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Identification of metabolites of PSORALEAE FRUCTUS in rats by ultra performance liquid chromatography coupled with quadrupole time-of-flight tandem mass spectrometry analysis

Pei-le Wang[a,1], Zhi-hong Yao[b,1], Feng-xiang Zhang[b], Xiu-yu Shen[a], Yi Dai[b,*], Ling Qin[c], Xin-sheng Yao[a,b,*]

[a] College of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University, Shenyang 110016, China
[b] Institute of Traditional Chinese Medicine and Natural Products, College of Pharmacy, Jinan University, Guangzhou 510632, China
[c] Department of Orthopaedics & Traumatology, The Chinese University of Hong Kong, Shatin, N.T. Hong Kong SAR, China

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ABSTRACT

The fruit of *Psoralea corylifolia* (PSORALEAE FRUCTUS) is a traditional Chinese medicine (TCM), which has been used to prevent and treat vitiligo, psoriasis, and osteoporosis in China for thousands of years. Phytochemical investigation on PSORALEAE FRUCTUS, as well as some metabolism research focused on pharmacokinetics of several single compounds from this plant, has been reported. However, the effective material of PSORALEAE FRUCTUS is still unknown. In the present study, the metabolic fate of multiple components of PSORALEAE FRUCTUS in rats was investigated by ultra performance liquid chromatography coupled with quadrupole time-of-flight tandem mass spectrometry (UPLC/Q-TOF-MS). Based on a three-step strategy, a total of 142 PSORALEAE FRUCTUS-related xenobiotics were identified or tentatively characterized in rat biofluids after oral administration of six representative single compounds and PSORALEAE FRUCTUS extract. All six different types of constituents of PSORALEAE FRUCTUS, including furocoumarin, coumestan, isoflavone, flavanone, chalcone and monoterpene phenol, could be absorbed into the circulation system. In addition, compared with the metabolism of six representative single compounds, different metabolic fate was observed after oral administration of PSORALEAE FRUCTUS extract, which indicated that the drug–drug interactions occurred when fed by multi-component herbal extract, and the investigations only focused on several main components were not sufficient to represent and reflect the overall efficacy of plants. The present study will be conducive to further pharmacological mechanism research on PSORALEAE FRUCTUS.

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1. Introduction

The mature and dry fruit of *Psoralea corylifolia* (PSORALEAE FRUCTUS), known as Buguzhi in China, is one of the commonly used traditional Chinese medicines (TCMs) [1]. Phytochemical study of PSORALEAE FRUCTUS resulted in the isolation of more than 60 compounds including of coumarins, flavonoids and monoterpene phenols [2]. Some of them were reported to possess a wide range of biological and pharmacological activities, such as immunomodulatory [3], antitumor [4], antioxidant [5], antifungal [6], antiplatelet [7], estrogenic [8] and stimulating osteoblastic proliferation [9] activities. The metabolism of PSORALEAE FRUCTUS has also been studied, which were mainly focused on pharmacokinetics of single compounds, such as bakuchiol, psoralenoside, isopsoralenoside, psoralen and isopsoralen [10,11]. As TCMs are complex chemical mixtures, the effect of herbal therapy is not the result of a single mechanism induced by a single ingredient, but a range of activities of multiple compounds working together to produce a medicinal benefit [12]. Therefore, the pharmacokinetics investigations only focused on several single components were not sufficient to represent and reflect the overall efficacy of PSORALEAE FRUCTUS. The clarification of the absorbed constituents and their metabolites is the basis for the pharmacokinetic and pharmacological mechanism study [13]. However, according to current knowledge about the metabolism of PSORALEAE FRUCTUS, researchers still do not know which ingredients are absorbed, what the fate of constituents in the body is, let alone which components, the prototype ones or the

* Corresponding authors to Prof. Dr. Xin-sheng Yao, College of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University, China. Tel: +86 24 23993994; fax: +86 24 23993994; Associate Prof. Dr. Yi Dai, College of Pharmacy, Jinan University, China. Tel: +86 20 85220785; fax: +86 20 85221559.
E-mail addresses: daiyi1004@163.com (Y. Dai), tyaoxs@jnu.edu.cn (X.-s. Yao).
[1] The first two authors contributed equally to this work.

