Fitoterapia 90 (2013) 57–64

Contents lists available at SciVerse ScienceDirect

Fitoterapia

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

A liquid chromatography–tandem mass spectrometry method for pharmacokinetics and tissue distribution of a camptothecin quaternary derivative in rats

Qingyong Li [a,b,*], Qiaochu Zhu [a], Xueke Zhang [a,**], Changlong Hua [a], Xiaoqiu Deng [a], Tengfei Zhao [a], Baihe Sun [a]

[a] The Key Laboratory of Forestry Plant Ecology, Northeast Forestry University, Ministry of Education, Harbin 150040, China
[b] College of Pharmaceutical Science, Zhejiang University of Technology, Hangzhou 310032, China

ARTICLE INFO

Article history:
Received 19 March 2013
Accepted in revised form 13 June 2013
Available online 27 June 2013

Keywords:
Camptothecin derivative
LC–MS/MS
Pharmacokinetics
Tissue distribution

ABSTRACT

A liquid chromatography–tandem mass spectrometry (LC–MS/MS) method was developed to identify and quantify the camptothecin quaternary derivative CPT8 for application in pharmacokinetics and tissue distribution studies. Rat plasma and tissue samples were extracted with methanol by using camptothecin as the internal standard (IS). Chromatographic separation of CPT8 and the IS was achieved using a Hypersil GOLD C_{18} column, with a flow rate of 1.0 mL/min followed by quantification with tandem mass spectrometry, operating with electrospray ionization in the positive ion mode and by applying multiple reaction monitoring. The MS/MS ion transitions were monitored at m/z 484.3–361.2 for CPT8 and m/z 349.0–305.2 for the IS (CPT). A calibration curve was constructed using CPT8 concentrations ranging from 2.5 ng/mL to 2500 ng/mL ($r > 0.993$). The efficiency of CPT8 extraction from plasma and tissue samples ranged from 91.23% to 105.4%. Intra- and inter-day precision (relative standard deviation) values were 0.21% and 7.25%, respectively. No matrix effects were observed. The freeze–thaw stability, post-extraction stability, and stability following short- and long-term storage at low temperatures ranged from 84.12% to 108.2%. The preclinical data obtained using this method is expected to facilitate future clinical investigations of CPT8.

1. Introduction

Camptothecin (CPT) is an alkaloid extracted from *Camptotheca acuminate* [1] and has antitumor activity. However, the in vivo use of CPT is limited by its poor water solubility and high toxicity [2]. Industrial and academic institutions have synthesized CPT derivatives with an aim to improve solubility in water, which has led to the development of pro-drugs [3–6]. In particular, topotecan and irinotecan, which appear to possess favorable water solubility, have been approved for marketing as antitumor drugs [7]. A number of novel water-soluble camptothecin derivatives are currently undergoing pre-clinical and clinical research trials.

Because camptothecin has shown promise as an anticancer agent, there has been an increasing demand for in vivo analytical studies on its pharmacokinetics and tissue distribution. There has been a great deal of interest in the development and optimization of analytical methods for the investigation of CPT and its derivatives in plant extracts and biological fluids by using high-performance liquid chromatography (HPLC) with ultraviolet [8], fluorescence detection [9,10], and mass spectrometry [11,12]. Several studies have quantified camptothecin derivatives in blood samples [13,14]. Nevertheless, to date, the methods for the quantification of

* Correspondence to: Q. Li, 332# No. 26 Hexing Road, Harbin City 150040, Heilongjiang Province, China. Tel.: +86 451 82192336.
** Corresponding author.
E-mail addresses: li_qingyong@126.com (Q. Li), 605764896@qq.com (X. Zhang).

0367-326X/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.fitote.2013.06.012

quaternary ammonium camptothecin derivatives have been described in very few reports. Compared with other camptothecin derivatives, these camptothecins showed better water solubility and potential cytotoxicity in vitro than 9-nitrocamptothecin and 9-aminocamptothecin. The structure of characteristics shows that the opening rate of the intact lactone ring E could be remarkably reduced, so that the toxicity of the newly modified CPT and the water solubility could be greatly decreased, but the Topo I inhibitory activity and antineoplastic activity could be without getting diminished.

CPT8 is a novel synthetic camptothecin derivative that has recently been investigated as an antitumor compound. It has demonstrated high antitumor potency and good water solubility. In vitro, the half-maximal inhibitory concentration of CPT8 is less than 0.005 μg/mL in human colon cancer HCT8 cells [15]. In rats, CPT8 (40 mg/kg) suppresses the growth of S180 xenograft tumors with an inhibition rate greater than 89.81%. These antitumor effects are better than other derivatives, such as 9-nitrocamptothecin and 9-aminocamptothecin, suggesting that it may be useful as an alternative clinical antitumor therapeutic agent.

