Journal of Pharmaceutical and Biomedical Analysis 120 (2016) 228–234

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

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

HPLC-MS/MS method for the simultaneous determination of MB07133 and its metabolites, cytarabine and arabinofuranosyluracil, in rat plasma

Dan Wang[a], Qingqing Xiao[b], Wanqiu Yang[a], Wei Qian[c,**], Jin Yang[a,*]

[a] Center of Drug Metabolism and Pharmacokinetics, China Pharmaceutical University, Nanjing 210009 China
[b] Department of Clinical Pharmacy, School of Pharmacy, China Pharmaceutical University, Nanjing 210009 China
[c] Department of Phase I Clinical Trials, Affiliated Nanjing University Medical College, Nanjing 210008 China

ARTICLE INFO

Article history:
Received 8 August 2015
Received in revised form
20 December 2015
Accepted 24 December 2015
Available online 29 December 2015

Keywords:
Prodrug
MB07133
Cytarabine
Arabinofuranosyluracil
HPLC-MS/MS

ABSTRACT

MB07133 is an intravenously administered cytarabine mononucleotide (araCMP) prodrug, for the treatment of hepatocellular carcinoma (HCC). A simple, selective and sensitive HPLC-MS/MS method using high pressure liquid chromatography (HPLC) coupled to triple-quadrupole mass spectrometer, was developed and validated for the detection of prodrug MB07133 and its metabolites, cytarabine (araC) and arabinofuranosyluracil (araU) in rat plasma. Protein precipitation using 3% trichloroacetic acid (TCA) was employed to extract analytes from 100 μL rat plasma. Adequate separation of araC and araU from their endogenous compounds was achieved on the Synergi® fusion-RP column (150 mm × 4.6 mm, 4 μm) by a gradient-elution with a mobile phase consisting of ammonium formate (1 mM) and methanol at a flow rate of 1 mL/min. Multiple reaction monitoring mode (MRM) was applied in the detection of MB07133, araC, araU and Ganciclovir (internal standard) with ion pairs 441.2/330.2, 244.2/112.2, 245.2/113.2 and 256.1/152.2, respectively. The assays were validated with respect to specificity, linearity (100–50000 ng/mL for MB07133, 2–1000 ng/mL for araC and araU), accuracy and precision, extraction recovery, matrix effect and stability. The validated method has been successfully applied to an intravenous bolus pharmacokinetic study of MB07133 in male Sprague-Dawley rats (18 mg/kg i.v.).

1. Introduction

Cytarabine (araC) is a pyrimidine nucleoside analogue used clinically for the treatment of acute and chronic leukemia. Cytarabine is converted initially to the cytarabine mononucleotide (araCMP) via phosphorylation catalyzed by deoxycytidine kinase (dCK) and then ultimately to its active triphosphate (araCTP) form to damage DNA by multiple mechanisms [1,2]. Unfortunately, therapy based on araC has limited effcacy against solid tumors due to the low abundance of nucleoside transporters and the rate-limiting phosphorylation (araC to araCMP), resulting in non-cytotoxic levels of araCTP in tumor cells at tolerable doses [3]. Therefore, bypassing the need for nucleoside transporters and the rate-limiting phosphorylation and delivering high concentration of araCMP directly into tumor cells could be an effective approach. However, araCMP is charged negatively at physiological pH and it cannot permeate through the lipid-rich cell membrane. As a result, a series of araCMP prodrugs have been developed by various groups [4,5]. MB07133 is an intravenously administered araCMP prodrug for the treatment of hepatocellular carcinoma (HCC) [6,7]. It is activated to araCMP by the cytochrome P450 3A (CYP3A), which is predominantly produced in the liver, but cannot be activated in plasma or non-CYP3A expressing tissues [6,8]. Then, araCMP is converted to araCTP by nucleotide kinases, to exert its antineoplastic activity. Subsequently, araCTP and araCMP are catabolized to araC, which can be released into the periphery circulation or further deaminated to arabinofuranosyluracil (araU). Therefore, targeting of the HCC tumor is achieved through tissue (liver) targeting providing a safe chemotherapy for HCC patients [3]. It has exhibited favorable pharmacokinetic characteristics and fewer adverse effects during in vivo and in vitro preclinical studies [3,6,7]. Moreover, a phase I clinical trial is under way in China. Here, a selective and accurate analytical method for the simultaneous determination of MB07133, araC and araU in rat plasma has been established to support pharmacokinetic study of MB07133.

* Corresponding author at: 24# Tong Jia Xiang, Nanjing, China. Fax: +86 25 83271386.
** Corresponding author at: 321# Zhongshan Road, Nanjing, China.
E-mail addresses: qwguyi708@126.com (W. Qian), yjcpu@yahoo.com (J. Yang).

http://dx.doi.org/10.1016/j.jpba.2015.12.049
0731-7085/

The quantitative and simultaneous determination of araC and araU in rat plasma is challenging for some reasons: endogenous compounds cytidine and uridine are isomers of araC and araU, respectively. Their structures are similar in every point to those of araC and araU, but the arabinose has been replaced by a ribose [9]. Because of the similar structure, identical precursor and product ions between the endogenous compounds and analytes (araC and araU), they can neither be separated during the pretreatment process nor distinguished by the mass spectrum [10]. Meanwhile, they are present in rat plasma at typical levels of 2500 ng/mL and 250 ng/mL, respectively, which may interfere in the detection of araC and araU [11]. Therefore, an adequate chromatographic separation is imperative. However, their high polarity results in a limited retention and difficult separation on traditional reversed-phase columns [12].