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metabolites, contribute to clinical efficacy [14]. Therefore, a systematic analysis of metabolites research on PSORALEAE FRUCTUS *in vivo* is of importance and necessity.

In a recent paper, by using LC-DAD/Q-TOF-MS, a total of 45 constituents have been identified or tentatively identified in PSORALEAE FRUCTUS [15]. As contained different type of structures, the related xenobiotics *in vivo* would be much more complex and diverse after oral administration of PSORALEAE FRUCTUS extract. To explore the metabolic fate of multiple components, we proposed a "representative structure based homologous xenobiotics identification" (RSBHXI) strategy based on UPLC/Q-TOF-MS and mass defect filter (MDF) technique, and applied to analysis *in vivo* metabolites profile of a PSORALEAE FRUCTUS containing TCM formula XLGB in rat biofluids after oral administration [16]. In order to reveal the metabolic pathway of different type of structures in PSORALEAE FRUCTUS in rats, in this paper, a similar three-step strategy was proposed to systematically characterize metabolites of PSORALEAE FRUCTUS *in vivo*. First, an in-house chemical database was established by literature retrieval, in which different structure types (furocoumarin, coumestan, isoflavone, flavanone, chalcone, monoterpene phenol) were sorted into six groups. And then, six representative single compounds were selected from each group, and *in vivo* metabolism of each component was identified by LC–MS, respectively. The MS fragmentation pattern and metabolic pathway were also be analyzed. At last, the metabolites of PSORALEAE FRUCTUS extract in rats were characterized by LC–MS.

2. Experimental

2.1. Materials and reagents

PSORALEAE FRUCTUS were provided by Tongjitang Pharm Co., Ltd. (Guizhou, China). Psoralen (PL), psoralidin (PLD), bavachin (BV), neobavaisoflavone (NBI), isobavachalcone (IBC) and bakuchiol (BK) were purchased from Shanghai Ronghe Medical Technological Limited Company (Shanghai, China). The other reference standards were isolated by various column chromatographies and were unambiguously identified by ^{1}H, ^{13}C-NMR technique in our laboratory. The purity of each compound was more than 98% determined by HPLC analysis. LC–MS grade acetonitrile and water were purchased from Fisher Scientific (Fair Lawn, New Jersey, USA). LC–MS grade formic acid was obtained from Sigma-Aldrich (St. Louis, USA). Water, methanol and ethanol were all of HPLC grade.

2.2. Sample preparation

For drug administration, 100 g of PSORALEAE FRUCTUS was extracted 3 times (each for 2 h) with 800 mL 70% (v/v) aqueous ethanol under reflux. The extract solution was concentrated in reduced pressure. The residue was suspended in 0.5% CMC-Na solution. The PSORALEAE FRUCTUS extract was orally administered to rats at 6 g/kg (equivalent to weight of PSORALEAE FRUCTUS). Psoralen, psoralidin, bavachin, neobavaisoflavone and isobavachalcone were also suspended in 0.5% CMC-Na solution and were given to rats ($n = 2$) orally at 40 mg/kg, respectively. As an exception, bakuchiol was given to rats ($n = 2$) at 100 mg/kg by oral gavage.

2.3. Animals and drug administration

Male Sprague-Dawley rats (220–250 g) were obtained from the experimental animal center of Guangdong province (Guangzhou, China). They were housed at ambient temperature of 20 ± 2 °C with 12-h light/dark cycles for two weeks before experiment and were fed a standard diet and water ad libitum. The animals were fasted with free access water in metabolic cages separately over night before experiment. The animal protocols were approved by the Guide for the Care and Use of Laboratory Animals of Jinan University. All procedures were in accordance with Guide for the Care and Use of Laboratory Animals (National Institutes of Health).

