Journal of Chromatography B 1060 (2017) 308–315

Contents lists available at ScienceDirect

Journal of Chromatography B

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

Simultaneous assessment of absorption characteristics of coumarins from Angelicae Pubescentis Radix: *In vitro* transport across Caco-2 cell and *in vivo* pharmacokinetics in rats after oral administration

Yan-Fang Yang, Lei Zhang, You-Bo Zhang, Xiu-Wei Yang*

State Key Laboratory of Natural and Biomimetic Drugs, Department of Natural Medicines, School of Pharmaceutical Sciences, Peking University, Beijing 100191, China

ARTICLE INFO

Keywords:
Angelicae Pubescentis Radix
Coumarins
Human intestinal Caco-2 cell
Pharmacokinetics
UPLC–MS/MS

ABSTRACT

Angelicae Pubescentis Radix (APR), a well-known traditional Chinese medicine, is widely used for the treatments of rheumatism and headache for centuries. To assess the absorption characteristics of coumarins from APR, a sensitive and reliable UPLC–MS/MS method was established for the simultaneous determination of sixteen coumarins from APR, including psoralen, xanthotoxin, bergapten, bergaptol, isoimperatorin, imperatorin, columbianetin, columbianetin acetate, columbianadin, oxypeucedanin hydrate, angelol B, umbelliferone, scopoletin, osthole, meranzin hydrate and nodakenetin. The specificity, linearity, sensitivity, precision, accuracy, recovery, matrix effect and stability of the method were all validated to be satisfactory. The method was then applied to the *in vitro* transport of APR extract (APRE) across human intestinal epithelial Caco-2 cell and *in vivo* pharmacokinetics in rats after oral administration of APRE. All of the tested coumarins were well or moderately absorbed across Caco-2 monolayers, and could be quickly absorbed into rat blood circulation after oral administration. Columbianetin was the most easily absorbed compound across Caco-2 cell, and also had extremely highest plasma concentration *in vivo*. Excellent correlation between *in vitro* absorption across Caco-2 cell monolayers and *in vivo* pharmacokinetics of coumarins from APRE was well verified. The results provided valuable information for the overall absorption characteristics of the coumarins from APR, as well as for its further studies of *in vivo* active substances in the further.

1. Introduction

Angelicae Pubescentis Radix (APR, known as Duhuo in Chinese), is the dried roots of *Angelica pubescens* Maxim. *f. biserrata* Shan et Yuan (family Umbelliferae). It is one of the most well-known traditional Chinese medicines and has been widely used for the treatments of rheumatism and headache for centuries in China [1]. Pharmacological activities of APR consistent with its traditional efficacy have been reported, such as its anti-inflammatory [2], analgesic [3] and anti-oxidative [4] effects. Phytochemical studies have revealed that coumarins are the most abundant ingredients of APR, and more than 60 coumarins have isolated and identified from APR [5]. Previous studies have reported that many of these coumarins possess multiple biological properties, such as the anti-inflammatory and analgesic properties of columbianadin, columbianetin acetate, bergapten, umbelliferone, osthole, xanthotoxin, isoimperatorin [2] and combianetin [6], anti-tumor effect of bergapten and xanthotoxin [7], neuro-protective effect of osthole [8], and regulation effect of imperatorin on lipid metabolism [9]. These studies suggest that coumarins are the prime ingredients contributing to the traditional efficiency of APR, so the absorption characteristics of these coumarins are much important since they may directly affect the pharmacological activities of APR.

Previously, *in vitro* intestinal absorptions across human colon adenocarcinoma cell line (Caco-2) cell monolayer of main coumarins from APR, such as umbelliferone, osthol, columbianadin, columbianetin acetate, psoralen, bergapten, xanthotoxin and isoimperatorin, have been studied in our group, and all of them were defined as well or moderately intestinal absorbed compounds [10–12]. In the aspect of *in vivo* pharmacokinetics of the coumarins, pharmacokinetics researches had been reported for single columbianetin after oral or intravenous administration of pure columbianetin [13], or oral administration of APR extract (APRE) [14], as well as the single columbianetin acetate after oral administration of APRE [15]. Also, the pharmacokinetics studies of columbianadin in rat plasma had been determined after intravenous administration of pure columbianadin [16,17], or after oral administration of APRE and formula Huo Luo Xiao Ling Dan [18]. To

