Journal of Functional Foods 40 (2018) 659–669

Contents lists available at ScienceDirect

Journal of Functional Foods

journal homepage: www.elsevier.com/locate/jff

Glucuronidation is the dominating *in vivo* metabolism pathway of herbacetin: Elucidation of herbacetin pharmacokinetics after intravenous and oral administration in rats

Beikang Ge[a], Liang Zhao[a], Xiaorong Li[a,b], Pingxiang Xu[a], Ming Xue[a,b,*]

[a] *Department of Pharmacology, Beijing Laboratory for Biomedical Detection Technology and Instrument, School of Basic Medical Sciences, Capital Medical University, Beijing 100069, China*

[b] *Beijing Engineering Research Center for Nerve System Drugs, Beijing 100053, China*

ARTICLE INFO

Keywords:
Herbacetin
Pharmacokinetics
Biotransformation
Glucuronidation
Hepatic metabolism
Colonic degradation

ABSTRACT

The promising potential benefits of herbacetin in human entail its pharmacokinetic investigations, but the metabolic fate of this natural compound *in vivo* remains a field of unknown so far. The current study, for the first time, identified and quantified seven herbacetin-metabolites from rat urine, feces and bile after administration of herbacetin to rats. It was found that herbacetin was excreted primarily from rat urine in the form of glucuronide-conjugations. Subsequent *in vitro* enzyme kinetic studies and *in vivo* pharmacokinetic investigations suggested an extensive hepatic metabolism of herbacetin and the high exposure of herbacetin-glucuronides in systemic circulation. The clearance, $t_{1/2}$ and bioavailability of herbacetin in rats were determined as 16.4 ± 1.92 ml·kg/min, 11.9 ± 2.7 min, and 1.32%, respectively. On basis of these findings, a comprehensive metabolic pathway of herbacetin in rats was composed, which was crucial for the further assessments of herbacetin therapeutic effects and mechanism of pharmacological action.

1. Introduction

Herbacetin is a novel flavanol compound stored in natural sources such as *Rhodiola rosea* L. and *Ephedrae herba* L. in the form of its glycosides. The quantity of herbacetin glycosides in these two herbs is around 3.13 mg/g DW and 0.03 mg/g DW, respectively (Amakura et al., 2013; Ma et al., 2013). Both *Rhodiola rosea* and *Ephedrae herba* have attracted substantial research interests due to their promising and diverse therapeutic effects such as anti-depression, anti-hypoxia and anti-tumor. Recent studies indicate that herbacetin and its glycosides, in a great degree, contribute to the pharmacological and health-beneficial effects of *Rhodiola rosea* (Choe et al., 2012). Previous investigations (Chen, Cui, Duan, Ma, & Zhong, 2006) and our preliminary findings suggest that flavonoid glycosides may not be absorbed in gastrointestinal (GI) tract before hydrolysis to their aglycones. Therefore, herbacetin, rather than its glycosides, has been supposed to be the major compound entering the systemic circulation and accounting for the pharmacological effects of *Rhodiola rosea.* Similarly, herbacetin is reported as a crucial constituent accounting for the anti-tumor activity of *Ephedrae herba* (Hyuga et al., 2013). Further studies via computer docking and modeling suggest that, among 7500 compounds in traditional Chinese database, the structure of herbacetin fits best to be used as the ornithine decarboxylase inhibitor which has a potential application in antitumor therapy. Subsequent investigations on the anti-tumor activity of herbacetin based on animal models also demonstrate supportive findings (Kim et al., 2016). Preclinical studies indicate that herbacetin is a natural, nontoxic compound which could be directly subjected to clinical trials for the evaluation of its therapeutic effect against colon cancer (Kim et al., 2016; Li, Sapkota, Kim, & Soh, 2016; Qiao et al., 2013). In addition, herbacetin and its diglucosides (HDG) have been previously reported as constituents of flaxseed and ephedrae herba, which are both regarded as functional foods to improve human nutritional and health status (Amakura et al., 2013; Fliniaux et al., 2014; Hyuga et al., 2013; Oomah, 2001; Struijs et al., 2007).

