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Research Paper

Effects of acute renal failure induced by uranyl nitrate on the pharmacokinetics of liquiritigenin and its two glucuronides, M1 and M2, in rats

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Abstract

Objectives Liver disease and acute renal failure (ARF) are closely associated. The pharmacokinetics of liquiritigenin (LQ), a candidate therapy for inflammatory liver disease, and its metabolites M1 and M2 were evaluated in rats with ARF induced by uranyl nitrate (U-ARF rats).

Methods LQ was administered intravenously (20 mg/kg) or orally (50 mg/kg) in U-ARF and control rats, and uridine diphosphate-glucuronosyltransferases (UGT) activity and uridine 5′-diphosphoglucuronic acid (UDPGA) concentrations were determined in the liver and intestine.

Key findings After intravenous LQ administration, U-ARF rats displayed significantly slower LQ renal clearance but no significant changes in the LQ area under the plasma concentration–time curve (AUC) compared with controls. This was because of similar hepatic UGT activity and UDPGA levels between two groups, which resulted in comparable non-renal clearance, as well as the limited contribution of LQ renal clearance to total LQ clearance. However, the AUC and AUC_M/AUC_{LQ} ratios of M1 and M2 were significantly increased in U-ARF rats because of decreased urinary excretion of M1 and M2. Similar results were observed following oral administration because of the comparable LQ intestinal metabolism in both groups and decreased urinary excretion of M1 and M2 in U-ARF rats.

Conclusions U-ARF rats displayed decreased urinary excretion of LQ glucuronides, resulting in significantly greater AUC and metabolite ratios of M1 and M2 following LQ administration.

Keywords acute renal failure; liquiritigenin; pharmacokinetics; UDPGA; UGT

Introduction

Liquiritigenin (2,3-dihydro-7-hydroxy-2-(4-hydroxyphenyl)-(S)-4*H*-1-benzopyran-4-one; LQ) is a liquiritin aglycone found in *Glycyrrhizae radix*. LQ exerts cytoprotective effects against heavy metal induced toxicity in rat hepatocyte-derived cultured cells.[1] Furthermore, LQ helps prevent paracetamol (acetaminophen) induced[2] or galactosamine/lipopolysaccharide induced[3] acute liver injury in rats, and carbon tetrachloride induced liver injury in mice.[4] Preclinical studies are currently evaluating LQ as an oral agent for the treatment of inflammatory liver disease. Recently, a phase II clinical study on a product containing LQ in patients with chronic hepatitis has been approved by the Korea Food and Drug Administration.

LQ is metabolised into five glucuronide and/or sulfate conjugated derivatives 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).[5] Following intravenous administration of LQ (5 mg/kg) in rats, only M1, M2 and M3 were detected in plasma, although all five conjugates were excreted in bile.[4] A study of various routes of LQ administration in male Sprague–Dawley rats showed that the unabsorbed fraction from the gastrointestinal tract up to 24 h was 1.07% of the oral dose, the extent of absolute oral bioavailability (*F*) was 6.68%, the hepatic first-pass extraction after absorption into the portal vein was 57.1%, and the gastrointestinal first-pass extraction was 92.5% of the oral dose.[6] A recent report

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examined the pharmacokinetics of LQ, M1 and M2 after intravenous and/or oral administration of various doses of LQ to four species (mice, rats, rabbits and dogs) and used these animal data to predict human LQ pharmacokinetics.[7] The effects of co-administered dimethyl-4,4′-dimethoxy-5,6,5′,6′-dimethylenedioxybiphenyl-2,2′-dicarboxylate, a synthetic hepatoprotective agent derived from schizandrin C,[8] acute hepatitis induced by D-galactosamine/lipopolysaccharide or carbon tetrachloride,[9] and diabetes mellitus induced by streptozotocin[10] on the pharmacokinetics of LQ, M1 and M2 in rats have also been reported.

Acute renal failure (ARF) is a life-threatening condition and a frequent complication of advanced liver disease.[11] Many studies have reported that renal failure and viral hepatitis are closely associated. For example, acute hepatitis A is a relatively common cause of ARF with hepatic dysfunction,[12,13] and hepatitis B related membranous nephropathy leads to renal failure in one-third of infected patients with low rates of spontaneous remission.[14] Furthermore, hepatitis C is both a cause and a complication of kidney disease.[15–17] Given the close association of renal dysfunction with liver disease, examining the changes in LQ, M1 and M2 pharmacokinetics due to ARF is a high priority.

In rats, LQ is metabolised into two primary glucuronide conjugates, M1 and M2, via uridine diphosphate-glucuronosyltransferases (UGTs) in the gastrointestinal tract and liver.[6] Although ARF-induced changes in hepatic cytochrome P450 isozyme expression and function have been reported,[18] the effects of ARF on hepatic UGTs and intestinal glucuronidation are not known. We therefore examined changes in the pharmacokinetics of LQ, M1 and M2 after intravenous or oral administration of LQ to rats with ARF induced by uranyl nitrate treatment (U-ARF rats). Pharmacokinetic parameters were evaluated with respect to changes in UGT activity in hepatic and intestinal microsomes, as well as in-vivo uridine 5′-diphosphoglucuronic acid (UDPGA) levels in liver and intestine.

