PŘÍLOHY

1. CHIRANAL 2018, UNIVERZITA PALACKÉHO V OLOMOUCI

UHPLC-MS/MS method for the determination of maraviroc in placental perfusions

Veronika Pilařová[1], Eliška Čapková Maxová[1], Zdeňka Němcová[1], Martina Čečková[2], Lenka Ťupová[2], Lucie Nováková[1]

[1]*Department of Analytical Chemistry, Faculty of Pharmacy, Charles University in Prague, Heyrovského 1203, 500 05 Hradec Králové, Czech Republic*

[2] *Department of Pharmacology and Toxicology, Faculty of Pharmacy, Charles University in Prague, Heyrovského 1203, 500 05 Hradec Králové, Czech Republic*

e-mail: *pilarovv@faf.cuni.cz*

Introduction

Maraviroc is an antiviral drug against human immunodeficiency virus type 1 (HIV-1). It blocks HIV-1 from entering human cells. This blocking is caused by a binding of maraviroc to transmembrane pocket of human C-C chemokine receptor CCR5, which is present on the membrane of CD4+ cells (T-cells) and macrophages. The CCR5 receptor interacts with HIV-1 glycoprotein 120 that enables the entry of HIV-1 into human cells. Maraviroc was approved for usage in adults and for children older than 2 years as a part of combination antiretroviral therapy (cART) in 2016. Up to date, there is sparse data about its administration during pregnancy. Some case reports indicate its limited passage across placenta barrier as umbilical-cord/maternal blood ratio measured after the administration. Thus, the aim of this work was to develop fast, selective, and sensitive method for the determination of maraviroc in human placental perfusions to extend current knowledge about maraviroc transplacental pharmacokinetics and its safety when it is used during pregnancy.

Experimental

UHPLC-MS/MS method using rapid high-throughput liquid-liquid extraction (LLE) as a sample preparation step was developed and used. Placental perfusion (500 μL) was extracted by dichloromethane (DCM). UHPLC using Acquity Ultra Performance LC ™ (UPLC) system (Waters, Milford, MA, USA) coupled with Micromass Quattro Micro ™ API benchtop triple quadrupole mass spectrometer (Waters, Milford, MA, USA) was used for the separation and detection. 2 μL of sample were injected in Acquity BEH

C18 analytical column (50 x 2.1 mm i.d.; particle size 1.7 μm). The analytes were separated using gradient elution with 0.1% aqueous formic acid (eluent A) and acetonitrile (eluent B) at a flow rate of 0.35 mL/min. The gradient started with 5 % of eluent B in A, and increased to 95 % B in 3 min. The percentage of eluent B was at the time 3.1 min reduced to the original value of 5 %. The total time of chromatographic separation including column equilibration was 5 min. Quantification of analyte was achieved via selected reaction monitoring (SRM) using the precursor ion $[M+H]^+$ and two selected fragment ions to increase method selectivity. Following MS parameters were used: capillary voltage 1.0 kV, extractor: 3.0 V, RF lens: 0.1 V, ion source temperature 130 °C, cone voltage: 35 V. The desolvation gas (nitrogen) flow was 1000 L/h and temperature 450 °C, Nitrogen was also used as a cone gas with a flow rate of 100 L/h. Collision energy was set individually for each SRM transitions.

Results

The LLE method for the selective isolation of maraviroc from human placental perfusion buffer was developed first. LLE was chosen due to the high lipophilicity of maraviroc, and to achieve the efficient removal of polar compounds from the sample. Various extraction agents including dichloromethane (DCM), terc-butylmethylether (TBME), ethylacetate, hexane, heptane, and their mixtures (DCM: hexane; DCM: heptane in ratio 10:90 and 20:80, v/v) were tested. The highest recoveries 90% and 78% were obtained using DCM and TBME, respectively. Further optimization was then carried out with these solvents. The effect of sample:solvent ratio (500, 1000 and 1500 μL of extraction agent) , extraction temperature (10 – 50 °C), and extraction intensity (250 – 1400 rpm) was determined.

The key factor in UHPLC-MS/MS method development was the separation of maraviroc and two known transporter inhibitors that are present in real-life samples, namely ritonavir and elacridar. The separation was successful when C18 was used. The mass spectrometer parameters including ionization mode, capillary voltage, RF lens, extractor voltage, ion source temperature, desolvation gas flow rate and temperature, cone gas flow rate, and collision energy for each SRM were optimized to obtain sufficient sensitivity.

Finally, optimized method was validated in the terms of precision, accuracy, calibration range, linearity, limit of detection and quantification, and matrix effects. The developed method provided sensitivity in ng/mL amounts with a high selectivity, accuracy, and precision.

