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Liquiritigenin pharmacokinetics in a rat model of diabetes mellitus induced by streptozotocin: greater formation of glucuronides in the liver, especially M2, due to increased hepatic uridine 5′-diphosphoglucuronic acid level

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Abstract

Liquiritigenin (LQ) is a candidate for the treatment of inflammatory liver disease. Many studies have confirmed that hepatic disease and diabetes mellitus are closely associated. Thus, the pharmacokinetic changes of LQ and its 2 glucuronides, M1 and M2, in a rat model of diabetes mellitus induced by streptozotocin (DMIS rats) were evaluated. Liquiritigenin was administered intravenously (20 mg/kg) or orally (50 mg/kg) in DMIS and control rats. Changes in in vitro activity and in vivo uridine 5′-diphosphoglucuronic acid level in the liver and intestine of DMIS rats compared with controls were also studied. After intravenous administration of LQ in DMIS rats, no significant changes in the pharmacokinetic parameters of LQ were observed. However, the AUC_{M2}/AUC_{LQ} ratio was significantly greater (by 53.0%) than that of controls. After oral administration of LQ, the AUC of LQ and metabolite ratios of M1 and M2 were comparable to controls. The increase in the formation of glucuronides of LQ, especially M2, after intravenous administration of LQ was due to the increased in vivo hepatic uridine 5′-diphosphoglucuronic acid level in DMIS rats as a result of alteration in carbohydrate metabolism in diabetes. The comparable pharmacokinetics of LQ, M1, and M2 after oral administration of LQ were mainly due to the comparable intestinal metabolism of LQ between the control and DMIS rats.
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1. Introduction

Liquiritigenin (LQ; 2,3-dihydro-7-hydroxy-2-[4-hydroxyphenyl]-[*S*]-4*H*-1-benzopyran-4-one), is an aglycone of liquiritin contained in *Glycyrrhizae radix* (licorice). Liquiritigenin is metabolized to 5 conjugates in rats: 4′-*O*-glucuronide (M1), 7-*O*-glucuronide (M2), 4′,7-*O*-disulfate (M3), 4′-*O*-glucuronide-7-*O*-sulfate (M4), and 7-*O*-glucuronide-4′-*O*-sulfate (M5) [1]. After intravenous administration of LQ at a dose of 5 mg/kg in rats, only M1, M2, and M3 were detected in the plasma, although 5 conjugates (M1-M5) were excreted in the bile [2]. After intravenous, oral, intraportal, intragastric, and intraduodenal administration of LQ at a dose of 20 mg/kg in male Sprague-Dawley rats, the unabsorbed fraction from the gastrointestinal tract up to 24 hours was 1.07% of the oral dose, the extent of absolute oral bioavailability (*F*) was only 6.68%, the hepatic first-pass effect after absorption into the portal vein was 57.1%, and the gastrointestinal first-pass effect was 92.5% of the oral dose [3]. Recently, the pharmacokinetics of LQ, M1, and M2 after intravenous and/or oral administration of various doses of LQ in 4 species (mice, rats, rabbits, and dogs) and animal scale-up of the pharmacokinetics of LQ to predict its pharmacokinetics in humans have been reported [4].

Liquiritigenin exerted cytoprotective effects against heavy metal–induced toxicity in rat hepatocyte-derived cultured cells [5] and showed protective efficacy in rats with acetaminophen-induced [6] or galactosamine/lipopolysaccharide-induced [7] acute liver injuries and in mice with carbon tetrachloride–induced liver injuries [2]. Liquiriti-

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genin is now being evaluated in preclinical studies as an oral agent for the treatment of inflammatory liver disease.

Many studies have confirmed that hepatic disease and diabetes mellitus are closely related. For example, chronic hepatitis C has been reported to be associated with type 2 diabetes mellitus and insulin resistance [8]. Imaeda and Nojiri [9] also reported that there is an important relationship between insulin resistance and hepatobiliary disease. The prevalence of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis is high in patients with diabetes mellitus [10,11]. Therefore, possible pharmacokinetic changes of LQ, M1, and M2 in the diabetic state should be studied.

Liquiritigenin was mainly metabolized to its 2 glucuronide conjugates, M1 and M2, via uridine diphosphate-glucuronosyltransferases (UGTs) in the gastrointestinal tract and liver of rats [3]. Although there have been many studies on the effects of diabetes mellitus on hepatic glucuronidation reactions [12], the results were controversial. For example, increase, decrease, or no changes in glucuronidation of 4-nitrophenol were reported in genetically diabetic animal and diabetic animal induced by streptozotocin or alloxan. Moreover, although intestinal metabolism is a major determinant of pharmacokinetics of LQ after its oral administration (gastrointestinal first-pass effect of LQ was 92.5% of the oral dose [3]), it seems that there have been few studies on the effects of diabetes mellitus on intestinal glucuronidation reaction.

Therefore, changes in the pharmacokinetics of LQ, M1, and M2 after intravenous (20 mg/kg) or oral (50 mg/kg) administration of LQ in streptozotocin-induced diabetes mellitus rats (DMIS rats) were evaluated with respect to changes in activities of UGTs in hepatic and intestinal microsomes and in vivo uridine 5′-diphosphoglucuronic acid (UDPGA) levels in the liver and intestine.