Materials and Methods

Chemicals

LQ was synthesised by Professor Jee W. Lee at the Seoul National University, College of Pharmacy (Seoul, South Korea). M1 and M2 were obtained from Dr Hye J. Chung at the Center for Chemoinformatics in the Life Sciences Research Division of the Korea Institute of Science and Technology (Seoul, South Korea). Chlorzoxazone, lamotrigine, theobromine, *p*-nitrophenol (*p*NP), *p*-nitrophenyl glucuronide (*p*NP-Glu), tetraethyl-ammonium bromide, UDPGA (as a trisodium salt), β-glucuronidase (type HP-1; from *Helixa pomatia* with a β-glucuronidase activity of 127 000 units/ml and a sulfatase activity of less than 7500 units/ml), and tris(hydroxymethyl)aminomethane (Tris)-buffer were all purchased from Sigma-Aldrich Corporation (St Louis, MO, USA). Uranyl nitrate and polyethylene glycol 400 were obtained from BDH Chemicals (Poole, England) and Duksan Pure Chemicals Company, Ltd (Ansan, South Korea), respectively. Other chemicals were of reagent or high-performance liquid chromatography (HPLC) grade.

Animals

The protocols for the animal studies were approved by the Institutional Animal Care and Use Committee of Seoul National University. Male Sprague-Dawley rats (8–10 weeks old, 260–325 g) were purchased from Charles River Company Korea (Seoul, South Korea). Animal housing and maintenance were similar to those in previous studies.[6–10]

Induction of acute renal failure in rats by uranyl nitrate injection

Rats were randomly divided into U-ARF and control groups. To induce ARF, we injected freshly prepared uranyl nitrate at a dose of 5 mg(in 1 ml)/kg via the lateral tail vein.[19] The same volume of vehicle (sterile 0.9% NaCl solution) was injected into control rats. Experiments were conducted on Day 5 following administration of uranyl nitrate or vehicle.

Induction of ARF in U-ARF rats is a well-documented phenomenon and liver function is generally not seriously impaired by this treatment.[19,20] In U-ARF rats, impaired kidney function has been observed based on the chemistry data and kidney microscopy:[19,20] significantly higher plasma level of urea nitrogen, significantly slower creatinine clearance and significantly heavier relative kidney weight compared with controls, and higher plasma level of urea nitrogen than the reported ranges (5.0–29.0 mg/dl) in control rats.[21] Extensive acute tubular necrosis involving the distal convoluted tubule in the kidney of U-ARF rats has been reported.[20]

Preparation of microsomes and extracts of rat liver and intestine

Preparation of hepatic and intestinal microsomes from control ($n = 6$) and U-ARF ($n = 5$) rats was performed as in previous studies.[22] Microsomal protein content in the hepatic and intestinal microsomes was measured using established methods.[23]

Liver and intestinal extracts for UDPGA measurement were prepared according to established methods[24] with a slight modification.[8,10] Briefly, 0.5 g liver tissue (or 0.25 g intestine) was placed in a test tube with 1.5 ml (0.75 ml for intestine) distilled water and boiled for 4 min, homogenised, then centrifuged at 3500*g* for 10 min. The resulting supernatant was collected and stored at −70°C (Revco ULT 1490 D-N-S; Western Mednics, Asheville, NC, USA).

Measurement of mean velocities for the disappearance of LQ and the formation of M1 and M2 in hepatic and intestinal microsomes

The procedures used for the measurement of maximum velocity (V_{max}) and the apparent Michaelis–Menten constant (K_m) for the disappearance of LQ and the formation of M1 and M2 were similar to previously reported methods.[8–10,25] Microsomes (0.1 and 0.2 mg total protein for hepatic and intestinal microsomes, respectively), 0.1 M Tris-HCl buffer (pH 7.4), 1 mM $MgCl_2$ in Tris-HCl (pH 7.4), and alamethicin (dissolved in 50% ethanol; 50 μg/mg protein) were mixed and placed on ice for 10 min. Next, 10 μl of a 50% methanol solution containing various concentrations of LQ was added (resulting in final LQ concentrations of 1, 2, 10, 20, 40, 100 and 200 μM), and the mixture was preincubated for 5 min in a

thermomixer (Thermomixer 5436; Eppendorf, Hamburg, Germany) at 37°C and 600 rev/min. To initiate the reaction, UDPGA (dissolved in Tris-HCl buffer, pH 7.4, for a final concentration of 3 mM) was added to produce a final volume of 500 μl. After incubating for 5 min, 50-μl aliquots were collected and added to an Eppendorf tube containing either 20 μg/ml chlorzoxazone (internal standard for LQ) in 50 μl methanol and 1 ml diethyl ether, or 7.5 μg/ml lamotrigine (internal standard for M1 and M2) in 100 μl acetonitrile. Tubes were vortexed to terminate the reaction. These incubation conditions were within the linear range of the reaction rate as determined in preliminary studies. The constants, K_m and V_{max}, were calculated using non-linear regression.[26] The unweighted kinetic data from microsomes were fitted to the single-site Michaelis–Menten equation, $V = V_{max} \times [S]/(K_m + [S])$, where [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 V_{max} by K_m.

Measurement of in-vivo hepatic and intestinal UDPGA levels

UDPGA can affect the metabolism of LQ in the liver and intestine via UGT. To determine whether in-vivo hepatic or intestinal UDPGA levels were changed in U-ARF rats, we used an enzymatic assay based on the formation of *p*NP-Glu from *p*NP and UDPGA present in tissue extracts according to established methods.[27] The UDPGA assay used an incubation mixture (final volume of 0.5 ml) similar to that used for the determination of V_{max} and K_m, with the following differences: 1 mmol/l *p*NP was used as the substrate; 200 μl hepatic or intestinal extract from control ($n = 6$) or U-ARF ($n = 5$) rats was used as an UDPGA donor, and the mixture was incubated for 60 min. A calibration curve for UDPGA quantification was obtained by adding known amounts of UDPGA (5, 10, 20, 30, 50 and 75 nmol dissolved in Tris-HCl buffer, pH 7.4; performed in triplicate) instead of hepatic or intestinal extracts. The reaction was terminated by adding 50 μl of the incubation mixture to Eppendorf tubes containing 20 μg/ml theobromine (internal standard for HPLC analysis of *p*NP-Glu) in 100 μl methanol and then vortexing.

