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Research Paper

Comparative metabolism of *Radix scutellariae* extract by intestinal bacteria from normal and type 2 diabetic mice *in vitro*

Jun Xu, Min Zhao, Dawei Qian, Er-xin Shang, Shu Jiang *, Jianming Guo, Jin-ao Duan [1], Leyue Du

Jiangsu Collaborative Innovation Center of Chinese Medicinal Resources Industrialization, Nanjing University of Chinese Medicine, 138 Xianlin Road, Nanjing 210023, PR China

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ABSTRACT

Ethnopharmacological relevance: Traditional Chinese medicine (TCM) has been used in clinical practice for several thousand years. TCM has played an indispensable role in the prevention and treatment of disease, especially the complicated and chronic ones. In TCMs, many ingredients which are known to have biological effects just pass through the gut, they do not get into the bloodstream. Study on interactions of these active ingredients with the intestinal bacteria is very helpful to unravel how TCM works. *Radix scutellariae* was widely used alone or in combination with other medicinal herbs to the treatment of type 2 diabetes mellitus for a long time in China even in Asia. Additionally, the incidence of type 2 diabetes is closely related to the changes of intestinal flora. In this paper, the metabolism of baicalin in *Radix scutellariae* extract by normal and type 2 diabetic mice intestinal bacteria were firstly investigated.
Materials and methods: Ultra performance liquid chromatography/quadrupole-time-of-flight mass spectrometry (UPLC/QTOF-MS) technique combined with MetabolynxTM software was used for analysis of the metabolic profile of baicalin in *Radix scutellariae* extracts by the intestinal bacteria from normal and type 2 diabetic mice.
Results: The amount of baicalin's aglycone (baicalein) in type 2 diabetic mice samples were remarkably more than that in normal mice samples and oroxylin A only existed in type 2 diabetic mice samples. Intestinal bacteria produced not only a small amount of baicalein, but also some conjugates such as hydrogenated baicalin and methylated baicalin.
Conclusions: We proposed that β-D-glucuronidases contributed to the deglycosylation prior to absorption. Intestinal bacteria from pathological state mice produced more baicalein, which was well absorbed contributing to the treatment of type 2 diabetes. Additionally, the pharmacological effects of oroxylin A were associated with type 2 diabetes. Hence, the production of metabolites of baicalin might influence the effects of traditional medicines. Thus the study on the metabolism of baicalin by intestinal bacteria from normal and type 2 diabetic mice was of great importance to understanding the effects of traditional medicines. Furthermore, this work demonstrated the potential of the ultra performance liquid chromatography/quadrupole time-of-flight mass spectrometry approach with MetaboLynx for quite rapid, simple, reliable and automated identification of metabolites of natural products.

1. Introduction

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 (Dunne, 2001; Bourlioux et al., 2003; Hooper and Gordon, 2011). Under normal conditions, the gut microbiome provides trophic (Hooper and Gordon, 2011) and protective functions (Umesaki and Setoyama, 2000). Alteration in the functions or signaling pathways of the commensal flora contributes to the pathogenesis of diverse illnesses such as inflammatory bowel disease (Frank et al., 2007), chronic inflammation, dyslipidemia, diabetes (Brugman et al., 2006), atopic disorders (Isolauri et al., 2008), cardiovascular diseases, neoplasms (Huycke and Gaskins, 2004) and obesity (Backhed et al., 2007). The biochemical milieu has a decisive part in shaping the structure, composition, and function of the microbial flora.

Diabetes mellitus is a metabolic disorder in the endocrine system, which is characterizes by hyperglycemia resulting from defects in insulin secretion, action or both. The prevalence of diabetes mellitus is

* Corresponding author. Tel./fax: +86 25 85811516.
E-mail addresses: jiangshu2000@163.com (S. Jiang), dja@njutcm.edu.cn (J.-a. Duan).
[1] Tel./fax: +86 25 85811116.

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rapidly increasing worldwide. Currently, 150 million people suffer from diabetes and the trend is expected to increase to over 300 million by 2025 (Zimmet et al., 2001; Shaw et al., 2010). There are two major types of diabetes, namely insulin-dependent diabetes mellitus (IDDM, type 1 diabetes) and non-insulin dependent diabetes mellitus (NIDDM, type 2 diabetes). More than 90% of patients are NIDDM, in which insulin resistance plays a key role in the development of the disease (Xie et al., 2003). The frequency of type 2 diabetes will escalate worldwide, due to an increased number of elderly people and a greater prevalence of obesity and sedentary lifestyles (Li et al., 2004a, 2004b). Therefore, the development of a more effective therapy to treat type 2 diabetes mellitus has become imminent. At present, available drugs for type 2 diabetes have several limitations. Drugs currently used to treat type 2 diabetes probably were divided into sulfonylureas, double quack drugs, glitazones, glinides and α-glucosidase inhibitors, insulin. According to their own circumstances with type 2 diabetes, doctors generally select for clinical use of these drugs combined. This is due to the current medical progress, a variety of drugs for treating diabetes were not able to reach the level of a drug alone for the treatment of diabetes. As a complementary approach, medicinal herbs with anti-hyperglycemic activities are increasingly sought after by diabetic patients and healthcare professionals (Deng et al., 2011a). In China, many compounds such as Huang-lian-jie-du decoction (HLJDD) and Da-chai-hu decoction (DCHD) are employed to treat type 2 diabetes mellitus (Lu et al., 2002; Chen et al., 2007; Yu et al., 2009; Deng and Wang, 2011b). *Radix scutellariae* (root of *Scutellaria baicalensis* Georgi) as one major herb contained in HLJDD and DCHD, was reported to possess anti-diabetic effect (Wang et al., 2007; Park et al., 2008). The active constituents in *Radix scutellariae* extract are mainly flavonoids such as baicalin, baicalein, wogonoside, and wogonin, and more than 60 structures have been identified (Li et al., 2004a, 2004b). Baicalin was one of the main components, which was proved to have the anti-diabetic effects (Gu et al., 2009; Waisundara et al., 2009a, 2009b; Li et al., 2011).