2. Materials and methods

2.1. Chemicals

Liquiritigenin was synthesized by Prof Jee W Lee (College of Pharmacy, Seoul National University, Seoul, South Korea). The purity of the synthesized LQ was comparable to that of the authentic LQ (Chromadex, Santa Ana, CA) using a high-performance liquid chromatographic (HPLC) analysis. M1 and M2 were obtained from Dr Hye J Chung (Center for Chemoinformatics, Life Sciences Research Division, Korea Institute of Science and Technology, Seoul, South Korea). Chlorzoxazone, lamotrigine, and theobromine (internal standards for the HPLC analysis of LQ, M1, and M2, and *p*-nitrophenyl glucuronide [*p*NP-Glu], respectively], *p*-nitrophenol (*p*NP), *p*NP-Glu, tetraethylammonium bromide, UDPGA (as a trisodium salt), β-glucuronidase (type HP-1; from *Helixa pomatia* having a β-glucuronidase activity of 127 000 U/mL and a sulfatase activity of <7500 U/mL), streptozotocin, and tris (hydroxymethyl)aminomethane (Tris)-buffer were purchased from Sigma-Aldrich (St Louis, MO). Polyethylene glycol 400 was a product from Duksan Pure Chemicals Company (Ansan, South Korea). Other chemicals were of reagent or HPLC grade.

2.2. Animals

Protocols for these animal studies were approved by Institutional Animal Care and Use Committee of Seoul National University. Male Sprague-Dawley rats (7-9 weeks old, weighing 230-295 g) were purchased from Charles River Korea (Orient, Seoul, South Korea). They were maintained in a clean room (Animal Center for Pharmaceutical Research, College of Pharmacy, Seoul National University) at a temperature of 20°C to 23°C with 12-hour light (7:00 AM to 7:00 PM) and dark (7:00 PM to 7:00 AM) cycles and a relative humidity of 50% ± 5%. The rats were housed in metabolic cages (Tecniplast, Varese, Italy) under filtered, pathogen-free air, with food (Agribrands Purina Korea, Pyeongtaek, South Korea) and water available ad libitum.

2.3. Induction of diabetes mellitus in rats by streptozotocin injection

Rats were randomly divided into 2 groups: control and DMIS rats. To induce diabetes mellitus, freshly prepared streptozotocin, 40 mg/kg, was injected (approximately 0.3 mL) in the overnight-fasted rats via the lateral tail vein [13]. The same volume of 0.9% NaCl injectable solution was injected into the control rats. On the seventh day after intravenous administration of streptozotocin (DMIS rats) or 0.9% NaCl injectable solution (control rats), blood glucose levels were measured using the Medisense Optium kit (Abbott Laboratories, Bedford, MA); and rats with blood glucose levels higher than 250 mg/dL were selected as being diabetic.

2.4. Preparation of microsomes or extracts of rat liver and intestine

The procedures used for the preparation of hepatic and intestinal microsomes from control (n = 5) and DMIS (n = 5) rats were similar to a reported method [13]. Protein contents in the hepatic and intestinal microsomes were measured using a reported method [14].

In the mean time, hepatic and intestinal extracts for the measurement of UDPGA level were prepared according to a reported method [15] with a slight modification. Briefly, a 0.5-g portion of the liver (or a 0.25-g portion of intestine) was placed in a test tube with 1.5 mL (or 0.75 mL for intestine) distilled water and put in the boiling water for 4 minutes, homogenized, and centrifuged at 3500*g* for 10 minutes. Resulting heat-treated supernatant was collected and stored at −70°C.

2.5. Measurement of the maximum velocity, the apparent Michaelis-Menten constant, and the intrinsic clearance for the disappearance of LQ and the formation of M1 and M2 in hepatic or intestinal microsomes of DMIS and control rats

The procedures used for the measurement of the maximum velocity (V_{max}) and the apparent Michaelis-Menten constant (K_m; the concentration at which the rate is one half of V_{max}) for the disappearance of LQ and the formation of M1 and M2 were similar to a reported method [16]. The above microsomes (equivalent to 0.1 and 0.2 mg proteins for hepatic and intestinal microsomes, respectively), 0.1 mol/L Tris-HCl buffer (pH 7.4), $MgCl_2$ (dissolved in Tris-HCl buffer of pH 7.4; 1 mmol/L in incubation), and alamethicin (dissolved in 50% ethanol; 50 μg/mg protein) were mixed and placed on the ice for 10 minutes. Afterward, 10 μL of 50% methanol containing LQ (to have final LQ concentrations of 1, 2, 10, 20, 40, 100, and 200 μmol/L in incubation) was added; and the mixture was preincubated for 5 minutes in a thermomixer (Thermomixer 5436; Eppendorf, Hamburg, Germany) kept at 37°C and at a rate of 600 rpm. To initiate the reaction, UDPGA (dissolved in Tris-HCl buffer of pH 7.4 to have a final concentration of 3 mmol/L in incubation) was added to give final volume of 500 μL. After 5-minute incubation, two 50-μL aliquots were collected and added to each Eppendorf tube containing 50 μL of methanol having 20 μg/mL of chlorzoxazone (internal standard for LQ) and 1 mL of diethyl ether, or containing 100 μL of acetonitrile having 7.5 μg/mL of lamotrigine (internal standard for M1 and M2), and vortex-mixed to terminate the reaction. All of the above microsomal incubation conditions were within linear range of reaction rate (preliminary experiments indicated that the reaction rates were in the linear ranges up to 10-minute incubation with the given microsomal protein contents). The kinetic constants (K_m and V_{max}) for the disappearance of LQ and the formation of M1 and M2 were calculated using a nonlinear regression method [17]. The unweighted kinetic data from microsomes were fitted to a single-site Michaelis-Menten equation: $V = V_{max} \times [S]/(K_m + [S])$, in which $[S]$ is the substrate concentration. The intrinsic clearance (CL_{int}) for the disappearance of LQ and the formation of M1 and M2 was calculated by dividing the V_{max} by the K_m.