Intravenous and oral administration of LQ

Cannulation of the carotid artery (for blood sampling) and the jugular vein (for drug administration in the intravenous study) was performed as in previous studies.[6–10] Rats were allowed to recover from light ether anaesthesia for 4–5 h before the study began, and were not restrained during drug administration or blood collection.

LQ dissolved in polyethylene glycol 400 and distilled water (40 : 60, v/v) was manually infused at a dose of 20 mg(in 2 ml)/kg over 1 min via the jugular vein of control ($n = 6$) and U-ARF ($n = 5$) rats, or administered orally using a gastric gavage tube at a dose of 50 mg(in 4 ml)/kg in control ($n = 9$) and U-ARF ($n = 7$) rats. For intravenous LQ infusion, blood samples (approx. 0.11 ml for time points for analysis of either LQ or its two metabolites, or 0.22 ml for time points for analysis of both LQ and its two metabolites) were collected via the carotid artery at 0 (baseline), 1 (immediately following infusion), 3, 5, 10, 20, 30, 45, 60, 120, 180, 240, 360 and 480 min. For oral studies, blood samples were collected at 0, 3, 7, 10, 15, 20, 30, 45, 60, 120, 240, 360, 480, 600 and 720 min after oral LQ administration. Cannulae were flushed between blood samples with heparinised 0.9% NaCl to prevent clotting. Blood samples were immediately centrifuged upon collection, and 50-μl plasma samples were stored at −70°C until HPLC analysis. Preparation and handling of urine samples ($Ae_{0-24\,h}$) and gastrointestinal tract samples (including its contents and faeces) at 24 h ($GI_{24\,h}$) was performed according to established methods.[6–10]

Measurement of rat plasma protein binding of LQ using equilibrium dialysis

LQ plasma protein binding values in control ($n = 4$) and U-ARF ($n = 5$) rats were determined using equilibrium dialysis.[6] We used a Spectra/Por 4 membrane (molecular weight cutoff 12–14 kDa; Spectrum Medical Industries, Los Angeles, CA, USA) to dialyse 1 ml plasma in a 1-ml dialysis cell against 1 ml isotonic Sørensen phosphate buffer (pH 7.4) containing 3% (w/v) dextran to reduce volume shift.[28] LQ was added into the plasma side to reduce the equilibrium time between buffer and plasma compartments.[29] After incubation for 6 h, two 50-μl aliquots were collected from each compartment and stored at −70°C until HPLC analysis. Kang *et al*.[6] reported that LQ binding to 4% human serum albumin (similar to albumin concentrations in rat plasma)[21] was constant at LQ concentrations ranging from 0.2 to 20 μg/ml. Thus, 5 μg/ml LQ was used for the plasma protein binding study.

HPLC analysis of LQ, M1, M2 and *p*NP-Glu

We measured LQ, M1 and M2 concentrations using reported HPLC methods.[7,30] Measurement of total LQ (unconjugated plus conjugated) in urine samples by incubation with β-glucuronidase was performed as in previous reports.[7] Detection limits of LQ in rat plasma and urine samples were 20 and 50 ng/ml, respectively. The detection limits of both M1 and M2 were 200 ng/ml. The mean within- and between-day coefficients of variation for the assay precision of LQ, M1 and M2 were all less than 5%.

Quantification of *p*NP-Glu was performed according to reported methods,[31] with a slight modification.[8,10] Briefly, 50 μl incubation mixture and 20 μg/ml theobromine (internal standard) in 100 μl methanol were vortexed, then centrifuged, and the supernatant 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 distilled water, and a 50-μl aliquot was injected directly onto a reversed-phase HPLC column (Nucleosil C_{18}; 4.6 mm i.d. × 150 mm length; particle size 5 μm). The mobile phase, methanol in distilled water (25 : 75, v/v) containing 5 mmol/l tetraethyl-ammonium bromide, was run at a flow rate of 0.7 ml/min, and a UV detector at 290 nm was used to monitor the column eluent. The retention times of *p*NP-Glu and theobromine were approximately 4.6 and 5.5 min, respectively. The limit of quantification of *p*NP-Glu in the microsomal incubation mixture was 0.1 μmol/l.

Pharmacokinetic analysis

Standard methods[32] and commercially available software (WinNonlin; professional edition version 2.1; Pharsight,

Mountain View, CA, USA) were used to calculate the following pharmacokinetic parameters using a non-compartmental analysis: the total area under the plasma concentration–time curve from time zero to infinity (AUC) or up to the last measured time, t, in plasma (AUC_{0-t});[33] time-averaged total body, renal and non-renal clearance (CL, CL_R and CL_{NR}, respectively); terminal half-life; apparent post-pseudodistribution volume of distribution (V_{area}); and F.

$$CL = dose/AUC \quad (1)$$

$$CL_R = Ae_{0-24\,h}/AUC \quad (2)$$

$$CL_{NR} = CL - CL_R \quad (3)$$

$$V_{area} = CL/k_{last} \quad (4)$$

$Ae_{0-24\,h}$ and k_{last} are the amount of LQ excreted in the 24-h urine and terminal phase rate constant, respectively. In the calculation of CL_R, the AUC instead of $AUC_{0-24\,h}$ was used, since the $AUC_{24\,h-\infty}$/AUC ratio of LQ was almost negligible.

