Journal of Pharmaceutical and Biomedical Analysis 115 (2015) 214–224

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

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

Simultaneous determination of thirteen flavonoids from *Xiaobuxin-Tang* extract using high-performance liquid chromatography coupled with electrospray ionization mass spectrometry

Meifeng Cen[a,1], Jinxiu Ruan[b,1], Lihua Huang[a], Zhenqing Zhang[b], Nengjiang Yu[b], Youzhi Zhang[b], Xuange Cheng[a], Xiaohong Xiong[a], Guixiang Wang[a], Linquan Zang[a], Sujun Wang[a,*]

[a] *School of Pharmacy, Guangdong Pharmaceutical University, Guangzhou 510006, People's Republic of China*
[b] *Key Laboratory of Drug Metabolism and Pharmacokinetics, Beijing Institute of Pharmacology and Toxicology, 27 Taiping Road, Beijing 100850, People's Republic of China*

ARTICLE INFO

Article history:
Received 5 May 2015
Received in revised form 16 July 2015
Accepted 17 July 2015
Available online 26 July 2015

Keywords:
HPLC-ESI–MS
Thirteen flavonoids
Intestinal absorption

ABSTRACT

A simple and reliable high performance liquid chromatography coupled with electrospray ionization mass spectrometry (HPLC-ESI–MS) analysis method was established to simultaneously determine thirteen flavonoids of *Xiaobuxing-Tang* in intestine perfusate, namely onpordin, 3′-*O*-methylorobol, glycitein, patuletin, genistein, luteolin, quercetin, nepitrin, quercimeritrin, daidzin, patulitrin, quercetagitrin and 3-glucosylisorhamnetin. Detection was performed on a quadrupole mass spectrometer equipped with an electrospray ionization (ESI) source operating in negative ionization mode. Negative ion ESI was used to form deprotonated molecules at *m*/*z* 315 for onpordin, *m*/*z* 299 for 3′-*O*-methylorobol, *m*/*z* 283 for glycitein, *m*/*z* 331 for patuletin, *m*/*z* 269 for genistein, *m*/*z* 285 for luteolin, *m*/*z* 301 for quercetin, *m*/*z* 477 for nepitrin, *m*/*z* 463 for quercimeritrin, *m*/*z* 461 for daidzin, *m*/*z* 493 for patulitrin, *m*/*z* 479 for quercetagitrin, *m*/*z* 477 for 3-glucosylisorhamnetin and *m*/*z* 609.2 for rutin. The linearity, sensitivity, selectivity, repeatability, accuracy, precision, recovery and matrix effect of the assay were evaluated. The proposed method was successfully applied to simultaneous determination of these thirteen flavonoids, and using this method, the intestinal absorption profiles of thirteen flavonoids were preliminarily predicted.

1. Introduction

Xiaobuxin-Tang (XBXT), a traditional Chinese herbal prescription used to remit depressive disorders, comprising *Haematitum*, *Flos Inulae*, *Folium Phyllostachydis Henonis* and *Semen Sojae Preparatum* four crude medicines, was originally recorded in the silk scroll manuscript of "*Fuxingjue Zangfu Yongyao Fayao*" [1]. We had found that the major chemical constituents of the extract (XBXT -2) from XBXT were flavones, flavonols, isoflavones and their glycosides [2]. Pharmacological investigations reveal that those compounds are responsible for significantly anti-depressant activity [3–5]. Onpordin, 3′-*O*-methylorobol, glycitein, patuletin, genistein, luteolin, quercetin, nepitrin, quercimeritrin, daidzin, patulitrin, quercetagitrin and 3-glucosylisorhamnetin are the thirteen main flavonoids in *XBXT-2*. However, quantification and pharmacokinetic study of these flavonoids of *XBXT-2* have not been conducted. High performance liquid chromatography coupled with electrospray ionization mass spectrometry (HPLC-ESI–MS) has been applied for in vivo analysis of glycitein [6], genistein [6], daidzin [7] and quercetin [8] of other plants with properties of high mass accuracies and high selectivity, nevertheless, it has not been developed and reported along with in vivo studies of patulitrin, nepitrin, patuletin, luteolin, onpordin, quercimeritrin, quercetagitrin, 3′-O-methylorobol and 3-glucosylisorhamnetin. Furthermore, HPLC-ESI–MS method has not been reported to quantification of 3-glucosylisorhamnetin, onpordin, quercimeritrin and quercetagitrin these four flavonoids in some plant materials.

