Liquiritigenin, a flavonoid aglycone from licorice, has a choleretic effect and the ability to induce hepatic transporters and phase-II enzymes

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Submitted 31 August 2008; accepted in final form 7 December 2008

KimYW, KangHE, LeeMG, HwangSJ, KimSC, LeeCH, KimSG. Liquiritigenin, a flavonoid aglycone from licorice, has a choleretic effect and the ability to induce hepatic transporters and phase-II enzymes. Am J Physiol Gastrointest Liver Physiol 296: G372–G381, 2009. First published December 12, 2008; doi:10.1152/ajpgi.00524.2008.—Liquiritigenin (LQ), an active component of licorice, has an inhibitory effect on LPS-induced inhibitory nitric oxide synthase expression. This study investigated the effects of LQ on choleresis, the expression of hepatic transporters and phase-II enzymes, and fulminant hepatitis. The choleretic effect and the pharmacokinetics of LQ and its glucuronides were monitored in rats. After intravenous administration of LQ, the total area under the plasma concentration-time curve of glucuronyl metabolites was greater than that of LQ in plasma, which accompanied elevations in bile flow rate and biliary excretion of bile acid, glutatione, and bilirubin. The expressions of hepatocellular transporters and phase-II enzymes were assessed by immunobots, real-time PCR, and immunohistochemistry. In the livers of rats treated with LQ, the protein and mRNA levels of multidrug resistance protein 2 and bile salt export pump were increased in the liver, which was verified by their increased localizations in canalicular membrane. In addition, LQ treatment enhanced the expression levels of major hepatic phase-II enzymes. Consistent with these results, LQ treatments attenuated galactosamine/LPS-induced hepatitis in rats, as supported by decreases in the plasma alanine aminotransferase, liver necrosis, and plasma TNF-α. These results demonstrate that LQ has a choleretic effect and the ability to induce transporters and phase-II enzymes in the liver, which may be associated with a hepatoprotective effect against galactosamine/LPS. Our findings may provide insight into understanding the action of LQ and its therapeutic use for liver disease.

choleresis; hepatocellular transporter; glucuronidation

LICORICE (GLYCYRRHIZAE RADIX) products have been consumed on a large scale (i.e., an annual amount of ~1.5 kg per person) not only in sweetening agents and beverages in the United States and European countries (e.g., United Kingdom and Belgium) but also in analgesic and antitussive remedies in Asian countries (16, 47). Liquiritigenin (7,4′-dihydroxyflavonane, LQ), an aglycone of liquoritirin in licorice, is a flavonoid with a cytoprotective effect. Studies from our laboratories showed that LQ exerts cytoprotective effects against heavy metal-induced toxicity in cultured hepatocytes (23) and has protective effects against liver injuries induced by acetaminophen and b互利he sulfoximine (BSO) in rats (26). Moreover, LQ has an anti-inflammatory effect as shown by the inhibition of nitric oxide (NO) and tumor necrosis factor-α (TNF-α) production induced by lipopolysaccharide (LPS) in macrophages (27).

Bile has diverse physiological functions that range from the pathway of toxic compound and lipid waste product elimination to the digestion and absorption of dietary lipids. Hepatic uptake of biliary components from blood occurs through sinusoidal transporters located in the basolateral membrane. The efflux of bile acid from hepatocytes is mediated by the members of the multidrug resistance protein (Mrp) family and the bile salt export pump (Bsep). In particular, Mrp2 mediates export of glucuronidated bile acids and bilirubin from hepatocytes into bile, whereas Bsep mainly excretes bile acids (20, 45). Hence, it is expected that malfunctioning of hepatic transporters may result in intracellular accumulation of bile acids and cause cholestasis, hepatocyte death, and parenchymal injury (2).