The peak plasma concentration (C_{max}) and time to reach C_{max} (T_{max}) were read directly from the experimental data.

Statistical analysis

We used an unpaired t-test to compare the two groups. P values below 0.05 were considered to be statistically significant. All results are expressed as means ± SD, except those for T_{max}, which are expressed as medians and ranges.

Results

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

The mean velocities for the disappearance of LQ and the formation of M1 and M2 in hepatic and intestinal microsomes from control and U-ARF rats are shown in Figure 1. V_{max}, K_m and CL_{int} values for the disappearance of LQ and the formation of M1 and M2 in hepatic and intestinal microsomes from

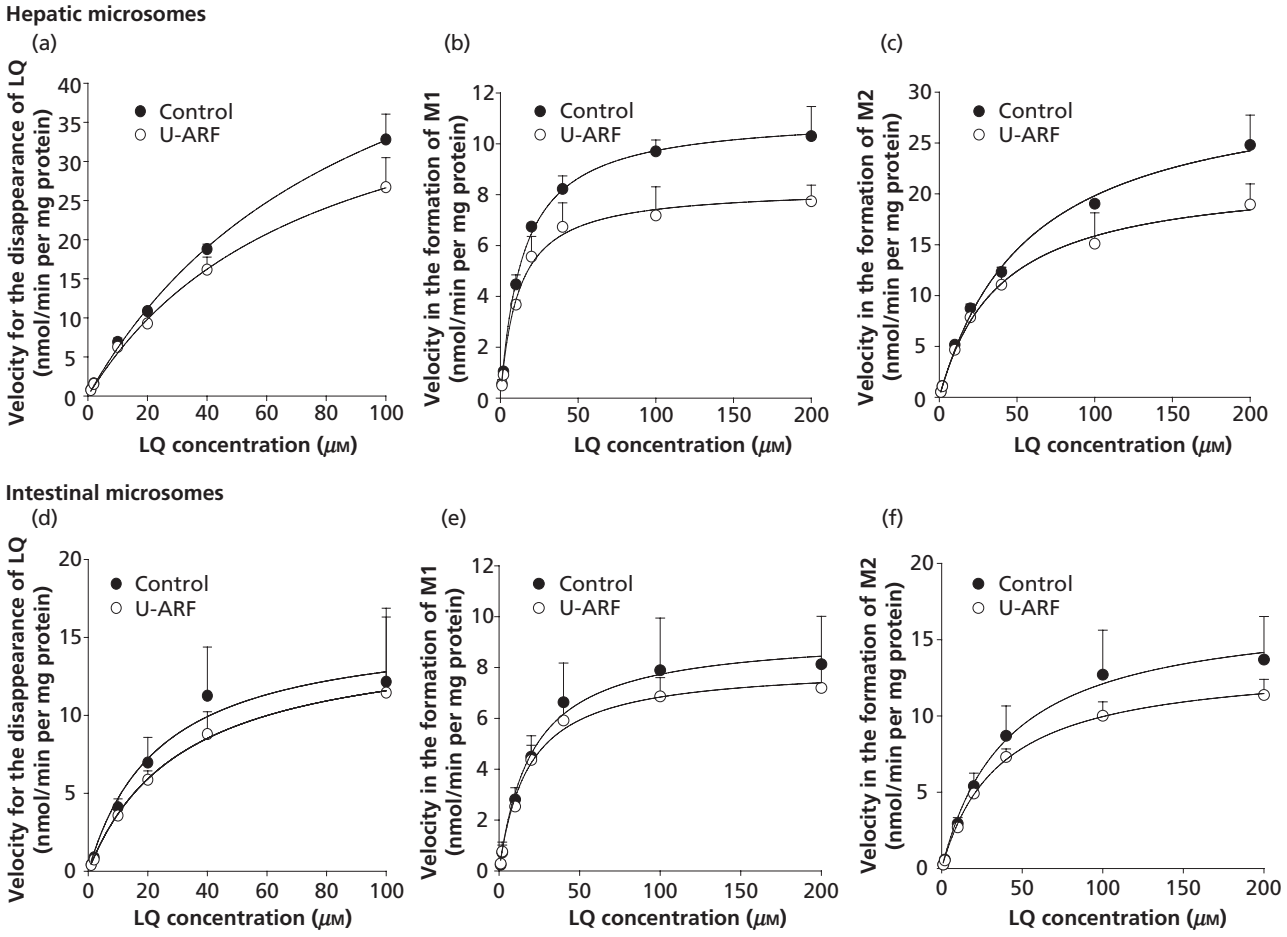

Figure 1 Mean velocities for the disappearance of liquiritigenin and the formation of M1 and M2 in hepatic and intestinal microsomes. Mean velocities were determined at each liquiritigenin (LQ) concentration in hepatic (a–c) and intestinal (d–f) microsomes from control rats ($n = 6$) and rats with acute renal failure induced by uranyl nitrate (U-ARF) ($n = 5$). Mean data for microsomes from each rat were fitted to a single-site Michaelis–Menten equation and shown as a solid line. Values are means ± SD.

