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Pharmacokinetic interaction between liquiritigenin (LQ) and DDB: Increased glucuronidation of LQ in the liver possibly due to increased hepatic blood flow rate by DDB

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ABSTRACT

It has been reported that both liquiritigenin (LQ) and dimethyl-4,4′-dimethoxy-5,6,5′,6′-dimethylenedioxybiphenyl-2,2′-dicarboxylate (DDB) have a hepatoprotective effect, and administration of both drugs together shows additive protective effect against acute liver injuries. Therefore, the pharmacokinetic interaction between LQ and DDB in rats was studied. LQ (20 and 50 mg/kg for the i.v. and p.o. administration, respectively), DDB (10 mg/kg for both i.v. and p.o. administration), and both drugs together were once administered intravenously or orally to rats. After the i.v. administration of both drugs together, the Cl_{nr} and AUC of LQ were significantly faster (by 30.5%) and smaller (by 22.5%), respectively, than those of without DDB due to the faster hepatic blood flow rate by DDB. After the p.o. administration of both drugs together, the AUC of LQ was comparable to that of without DDB due to negligible effect of DDB on intestinal metabolism of LQ. The pharmacokinetic parameters of DDB after both i.v. and p.o. administration were not altered by LQ, indicating that LQ did not considerably affect the pharmacokinetics of DDB in rats.

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1. Introduction

Liquiritigenin [LQ; 2,3-dihydro-7-hydroxy-2-(4-hydroxyphenyl)-(S)-4*H*-1-benzopyran-4-1], is an aglycone of liquiritin contained in *Glycyrrhizae radix* (licorice). It exerted cytoprotective effects against heavy metal-induced toxicity in rat hepatocyte-derived cultured cells (Kim et al., 2004), and showed protective efficacy in rats with acetaminophen-induced (Kim et al., 2006) or galactosamine/lipopolysaccharide (GalN/LPS)-induced (Kim et al., 2009) acute liver injuries and in mice with carbon tetrachloride-induced liver injuries (Shimamura et al., 1993). LQ was metabolized to five 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) (Shimamura et al., 1990). After intravenous (i.v.) administration of LQ at a dose of 5 mg/kg to rats, only M1, M2, and M3 were detected in the plasma although five conjugates (M1–M5) were excreted in the bile (Shimamura et al., 1993). Recently, the pharmacokinetics of LQ, M1, and M2 after i.v. and/or p.o. administration of various doses of LQ to four species (mice, rats, rabbits, and dogs) and animal scale-up of the pharmacokinetics of LQ to predict its pharmacokinetics in humans have been reported (Kang et al., 2009a). LQ is now being evaluated in preclinical studies as a p.o. agent for the treatment of inflammatory liver disease.

DDB (dimethyl-4,4′-dimethoxy-5,6,5′6′-dimethylene dicarboxybiphenyl-2,2′-dicarboxylate) is a synthetic hepatoprotective agent derived from Schizandrin C, a component of *Fructus schizandrae*. It was active against toxin-induced liver injuries in animals. For example, it protects the liver against CCl_4-, D-galactosamine-, thioacetamide-, or prednisolone-induced injuries (Liu et al., 1979, 1981, 1982; Liu and Lesca, 1982; Kim et al., 1995). It also prevented aflatoxin B1-induced hepatotoxicity in rats (Liu et al., 1995), and improved liver function in patients with hepatitis B virus (Lee et al., 1991). Human liver microsomal studies have shown that DDB was metabolized to 5 metabolites via the hepatic cytochrome P450 (CYP) 1A2, 2C9, and 3A4 (Baek et al., 2001). DDB is marketed as a curative agent for patients with viral hepatitis in several countries including China, South Korea, Vietnam, and Pakistan.

Since both LQ and DDB have a hepatoprotective effect as mentioned above, it is quite possible that these two drugs could be administered together. Moreover, combined treatments of rats with LQ and DDB showed some additive protective effect against

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acute liver injuries induced by acetaminophen or acetaminophen plus buthionine sulfoximine (Kim et al., 2006).

The purpose of this study is to report the possible pharmacokinetic interaction between LQ and DDB after single i.v. and p.o. administration of both drugs together to rats and the possible reason for the interaction.

2. Materials and methods

2.1. Chemicals

LQ was synthesized by Prof. Jee W. Lee (College of Pharmacy, Seoul National University, Seoul, South Korea). 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). DDB was supplied from PharmaKing Company, Ltd. (Seoul, South Korea). Chlorzoxazone, lamotrigine, flutamide, and theobromine [internal standards for the high-performance liquid chromatographic (HPLC) analysis of LQ, M1 and M2, DDB, and *p*-nitrophenyl glucuronide (*p*NP-Glu), respectively], *p*-nitrophenol (*p*NP), *p*NP-Glu, tetraethyl-ammonium bromide (TEAB), dimethylacetamide (DMA), uridine 5′-diphosphoglucuronic acid (UDPGA; as a trisodium salt), tri(hydroxymethyl)aminomethane (Tris)-buffer, and β-glucuronidase (type HP-1; from *Helixa pomatia* having a β-glucuronidase activity of 127,000 units/ml and a sulfatase activity of below 7500 units/ml) were purchased from Sigma–Aldrich Corporation (St. Louis, MO). Polyethylene glycol 400 (PEG 400) was a product from Duksan Pure Chemicals Company, Ltd. (Ansan, South Korea). Other chemicals were of reagent or HPLC grade.

