Journal of Chromatography B, 981–982 (2015) 19–26

Contents lists available at ScienceDirect

Journal of Chromatography B

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

Pharmacokinetics of Rac inhibitor EHop-016 in mice by ultra-performance liquid chromatography tandem mass spectrometry☆

Tessa Humphries-Bickley[a], Linette Castillo-Pichardo[a,b], Francheska Corujo-Carro[c], Jorge Duconge[d], Eliud Hernandez-O'Farrill[d], Cornelis Vlaar[d], Jose F. Rodriguez-Orengo[a,c], Luis Cubano[e], Suranganie Dharmawardhane[a,*]

[a] Department of Biochemistry, School of Medicine, University of Puerto Rico, San Juan, PR, United States
[b] Department of Pathology and Laboratory Medicine, Universidad Central del Caribe, Bayamon, PR, United States
[c] Fundacion de Investigacion, Rio Piedras, PR, United States
[d] Department of Pharmaceutical Sciences, School of Pharmacy, University of Puerto Rico, San Juan, PR, United States
[e] Department of Anatomy and Cell Biology, Universidad Central del Caribe, Bayamon, PR, United States

ARTICLE INFO

Article history:
Received 14 September 2014
Accepted 21 December 2014
Available online 2 January 2015

Keywords:
EHop-016
Pharmacokinetics
UPLC/MS/MS
Rac
Cancer

ABSTRACT

The Rho GTPase Rac is an important regulator of cancer cell migration and invasion; processes required for metastatic progression. We previously characterized the small molecule EHop-016 as a novel Rac inhibitor in metastatic breast cancer cells and recently found that EHop-016 was effective at reducing tumor growth in nude mice at 25 mg/kg bodyweight (BW). The purpose of this study was to compare the pharmacokinetics and bioavailability of EHop-016 at different dosages in a single dose input scheme (10, 20 and 40 mg/kg BW) following intraperitoneal (IP) and oral gavage (PO) administration to nude mice. We developed and validated a rapid and sensitive method for the quantitation of EHop-016 in mouse plasma by ultra high performance liquid chromatography coupled with electrospray ionization tandem mass spectrometry (UPLC/MS/MS). Separation was carried out on an Agilent Poroshell 120 EC-C18 column (3.0 mm × 50 mm) using organic and aqueous mobile phases. EHop-016 was identified from its accurate mass and retention time from the acquired full-scan chromatogram and quantified by its peak area. The validated method was linear ($R^2 > 0.995$) over the range of 5–1000 ng/mL ($1/x^2$ weighting). Pharmacokinetic parameters were obtained by non-compartmental analysis using WinNonlin®. The area under the curve ($AUC_{0-\infty}$) ranged from 328 to 1869 ng h/mL and 133–487 ng h/mL for IP and PO dosing, respectively. The elimination half-life ($t_{1/2}$) ranged from 3.8–5.7 h to 3.4–26.8 h for IP and PO dosing, respectively. For both IP and PO administration, the $AUC_{0-\infty}$ values were proportional to the tested doses demonstrating linear PK profiles. The relative bioavailability of EHop-016 after oral gavage administration ranged from 26% to 40%. These results support further preclinical evaluation of EHop-016 as a new anti-cancer therapy.

Published by Elsevier B.V.

Abbreviations: IP, intraperitoneal; ROA, route of administration; AUC, area under the curve; BW, bodyweight; V_z, volume of distribution; ACN, acetonitrile; MeOH, methanol; UPLC/MS/MS, ultra performance tandem mass spectrometry; DMSO, dimethyl sulfoxide; CAV, cell accelerator voltage; EMV, electron multiplier voltage; PO, per oral; MRT, mean residence time; F_{rel}, relative bioavailability; $t_{1/2}$, half-life; C_{max}, maximum concentration; PK, pharmacokinetics.