The phase-II detoxification enzymes are a group of antioxidant systems that function against oxidative stress, carcinogenesis, mutagenesis, and other forms of toxicity (37). The induction of phase-II enzymes, including UDP-glucuronosyltransferase (UGT), may lower susceptibility to free radicals and carcinogens, whereas their inhibition enhances susceptibility to toxic stress (13, 30, 42). In particular, UGT glucuronidation is a major detoxification process in the liver. Hence, selective induction of phase-II enzymes is a highly effective strategy for preventing and reducing cellular toxicity (43). UGT glucuronidation is the most important metabolic pathway of flavonoids such as LQ. Therefore, glucuronides exist in blood circulation after flavonoid administration. Comprehensive mechanistic studies also suggest that the cytoprotective effect of chemopreventive agents is associated with the induction of other phase-II enzymes, such as heme oxygenase 1 (HO-1) and glutathione S-transferase (GST). In animal models, deficiency of HO-1 or GST augments carcinogenesis or aggravates vascular disease, respectively (8, 13). Although glucuronosyl metabolism of LQ has been studied (38, 39), the effects of LQ on the expression of phase-II enzymes have not been explored.

In view of the importance of phase-II glucuronosyl metabolism, biliary excretion of glucuronides, and the potential roles of transporters and phase-II enzymes in liver protection, this study investigated the effect of LQ on choleresis and the expression of hepatocellular transporters and hepatic phase-II enzymes in rats. Galactosamine (GalN) and LPS induce liver injury as a consequence of UTP depletion in parenchymal...
cells, the impairment of hepatic glucuronidation, and the activation of Kupffer cells, causing the subsequent release of toxic cytokines (11, 15). In the present study, we further examined the ability of LQ to protect the liver in a GalN/LPS rat model.

MATERIALS AND METHODS

Reagent. LQ was synthesized by Dr. J. W. Lee (Seoul National University). 4’-O-glucuronide (M1) and 7-O-glucuronide (M2) were obtained from Dr. H. J. Chung (Life Sciences Research Division, Korea Institute of Science and Technology). Anti-Mrp2 (M2III-6) and anti-HO-1 antibodies were purchased from Alexis (Lausen, Switzerland) and Stressgen (San Diego, CA), respectively. Anti-UGT1A and anti-Bsep antibodies were supplied from Becton Dickinson (Franklin Lakes, NJ) and Stressgen, respectively. Anti-UGT1A and anti-Bsep antibodies were supplied from Dr. H. J. Chung (Life Sciences Research Division, Korea Institute of Science and Technology), respectively. Vectashield was supplied from Vector Laboratories (Burlingame, CA). D(+)-galactosamine hydrochloride and LPS (Escherichia coli 026:B6) were purchased from Sigma (St. Louis, MO).

Animals. The protocols for the animal studies were approved by the Animal Care and Use Committee of Seoul National University. Male Sprague-Dawley rats (6 wk old and weighing 140–160 g) were purchased from Samtako (Osan, Korea) and housed at 20 ± 5°C with a 12-h light/dark cycles and a relative humidity of 50 ± 5% (Tecniplast, Varese, Italy) under filtered, pathogen-free air, with food (Purina, Korea) and water available ad libitum.

Intravenous study. LQ [dissolved in polyethylene glucol 400: distilled water = 40:60 (vol/vol)] at a dose of 50 mg/kg was infused over 1 min via the jugular vein of rats (n = 4). A blood sample (~0.22 ml) was collected via the carotid artery at designated times. Other procedures used are similar to a reported method (24).

Biliary excretion of LQ, M1, and M2 and chemistry data. LQ at a dose of 50 mg/kg was intravenously administered to rats (n = 6) with bile duct cannulation, and bile samples were collected for the following time points (in hours): 1-0, 0-1, 1-2, 2-3, 3-4, and 4-6. Bile flow rate was also measured. Reduced GSH contents in the bile and liver tissue were quantified as previously described (23). Total bilirubin and bile acid were determined using assay kits (Asan Pharmaceutical, Seoul, Korea and Trinity Biotech, Wicklow, Ireland).