Table 1 Hepatic and intestinal uridine diphosphate-glucuronosyltransferase activity and uridine 5′-diphosphoglucuronic acid level

Parameters	Liver		Intestine	
	Control ($n = 6$)	U-ARF ($n = 5$)	Control ($n = 6$)	U-ARF ($n = 5$)
UGT activity				
Disappearance of LQ				
V_{max} (nmol/min per mg protein)	59.2 ± 14.3	46.5 ± 10.9	16.3 ± 7.08	12.4 ± 2.31
K_m (μmol/l)	83.6 ± 27.2	74.1 ± 22.0	24.1 ± 10.4	22.0 ± 5.70
CL_{int} (ml/min per mg protein)	0.725 ± 0.0633	0.644 ± 0.0941	0.694 ± 0.129	0.578 ± 0.0921
Formation of M1				
V_{max} (nmol/min per mg protein)	11.1 ± 0.794	8.37 ± 1.01***	9.35 ± 2.49	8.08 ± 0.963
K_m (μmol/l)	14.3 ± 1.84	11.7 ± 1.77**	20.4 ± 6.08	18.1 ± 2.78
CL_{int} (ml/min per mg protein)	0.782 ± 0.0612	0.720 ± 0.0733	0.472 ± 0.102	0.452 ± 0.0598
Formation of M2				
V_{max} (nmol/min per mg protein)	28.6 ± 4.83	22.0 ± 2.81*	17.1 ± 3.94	13.5 ± 1.17
K_m (μmol/l)	49.8 ± 13.5	39.3 ± 4.34	40.9 ± 6.75	35.8 ± 1.96
CL_{int} (ml/min per mg protein)	0.585 ± 0.0552	0.568 ± 0.103	0.419 ± 0.0758	0.378 ± 0.0307
UDPGA level (nmol/g tissue)	325 ± 95.9	252 ± 125	289 ± 109	278 ± 56.1

CL_{int}, intrinsic clearance; K_m, apparent Michaelis–Menten constant (concentration at which the rate is half the V_{max}); UDPGA, uridine 5′-diphosphoglucuronic acid; UGT, uridine diphosphate-glucuronosyltransferase; V_{max}, maximum velocity. In-vitro V_{max}, K_m and CL_{int} for the disappearance of liquiritigenin (LQ) and the formation of M1 and M2 in hepatic and intestinal microsomes, and in-vivo hepatic and intestinal UDPGA levels were determined in control rats and rats with acute renal failure induced by uranyl nitrate (U-ARF). Values are means ± SD. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, significantly different compared with the control group.

both groups are listed in Table 1. V_{max}, K_m and CL_{int} values in U-ARF rats were similar to those in controls for the disappearance of LQ in both hepatic and intestinal microsomes, and for the formation of M1 and M2 in intestinal microsomes. However, V_{max} values for the formation of M1 and M2, and K_m values for the formation of M1, in hepatic microsomes were significantly slower (by 24.6 and 23.1%, respectively) and lower (by 18.2%) in U-ARF rats compared with controls. CL_{int} values for the formation of M1 and M2 in hepatic microsomes of U-ARF rats were also similar to control values. These data suggest that UGT activity in hepatic and intestinal microsomes of U-ARF rats did not differ considerably from that in controls.

In-vivo hepatic and intestinal UDPGA levels

The linear calibration curves for hepatic and intestinal microsomes were $y = 0.9455x + 0.6932$ ($r = 0.999$) and $y = 0.1582x - 0.7603$ ($r = 0.997$), respectively. In these equations, y is the concentration of *p*NP-Glu formed (μmol/l) and x is the amount of UDPGA in the incubation mixture (nmol). The amount of UDPGA used for calibration ranged from 5 to 50 nmol for hepatic microsomes, or from 10 to 75 nmol for intestinal microsomes. Measured amounts of UDPGA in hepatic (6.99–22.6 nmol) or intestinal (11.1–22.4 nmol) extracts were all within the linear range of the assay. UDPGA levels in the liver or intestine of control and U-ARF rats are listed in Table 1. There were no significant differences between the groups.

Pharmacokinetics of LQ, M1 and M2 after intravenous LQ administration

Mean arterial plasma concentration–time profiles of LQ, M1 and M2 after intravenous administration of 20 mg/kg LQ are shown in Figure 2. The relevant pharmacokinetic parameters are listed in Table 2. U-ARF rats showed the following changes in LQ pharmacokinetic parameters compared with controls: significantly longer terminal half-life (by 19.7%), slower CL_R (by 87.0%), larger V_{area} (by 29.1%), and a smaller percentage of the LQ dose excreted in urine as unconjugated LQ ($Ae_{0-24\,h}$) and total (conjugated + unconjugated) LQ ($Ae_{0-24\,h}$ of total LQ; by 88.3 and 92.1%, respectively). U-ARF rats also displayed changes in the pharmacokinetic parameters of M1 and M2. Compared with controls, U-ARF rats showed significantly greater AUC for both M1 and M2 (by 67.6 and 120%, respectively), longer terminal half-life of M1 (by 106%), significantly lower C_{max} of M1 (by 27.2%), significantly smaller $Ae_{0-24\,h}$ of M1 and M2 (by 91.5 and 89.8%, respectively), and significantly greater AUC_{M1}/AUC_{LQ} and AUC_{M2}/AUC_{LQ} ratios (by 76.8 and 124%, respectively).

Pharmacokinetics of LQ, M1 and M2 after oral administration of LQ

The mean arterial plasma concentration–time profiles of LQ, M1 and M2 after oral administration of 50 mg/kg LQ to both groups of rats are shown in Figure 3. The relevant pharmacokinetic parameters are listed in Table 3. U-ARF rats showed significantly smaller $Ae_{0-24\,h}$ for LQ and total LQ (by 92.0 and 96.4%, respectively) compared with controls. U-ARF rats also showed significantly greater AUC for both M1 and M2 (by 42.9 and 271%, respectively), significantly higher C_{max} of M2 (by 56.8%), significantly smaller $Ae_{0-24\,h}$ of M1 and M2 (by 97.5 and 98.3%, respectively), and a significantly greater AUC_{M2}/AUC_{LQ} ratio (by 204%) than controls.

