Journal of Chromatography B 1061–1062 (2017) 256–262

Contents lists available at ScienceDirect

Journal of Chromatography B

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

High-performance liquid chromatography with time-programmed fluorescence detection for the quantification of Levofloxacin in human plasma and cerebrospinal fluid in adults with tuberculous meningitis

Pham Van Toi[a,*], Thomas Pouplin[d], Nguyen Duc Khanh Tho[a], Pham Nguyen Phuong[a], Tran Thi Hong Chau[c], Nguyen Thuy Thuong Thuong[a], Dorothee Heemskerk[a], Tran Tinh Hien[a,b], Guy E. Thwaites[a,b]

[a] Oxford University Clinical Research Unit, Wellcome Trust Major Oversea Programme, Ho Chi Minh City- In Partnership with Hospital for Tropical Diseases Ho Chi Minh City, Viet Nam
[b] Centre for Tropical Medicine and Global Health, Nuffield Department of Clinical Medicine, University of Oxford, United Kingdom
[c] Hospital for Tropical Diseases Ho Chi Minh City, Viet Nam
[d] Mahidol-Oxford Tropical Medicine Research Unit, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand

ARTICLE INFO

Keywords:
Levofloxacin
High-performance liquid chromatography
Clinical pharmacokinetics
Human plasma
Human cerebrospinal fluid

ABSTRACT

An accurate and reliable high-performance liquid chromatography with time-programmed fluorescence detection was developed and validated to measure levofloxacin in human plasma and cerebrospinal fluid (CSF). After solid phase extraction process using Evolute® ABN 96 fixed well plate; levofloxacin and internal standard-enoxacin were separated using a mobile phase consisting of phosphate buffer 10 mM with 0.025% triethylamine pH 3.0 - acetonitrile (88:12, v/v) on a Purosphere RP-8e column (5 µm, 125 × 4.0mm) at a flow rate of 1.2 mL/min at 35 °C. The excitation/emission wavelengths were set to 269/400 nm and 294/500 nm, for enoxacin and levofloxacin, respectively. The method was linear over the concentration range of 0.02 to 20.0 µg/mL with a limit of detection of 0.01 µg/mL. The relative standard deviation of intra-assay and inter-assay precision for levofloxacin at four quality controls concentrations (0.02, 0.06, 3.0 and 15.0 µg/mL) were less than 7% and the accuracies ranged from 96.75% to 101.9% in plasma, and from 93.00% to 98.67% in CSF. The validated method was successfully applied to quantify levofloxacin in a considerable quantity of plasma (826) and CSF (477) samples collected from 232 tuberculous meningitis patients, and the preliminary intensive pharmacokinetics analysis from 14 tuberculous meningitis patients in Vietnam is described in this paper.

1. Introduction

Tuberculous meningitis (TBM) is the most dangerous form of tuberculosis, causing severe morbidity and mortality in both children and adults. Even with the introduction of antibiotic treatment for tuberculosis, the death rate for TBM remains high at around 67% and 25% respectively for human immunodeficiency infected (HIV +) and uninfected (HIV-) Vietnamese adult patients [1,2]. Outcome from TBM might be improved by antibiotics with efficient cerebrospinal fluid (CSF) penetration and strong bactericidal activity against *Mycobacterium tuberculosis*.

To address this hypothesis we conducted a randomized controlled trial (RCT) at two sites in Ho Chi Minh City, Viet Nam, to assess the efficacy of an intensive anti-tuberculosis treatment regimen for TBM [3]. The regimen consisted of standard doses of isoniazid (INH), ethambutol or streptomycin and pyrazinamide (PZA) in combination with high dose of rifampicin (RIF; 15 mg/kg) and an added fifth drug, levofloxacin (LEV; 20 mg/kg). This recently completed RCT shown that the intensified regimen was not associated with an improvement in treatment outcomes [4]. To understand the reasons for the lack of clinical effect, we wanted to analyze the relationship between clinical outcomes and drug concentrations in blood and CSF. Knowledge of the drug exposure concentration at the infectious site (brain) and the ratio of AUC_{LEV} in CSF to AUC_{LEV} in plasma should help to elucidate the