2.6. Measurement of the in vivo hepatic and intestinal UDPGA levels in control and DMIS rats

To find whether in vivo hepatic or intestinal UDPGA level is changed in DMIS rats, which can affect the metabolism of LQ via UGTs in the liver or intestine, in vivo hepatic and intestinal UDPGA levels were measured according to the reported enzymatic assay based on the formation of *p*NP-Glu from *p*NP and UDPGA present in the extracts [18]. The above-mentioned incubation mixture (a final volume of 0.5 mL) was used except for 1 mmol/L *p*NP as a substrate and 200 μL of hepatic or intestinal extract from control (n = 5) and DMIS (n = 5) rats (or known amounts of UDPGA in Tris-HCl buffer of pH 7.4) as a UDPGA donor, and incubated in the same condition for 60 minutes. The calibration curve for UDPGA quantification was obtained by adding known amounts of UDPGA (5, 10, 20, 30, 50, and 75 nmol dissolved in Tri-HCl buffer of pH 7.4; 3 times repetition for each concentration) instead of liver or intestinal extracts. The reaction was terminated by adding 50 μL of each incubation mixture to each Eppendorf tube containing 100 μL of methanol having 20 μg/mL of theobromine (internal standard for the HPLC analysis of *p*NP-Glu) and vortex-mixed.

2.7. Measurement of the affinity of hepatic microsomes from control and DMIS rats to UDPGA for the disappearance of LQ and the formation of M1 and M2

To prove whether changes in in vivo hepatic UDPGA level can affect the metabolism of LQ via UGTs in the liver, the disappearance of LQ and the formation of M1 and M2 were evaluated in hepatic microsomes from control (n = 4) and DMIS (n = 4) rats with various concentrations of UDPGA. The procedures used were similar to the above-mentioned in vitro study to measure the V_{max} and K_m for the disappearance of LQ and the formation of M1 and M2; 200 μmol/L of LQ, as a substrate, and UDPGA (dissolved in Tris-HCl buffer of pH 7.4 to have final concentrations of 100, 200, 400, 800, 1500, and 3000 nmol/mL in incubation) were applied.

2.8. Intravenous and oral studies

The procedures used for the pretreatments of rats including the cannulation (early in the morning) of the carotid artery (for blood sampling) and the jugular vein (for drug administration in the intravenous study) on the seventh day after intravenous administration of streptozotocin (DMIS rats) or 0.9% NaCl injectable solution (control rats) were similar to reported methods [3,4,13]. Each rat was allowed to recover from light ether anesthesia for 4 to 5 hours before the study began. They were not restrained during the present study.

Liquiritigenin (dissolved in polyethylene glycol 400: distilled water = 40:60 [vol/vol]) was manually infused for 1 minute at a dose of 20 mg (2 mL)/kg via the jugular vein of control (n = 9) and DMIS (n = 11) rats, or administered orally at a dose of 50 mg (4 mL)/kg using a gastric gavage tube to control (n = 8) and DMIS (n = 9) rats. A blood sample was collected via the carotid artery (approximately 0.11 mL for time points for analysis of either LQ or its 2 metabolites, or 0.22 mL for time points for analysis of both LQ and its 2 metabolites) at 0 (control), 1 (end of the infusion), 3, 5, 10, 20, 30, 45, 60, 120, 180, 240, 360, and 480 minutes after the start of intravenous administration of LQ and at 0, 3, 7, 10, 15, 20, 30, 45, 60, 120, 240, 360, 480, 600, and 720 minutes after oral administration of LQ. A heparinized 0.9% NaCl injectable solution (20 U/mL), 0.3 mL, was used to flush the cannula immediately after each blood sampling to prevent

blood clotting. A blood sample was immediately centrifuged and one (or two) 50 μL of a plasma sample was stored at −70°C (Revco ULT 1490 D-N-S; Western Mednics, Asheville, NC) until the HPLC analysis of LQ, M1, and M2. The procedures used for the preparation and handling of the 24-hour urine sample ($Ae_{0\text{-}24\ h}$) and the gastrointestinal tract (including its contents and feces) sample at 24 hours ($GI_{24\ h}$) were similar to reported methods [3,4,13].

2.9. Measurement of rat plasma protein binding of LQ using equilibrium dialysis

Protein binding values of LQ in fresh plasma from control (n = 5) and DMIS (n = 5) rats were determined using equilibrium dialysis [3]. One milliliter of plasma was dialyzed against 1 mL of isotonic Sorensen phosphate buffer (pH 7.4) containing 3% (wt/vol) dextran ("the buffer") to reduce volume shift [19] in a 1-mL dialysis cell (Spectrum Medical Industries, Los Angeles, CA) using a Spectra/Por 4 membrane (molecular weight cutoff, 12-14 kd; Spectrum Medical Industries). To reduce equilibrium time between "the buffer" and plasma compartments, LQ was spiked into the plasma side [20]. After 6-hour incubation, two 50-μL aliquots were removed from each compartment and stored at −70°C until used for the HPLC analysis of LQ. Kang et al [3] reported that binding of LQ to 4% human serum albumin, similar to the ratio of albumin in rat plasma [21], was constant at LQ concentrations ranging from 0.2 to 20 μg/mL; the mean value was 90.9%. Thus, an LQ concentration of 5 μg/mL was chosen for this plasma protein binding study.

2.10. HPLC analysis of LQ, M1, M2, and pNP-Glu

The procedures used for the measurement of LQ, M1, and M2 in the samples were similar to reported HPLC methods [4,22]. Measurement of unconjugated LQ and total (unconjugated plus conjugated) LQ in the urine samples before and after incubation with β-glucuronidase was performed as in previous reports [4]. Detection limits of LQ in rat plasma and urine samples were 20 and 50 ng/mL, respectively. For the 2 metabolites, the detection limits were all 200 ng/mL. The mean within- and between-day coefficients of variation of the analysis of the same samples within the same day and on consecutive 3 days for LQ, M1, and M2 were all less than 5%.