Table 1. Primers used for real-time PCR analyses

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward Primers</th>
<th>Reverse Primers</th>
<th>Size, bp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bsep</td>
<td>5'-TCT ATG TAT GCC TGC CTT TTG GCT 5'-TTT-3'</td>
<td>5'-GCC CAG ACC GTC TTA GGC TA-3'</td>
<td>101</td>
</tr>
<tr>
<td>Oatpl1a</td>
<td>5'-TCT GCC TGG CTT GCT TGG TTC TCC TTG TC-3'</td>
<td>5'-GTC TGC TGG CTT TCC TGC-3'</td>
<td>177</td>
</tr>
<tr>
<td>Ntcp</td>
<td>5'-CCT CCC TGA TGC CTT CT-3'</td>
<td>5'-GAA TCC GTA GAT GCT GAT G-3'</td>
<td>104</td>
</tr>
<tr>
<td>Mrp1</td>
<td>5'-AGA AAT TCG GGA GCA TGG TGG TG-3'</td>
<td>5'-CAG ACG CCA GAC GTC TTT GA-3'</td>
<td>321</td>
</tr>
<tr>
<td>Mrp2</td>
<td>5'-GGC AGG AGA GGA GCA TTA TGC AG-3'</td>
<td>5'-GAG GAG GAG CTC TGG GTT GTT GC-3'</td>
<td>306</td>
</tr>
<tr>
<td>Mrp3</td>
<td>5'-ACA AGC CCG AGC CCA TAT ACAC-3'</td>
<td>5'-GAT CTT GGG CGG ATC AC-3'</td>
<td>301</td>
</tr>
<tr>
<td>HO-1</td>
<td>5'-TGC TCG CAT GAA CAC TCT G-3'</td>
<td>5'-TCC TCT GTC AGC GAG TAC G-3'</td>
<td>123</td>
</tr>
<tr>
<td>UGT1A1</td>
<td>5'-ACA CAG ATC GCA TGA ACT TGC TTG TG-3'</td>
<td>5'-AGG ACT CAG AAG GTC TGT GAC AGT C-3'</td>
<td>152</td>
</tr>
<tr>
<td>mEH</td>
<td>5'-GCC ACT TCT GTC CCG AGG AGA A-3'</td>
<td>5'-TAA GGT TCC CTT CTT GTC GAG G-3'</td>
<td>145</td>
</tr>
<tr>
<td>GST-a</td>
<td>5'-GGA GAA CAT GAA GAA GAG ACC GC-3'</td>
<td>5'-TGT TGG CTA GAT TCG TGT GTC ATC-3'</td>
<td>397</td>
</tr>
</tbody>
</table>

Bsep, bile salt export pump; Oatpl1a, organic anion-transporting polypeptide 1a1; Ntcp, Na‘-taurocholate cotransporting polypeptide; Mrp, multidrug resistance protein; HO-1, heme oxygenase 1; UGT, UDP-glucuronosyltransferase; mEH, microsomal epoxide hydrolase; GST, glutathione S-transferase.
HPLC analysis of LQ, M1, and M2. For the analysis of LQ, chloroxazone (internal standard) was added to a 50-μl aliquot of biological sample. Lamotrigine was used as an internal standard for the analysis of M1 and M2. The mixture was extracted and subjected to HPLC analysis. The mobile phase for the analysis of LQ, 15 mM KH2PO4:acetonitrile at a ratio of 72:28 (vol/vol), and for the analysis of M1 and M2, 0.05 M sodium acetate buffer:acetonitrile at a ratio of 84:16 (vol/vol), were run at a flow rate of 1.2 ml/min, and the eluent was monitored using an ultraviolet detector at 276 nm at room temperature.

Real-time RT-PCR. To determine the levels of transporters and phase-II enzymes, LQ (15 mg/kg per day for 3 days) was intravenously administered to six rats, which were euthanized 1 h after the last treatment. Total RNA was isolated from liver using Trizol reagent (Invitrogen, Carlsbad, CA). RNA (2 μg) was reverse transcribed using an oligo-dT16 primer to obtain cDNA. The crossing point (Cp) values were normalized on the basis of GAPDH with the use of the Light-cycler software (Roche, Mannheim, Germany). A melting curve analysis was employed after amplification to verify the accuracy of the amplicon. Real-time RT-PCR was carried out according to the manufacturer’s instructions (Light-Cycler 2.0, Roche) (Table 1).

Subcellular fractionation and immunoblot analysis. Liver samples were prepared from three randomly selected animals in each treatment group and subjected to immunoblot analyses (3 determinations per sample). Liver homogenates were fractionated and subjected to immunoblot analyses (5, 22). A representative blot was chosen for each figure, and three different samples per treatment were included in each blot.

