Journal of Chromatography B, 935 (2013) 1–9

Contents lists available at ScienceDirect

Journal of Chromatography B

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

Identification of the absorbed constituents after oral administration of Yuanhu Zhitong prescription extract and its pharmacokinetic study by rapid resolution liquid chromatography/quadrupole time-of-flight☆

Ye Tao[a,b,1], Haiyu Xu[a,1], Shanshan Wang[c], Bing Wang[d], Yingchun Zhang[a], Weihao Wang[a], Bin Huang[a], Hongwei Wu[a], Defeng Li[a], Yi Zhang[a], Xuefeng Xiao[b], Yubo Li[b], Hongjun Yang[a,*], Luqi Huang[e,*]

[a] Institute of Chinese Materia Medica, China Academy of Chinese Medical Sciences, Beijing 100700, PR China
[b] Tianjin University of Traditional Chinese Medicine, Tianjin 300193, PR China
[c] Analysis and Test Center, Shandong Academy of Sciences, Jinan 250014, PR China
[d] Department of Anesthesiology and Critical Care Medicine, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA
[e] National Resource Center for Chinese Materia Medica, China Academy of Chinese Medical Sciences, No. 16 Nanxiaojie, Dongzhimennei, Beijing 100700, PR China

ARTICLE INFO

Article history:
Received 16 January 2013
Accepted 17 July 2013
Available online 25 July 2013

Keywords:
RRLC-ESI-Q-TOF-MS
Yuanhu Zhitong prescription
Cerebrospinal fluid
Pharmacokinetic study

ABSTRACT

Yuanhu Zhitong prescription (YZP) is well known for its analgesic effect. However, its multiple bioactive components *in vivo* remain unclear. In this paper, a rapid resolution liquid chromatography/quadrupole time-of-flight (RRLC-ESI-Q/TOF) was employed to identify the bioactive components and partial metabolites after oral administration of YZP extracts. Meanwhile, a RRLC-ESI-Q/TOF method was established and validated for the simultaneous quantification of protopine, α-allocryptopine, tetrahydropalmatine, corydaline, tetrahyberberine and byakangelicin in rat plasma and applied for their pharmacokinetic research. The results showed that twenty-one bioactive components of YZP were absorbed into the blood circulation and seventeen components were detected in cerebrospinal fluid (CSF). Moreover, the kinetic profiles of six analytes were obtained and the results suggested that the six analytes peaked between 3.5 and 5.0 h and C_{max} ranged from 214.6 to 858.3. The works could provide key information for identification of bioactive constituents and understanding the metabolism as well as pharmacological actions for YZP.

1. Introduction

Traditional Chinese medicine (TCM) has been used in China for thousands of years and has played an indispensable role in prevention and treatment of diseases, especially for complicated and chronic conditions [1,2]. The herbs used in TCM are usually a complex prescription containing hundreds or even thousands of chemically distinct components in each ingredient, which become a huge challenge to determine which one is responsible for a certain therapeutic effect [3]. Most of TCM formulations are taken orally, and some constituents persist at detectable levels, along with their metabolites, after digestion and absorption. Therefore, it is necessary to trace the constituents of TCM formulation *in vivo* and evaluate their pharmacokinetic profiles which can gain more in-depth insights into the active components and the therapeutic mechanisms for TCM formulation, especially for the drugs working in the brain system [4]. Recently, a RRLC-ESI-Q/TOF method has been reported to boast a higher sensitivity than other methods for identifying known and unknown compounds in complex matrices [5,6]. Thus, it could be a valuable analytical technique for identifying active constituents and evaluating their pharmacokinetic properties of TCM herbs *in vivo*.