The promising potential benefits of herbacetin in human entail a better understanding of its pharmacokinetics for assessments of its biological activity, mechanism of action and clinical application such as dose-finding and dose-escalation. Preliminary study in our laboratory found that after oral administration of herbacetin at 4 mg/kg, the plasma concentration of herbacetin in rat was undetectable and it is infeasible to draw a complete plasma concentration *vs.* time curve. These findings suggest that herbacetin may undergo extensive first-pass metabolism or GI tract degradation in rats. In consideration of this, it is of vital importance to elucidate the biotransformation of herbacetin *in*

* Corresponding author at: Department of Pharmacology, Capital Medical University, Beijing, China.
E-mail address: xuem@cpums.edu.cn (M. Xue).

https://doi.org/10.1016/j.jff.2017.12.006
Received 11 May 2017; Received in revised form 15 November 2017; Accepted 3 December 2017

vivo and to simultaneously monitor the pharmacokinetic process of both parent drug and its major metabolites after administration of herbacetin. However, researches concerning the metabolic fate of herbacetin *in vivo* are unavailable. To date, the pharmacokinetic behaviors of herbacetin and herbacetin metabolites including their absorption, distribution, metabolism and excretion are still a field of unknown primarily due to the lack of analytical method.

With an aim to map a comprehensive metabolic pathway of herbacetin *in vivo*, liquid chromatography/ion trap mass spectrometry (LC/MS^n) and ultra-liquid chromatography coupled with mass spectrometry (UPLC/MS) are combined in the current study for qualitative and quantitative determinations of herbacetin and its metabolites in bile, urine and feces after both oral and intravenous administration of herbacetin to rats. Enzyme kinetic studies on the intestinal and hepatic metabolism of herbacetin are further conducted to elucidate metabolic profiles of herbacetin in rat tissues and organs. Additionally, plasma concentration profiles of herbacetin and herbacetin metabolites in rats are obtained to characterize the overall pharmacokinetic behavior of herbacetin.

2. Materials and methods

2.1. Chemicals and reagents

Herbacetin (bench number Y07M6H2) and puerarin (bench number 1,10,752–2,00,511) with purity over 98% were purchased from National Institutes for Food and Drug Control (Beijing, China). Oasis hydrophilic–lipophilic-balanced copolymer extraction cartridge (HLB, 1 ml) used for solid phase extraction (SPE) was supplied by Waters (Milford, USA). Acetonitrile and methanol with HPLC grade were supplied by Thermo Fisher Scientific UK Limited (Leicestershire, UK). Formic acid with HPLC grade and ascorbic acid were bought from BDH Laboratory (Poole, England). Distilled and deionized water was used for the preparation of all solutions. Uridine-5′-diphosphoglucuronic acid (UDPGA), 3′-phosphoadenosine-5′-phosphosulfate (PAPS) and S-Adenosyl methionine (SAM) were obtained from Sigma-Aldrich Chem. Co. (Milwaukee, WI, USA). Other reagents were all of analytical grade.

2.2. Animals

The male Sprague-Dawley (SD) rats (250 g ± 20 g) were supplied by the Animal Center of Capital Medical University (ACCMU, Beijing, China). Rats were housed in individual cages with free access to food and water in a room with controlled illumination (a 12 h light and dark cycle), temperature (22 ± 2 °C) and relative humidity. All the rat experiments were operated in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the National Health Ministry of China. The procedures of rat experiments had been approved by the Animal Center of Capital Medical University.

2.3. The development and validation of an UPLC-MS/MS method for quantifying herbacetin

The quantity of herbacetin in rat biological matrices after administration, including plasma, urine, feces and bile, was determined by the current UPLC-MS/MS method.

