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Quantification of darunavir and etravirine in human peripheral blood mononuclear cells using high performance liquid chromatography tandem mass spectrometry (LC–MS/MS), clinical application in a cohort of 110 HIV-1 infected patients and evidence of a potential drug–drug interaction

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ABSTRACT

Objectives: To describe the validation of a sensitive high performance liquid chromatography tandem mass spectrometry (LC–MS/MS) method allowing the simultaneous quantification of darunavir (DRV) and etravirine (ETR) in peripheral blood mononuclear cells (PBMCs) and its application in a cohort of HIV-1 infected patients.

Methods: Blood samples were obtained from 110 patients. PMBCs were isolated using density gradient centrifugation. Drug extraction from PBMCs was performed with a 60:40 methanol–water (MeOH–H_2O) solution containing deuterated IS (DRV-d9 and ETR-d8). The chromatographic separation was performed on a RP18 XBridge™ column.

Results: The geometric mean (GM) of cell associated concentration ($[DRV]_{CC}$) and plasmatic concentration ($[DRV]_{plasma}$) were 360.5 ng/mL (CI95%:294.5–441.2) and 1733 ng/mL (CI95%:1486–2021), respectively. A geometric mean intracellular (IC)/plasma ratio (GMR) of 0.21 (CI95%:0.18–0.24) was calculated. Adjusted for dose/body surface area and post-intake time, a statistically significant correlation was observed between $[DRV]_{Plasma}$ and the eGFR ($p = 0.002$) and between $[DRV]_{Plasma}$ and the concomitant use of ETR ($p = 0.038$). For the 10 patients receiving ETR in addition to DRV, the GM of $[ETR]_{Plasma}$ (available for 8 out of 10 patients) and $[ETR]_{CC}$ were 492.3 ng/mL and 2951 ng/mL respectively. The GMR of ETR was 7.6 (CI95%: 3.61–13.83).

Conclusions: A handy and sensitive high performance LC–MS/MS method allowing the simultaneous quantification of DRV and ETR in PBMCs has been described and successfully applied in the largest cohort of DRV-treated patients reported to date. ETR accumulates more efficiently in PBMCs compared to DRV. We have also highlighted a possible impact of ETR on DRV plasma concentrations requiring further investigations.

1. Introduction

Darunavir (Prezista® DRV) is a potent protease inhibitor (PI) used as a component of highly active antiretroviral therapy (HAART) in combination with the pharmacokinetic (PK) booster ritonavir for the treatment of Human Immunodeficiency Virus-1 (HIV-1) infected patients [1,2]. Etravirine (Intelence® ETR) is another anti-HIV-1 drug belonging to the non-nucleoside reverse transcriptase inhibitor (NNRTI) class employed for the management of HIV-1 infections in the presence of viral mutations associated with NNRTI resistance [3].

Therapeutic drug monitoring (TDM) of antiretrovirals (ARV) based on drug plasma concentrations is currently widely available to control anti-HIV therapy [4–6]. This reactive strategy allows clinician to better manage the risk of drug under- and over-exposure. Nevertheless, while drug overshoot is thought to be directly linked to the occurrence and the severity of adverse drug reactions (ADRs) [5], it is not clear whether drug plasma level is an indicator of its effectiveness. Indeed, the targets of PIs and NNRTIs are viral proteins located within the infected cells. Thus, at least intuitively, it appears that intracellular (IC) concentrations of ARV drugs should better correlate with their efficacy

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than plasma concentrations. Therefore, quantitative determination of DRV and ETR in human peripheral blood mononuclear cells (PBMCs) may more adequately reflect treatment effectiveness than quantification of these drugs in plasma [7–12].

Previously, only few methods have been developed for the quantification of both antiretroviral drugs in PBMCs [13,14]. The present paper describes the development and the full validation of a handy and sensitive high performance liquid chromatography tandem mass spectrometry (LC–MS/MS) method allowing the simultaneous quantification of DRV and ETR in PBMCs and its clinical application in a cohort of 110 naïve and experienced HIV-1 infected patients.

2. Material and methods

2.1. Study design

HIV-1 infected patients were recruited from the AIDS Reference Centre of the Cliniques Universitaires Saint-Luc (Brussels, Belgium) between November 2012 and February 2014. Patients of 18 years and older with confirmed HIV-infection and treated by DRV with/without ETR for at least one month prior to inclusion were eligible for the study.

In addition to the samples routinely collected for the clinical follow-up of the patient (viral load, CD4 cell count), two additional samples used for determination of trough plasma and IC concentrations were drawn immediately before next drug intake, with the highest timing precision conceivable given the ambulatory context of the study recruitment. In order to obtain a post-intake delay as close as possible to the trough concentration sampling time, each patient was contacted by phone a few days before the day of the visit for the study to be asked not to take the medication prior to blood sampling.

The protocol (NCT02514369) was approved by the local ethical committee (B403201214460) and a written informed consent was obtained from each patient taking part to the study.

