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Effects of licorice extracts on the pharmacokinetics of brucine in rats and its possible mechanism

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Abstract: The detoxification effects of licorice are believed to be related to its pharmacokinetic (PK) interference. This paper aimed to evaluate the effects of licorice water extracts (LWE) on the pharmacokinetics of brucine. Rats were administered brucine and/or LWE. The pharmacokinetic behavior of brucine and bioactive components of licorice were quantified by HPLC-MS/MS. P-glycoprotein (P-gp) inhibitor verapamil, real time PCR, vesicular transport assay and everted gut sacs were employed to investigate its possible mechanism. We found LWE reduced the C_{max} and AUC of oral brucine in a dose-dependent way. In contrast, the AUC values of intraperitoneal brucine showed no significant difference between LWE treated and untreated rats, which indicating the intestinal absorption of brucine was influenced by LWE. We found that high dose of LWE activated the transport activity of P-gp in vesicular transport assay, while the mRNA level of P-gp in the intestinal was not affected by licorice. Moreover, high dose of LWE decreased the intestinal absorption of brucine in the everted gut sacs model, which could over turned by verapamil. These results suggested that a single high dose of LWE could impair the intestine absorption of brucine, and its potential mechanism may be mediated by P-gp in intestine.

Keywords: Pharmacokinetics interaction, licorice, brucine, Intestinal absorption, P-gp.

INTRODUCTION

Licorice (Glycyrrhiza), the root of *Glycyrrhiza uralensis* (Ji *et al.,* 2016), has been shown to exert numerous beneficial effects, such as anti-inflammation (Yu *et al.,* 2015), antiulcer, gastroprotective (Nugroho *et al.,* 2016), immunomodulating and antioxidant effects (Zhao *et al.,* 2015). More importantly, it appears in more than half of the traditional Chinese medicine (TCM) prescriptions and is co-administrated with other herbs to moderate toxicity or enhance the activity of other herbs (Gong *et al.,* 2014). Recent studies demonstrated that there was a remarkable interaction between strychnine, brucine and licorice (Gu *et al.,* 2014), which could be an underlying detoxification mechanism of licorice. Phytochemical investigations reveal that the main active components of licorice include glycyrrhizic acid, glycyrrhetinic acid, liquiritigenin, isoliquiritigenin, liquirtin and isoliquiritin (Wang *et al.,* 2015).

The co-existence of multiple compounds may potentially alter the pharmacokinetics of co-administered prescription drugs and/or herbs, especially absorption and oxidative metabolism (Qiao *et al.,* 2012). This is possibly through inhibition or induction of intestinal and hepatic drug metabolizing enzymes like CYP as well as efflux transport proteins (Chu *et al.,* 2013; Zhang *et al.,* 2017). Recent studies have confirmed that licorice and its active constituent were able to modulate the expression of drug-metabolizing enzymes and transporters (Awad *et al.,* 2016; Gong *et al.,* 2014; Hou *et al.,* 2012; Tai *et al.,* 2014). For example, *in vivo* studies have demonstrated that glycyrrhizic acid induced the activity of CYP3A, CYP1A2 and CYP2B1, but the activity of CYP2E1 and CYP1A1 decreased after glycyrrhizic acid received over 4-10 days (Feng *et al.,* 2015). In addition, glycyrrhizic acid induced the activity of P-gp after long periods of intake (Feng *et al.,* 2015). However, there were some experiments indicated diverse results (Yan *et al.,* 2013). Therefore, further studies on the drug-drug interactions and temporary effects of LWE on drug metabolizing enzymes and transports were required.

