; van Rossum et al., 1998; Kumada, 2002). GL treatment has led to a significant decrease in serum alkaline aminotransferase (ALT) levels in patients with chronic hepatitis C (van Rossum et al., 2001).

An excessive quantity of licorice extract or GL preparation induces peripheral edema, hypokalemia, myopathy, and hypertension, which are the symptoms of pseudoaldosteronism; it can be potentially lethal (Morimoto and Nakajima, 1991). The frequency of pseudoaldosteronism caused by Kampo medicines depends on the dosage and the duration of licorice treatment (Homma et al., 2006). It was reported that administration of licorice extract corresponding to a daily dosage of 814 mg of GL (equivalent to 466 mg of GA) induced pseudoaldosteronism in healthy volunteers (Bernardi et al., 1994). Long-term (4 weeks) ingestion of licorice extract also induced a rise in blood pressure, even at low dosage (50 g/day; corresponding to 75 mg/day GA) in healthy volunteers (Sigurjónsdóttir et al., 2001). Repeated i.v. infusion of GL (200 mg/day) for the treatment of liver disease for 5 weeks induced hypotension with hypokalemia (Kanaoka et al., 1986). The pseudoaldosteronism caused by GL is thought to be due to the inhibition of type 2 11β-hydroxysteroid dehydrogenase (11β-HSD2) in renal tubular epithelial cells by GA, a major metabolite of GL (Tanahashi et al., 2002). The inhibition leads to an elevated level of renal cortisol, a potent agonist of mineralocorticoid receptors, leading to sodium retention, hypokalemia, hypertension, and, eventually, to suppression of renin and aldosterone (Conn et al., 1968).

ABBREVIATIONS: GL, glycyrrhizin; GA, glycyrrhetinic acid; ALT, alkaline aminotransferase; 11β-HSD2, type 2 11β-hydroxysteroid dehydrogenase; 3MGA, 3-monoglucuronyl-glycyrrhetinic acid; CDAA, choline-deficient L-amino acid-defined; Mrp, multidrug resistance-associated protein; EHBR, Eisai hyperbilirubinemic rat; SD, Sprague-Dawley; C3AA, choline-supplemented L-amino-defined; ALP, alkaline phosphatase; TBA, total bile acid; AUC, area(s) under the plasma concentration-time curve; IS, internal standard, carbenoxolone sodium; LC/MS/MS, liquid chromatography/tandem mass spectrometry; HPLC, high-performance liquid chromatography; MRM, multiple reaction monitoring.
Orally administered GL was enzymatically hydrolyzed to GA by intestinal bacterial flora before absorption into the bloodstream (Akae, 1998). The circulated GA was further metabolized to 3-monogluco-uronyl-glycyrrhetinic acid (3MGA) in the liver by UDP-glucuronosyltransferase and then excreted with bile into the intestine (Ploeger et al., 2001). When GL was injected i.v., it was partially metabolized to 3MGA in the liver by lysosomal β-d-glucuronidase, and GL and 3MGA could be excreted with bile. The biliary excreted GL and 3MGA were hydrolyzed by intestinal bacteria into GA, which was then reabsorbed into the bloodstream (Akae et al., 1991; Akae, 1998).

It was reported that the plasma concentration of 3MGA was significantly higher in the hypokalemia group than in the normal group in rats fed the CDAA diet to that in rats fed a CSAA diet for 4 and 12 weeks were then calculated (n = 6).

**Materials and Methods**

**Animals.** Male Wistar rats weighing 120 to 140 g, male Sprague-Dawley (SD) rats weighing 160 to 180 g, or male EHBRs weighing 160 to 250 g were purchased from Japan SLC (Hamamatsu, Japan). SD strain rats were used as the control of EHBRs because EHBRs have the backgrounds of the SD strain (Ito et al., 1997). Animals were housed three per cage and received food and water ad libitum under controlled temperature (25°C), humidity, and lighting (12 h of light, 12 h of dark) in accordance with the guidelines for animal care at our institution.