Plasma protein binding of LQ

Plasma protein binding values of LQ did not differ significantly between control and U-ARF rats (85.3 ± 6.65 and 81.6 ± 3.15%, respectively).

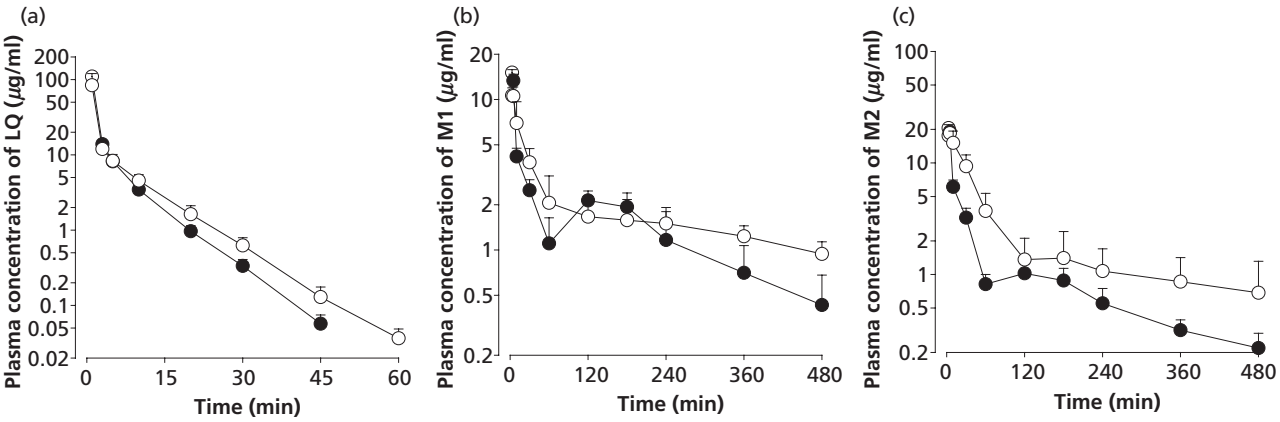

Figure 2 Concentration–time profiles of liquiritigenin, M1 and M2 after intravenous administration. Mean arterial plasma concentration–time profiles were determined after intravenous infusion of 20 mg/kg liquiritigenin (LQ) to control rats (●; $n = 6$) and rats with acute renal failure induced by uranyl nitrate (U-ARF) (○; $n = 6$). Values are means ± SD.

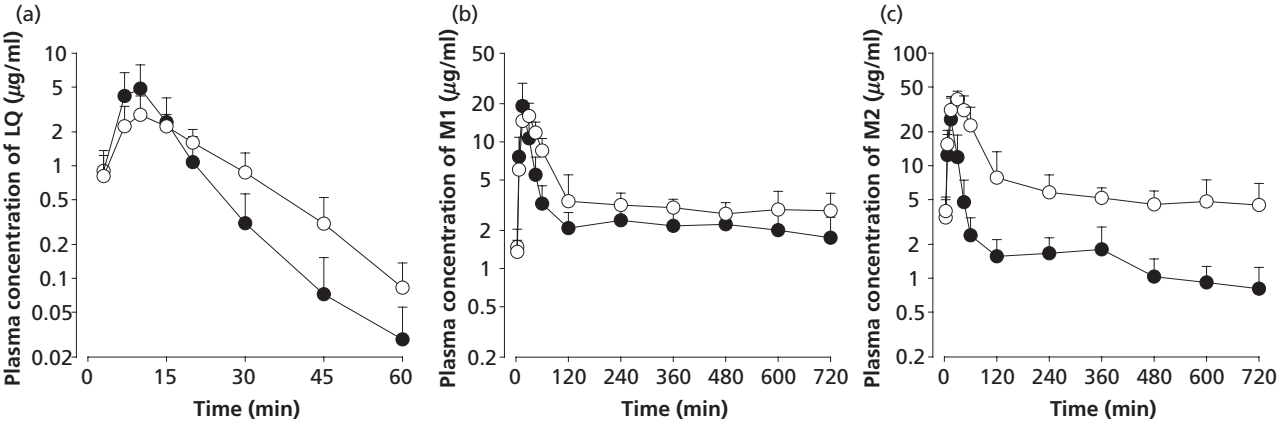

Figure 3 Concentration–time profiles of liquiritigenin, M1 and M2 after oral administration. Mean arterial plasma concentration–time profiles were determined after oral administration of 50 mg/kg liquiritigenin (LQ) to control rats (●; $n = 9$) and rats with acute renal failure induced by uranyl nitrate (U-ARF) (○; $n = 7$). Values are means ± SD.

Discussion

As the primary target organ of uranyl nitrate is the kidney,[34] we chose uranyl nitrate to induce ARF. The AUC values of LQ in rats are dose-proportional following intravenous and oral administration.[7] Moreover, an intravenous dose of 15 mg/kg and an oral dose of 50 mg/kg of LQ were hepatoprotective in rats with acute liver injury.[2,3] Therefore, intravenous and oral LQ doses of 20 and 50 mg/kg, respectively, were used in the present study.