The interaction is important especially for drugs that have narrow therapeutic window, as the consequences are life threatening. Brucine was chosen as a model drug in this work. As the second abundant alkaloids in *Semen Strychni*, brucine is much less virulent than the most abundant alkaloid-strychnine (Patel *et al.,* 2017; Saraswati *et al.,* 2013). Brucine was mainly responsible for the analgesic, anti-inflammatory and anti-tumor effect produced by *Semen Strychni* (Chen *et al.,* 2012), which was frequently prescribed in many prescriptions in TCM. Brucine was proved to repress the activity of CYP3A and be the substrate of P-gp (Li *et al.,* 2013). In addition, more than 60 formulations containing *Semen Strychni* have been reported on the medicine literatures of Indian (Chen *et al.,* 2013). However, *Semen Strychni* is limited by the fatal

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neurotoxicity of these alkaloids (Gu *et al.,* 2016). Licorice could alleviate the toxicity of *Semen Strychni* through several aspects. On the one hand, licorice was noted to have detoxified effect via compatibility decoction (Zhou *et al.,* 2015). Besides, a Chinese report has shown that a single megadose protocol of licorice treatment could relieve the poisonousness symptom causing by *Semen Strychni*. However, the underlying mechanism remains unknown.

Thus, we hypothesized that the herb–drug interactions may occur between licorice and brucine, leading to altered pharmacokinetics and subsequently decreased toxicity of brucine *in vivo*. In the present study, pharmacokinetic studies were carried out to explore the effects of LWE on brucine. Whether P-gp transporter played a vital role in the pharmacokinetic interactions was further confirmed by mRNA level determination, *in vitro* transport activity assay and everted gut sacs absorption evaluation.

MATERIALS AND METHODS

Herbal materials and reagents

Glycyrrhiza uralensis Fisch (Licorice) was purchased from Hunan Sanxiang Pharmaceutical Co. Ltd (Hunan, China), which was authenticated by Prof Li Jin-Ping (School of XiangYa Pharmaceutical Sciences, Central South University, Changsha, China). Verapamil was provided by Sigma-Aldrich Ltd. (St. Louis, MO). Standards including brucine and moclobemide were obtained from the National Institute for Food and Drug Control (Beijing, China). Methanol, acetonitrile (HPLC grade) were from ACS Company (Poole, UK). Formic acid (HPLC grade) was from ROE scientific INC (Network, USA). Ultra-pure water was acquired from a Milli-Q system (Millipore, Bedford, MA, USA). All other reagents were of analytical grade.

Preparation of licorice extracts

Licorice was soaked for 12h and extracted with five-fold mass of water for 2h twice in a reflux condenser. Then the decoctions were filtered, the filtrate was collected and concentrated. The final concentration of the LWE was 1.8g of crude drug per milliliter. The contents of glycyrrhizic acid, glycyrrhetinic acid, liquiritigenin, isoliquiritigen and liquiritin in LWE were determined to be 17.75mg/mL, 3.55μg/mL, 0.87mg/mL, 0.26mg/mL and 5.26 mg/mL, respectively. The fingerprints of licorice was also explored (Supplement fig. 1). Part of the LWE was dilute to 0.045 g and 0.018 g of crude drug per milliliter.

Animal treatment

Male pathogen-free Sprague-Dawley rats (300±20g) were purchased from Hunan SJA Laboratory Animal Co. Ltd (Hunan, China). The animals were acclimatized to a temperature-controlled environment under a 12/12h light/dark cycle for 7 days, water and food allowed ad libitum. The rats were randomly divided into several groups (n=8) as follows. The brucine group: received intragastric (B) or intraperitoneal (IB) administration of brucine (40mg/kg). LWE treatment groups: rats were given oral brucine co-administrated with 18g/kg (HLT), 0.45g/kg (MLT) or 0.18g/kg (LLT) LWE, respectively; rats received intraperitoneal (*ip*) administration of brucine and 18g/kg (IHLT), 0.45g/kg (IMLT) or 0.18g/kg (ILLT) LWE. Except for pharmacokinetic study, another five rats in vehicle (C), oral brucine (B), *ip* brucine (IB), oral brucine and 18g/kg LWE (HLT), *ip* brucine and 18g/kg LWE (IHLT) groups were used for real-time PCR. The rats were fasted overnight before drug administration. The study was approved by the Animal Care & Use Committee of Central South University. All experiments were carried out in accordance with the Guide for Care and Use of Laboratory Animals.