Currently, a number of methods have been reported for the determination of araC and araU levels, separately or simultaneously, in plasma or other matrices. A series of methods were reported using ion-pairing liquid chromatography, mixed-mode liquid chromatography and porous graphitic carbon chromatography-tandem mass spectrometric for the determination of araC in mouse plasma; the lower limit of quantification (LLOQ) of these methods were 50 ng/mL [13–15]. Sun et al. developed a UPLC-MS/MS method to quantify araC and valcytarabine in rat plasma using cation exchange SPE as extraction technique with the LLOQ of 10 ng/mL [10]. A UPLC-MS/MS strategy to evaluate araC level in human plasma also was described (LLOQ 0.5 ng/mL), which separated araC from interfering endogenous compounds by applying a 100 mm column with 1.8 μm particles [16]. AraC and araU were quantified separately by Liao et al. because of the different extraction procedure and the LLOQs were 5 ng/mL and 1 ng/mL, respectively [17]. More recently, a simultaneous detection of araC and araU in leukemic cell was achieved by using an ion-pairing liquid chromatography, with a LLOQ 3.45 ng/mL for araC and 1.12 ng/mL for araU [18].

In the present study, we firstly developed a selective, sensitive and convenient HPLC-MS/MS method to detect MB07133, araC and araU in rat plasma, simultaneously under the reversed-phase conditions after one-step precipitation preparation. The method was validated and successfully applied to the preclinical studies of MB07133.

2. Materials and methods

2.1. Reagents and chemicals

HPLC-grade methanol, acetonitrile and tetrahydrouridine (THU) were obtained from Merck (Darmstadt, Germany). Trichloroacetic acid (TCA) and ammonium formate were purchased from Shanghai Lingfeng Chemical Reagent Co., Ltd. (Shanghai, China) and Nanjing Chemical Reagent Co., Ltd, respectively. MB07133, araC, araU and the injection of MB07133 were kindly provided by Xintong Pharmacy Co., Ltd. (Xi'an China). Ganciclovir (GCV) was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China) as an internal standard (IS). Blank rat whole blood and plasma were freshly collected and used throughout the validation and application of method. Ultrapure water was obtained from a UPH Ultrapure Water System (UPH-II-5T, Chengdu, China).

2.2. Instrumentation

A Shimadzu LC-20A series chromatographic system (Shimadzu Corporation UFLC XR, Kyoto, Japan) was employed, which consisted of two LC-20AD binary pumps, a DGU-20A3 degasser, a SIL-20AC autosampler and a CTO-20A column oven. An Applied Biosystem/Sciex (Foster City, CA, USA) API4000 triple-quadrupole mass spectrometer was used for detection. Chromatographic separation was achieved using a Synergi® fusion-RP column (150 mm × 4.6 mm internal diameter, 4 μm particles, 80 Å, Phenomenex, Torrance, CA, USA). All data were acquired and processed using Analyst 1.5.2 software (Applied Biosystems/Sciex).

2.3. HPLC-MS/MS analytical conditions

For the simultaneous analysis of MB07133, araC and araU, a step-gradient elution program was applied, consisting of mobile phases ammonium formate (1 mM) and methanol as follows: 0% B from 0.0 to 3.0 min; 0-30% B from 3 to 4.5 min; 30% B from 4.5 to 6 min; 30-0% B from 6 to 6.1 min; and, 0% B from 6.1 to 9 min (equilibration). The flow rate was set to 1 mL/min. The analytical column temperature was maintained at 40 °C.

The API4000 MS was operated in positive, multiple reaction monitoring (MRM) mode with an electrospray ionization (ESI) interface. MRM transitions and MS conditions for each analyte are summarized in Table 1.

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

Stock solutions were individually prepared in methanol: water (1:1, v/v) at a concentration of 10 mg/mL for MB07133, 1 mg/mL for araC, araU and GCV, then stored at −20 °C. Three stock solutions (MB07133, araC and araU) were mixed and diluted with water to generate a final mixed standard solution containing 5 mg/mL of MB07133, 100 μg/mL of araC and araU. Nine-point working standard solutions of MB07133, araC and araU and were diluted from the mixed standard solutions with water. A working IS solution (500 ng/mL) was prepared in a similar way. All the solutions were stored at −20 °C until use.

Blank rat plasma was prepared from whole blood after addition of 25 μg THU per mL. Then, calibration samples (MB07133: 100, 200, 500, 1000, 2000, 5000, 10000, 20000, 50000 ng/mL; araC and araU: 2, 5, 10, 20, 50, 100, 200, 500, 1000 ng/mL) were prepared by spiking 95 μL blank rat plasma with 5 μL appropriate working standard solution. QC samples were also prepared in a similar approach at low (LQC), middle (MQC) and high (HQC) concentrations of 200, 5000, 40000 ng/mL for MB07133, 4, 100, 800 ng/mL for araC and araU. The three QC concentrations for analytes are uniformly distributed in the wide linear ranges, though the selection of MQCs is neither in line with current FDA bioanalytical guidance [19] nor EMA guideline [20].

2.5. Sample preparation

IS working solution (10 μL) was added to100 μL of plasma and vortexed for 0.5 min in a 1.5 mL micro-centrifuge tube. After the addition of 3% aqueous TCA (300 μL), the mixture was vortexed for 1 min and centrifuged at 13000 × g for 6 min at 4 °C to remove precipitated proteins. The supernatant was transferred into a clean tube and centrifuged at 13000 × g for 6 min again. Then, 10 μL of the supernatant was injected into the HPLC-MS/MS system for analysis.

2.6. Method validation

2.6.1. Selectivity and carry-over

Selectivity was assessed by analyzing six different sources of blank plasma, a blank sample spiked with analytes at LLOQ level and IS and a plasma sample from rat injected with MB07133. Carryover was determined by injecting a blank sample after the upper limit of quantification (ULOQ) on the calibration curve.