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Metabolic profile of irisolidone in rats obtained by ultra-high performance liquid chromatography/quadrupole time-of-flight mass spectrometry

Guozhe Zhang[a], Jiahong Sun[b], Yoshihiro Kano[a], Dan Yuan[a,*]

[a] Department of Traditional Chinese Medicine, Shenyang Pharmaceutical University, 103 Wenhua Road, Shenyang 110016, China
[b] Department of Pharmacology, West Virginia University, PO Box 6001, Morgantown, WV 26506-0002, USA

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ABSTRACT

Irisolidone, a major isoflavone found in *Pueraria lobata* flowers, exhibits a wide spectrum of bioactivities, while its metabolic pathway *in vivo* has not been investigated. In this study, an ultra-high performance liquid chromatography/quadrupole time-of-flight mass spectrometry (UHPLC/Q-TOF MS) method was employed to investigate the *in vivo* metabolism of irisolidone in rats. Plasma, bile, urine, and feces were collected from rats after a single 100 mg/kg oral dose of irisolidone. Protein precipitation, solid phase extraction (SPE) and ultrasonic extraction were used to prepare samples of plasma, bile/urine, and feces, respectively. A total of 46 metabolites were detected and tentatively identified based on the mass spectral fragmentation patterns, elution order or confirmed using available reference standards. The metabolic pathways of irisolidone in rats included decarbonylation, reduction, demethylation, demethoxylation, dehydroxylation, hydroxylation, sulfation, and glucuronidation. The relative content of each metabolite was also determined to help understand the major metabolic pathways of irisolidone in rats.

1. Introduction

Flavonoids, as plant secondary metabolites, include many structurally similar classes such as flavones, isoflavones, flavans, anthocyanins, proanthcyanidins, flavanones, chalcones, and aurones. Flavones and isoflavones are of ecotoxicological importance since they are present in the heartwood of tree species used for wood pulp [1,2], and are to be found in a variety of fruits and vegetables. Plants of the Leguminosae family (*e.g.* soy, lupin) contain isoflavones that are important components in the diets of humans and animals.

Flos Puerariae, as a traditional Chinese medicine, has been used in China since ancient times to help with recovery from alcohol intoxication. In China and Japan, phytochemicals extracted from Flos Puerariae have recently become popular herbal medicines for treating alcohol intoxication and liver injury. The content of kakkalide (irisolidone-7-*O*-β-D-xylosylglucoside, KA) in the *P. lobata* flower and in its aqueous extract accounts for more than 2 and 10%, respectively. It has been found that human fecal bacteria can transform KA into irisolidone (Ir, Fig. 1) in anaerobic medium [3]. Whether given orally or intraperitoneally, Ir exhibits more potent bioactivity than KA [4–6]. In addition, some isoflavone aglycone metabolites, such as tectorigenin, glycitein, and genistein, also exhibited more potent bioactivity than their glycoside precursors [4]. These results indicate that flavone glycoside is in essence a prodrug, while aglycone may be the real active component *in vivo*.

Irisolidone, as the aglycone of kakkalide, exhibits a wide spectrum of bioactivities such as anti-inflammatory [7], antioxidative [8], antiviral [9], anti-tumor [10], and estrogenic effects [6] and protecting against ethanol-induced damage and hepatic injury [3]. Compared with tectorigenin (Te) and genistein, Ir has the most potent inhibitory effect on the growth of *Helicobacter pylori* (HP) [11], suggesting that the C-4′-OCH_3 group may be the active group involved. Although it has been reported that Ir is produced from the metabolism of KA both *in vitro* [3,6] and *in vivo* [12], the metabolism of Ir has not yet been studied in detail either *in vitro* or *in vivo*. Accordingly, there is a need to study the detailed metabolism of Ir.

In this paper, an UHPLC/Q-TOF MS method was employed to characterize the metabolites of Ir in rat biological samples, including plasma, bile, urine, and feces, in order to provide evidence of the metabolic pathways of isoflavones *in vivo*.

Abbreviations: BiA, biochanin A; ESI, electrospray ionization; CLog *P*, calculated 1-octanol/water partition coefficient; Ir, irisolidone; KA, kakkalide; SPE, solid phase extraction; Te, tectorigenin; UHPLC/Q-TOF MS, ultra-high performance liquid chromatography/quadrupole time-of-flight mass spectrometry.

* Corresponding author. Tel.: +86 024 23986502; fax: +86 024 23986502.
E-mail addresses: yuandan_kampo@163.com, 4083448@qq.com (D. Yuan).

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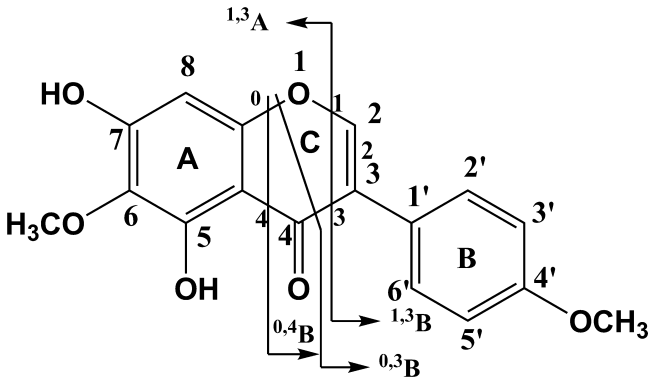

Fig. 1. Structure, nomenclature and diagnostic fragmentations of irisolidone.

2. Experimental

2.1. Chemicals and reagents

Irisolidone (purity >98%) and other authentic standards (purity >95%) were separated in our laboratory. Their structures were determined using ultraviolet (UV), infrared, ^{1}H and ^{13}C NMR, and mass spectrometry (MS) methods. The formic acid and acetonitrile used were of HPLC grade (Fisher Scientific) and ultra-pure water (18.2 MΩ) was prepared with a Milli-Q water purification system (Millipore, France).

