Available online at www.sciencedirect.com
ScienceDirect
Chinese Journal of Natural Medicines 2016, **14**(7): 0534–0540

Chinese Journal of Natural Medicines

Effects of diammonium glycyrrhizinate on hepatic and intestinal UDP-Glucuronosyltransferases in rats: Implication in herb-drug interactions

LI Fei-Yan [1, 2], XIE Hao [1], WENG Lin [2], WANG Hong [1], CAO Li-Juan [1], HAO Hai-Ping [1*], WANG Guang-Ji [1*]

[1] *State Key Laboratory of Natural Medicines, Key Laboratory of Drug Metabolism and Pharmacokinetics, China Pharmaceutical University, Nanjing 210009, China;*

[2] *Department of Good Clinical Practice, Zhejiang Cancer Hospital, Hangzhou 310022, China*

Available online 20 Jul., 2016

[ABSTRACT] Glycyrrhizin is a major bioactive component of liquorice, which exerts multiple biochemical and pharmacological activities and is frequently used in combination with other drugs in the clinic. Mycophenolate mofetil (MMF), an immunosuppressant widely used in transplant patients, is metabolized by UDP-glucuronyltransferases (UGTs). Although significant evidence supports that glycyrrhizin could interact with the cytochrome P450s (CYPs), few studies have addressed its effects on UGTs. The present study aimed at investigating the regulatory effects of diammonium glycyrrhizinate (GLN) on UGTs *in vitro* and *in vivo*. We found that long-term administration of GLN in rats induced overall metabolism of MMF, which might be due to the induction of UGT1A protein expression. Hepatic UGT1A activity and UGT1A mRNA and protein expression were significantly increased in GLN-treated rats. UGT1A expression levels were also increased in the intestine, contradicting with the observed decrease in intestinal UGT1A activities. This phenomenon may be attributed to different concentrations of glycyrrhetinic acid (GA) in liver and intestine and the inhibitory effects of GA on UGT1A activity. In conclusion, our study revealed that GLN had multiple effects on the expression and activities of UGT1A isoforms, providing a basis for a better understanding of interactions between GLN and other drugs.

[KEY WORDS] Diammonium glycyrrhizinate; UDP-glucuronosyltransferase; Sprague-Dawley rat; Drug-drug interaction

[CLC Number] R965 **[Document code]** A **[Article ID]** 2095-6975(2016)07-0534-07

Introduction

Liquorice, one of the most ancient medicinal plants, has been used in traditional Chinese medicine to complement other drugs to reduce toxicity and increase efficacy [1]. Glycyrrhizin is a major water-soluble bioactive triterpene glycoside of licorice [2]. After oral intake, glycyrrhizin is hydrolyzed by intestinal bacteria and then absorbed in the form of glycyrrhetinic acid (GA), aglycone of glycyrrhizin [3]. GA is responsible for the main pharmacological effects of glycyrrhizin, such as anti-inflammatory [4], anti-viral [5], and anti-carcinogenic activities [6]. It is commonly used for the treatment of hepatic steatosis [7], acute and chronic liver injury [8], and cancer [9].

It is widely known that cytochrome P450 (CYPs) and UDP-glucuronyltransferases (UGTs) are the two main enzymatic families affecting the pharmacokinetic and pharmacodynamic properties of xenobiotics, especially with regard to drug-drug interactions (DDIs). Prolonged administration of glycyrrhizin has been reported to induce the

[Received on] 28-Dec.-2015

[Research funding] This work was supported by the Key Lab of Drug Metabolism and Pharmacokinetics of Jiangsu Province (No. BM2012012).

[*Corresponding author] E-mail: guangjiwang@hotmail.com (WANG Guang-Ji), hhp_770505@hotmail.com (HAO Hai-Ping).

ABBREVIATIONS: GLN, diammonium glycyrrhizinate; GA, glycyrrhetinic acid; DDI, drug-drug interaction; CYPs, cytochrome P450s; UGTs, UDP-glucuronosyltransferases; MMF, mycophenolate mofetil; MPA, mycophenolic acid; 4-MU, 4-methylumbelliferone; MPAG, MPA 7-O-glucuronide; E_2, 17beta-estradiol; Tris-HCl, tris-droxymethyl aminomethane- hydrochloric acid

These authors have no conflict of interest to declare.

Published by Elsevier B.V. All rights reserved

activities of various CYP subtypes in rodents and humans [10-11]. Conversely, GA is shown to have a modest inhibitory effect on CYP3A4 and CYP2D6 both *in vitro* and *in vivo* [12]. Glycyrrhizae radix is also found to inhibit the UGT1A1, UGT1A3, and UGT2B7 activities when incubated with human liver microsomes or recombinant human UGTs *in vitro* [13-15]. However, these UGTs-mediated GA-related DDI studies have not been explored in depth and *in vivo* effects are not mentioned.