Yuanhu Zhitong prescription (YZP) consists of *Radix Angelicae dahuricae* and *Rhizoma corydalis* (processed with vinegar) and is widely used for the treatment of gastralgia, costalgia, headache, and dysmenorrhea caused by qi stagnancy and blood stasis, as recorded in the Chinese Pharmacopoeia [7]. Previous studies indicated that YZP exhibited therapeutic effects through a wide variety of actions, including anti-nociceptive [8], anti-inflammatory [9], anxiolytic [10], spasmolysis [11], and vasorelaxation [12]. Furthermore, *Radix Angelica dahuricae* extracts produced synergistic

* Corresponding authors. Tel.: +86 10 84035184; fax: +86 10 64013996.
E-mail addresses: hongjun0420@vip.sina.com, hyxu627@sohu.com (H. Yang), huangluqi@263.net (L. Huang).
[1] These authors contributed to the work equally.

1570-0232/$ – see front matter © 2013 The Authors. Published by Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.jchromb.2013.07.015

actions on the analgesic effect of *Rhizoma corydalis* alkaloid by increasing plasma concentrations of DL-tetrahydropalmatine [13]. Alkaloids in *Rhizoma corydalis* and coumarins in *Radix Angelicae dahuricae*, had been identified as the active components; moreover, alkaloids, such as DL-tetrahydropalmatine, protopine and berberine, and coumarins, such as imperatorin and isoimperatorin, had been studied to determine their pharmacological effects [14–18]. Recently, we had developed a system to detect 17 constituents of YZP tablet using a RRLC technique coupled with a triple quadrupole mass spectrometry (RRLC-QQQ) [19].

With the recent advances in new mass analyzers, hyphenated technologies and data acquisition software, the qualitative and quantitative analysis of bioactive compounds in TCM herb *in vivo* are more easy to carry out. In addition, this could be applied to pharmacokinetic study of YZP based on quantitative analysis that would identify potentially effective constituents in this herbal formula. These studies might play a very significant role in certain therapeutic effects and warrant further study.

2. Experiments

2.1. Chemicals and materials

HPLC-grade acetonitrile, formic acid, and methanol were obtained from Merck (Darmstadt, Germany). Water was purified by a Milli-Q system (Millipore, Billerica, MA, USA). The standards of imperatorin, isoimperatorin, psoralen, protopine, and DL-tetrahydropalmatine were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Byakangelicin, byakangelicol, and coptisine were acquired from Chengdu Herbpurify Co. (Chengdu, China). Xanthotoxin, oxypeucedanin, and tetrahydroberberine were purchased from Shanghai Winherb Co. (Shanghai, China). Bergapten was obtained from Shanghai Baiyi Biotech (Shanghai, China). Corydaline was purchased from Sigma–Aldrich Co. (St. Louis, MO, USA). α-Allocryptopine was supplied by Shenzhen Meihe Biotech (Shenzhen, China). The purities of all standards were no less than 98% and suitable for RRLC-ESI-Q-TOF analysis.

Angelica dahurica (Fisch. ex Hoffm.) Benth. et Hook. f. and *Corydalis yanhusuo* W. T. Wang were obtained from the Anguo Medicinal Material Market (Hebei, China) in February 2011, and the drugs were identified by Pharmacist Xirong He (research assistant at the China Academy of Chinese Medical Sciences). Voucher specimens were deposited in the Institute of Chinese Materia Medica (China Academy of Chinese Medical Sciences).

2.2. Instrument and RRLC-ESI-Q/TOF conditions

Chromatographic experiments were performed on an Agilent 1200 Rapid Resolution Liquid Chromatography System (Agilent Crop., Santa Clara, CA, USA) equipped with a quaternary pump, an online vacuum degasser, an autosampler and an automatic thermostatic column oven. The separation was carried out on Poroshell DB C_{18} column (3.0 mm × 150 mm, 2.7 μm, Agilent) at 35 °C with a flow rate of 0.4 mL/min and injection volume was 5 μL. Mobile phase was a mixture of 0.1% formic acid–water (A) and acetonitrile (B). The gradient program of mobile phase was carried out as follows: 0–10 min, 20–30% B; 10–25 min, 30–60% B; 25–35 min, 60–100% B; 35–40 min, 100% B.