Conclusion

Selective and sensitive method for the high-throughput determination of maraviroc in human placental perfusion samples using LLE-UHPLC-MS/MS was developed and fully validated. The method is suitable for the fast analysis of samples containing maraviroc and it will be used for the quantification of this antiviral drug to explain its transport across human placenta in materno-fetal and feto-maternal direction.

Acknowledgements

The authors gratefully acknowledge the financial support of the Charles University Grant Agency for Grant GAUK no. 616216/C/2016, the financial support of the Project

of Specific research, SVV 260 412 (2017), and the project STARSS reg. no.: CZ.02.1.01/0.0/0.0/15_003/0000465 funded by EFRR.

UHPLC-MS/MS METHOD FOR THE DETERMINATION OF MARAVIROC IN PLACENTAL PERFUSATE

ČAPKOVÁ MAXOVÁ, E.[1], PILAŘOVÁ, V.[1], NĚMCOVÁ, Z.[1], ŤUPOVÁ, L.[2], ČEČKOVÁ, M.[2], NOVÁKOVÁ, L.[1]

[1]Department of Analytical Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

[2]Department of Pharmacology and Toxicology, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

e-mail: maxovae@faf.cuni.cz

Maraviroc is an antiretroviral drug acting as an entry inhibitor blocking the chemokine co-receptor 5 (CCR5). It is used as the second choice therapy in HIV-1- positive patients; the lack of knowledge on its safety in pregnancy and transplacental transfer, however, limit its administration to pregnant women so far. The goal of this work was to develop fast and selective method for the analysis of maraviroc in perfusates of human placental cotyledon performed in order to clarify its transfer from mother to fetus. The ultra-high performance liquid chromatography with tandem mass spectrometry (UHPLC-MS/MS) was the method of choice. BEH C18 column and gradient elution with the mobile phase A (water with 0.1% formic acid) and B (acetonitrile) at 0.35 mL/min flow-rate and 40 °C temperature were used for the separation. The mass spectrometry conditions were set up as follows: electrospray ionization in positive mode, capillary voltage 1.0 kV, RF lens 0.1 V, extractor 3.0 V, ion source temperature 130 ºC, cone voltage 35 V, desolvation gas flow 1000 L/h, and temperature 450 ºC. Selected reaction monitoring (SRM) mode was used for quantitation with collision energy 20 eV (SRM 1, quantifier transition) and 30 eV (SRM 2, qualifier transition). Liquid-liquid extraction (LLE) was chosen for sample preparation due to the high lipophilicity of maraviroc. The LLE optimization included optimization of solvent type, solvent to sample ratio, extraction temperature, shaking intensity, and extraction time. The best results were obtained when dichloromethane was used as the extraction agent (recovery > 90%). The optimized method was fully

validated in the calibration range 1-1000 ng/mL at five concentration levels (1 ng/mL, 2.5 ng/mL, 50 ng/mL, 500 ng/mL and 1000 ng/mL) with the lower limit of quantification (LLOQ) 1 ng/mL and limit of detection (LOD) 0.33 ng/mL. Precision (RSD %) and accuracy (% bias) was determined for each concentration level: 1 ng/mL (RSD = 8.1%, bias = +17.5%), 2.5 ng/mL (RSD = 13.0%, bias = +13.5%), 50 ng/mL (RSD = 2.6%, bias = +3.3%), 500 ng/mL (RSD = 2.7%, bias = +0.4%), 1000 ng/mL (RSD = 1.7%, bias = -0,6%). Further parameters of validation were linearity (R^2 = 0.9994) and matrix effects (99.1% - 109.2%) for four concentration levels: 1 ng/mL, 50 ng/mL, 500 ng/mL and 1000 ng/mL. The internal standard of maraviroc, maraviroc-d_6, was used for quantification in all experiments. The method finally enabled sensitive and selective determination of maraviroc in placental perfusion samples. We believe these data could help gain better knowledge on transplacental transport of maraviroc and its safety in pharmacotherapy of HIV-1 positive pregnant women.

The study was supported by Grant GAUK no. 616216/C/2016, SVV 260 412, and the project STARSS reg. no.: CZ.02.1.01/0.0/0.0/15_003/0000465 funded by EFRR.


Farmaceutická fakulta v Hradci Králové, Univerzita Karlova
a Spolek českých studentů farmacie

XXVI. STUDENTSKÁ VĚDECKÁ KONFERENCE

Diplom za 2. místo

Eliška Čapková Maxová

Chemická sekce - farmaceutická analýza

doc. PharmDr. Jaroslav Roh, Ph.D.
proděkan pro vědeckou činnost, doktorské studium a akademické kvalifikace

prof. PharmDr. Tomáš Šimůnek, Ph.D.
děkan Farmaceutické fakulty

Josef Kunrt
prezident Spolku českých studentů farmacie

Hradec Králové
18. - 19. 4. 2018