* Corresponding author at: Oxford University Clinical Research Unit, Wellcome Trust Major Overseas Programme, Ho Chi Minh City- In Partnership with Hospital for Tropical Diseases Ho Chi Minh City-Viet Nam. 764 Vo Van Kiet Street, Ward 1, District 5, Ho Chi Minh City, Viet Nam.
E-mail addresses: toipv@oucru.org (P. Van Toi), thomas@tropmedres.ac (T. Pouplin), nguyenduckhanhtho@gmail.com (N.D.K. Tho), pnphuong87@gmail.com (P.N. Phuong), chauth@oucru.org (T.T.H. Chau), thuongntt@oucru.org (N.T. Thuong Thuong), a.heemskerk@vumc.nl (D. Heemskerk), hientt@oucru.org (T.T. Hien), gthwaites@oucru.org (G.E. Thwaites).

http://dx.doi.org/10.1016/j.jchromb.2017.07.032
Received 25 April 2017; Received in revised form 17 July 2017; Accepted 18 July 2017
Available online 20 July 2017

relationship between pharmacokinetics and pharmacodynamics of LEV in TBM patients, and explain the clinical results.

LEV is a synthetic broad-spectrum antibacterial agent for oral and intravenous administration and is a concentration-dependent antibiotic whose effective bacteria-killing activity depends on the ratio of maximum concentration (C_{max}) to minimum inhibitory concentration (MIC) [5]. Measuring LEV concentration in plasma and CSF is not only helpful in making effective drug dosage regimens but also in assessing the CSF penetration of LEV in patients with TBM.

Many studies have described high-performance liquid chromatography (HPLC) methods with ultraviolet (UV) [6–9] or fluorescence (FL) detection [10–17] for the determination of LEV or other fluoroquinolones (FQ) simultaneously in human plasma [6–10,12,13,16–18]; serum [11,14,15,19]; CSF [9,18]; broncho-alveolar lavage [8]; dialysate [12,17]; soft tissue [11]; and urine [20]. Recently, Sung Joong Lee, Sung Chul Shin et al. developed a liquid chromatography tandem mass spectrometry method, operating in a positive electro spray ionization mode (LC-ESI–MS/MS) for the analysis of moxifloxacin and levofloxacin in the serum of multidrug-resistant tuberculosis patients, which was applied on five separate samples for analysis of each analyte [19]. Ultra-high-performance liquid chromatographic (U-HPLC) technique was also applied to determine LEV in human plasma and prostate tissue [21]. For samples from laboratory animals, Christopher J. Destache et al. has described an HPLC-UV method to measure LEV concentration in plasma and CSF samples from a rabbit pneumococcal meningitis model [22]. In addition, Fang et al. has developed a LC–MS/MS method for the high throughput and simultaneous determination of LEV and two anti-tuberculosis drugs (INH and RIF) in mouse plasma and different tissues including brain, lung, liver, kidney and small intestine [23]. A large number of sample preparation techniques were applied: direct injection [12,15]; protein precipitation [6,10–12,14,16,17]; ultrafiltration [7]; liquid–liquid extraction [13]; and solid phase extraction (SPE) [8].

Generally, in contrast to numerous studies to determine LEV in plasma samples, there are only a few publications with a limited number of clinical samples describing the quantification of LEV in human CSF samples [9,18,24,25]. Additionally, to the best of our knowledge, there was only one study [18] has reported the measurement of LEV concentration in both human plasma and CSF samples from TBM patients, using LC/MS and LC/MS/MS.

Therefore, we describe a sensitive and reliable HPLC–FL method, in combination with a SPE process, to determine LEV in a small volume (100 µL) of plasma and CSF from TBM patients. The method can be helpful in both pharmacokinetic studies and routine analysis of LEV in clinical specimens.

2. Materials and methods

2.1. Reagents and solutions

All reagents and solvents used were of analytical grade. Potassium dihydrogen phosphate (KH_2PO_4), phosphoric acid (H_3PO_4), formic acid (HCO_2H), triethylamine (TEA), HPLC-grade acetonitrile (ACN) and methanol (MeOH) were purchased from Merck (Darmstadt, Germany). Water was provided by a Purelab UHQ system (ELGA, Marlow, UK). The reference standards levofloxacin (LEV) and internal standard (IS) enoxacin (ENO) were purchased from Fluka-Sigma Aldrich (Singapore). The different lots of blank human plasma samples from healthy people were supplied by the Blood Transfusion and Haematology Hospital in Ho Chi Minh City. Blank CSF samples were provided by the microbiology laboratory at the Hospital for Tropical Diseases in Ho Chi Minh City as spare aliquots, after routine laboratory investigation, from suspected meningitis patients who had negative diagnosis of bacterial meningitis. CSF blank samples were tested for drug-free LEV before pooling by using the validated method.