Chinese herbal medicines (TCM) are usually used through oral administration, this renders intestinal absorption is significantly

* Corresponding author at: School of Pharmacy, Guangdong Pharmaceutical University, Guangzhou Higher Education Mega Center, 280 Wai Huan East Road, Guangzhou 510006, People's Republic of China.
E-mail address: wsj7272@126.com (S. Wang).
[1] These authors contribute equally to this work.

http://dx.doi.org/10.1016/j.jpba.2015.07.015
0731-7085/

crucial to evaluate the bioactivity of these flavonoids. Although investigations on intestinal absorption of flavonoids have been performed to a great extent with several methods included Caco-2 cell monolayer model [9], in situ single-pass intestinal perfusion [10], in vitro everted gut sac model [11], and in vivo pharmacokinetic study [12], the in situ vascularly perfused rat intestine preparation method available is scarce and has not been used to study these thirteen flavonoids as well.

In this study, a sensitive, precise and convenient HPLC-ESI–MS method for simultaneous determination of thirteen flavonoids compounds in *XBXT-2* was established and used to determine them in the intestinal perfusate for the first time. The in situ vascularly perfused rat intestine preparation was used to preliminarily investigate their intestinal absorption characteristic, and the obtained results would provid a meaningful basis for their in vivo pharmacokinetic studies.

2. Experimental

2.1. Chemicals, reagents and materials

The extract (*XBXT-2*) from *Xiaobuxing-Tang* was provided by Professor Yimin Zhao (Laboratory of phytochemistry, Beijing Institute of Pharmacology and Toxicology, Beijing, China) and the method of extracting flavonoids was according to the literature previously described [13]. Reference substances of onpordin, 3′-O-methylorobol, glycitein, patuletin, genistein, luteolin, quercetin, nepitrin, quercimeritrin, daidzin, patulitrin, 3-glucosylisorhamnetin, quercetagitrin and rutin (≥96.5%, purity) were purchased from Shanghai Forever Biotech Co., Ltd. (Shanghai, China). 5% Bovine serum albumin (≥98.0%, purity) was purchased from Shanghai Junruishengwu Biomart Co., Ltd. (Shanghai, China), 10% washed rat red blood cells were taken from the healthy partial adult male sprague-dawley rat blood withdrawn within one week. 3% dextran (T-40), 0.02% dexamethasone and 0.004% noradrenalin were obtained from National Institute for the Control of Pharmaceutical and Biological Products. The Krebs-Ringer solution (intestinal perfusate, PH 7.4) consisted of NaH_2PO_4 (0.22 g), NaCl (7.8 g), $CaCl_2$ (0.37 g), $MgCl_2$ (0.22 g), KCl (0.35 g), $NaHCO_3$ (1.37 g), glucose (3.0 g), 3% dextran (T-40), 5% bovine serum albumin, 10% washed rat erythrocyte, 0.02% hexadecadrol and 0.004% noradrenalin dissolved in 1.0 L of deionized water. Acetonitrile and methanol were of HPLC grade (Fisher Scientific, Fair Lawn, NJ, USA). All other chemicals were of analytical reagent grade. Distilled, deionized water was produced by a Milli-Q Reagent Water System (Millipore, MA, USA).