The AUCs of intravenous LQ were similar in U-ARF rats and controls because of comparable CL values of LQ between the two groups (Table 2). The slower CL_R of LQ in U-ARF rats was most likely due to the significantly smaller $Ae_{0-24\,h}$ value of LQ, which in turn might be due to impaired kidney function compared with controls. Impaired kidney function in U-ARF rats was supported by the blood and urine chemistry data, creatinine clearance and kidney microscopy.[19,20] The small contribution of CL_R to the total CL of LQ (8.18 and 0.993% for controls and U-ARF rats, respectively; Table 2) indicates that most of the intravenous LQ dose is eliminated via the non-renal route (CL_{NR}). Therefore, the significantly slower CL_R of LQ in U-ARF rats did not result in significant changes of CL and AUC of LQ.

It has been reported that CL_{NR} of LQ could represent its metabolic clearance (primarily glucuronide formation).[8,10] Therefore, similar CL_{NR} values of LQ in U-ARF and control rats suggest that they have similar metabolic clearance.

LQ has an intermediate hepatic extraction ratio (57.1%),[6] and therefore its hepatic clearance depends on hepatic intrinsic clearance (CL_{int}), free LQ fraction in plasma and the hepatic blood flow rate.[35] The CL_{int} values for the disappearance of LQ in hepatic microsomes were comparable in control and U-ARF rats (Table 1; Figure 1), suggesting that UGT activity was not altered considerably in U-ARF rats. Similar results have been reported in the 5/6th nephrectomised rat model of chronic renal failure; [36] they showed no significant differences in hepatic and renal activity and expression of UGT1A1, UGT2B3, UGT1A6 and UGT2B1 compared with sham-operated rats. In addition, in-vivo liver UDPGA levels were comparable in U-ARF and control rats (Table 1). The plasma protein binding values of LQ were also similar in the

Table 2 Pharmacokinetic parameters of liquiritigenin, M1 and M2 after intravenous administration

Parameter	Control ($n = 6$)	U-ARF ($n = 6$)
Initial bodyweight (g)[a]	311 ± 8.61	313 ± 6.83
Final bodyweight (g)[b]	319 ± 12.8	309 ± 15.6
LQ		
AUC (μg min/ml)	225 ± 20.2	212 ± 37.2
Terminal half-life (min)	6.13 ± 0.538	7.34 ± 0.628*
CL (ml/min per kg)	89.7 ± 8.21	96.4 ± 14.7
CL_R (ml/min per kg)	7.34 ± 2.41	0.957 ± 0.812***
CL_{NR} (ml/min per kg)	82.3 ± 6.14	95.4 ± 14.2
V_{area} (ml/kg)	790 ± 89.7	1020 ± 182*
$Ae_{0-24\,h}$ (% of LQ dose)	8.06 ± 1.98	0.941 ± 0.703***
$Ae_{0-24\,h}$ of total LQ (% of LQ dose)[c]	26.6 ± 3.30	2.11 ± 1.17***
$GI_{24\,h}$ (% of LQ dose)	0.147 ± 0.0846	0.378 ± 0.191
M1		
AUC (μg min/ml)	883 ± 248	1480 ± 291**
Terminal half-life (min)	208 ± 120	429 ± 209*
C_{max} (μg/ml)	15.1 ± 0.724	11.0 ± 1.37***
T_{max} (min)	3 (3)	3 (3–5)
$Ae_{0-24\,h}$ (% of LQ dose)	13.1 ± 4.78	1.11 ± 0.646***
AUC_{M1}/AUC_{LQ} ratio	3.96 ± 1.81	7.00 ± 1.16**
M2		
AUC (μg min/ml)	644 ± 63.1	1420 ± 725*
Terminal half-life (min)	211 ± 91.1	260 ± 147
C_{max} (μg/ml)	20.5 ± 1.52	18.6 ± 2.33
T_{max} (min)	3 (3–5)	5 (3–5)
$Ae_{0-24\,h}$ (% of LQ dose)	6.31 ± 3.96	0.642 ± 0.307**
AUC_{M2}/AUC_{LQ} ratio	2.89 ± 0.418	6.48 ± 2.22**

$Ae_{0-24\,h}$, percentage of the dose excreted in the 24-h urine; AUC, total area under the plasma concentration–time curve from time zero to infinity; CL, time-averaged total body clearance; CL_{NR}, time-averaged non-renal clearance; CL_R, time-averaged renal clearance; C_{max}, maximum plasma concentration; $GI_{24\,h}$, percentage of the dose recovered from the gastrointestinal tract (including its contents and faeces) at 24 h; T_{max}, time to reach C_{max}; V_{area}, apparent post-pseudodistribution volume of distribution. Pharmacokinetic parameters were determined after intravenous administration of 20 mg/kg liquiritigenin (LQ) to control rats and rats with acute renal failure induced by uranyl nitrate (U-ARF). [a]Measured just before treatment. [b]Measured just before experiment. [c]$Ae_{0-24\,h}$ of conjugated plus unconjugated LQ. Values are means ± SD except T_{max}, which is given as the median (range). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, significantly different compared with the control group.