Quantification of *p*NP-Glu was performed according to a reported method [23] with a slight modification. The supernatant after centrifugation of vortex-mixed 50 μL of incubation mixture and 100 μL of methanol containing 20 μg/mL of theobromine (internal standard for the HPLC analysis of *p*NP-Glu) was transferred to a clean tube and evaporated under a gentle stream of nitrogen gas at 50°C. The residue was reconstituted in 100 μL of distilled water, and a 50-μL aliquot was directly injected onto a reversed-phase HPLC column (Nucleosil C_{18}; 4.6 mm [internal diameter] × 150 mm [length]; particle size, 5 μm). The mobile phase (methanol:distilled water [25:75, vol/vol] containing 5 mmol/L tetraethylammonium bromide) was run at a flow rate of 0.7 mL/min, and an ultraviolet detector at 290 nm was used to monitor the column eluent. The retention times of *p*NP-Glu and theobromine (internal standard) were approximately 4.6 and 5.5 minutes, respectively. The limit of quantification of *p*NP-Glu in the microsomal incubation mixture was 0.1 μmol/L.

2.11. Pharmacokinetic analysis

Standard methods [24] were used to calculate the following pharmacokinetic parameters using a noncompartmental analysis (WinNonlin; professional edition version 2.1; Pharsight, Mountain View, CA): the total area under the plasma concentration–time curve from time zero to time infinity (AUC) or up to the last measured time, *t*, in the plasma ($AUC_{0\text{-}t}$) [25]; the time-averaged total body, renal, and nonrenal clearances (CL, CL_R, and CL_{NR}, respectively); the terminal half-life; the mean residence time, the apparent volume of distribution at steady state, and the *F*. The peak plasma concentration (C_{max}) and time to reach C_{max} (T_{max}) were directly read from the experimental data.

2.12. Statistical analysis

We used unpaired *t* tests to compare the 2 groups. *P* values < .05 were considered to be statistically significant. All results are expressed as means ± SDs except those for T_{max}, which are expressed as medians (ranges).

3. Results

3.1. V_{max}, K_m, and CL_{int} for the disappearance of LQ and the formation of M1 and M2 in hepatic and intestinal microsomes of control and DMIS rats

The V_{max}, K_m, and CL_{int} for the disappearance of LQ and the formation of M1 and M2 in the hepatic and intestinal microsomes of control and DMIS rats are listed in Table 1. The V_{max}, K_m, and CL_{int} for the disappearance of LQ and the formation of M1 and M2 in the hepatic microsomes were comparable between control and DMIS rats, except for the significantly slower (by 19.6%) V_{max} for the formation of M1 in DMIS rats. In DMIS rats, the V_{max} for the disappearance of LQ and the formation of M1 and M2 in the intestinal microsomes were significantly slower (by 55.0%, 47.4%, and 37.6%, respectively) than controls; and the K_m values for the formation of M1 and M2 in the intestinal microsomes were significantly smaller (by 48.6% and 30.8%, respectively) than controls. As a result of same-directional change of the V_{max} and the K_m, the CL_{int} for the disappearance of LQ and the formation of M1 and M2 in the intestinal microsomes of DMIS rats were comparable to controls. The above data suggested that the UGTs activity in hepatic and intestinal microsomes for the metabolism (glucuronidation) of LQ was not changed in DMIS rats.

Table 1
In vitro V_{max}, K_m, and CL_{int} for the disappearance of LQ and the formation of M1 and M2 in hepatic or intestinal microsomes and in vivo hepatic and intestinal UDPGA levels of control and DMIS rats

Parameter	Liver		Intestine	
	Control (n = 5)	DMIS (n = 5)	Control (n = 5)	DMIS (n = 5)
UGTs activity				
Disappearance of LQ				
V_{max} (nmol/[min mg protein])	60.6 ± 15.6	65.1 ± 7.79	17.1 ± 7.62	7.70 ± 4.12*
K_m (μmol/L)	85.8 ± 29.8	101 ± 10.4	26.0 ± 10.4	13.5 ± 7.68
CL_{int} (mL/[min mg protein])	0.726 ± 0.0708	0.646 ± 0.0498	0.664 ± 0.120	0.572 ± 0.111
Formation of M1				
V_{max} (nmol/[min mg protein])	11.3 ± 0.680	9.09 ± 0.951**	9.64 ± 2.68	5.07 ± 1.39**
K_m (μmol/L)	14.5 ± 1.99	14.3 ± 2.47	22.0 ± 5.40	11.3 ± 1.36**
CL_{int} (mL/[min mg protein])	0.789 ± 0.0665	0.653 ± 0.134	0.443 ± 0.0823	0.446 ± 0.112
Formation of M2				
V_{max} (nmol/[min mg protein])	28.6 ± 4.83	35.5 ± 6.77	17.0 ± 4.40	10.6 ± 2.55*
K_m (μmol/L)	49.8 ± 13.5	65.3 ± 15.0	41.6 ± 7.28	28.8 ± 2.57**
CL_{int} (mL/[min mg protein])	0.585 ± 0.0552	0.552 ± 0.0660	0.409 ± 0.0800	0.367 ± 0.0793
UDPGA level (nmol/g tissue)	309 ± 98.2	744 ± 156***	319 ± 88.6	475 ± 201

Values are expressed as means ± SD.
* Significantly different from control: $P < .05$.
** Significantly different from control: $P < .01$.
*** Significantly different from control: $P < .001$.

3.2. In vivo hepatic and intestinal UDPGA levels in control and DMIS rats

The linear calibration curve ($y = 0.9455x + 0.6932$ [$r = 0.999$] and $y = 0.1582x - 0.7603$ [$r = 0.997$] for hepatic and intestinal microsomes, respectively; in which y is the concentration of *p*NP-Glu formed [in micromoles per liter] and x is the amount of UDPGA in the incubation mixture [in nanomoles]) for the enzymatic assay of UDPGA level based on the formation of *p*NP-Glu from *p*NP was obtained from the amount of UDPGA in the assay mixture ranging from 5 to 50 nmol (for hepatic microsomes) or 10 to 75 nmol (for intestinal microsomes) (accuracies lay between 91.7% and 106%). Measured amounts of UDPGA in each hepatic (8.49-43.9 nmol) or intestinal (10.6-33.7 nmol) extract were all in the linear ranges of the assay. The obtained UDPGA levels in the liver and intestine of control and DMIS rats are also listed in Table 1. In DMIS rats, the UDPGA level in the liver was significantly higher (by 141%) than the controls, whereas that in the intestine was similar to controls.