Pharmacokinetic study and sample collection

Blood samples (0.25mL) were collected under light ether anesthesia via ophthalmic venous plexus at 0.0833, 0.25, 0.5, 0.75, 1, 2, 3, 5, 7, 9, 11, 24h after oral or *ip* administration of brucine. The predose blood was collected 2 days before experiment. The rats were euthanasia after experiment. The plasma samples were centrifuged at 3700rpm for 10 min. The intestine were collected 24 hours after drug administration. All these samples were stored at -70.

Drug concentration analysis

Brucine was quantified by an HPLC-MS/MS method described previously (Zhang *et al.,* 2018). Briefly, 50μL of internal standard working solution (moclobemide) was added into 50μL rat plasma samples. The agent for protein precipitation was methanol (450μL). After vortex and centrifugation at 15000 rpm (20627g), the supernatant was transferred and evaporated to dryness. The residue was dissolved with 50% acetonitrile and followed with blending and centrifugation. Finally, the supernatant was injected into HPLC-MS system for analysis. The HPLC-MS/MS analysis was carried out on an LC-20A HPLC system (SHIMADZU, Kyoto, Japan), coupled with a 4000 triple-quadrupole mass spectrometer (AB SCIEX, Framingham, MA, USA).

The chromatographic separation was achieved on Ultimate AQ-C18 column (3.0μm, 3.0×100mm, Welch Materials Inc, MD, USA). The mobile phase was a gradient established between solvent A (0.07% ammonium acetate and 0.12% formic acid aqueous) and solvent B (acetonitrile). The elution program was as follows: 0-0.5 min, 20% B; 0.5-1min, 20-60% B; 1-4min, 60-75% B. The mass spectrometer was operating in ESI^+ with following parameters: curtain gas at 30; ionspray voltage at 4500 V; nebulizer gas at 50; cell exit potential at 10; entrance potential at 10; declustering potential: 114.0 eV, collision energy: 44 eV. The selected reaction monitoring transitions were 395.2→324.3 for brucine, and 269.2→181.9 for moclobemide.

Table 1: Effects of LWE on pharmacokinetic parameters of brucine in rats following oral administration of brucine (n=8, mean ±SD).

Parameters	T_{max} (h)	C_{max} (ng/mL)	$T_{1/2}$ (h)	$AUC_{0\rightarrow t}$ (ng·h/mL)	$AUC_{0\rightarrow \infty}$ (ng·h/mL)	MRT (h)	CL (L/kg/h)
B	0.4±0.3	1628.0±147.2	2.2±0.6	2355.4±780.2	2371.5±782.7	3.1±0.9	18.4±5.3
HLT	0.2±0.1	426.5±224.9***	2.4±0.4	1396.8±425.3 *	1484.0±436.5*	3.4±0.6	25.5±3.32
MLT	0.3±0.1	1053.9±231.8**	2.2±0.8	2109.8±350.0	2143.3±379.0	3.1±1.1	19.3±2.9
LLT	0.4±0.3	1569.7±329.5	2.1±0.4	2684.7±441.4	2806.2±375.5	3.0±0.6	15.1±2.5

B: brucine alone, HLT: high dose of LWE, MLT: medium dose of LWE, LLT: low dose of LWE. Values are expressed as Mean ± SD, significant was considered when *p< 0.05,**p< 0.01, ***p< 0.001 versus B group.

Table 2: Pharmacokinetic parameters of brucine in rats given *ip* administration of brucine with or without LWE (n=8, mean ±SD).

Parameters	T_{max} (h)	C_{max} (ng/mL)	$T_{1/2}$ (h)	$AUC_{0\rightarrow t}$ (ng·h/mL)	$AUC_{0\rightarrow \infty}$ (ng·h/mL)	MRT (h)	CL (L/kg/h)
IB	0.2±0.1	4561.5±485.3	2.8±0.8	6140.1±697.4	6296.7±875.1	4.1±1.2	6.6±0.7
IHLT	0.1±0.1	3791.3±388.5*	1.8±0.7*	6131.1±1614.7	6168.2±1643.6	2.6±1.0*	6.9±2.1
IMLT	0.2±0.1	3788.9±456.1*	1.7±0.5*	6187.3±871.1	6222.7±882.8	2.5±0.7*	6.7±1.4
ILLT	0.2±0.1	4171.1 ±679.1	2.3±0.3	6115.2±1533.9	6159.2±1538.8	3.3±0.5	7.0 ±2.1

IB: brucine alone, IHLT: high dose of LWE, IMLT: medium dose of LWE, ILLT: low dose of LWE.Values are expressed as Mean ± SD, significant was considered when *p< 0.05 versus IB group.