Immunohistochemistry. Liver specimens were fixed in 10% formalin, embedded in paraffin, cut into 4-μm-thick sections, and mounted on slides. Tissue sections were immunostained with antibodies directed against Mrp2 or Bsep. Briefly, tissue sections were deparaffinized and were incubated with mouse anti-Mrp2 (1:200) and goat anti-Bsep (1:50) antibodies for 16 h, followed by incubation with Cy3-conjugated donkey anti-goat and Alexa 488-conjugated donkey anti-mouse antibodies for 2 h.

GalN/LPS-induced fulminant hepatitis. LQ (at the indicated daily doses) was intravenously or orally administered to rats (n = 8) once a day for three consecutive days. To induce acute liver injury, rats were intraperitoneally administered with a single dose of GalN (600 mg/kg) and LPS (1 μg/kg) 1 h after the final injection of LQ and euthanized 24 h posttreatment.

Blood chemistry. Plasma levels of alanine aminotransferase (ALT) and TNF-α were analyzed as described previously (26, 27).

Histopathology. Hepatic morphology was assessed by light microscopy. Liver samples were stained with hematoxylin and eosin (H & E) (26). TUNEL staining was performed with an in situ cell death detection kit (Chemicon International, Temecula, CA). The number of TUNEL-positive cells was determined in five randomly selected fields from each slide at a magnification of ×100.

Caspase-3 activity assay. Caspase-3 activity was determined by a commercially available caspase assay kit (Promega, Madison, WI).

Statistical analysis. One-way ANOVA was used to assess the statistical significance of differences among treatment groups. For each statistically significant effect of treatment, the Newman-Keuls test was used for comparisons between multiple group means.

RESULTS

Pharmacokinetics of LQ, M1, and M2. LQ was metabolized to glucuronide conjugates including M1 and M2 (38). First, we evaluated the plasma concentration-time profiles of LQ, M1, and M2 after a dose of 50 mg/kg LQ, a dose shown to be effective against acetaminophen intoxication (26). After 1-min intravenous administration of LQ, the plasma concentrations of LQ declined rapidly with a terminal half-life of 8.77 min and...
were detected for up to 75 min in the plasma (Fig. 1B). The time profiles for the LQ metabolites indicated that the levels of M1 and M2 rapidly increased 1 min after injection of LQ, followed by decreases from the maximum at 5–30 min (Fig. 1C). The plasma concentrations of M1 and M2 then further increased at 3–6 h, and the increased concentrations of the glucuronides remained for up to 8 h posttreatment. The total area under the plasma concentration-time curves of M1 and M2 were greater than that of LQ [1,650 ± 214 and 1,780 ± 320 (AUC$_{0-8}$ of M1 and M2, respectively) vs. 999 ± 161 (AUC$_{0-8}$ of LQ) μg·min$^{-1}$·ml$^{-1}$].

**Choleretic effect of LQ.** It has been shown that the metabolites of LQ were excreted into bile after injection of 5 mg/kg LQ, but the bile flow rate was not changed at this dose (39). To characterize the possible choleretic effect of LQ at the dose of 50 mg/kg (the most effective dose for hepatoprotection or anti-inflammation), LQ was intravenously administered to rats. At 1 h after injection of LQ, the bile flow rate significantly increased (1.9 ml/h) compared with vehicle treatment (1.0 ml/h), which was then slightly decreased from the maximum 2 h posttreatment and restored to control rate at later times (Fig. 2A). The biliary excretion of LQ and M1 were almost completed within 1 h and M2 within 2 h (Fig. 2B), suggesting that biliary excretion of LQ, M1, and M2 were independent of bile flow rate. Moreover, LQ treatment increased the biliary excretion rate of bile acid, the main component in bile, as a function of time and reached a maximum plateau at 4 h (Fig. 2C).