Since pharmacokinetic analysis and tissue distribution using an appropriate animal model are critical steps in the development of novel therapeutic drugs, a robust method is essential for identifying and quantifying CPT8 in various body compartments. To this end, we established a sensitive, valid, specific, and rapid LC–MS/MS method for determining the concentration of CPT8 in plasma and tissues. Conditions for sample preparation and analysis were optimized. The novel methods described herein were applied in the evaluation of pharmacokinetics and tissue distribution of CPT8 following its oral administration.

2. Experimental

2.1. Reagents and chemicals

CPT8 (Fig. 1) was synthesized as previous described [15]. CPT (Fig. 1) was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). The purity of both agents was greater than 99.5%. HPLC-grade methanol was obtained from J&K Chemical Ltd. (Beijing, China) and HPLC-grade formic acid was obtained from Dima Technology Inc. (Beijing, China). All other solvents or chemicals were of analytical grade. Water used in all experiments was purified by filtration through ion exchange columns using a 0.22 μm filter (Milli-Q purification system, Millipore Corporation, Bedford, MA, USA).

2.2. Instrumentation and chromatographic conditions

An Agilent 1100 series HPLC system (Agilent Technologies, San Jose, CA, USA) was used for chromatographic analyses. The whole system consisted of a G1312A HPLC binary pump, a 7725i manual injector, and a G1379A degasser. The effluent was monitored using an API3000 triple-stage quadruple mass spectrometer (Applied Biosystems, Concord, Canada) equipped with an electrospray ionization (ESI) source. The separation was achieved with a Hypersil GOLD C_{18} column (200 × 4.6 mm, 5 μm, Thermo, USA) maintained at 20 °C. The mobile phase consisted of methanol and water 80:20 (v/v) containing 0.1% formic acid. The flow rate was maintained at 1.0 mL/min. The sample injection volume was 10 μL. Typical parameters for the Turbo Ionspray source set were: curtain gas, 10 psi; nebulizer gas, 12 psi; ion spray voltage, 5500 V; temperature, 300 °C; entrance potential, 10 V; focusing potential, 400 V; collision cell exit potential, 8 V; and declustering potential, 80 V. The protonated molecular ions of CPT8 and IS were fragmented at a collision energy of 40. The mass spectrometer was operated in ESI positive ion mode, and the detection of ions was performed in the multiple reaction monitoring (MRM) mode. The optimized transitions were monitored at m/z 484.3–361.2 for CPT8 and m/z 349.0–305.2 for the IS (CPT). Analyst software Version 1.4 (Applied Bio-systems/MDSSCIEX, Foster City, CA, USA) was used to integrate the peak areas for all components.

2.3. Animals

Female Sprague Dawley (SD) rats weighting 200–220 g (8 weeks old) were obtained from National Rodent Laboratory Animal Resources (Shanghai, China). Upon arrival, animals were acclimated for 4 days to a 12 h light/dark cycle in a temperature-controlled environment with appropriate humidity, in accordance with the National Institutes of Health publication Guide for the Care and Use of Laboratory Animals.

2.4. Preparation of standards and quality control samples

Stock solutions of CPT8 and CPT (1 mg/mL and 500 μg/mL, respectively) were prepared in methanol. The solutions were then serially diluted with methanol to obtain working solutions for CPT8 at concentrations ranging from 0.025 to 50 μg/mL, along with a working solution of CPT as the IS (5 μg/mL). All working solutions were stored at 4 °C and

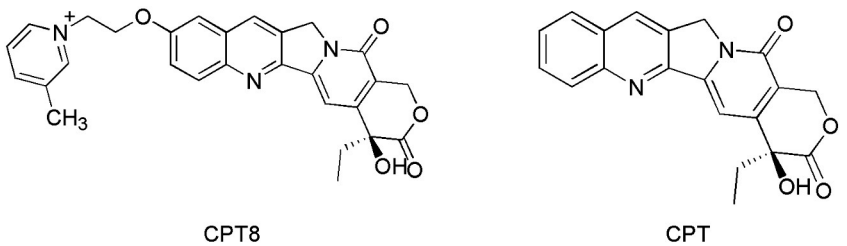

Fig. 1. Chemical structures of CPT8 and CPT (IS).