2.2. Animals

The protocols for this animal studies were approved by Institute of Laboratory Animal Resources of Seoul National University. Male Sprague–Dawley rats (7–9 weeks old, weighing 250–310 g) were purchased from Charles River Company Korea (Orient, Seoul, South Korea). The methods of housing and handling of the rats were similar to a reported method (Kang et al., 2009a). Each rat was randomly divided into three groups: DDB, LQ, and both drugs together.

2.3. I.v. and p.o. studies

The procedures used for pretreatment 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 i.v. study) were similar to a reported method (Kang et al., 2009a).

LQ (20 mg/kg; $n = 7$), DDB (10 mg/kg; $n = 7$), and both drugs together ($n = 8$) were manually infused via the jugular vein over 1 min (both drugs were dissolved in DMA:PEG 400:distilled water = 3:1:2, v/v/v; total injection volume of 2 ml/kg). A blood sample was collected via the carotid artery (approximately 0.11 ml for time points for the analysis of either LQ, its two metabolites, or DDB, 0.22 ml for time points for the analysis of both LQ and its two metabolites or either of them and DDB, or 0.33 ml for time points for the analysis of LQ, its two metabolites, and DDB in LQ plus DDB group) at 0 (control), 1 (end of the infusion), 3, 5, 10, 20, 30, 45, 60, 120, 180, 240, 360, and 480 min after the start of the infusion. For LQ plus DDB group, a blood sample (approximately 1.5 ml) collected from the untreated rats was infused manually via the carotid artery after the 60-min blood sampling to replace the blood loss due to the blood sampling. A blood sample was immediately centrifuged and one (two or three) 50-μl of a plasma sample was stored at −70 °C (Revco ULT 1490 D-N-S, Western Mednics, Asheville, NC) until used for the HPLC analysis of LQ, M1, M2, and DDB. The procedures used for preparation and handling of the 24-h urine sample ($Ae_{0–24\,h}$) and gastrointestinal tract (including its contents and feces) sample at 24 h ($GI_{24\,h}$) were similar to a reported method (Kang et al., 2009a).

LQ (50 mg/kg; $n = 6$), DDB (10 mg/kg; $n = 8$), and both drugs together ($n = 7$) were administered orally using a gastric gavage tube (the same solution used in the i.v. study; total p.o. volume of 2 ml/kg). A blood sample was collected via the carotid artery at 0, 3, 7, 10, 15, 20, 30, 45, 60, 120, 240, 360, 480, 600, and 720 min after the p.o. administration. Other procedures were similar to those in the i.v. study.

2.4. Preparation of liver (or intestinal) microsomes and liver (or intestinal) extracts from control and i.v. (or p.o.) DDB-treated rats

Rats were treated with the vehicle (DMA:PEG 400:distilled water = 3:1:2, v/v/v; control rats; $n = 6$ and 5 for i.v. and p.o., respectively) or DDB (10 mg/kg; DDB-treated rats; $n = 6$ and 5 for i.v. and p.o., respectively) in the same manner as described in the i.v. and p.o. studies. Twenty minutes after the start of the infusion (or p.o. administration), the liver (or intestine) was rapidly excised after exsanguination. The procedures used for preparation of hepatic (or intestinal) microsomes were similar to a reported method (Lee and Lee, 2008). Protein contents in the hepatic (or intestinal) microsomes were measured using a reported method (Bradford, 1976). In the mean time, liver (or intestine) extracts for the measurement of UDPGA level were prepared according to a reported method (Watkins and Klaassen, 1982) 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 min, homogenized, and centrifuged at 3500 × *g* for 10 min. Resulting heat-treated supernatant was collected and stored at −70 °C.

2.5. Measurement of V_{max}, K_m, and Cl_{int} for the disappearance of LQ and the formation of M1 and M2 in hepatic or intestinal microsomes from control and DDB-treated rats

To find the effect of i.v. DDB on the hepatic UGT activity (or p.o. DDB on the intestinal UGT activity) for the metabolism of LQ, the *in vitro* metabolism studies of LQ in hepatic (or intestinal) microsomes from control and i.v. (or p.o.) DDB-treated rats were performed. The procedures used for the measurement of $V_{\max}$ (the maximum velocity) and $K_{\rm m}$ (the apparent Michaelis–Menten constant; 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 (Fisher et al., 2000). The above microsomes (equivalent to 0.1 and 0.2 mg proteins for hepatic and intestinal microsomes, respectively), 100 mM Tris–HCl buffer (pH 7.4), $MgCl_2$ [1 mM in incubation dissolved in Tris–HCl buffer (pH 7.4)], and alamethicin (50 μg/mg protein dissolved in 50% ethanol) were mixed and placed on the ice for 10 min. Then, 10-μl of 50% methanol containing LQ (to have final LQ concentrations of 1, 2, 10, 20, 40, 100, and 200 μM in incubation) was added, and the mixture was preincubated for 5 min in a thermomixer (Thermomixer 5436; Eppendorf, Hamburg, Germany) kept at 37 °C and 600 revolutions/min (rpm). To initiate the reaction, UDPGA [dissolved in 100 mM Tris–HCl buffer (pH 7.4) to have a final concentration of 3 mM in incubation] was added to give a final volume of 500 μl. After 5-min incubation, two 50-μl of incubation mixture 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 micro-

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