2.2. Instrument and chromatographic conditions

The molecular weights of thirteen flavonoids were determined performing on Agilent LC–MSD quadrupole mass spectrometer, equipped with a series 1100HPLC system consisting of a binary pump, automatic solvent degasser and autosampler. Intersil C_8-3 column (250 mm × 2.1 mm, 5 μm, DIKMA, Japan) was used and the column temperature was maintained at 25 °C. The binary solvent system was consisted of A (acetonitrile) and B (water with 5 mM ammonium formate) using a gradient elution at a flow of 0.2 mL/min. The gradient conditions of the mobile phase were as follows: 0–10 min, 20% A, 10–15 min, 25% A, 15–20 min, 40% A, 20–25 min, 55% A, 25–40 min, 20% A. The Agilent MS system operated in negative ion electrospray ionization was used to form deprotonated molecules for all compounds. Nitrogen was used as nebulization gas and was set to 10 L/min at a temperature of 350 °C. The capillary voltage was set at 4.0 kV and the nebulizer pressure was 40 psi.

2.3. In situ vascularly perfused rat intestine preparation

The surgical procedure for the in situ vascularly perfused rat intestine preparation was similar to that for the in situ vascularly perfused rat intestine–liver preparation with modification [14]. Adult male SD rats (180–220 g) were anesthetized with intraperitoneal injection of sodium pentobarbital solution following overnight fasting. The abdomen was opened with a midline incision to expose the organs, pyloric vein, celiac artery, gastric artery, splenic blood vessels, and the lower aorta were tied before and after the common juncture of the superior mesenteric artery and right renal artery. The surgical cannulation for intestinal perfusion was illustrated in Fig. 1 and performed on peristaltic pump (HL-2, Luxi Analysis Instrument Co., Ltd., Shanghai, China). The intestine was perfused via the superior mesenteric artery, and its venous outflow into the portal vein, therefore, the diaphragm was cut to allow the outflow catheter to be in a straight line with the portal vein. Perfusion was initiated at a constant rate of 2–3 mL/min after the insertion of the cannula and was gradually increased to the desired value of 7.5 mL/min upon completion of the outflow circuit after the completion of surgery. The perfusate was oxygenated with carbogen (95% oxygen and 5% carbon dioxide) at 1.0 L/min. The surgical procedure for initial 15 min to wash the residual blood in the vessel and balance the whole perfusion path was carried out with perfusate that was not appended 3% dextran (T-40) and 10% washed rat erythrocyte, and then the perfused flow was increased to 10 mL/min before intraduodenal adminstration. The incisions were made on the duodenum to allow for insertion of the cannulas and on the ileocecal junction for collection of intestinal perfusate. Perfusion was started at a flow of 10 mL/min with the perfusate (PH 7.4) contained 3% dextran (T-40) and 10% washed rat erythrocyte, and the experimental time lasted for 2 h. Effluent perfusate samples were collected quantitatively from the reservoir before drug administration (zero time) and other 9 intervals (5, 15, 30, 40, 60, 75, 90, 105 and 120 min) after intraduodenal administration. The rats were placed on a flat plate under a heating pad to maintain the temperature at 37 °C throughout the experiment and a cotton gauze moistened with isotonic saline solution was used to cover the surgical incision to prevent the intestine drying.

2.4. Animals and analysis sampling

Adult male Sprague-Dawley (SD) rats, weighing 180–220 g, were purchased from the Experimental Animal Center of Guangzhou University of Chinese Medicine (Guangzhou, China). The rats were housed in the stainless steel metabolic cages equipped with an automated watering valve and cared for according to the regulations of the animal committeen under a constant temperature at (22 ± 1 °C), humidity at (50 ± 20%), 12 h light/12 h dark cycle for one week. The experimental protocol was approved by the Ethics Committee of Guangdong Pharmaceutical University. The anaimls were fasted for 12 h prior to study and anesthetized with sodium pentobarbital solution via intraperitoneal injection. Each rat was given *XBXT-2* at a single does of 0.2 g through intraduodenal administration, and perfused 2 h with the in situ vascularly perfused rat intestine preparation. The samples (500 μL) were withdrawn at different time intervals (0, 5, 15, 30, 40, 60, 75, 90, 105 and 120 min).

2.5. Sample preparation

2.5.1. Stock solutions, calibration standards and quality control (QC)

Standard stock solutions of the thirteen flavonoids and rutin (internal standard, IS), were separately prepared by amounts