2.2. Chemicals and reagents

DRV, ETR, and their respective deuterated internal standards (IS) DRV-d9 and ETR-d8 were purchased from Toronto Research Chemicals (Ontario, Canada); methanol HPLC grade from Biosolve (Dieuze, France); formic acid from Sigma-Aldrich (Seelze, Germany) and the Dulbecco's phosphate buffered saline (DPBS) solution from Invitrogen (Life Technologies, Gent, Belgium). HPLC grade water was produced with Milli-Q water purification system by Millipore (Darmstadt, Germany). Blank PBMCs used for calibration curves and quality control (QC) samples were isolated from the blood of healthy volunteers. PBMCs count was performed on a Sysmex K-1000 haematology analyzer (Norderstedt, Germany).

2.3. LC–MS/MS system and chromatographic conditions

DRV and ETR measurements were performed by LC–MSMS, using a Quattro micro tandem-mass spectrometer (Micromass UK, Manchester, UK) fitted with a Z-spray ion source. The instrument was directly coupled to a Waters 2795 Alliance (high throughput) HT LC system, with an integrated auto sampler thermostated at 10 °C (Waters, Milford, USA). The chromatographic separation was performed on RP18 2.5 μm column (2.1 × 75 mm) (Waters, Milford, MA, USA) maintained at 35 °C. The 20 μL injected aliquot was eluted at a mobile phase flow rate of 0.3 mL/min. The mobile phase consisted of water (eluent A) and methanol (eluent B), both containing 10 mM formic acid. Initial eluent composition was 90% A directly followed by a linear 1.0 min ramp to 10% A, which was maintained for 4.49 min. The mobile phase returned to 90% A at 5.5 min. Equilibration time was 1 min. Ionization was in the positive ion mode using the following settings: capillary voltage 3.5 kV, cone voltage 22/22/54/54 V (DRV/DRV-d9/ETR/ETR-d8), source temperature 125 °C, desolvation temperature 300 °C at a nitrogen flow of approximately 650 L/h, and collision gas (high-purity argon) pressure $3 \cdot 10^{-3}$ bar. DRV, DRV-d9, ETR, and ETR-d8 were monitored in multiple reaction monitoring mode (MRM) by detecting specific product ions: DRV m/z 548.24 > 113.2, DRV-d9 m/z 557.36 > 401.26, ETR m/z 435.09 > 144.4, ETR-d8 m/z 443.18 > 304.33. Collision energies were 21/15/49/45 for DRV/DRV-d9/ETR/ETR-d8 respectively.

2.4. Collection of PBMCs from patients

About 8 mL of blood was collected in cell preparation tube (CPT) from Becton Dickinson Vacutainer® systems. Blood samples were first centrifuged at 1850 ×*g* for 15 min at room temperature (RT). The cell layer was collected with a Pasteur pipette and transferred to a 15 mL size conical centrifuge tube. The volume was completed to 15 mL with cold DPBS to block enzymatic activity and to avoid active transport out of the cells [15]. PBMCs were then washed 2 times according to the manufacturer's instructions (at 650 ×*g* for 10 min at 4 °C). The cell pellet was resuspended in 1 mL of DBPS. 20 μL aliquot was diluted in 180 μL of DBPS and directly used for cell counting using a Sysmex analyzer. After a final centrifugation of the remaining 980 μL at 650 ×*g* (10 min at 4 °C), the supernatant was aspirated and the cell pellet was stored immediately at −80 °C until the day of drug extraction. This method was adapted from Elens et al. [8].

2.5. Preparation of blank PBMCs

Approximately 8 mL of blood from healthy volunteers was collected in heparin tubes (Sarstedt Monovette) and was transferred in LeucoSepTM tubes (Greiner Bio-One, Kremsmünster, Austria). Blood samples were centrifuged at 800 ×*g* for 20 min according to the manufacturer's instructions. The thin mononuclear layer was collected and successively washed twice with DPBS (at 250 ×*g* for 10 min). The final dry cell pellet was stored at −20 °C.

2.6. Stock solutions, calibrators and quality controls

DRV and DRV-d9 stock solutions were obtained by diluting the compounds in methanol to reach a final concentration of 0.5 mg/ml and 1 mg/ml, respectively. ETR and ETR-d8 were diluted in chloroform to obtain a concentration of 1 mg/ml. All stock solutions were then kept at −20 °C. A working solution of DRV and ETR at a concentration of 1250 ng/ml was prepared in 60:40 MeOH:H_2O. Similarly, a solution of DRV-d9 and ETR-d8 at a concentration of 4000 ng/ml was also prepared.

DRV and ETR working solutions were diluted in 60:40 MeOH:H_2O to obtain 6 calibrators (1.25, 6.25, 12.5, 62.5, 93.75, 125 ng/ml) and 3 QC solutions (2.5, 25, 75 ng/ml) containing all DRV-d9 and ETV-d8 at a fixed concentration of 20 ng/ml.

2.7. Sample processing

Blank PBMCs were thawed at RT and resuspended in 400 μL of the calibrators or QC diluted in the extraction solution (60:40 MeOH:H_2O solution containing DRV-d9 and ETR-d8 at 20 ng/ml). PBMCs from HIV-infected patients were resuspended with 400 μL of the cell extraction solution. After vortex mixing (10 s), the resuspended pellets were sonicated for 5 min and subsequently placed on a horizontal shaker at 250 rpm for 6 h. The mixture was finally centrifuged (10,500 ×*g*, 10 min) and the supernatant was transferred to an automatic autosampler vial.

2.8. Analytical method validation

The assay was fully validated according to the U.S. Food and Drug Administration (FDA) [16].