2.2. Animal experiments

Male Sprague-Dawley rats (220–250 g) purchased from the Animal Center of Shenyang Pharmaceutical University (Shenyang, China) were housed in an animal room with a standardized temperature (25–28 °C), humidity (50–60%) and a 12 h light/dark cycle, with free access to a soy-free diet and tap water for one week. Rats were fasted for 12 h before the experiments, and were allowed free access to water and sugar over the period of sample collection. Ir was dispersed in 0.5% carboxymethylcellulose solution at 10.0 mg/mL, and then sonicated for 5 min to obtain a homogeneous suspension. Whole blood samples were collected from the suborbital vein and placed in heparinized polythene tubes at 0, 1, 2, 4, 8, 12, 24, 36 and 48 h after oral administration of Ir at a dose of 100 mg/kg BW, then immediately centrifuged at 3500 rpm for 10 min at 4 °C to obtain plasma. Urine and feces were collected for 0–48 h after administration. Urine samples were made acidic by adding 1% acetic acid and then immediately stored at −20 °C; feces samples were also stored at −20 °C after drying. For the study of bile, rats were anaesthetized by intraperitoneal administration of urethane, and then a plastic cannula was surgically inserted into the bile ducts. After collecting blank bile for one hour, bile was collected for 0–36 h after the oral dose. All the biological samples were stored at −20 °C. The experimental protocol was approved by the Ethics Review Committee for Animal Experimentation of Shenyang Pharmaceutical University.

2.3. Sample preparation

2.3.1. Plasma samples

Two hundred microliter of mixed plasma was diluted with 600 μL acetonitrile containing 1% acetic acid, and vortex-mixed for 2 min. After centrifuging for 10 min at 10,000 rpm to precipitate proteins, the supernatants were transferred to other tubes and evaporated to dryness under a stream of nitrogen gas at room temperature. The residue obtained was reconstituted with 100 μL methanol–water (80:20, v/v) and centrifuged at 13,000 rpm for 10 min. Then, 5 μL samples of the supernatants were injected into the UHPLC/Q-TOF MS system for analysis.

2.3.2. Urine samples

Solid phase extraction (SPE) was used for extracting the metabolites from urine with Bond Elut C_{18} cartridges (3 mL, 500 mg). Each cartridge was conditioned with 3 mL methanol followed by 2 mL distilled water. Spiked urine samples were centrifuged at 3500 rpm for 10 min, and then 100 μL of these supernatants were loaded on to the cartridges. The SPE cartridges were washed with 2 mL 1% acetic acid in water to elute the matrix and then with 2 mL methanol to elute the metabolites at a rate of 30 drops/min. The methanol layer was evaporated to dryness under a stream of nitrogen gas at room temperature. The residue of urine was reconstituted with 600 μL methanol–water (80:20, v/v), and then centrifuged at 13,000 rpm for 10 min, and 2 μL samples of the supernatants were injected into the UHPLC/Q-TOF MS system for analysis.

2.3.3. Bile samples

The method for preparing the bile samples was the same as that for preparing urine samples, except that 100 μL bile extract was reconstituted with 300 μL methanol–water (80:20, v/v).

2.3.4. Feces samples

Feces samples weighing 1 g were extracted with 20 mL methanol–water (80:20, v/v). Then, after ultrasonic extraction for 20 min and centrifugation at 3500 rpm for 10 min, the supernatants were passed through a 0.22 μm membrane filter. Finally, 2 μL samples of the filtrates were injected into the UHPLC/Q-TOF MS system for analysis.

2.4. UHPLC conditions

Separations were performed on an Acquity UPLC system (Waters) with an Acquity UPLC column (HSS C_{18} 100 mm × 2.1 mm, 1.8 μm) at 40 °C and at a flow rate of 0.45 mL/min. Biological samples were maintained at 4 °C in the auto sampler. A VanGuard (Waters) pre-column (HSS C_{18}, 5 mm × 2.1 mm, 1.8 μm) was used with a mobile phase consisting of (A) 0.2% formic acid in water (v/v) and (B) 0.2% formic acid in acetonitrile (v/v). The gradient elution was as follows: 0–0.5 min, a linear gradient from 5 to 15% B; 0.5–4 min, 15 to 25% B; 4.1–5 min, 35 to 42% B; 5–7 min, 42 to 70% B; 7–7.1 min, 70 to 100% B; 7.1–9 min, 100% B, a linear gradient back to 5% B. The injection volume was 5 μL for plasma and 2 μL for other fluids.

2.5. Q-TOF/MS^{E} parameters

Analyses were performed using a Micromass-Q-TOF Premier mass spectrometer (Waters) coupled with an electrospray ionization (ESI) source operated in positive ion mode. There are sensitivity and resolution mode available for acquiring mass data. For ions originating from a given source and accelerated by a fixed potential, the mass resolving power of a TOF MS will increase as the flight path is lengthened. However, a longer flight path will sure reduce the total signal as fewer ions strike the detector. Therefore, the sensitivity mode is more sensitive, but the resolution mode offers higher mass resolution. In the present study, the sensitivity mode was used for there are some trace amount of metabolites in biological samples. The MS tune parameters were as follows: the cone and desolvation gas flow were 50 L/h and 700 L/h, respectively; the temperature of the source and desolvation were set at 130 °C and 350 °C, respectively; the capillary and the cone voltage were set at 3.0 kV and 40 eV, respectively; the micro-channel plates (MCPs) were operated at 1750 V and the Q-TOF mass spectrometer was operated in MS^{E} mode with a low collision energy set at 6 eV in the