Biliary excretion of reduced GSH is considered to be an important determinant of bile acid-independent bile flow. Next, we measured the effect of LQ on the biliary excretion of GSH. LQ treatment substantially enhanced GSH secretion 1 h after treatment (Fig. 2D). The excretion of glucuronide conjugates of bilirubin as well as xenobiotics is known to be mediated by hepatocellular transporters (41). Total bilirubin concentration in the bile specimen was significantly higher in the LQ-treated group than in vehicle-treated group (Fig. 2E). Our results
demonstrate that the choleretic action of LQ may be both bile acid-dependent and bile acid-independent.

**Induction of hepatocellular transporters.** Canalicular bile transport represents the rate-limiting step in hepatic excretion and bile formation (28, 29). In an effort to find the mechanistic basis of the choleretic effect of LQ, we next examined the expression of major hepatic transporters, including canalicular transporters (Mrp2 and Bsep) and basolateral transporters organic anion-transporting polypeptide 1a1 (Oatp1a1) and Na+/taurocholate cotransporting polypeptide (Ntcp). Real-time RT-PCR analysis revealed that LQ treatments (15 mg/kg per day iv, for 3 days) significantly increased the mRNA levels of Mrp2, Bsep, Oatp1a1, and Mrp3 but not Mrp1 in the liver (Fig. 3A). The data are consistent with the induction of Mrp2 and Bsep by LQ, as shown by immunoblottings (Fig. 3B). The increased expression of Mrp2 and Bsep after LQ treatments was also demonstrated in the double-labeling immunohistochemistry. Immunoreactivity for both Mrp2 (green) and Bsep (red) in the hepatic canaliculi was more intensive in the liver of LQ-treated rats than those of vehicle-treated control rats. Colocalization of Mrp2 and Bsep was confirmed by yellow color in merged images (Fig. 3C).

**Induction of phase-II enzymes.** One of the major mechanisms of organ protection against toxic stress is the induction of phase-II enzymes (36, 37, 42). To assess changes in the expression of phase-II enzymes in the liver, we examined the effect of LQ treatments on UGT1A, HO-1, GST-α, and mEH. Real-time RT-PCR assays demonstrate that the expression of major phase-II enzyme transcripts was enhanced after treating rats with LQ (15 mg/kg per day iv, for 3 days) (Fig. 4A). LQ-induced expression of the phase-II enzymes was also verified by immunoblottings (Fig. 4B).

**Effects on GalN/LPS-induced fulminant hepatitis.** GalN inhibits glucuronidation by depleting UTP and UDP-glucuronic acid and thereby induces fulminant hepatitis (6, 11, 21). As a functional study, we evaluated the effect of LQ on GalN/LPS-induced liver injury. ALT activity was markedly increased 24 h after GalN/LPS treatment. LQ pretreatments (15 mg/kg per day iv or 15–50 mg/kg per day po, for 3 days) attenuated the increase in ALT activity by GalN/LPS (Fig. 5A). Dimethyl-4,4’-dimethoxy-5,6,5’,6’-dimethylene dioxybiphenyl-2,2’-dicarboxylate treatment (15 mg/kg per day po, for 3 days), a positive control, also inhibited the increase in ALT (10).

Our study was extended to histopathologically examine the extent of liver injury. GalN/LPS treatment resulted in hemorrhage, acidophilic degeneration and necrosis of the hepatocytes, and infiltration of inflammatory cells. These changes were attenuated by LQ pretreatments (Fig. 5B). The TUNEL assay identified apoptotic cells in the liver of rats treated with GalN/LPS (Fig. 6A). The number of cells stained with TUNEL was inhibited by LQ pretreatments, resulting in a decrease in apoptotic scores from 17 ± 1.5 (GalN/LPS) to 4.6 ± 2.2 (LQ iv) or 5.6 ± 3.7 (LQ po) (Fig. 6B). In addition, the result of caspase-3 activity assay confirmed hepatocyte apoptosis in the liver of rats treated with GalN/LPS (Fig. 6C). As expected, the induction of caspase-3 activity was significantly inhibited by LQ pretreatment (Fig. 6C). The decreases in TUNEL-positive cells and caspase-3 activity in the liver along with the immunohistochemical improvement strongly support the protective effect of LQ against GalN/LPS-induced hepatitis.