☆ Grant numbers: This study was supported by National Institute on Minority Health and Health Disparities of the National Institutes of Health (NIMHHD/NIH) U54MD008149 to SD; Title V PPOHA P031M10505 and Title V Cooperative P031S130068 from U.S. Department of Education to UCC; and UPR RCM NIH/NIMHHD grants 5U54CA096297 and R25GM061838 to THB.

* Corresponding author at: Department of Biochemistry, University of Puerto Rico Medical Sciences Campus, P.O. Box 365067, San Juan, PR 00936-5067, United States. Tel.: +1 7877582525x1630.
E-mail address: su.d@upr.edu (S. Dharmawardhane).

http://dx.doi.org/10.1016/j.jchromb.2014.12.021
1570-0232/Published by Elsevier B.V.

1. Introduction

The small molecule EHop-016 (Table 1) is an effective Rho GTPase Rac-specific inhibitor. We previously reported that EHop-016 inhibits Rac activity of metastatic cancer cells with an IC_{50} of 1 μM. EHop-016 was found to disrupt the interaction of Rac with the guanine nucleotide exchange factor Vav2 and to inhibit the activity of the Rac downstream effector p21-activated kinase 1 (PAK1), Rac-directed lamellipodia formation and directed cell migration. Additionally, EHop-016 decreased Akt and Jun kinase (JNK) activities and the expression of c-Myc and cyclin D in breast and prostate cancer cell lines. At higher concentrations (>10 μM) EHop-016 also inhibited the activity of the Rho GTPase Cdc42, reduced cell viability, and activated apoptosis [1,2]. Other reports confirm our results and show that EHop-016 inhibits human T-lymphocyte and murine melanoma cell migration, as well as human and murine leukemic cell growth [3–5]. Since Rac and Cdc42 mediated cell signaling and migration/invasion are integral to cancer metastasis as well as immune and stromal cell function, in addition to being a potential anticancer compound, EHop-016 is a valuable tool for probing Rac/Cdc42/Vav activities in biological systems.

To validate an in vivo anticancer role for EHop-016, we recently characterized the pharmacological effects of EHop-016 in a mouse model of experimental metastasis. At 25 mg/kg body weight (BW), EHop-016 was effective at reducing mammary fat pad tumor growth, metastasis, and angiogenesis in athymic nude mice. A role for EHop-016 in angiogenesis inhibition was confirmed in vitro by demonstrating inhibition of Rac activity and capillary tube formation of endothelial cells [2]. There are currently no reports describing the pharmacokinetics (PK) of EHop-016 in an experimental mouse model. Such PK data are essential to explain the mechanisms of drug distribution and elimination to attain a better understanding of the pharmacology of EHop-016. To address this question, we first developed and fully validated a rapid and sensitive method for the quantification of Ehop-016 in mouse plasma by ultra high performance liquid chromatography coupled with electrospray ionization tandem mass spectrometry (UPLC/MS/MS). UPLC/MS/MS is the standard of choice for pharmacokinetic studies of new preclinical drug compounds because of its high sensitivity and specificity [6]. In particular, multiple reaction monitoring (MRM) using a triple quadrupole detector is a highly specific detection method with very low background interference. Therefore, this method was applied to evaluate the pharmacokinetics of EHop-016 in nude mice.

The aim of this study was to develop and validate an UPLC/MS/MS bioanalytical method to quantify the Rac inhibitor EHop-016 and evaluate its pharmacokinetic parameters in healthy mice. Therefore, we determined the time-course of plasma concentrations after intraperitoneal (IP) injection and oral gavage (per oral, PO) administration after a single dose input scheme at 10 mg/kg, 20 mg/kg, and 40 mg/kg BW EHop-016.