**Compounds and Diets.** GL (purity > 99%), GA (97%), and 3MGA (99%) were purchased from Calbiochem (San Diego, CA), Tokyo Kasei Kogyo (Tokyo, Japan), and Nacalai Tesque (Kyoto, Japan), respectively. Carbenoxolone sodium was purchased from LKT Laboratories, Inc. (St. Paul, MN). The CDAA and control, choline-supplemented t-αmino-defined (CSAA) diets were obtained from Dyets, Inc. (Bethlehem, PA). The compositions of these diets were previously described (Nakae et al., 1992; Endoh et al., 1996; Sakaia et al., 1996).

**Biochemical Analysis.** ALT, alkaline phosphatase (ALP), total bile acids (TBAs), and phospholipids in plasma were measured using Biochemical Analyzer TBA-40FR (Toshiba Medical Systems Co., Tokyo, Japan). Plasma hyaluronic acid was estimated using a human ELISA kit (Seikagaku Co., Tokyo, Japan).

**Liver Histology.** Liver tissue was fixed in 10% formalin for 48 h and embedded in paraffin. Sections of 3-μm thickness were examined under light microscopy after staining with hematoxylin and eosin, oil red O, or Sirius red.

**Immunoblot Analysis.** Liver tissue sections (each 50 mg) were frozen in Freon/liquid nitrogen, embedded in OCT Compound (Sakura Finetech Co., Ltd., Tokyo, Japan), cut into 6-μm-thick sections, and mounted on slides. The tissue sections were immunostained with monoclonal antibodies against Mrp2 as previously described (Okada et al., 2007). The Mrp2 staining area in 20 hyperfields of the specimen was measured by NIH Image (National Institutes of Health, Bethesda, MD), and the average was determined as the value of Mrp2 level in each specimen. Data were expressed as the ratio of Mrp2 levels in the specimens of CDAA rats to those in CSAA rats (n = 6).

**Bile Secretion.** Rats were anesthetized with urethane (1 g/kg body weight, i.p. injected), and the common bile duct was cannulated with a polyethylene tube (outer diameter, 0.6 mm; SP10; Natsume, Tokyo, Japan). Starting from 10 min after cannulation, bile was collected into preweighed tubes at 15- to 30-min intervals over 120 min. Cumulative amounts of bilirubin excretion in bile were calculated based on the weight and total concentration of bilirubin of each specimen.

**Pharmacokinetic Analysis.** GL (100 mg/kg body weight) was dissolved in distilled water containing 0.1% (v/v) NH₄OH and orally administered to rats fed the CDAA or CSAA diet. Blood (0.3 ml) was withdrawn via the right jugular vein 1, 2, 4, 8, 12, 24, and 48 h after treatment under light anesthesia with ether. Urine was collected over a period of 24 h using a metabolic cage after GL treatment. For studying the biliary excretion of 3MGA in rats fed the CDAA or CSAA diet, and in SD rats and EHBRs, the animals were anesthetized with urethane, and the common bile duct was cannulated with a polyethylene tube as mentioned above; the right jugular vein and carotid artery were cannulated with a polyethylene tube (SP10). 3MGA (5 mg/kg body weight) was dissolved in a mixture of polyethylene glycol 400/ethanol (4:1) and infused to the catheter of the jugular vein 10 min after cannulation. Blood (0.3 ml) was withdrawn via the carotid catheter at 5, 20, 40, 90, and 120 min and was centrifuged (1200g, 20 min, 4°C) to obtain plasma. Samples of plasma, bile, and urine were stored at −80°C until analysis. The area under the plasma concentration-time curve (AUC) was calculated using the trapezoidal rule from the first measurement point to the last measurement point.

**Preparation of Sample for Liquid Chromatography Analysis.** Fifty microliters of each sample and standard was spiked with internal standard, carboxenolone sodium (IS). The sample was loaded onto an Oasis HLB μElution plate (Waters, Milford, MA), which was preconditioned with methanol followed by 0.1 M HCl solution. The plate was washed with 0.1 M HCl solution, and the analytes were eluted with 200 μl of methanol. The eluate was evaporated to dryness under nitrogen gas and redissolved in 100 μl of mixture of 2% acetic acid in water and acetonitrile (1:1). Aliquots of the sample solutions were analyzed by LC/MS/MS (10 μl) and HPLC/CV (30 μl).

