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Original article

UPLC-Q-TOF/MS-based Metabolic Profiles of Bioactive Components in *Rehmannia glutinosa* and *Cornus officinalis* Herb Pair by Rat Intestinal Bacteria

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ABSTRACT

Objective To investigate the metabolic routes and metabolites of *Rehmannia glutinosa* and *Cornus officinalis* herb pair produced by gut microbiome from rats. **Methods** A rapid and sensitive ultra-performance liquid chromatography/quadrupole-time-of-flight mass spectrometry (UPLC-Q-TOF/MS) technique combined with Metabolynx™ software was established and successfully applied to identify the metabolites of the main bioactive components in the herb pair extract by rat intestinal bacteria. **Results** Four parent compounds (loganin, morroniside, catalpol, and acteoside) and their eight corresponding metabolites were detected and tentatively identified by the characteristics of their protonated ions. Hydrogenated and demethylated loganetin, dehydroxylated morronisid aglycone, caffeic acid, and its methylated product were the main metabolites. These metabolites suggested that the glycosides were firstly hydrolyzed to their aglycones by hydrolytic enzymes of the enteric microbial flora and subsequently to the other metabolites through hydrogenation, (de)-methylation, and de-hydroxylation. **Conclusion** The results may be helpful for the further investigation of the pharmacokinetic study of *R. glutinosa* and *C. officinalis* herb pair *in vivo.*

Key words

Cornus officinalis; herb pair; intestinal bacteria; *Rehmannia glutinosa*; UPLC-Q-TOF/MS

1. Introduction

The present world population is aging at the fastest pace. The population trends are reflected in demographic profiles of patients with diseases such as chronic kidney disease (CKD) that are common in the elderly (Aydede et al, 2014; Chan et al, 2014). CKD is clinically frequently-occurring and common diseases affecting the health of people (Rong et al, 2014; Wang et al, 2014). The CKD is regulated by multiple pathogenic factors and thus is hard to cure. Traditional Chinese medicine (TCM), used in the clinical practice for thousands of years, has been taken as a new way to tackle chronic diseases. The potency of a single herb is modest and cannot address the complicated and multivariate

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conditions of patients. To achieve better curative effect, several herbs are combined according to their properties to extend their bioactivities. Herb pair, the unique combination of two relatively fixed herbs in the clinical treatment, is the most fundamental and simplest form of multi-herb therapy. Because its appearance broadens the application of Chinese herbal medicine (CHM) and lays the foundation for the organizing principles of herb formulae, herb pair has played an important role in development of TCM (Zhao et al, 2007).

Rehmannia glutinosa Libosch and *Cornus officinalis* Sieb are two main crude herbs in the valid prescription Liuwei Dihuang Pill, which has been applied for the treatment of CKD for more than thousands of years in China and Japan (Aydede et al, 2014; Wang et al, 2010; Wu et al, 2007; Liu et al, 2013). The roots of *R. glutinosa* are used in oriental medicine as antianemic, an antipyretic and a tonic to nourish the *yin* deficiency of liver, kidney, and heart (Fu et al, 2011; Park et al, 2011). *C. officinalis* also has been clinically applied for more than 2000 years for its tonic, diuretic, and analgesic effects. The fruits of *C. officinalis* exhibit various bioactivities such as anti-microbial, anti-oxidant, anti-aging, and immunoregulatory effects (Ma et al, 2014; Liang et al, 2013). According to previous researches, the main bioactive constituents in *R. glutinosa* and *C. officinalis* herb pair include loganin, morroniside, catalpol, and acteoside, which can improve the kidney function and ameliorate the renal pathology state (Wang et al, 2014; 2009; Tarko et al, 2013).