2.2. Equipment

The liquid chromatography system was a LaChrom Elite (Merck–Hitachi, Japan) composed by an organizer, an autosampler L-2200, 2 pumps L-2130, a column Oven L-2350 and a Fluorescence Detector (FD) L-2480. The system was controlled by EZchrom Elite version 3.18 HPLC System Manager Software (Merck–Hitachi, Japan). The analysis was performed on a LichroCart® Purospher Star reversed-phase C8 end-capped column (125 mm × 4 mm i.d., particle size 5 µm; Merck, Darmstadt, Germany), which was equipped with a LichroCart® (4 mm × 4 mm i.d., particle size 5 µm) guard column (Merck, Darmstadt, Germany). The SPE was performed on Evolute® ABN, 25 mg/1 mL, 96 fixed well plates (Biotage AB, Uppsala, Sweden).

2.3. HPLC analytical conditions

The mobile phase consisted of a mixture of 10 mM KH_2PO_4 and 0.025% of TEA adjusted to pH 3.0 using H_3PO_4 − ACN (88:12, v/v), filtered through 0.20 µm, regenerated cellulose membrane, (Sartorius, Goettingen, Germany) and degassed for 30 min in a sonic bath (AL 04-12, Advantage Lab, Switzerland). The chromatography was performed at 35 °C for seven minutes at a flow rate of 1.2 mL/minute and the autosampler was set at an ambient temperature. The excitation/emission wavelengths (EX/EM) were set to 269/400 nm, 294/500 nm for ENO and LEV respectively. A system suitability test was performed prior to each sequence by injecting six consecutive aqueous standard solutions (3 µg/mL). The tolerated variation was assessed on area response and retention time with an accepted variation of no more than 2%.

2.4. Standard solutions preparation

Stock solutions of LEV (1 mg/mL), ENO (1 mg/mL) were prepared by dissolving the standards in mixture of MeOH and water (1:1, v/v). The different LEV stock solutions were further diluted with formic acid pH 3.0 to obtain fresh working solutions ranging from 0.4 to 400 µg/mL, and 0.4, 1.2, 60 and 300 µg/mL for preparing calibration curve and quality control samples, respectively. ENO/IS solution at concentration of 10 µg/mL was obtained by diluting ENO stock solution (1 mg/mL) in formic acid pH 3.0 solution.

Plasma and CSF calibration curve (CC) and quality control (QC) were prepared by diluting the respective working solutions with blank plasma or CSF with the ratio of 1:20 to give eight CC points at 0, 0.02, 0.1, 0.5, 2.0, 5.0, 10.0, and 20.0 µg/mL. Four QC points were prepared separately in the same way to give the limit of quantification (LOQ), low (QCL), medium (QCM) and high (QCH) concentrations with 0.02, 0.06, 3.0 and 15.0 µg/mL, respectively.

2.5. Samples preparation

Aliquots of thawed plasma or CSF samples were mixed for 20 s and put in a steam bath at 56 °C for 60 min for the purpose of inactivation to prevent any risk of bacterial or viral infection [26,27]. After cooling down on the bench for 10 min, 300 µL of internal standard/ENO solution (10 µg/mL in formic acid, pH 3.0) were added to 100 µL of sample. The resulting mixture was then vortex mixed for 15 s and rested for two minutes. Finally, the mixture was centrifuged at 9600 × *g* for five minutes at room temperature.

The supernatant was loaded into Isolute® C18 50 mg or Evolute® ABN 25 mg, 96 fixed well plates (pre-treated with 2 mL of MeOH and 1 mL of formic acid pH 3.0 solution). The SPE plate was washed with 1 mL of formic acid pH 3.0 solution, 1 mL formic acid pH 3.0 solution-MeOH (97:3, v/v) and then dried for two minutes. LEV and ENO (IS) were next eluted with 600 µL of formic acid pH 3.0 solution-MeOH (60:40, v/v) into a 96-collection plate. And then we injected 30 µL of each eluate into the equilibrated chromatographic system.