Table 3: Apparent permeability coefficients, Papp ($cm/s\times10^{-5}$) of brucine transportation for 60 min in the everted gut sacs.

	B	HLT	MLT	LLT	VER	HLT+VER
jejunum	1.39±0.12	$0.71\pm0.10^{***}$	1.16±0.10	1.41±0.15	$2.41\pm0.16^{***}$	1.37±0.09
ileum	1.73±0.24	0.93±0.15***	1.30±0.12*	1.67±0.13	2.79±0.27***	1.69±0.25

B: brucine alone, HLT: high dose of LWE, MLT: medium dose of LWE, LLT: low dose of LWE; VER: verapamil; HLT+VER: verapamil and high dose of LWE. Values are expressed as Mean ± SD, significant was considered when**p< 0.01, ***p< 0.001 versus B group.

SUPPLEMENTARY MATERIALS

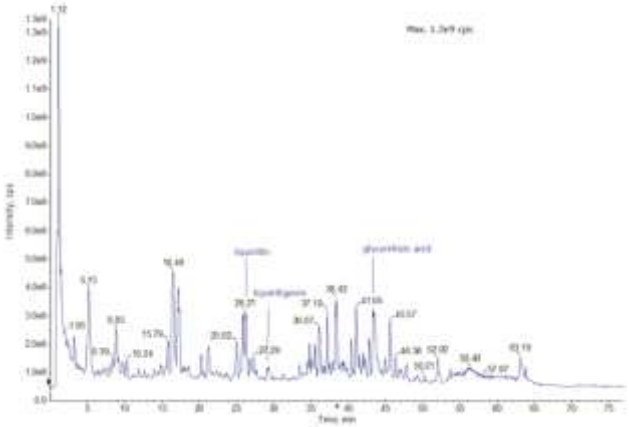
Supplement Table 1: Primer sequences used for the real-time PCR analysis.

Gene	Sense Primer (5'-3')	Antisense Primer (5'-3')	Size (bp)
MDR1	CTCCTATGCTGCTTGTTTCCG	TCAATGATCCTGATGATGTGGG	186
β-Actin	CATCCTGCGTCTGGACCTGG	TAATGTCACGCACGATTTCC	116

Supplement Table 2: The effects of LWE on relative transport of NMQ.

LWE(%)	Relative transport (% of control)
0	100±11.9
0.002	100±13.2
0.02	118±9.78
0.2	109±4.59
2	155±13.4**
20	203±12.5***

The uptake activity of NMQ in the control group was set as 100%. The Relative transport of LWE group=uptake activity in LWE group/uptake activity in control group ×100%.



Supplement Fig. 1: Chromatographic fingerprints of licorice water extract.

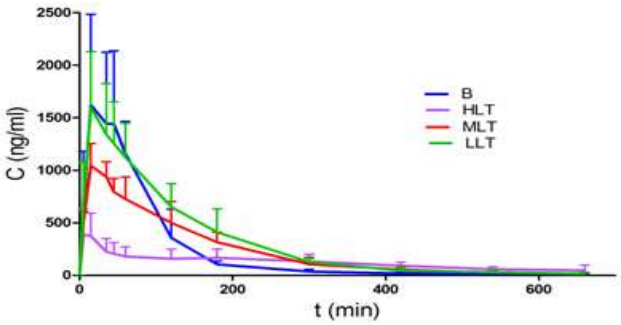


Fig. 1: Effects of LWE on mean plasma concentration-time curves of brucine in rats following oral administration of brucine at a single dose of 40mg/kg.