MS detection was conducted on an Agilent 6520 quadrupole time-of-flight mass spectrometer equipped with an electrospray ionization source (ESI). Ionization was performed in the positive electrospray mode. On the basis of the best response for most compounds, the final parameters were as follows: fragmentor (150 V), Vcap (3500 V), nebulizer (30 psi), drying gas (N_2, 10 L/min, 350 °C). The TOF-MS was calibrated daily, according to the manufacturer's recommendations. The testing mass range was set from m/z 50 to 1000 with a scanning rate of 2 s^{-1}. Reference masses at m/z 121.05087 (purine, 1.125×10^{-6} mol/L, Agilent Corp.) and m/z 922.00980 (hexakis (1H, 1H, 3H-tetrafluoropropoxy)-phosphazine, 5×10^{-6} mol/L, Agilent Corp.) were continually introduced, along with the LC stream for accurate mass calibration. The collision energy for each compound varied according to this formula: [5 × (mass/100)] + 5. For example, the collision energy for an ion with nominal m/z of 300 would be 20 V.

2.3. Preparation of YZP extract and the solutions for analysis in vitro

The procedures of YZP extract preparation were as follows: Herbs were ground into powders, respectively. Based on the optimum extraction conditions in the previous research [12], a total of 400 g of *Corydalis yanhusuo* and 200 g of *Radix Angelica dahuricae* were macerated with 2.4 L of 70% ethanol for 2 h, and then extracted twice under thermal reflux for 2 h. The extracts were filtrated out by running through absorbent cotton inserted in a funnel. The two extracts were combined and evaporated to less than 200 mL in a rotary evaporator R-210 (Buchi Ltd., Labortechnik AG, Switzerland) at 50 °C under reduced pressure. Subsequently, the concentrated extracts were freeze-dried using a freeze dryer (TF-FD-1; Tianfeng Instrument Co., Shanghai, China) at −30 °C, and then pressed into powder with a 60-mesh sieve.

2.4. Preparation of standards, calibration standard and quality control (QC) samples

Standard stock solutions for 15 components (protopine, α-allocryptopine, DL-tetrahydropalmatine, coptisine, berberine, tetrahydroberberine, corydaline, bergapten, byakangelicin, xanthotoxin, oxypeucedanin, byakangelicol, imperatorin, psoralen, and isoimperatorin) were prepared respectively by weighing the required amounts into volumetric flasks and dissolving in 75% methanol.

Calibration curve stock solutions of six analytes including protopine, α-allocryptopine, DL-tetrahydropalmatine, tetrahyberberine, corydaline and byakangelicin were prepared in 75% methanol at 1 mg/mL. Prior to use, all stock solutions were diluted with 75% methanol to obtain the combined working solution with the concentration of 10 μg/mL for the six analytes. The combined solution was further diluted with methanol in formation of the following standard solutions, with the concentration of 50, 100, 250, 500, 1000, 2000 and 5000 ng/mL respectively for five standards including protopine, α-allocryptopine, DL-tetrahydropalmatine, tetrahyberberine, corydaline and 15, 30, 75, 150, 300, 600 and 1500 ng/mL for byakangelicin. All the mixed standard solutions were stored at 4 °C.

100 μL combined working solution was evaporated to dryness at 37 °C under the stream of nitrogen, to which 200 μL blank rat plasma were spiked sequentially, and were vigorously vortex mixed for 30 s. For method validation, QC samples were prepared independently according to the same sample preparing protocol, with a spiked concentration of 250 ng/mL for protopine, α-allocryptopine, DL-tetrahydropalmatine, tetrahyberberine, corydaline and 75 ng/mL for byakangelicin.

2.5. Preparation of plasma and CSF samples

The rat plasma samples were prepared by liquid–liquid extraction with ethyl acetate. A 200 μL aliquot of ethyl acetate was added to a clean tube containing 100 μL of plasma sample, thoroughly mixed by vortex for 3 min, then centrifuged at 2000 × *g* for 15 min