Table 3 Pharmacokinetic parameters of liquiritigenin, M1 and M2 after oral administration

Parameter	Control ($n = 9$)	U-ARF ($n = 7$)
Initial bodyweight (g)[a]	274 ± 5.27	269 ± 6.27
final bodyweight (g)[b]	274 ± 7.41	249 ± 3.78***
LQ		
AUC (μg min/ml)	59.8 ± 31.5	60.6 ± 10.3
Terminal half-life (min)	8.26 ± 2.13	9.59 ± 4.53
C_{max} (μg/ml)	4.93 ± 2.98	2.99 ± 1.15
T_{max} (min)	10 (7–15)	10 (10–30)
$Ae_{0-24\,h}$ (% of LQ dose)	4.57 ± 4.27	0.366 ± 0.363*
$Ae_{0-24\,h}$ of total LQ (% of LQ dose)[c]	23.4 ± 4.79	0.853 ± 0.778***
$GI_{24\,h}$ (% of LQ dose)	1.77 ± 2.12	1.52 ± 0.641
F (%)	10.6	11.4
M1		
$AUC_{0-12\,h}$ (μg min/ml)	1960 ± 474	2800 ± 390**
C_{max} (μg/ml)	19.2 ± 9.86	17.5 ± 4.72
T_{max} (min)	15 (15)	30 (15–45)
$Ae_{0-24\,h}$ (% of LQ dose)	11.3 ± 4.44	0.286 ± 0.262***
$AUC_{M1,\,0-12\,h}/AUC_{LQ}$ ratio	46.3 ± 37.7	46.1 ± 13.6
M2		
$AUC_{0-12\,h}$ (μg min/ml)	1520 ± 491	5640 ± 1660***
C_{max} (μg/ml)	25.7 ± 15.5	40.3 ± 7.70*
T_{max} (min)	15 (15)	30 (15–45)
$Ae_{0-24\,h}$ (% of LQ dose)	15.8 ± 8.89	0.265 ± 0.149***
$AUC_{M2,\,0-12\,h}/AUC_{LQ}$ ratio	31.0 ± 15.7	94.2 ± 39.6***

$Ae_{0-24\,h}$, percentage of the dose excreted in the 24-h urine; AUC, total area under the plasma concentration–time curve from time zero to infinity; $AUC_{0-12\,h}$, total area under the plasma concentration–time curve from time zero to the last measured time, 12 h, in plasma; C_{max}, maximum plasma concentration; F, extent of absolute oral bioavailability; $GI_{24\,h}$, percentage of the dose recovered from the gastrointestinal tract (including its contents and faeces) at 24 h; T_{max}, time to reach C_{max}. Pharmacokinetic parameters were determined after oral administration of 50 mg/kg liquiritigenin (LQ) to control rats and rats with acute renal failure induced by uranyl nitrate (U-ARF). [a]Measured just before treatment. [b]Measured just before experiment. [c]$Ae_{0-24\,h}$ of conjugated plus unconjugated LQ. Values are means ± SD except T_{max}, which is given as median (range). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, significantly different compared with the control group.

two groups. Moreover, hepatic blood flow rate was reported to be unchanged in U-ARF rats.[37] Therefore, the comparable AUC values of intravenous LQ in U-ARF and control rats (Table 2) were likely due to the comparable hepatic CL_{int} for LQ disappearance, free LQ fraction in plasma, and hepatic blood flow rate in the two groups.

In U-ARF rats, the V_{area} of intravenous LQ was significantly larger than in controls (Table 2). Although the exact reason for this effect is not clear, it was not due to increases in the free LQ fraction in the plasma of U-ARF rats, as both groups showed similar plasma protein binding values of LQ.

Following intravenous administration of LQ, both AUC_{M1}/AUC_{LQ} and AUC_{M2}/AUC_{LQ} ratios (metabolite ratios) were significantly greater in U-ARF rats than in controls (Table 2). However, this was not due to greater M1 and M2 formation in U-ARF rats, as CL_{int} values for the formation of M1 and M2 in hepatic microsomes and in-vivo liver UDPGA levels were not altered compared with controls (Table 1; Figure 1). As a result of impaired kidney function in U-ARF rats, the $Ae_{0-24\,h}$ values of both M1 and M2 were significantly smaller in U-ARF rats (by 91.5 and 89.8%, respectively) than in controls (Table 2). Therefore, in U-ARF rats, the greater metabolite ratios of M1 and M2 following LQ administration were likely due to decreased urinary excretion.

The AUC of oral LQ did not differ between the two groups. However, the $AUC_{0-12\,h}$ of both M1 and M2, and the $AUC_{M2,\,0-12\,h}/AUC_{LQ}$ ratios were significantly greater (by 42.9, 271 and 204%, respectively) in U-ARF rats than in controls. The reported hepatic first-pass effect for LQ in rats was trivial (3.67% of the oral dose) because of the extensive gastrointestinal first-pass effect (92.5% of the oral dose) preceding liver metabolism.[6] Thus, intestinal metabolism is a major determinant of the AUC of LQ and the metabolite ratios of both M1 and M2 following oral administration of LQ. UGT activity and UDPGA levels in the intestine did not differ between the

two groups (Table 1; Figure 1), suggesting that intestinal LQ metabolism is also similar. However, $Ae_{0-24\,h}$ values of both M1 and M2 after oral administration of LQ were significantly smaller in U-ARF rats (by 97.5 and 98.3%, respectively) than in controls (Table 3). Therefore, the increase in the AUCs of M1 and M2 and the M2 metabolite ratio in U-ARF rats were again due to decreased urinary excretion as a result of impaired kidney function and not due to increased intestinal formation of M1 and M2.

Conclusions

Hepatic and intestinal metabolism of LQ and the formation of M1 and M2 were not altered in U-ARF rats compared with controls, as evidenced by similar UGT activity and UDPGA levels. The slower CL_R of LQ in U-ARF rats did not significantly affect the AUC of LQ because of the small contribution of CL_R to the total CL of LQ. However, U-ARF rats displayed decreased urinary excretion of glucuronide conjugates of LQ, resulting in significantly greater AUC and metabolite ratios of M1 and M2 following intravenous and oral LQ administration. These pharmacokinetic changes of M1 and M2 in ARF are meaningful because LQ glucuronides may cause choleretic action, which is one of the mechanisms of hepatoprotective action of LQ.[3]

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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