**Analysis of GL and Its Metabolites.** The concentrations of GL, 3MGA, and GA in plasma and urine were measured by LC/MS/MS analysis when GL
Plasma characteristics of rats fed the CSAA or CDAA diet

Wistar rats were fed CSAA or CDAA diet for 4 or 12 weeks and then sacrificed. Plasma markers were measured as described under Materials and Methods.

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<tr>
<td></td>
<td>4 Weeks</td>
<td>12 Weeks</td>
<td>4 Weeks</td>
<td>12 Weeks</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>274 ± 13.9</td>
<td>248 ± 14.2**</td>
<td>361 ± 35</td>
<td>293 ± 18**</td>
</tr>
<tr>
<td>Liver weight (g)</td>
<td>7.3 ± 0.7</td>
<td>16.3 ± 1.6***</td>
<td>8.1 ± 0.8</td>
<td>12.7 ± 1.4***</td>
</tr>
<tr>
<td>ALT (IU/l)</td>
<td>37.8 ± 6.5</td>
<td>568 ± 81****</td>
<td>34.3 ± 6.1</td>
<td>422 ± 85****</td>
</tr>
<tr>
<td>ALP (IU/l)</td>
<td>617 ± 41</td>
<td>965 ± 97****</td>
<td>369 ± 22</td>
<td>723 ± 88****</td>
</tr>
<tr>
<td>Total bilirubin (µg/ml)</td>
<td>0.18 ± 0.06</td>
<td>0.04 ± 0.13**</td>
<td>0.23 ± 0.04</td>
<td>0.08 ± 0.19**</td>
</tr>
<tr>
<td>Bile acids (µmol/l)</td>
<td>5.33 ± 0.44</td>
<td>46.0 ± 5.10***</td>
<td>4.40 ± 1.04</td>
<td>49.8 ± 11.4***</td>
</tr>
<tr>
<td>Phospholipids (mg/dl)</td>
<td>143 ± 15</td>
<td>88.7 ± 8.55***</td>
<td>125 ± 7</td>
<td>77.3 ± 11.6***</td>
</tr>
<tr>
<td>Hyaluronic acid (ng/ml)</td>
<td>79.7 ± 9.0</td>
<td>112 ± 39</td>
<td>70.0 ± 6.1</td>
<td>92.1 ± 7.2**</td>
</tr>
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</table>

**p < 0.01, significantly different from the CSAA rats (n = 6).
***p < 0.001, significantly different from the CSAA rats (n = 6).

Bilirubin excretions in bile and urine in rats fed the CSAA or CDAA diet

Wistar rats were fed CSAA or CDAA diet for 12 weeks. Bile was collected from rats under anesthesia with urethane over a 60-min period to estimate the choleretic activity, and urine was collected over a 24-h period using a metabolic cage. The total bilirubin levels of bile and urine were determined as described under Materials and Methods.

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<tr>
<td></td>
<td>4 weeks</td>
<td>12 weeks</td>
<td>4 weeks</td>
<td>12 weeks</td>
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<tr>
<td>Bile flow (µl/min/g liver)</td>
<td>0.8 ± 0.1</td>
<td>0.8 ± 0.1</td>
<td>0.1 ± 0.1</td>
<td>0.1 ± 0.1</td>
</tr>
<tr>
<td>Total bilirubin concentration (µg/ml)</td>
<td>85.4 ± 18.9</td>
<td>51.2 ± 6.0**</td>
<td>89.0 ± 13.7</td>
<td>42.0 ± 7.2**</td>
</tr>
<tr>
<td>Bilirubin excretion (ng/min/g liver)</td>
<td>69.0 ± 13.7</td>
<td>42.0 ± 7.2**</td>
<td>0.9 ± 0.8</td>
<td>1.4 ± 0.8</td>
</tr>
<tr>
<td>Total bilirubin concentration (µg/ml)</td>
<td>0.1 ± 0.1</td>
<td>0.5 ± 0.1***</td>
<td>0.1 ± 0.1</td>
<td>0.7 ± 0.6***</td>
</tr>
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</table>

* p < 0.05, significantly different from CSAA rats (n = 5–6).
** p < 0.01, significantly different from CSAA rats (n = 5–6).
***p < 0.001, significantly different from CSAA rats (n = 5–6).

