SHORT COMMUNICATION

Biliary Excretion of Glycyrrhetinic Acid: Glucuronide-Conjugate Determination Following a Pharmacokinetic Study of Rat Bile

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Liquorice is a commonly prescribed herb in traditional Chinese medicine with the primary constituent, glycyrrhetinic acid (GA) responsible for the toxic effects arising from its chronic consumption. Hepatic transformation and biliary excretion of GA are significant and well-documented pharmacokinetic pathways in humans, while glucuronide conjugates are the major identified metabolites. Here we report the role of bile in GA bioconversion in rats; this being achieved following intravenous administration of GA to Sprague–Dawley rats at a dose of 2 mg/kg with bile fluid analyzed for 3 h post-injection using HPLC. The maximum concentration of glucuronides was detected about 30 min post-administration, while the cumulative biliary excretion of glucuronides after 3 h was found to be 63.6 ± 6.4%. Our findings indicate a relatively high rate of biliary excretion for GA via the formation of glucuronide conjugates, and as a result of these findings, glucuronidation can be firmly regarded as a primary detoxification pathway for GA in rats. Copyright © 2014 John Wiley & Sons, Ltd.

Keywords: glycyrrhetinic acid; glucuronide metabolite; biliary excretion.

INTRODUCTION

Liquorice (Glycyrrhiza glabra) is one of the oldest and most commonly prescribed herbs in traditional Chinese medicine (Ren et al., 2010). It has been widely used in the treatment of numerous ailments involving those of the liver and cardiovascular system, as well as conditions such as rheumatoid arthritis, asthma and gastric ulceration (Asl and Hosseinzadeh, 2008). The principal constituent within liquorice is glycyrrhizin, which is responsible for the characteristic sweetness and typical taste associated with liquorice products. After oral administration of liquorice, the glycyrrhizin within is preferentially hydrolyzed to an aglycone derivative, glycyrrhetinic acid (GA) through the action of β-glucuronidases present in human intestinal commensal bacteria (Hattori et al., 1983). GA undergoes glucuronidation reaction, rendering it polar and so a candidate for renal excretion (Lu et al., 2009). Despite its wide ranging curative properties, its use has been hampered by toxic symptoms (such as pseudoaldosteronism, hypertension and hypokalemia) arising from its chronic use, known to stem from elevated GA levels in the circulation (van Rossum et al., 1998; Isbrucker and Burdock, 2006). The mechanism underlying the toxicity is attributed to its potent and direct mineralocorticoid activity, through inhibition of renal 11β-hydroxysteroid dehydrogenase, resulting in significant sodium retention (Stewart et al., 1990). Glucuronides are the major identified inactive metabolites of GA. Since glucuronide conjugates are catalyzed by enzymes responsible for phase II metabolism and are mainly excreted through transporters located in liver, it follows that detailed investigation and understanding of GA’s biliary excretion profile, in a quantitative manner, will assist in better predicting potential drug–drug interactions occurring through phase II enzymes and their hepatic transporters. The primary purpose of this pharmacokinetic study was to quantitatively analyze glucuronidated by-products of GA arising from biliary excretion.

MATERIALS AND METHODS

Chemicals. GA (purity >99%) was supplied by Henan Shuaike Pharmaceutical Co. Ltd (Henan, China). Methanol and acetonitrile of HPLC grade were purchased from Tedia Company (Fairfield, OH, USA). All other reagents were of analytical grade.

Preparation of stock solutions and working solutions. A reference standard of GA was accurately weighed and dissolved in methanol to a final concentration of 2000 μg/mL (stock solution). The stock solution of GA was diluted serially with methanol to concentrations of 8, 16, 40, 160, 400, 800 and 2000 μg/mL (working solutions), and each was stored at 4 °C prior to use.

Received 29 January 2014
Revised 12 April 2014
Accepted 18 April 2014
**Determination of GA.** Analysis was performed on a Shimadzu SPD-10Avp series HPLC-UV system and a HW-2000 Workstation. The detection wavelength was 254 nm, and the analytical column was a Shimadzu VP-ODS column (150 mm × 2.0 mm, i.d. 5 μm). The mobile phase consisted of methanol and 5 mM ammonium acetate buffer adjusted to pH 4.0 with 1% acetic acid (85:15, v/v) at a flow rate of 1 mL/min, and the injection volume was 20 μL.

**Animal experiments.** All animal studies were approved by the Animal Ethics Committee of China Pharmaceutical University. Male Sprague–Dawley rats (weight 200 ± 20 g) were obtained from the Laboratory Animal Centre of Southeast University (Nanjing, China). Four rats were fasted overnight, and each had access to water throughout the experimental period. The abdomen was opened by median incision under anesthesia with i.p. administration of urethane (1 g/kg). The common bile duct was cannulated with PE10 polyethylene tubing having an inner diameter of 0.28 mm and an outer diameter of 0.61 mm (product of BD Company, Parsippany, NJ, USA). Two hours after the surgery, the rats were dosed intravenously via the caudal vein with GA (2 mg/kg dose). Bile samples were collected in pre-weighed vials over the periods of 0–15, 15–30, 30–45, 45–60, 60–90, 90–120 and 120–180 min after administration. All samples were frozen over dry ice prior to being sequentially analyzed on the same day.

