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Metabolic regulatory effects of licorice: A bile acid metabonomic study by liquid chromatography coupled with tandem mass spectrometry

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ABSTRACT

Licorice is one of the most popular herbal medicines worldwide, and is mainly used to moderate the characteristics of other herbs in Traditional Chinese Medicine. It is hypothesized that licorice exerts this role by regulating systemic metabolism. Bile acids play a critical role in lipid digestion and cholesterol metabolism, and are sensitive biomarkers for hepatic function. In this study, the regulatory effects of licorice on bile acid metabonome in rats were investigated using liquid chromatography coupled with tandem mass spectrometry. After oral administration of a clinical dosage of licorice water extract, the levels of 21 fully identified and 41 tentatively characterized bile acid analogs in rat plasma were determined by a fully validated method. Following partial least squares discriminant analysis, the results showed that licorice treatment led to dose-dependent up-regulation of free and glycine-conjugated bile acids excretion. Particularly, the plasma levels of cholic acid (1465.33 ± 915.93–7156.46 ± 3490.49 ng/mL, $p = 0.0027$) and β-muricholic acid (228.19 ± 163.95–1284.40 ± 775.62 ng/mL, $p = 0.0045$) increased significantly 48 h after administration. As licorice is widely used as a detoxifying drug, the regulation of plasma bile acids may be an important evidence to interpret its mechanism.

1. Introduction

Bile acids (BAs) are synthesized in liver, excreted into mammal bile mainly as glycine and taurine conjugates, and distributed to liver, blood, urine, feces and gallbladder maintaining a balance between free and conjugated forms. Recent studies suggested that BAs are closely related to hepatic dysfunction, intestinal ailments, cardiovascular function [1–5], as well as glucose homeostasis and nuclear receptor activation [6–7]. Particularly, BAs are involved in a number of metabolic processes, and play a critical role in lipid digestion and cholesterol metabolism [1]. Bile acids levels in plasma have been proved to be sensitive biomarkers for the evaluation of liver function and for the diagnosis of diseases affecting liver metabolism [2,3].

Given the diagnostic significance of BAs, a number of analytical methods have been reported to determine total BA amounts in biological matrices [8]. Recently, it has been further realized that different bile acids, even those only differing slightly in hydroxylation patterns, may represent diverse biological and clinical relevant information [9–12]. Therefore, bile acid metabonome was developed to profile all bile acid analogs in a specific organism both qualitatively and quantitatively, offering untargeted and unbiased view of processes (toxic response, for instance) occurring at the systemic level [13–16]. For the analysis of bile acid metabonome, mass spectrometry (including LC/MS/MS and GC/MS) is one of the most powerful tools.

Licorice, derived from the roots and rhizomes of *Glycyrrhiza* species, is a world-known and frequently used herbal medicine. It is popular as a therapeutic and flavoring agent in western countries, and it appears in about 60% of Traditional Chinese Medicine (TCM) prescriptions [17–19]. According to TCM theory, licorice is mainly used to moderate and harmonize the characteristics of other herbs [20]. This function could be partly interpreted as detoxification, in which licorice reduces adverse reactions of co-existing herbs to achieve maximal therapeutic effects of a multi-herb prescription. Indeed, a number of studies have revealed that licorice could alter the bioavailability or pharmacokinetics of co-administered herbal medicines or chemical drugs [21,22]. Considering the popular use of licorice in TCM clinical practice, the metabolic regulatory effects of licorice should be general instead of specific. As the major organ for drug metabolism, the function of liver may be highly relevant. The concentrations and profiles of bile acids in plasma could be determined as markers to reflect changes in liver function.

In the present study, we conducted a bile acid metabonomic study on rats after oral administration of licorice water extract (LWE). The experiment was designed to mimic a clinical situation, where the animals received an oral, single and clinical dose of LWE,

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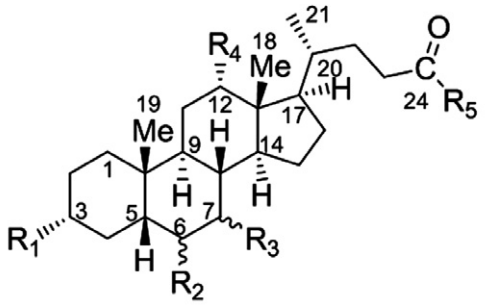
and were not fasted to maintain regular healthy state. Plasma concentrations of 21 fully identified and 41 tentatively characterized bile acids were measured by a segmented selected reaction monitoring (SSRM) LC/MS/MS method, and the data were analyzed by partial least squares discriminant analysis (PLS-DA).

2. Experimental

2.1. Reagents and reference compounds

Acetonitrile, methanol, formic acid (Mallinkrodt Baker, NJ, USA) and ammonium acetate (Sigma–Aldrich, MO, USA) were of HPLC grade. De-ionized water was prepared by a Milli-Q system (Millipore, MA, USA). High-purity nitrogen (99.9%) and helium (99.99%) were from Gas Supply Center of Peking University Health Science Center (Beijing, China).

