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Pharmacokinetics of irisolidone and its main metabolites in rat plasma determined by ultra performance liquid chromatography/quadrupole time-of-flight mass spectrometry

Guozhe Zhang[a,b], Wen Qi[a], Liangyu Xu[a], Yoshihiro Kano[a], Dan Yuan[a,*]

[a] Department of Traditional Chinese Medicine, Shenyang Pharmaceutical University, 103 Wenhua Road, Shenyang 110016, China
[b] Department of Translational Medicine, Yancheng Institute of Health Scinces, 263 Jiefang Road, Yancheng 224005, China

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ABSTRACT

Irisolidone, a major isoflavone found in *Pueraria lobata* flowers, exhibits a wide spectrum of bioactivities, while its metabolic pathways and the pharmacokinetics of its metabolites *in vivo* have not been investigated yet. In the present study, an ultra performance liquid chromatography/quadrupole time-of-flight mass spectrometry (UHPLC/Q-TOF MS) method was employed to investigate the metabolic pathways of irisolidone and the pharmacokinetics of its main metabolites in rats, after a single 100 mg/kg oral dose of irisolidone. Protein precipitation method was used to prepare plasma samples. A total of 15 metabolites included irisolidone were detected and tentatively identified based on the mass spectral fragmentation patterns, elution order or confirmed using available reference standards. The pharmacokinetics of the main metabolites included three glucuronide metabolites tectorigenin-7-*O*-glucuronide (Te-7G), 6-hydroxybiochanin A-6-*O*-glucuronide (6-OH-BiA-6G), irisolidone-7-*O*-glucuronide (Ir-7G), and three sulfate metabolite tectorigenin-7-*O*-sulfate-4′-*O*-sulfate (Te-7S-4′S), tectorigenin-7-*O*-sulfate (Te-7S) and irisolidone-7-O-sulfate (Ir-7S), and aglycone tectorigenin (Te), and irisolidone (Ir) were evaluated. The plasma concentrations reached maximal values of 0.297 μmol/L at 10.3 h for Te-7S-4′S, 0.199 μmol/L at 21.7 h for Te-7G, 0.154 μmol/L at 8.00 h for Te-7S, 4.10 μmol/L at 15.3 h for 6-OH-BiA-6G, 10.7 μmol/L at 9.71 h for Ir-7G, 0.918 μmol/L at 11.3 h for Te, 0.150 μmol/L at 8.67 h for Ir-7S, and 0.843 μmol/L at 9.67 h for Ir, respectively. Since the total plasma concentrations of conjugated metabolites were much higher than that of the irisolidone aglycone, an extensive phase II metabolism plays an important role in the pharmacokinetics of irisolidone *in vivo*.

1. Introduction

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 become popular herbal medicines for treating alcohol intoxication and liver injury recently. The content of kakkalide (irisolidone-7-*O*-β-D-xylosylglucoside, KA) in the *Pueraria lobata* flower and in its aqueous accounts for more than 2% and 10%, respectively [1]. It is well known that KA can be transformed into irisolidone (Ir, Fig. 1) by human fecal bacteria in anaerobic medium [2]. Ir exhibits more potent estrogenic effect [3], anti-inflammatory effect [4], and more effect on prostaglandin E2 production than KA [5]. When 100 mg/kg kakkalide was orally administered to mice injured by *t*-BHP, it significantly inhibited the increase in plasma alanine aminotransferase and aspartate aminotransferase activities, which was much more potent than that of silybin, a hepatoprotective agent. However, intraperitoneally administered kakkalide did not exhibit hepatoprotective activity. When irisolidone was intraperitoneally administered to mice, it exhibited potent hepatoprotective activity [6]. Orally administering KA and Ir to hyperlipidemic mice could potently lower total cholesterol (TC) and triglyceride (TG) in serum; however, after intraperitoneally administering KA and Ir to mice, only Ir could

Abbreviations: Te, tectorigenin; Te-7G-4′S, tectorigenin-7-*O*-glucuronide-4′-*O*-sulfate; Te-7S-4′S, tectorigenin-7-*O*-sulfate-4′-*O*-sulfate; Te-7G, tectorigenin-7-*O*-glucuronide; Te-7S, tectorigenin-7-*O*-sulfate; Te-7G-4′G, tectorigenin-7-*O*-glucuronide-4′-*O*-glucuronide; 6-Hydroxygenistein-G, 6-hydroxygenistein-*O*-glucuronide; BiA, biochanin A; 6-OH-BiA-6G, biochanin A-6-*O*-glucuronide; 6-OH-BiA-6S, biochanin A-6-*O*-sulfate; BiA-7G, biochanin A-7-*O*-glucuronide; Ir, irisolidone; ISO-Ir, isomer of irisolidone; Ir-7G, irisolidone-7-*O*-glucuronide; Ir-7S, irisolidone-7-*O*-sulfate; KA, kakkalide.

* Corresponding author. Fax: +86 24 23986502.
E-mail address: yuandan_kampo@163.com (D. Yuan).

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decrease the serum level of TC and TG [7]. Based on these findings, it indicates that KA is in essence a prodrug, while the aglycone Ir may be the real active component *in vivo*.