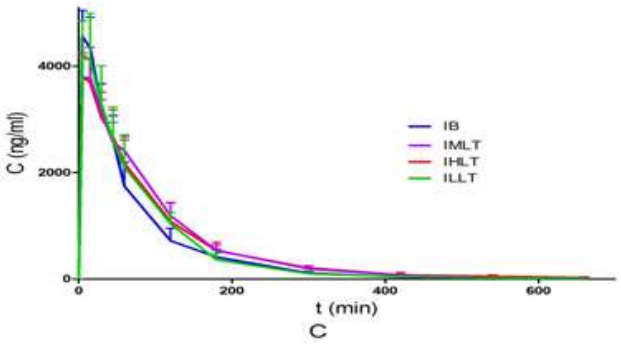


Fig. 2: Effects of LWE on mean plasma concentration-time curves of brucine in rats following *ip* administration of brucine at a single dose of 40 mg/kg.

Real-time PCR analysis

Total intestine RNA was homogenized using Trizol reagent (invitrogen, USA) according to the manufacturer's instructions. Quantitative real-time polymerase chain reaction was performed on ABI 7900HT Detection System (Lifetech, USA) using SyBr Green reagent kit (Takara RR820A, China) and gene-specific primers. Relative quantitation for PCR product was normalized to β-actin mRNA values. Sequences of the primers are provided in Supplement table 1.

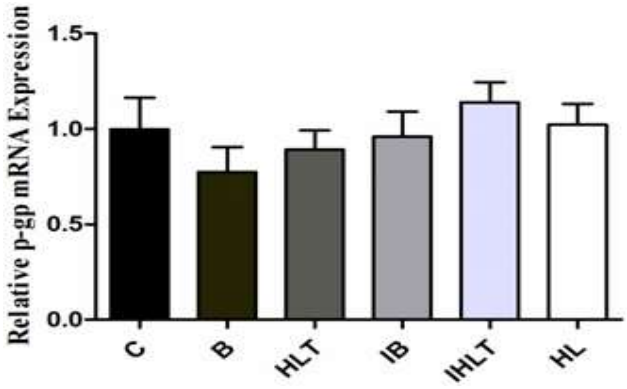


Fig. 3: Effects of LWE and brucine on mRNA levels of P-gp (n=5, mean±SD).

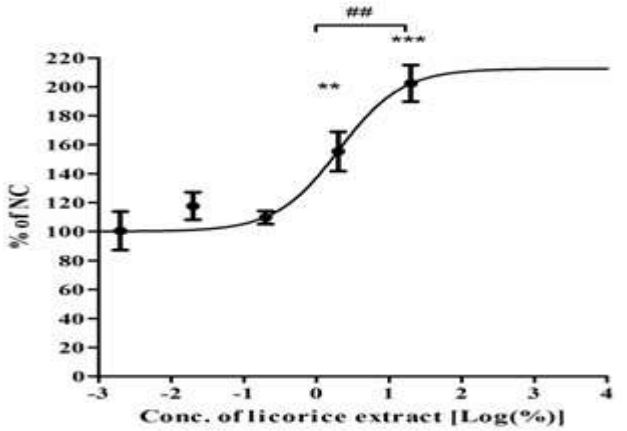


Fig. 4: Induction effect of LWE on the transport activity of P-gp (n=3).
***P<0.001, **P<0.01 versus NC group; ##P<0.01 versus treatment../../../360Downloads/Youdao/Dict/7.5.2.0/result ui/dict/?keyword=group; EC50: 2.026, 95% confidence interval: 0.9704 - 4.232.

Vesicular transport (VT) assay

To determine whether LWE was a activation of P-gp and could promote the transport of brucine, VT assay was performed with MDR1-overexpressing membranes and vesicular transport kit (GenoMembrane, Yokohama, Japan) according to the manufacturer's protocol. Briefly, [3H]-Nmethyl- quinidine (NMQ) or brucine (1μM and 6 μM) was incubated with LWE (0, 0.002%, 0.02%, 0.2%, 2%, 10% and 20% volume ratio) with or without 4mM ATP at 37℃ for 5 min. The vesicular were incubated with the buffer at 37℃ for 5 min. Then the vesicular and drugs were mixed and incubated for 5 min. Reactions were stopped by ice-cold washing buffer, followed by vacuum filtration and repeating wash. Methanol (80%) was used to dissolve the vesicular, then isometric H_2SO_4 (0.1 N) was added. NMQ was analyzed by fluorescence measurement at λex 355nm and λem 460nm (Molecular Devices i3-R, CA, United States). Brucine was detected by the HPLC-MS/MS method described above.