The intestinal tract contains more than 400 bacterial species, in which bacteroides, streptococcus, lactobacillus are major species and most of them are strictly anaerobic (Rambaud, 1992; Lee et al., 2011). Most herbal medicines are administrated orally, after which ingredients such as glycosides are metabolized by intestinal bacteria in the gastrointestinal tract before absorption by the small intestine (Hattori et al., 1986). These bacteria have excellent enzymatic systems, which contribute to their enormous catalytic and hydrolytic potential. Due to their significance, the anaerobic bacterial metabolism of baicalin by gut microflora in normal animals has been fairly studied. It is generally assumed that baicalin is poorly absorbed from the gastrointestinal tract in its native form and must be hydrolyzed by intestinal microflora in the intestine to its aglycones in healthy humans and rats (Akao et al., 2000; Yim et al., 2004). However, drugs are used to treat diseases and only patients are the ultimate consumers of drugs. Therefore, it is necessary to investigate the metabolites of baicalin by intestinal bacteria in the pathological state of type 2 diabetes mellitus. In order to determine the metabolites of baicalin in *Radix scutellariae* extract by the isolated intestinal bacteria, ultra performance liquid chromatography/quadrupole time-of-flight mass spectrometry (UPLC-Q-TOF/MS) with automated data analysis (MetaboLynx™) was utilized (Guo et al., 2011).

2. Materials and methods

2.1. Chemicals and reagents

AnaeroPack Rectangular Jars were purchased from Mitsubishi Gas Chemical Company INC (Japan). Baicalin standard substance was purchased from Shanghai Winherb Medical S & T Development Co. Ltd. (Shanghai, China). The HPLC-grade acetonitrile was purchased from TEDIA Company Inc. (Fairfield, USA). Formic acid was obtained from Merck KGaA (Darmstadt, Germany). The distilled water was purified by an EPED super purification system (Nanjing, China). Other reagents were of analytical grade.

2.2. Radix scutellariae extract preparation

The roots of *Radix scutellariae* were purchased from Weiyuan county of Gansu Province, China. A total of 15.0 g *Radix scutellariae* roots which were chopped into pieces about 1 cm long were immersed into 150 mL water for 1 h, and then extracted under thermal reflux for 1 h twice. The extract was filtrated out by analytical filter paper and evaporated to dryness in a rotary evaporator R-210 (BUCHI ltd., Labortechinik AG, Switzerland) at 60 °C under reduced pressure. Finally, the dried residue was dissolved in water to form a final concentration of 0.30 g/mL (equivalent to dry weight of raw materials). The supernatant was filtered through a 0.22 μm membrane, and an aliquot of 5.0 μL was injected for UPLC-Q-TOF/MS analysis.

2.3. Preparation of standard solution of baicalin

The standard solution of baicalin was prepared by dissolving accurately weighed baicalin in MeOH to give a final concentration of 5.0 mg/mL. The solution was stored in a refrigeration at 4 °C before analysis.

2.4. Preparation of the general anaerobic medium broth

The general anaerobic medium (GAM) was prepared as follows: 10.0 g tryptone, 3.0 g soya peptone, 10.0 g proteose peptone, 13.5 g digestibility serum powder, 5.0 g yeast extract, 2.2 g beef extract, 1.2 g beef liver extract powder, 3.0 g glucose, 2.5 g KH_2PO_4, 3.0 g NaCl, 5.0 g soluble starch, 0.3 g L-cysteine hydrochloride, and 0.3 g sodium thioglycolate, and 1000 mL distilled water, then the pH was adjusted to 7.3 with NaOH aqueous solution before adjusting autoclaving to a total volume of 1000 mL. The obtained anaerobic medium was then autoclaved at 121 °C for 20 min.

2.5. Preparation of mice intestinal bacterial mixture

Fresh feces obtained from type 2 diabetic mice were thoroughly suspended in the general anaerobic medium broth under CO_2 atmosphere and then cultured in the anaerobic incubator at 37 °C for 24 h. The obtained bacterial mixture was then used as mice intestinal bacterial mixture. A concentration of 0.30 g/mL of *Radix scutellariae* extract (1 mL) added to 10 mL mice intestinal bacterial mixture, respectively. Then the mixture was anaerobically incubated for 48 h at 37 °C.

2.6. Preparation of sample solutions for UPLC-Q-TOF/MS

After termination of the incubation, the incubated solution was extracted with ethyl acetate three times. The supernatant was removed and dried at 50 °C. The residues were dissolved in 0.2 mL MeOH, centrifuged at 13,000*g* for 10 min, and the supernatant was analyzed by UPLC-Q-TOF/MS.

2.7. UPLC and MS conditions

Analysis was performed using an ACQUITY UPLC system (Waters Corp., Milford, MA, USA) with a conditioned autosampler at 4 °C. The separation was carried out on a Syncronis C 18 column (100×2.1 mm^2 i.d., 1.7 μm; Thermo, USA). The column temperature was maintained at 35 °C. The column was eluted with a gradient mobile phase of acetonitrile (solvent system A) and 0.1% formic acid