Generally, the classic and traditional medicine is orally administered as decoction. To our knowledge, components of the oral administration medicine will inevitable contact with the bacteria in gut (Chen et al, 2014; Maynard et al, 2012). The large community of microbes residing in the intestinal tract (microbiome) constitutes a dynamic and symbiotic ecosystem that is in constant interaction with the host metabolism (Clemente et al, 2012; Robles and Guarner 2013; Ley et al, 2006). Under normal conditions, the gut microbiome provides trophic and protective functions. Additionally, the normal microbial flora could affect energy metabolism by facilitating absorption of complex carbohydrates and contribute to the nitrogen and micronutrient homeostasis via synthesis of amino acids and various vitamins (Olszak et al, 2012; Fukuda et al, 2011; Kau et al, 2011; Gonzalez et al, 2011; Jess, 2014). It has been reported that the intestinal bacteria are related to a variety of diseases such as diabetes and obesity (Haiser and Turnbaugh, 2013; Hullar et al, 2013; Hansen et al, 2014; Possemiers et al, 2011).

The complex microbial ecosystem with a metabolic capacity which exceeds the liver with a factor 100 is closely involved in the first-pass metabolism of oral administration/ consumption medicines and dietary compounds (Sousa et al, 2008; Zhou et al, 2013). The medicines are used to treat diseases and only the patients are ultimate consumers. Therefore, it is necessary to investigate the metabolic profiles of the main active components in the herb pair by rat intestinal bacteria. In this work, to identify the metabolites, a rapid and sensitive ultra-performance liquid chromatography/ quadrupole time-of-flight mass spectrometry (UPLC-Q-TOF/MS) with automated data analysis (MetaboLynx^{TM}) was established and successfully applied.

2. Materials and methods

2.1 Reagents and chemicals

Rehmannia glutinosa Libosch and *Cornus officinalis* Sieb were obtained from Nanjing Guoyao Pharm. Co. Ltd (China). Loganin, morroniside, catalpol, and acteoside standard substances were purchased from Shanghai Winherb Medical S&T Development Co., Ltd. (China). Formic acid was obtained from Merck KGaA (Darmstadt, Germany). The distilled water was purified by an EPED super purification system (Nanjing, China). The acetonitrile of HPLC-grade was purchased from TEDIA Company Inc. (Fairfield, USA). The other reagents were of analytical grade. AnaeroPack Rectangular Jars were purchased from Mitsubishi Gas Chemical Company INC (Japan).

General anaerobic medium (GAM, 1000 mL) contains 10.0 g tryptone, 3.0 g soya peptone, 10.0 g proteose peptone, 3.0 g glucose, 13.5 g digestive serum powder, 5.0 g yeast extract, 5.0 g soluble starch, 2.2 g beef extract, 1.2 g beef liver extract powder, 2.5 g KH_2PO_4, 3.0 g NaCl, 0.3 g *L*-cysteine hydrochloride, 0.3 g sodium thioglycolate, and 1000 mL distilled water. The pH was adjusted to 7.3 with NaOH aqueous solution before autoclaving, and the obtained anaerobic medium was autoclaved at 121 ^{o}C for 20 min.

2.2 Preparation of herb pair extract

C. officinalis (100 g) and *R. glutinosa* (200 g) crude material mixture was decocted with 1000 mL water for 2 h. Then, the filtrate was collected and the residue was decocted with 1000 mL water for another 2 h. Finally, the two batches of filtrates were combined and concentrated to 100 mL.

2.3 Preparation samples for analysis

The rat fresh feces were respectively suspended in GAM and anaerobically cultured in the incubator at 37 ^{o}C for 24 h. Then, the suspension culture was used as rat intestinal bacterial mixture in the later metabolism experiment. The medicine extract (1.0 mL) was added into the rat intestinal bacterial mixture and anaerobically incubated at 37 ^{o}C for 48 h. Then, the incubated solution was precipitated with three times methanol. After vortexing for 60 s, the sample was centrifuged at 3000 r/min for 10 min. The organic layer was evaporated to dryness at 35 ^{o}C. The residue was reconstituted in 300 μL methanol and centrifuged at 13,000 rpm for 10 min. Then, 5 μL of the supernatant was injected into the chromatographic system for analysis.

2.4 UPLC-MS analysis

Loganin, morroniside, catalpol, acteoside, and their