3.3. Affinity of the hepatic microsomes from the control and DMIS rats to UDPGA for the formation of M1 and M2

The mean velocities for the disappearance of LQ and the formation of M1 and M2 in the hepatic microsomes from control and DMIS rats with various UDPGA concentrations are shown in Fig. 1A, B, and C, respectively. We could not

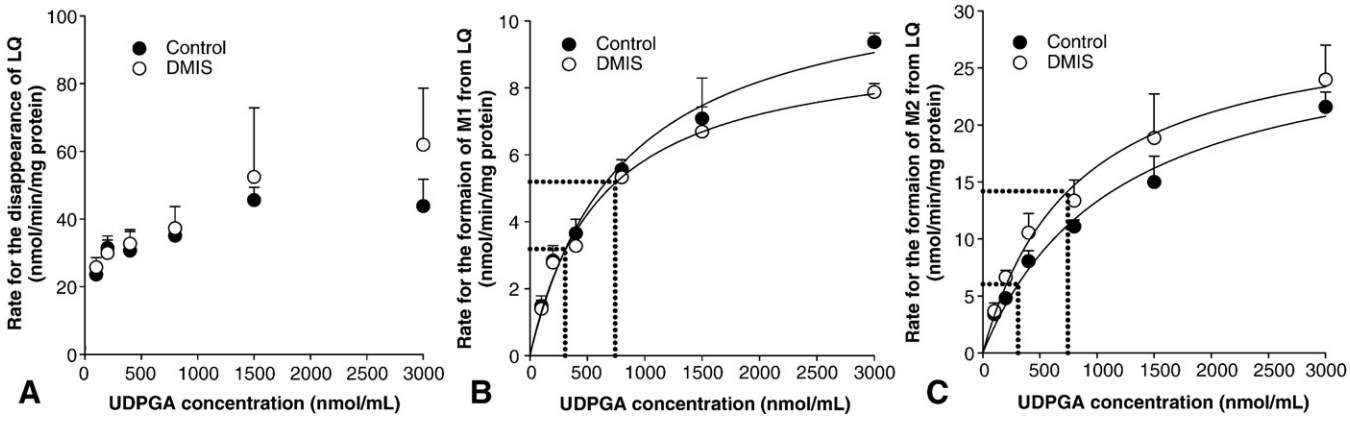

Fig. 1. Mean velocities for the disappearance of LQ (A) and the formation of M1 (B) and M2 (C) from 200 μmol/L of spiked LQ in hepatic microsomes of control (n = 4) and DMIS (n = 4) rats with various concentrations of UDPGA. Data from each group were fitted to a single-site, Michaelis-Menten equation (solid line); and extrapolated rates for the formation of M1 and M2 of each group based on the mean in vivo hepatic UDPGA level are also shown (dotted line). Values are means ± SD.

obtain the Michaelis-Menten–type relationship between the disappearance of LQ and the concentrations of UDPGA (Fig. 1A). On the other hand, the formation of both M1 and M2 showed UDPGA concentration dependency (Fig. 1B and C). The kinetic data for the formation of M1 and M2 in hepatic microsomes from control and DMIS rats were fitted to a single-site Michaelis-Menten equation: $V = V_{max} \times [S]/(K_m + [S])$, in which $[S]$ is the UDPGA concentration (Fig. 1B and C). The estimated V_{max} and K_m values for the formation of M1 were 11.5 nmol/(min mg protein) and 807 nmol/mL in controls, and 9.43 nmol/(min mg protein) and 616 nmol/mL in DMIS rats. The corresponding values for the formation of M2 were 28.9 nmol/(min mg protein) and 1170 nmol/mL in controls, and 29.7 nmol/(min mg protein) and 812 nmol/mL in DMIS rats. Extrapolated formation rates of M1 and M2 in each group of rats based on the measured mean in vivo hepatic UDPGA level (Table 1) are also shown in Fig. 1B and C, respectively, and suggest that increased hepatic UDPGA level in DMIS rats could lead to increase in the formation of M1 and M2 in DMIS rats.

3.4. Pharmacokinetics of LQ, M1, and M2 after intravenous administration of LQ to rats

After intravenous administration of LQ at a dose of 20 mg/kg in control and DMIS rats, the mean arterial plasma concentration–time profiles of LQ, M1, and M2 are shown in Fig. 2A, B, and C, respectively. The relevant pharmacokinetic parameters are listed in Table 2. In DMIS rats, the pharmacokinetic parameters of LQ were comparable to controls, except for the significantly greater (by 53.8%) percentages of the intravenous dose of LQ excreted in the 24-hour urine as total (conjugated + unconjugated) LQ ($Ae_{0\text{-}24\ h}$ of total LQ) than controls. Formation of both M1 and M2 was rapid; both M1 and M2 were detected in plasma at the second blood sampling time point (3 minutes) and rapidly reached T_{max} (3-5 minutes) in all rats studied. The DMIS rats showed the following changes in the pharmacokinetic parameters of M1 and M2 compared with controls: significantly shorter terminal half-life (by 31.8%) of M1, significantly higher C_{max} (by 44.0% and 56.9%, respectively) of both M1 and M2, and significantly greater AUC_{M2} and AUC_{M2}/AUC_{LQ} ratio (by 57.2% and 53.0%, respectively).