Mycophenolate mofetil (MMF) is an immunosuppressant prodrug indicated to prevent solid organ transplant rejection [16]， which is rapidly absorbed and de-esterfied into mycophenolic acid (MPA) *in vivo*. MPA is further metabolized in the liver by UGTs to form the pharmacologically inactive compound MPA 7-*O*-glucuronide (MPAG) [17]. As MPA pharmacokinetics is closely related with its drug-related toxicity and patients' clinical outcomes [18-20], based on the aforementioned regulation of glycyrrhizin on UGTs, glycyrrhizin was suspected to alter the pharmacokinetics and pharmacodynamics of MMF when the two agents are combined in the clinic. Therefore, the concomitant use of glycyrrhizin and MPA in the treatment of diseases such as atopic dermatitis [21-22] bears possible herb-drug interactions that may affect therapeutic outcome and safety.

The present study aimed at investigating the effects of diammonium glycyrrhizinate (GLN) on hepatic and intestinal UGTs activities in rats. The effect of GLN on the activities of UGTs *in vivo* was evaluated by measuring the pharmacokinetic parameters of MMF. Meanwhile, *in vitro* experiments were conducted in isolated microsomal fractions using using 17beta-estradiol (E_2), 4-methylumbelliferone (4-MU), and MPA, which are typical substrates for UGT1A1, UGT1A6 and UGT1A7, respectively [23-25]. Real-time PCR and Western blotting analyses were used to determine the expression levels of UGT isoforms in the liver and intestine. To explain the differential effects of GA on intestinal and hepatic UGT activities, concentrations of GA in the liver and intestine were detected. Our findings from the present study may be useful for the safe and effective combination therapy of GLN and other prescription drugs in the clinic.

Materials and Methods

Chemicals and reagents

GLN and the corresponding standard substance were provided by Chia-Tai Tianqing Pharma-ceutical Co. Ltd. (Jiangsu, China, purity 98%). Ursolic acid was purchased from Zelang Ltd. Co. (Nanjing, China). Uridine 5′-diphosphate-glucuronic acid (UDPGA), d-saccharic acid 1, 4-lactone, alamethicin, 4-MU, MMF, MPA, E_2, and estradiol -3-glucuronide were purchased from Sigma-Aldrich (Shanghai, China). Goat anti-rat polyclonal antibodies against UGT 1A, UGT 1A1, and UGT 1A6 were obtained from Santa Cruz, Inc. (Santa Cruz, CA,USA). Secondary antibodies were purchased from Bioworld technology Company (Nanjing, China). BCA Protein Assay Kit was purchased from Beyotime Institute of Biotechnology (Haimen, China). Prime Script Real time reagent kit for RT-PCR was purchased from Biotechnology Co. Ltd. (Dalian, China). HPLC-grade acetonitrile and methanol were from Merck (Darmstadt, Germany). Deionized water was purified using a Milli-Q system (Millipore Corporation, Billerica, MA, USA).

Animals

Adult male Sprague-Dawley rats (weighing 180–220 g) were purchased from Sino-British Sippr/BK Lab Animal Ltd. (Shanghai, China)and housed in an air-conditioned room at a temperature of 25 ± 2 °C with a 12 h/12 h light/dark cycle, with free access to food (standard rodent chow diet) and tap water. The rats were allowed to acclimatize for one week and fasted for 12 h prior to the experiments. The study protocol was approved by the Animal Care and Use Committee of the College of Pharmacy, China Pharmaceutical University, Nanjing, China. Animals were randomly allocated to two groups (6/group). For 15 consecutive days, GLN dissolved in physiological saline was administered by oral gavage at a dose of 40.5 mg·kg^{-1} body weight. Control animals received physiological saline only. On the 16th day, the animals were sacrificed and the liver and intestine were removed, weighed, and frozen in liquid nitrogen for further analysis.

Determination of the effects of GLN on pharmacokinetics of MMF in rats

In this experiment, the rats were randomly allocated into two groups (n = 6). For 15 consecutive days, control group received saline and GLN group received 40.5 mg·kg^{-1} of GLN. The drug doses were based on data available in literature [26-27]. On the 16th day, both groups were intragastrically administered MMF at 20 mg·kg^{-1} 2 hours after GLN pretreatment. The dosing time interval was designed based on the previous pharmacokinetic study of GLN, so as to ensure maximal exposure of MMF to GLN. Blood samples were collected in heparinized tubes at 0, 0.083, 0.167, 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 6, 8, 12, and 24 h after intragastric administration of MMF to determine plasma MPAG (glucuronidation metabolite of MPA) concentrations. Blood samples were immediately centrifuged and stored at –20 °C until analysis.

Enzyme assay

Differential centrifugation was used to prepare rat hepatic and intestinal microsomes as described by Hao *et al.* [28]. Protein concentrations were determined by BCA Protein Assay kit according to the manufacturer's instructions. The prepared microsomes were frozen and stored at –80 °C until analysis. UGT activities were determined using the typical substrate E_2 for UGT1A1, 4-MU for UGT1A6, MPA for UGT1A7 [25, 29]. For the UGT1A1 test, incubations were performed in 1.5-ml polypropylene tubes. First, microsomes were pretreated with alamethicin (2 mg·mL^{-1} of protein) for 20 min to diminish the latency of UGTs activity. After incubating with 10 mmol·L^{-1} of $MgCl_2$, 20 mmol·L^{-1} of d-saccharic acid 1, 4-lactone, 500 μmol·L^{-1} of E_2 and 50

Download English Version:

https://daneshyari.com/en/article/2526063

Download Persian Version:

https://daneshyari.com/article/2526063

Daneshyari.com