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A rapid and sensitive UHPLC–MS/MS method for quantification of 83b1 in plasma and its application to bioavailability study in rats

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ABSTRACT

Great attentions have been drawn by quinoline for its broad bioactivity as anti-fungal, anti-bacterial and anti-tumor activities. Compared with cisplatin, 83b1, a quinoline derivative, showed equal activity in anti-tumor and lower cyctotoxicity in normal cell. In this study, a simple, rapid and sensitive method for determination of 83b1 in rat plasma using UHPLC–MS/MS was developed for the first time. Loratadine was used as an internal standard (IS). Separation was performed on an Xterra MS C_{18} column by isocratic elution using acetonitrile: water solution with 1‰ formic acid (90:10, v/v) as mobile phase at a flow rate of 0.3 mL/min. A triple quadrupole mass spectrometer operating in the positive ion-switching electron spray ionization mode with selection reaction monitoring (SRM) was employed to determine 83b1 and IS transitions of m/z 321.82 → 147.84, 382.71 → 258.76 for 83b1 and Loratadine, respectively. The values of specificity, linearity and lower limit of quantification, intra- and inter- day precision and accuracy, extraction recovery, matrix effect and stability for this method satisfied the acceptable limits. The lower limit of quantification was 0.5 ng/mL with a linear range of 0.5–1500 ng/mL. The validated method was employed to study the bioavailability of 83b1 in rat by dosing with intravenous injection (1 mg/kg) and gavage (10 mg/kg), and the oral bioavailability of 83b1 in rat was calculated as 20.9 ± 8.8%.

1. Introduction

Esophageal cancer is a life-threaten disease puzzling us for decades and the chemotherapy was employed as a major strategy to treat cancers. Although there are some anti-esophageal cancer drugs used in clinic, the undesirable side effects as well as limited scope of activity is major cause of the limitations in treatment. Further more, drug resistance showed grave medical problems [1,2]. Therefore, the discovery of novel drugs with capability to anti-cancer and minimum side effects is imminently needed[3].

Quinoline derivatives which can be isolated from different plant sources are famous for its broad biological activities such as anti-fungal, antibacterial and HIV-1 replication inhibitors that have been used in traditional medicine as a remedy [4–6]. For example, the firstly reported natural quinoline alkaloids such as Kynurenic acid, 6-methoxykynurenic acid and 6-hydroxykynurenic acid can be isolated from plant *Ephedra pachyclada* [7]. Recently, quinoline-based compounds as tetrazole and benzothiazole have been reported to show cytotoxicity in breast cancer cells *in vitro* [8]. Furthermore,

Abbreviations: UHPLC–MS/MS, ultra-high performance liquid chromatography tandem mass spectrometry; ESCC, esophageal squamous cell carcinoma; PPAR, peroxisome proliferator-activated receptor; CDDP, cisplatin; COX-2, cyclooxygenase-2; PGH, prostaglandin H; MTS, 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfohenyl)-2*H*-tetrazolium; MTBE, methyl *tert*-butyl ether; SRM, selection reaction monitoring; QC, quality control; T_{max}, Time to maximum plasma concentration; C_{max}, maximum of plasma concentration; AUC_{0-t}, the area under the concentration-time curve 0 to time; AUC, $_{0-\infty 0-\infty}$the area under the concentration-time curve 0 to infinity; CL, apparent total body clearance; $t_{1/2}$, terminal elimination half-life; Vd, apparent volume of distribution; MRT, mean residence time 0 to time infinity; *F*, the absolute oral bioavailability.

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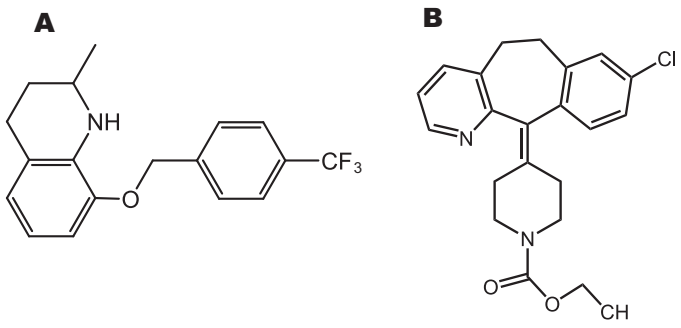

Fig. 1. The chemical structure of 83b1 (A) and Loratadine (B).

8-hydroxyquinoline derivatives exhibited strong anti-tumor activities against human cancer cell lines and hepatocellular carcinoma Hep3 B xenograft in athymic nude mice model and there was no observable damage on the vital organs at histological level [9]. These facts inspired researchers to further improve the efficacy and potency of quinoline by modifying its structure.

8-(4-(Trifluoromethyl) benzyloxy)-1,2,3,4-tetrahydro-2-methylquinoline named 83b1 (Fig. 1A) is a novel quinoline derivative and the activity for anti-esophageal cancer was evaluated *in vitro* and *in vivo*. 83b1 reveals the significant anti-tumor effects on a series of ESCC cell lines compared to the widely used anti-cancer drug CDDP for that the ratio of MTS_{50} for 83b1 versus CDDP was 0.73, 1.63, 0.80, 1.53, 0.33 and 2.73 in the ESCC cell lines KYSE-150, KYSE-450, KYSE-520, SLMT-1, HKESC-2 and HKESC-4 respectively. However, 83b1 shows a much lower cytotoxic effect on all non-tumor cells (NE-1, NE-3, HEK001) than CDDP for that the ratio of MTS_{50} for 83b1 versus CDDP was 0.2, 0.0004 and 0.015 in the non-tumor cells NE-1, NE-3 and HEK001 respectively. The following mechanism study demonstrated that the 83b1 could target the PPARδ that resulted in down regulation of COX-2 mRNA expression and reduced production of PGE2 in ESCC cell, and this pathway has been reported to induce tumor growth [10,11]. Furthermore, *in vivo* study also showed that 83b1 can significantly suppress the tumor in animal by 19th day and the tumor inside of the nude mice almost disappeared at the dose of 10 mg/kg. These facts showed that the compound 83b1 has the potential to develop as a novel anti- esophageal cancer drug [12].