Table 2
Pharmacokinetic parameters of LQ, M1, and M2 after intravenous administration of LQ at a dose of 20 mg/kg in control and DMIS rats

Parameter	Control (n = 9)	DMIS (n = 11)
Initial body weight (g)[a]	271 ± 14.5	275 ± 10.1
final body weight (g)[b]	331 ± 23.0	282 ± 18.2***
Blood glucose (mg/dL)	109 ± 24.7	372 ± 43.1***
Urine output (mL/24 h)	16.0 ± 7.94	25.9 ± 11.1*
LQ		
AUC (μg · min/mL)	384 ± 127	355 ± 115
Terminal half-life (min)	6.77 ± 1.10	7.71 ± 1.36
MRT (min)	2.97 ± 1.06	2.56 ± 0.570
CL (mL/[min kg])	57.9 ± 20.8	61.0 ± 16.3
CL_R (mL/[min kg])	8.04 ± 3.71	10.1 ± 6.10
CL_{NR} (mL/[min kg])	49.8 ± 19.7	50.9 ± 14.4
V_{ss} (mL/kg)	186 ± 123	161 ± 65.8
$Ae_{0\text{-}24\ h}$ (% of LQ dose)	14.5 ± 6.27	16.4 ± 8.32
$Ae_{0\text{-}24\ h}$ of total LQ (% of LQ dose)	26.0 ± 5.66	40.0 ± 16.2*
$GI_{24\ h}$ (% of LQ dose)	0.158 ± 0.151	0.159 ± 0.0781
M1		
AUC (μg · min/mL)	786 ± 181	925 ± 146
Terminal half-life (min)	211 ± 70.6	144 ± 59.1*
C_{max} (μg/mL)	13.4 ± 1.82	19.3 ± 3.52***
T_{max} (min)[c]	3 (3)	3 (3–5)
AUC_{M1}/AUC_{LQ} ratio	2.32 ± 1.12	2.76 ± 0.619
M2		
AUC (μg · min/mL)	738 ± 160	1160 ± 511*
Terminal half-life (min)	207 ± 81.2	143 ± 73.0
C_{max} (μg/mL)	24.6 ± 3.62	38.6 ± 12.4**
T_{max} (min)[c]	3 (3–5)	3 (3–5)
AUC_{M2}/AUC_{LQ} ratio	2.15 ± 0.874	3.29 ± 0.973*

Values are expressed as means ± SD. MRT indicates the mean residence time; V_{ss}, the apparent volume of distribution at steady state.
[a] Measured just before treatment.
[b] Measured just before experiment.
[c] T_{max} was expressed as median (ranges).
* Significantly different from control: $P < .05$.
** Significantly different from control: $P < .01$.
*** Significantly different from control: $P < .001$.

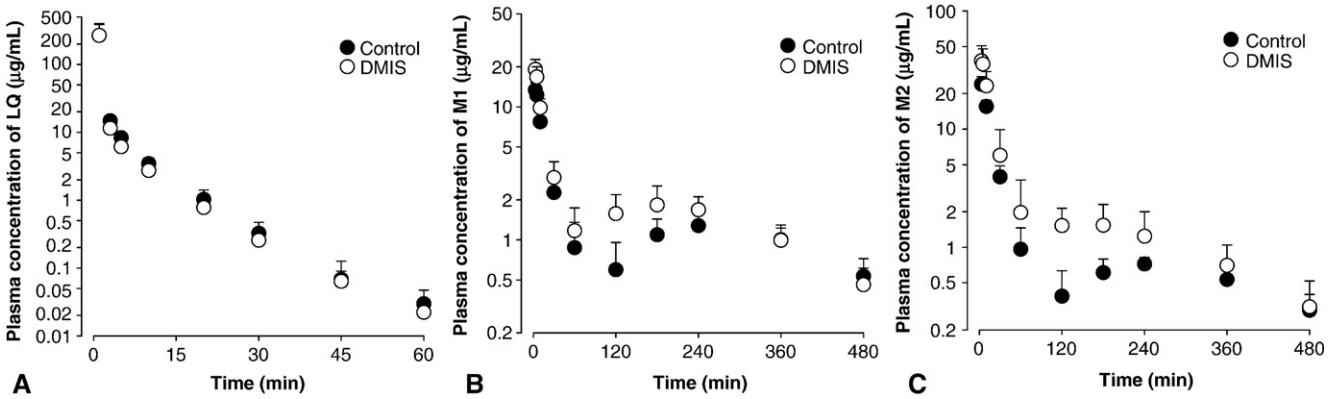

Fig. 2. Mean arterial plasma concentration–time profiles of LQ (A), M1 (B), and M2 (C) after intravenous infusion of LQ at a dose of 20 mg/kg in control (n = 9) and DMIS (n = 11) rats. Values are means ± SD.

3.5. Pharmacokinetics of LQ, M1, and M2 after oral administration of LQ to rats

For the oral administration of LQ at a dose of 50 mg/kg in control and DMIS rats, the mean arterial plasma concentration–time profiles of LQ, M1, and M2 are shown in Fig. 3A, B, and C, respectively. The relevant pharmacokinetic parameters are listed in Table 3. Absorption of LQ from the rat gastrointestinal tract was rapid; LQ was detected in plasma from the first blood sampling time point (3 minutes) and rapidly reached T_{max} (3-15 minutes) in all rats studied. Formation of both M1 and M2 was also rapid; both M1 and M2 were detected in the plasma at the first blood sampling time point (3 minutes). The pharmacokinetic parameters of LQ, M1, and M2 listed in Table 3 were comparable (not significantly different) between control and DMIS rats, except for the significantly shorter terminal half-life of LQ (by 38.8%), significantly greater $Ae_{0\text{-}24\ h}$ of total LQ (by 33.5%), and significantly higher C_{max} of both M1 (by 125%) and M2 (by 126%) in DMIS rats than controls.

3.6. Rat plasma protein binding of LQ

Protein binding values of LQ to fresh rat plasma from control and DMIS rats were 85.0% ± 5.79% and 81.9% ± 4.01%, respectively; they were not significantly different.