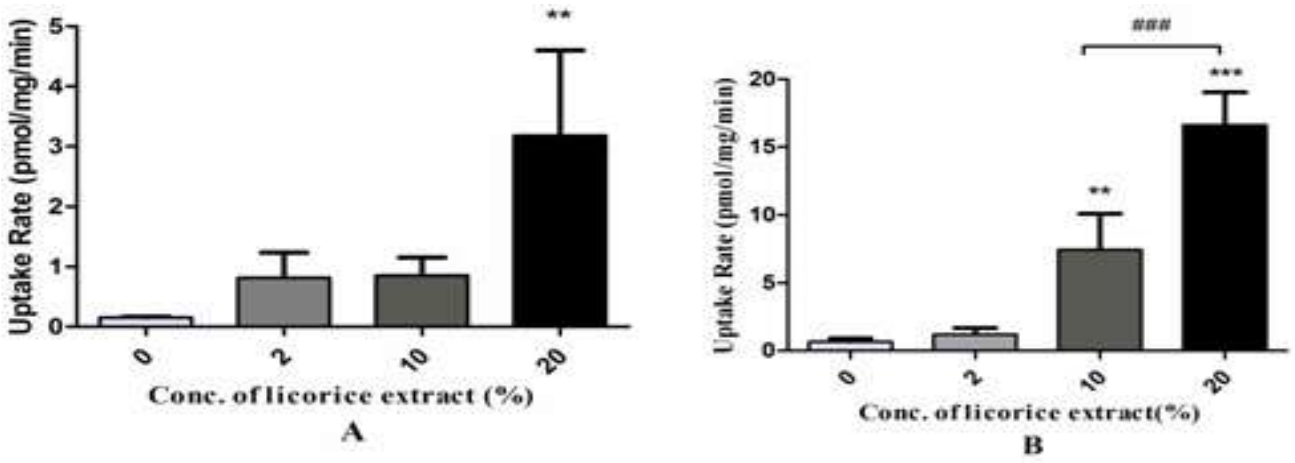


Fig. 5: Effects of LWE on the uptake rate of brucine (n=3).

A: the concentration of brucine was 1 μM; B: the concentration of brucine was 6 μM.
***P<0.001, **P<0.01 versus NC group; ###P<0.001versus treatment.

Everted intestinal sac model

The everted intestinal sac model was performed as previous descriptions (Chan *et al.,* 2006). In short, adult male Sprague-Dawley rats were starved overnight before anesthetized. Segments (10cm) at the jejunum and ileum were carefully collected and washed with warm Tyrode's solution. The intestinal segments were everted with one extremity clamped, and the other extremity ligated to a sampler. A 3mL volume of blank solution was introduced into the sacs.

Each sac was placed in a 35mL of oxygenated Tyrode's solution at 37℃ containing brucine (6μM), LWE (2%, 10%, 20%) or verapamil (50μM). The prepared gut sacs were incubated for 60 min, then 1mL solution were taken out. The sample was filtered by 0.45mm millipore membrane, then detected by HPLC-MS/MS. The length and width of the intestinal segments were measured, the P_{app} of brucine was calculated.

Pharmacokinetic analysis

Pharmacokinetic parameters were assessed by non-compartmental method using DAS 3.2.8. The C_{max} and T_{max} were obtained directly from the plasma concentration-time curve. The area under the plasma concentration-time curve from zero to infinite ($AUC_{0-\infty}$) was calculated with formula: $AUC_{0-\infty} = AUC_{0-t} + C_t/K_e$, where AUC_{0-t} was measured by trapezoidal method; K_e was determined from the slope of the regression line. The elimination half-life ($t_{1/2}$) was obtained from $0.693/K_e$. The clearance rate (CL) was calculated as dose/$AUC_{0-\infty}$. The mean residence time (MRT) was estimated by the following equation: MRT =AUMC/AUC.