2.3.1. Preparation of stock solutions, calibration standards and quality control (QC) samples

Stock solution of herbacetin was prepared by dissolving the authentic compound in methanol to yield a final concentration of 10 mM. The stock solution was stored at 4 °C and further diluted serially by methanol to prepare working standard with concentrations of 1000, 500, 100, 50, 10, 1 and 0.1 μM, respectively. Puerarin at the concentration of 5 μM was used as internal standard (IS). Calibration standards and QC samples were prepared by spiking working standards to drug-free biological matrices. Specifically, to a 100-μl aliquot of rat plasma, 5 μl of IS, 5 μl of 0.1% formic acid and 10 μl of working standard were spiked to yield the concentrations of 100, 50, 10, 5, 1, 0.1 and 0.01 μM for herbacetin as calibration standards. QC samples were prepared at three concentrations (low, medium and high) including 0.02, 20, 80 μM. The calibration standards and QC samples of herbacetin in urine and bile were prepared in the same way with that in plasma described above. As to the treatment of feces, 2000 μl of methanol were used for diluting 2 g feces. After thorough vortex for 10 min, the feces mixtures were sonicated and thereafter centrifuged at 13,200 rpm for 10 min. To a 100-μl aliquot of obtained feces supernatant, 5 μl of IS, 5 μl of 0.1% formic acid and 10 μl of working standard was spiked to yield the concentrations of 100, 50, 10, 5, 1, 0.1 and 0.05 μM for herbacetin as calibration standards in feces. QC samples in feces were prepared at three concentrations (low, medium and high) including 0.1, 40, 80 μM. All calibration and QC samples were subjected to the sample extraction and preparation as described in Section 2.3.2.

2.3.2. Sample extraction and preparation

To a 100-μl aliquot of plasma, urine or bile obtained from herbacetin-administered rats, 5 μl of IS, 5 μl of 0.1% formic acid and 10 μl of methanol were added immediately. 50-μl aliquot of methanol in water (1:1, *v/v*, with 1% ascorbic acid and 0.1% formic acid) was then spiked into above acidified biological samples or calibration and QC samples prepared in Section 2.3.1. After diluting the mixture to 1 ml with 25% methanol in water (with 1% ascorbic acid and 0.1% formic acid), the sample was centrifuged at 21,000*g* for 10 min. The supernatant was collected and loaded onto the Oasis®HLB cartridge (1 ml) which had been preconditioned with 1 ml methanol and 1 ml 0.1% formic acid in water. The cartridge was then rinsed with 1 ml of 0.1% formic acid, followed by 1 ml of 25% methanol in water (with 1% ascorbic acid and 0.1% formic acid). After the cartridge was dried under vacuum for 20 min, the analytes were eluted by 1 ml of methanol from the cartridge. The obtained eluent was then evaporated to dryness in a Labconco Centrivap Concentrator (Model 78,100–01, Labconco Corp., Kansas City, MO) at 40 °C for 4 h under vacuum, and the residue was reconstituted by 100 μl of methanol. After centrifugation at 21,000*g* for 10 min, 5 μl of the supernatant was injected into the UPLC-MS/MS system for analysis.

2.3.3. UPLC-MS/MS conditions

The UPLC-MS/MS system consisted of Agilent 1200 series LC pumps and auto-sampler (Agilent, CA, USA), coupled with a Waters ACQUITY TQD triple quadrupole mass spectrometer with an electrospray ionization source (Waters, CA, USA). Chromatographic separation was achieved by Waters BEH C18 (50 mm × 2.7 mm i.d., 1.7 μm particle size). The mobile phase was composed by acetonitrile (solvent A) and 0.2% formic acid in water (solvent B). Gradient elution was performed according to the following program: solvent B started at 25% for 1 min and linear increased to 40% in the following 6 min; after maintenance at 40% for 1 min, the percentage of B was reduced to 25% in 1 min and equilibrated for another 1 min for the next injection. The flow rate was set at 0.4 ml/min and the total running time was 10 min. The temperatures of the auto-sampler and the column were set at 4 °C and ambient, respectively. The MS/MS system was operated under negative mode with an optimized condition as follow: ion spray voltage at +5.5 kV; nitrogen as nebulizer gas, auxiliary gas and curtain gas at 30, 60 and 10 psi, respectively; auxiliary gas temperature at 225 °C and interface heater temper at 110 °C. The multiple reaction monitoring (MRM) analysis was conducted by monitoring the precursor ion to product ion transitions *m/z* 301.1 → 255.2, 301.1 → 229.1 for the identifying and quantifying of herbacetin; *m/z* 417.1 → 299.9 for puerarin (IS).

2.3.4. UPLC-MS/MS method validation

According to the FDA guidance of bio-analytical method validation,