Table 3
Pharmacokinetic parameters of LQ, M1, and M2 after oral administration of LQ at a dose of 50 mg/kg in control and DMIS rats

Parameter	Control (n = 8)	DMIS (n = 9)
Initial body weight (g)[a]	246 ± 7.29	250 ± 7.25
final body weight (g)[b]	285 ± 7.07	247 ± 14.4***
Blood glucose (mg/dL)	93.9 ± 9.83	335 ± 39.4***
Urine output (mL/24 h)	11.0 ± 6.36	16.9 ± 1.96*
LQ		
AUC (μg · min/mL)	17.8 ± 9.12	26.6 ± 21.6
Terminal half-life (min)	16.3 ± 3.09	9.98 ± 6.82**
C_{max} (μg/mL)	1.56 ± 1.04	2.98 ± 2.45
T_{max} (min)[c]	7 (3–10)	5 (3–5)
$Ae_{0\text{-}24\ h}$ (% of LQ dose)	10.5 ± 5.74	11.1 ± 4.65
$Ae_{0\text{-}24\ h}$ of total LQ (% of LQ dose)	19.4 ± 4.06	25.9 ± 6.65*
$GI_{24\ h}$ (% of LQ dose)	1.24 ± 2.16	0.109 ± 0.118
F (%)	1.85	3.00
M1		
$AUC_{0\text{-}12\ h}$ (μg · min/mL)	2090 ± 810	1940 ± 310
C_{max} (μg/mL)	7.97 ± 3.46	17.9 ± 10.6*
T_{max} (min)[c]	15 (15–720)	15 (15–360)
$AUC_{M1,\ 0\text{-}12\ h}/AUC_{LQ}$ ratio	174 ± 184	149 ± 185
M2		
$AUC_{0\text{-}12\ h}$ (μg · min/mL)	1920 ± 688	1980 ± 580
C_{max} (μg/mL)	14.8 ± 6.71	33.5 ± 18.9*
T_{max} (min)[c]	15 (7–60)	15 (7–15)
$AUC_{M2,\ 0\text{–}12\ h}/AUC_{LQ}$ ratio	158 ± 156	131 ± 127

Values are expressed as means ± SD.
[a] Measured just before treatment.
[b] Measured just before experiment.
[c] T_{max} was expressed as median (ranges).
* Significantly different from control: $P < .05$.
** Significantly different from control: $P < .01$.
*** Significantly different from control: $P < .001$.

4. Discussion

Induction of diabetes mellitus in rats by streptozotocin was evident based on the significantly higher blood glucose level, larger 24-hour urine output, and decrease in body weight gain (Tables 2 and 3).

The AUC values of LQ were dose-proportional following its intravenous (5, 10, 20, and 50 mg/kg) and oral (20, 50, and 100 mg/kg) administration in rats. Moreover, an intravenous dose of 15 mg/kg and an oral dose of 50 mg/kg showed hepatoprotective effects in rats with acute liver injuries [6,7]. Thus, the intravenous and oral doses of LQ, 20 and 50 mg/kg, respectively, were chosen in the present study.

The AUCs of intravenous LQ were similar in DMIS rats and controls because of the comparable CLs between the 2 groups of rats (Table 2). The comparable CLs could have been due to the comparable CL_Rs and CL_{NR}s (Table 2). The contribution of the gastrointestinal (including the biliary) excretion of unchanged LQ to its CL_{NR} was almost

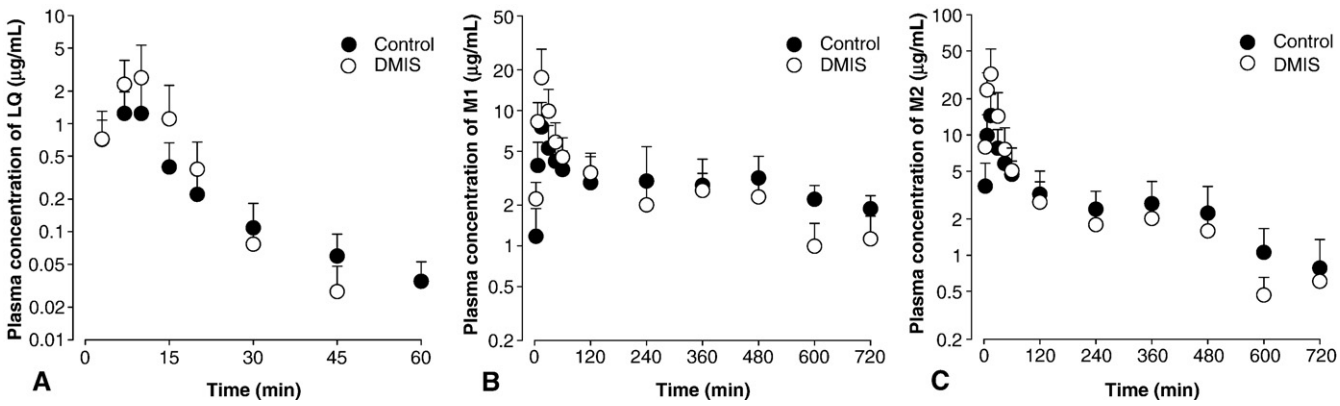

Fig. 3. Mean arterial plasma concentration–time profiles of LQ (A), M1 (B), and M2 (C) after oral administration of LQ at a dose of 50 mg/kg in control (n = 8) and DMIS (n = 9) rats. Values are means ± SD.

negligible; the $GI_{24\ h}$s of LQ were less than 0.159% of the intravenous dose for the 2 groups of rats (Table 2). However, the negligible value of $GI_{24\ h}$, less than 0.159% of the dose, was not due to the degradation of LQ in rats' gastric juices; more than 97.5% of the spiked amounts of LQ were recovered after 4-hour incubation in 5 rats' gastric juices (pHs 1-2) (our unpublished data). Moreover, the 6-hour biliary excretion of LQ was almost negligible (only 0.0327% of the dose) after intravenous administration of 20 mg/kg of LQ to control rats with bile duct cannulation (our unpublished data). These data indicate that LQ CL_{NR}s listed in Table 2 may be primarily due to metabolism and suggest that DMIS and control rats have similar metabolic clearance.