It is necessary to develop a bioanalysis method to assay the compound concentration in biosample in the following druggability evaluation as the pharmacokinetics and toxicity study. Therefore, in this study, an ultra-high performance liquid chromatography tandem mass spectrometry (UHPLC–MS/MS) method was developed, validated and successfully applied to characterize the pharmacokinetic property of 83b1 in SD rat plasma after intravenous injection and oral administration for the first time.

2. Materials and methods

2.1. Chemicals and reagents

83b1 was kindly provided by Prof. Lu Gui, Institute of Drug Synthesis and Pharmaceutical process, School of Pharmaceutical Sciences of Sun Yat-sen University; Loratadine was purchased from Sigma-Aldrich (St. Louis, Missouri, US); HPLC grade acetonitrile, formic acid, Methyl *tert*-butyl ether (MTBE) and *n*-hexane were purchased from Tedia Company Inc. (Fairfield, OH, USA). Ultrapure water (18.2 MΩ cm at 25 °C) was obtained from a Millipore Direct-Q® ultra pure water system (Billerica, MA, USA). Blank rat plasma was obtained from healthy male SD rats with heparin as anticoagulant; Heparin was from the first affiliated hospital of Sun Yat-sen University.

2.2. Instrumentation and analytical conditions

The UHPLC (Thermo Fisher Scientific Inc., Boston, USA) system consisting of Ultimate 3000 RSLC system with binary pumps and S surveyor autosampler (Thermo Scientific Inc., San Jose, CA, USA) coupled with a TSQ Ultra triple-quadrupole mass spectrometer was used to analyze 83b1's concentration in rat plasma.

Samples were separated on an Xterra MS C_{18} column (150 mm × 2.1 mm, 5 μm; Waters, USA). The column temperature was set at 40 °C. The mobile phase was acetonitrile: 1‰ formic acid water solution (90:10, v/v) as an isocratic mobile phase with a flow rate of 0.3 mL/min and total run time was 2.0 min.

Mass spectrometric detection was performed on a TSQ Quantum Ultra triple-quadrupole mass spectrometer equipped with an electrospray ionization (ESI) interface. Both the 83b1 and Loratadine were monitored under positive ion-switching ESI conditions and quantified in selection reaction monitoring (SRM) mode with transitions of m/z 321.82 → 147.84, 382.71 → 258.76 for 83b1 and Loratadine, respectively. The source-dependent parameters for both compounds were as following: Spray Voltage, 4000 V; Vaporizer Temperatrue, 300 °C; Sheath Gas Pressure: 45 Psi; Ion Sweep Gas Pressure: 0; Aux Gas Pressure: 15 Psi; Capillary Temperature: 350 °C; Tbube Lens offset: 139; collision gas pressure 1.5 mTorr; collision energy (CE) 17 eV for 83b1 and 24 eV for Loratadine.

2.3. Preparation of stock and working solutions, calibration standards and quality control samples

Stock solution of 83b1 (2 mg/mL) and IS (200 μg/mL) were prepared and dissolved in acetonitrile. The IS working solution was diluted with acetonitrile to 0.4 μg/mL. The stock solution of 83b1 was then serially diluted in acetonitrile: water = 1:1 (v/v) to prepare standard working solutions at concentrations 5, 10, 50, 250, 1000, 2500, 5000 and 15,000 ng/mL.

The calibration standards solution at the concentration 0.5, 1, 5, 25, 100, 250, 500, 1500 ng/mL were made by spiking 10 μL standard working solution with 90 μL rat blank plasma, and the quality control (QC) samples of 83b1 were prepared from blank plasma at high, medium and low concentrations of 1200 (HQC), 100 (MQC) and 1 (LQC) ng/mL, respectively. All solutions were stored at −20 °C prior to analysis.

2.4. Sample preparation

100 μL rat plasma sample and 10 μL IS were put into a 1.5 mL Eppendorf tube and vortex-mixed for 1 min. Then, 500 μL extraction solvent (methyl *tert*-butyl ether: *n*-hexane = 7:3, v/v) was added into the tube and voxtex-mixed for 3 min, the mixture was deposited for 5 min and then centrifuged at 15,000*g* at 4 °C for 10 min. The supernatant organic phase was transferred into another Ependorf tube and evaporated to dryness using a vacuum concentrator at room temperature. Finally, the resultant residue was dissolved in 100 μL 90% acetonitrile water solution and vortex-mixed for 2 min, and centrifuged at 15,000*g* at 4 °C for 5 min. The corresponding supernatant was transferred to an autosampler vial with an insert and 5 μL aliquot was injected into the UHPLC–MS/MS system for analysis

2.5. Method validation

The specificity, linearity, lower limit of quantification, intra- and inter-batch precision and accuracy, extraction recovery, matrix effect and stability of this assay method were validated according to the US Food and Drug Administration[13].