2. Materials and methods

2.1. Materials

Organic solvents acetonitrile (ACN), methanol (MeOH), and dimethyl sulfoxide (DMSO) were purchased from Sigma. Formic acid was from Agilent. Ammonium fluoride and ultrapure water were from Sigma. Non-sterile mouse plasma containing sodium citrate was from Equitech-Bio, Inc. EHop-016 and EHop-0141 were used as analytical standards, and EHop-016 was synthesized as previously described by us [1]. The synthesis of EHop-0141 is described in supplementary data. A primary stock solution of EHop-016 analyte (2 mg/mL) was prepared by dissolving 10 mg of analyte in 4 mL of DMSO and 1 mL of MeOH. A stock solution of EHop-0141 internal standard (21.9 mg/mL) was prepared by dissolving 7.3 mg of analyte in 334 μL of DMSO. Analyte stock solutions were stored in the dark at −20 °C. Standard solutions were prepared by diluting primary stock solutions in either mouse plasma or a solution of 65% deionized water (dH_2O) and 35% organic (50% ACN:50% MeOH).

2.2. Instrumentation

The analysis was performed on an Agilent automated UPLC system coupled to a triple quadrupole MS/MS. The data was collected and analyzed by the Agilent MassHunter software package (Version B.05.01). The UPLC separations were performed on a Poroshell 120 EC-C18 column (3 mm × 50.0 mm), 2.7 μm particle size (Agilent, CA), maintained at 40 °C, under gradient conditions. The mobile phases were 1 mM ammonium fluoride in dH_2O (Solution A) and 50% ACN/50% MeOH/0.1% formic acid (Solution B) and were equilibrated at an initial composition of 65% A:35% B. Subsequently, the percentage of B was increased by a linear gradient to 98% from 2.5 min to 3.0 min. The content of B was decreased by a linear gradient to 35% from 4.5 min to 5.0 min. The column was then stabilized for 1.5 min prior to the next injection. The total run time for analysis was 6.5 min and the injection volume was 1.0 μL. The 6460 triple quadrupole MS/MS with an electrospray ionization source was operated in the positive ionization mode using the following parameters: electrospray voltage +3848 V, gas temperature 300 °C, gas flow rate 8 L/min, sheath gas temperature 375 °C, sheath gas flow rate 11 L/min, nebulizer pressure 30 psi, and capillary current 4000 nA. An instrument autotune was performed prior to each analysis or when necessary using a certified calibration solution. The mass spectra of $[M+H]^+$ ions were recorded from 100 to 800 *m/z*. EHop-016 with precursor ion $[M+H]^+ = 431.6$ and EHop-0141 with precursor ion $[M+H]^+ = 443.6$ were used as analytical standards monitoring the MRM transitions $[M+H]^+ = 179.1$ and $[M+H]^+ = 124.1$ for EHop-016 and EHop-0141, respectively. Nitrogen (99.95%) was used as sheath gas.

2.3. Sample extraction procedure

Plasma samples were extracted by a standard protein precipitation method as follows: 100 μL of plasma was transferred to a 1.5 mL eppendorf tube. When appropriate, 50 μL of internal standard (500 ng/mL prepared in 50% dH_2O and 50% MeOH) was added to sample. Proteins in the matrix were precipitated by addition of 450 μL of ACN followed by vortexing for 10 min using a DVX-2500 at 2500 rpm. Samples were then centrifuged for 10 min at 3300 rpm at 4 °C using a Sorvall Legend 17R. Supernatant was transferred to borosilicate glass tubes and dried using a Savant Speed Vac. Dried samples were reconstituted by adding 200 μL of 65% dH_2O/35% organic (50% ACN:50% MeOH) solution and vortexed for 10 min at 2500 rpm. Samples were transferred to autosampler vials, sealed, and centrifuged at 3000 rpm on a Sorvall ST 16R centrifuge for 3 min prior to injection onto the UPLC system.

2.4. Method validation

Calibration curves were prepared using working solutions of standards at 5, 20, 50, 60, 100, 250, 500, and 1000 ng/mL EHop-016 in mouse plasma. Quality control samples were prepared using working solutions of 10, 30, 75, and 750 ng/mL. The lower limit of quantification (LLOQ) was defined as the lowest concentration (5 ng/mL) that could be quantified with acceptable accuracy and precision. The upper limit of quantification (ULOQ) was defined as the highest concentration (1000 ng/mL) that could be quantified with acceptable accuracy and precision. Intra-day precision and accuracy were measured by comparing