GalN suppresses protein synthesis in hepatocytes and thus exhausts cellular GSH (6, 21, 46). The hepatic GSH content was measured to verify the improvement in liver function. LQ treatments enabled the liver to restore the reduced GSH content (Fig. 7A). Moreover, LQ blocked the production of TNF-α (a representative hepatitis marker) in the plasma of rats challenged with GalN/LPS (Fig. 7B).

**DISCUSSION**

This study demonstrates the metabolism of LQ to glucuronide conjugates after intravenous administration to rats and the ability of LQ to induce hepatic UGT1A, an enzyme most predominantly involved in the glucuronidation of bilirubin and xenobiotics (e.g., polyphenols) (49). Following the rapid disappearance of LQ from plasma, two glucuronide metabolites were sequentially observed at least up to 8 h, indicating that LQ glucuronidation may be immediate and sustained for a long period of time. Our pharmacokinetic results strongly support the idea that LQ is conjugated to its glucuronides in the liver. Since the conjugation of LQ depends on the expression of hepatic UGT, LQ glucuronidation may be associated with the ability of LQ to induce UGT in the liver. In addition, we...
observed that NF-E2-related factor 2 (Nrf2) LQ treatment significantly increased nuclear activation of Nrf2 in the liver (data not shown), which as a key transcription factor may be responsible for the induction of UGT. Glucuronide formation is greater after oral administration of LQ than after intravenous administration of LQ to rats (data not shown), indicating that UGT is also the important metabolic pathway for LQ in the intestine. Quercetin and catechin glucuronides also accumulate in the plasma after oral administration, which may result from the predominant absorption of glucuronide conjugates in the intestine (9, 35).

Mrp2 is a representative organic anionic transporter of the canalicular domain and arbitrates the efflux of a series of organic anions including GSH, GSH conjugates, and bilirubin glucuronides as well as bile acids. Mrp2-dependent excretion of bile provides the bile acid-independent bile flow, which
explains approximately one-half of the bile flow (20, 45). In fact, impairment of Mrp2 function leads to a reduction in bile flow, which may result in diminished GSH excretion (15) and thus induce cholestatic liver disease. Bsep is the major transporter of canalicular membrane responsible for the secretion of bile acids into bile, and inactivation of this protein therefore results in impaired bile secretion and mild intrahepatic cholestasis (1). Our RT-PCR results indicated that LQ increased the mRNA levels of canalicular efflux transporters (Mrp2 and Bsep) and hepatic basolateral uptake transporters (Ntcp and Oatp1a1). Moreover, the results of immunoblot and immunohistochemical assays verified the bona fide induction of Mrp2 and Bsep by LQ. Because an increase in bile flow comprising bile components largely depends on the efflux transporters, the significant elevation in bile flow by LQ observed in the present study may result from the induction of Mrp2 and Bsep. Another important finding is that LQ treatment promoted transport of GSH and bilirubin to bile. Our results imply that LQ may have a beneficial effect on cholestasis.

Conjugation of xenobiotics and their metabolites increases solubility and facilitates excretion of the conjugates. Although glucuronidation is an ordinary inactivation pathway, the gluc-
Choleretic Effect of Liquiritigenin

Liquiritigenin (LQ) is a flavonoid derived from the roots of licorice and has been shown to have hepatoprotective effects. It can induce phase-II enzymes in the liver, which are involved in the detoxification of drugs and other xenobiotics. The induction of phase-II enzymes may be commonly regulated by LQ, which remains to be established.