2.4. Biological samples collection and pretreatment

Plasma samples: The rats ($n = 2$) were anesthetized by intraperitoneal injection of 10% aqueous chloral hydrate. The blood samples were collected from external jugular vein in heparinized tube at 0.5 h, 1 h, 2 h, 4 h and 6 h and were centrifuged at 12,000 rpm for 10 min, respectively. The blood samples were mixed, and an aliquot of 2 mL was treated with 8 mL acetonitrile to precipitated protein. After centrifuging at 12,000 rpm for 10 min, the supernatant was dried under nitrogen gas at room temperature. The residue was dissolved in 300 μL methanol.

Urine and feces samples: Urine and feces samples were collected for 0–24 h. The urine sample (2 mL) was loaded on a HLB column (6 m^3, 200 mg, Waters Oasis, Ireland) directly, and then eluted by 6 mL of 5% methanol and 6 ml of methanol successively. The methanol eluate was collected and dried under nitrogen gas at room temperature. The residue was reconstituted in 300 μL methanol. Feces were dried in air and then crushed into crude powder. The powder (1.0 g) was extracted by 10-fold of methanol in an ultrasonic bath for 30 min. After centrifuging at 12,000 rpm for 10 min, the supernatant was dried under reduced pressure. The residue was reconstituted in 300 μL methanol.

Bile samples: Under light anesthesia, bile duct (two rats) was inserted with polyethylene tubing for collection of bile samples. Bile samples were collected for 0–12 h and were treated by the same way as the urine samples.

2.5. UPLC-Q/TOF-MS instrumentation

UPLC analyses were performed using an ACQUITY^{TM} UPLC I-Class system on an Acquity UPLC BEH C_{18} Column (2.1 mm × 100 mm, 1.7 μm) at a temperature of 35 °C. The mobile phases consisted of eluent A (0.1% formic acid in water, v/v) and eluent B (0.1% formic acid in acetonitrile, v/v) was delivered at a flow rate of 0.4 mL/min by using a liner gradient program as follow: 10% B from 0 to 0.5 min, 10–25% B from 0.5 to 8 min, 25–55% B from 8 to 13 min, 55–70% B from 13 to 17 min, 70–100% B from 17 to 20 min. After holding 100% B for next 5 min, the column was returned to its starting condition. The injection volume was 2 μL for all the samples. UPLC system was coupled to a quadrupole orthogonal time-of-flight (Q-TOF) tandem mass spectrometer (SYNAPT^{TM} G2 HDMS, Waters, Manchester, U.K.) equipped with electrospray ionization (ESI). The operating parameters were set as follow: Capillary voltage of 3 kV (ESI+) or −2.2 kV (ESI-); Sample cone voltage of 35 V; Extraction cone voltage of 4 V, source temperature of 100 °C, desolvation temperature 400 °C, cone gas flow of 50 L/h and desolvation gas flow of 800 L/h. In MS^E mode, trap collision energy was 5 eV for low energy function and 20–50 eV for high energy function. Argon was used as collision gas for CID in both MS^E and MS^2 mode. The mass spectrometer was calibrated over a range of 50–1200 Da using solution of sodium formate. Leucine-enkephalin (m/z 556.2771 in positive ion mode; m/z 554.2615 in negative ion mode) was used as external reference of LockSpray ^{TM} infused at a constant flow of 2 μL/min. Argon was used as collision gas. All data were processed with Metabolynx XS software under the operating interface of Masslynx (V4.1, Waters, Milford, MA, USA). As the mass defects of phase I and phase II metabolite ions almost fall within 50 mDa relative to that of the parent compound [17], so the Δm used for the mass defect filter was set as 50 mDa.

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