STATISTICAL ANALYSIS

Results from the experiments were calculated with SPSS 19.0. Differences between groups were determined by one-way analysis of variance followed by Tukey's test. The prior level of significance was established at P<0.05.

RESULTS

Effects of LWE on the pharmacokinetic profiles of brucine in oral administration

As shown in the plasma concentration-time profiles (fig. 1), brucine exhibited a fast absorption phase and lasting elimination phase. Interestingly, compared with brucine group, LWE reduced the C_{max} and AUC of brucine in a dose-dependent way (table 1). In high dose of LWE treatment group, the C_{max}, $AUC_{0\rightarrow t}$ and $AUC_{0\rightarrow\infty}$ of brucine were significantly decreased by 73.8%, 40.7% and 37.4%, respectively. Medium dose of LWE could markedly declined the C_{max} of brucine (P<0.01), accompanied by lower exposure. The low dose of LWE had little effect on the pharmacokinetics of brucine.

Effects of LWE on the pharmacokinetic profiles of brucine in ip administration

The pharmacokinetic profiles of brucine in *ip* administration were illustrated in fig. 2 the pharmacokinetic parameters were listed in table 2. Compared with oral administration, the plasma concentrations of brucine in *ip* administration increased sharply, accompanied by higher exposure and lower clearance values. Distinct from the results in oral administration, there was no statistical difference (P>0.05) between brucine and LWE treatment groups in the AUC. The C_{max} and $t_{1/2}$ of brucine exhibited 16.9% and 35.7% (P<0.05) decrement in rats after high dose of LWE treatment. Analogously, medium dose of LWE treatment had the same influence on the C_{max} and $t_{1/2}$ of brucine, which decreased by 16.9% and 39.3%, respectively. Low dose of LWE had no significant difference from control rats in the main pharmacokinetic parameters.

The mRNA expressions of P-gp in intestine

P-gp mRNA samples have been shown to be in good concordance with the protein expression (MacLean, *et al.*, 2008). The mRNA expressions of P-gp in the intestine were measured by real-time PCR. Our results showed that the mRNA expression of P-gp were unaltered after brucine or/and LWE treatment (fig. 3).

Effects of LWE on the transport activity of P-gp

After determining changes in the mRNA expressions of P-gp, we further investigated whether LWE induced the activity of P-gp. The uptake ratio of the NMQ in the VT systems was 5.47, indicating that the VT systems were in good condition. The relative transport of NMQ and the induction effect of LWE was presented in supplement table 2 and fig. 4. The relative transport of the NMQ was induced by 2% and 20% LWE co-incubation, while not influenced by 0.002%, 0.02% or 0.2% LWE co-incubation. The EC_{50} of LWE was in volume ratio at 2.026%, which was 37.08mg/mL. The 95% confidence interval of EC_{50} was 0.9704 to 4.232.

Effects of LWE on the transport of brucine

Effects of LWE on the uptake rate of brucine were evaluated (fig. 5). Brucine at 1μM or 6μM was incubated with 0, 2%, 10% or 20% LWE. High dose of LWE accelerate the transportation of brucine significantly in both 1μM and 6μM. 10% LWE could facilitate the uptake rate of brucine when brucine was at 6μM.

Effects of LWE on the intestinal absorption of brucine in the everted gut sacs

The everted gut sacs model was employed to examine the intestinal absorption of brucine. We compared the transport of brucine with or without LWE (table 3). 20% LWE decreased the intestinal absorption of brucine significantly in the jejunum and ileum. 10% LWE obviously reduced the absorption of brucine in the ileum but not in the jejunum. 2% LWE had little impact on the intestinal absorption of brucine. The absorption of brucine in the ileum was higher than that in the jejunum.

Effects of P-gp inhibitor verapamil on the absorptive profile of brucine

Verapamil was selected as a P-gp inhibitor. To determine the possible mechanism of intestinal P-gp in the function of LWE, a co-incubative test with verapamil, brucine and LWE was conducted. Co-treatment with verapamil significantly increased the accumulative absorptive amount per area of brucine in both the jejunum and ileum compared with that of brucine treated alone (table 3). Co-incubated with verapamil counteracted the role of 20% LWE in both the jejunum and ileum (table 3).