In the present study, we demonstrated that LQ has the ability to induce phase-II enzymes in the liver. Many studies have indicated that the induction of phase-II enzymes by natural or synthetic compounds is a sufficient condition for obtaining chemoprevention and antioxidant activity (30, 36, 37, 42). Acetaminophen is metabolized chiefly to glucuronide and sulfate conjugates (3). Therefore, the induction of phase-II enzymes, along with GSH-conjugation of its metabolites, contributes to hepatoprotection against acetaminophen. Previously, we found a liver-protective effect of LQ in an acetaminophen and BSO-induced toxicity model. LQ treatment efficaciously prevented acute liver injuries induced by the toxicants as shown by decreases in hepatic necrosis and plasma ALT activities (26). The hepatoprotective effects of LQ against acetaminophen or acetaminophen plus BSO might be related to its ability to induce UGT and hepatocellular transporters identified in the present study. In addition to the induction of UGT, LQ was capable of inducing HO-1. It has been recognized that the induction of HO-1 contributes to protecting hepatocytes from inflammatory liver damage including GalN/LPS-induced hepatitis (48); the products of heme degradation such as bilirubin and CO may exert a hepatoprotective effect against toxic stress and reactive oxygen species (8). Moreover, comprehensive mechanistic studies suggest that the induction of GST accounts for the cytoprotective effect against toxicant-induced injury and thereby contributes to chemopreventive effects (17, 25). Therefore, our results showing that LQ significantly induces hepatocellular transporters and major phase-II enzymes may account, at least in part, for the hepatoprotective effect of LQ. The expression of the genes encoding for these proteins may be commonly regulated by LQ, which remains to be established.

Although the primary purpose of the functional studies was to examine biliary flow and cholestasis, we further sought to determine the effect of LQ against GalN/LPS. GalN-induced liver damage resembles the drug-induced hepatitis in humans characterized by necrosis and parenchymal inflammation (14, 19, 32). Studies have shown that GalN inhibits glucuronidation through its rapid conversion to UDP-GalN and subsequent depletions of UDP and UDP-glucuronic acid and depletes GSH by suppressing protein synthesis in hepatocytes (6, 11, 21). Our supposition is that the ability of LQ to induce UGT1A contributes to the cytoprotective effect by facilitating UGT-dependent metabolism of endobiotics and toxicants. The induction of canalicular transporter activity and cholestasis by LQ may also be associated with the hepatoprotective effect of LQ. Our finding that LQ enabled hepatocytes to almost completely restore reduced GSH content strengthens its hepatoprotective effect.

It is well recognized that hepatocyte damage leads to the recruitment of inflammatory cells. In particular, GalN treatment increases the absorption of endotoxin from the gut into the portal blood and resultant activation of Kupffer cells and the production of TNF-α(12, 34, 40). Moreover, inflammation represses the expression of hepatic transporters by disrupting critical physiological processes (4, 31), which may explain the role of inflammation in the pathophysiology of cholestasis. Our present findings that LQ attenuates TNF-α production elicited by GalN/LPS supports its anti-inflammatory effect in the liver and corroborates our previous findings regarding the inhibition of LPS-induced NO and TNF-α production by LQ in macrophages (27). Moreover, GalN/LPS treatment slightly, but significantly, increased the activities of alkaline phosphatase and γ-glutamyl transferase in plasma, the known markers of obstructive liver diseases, which were also abrogated by LQ treatment (data not shown). All of these results lend support to the conclusion that LQ prevents the ability of GalN/LPS to induce hepatitis involving macrophage-mediated release of proinflammatory cytokines.

The final purpose of pharmacokinetic studies in experimental animals is to extrapolate pharmacokinetic parameters for use in humans. On the basis of the differences of body surface area between rat and human, a dose of 50 mg/kg in rats would be equivalent to less than 1 g in humans. Human dose can be assessed more precisely by animal scale-up study. A preliminary animal scale-up study indicated that the total body clearance, apparent volume of distribution, and plasma concentration of LQ after an intravenous administration were in the linear range. The exact dose will be predicted by the complete animal scale-up study in the future.

Research on flavonoids may warrant exploration into novel mechanisms for therapeutic intervention. The results of this study demonstrate that LQ has a choleretic effect, significantly promoting hepatic bile flow and the induction of hepatocellular transporters. The induction of transporters as well as phase-II enzymes may be associated with a hepatoprotective and anti-inflammatory effect in a GalN/LPS-induced hepatitis model. Our findings may provide insight into understanding the pharmacological action of LQ and its therapeutic use in liver disease.

ACKNOWLEDGMENTS

We thank Dr. S. C. Brooks III for helpful discussion and editing.

GRANTS

This work was supported by the Korea Science and Engineering Foundation (KOSEF) grant funded by the Korea government (MEST) (No.R11-2007-107-01001-0).

REFERENCES