HPLC/UV analysis was done using the Shimadzu LC-10AD system (Shimadzu, Kyoto, Japan) with a photodiode array detector. The analytical column was a Symmetry Shield RP-18, 75 × 4.6 mm, 3.5 µm (Waters), and column temperature was kept at 40°C. The mobile phase was delivered with a linear gradient elution system acetonitrile (A)/0.5% (v/v) acetic acid (B) at a flow rate of 1.0 ml/min. The gradient profile was as follows: 45% A increasing to 60% over 20 min and then A/B (60:40) maintained for 5 min. The UV detector was set at 254 nm.

Statistical Analysis. Statistical analysis was carried out using the Student’s t test. Statistical significance was considered at a p value of less than 0.05. Data were expressed as mean ± S.D.

Results

Characteristics of the Rats Fed CDAA Diets. Chronic liver injury model was made using Wistar strain rats fed CDAA diet (CDAA rats) for 4 or 12 weeks. For the control, rats fed CSAA diet (CSAA rats) were prepared. Plasma levels of ALT, ALP, total bilirubin, and TBA were significantly higher in CDAA rats than in CSAA rats, whereas phospholipid concentration was significantly decreased (Table 1). Liver weights in CDAA rats were markedly greater than those in CSAA rats (Table 1). Hepatic steatosis was observed in the liver tissues of rats fed the CDAA diet for 4 and 12 weeks (Fig. 2). Plasma hyaluronic acid concentration, a plasma parameter of tissue fibrosis, was significantly higher only in rats fed the CDAA diet for 12 weeks compared with CSAA rats. The change in the plasma hyaluronic acid level was consistent with the histological observation, indicating severe hepatic fibrosis only in rats fed the CDAA diet for 12 weeks (Fig. 2). Bilirubin excretion into bile was lower in CDAA rats than in CSAA rats, although bile flow did not change. Urinary bilirubin excretion was higher in the rats fed the CDAA diet for 12 weeks than CSAA rats (Table 2).
Materials and Methods

Pharmacokinetics of GL and Its Metabolites in CDAA Rats. GL was orally administered to rats fed the CDAA or CSAA diet for 4 or 12 weeks, and samples of blood and urine were collected. Time course of changes in the plasma concentration of GL, 3MGA, and GA in rats fed the CDAA diet for 12 weeks are shown in Fig. 3. The plasma concentrations of GL and 3MGA in CDAA rats were higher than those in CSAA rats until 12 h for GL and 4 h for 3MGA after GL administration. The plasma concentration of GA for all the measurement periods after GL treatment was almost identical in CDAA and CSAA rats.

Area under plasma concentration-time curve from 1 to 24 h (AUC \(_{1-24}\) h) of GL and its metabolites when GL was orally administered to rats fed the CSAA or CDAA diet

Wistar rats were fed CSAA or CDAA diet for 4 or 12 weeks. GL (100 mg/kg) was orally administered to the rat, and the blood samples were collected 1, 2, 4, 8, 12, and 24 h after the treatment. Plasma concentrations of GL and its metabolites, 3MGA and GA, were measured as described under Materials and Methods. AUC \(_{1-24}\) h was calculated from the plasma measurements using the trapezoidal rule from the first point to the last point.

<table>
<thead>
<tr>
<th>Compound</th>
<th>4 Weeks</th>
<th>12 Weeks</th>
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<tr>
<td></td>
<td>CSAA</td>
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<td></td>
<td>CSAA</td>
<td>CDAA</td>
</tr>
<tr>
<td>GL (µg/h/ml)</td>
<td>2.58 ± 2.11</td>
<td>18.6 ± 8.8***</td>
</tr>
<tr>
<td>3MGA (ng/h/ml)</td>
<td>102 ± 30</td>
<td>404 ± 34***</td>
</tr>
<tr>
<td>GA (µg/h/ml)</td>
<td>30.9 ± 9.8</td>
<td>34.4 ± 18.4</td>
</tr>
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</table>

**p < 0.01, significantly different from the CSAA rats (n = 5–6).***p < 0.001, significantly different from the CSAA rats (n = 5–6).

Urinary excretion of GL and its metabolites when GL was orally administered to the rats fed the CSAA or CDAA diet

Wistar rats were fed with CSAA or CDAA diet for 13 weeks; then, 100 mg/kg GL was orally administered. Urine samples were collected 24 h using metabolic cages. The collected urinary volume and the concentrations of GL and its metabolites, 3MGA and GA, were measured as described under Materials and Methods. Excretive amounts of GL, 3MGA, and GA were calculated from the urinary volume and these concentrations.