DISCUSSION

The pharmacokinetic interactions between brucine and licorice were systematically analysed in rats. Different dosages of LWE and different drug-delivery ways of brucine were employed. LWE at high dose (200g) was commonly used for detoxication, whereas the medium (5g) and low dose (2g) were usual dosage for compatibility according to Chinese Pharmacopoeia (National Pharmacopoeia Committee. 2010). In accordance with our results, plenty of studies had reported the pharmacokinetic profile of brucine (Chen *et al.*, 2011; Li *et al.*, 2013), which was absorbed and eliminated quickly. Brucine has shown to possess a variety of biological activities. However, severe central nervous system toxicity and nephrotoxicity limited its clinical application (Yang *et al.*, 2011). Our results demonstrated that co-admininstration with LWE reduced the C_{max} and AUC of brucine in a dose-dependent way, indicating that high dosage of LWE might have outstanding clinical value, since the traditional ways using oil or sand bath treatment for brucine preparation were inconvenient (Saraswati *et al.*, 2013) and carried by liposomes was too costly (Chen *et al.*, 2012).

To research whether the decreasing exposure of brucine in LWE treated rats was resulted from the accelerated metabolism in the liver, the pharmacokinetic behavior of brucine in *ip* administration was studied. Different from the results in oral administration, LWE has limited effect on C_{max} and AUC of brucine. This results indicated that metabolism of brucine was not the main target for LWE to reduce the exposure of brucine. Therefore, the alterative exposure of brucine might be attributed to the decreased intestinal absorption. Efflux transporters and metabolizing enzymes play essential roles in disposition and toxicology of orally administrated drugs (Li *et al.*, 2016). Among the efflux transporters, P-gp plays a central role in the absorption of relevant drugs, and subsequently affected their exposure (Zhou *et al.*, 2008). In the intestine, P-gp and CYP3A are believed to work coordinately as to reduce the intracellular concentrations of xenobiotics and the absorption of orally taken drugs (Cousein *et al.*, 2007).

The influence of P-gp on the pharmacokinetics and pharmacodynamics of drugs have been widely studied. But drug-drug interactions based on P-gp required pre-clinical investigation. Licorice was documented to activate the nuclear receptor PXR to induce CYP3A(Mu *et al.*, 2005). Particularly, glycyrrhizic acid could significantly increased the efflux of P-gp (Feng *et al.*, 2015; Tai *et al.*, 2014). It was found that glycyrrhetinic acid significantly increased the P-gp mediated efflux of Rhodamine 123 (Hou *et al.*, 2012). However, glycyrrhetinic acid significantly inhibited P-gp in MDR1-MDCKII and Caco-2 cells (Li *et al.*, 2014).

In our results, it was worth mentioning that high dose of LWE activated the transport activity rather than the mRNA expression of P-gp. It was contradictory with our previous study (Hou *et al.*, 2018). It was believed that the induction of mRNA or protein required approximately 7

days, therefore, the P-gp mRNA was not induced in our experiment. It is well known that TCMs exert the therapeutic efficacy based on the synergic effects from their multi-components and multi-targets. According to the previous findings, licorice might have interactions with herbs like *paeonia lactiflora* (Ding *et al.*, 2016; Xu *et al.*, 2013), *laminaria japonica* (Zhao *et al.*, 2015), and *Sophorae flavescentis radix* (Shi *et al.*, 2015). More important, the effects of glycyrrhetinic acid and glycyrrhizin on intestinal absorption was through its effects on drug-metabolizing enzymes and transporters (He *et al.*, 2017). Our research adopted *in vitro* vesicular transport assay and everted gut sacs model to measure the effects of LWE on the brucine intestinal absorption. We uncovered that high dose of LWE activated the transport activity of P-gp, decreased the intestinal absorption of brucine. These results suggested that the activation of P-gp maybe the main mechanism of licorice to alter the pharmacokinetics of brucine. In conclusion, the present study illuminated the pharmacokinetic interactions of licorice on brucine. LWE co-administration obviously slowed down the absorption; reduced the exposure of brucine. And its potential mechanism was proved mainly relied on P-gp in intestinal absorption.

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