**Sample preparation of GA-derived glucuronide in rat bile.** Alkaline hydrolysis of GA-derived glucuronide to transform it to GA was undertaken for the purpose of quantitative analysis according to a method previously described (Jing et al., 2008). Briefly, 20 μL bile sample was mixed with 20 μL NaOH (2 M), and hydrolysis was performed at 90°C for 1 h. After cooling to room temperature, 100 μL methanol was added to the mixture, followed by sonication for 30 min with the water bath maintained at room temperature. The mixture was centrifuged at 3000 g for 5 min, followed by filtering of supernatant through a 0.45-μm RC filter.

**RESULTS AND DISCUSSION**

**Determination of GA-derived glucuronide in rat bile**

The calibration curves were prepared by adding 5 μL of each GA working solution to 15 μL of drug-free rat bile, which achieved final concentrations of GA in bile of 2, 4, 10, 40, 100, 200 and 500 μg/mL; then centrifuged at 3000 g for 5 min and filtered through a 0.45-μm RC filter prior to HPLC analysis. Linearity regression was assessed by plotting the peak areas of GA versus known concentrations (C). A good linearity profile \((r^2 = 0.9995)\) was obtained over the entire concentration range of 2–500 μg/mL, and the regression equation of the curves was then determined \((C = 12.302C + 47.286)\). As is the peak areas of GA. The lower limit of quantification was calculated to be 2 μg/mL.

**Pharmacokinetics of GA-derived glucuronide in rat bile**

The time course of the mean concentrations of GA-derived glucuronide in bile fluid of the four SD rats after intravenous injection of 2 mg/kg GA is shown in Fig. 1 (dashed line). Excretion of the GA equivalents in rat bile after intravenous administration of GA was calculated from the GA concentration in bile, the bile flow rate and the body weight of each rat. The cumulative biliary excretion of GA-derived glucuronide in 3 h is also shown in Fig. 1 (solid line). The recovery of administrated dose was calculated as follows:

\[
\text{Recovery of the administrated dose (\%)} = \frac{C_{\text{bile}} (\mu g/mL)}{V_{\text{bile}} (mL)/\text{Dose} (mg)/10}
\]

where \(C_{\text{bile}}\) is the concentration of GA in the bile and \(V_{\text{bile}}\) is the volume of the bile.

In the present study, we aimed to quantitatively identify GA glucuronide to facilitate the better prediction of the potential drug–drug interactions occurring through phase II enzymes and their hepatic transporters. A dose of 2 mg/kg was selected according to the dose in our previous report (Jing et al., 2008) which can allow us to make comparison between the previous results and this current study. Preliminary studies showed that GA and its major metabolites can be detected after receiving this dose during the whole experimental process. The plasma pharmacokinetic behavior of GA was also studied, and no enterohepatic cycling of GA was observed after intravenous injection of 2 mg/kg GA (data not shown).

Phase II metabolism is the major pathway of GA detoxification in rats as demonstrated in our previous work (Jing et al., 2008). Through meticulous analysis using LC-MS we went on to report the detection of various metabolites, and these included glucuronides, sulfates and the novel glucuronide–sulfate conjugate (Jing et al., 2008). Along the premise of our previous work, GA glucuronides can be transformed to GA (through base hydrolysis) and reach steady-state...
concentrations within 1 h (data not shown). In this quantitative study of glucuronide biotransformation, we revealed that the glucuronide conjugate was indeed the major metabolite, accounting for >60% of total GA administered to rats. Given that it is likely other metabolites such as amino acid conjugates might also be hydrolyzed by base, we further confirmed the results by using the linear curve of GA and quantified the biliary excretion of GA-glucuronides by the method previously described (Lu et al., 2009), the results of which are similar to our findings here. Thus, GA-glucuronide can be generally considered the only source for GA under base analysis.

In summary our findings confirm that glucuronidation of GA is the most important pathway for GA detoxification in rats. The LC-MS method we developed previously and the method utilized here is versatile in that it can also be used in future studies towards the identification of GA metabolites and compounds following a similar metabolic pathway.

### CONCLUSION

The high biliary excretion of GA within 3 h indicates that glucuronidation can be regarded as the predominant detoxification pathway of GA in rats.

### Acknowledgement

This study was supported by the 863 Hi-tech Program of China (No. 2007AA02Z171).

### Conflict of Interest

The authors have declared that there is no conflict of interest.

### REFERENCES


Table 1. Pharmacokinetic parameters of GA-derived glucuronide in SD rat bile after intravenous administration of GA (*n* = 4)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>$C_{\text{max}}$ (μg/mL)</th>
<th>$T_{\text{max}}$ (min)</th>
<th>$t_{1/2}$ (min)</th>
<th>AUC (μg min/mL)</th>
<th>Three-hour cumulative biliary excretion (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>22.5</td>
<td>33.8</td>
<td>46.4</td>
<td>1434.3</td>
<td>63.6</td>
</tr>
<tr>
<td>SD</td>
<td>3.5</td>
<td>7.5</td>
<td>16.5</td>
<td>202.1</td>
<td>6.4</td>
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</tbody>
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