<table>
<thead>
<tr>
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<th>CSAA</th>
<th>CDAA</th>
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<tr>
<td>Urinary volume (ml/100 g body weight)</td>
<td>1.2 ± 0.5</td>
<td>1.4 ± 0.4</td>
</tr>
<tr>
<td>GL (ng/100 g body weight)</td>
<td>75.2 ± 55.3</td>
<td>408 ± 170**</td>
</tr>
<tr>
<td>3MGA (ng/100 g body weight)</td>
<td>35.8 ± 14.5</td>
<td>145 ± 37.5*</td>
</tr>
<tr>
<td>GA (ng/100 g body weight)</td>
<td>1.1 (1/6)*</td>
<td>3.3 ± 3.9 (4/5)*</td>
</tr>
</tbody>
</table>

**p < 0.05, significantly different from the CSAA rats (n = 5–6).***p < 0.001, significantly different from the CSAA rats (n = 5–6).

*The incidence rate of detection (limit, 1 ng/100 g body weight).

Immunoblot and Immunohistochemical Analyses of Liver Mrp2.

Levels of Mrp2 in the livers of CDAA rats were evaluated by Western blotting and immunohistochemical analysis. Mrp2 levels in the plasma membrane fraction from liver homogenate of the rats fed the CDAA diet for 12 weeks were lower than those of the rats fed the CSAA diet (Fig. 4A). Densitometric analysis revealed that Mrp2 levels in the CDAA diet-fed rats for 4 and 12 weeks were 40.8 ± 6.5% (p < 0.01) and 37.0 ± 5.5% (p < 0.001), respectively, compared with those of CSAA rats. Mrp3 levels were down-regulated only in the rats treated with the CDAA diet for 12 weeks, and Mrp4
levels were unaffected by CDAA diets (data not shown). Immunohistochemical analysis of Mrp2 in liver tissue sections confirmed remarkable differences between the rats fed the CSAA diet and those fed the CDAA diet for 12 weeks (Fig. 4B). The Mrp2-derived signals were much lower in rats fed the CDAA diet than those fed the CSAA diet for 4 (27.7 ± 6.3%, p < 0.001) and 12 (23.6 ± 5.3%, p < 0.001) weeks.

Pharmacokinetics of i.v. Administered 3MGA in Rats Fed the CDAA Diet and in EHBRs. 3MGA was i.v. injected into rats fed the CSAA or CDAA diet for 13 weeks, and the plasma and bile concentrations of 3MGA were traced (Fig. 5, A and B). In CSAA rats, 3MGA disappeared quickly from the plasma, and about 90% of 3MGA was excreted in the bile within 120 min; the rate of disappearance of 3MGA from plasma was slower in CDAA rats than in CSAA rats. The plasma concentration of 3MGA in CDAA rats was significantly augmented compared with that in CSAA rats. The excretion of 3MGA into bile of CDAA rats was markedly suppressed by liver injury. The pharmacokinetics of i.v. injected 3MGA were also evaluated using EHBRs, which are expressing dysfunctional Mrp2 protein (Fig. 5, C and D). The plasma concentration of 3MGA was immediately decreased, and approximately 85% of 3MGA was excreted into the bile 40 min postinjection. In contrast, biliary excretion of 3MGA was significantly slower in EHBR than in SD rats, and plasma concentrations of 3MGA were significantly higher by 120 min postinjection in EHBRs than in SD rats (Fig. 5, C and D).

Discussion

Feeding CDAA diets to rats is known to induce hepatic fibrosis and cirrhosis and has been used to establish an experimental model of hepatitis in animals (Jin et al., 2007; Ustundag et al., 2007). Biochemical analysis and histological observation in this study revealed that severe liver injury was induced by feeding the CDAA diet to Wistar rats for as little as 4 weeks. For CDAA rats given GL orally, the plasma concentration, AUC, and urinary excretion of GL and 3MGA were significantly increased, whereas those of GA were not affected.