Ir, as aglycone of kakkalide, exhibits a wide spectrum of bioactivities such as anti-inflammation [8], antioxidation [9], antiviral propertiy [10], anti-tumor effect [11], estrogenic effect [3], protecting against ethanol-induced lethality and hepatic injury [2], *etc.* Compared with tectorigenin (Te) and genistein, Ir has the most potent inhibitory effect on the growth of *Helicobacter pylori* (HP) [12]. KA could be metabolized *in vitro* by intestinal bacteria not only into Ir but also into 6-hydroxybiochanin A (6-OH-BiA) and Ir-7-*O*-glucoside [13]. Three glucuronide metabolites, irisolidone-7-*O*-glucuronide (Ir-7G), tectorigenin-7-*O*-glucuronide (Te-7G) and 6-OH biochanin A glucuronide (6-OH-BiA-G), as well as KA and trace amount of Ir were detected in plasma, after oral administration of KA to rats, and the pharmacokinetics of KA and its metabolites except Ir were also evaluated [14]; however, the metabolism of Ir and the pharmacokinetics of its metabolites have not been investigated and, so, the direct metabolism and the pharmacokinetics of Ir and its metabolites should be elucidated.

In this paper, an UHPLC/Q-TOF MS method was employed to characterize the metabolites of Ir in rat plasma, whilst the pharmacokinetics of Ir and its main metabolites were also evaluated, in order to provide evidence of the metabolic pathways of isoflavones *in vivo*.

2. Material and methods

2.1. Chemicals and reagents

Irisolidone (purity > 98%), and other standards (purity > 95%) Te, Te-7G, tectorigenin-7-*O*-glucuronide-4′-*O*-sulfate (Te-7G-4′S), tectorigenin-7-*O*-sulfate (Te-7S), irisolidone-7-*O*-glucuronide (Ir-7G), 6-hydroxybiochanin A-6-*O*-sulfate (6-OH-BiA-6S) and biochanin A (BiA) were separated in our laboratory. Their structures were determined using NMR, MS, UV and IR methods [15,16]. Acetonitrile (HPLC grade) and formic acid used were purchased from Thermo Fisher Scientific (USA). 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 experiment, and were allowed free access to water and sugar for the sample collection. Ir was dispersed in 0.5% carboxymethyl cellulose solution at 10.0 mg/mL, and then sonicated for 5 min to obtain a homogeneous suspension. The whole blood samples were collected from the suborbital vein and placed in heparinized polythene tubes for 0, 0.25, 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 24, 36, 48, 60 and 72 h after oral administration of Ir at a dose of 100 mg/kg body weight, and immediately centrifuged at 3500 rpm for 10 min at 4 °C to obtain plasma. About 0.5 mL blood was taken from each rat at each time point, and the plasma samples were disposed and detected separately. The experimental protocol was approved by the Ethics Review Committee for Animal Experimentation of Shenyang Pharmaceutical University.

2.3. Sample preparation

Plasma (combined plasma for characterization and each time point for quantification) was diluted by adding three times volume of acetonitrile containing 1% acetic acid, vortex-mixed for 2 min. After centrifuging for 10 min at 10,000 rpm to precipitate proteins, the supernatants were transferred to another tube and evaporated to dryness under a stream of nitrogen gas at room temperature. The residue obtained was reconstituted with 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 UHPLC/Q-TOF MS system for analysis.

2.4. Ultra-performance liquid chromatography/mass spectrometry (UHPLC/MS)

Separations were performed on an Acquity UPLC system (Waters) with Acquity UPLC columns (HSS C_{18} 100 × 2.1 mm, 1.8 μm) at 40 °C. The flow rate was 0.45 mL/min. Auto sampler was maintained at 4 °C. The mobile phase was (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 to 4 min, 15 to 25% B; 4.1 to 5 min, 35 to 42% B; 5 to 7 min, 42 to 70% B; 7 to 7.1 min, 70 to 100% B; 7.1 to 9 min, 100% B, 9.0 to 9.1 min, 100 to 5% B; 9.1 to 11 min, 5% B. The injection volume was 5 μL for both control and samples. Micromass-Q-Tof Premier mass spectrometer (Waters) coupled with an electro spray ionization (ESI) source was operated in positive ion mode. Data was acquired in sensitivity mode. MS tune parameters were set as follows: The temperature of the source and desolvation was set at 130 °C and 350 °C, respectively. The cone and desolvation gas flow were 50 L/h and 700 L/h, respectively. The capillary voltage was set at 3.0 kV, and the cone voltage at 40 eV. The micro-channel plates (MCPs) were operated at 1750 V with 3.6 GHz. Data was acquired in MS^E mode for metabolite identification with a low collision energy set at 6 eV in the first function and a collision energy ramp from 20 to 40 eV in the second function, while MS mode was selected for metabolites quantification. Centroid mode data was collected over the range of *m/z* 100–1,000, and scan time was 0.2 s with an interscan delay of 0.02 s. A 2-ng/mL solution of leucine-enkephalin generating an $[M+H]^+$ ion (*m/z* 556.2771) was infused through the Lock Spray probe at 10 μL/min.

2.5. Identification of the metabolites

Plasma samples were analyzed by TOF MS method. MetaboLynx™ software was used to identify metabolites automatically by comparing the samples with the controls, with minimal manual interference, and the metabolic pathways were also provided. Metabolites characterization could be achieved in a single analytical run in MS^E mode, and then the data used for quantification was acquired in MS mode.

2.6. Standard solution, calibration curves, and quality control (QC) samples

Primary stock solutions of Ir and Te were prepared at the final concentrations of 5.0 and 6.0 μg/mL, respectively, by

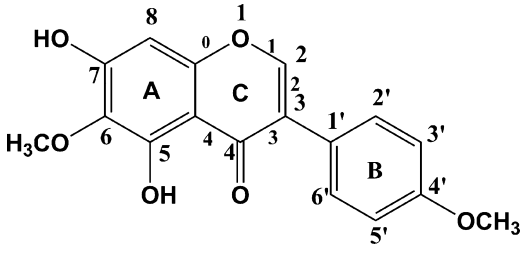

Fig. 1. Structure and nomenclature of irisolidone.