Cholic acid (CA), chenodeoxycholic acid (CDCA), deoxycholic acid (DCA) and taurochenodeoxycholic acid (TCDCA) were purchased from China National Institutes for Food and Drug Control (Beijing, China). Glycochenodeoxycholic acid (GCDCA) and lithocholic acid (LCA) were from Sigma–Aldrich (MO, USA). Hyodeoxycholic acid (HDCA) and dehydrocholic acid (dhCA) were from Tianqi Chemical Engineering (Anhui, China). Ursodeoxycholic acid (UDCA) was from Bio Basic Inc. (Ontario, Canada). α-Muricholic acid (αMCA), β-muricholic acid (βMCA) and tauro-β-muricholic acid (TβMCA) were from Steraloids Inc. (RI, USA). The other BAs were synthesized by the authors following the method described by Willemen et al. after minor modification [23,24] as reported in our previous study [25]. The synthesized reference compounds were taurine conjugates, including taurocholic acid (TCA), taurodeoxycholic acid (TDCA), taurohyodeoxycholic acid (THDCA), taurolithocholic acid (TLCA), tauroursodeoxycholic acid (TUDCA); and glycine conjugates, including glycocholic acid (GCA), glycodeoxycholic acid (GDCA), glycohyodeoxycholic acid (GHDCA), glycolithocholic acid (GLCA), glycoursodeoxycholic acid (GUDCA). Their purities were above 98%. The structures are shown in Fig. 1.



Compd.	R_1	R_2	R_3	R_4
Free BAs: R_5=OH				
CA	OH	H	α-OH	OH
CDCA	OH	H	α-OH	H
DCA	OH	H	H	OH
HDCA	OH	α-OH	H	H
LCA	OH	H	H	H
UDCA	OH	H	β-OH	H
αMCA	OH	β-OH	α-OH	H
βMCA	OH	β-OH	β-OH	H
dhCA	ΔO	H	ΔO	ΔO
Glycine-conjugated BAs: R_5=$NHCH_2CO_2H$				
GCA	OH	H	α-OH	OH
GCDCA	OH	H	α-OH	H
GDCA	OH	H	H	OH
GHDCA	OH	α-OH	H	H
GLCA	OH	H	H	H
GUDCA	OH	H	β-OH	H
Taurine-conjugated BAs: R_5=$NH(CH_2)_2SO_3H$				
TCA	OH	H	α-OH	OH
TCDCA	OH	H	α-OH	H
TDCA	OH	H	H	OH
THDCA	OH	α-OH	H	H
TLCA	OH	H	H	H
TUDCA	OH	H	β-OH	H
TβMCA	OH	β-OH	β-OH	H

Fig. 1. Structures of bile acids analyzed in this study.

2.2. Preparation of licorice water extract

Licorice (dried roots and rhizomes of *Glycyrrhiza uralensis* Fisch.) was purchased from Elion Resources Group Company (Inner Mongolia, China) and was authenticated by comparing its HPLC fingerprint with a reference sample from China National Institutes for Food and Drug Control (Beijing, China). Licorice water extract was prepared with the following procedure: crude drug materials of licorice (100 g) were decocted in boiling water (500 mL) for 1 h twice. The decoctions were filtered, combined and concentrated in vacuum at 50 °C. The concentrated decoction was then diluted with water to produce a series of LWEs equivalent to 0.2, 1.0 and 2.0 g of crude drug/mL, respectively.

2.3. Animals, administration, and sample collection

Male Sprague–Dawley (SD) rats (200–230 g, 6–8 weeks of age) were obtained from the Experimental Animal Center of Peking University Health Science Center (Beijing, China). Animal welfare and experimental protocols complied with the Guide for the Care and Use of Laboratory Animals (U.S. National Research Council, 1996). The rats were housed in a cage (465 × 300 × 200 mm) in a breeding room and were given access to water and commercial pellet diet *ad libitum* before and during the experiments. Prior to treatment, the rats were kept in the breeding room for three days and were randomly divided into four groups (n = 9 for each group). Group I was orally administrated with 1.0 mL of water as the vehicle. Group II, III and IV were orally administrated with 1.0, 5.0 and 10.0 g/kg of licorice water extract, respectively. The dosage of 1.0 g/kg was equivalent to normal clinical dosage (9 g of crude drug/day for a 60-kg human) [19]. Retro-orbital blood samples were collected into heparinized tubes before and at 4, 8, 12, 24, 48 h after treatment. The blood samples were immediately centrifuged at 6000 rpm (4 °C) for 20 min to separate the plasma. The collected plasma samples were stored at −80 °C until analysis.

2.4. Preparation of calibration standards, quality control and internal standard solutions

Twenty-one bile acid reference standards (Table 1) were separately dissolved in methanol to prepare individual stock solutions. These stock solutions were mixed and then serially diluted to produce calibration standard solutions (25, 15, 10, 5, 3, 1, 0.5, 0.3, 0.1, 0.05, 0.03, 0.01, 0.005 and 0.003 μg/mL for each compound). Quality control (QC) samples were prepared at three concentration levels as high QC (HQC), middle QC (MQC) and lower limit of