![Fig. 4. Western blot and immunohistological analysis of Mrp2 in the rats fed the CSAA or CDAA diet. A, immunoblot analysis of Mrp2 in crude plasma membrane fraction isolated from liver of Wistar rats treated with CSAA or CDAA diet for 12 weeks. B, immunohistological localizations of Mrp2 in the livers were analyzed using light microscopy at 100× magnification. Brownish area shows Mrp2-positive staining.](image)

![Fig. 5. Plasma concentration profiles and biliary excretion of 3MGA when 3MGA was i.v. infused to Wistar rats fed CSAA or CDAA diet for 13 weeks (A and B) and SD rats or EHBRs (C and D). 3MGA (5 mg/kg) was i.v. infused to the rats, blood samples were collected at 5, 20, 40, 60, 90, and 120 min (A and C), and bile samples were collected over a period of 120 min (B and D). Plasma and bile concentrations of 3MGA were measured as described under Materials and Methods. Open square, CSAA rats; closed circle, CDAA rats (A and B); open circle, SD rats; closed square, EHBRs (C and D); (n = 6).](image)
In contrast, excretion of 3MG into bile was significantly reduced in CDAAs rats when 3MG was i.v. injected. These results clearly indicate that the pharmacokinetics of GL and its metabolites are altered when severe liver injury is induced. They are consistent with a clinical report stating that the elimination of GL from plasma was significantly slower when GL was injected in patients with chronic hepatitis than when it was injected in healthy individuals (Yamamura et al., 1995).

The increased plasma concentrations of GL and 3MG in the hepatic fibrosis model of rats may be related to the decreased level of Mrp2 in their livers. Mrp2 is an organic anion transporter located on the bile canalicular membrane and is responsible for the excretion of various organic anions, including glutathione conjugates and glucuronide conjugates of xenobiotics into bile (Kepler and Konig, 1997; Gotot et al., 2000). The Mrp2 level in the livers of CDAAs rats was drastically down-regulated, which might lead to a decrease in excretion of 3MG in bile and a subsequent increase in tubular secretion (as shown in the increased urinary concentration of 3MG in CDAAs rats). EHBRs were originally found as mutant rats with chronic conjugated hyperbilirubinemia (Hosokawa et al., 1992). They were subsequently confirmed to express the dysfunctional Mrp2 protein by a point mutation in the open reading frame (Ito et al., 1997). Excretion of 3MG into bile was shown to be suppressed in EHBRs, which also supports the expectation that Mrp2 plays a role in the biliary excretion of 3MG and suggests that the reduction and/or dysfunction of Mrp2 by liver injury or mutation led to a decrease in bile excretion of 3MG. Suppression of biliary excretion of GL and 3MG resulted in increased plasma concentrations and eventually increased urinary excretion of GL and 3MG through renal tubular epithelial cells.

The inhibitory effect of GL on 11β-HSD2 activity was reported to be 200- to 1000-fold less potent than GA (Bühler et al., 1994). In contrast, 3MG exhibited potent inhibition of 11β-HSD2 activity in kidney microsomes at a similar extent similar to GA (Kato et al., 1995). It was reported that patients with GL-induced hypokalemia exhibited higher plasma concentration of 3MG than GL-treated patients without hypokalemia, although the plasma concentrations of GA between these two groups did not differ (Kato et al., 1995). GA would not be transported into renal tubular epithelial cells from the circulation because GA is scarcely eliminated into urine as rats as well as in humans (Ploeger et al., 2001). Therefore, it is predicted that the inhibitory effects of 3MG on 11β-HSD2 in renal tubular epithelial cells might be much more potent than the effect of GA in vivo. Indeed, i.v. injection of 3MG into guinea pigs was shown to decrease the plasma concentration of potassium (Ohtake et al., 2007). These studies suggest that 3MG, not GA, is likely to play a central part in inducing pseudoaldosteronism by inhibiting 11β-HSD2. Therefore, the accumulation of 3MG in renal tissues by reduced biliary excretion due to liver dysfunction might be involved in the pathogenesis of pseudoaldosteronism caused by ingestion of licorice and GL. Further investigations of Mrp2 function for the biliary transportation of 3MG and its transport mechanisms through tubular epithelium are required, but the present study provides basic but important information for the clinical use of GL and GL-containing herbal medicines because they are often prescribed to patients with liver dysfunction.

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