* Corresponding author at: State Key Laboratory of Natural and Biomimetic Drugs, Department of Natural Medicines, School of Pharmaceutical Sciences, Peking University, Xueyuan Road 38, Haidian District, Beijing 100191, China.
E-mail address: xwyang@bjmu.edu.cn (X.-W. Yang).

http://dx.doi.org/10.1016/j.jchromb.2017.06.020
Received 25 April 2017; Received in revised form 29 May 2017; Accepted 12 June 2017
Available online 13 June 2017

study the *in vivo* absorptions of APR coumarins as many as possible, it is necessary to develop a simultaneous quantitative method for the multicomponent pharmacokinetics determination of APR.

In the present study, a sensitive and reliable ultra performance liquid chromatography–tandem mass spectrometry (UPLC–MS/MS) method was established for the simultaneous determination of sixteen coumarins from APR, and then applied to examine their *in vitro* transports across Caco-2 cell monolayer and *in vivo* pharmacokinetics in rats after oral administration of APRE. The aim of the study was to systematically reveal the absorption characteristics of the major bioactive components from APR, and compare the coincidence degrees of their *in vitro* and *in vivo* absorption behaviors.

2. Materials and methods

2.1. Chemicals and reagents

APR (No. ES20150411) was collected from 'the national good agricultural practice planting bases of Chinese medicinal materials for *Angelica pubescens* f. *biserrata*' at Enshi County (Hubei, China). The crude drug was identified by Prof. Xiu-Wei Yang from Peking University.

Reference standards for psoralen (**1**), xanthotoxin (**2**), bergapten (**3**), bergaptol (**4**), isoimperatorin (**5**), columbianetin (**7**), columbianetin acetate (**8**), columbianadin (**9**), osthole (**14**) [19], angelol B (**11**) and scopoletin (**13**) [20] were prepared from the APR, imperatorin (**6**), oxypeucedanin hydrate (**10**) and umbelliferone (**12**) [21] from the roots of *Angelica dahurica*, and nodakenetin (**16**) [22] from the roots and rhizomes of *Notopterygium incisum* by our research group. Meranzin hydrate (**15**) was obtained from Shanghai Yuanye Bio-Tech. Co. Ltd. (Shanghai, China). Daidzein (internal standard, IS) was purchased from the National Institutes for Food and Drug Control (Beijing, China). The purities of all standards were above 98.0% and suitable for UPLC–MS/MS analysis. Their chemical structures were shown in Fig. 1.

Methanol (MeOH) and acetonitrile (MeCN) of LC–MS grade were purchased from J. T. Backer (Center Valley, PA, USA). Formic acid (FA) of HPLC grade (Dikma, Lake Forest, CA, USA) and Milli-Q water (Millipore, Bedford, MA, USA) were used throughout the study. All other chemicals and solvents used were of analytical grade.

2.2. Preparation of APR extract

The powdered APR (500 g) was extracted 5 times under reflux with 1.5 L of ethanol–water (75:25, v/v) for 2 h each time. The extract of APR was concentrated to dryness under reduced pressure. The final weight of APR extract (APRE) was 215.6 g (yield 43.1%).

2.3. Preparation of standard and quality control samples

Stock solutions of tested coumarins and daidzein (IS) were prepared in MeOH at a concentration of 1 mg/mL and 1 μg/mL, respectively. Appropriate aliquots of individual stock solutions were mixed together to prepare a mixed stock solution. All of the stock solutions were stored at 4 °C.

To prepare the calibration standards, the mixed stock solution was diluted with the blank plasma or Hank's balanced salts solution medium (HBSS, pH 7.4) to different concentrations with IS (20 ng/mL finally). Quality control (QC) samples at high, medium and low concentrations with IS (20 ng/mL finally) were prepared in the same way.