Liquiritigenin is a drug with an intermediate hepatic extraction ratio (hepatic first-pass effect after absorption into the portal vein was 57.1% [3]); therefore, its hepatic clearance depends on the CL_{int}, the free (unbound to plasma proteins) fractions of LQ in the plasma, and the hepatic blood flow rate in rats [26]. The CL_{int}s for the disappearance of LQ in the hepatic microsomes were comparable in control and DMIS rats (Table 1), suggesting that activities of UGTs for the disappearance of LQ in DMIS rats were not altered. The plasma protein binding of LQ in plasma were also comparable between the control and DMIS rats as mentioned earlier. Although Sato et al [27] reported that the hepatic plasma flow rate was faster (by 67.0%) in male Wistar DMIS rats, this seemed to have little effect on the hepatic clearance of LQ. Therefore, the comparable CL_{NR}s of LQ between DMIS and control rats after its intravenous administration were due to the comparable hepatic CL_{int} of LQ and the comparable free fractions of LQ in plasma. Note that the in vivo UDPGA level in the liver of DMIS rats was significantly higher (by 141%) than controls (Table 1). However, we could not obtain the in vitro Michaelis-Menten–type relationship between the disappearance of LQ and the concentrations of UDPGA (Fig. 1A); one of the possible reasons for this is that measuring the small changes in high concentration of LQ was less accurate than measuring the formation of glucuronide conjugates. Although the hepatic UDPGA level was elevated in DMIS rats, the in vivo hepatic CL_{int} of LQ in DMIS rats appeared comparable to that of controls possibly due to presence of other in vivo metabolic pathway of LQ such as sulfate conjugation.

Although activities of UGTs for the formation of both M1 and M2 in hepatic microsomes from DMIS rats were also not altered (comparable CL_{int}s for the formation of M1 and M2; Table 1), the in vivo UDPGA levels in liver of DMIS rats were significantly higher (by 141%) than controls (Table 1); and the formation rate of both M1 and M2 in the hepatic microsomes increased with increase in UDPGA concentrations (Fig. 1B and C). It was also reported that increased UDPGA augmented glucuronidation even without a detectable increase in enzyme activity [28]. Thus, the increase in the formation of M1 and M2 in DMIS rats was expected. As a result, after intravenous administration of LQ to DMIS rats, the formation of M2 was increased compared with controls; the AUC_{M2}/AUC_{LQ} ratio was significantly greater (by 53.0%) than controls (Table 2). Although DMIS rats showed significantly higher C_{max} and considerably greater AUC (by 17.7%, $P = .0732$) of M1 after intravenous administration of LQ, their AUC_{M1}/AUC_{LQ} ratio was comparable to controls. This suggested that increase in formation of M1 in DMIS rats was not sufficient to show statistically significant increase in AUC_{M1}/AUC_{LQ} ratio; the magnitude of increase in estimated formation rate of M1 in DMIS rats compared with controls (1.63 times faster) was smaller than that of M2 (2.35 times faster) (Fig. 1B and C).

The increase in hepatic UDPGA level in DMIS rats is consistent with previous reports. Carbohydrate metabolism in hepatocytes of diabetes mellitus rats may have been altered in a way to increase dehydrogenation of UDP-glucose to UDPGA, resulting in greater rates of glucuronidation [29]. Glucose metabolism was shunted through insulin-insensitive pathways when carbohydrate metabolism in insulin-sensitive pathways was inhibited by diabetes [30]. Anderson [31] suggested that UDP-glucose conversion to UDPGA was accelerated primarily because its conversion to glycogen was severely limited in the diabetic liver. The UDP-glucose dehydrogenase was reported to increase in diabetes mellitus [32]. Increase in conjugated bilirubin in patients with non–insulin-dependent diabetic mellitus possibly due to increase in levels of glucuronic acid as a result of alteration of UDP-glucose pathway has also been reported [33]. Therefore, the increase in hepatic UDPGA levels in DMIS rats and the increase in the formation of M2 after intravenous administration of LQ were caused by diabetic state and not by streptozotocin itself.

After oral administration of LQ to DMIS rats, the AUC of LQ and metabolite ratios of both M1 and M2 were comparable with controls. It has been reported that the hepatic first-pass effect of LQ, 57.1% after absorption into the portal vein, was trivial (only 3.67% of the oral dose) in rats because most of the orally administered LQ was first undertaken by the extensive gastrointestinal first-pass effect (92.5% of the oral dose) before getting into the liver through portal vein [3]. Thus, intestinal metabolism is the major determinant of AUC of LQ and metabolite ratios of both M1 and M2 after oral administration of LQ. Both the activities of UGTs (comparable CL_{int}s for the disappearance of LQ and the formation of M1 and M2 in the intestinal microsomes; Table 1) and UDPGA level in the intestine (Table 1) were comparable between the control and DMIS rats, suggesting that intestinal metabolism of LQ may be comparable between the 2 groups of rats.

In summary, after intravenous administration of LQ in DMIS rats, the AUC and CL_{NR} of LQ were comparable with controls because of the comparable hepatic CL_{int}s of LQ and comparable free fractions of LQ in plasma, whereas the formation of its glucuronides, especially M2, increased significantly because of significantly higher hepatic UDPGA level than controls. After oral administration of LQ, the AUCs of LQ and metabolite ratios of both M1 and M2 were

comparable between the 2 groups of rats because of the comparable intestinal metabolism of LQ. Liquiritigenin is designed to be orally administered to patients. If the present rat data could be extrapolated to humans, modification of the oral dosage regimen of LQ does not seem to be necessary in patients with diabetes mellitus. Human studies are required to prove the above hypothesis.

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