2.4. UPLC–MS/MS apparatus and operation conditions

UPLC experiments were performed with a Shimadzu Nexera UPLC (Shimadzu Corp., Kyoto, Japan) equipped with LC-30AD Pump, CTO-30A column oven, and SIL-30AC Autosampler. An ACQUITY UPLC BEH RP18 column (2.1 × 100 mm, 1.7 μm, Waters, MA, USA) equipped with a BEH RP18 guard column (2.1 × 5 mm, 1.7 μm, Waters, MA, USA) was used for chromatographic separation. The mobile phase was 0.1% (*v/v*) formic acid in water as eluent A and MeCN as eluent B. The elution program was as follows: 0.0–3.0 min, 22%–35% B; 3.0–4.0 min, 35–52% B; 4.0–8.0 min, 52% B; 8.0–8.1 min, 52–95% B; 8.1–9.0 min, 95% B; 9.0–9.1 min, 95–22% B; 9.1–12 min, 22% B. The column temperature and flow rate were set at 30 °C and 0.4 mL/min, respectively. The sample injection volume was 2 μL.

Mass spectrometry analysis was performed on a Shimadzu LCMS-8050 system, equipped with an electrospray ionization (ESI) source and Labsolutions 5.65 software (Shimadzu Corp., Kyoto, Japan). The MS conditions for quantification analysis were optimized and finally performed in positive ion mode (ESI^+) in multiple reaction monitoring

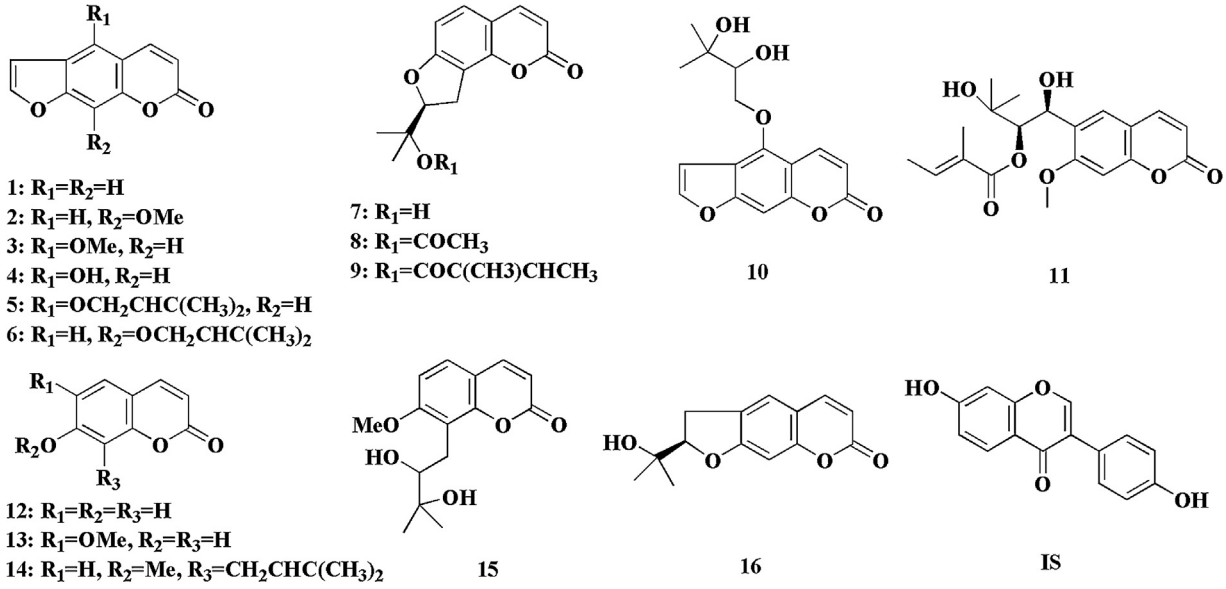

Fig. 1. Chemical structures of main coumarins from APR and the internal standard. (**1**) psoralen, (**2**) xanthotoxin, (**3**) bergapten, (**4**) bergaptol, (**5**) isoimperatorin, (**6**) imperatorin, (**7**) columbianetin, (**8**) columbianetin acetate, (**9**) columbianadin, (**10**) oxypeucedanin hydrate, (**11**) angelol B, (**12**) umbelliferone, (**13**) scopoletin, (**14**) osthole, (**15**) meranzin hydrate, (**16**) nodakenetin, (IS) daidzein.