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Development and validation of a UHPLC diode array detector method for meropenem quantification in human plasma

Gregori Casals [a,*], Cristina Hernández [b], Susana Hidalgo [a], Blai Morales [a], Yolanda López-Púa [c], Pedro Castro [d], Virginia Fortuna [a], José Antonio Martínez [b], Mercè Brunet [a]

[a] Pharmacology and Toxicology Laboratory, Biochemistry and Molecular Genetics, Centro de Diagnóstico Biomédico, Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBERehd), IDIBAPS, Hospital Clínic de Barcelona, Barcelona University, C/Villarroel 170, 08036 Barcelona, Spain
[b] Department of Infectious Diseases, Hospital Clínic-IDIBAPS, Barcelona Centre for International Health Research (CRESIB, Universitat de Barcelona), Barcelona, Spain
[c] Direcció de Qualitat i Seguretat Clínica, Hospital Clínic, Barcelona, Spain
[d] Medical Intensive Care Unit, Hospital Clínic, IDIBAPS, Barcelona Centre for International Health Research (CRESIB, Universitat de Barcelona), Barcelona, Spain

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ABSTRACT

Objectives: Meropenem is a β-lactam antibiotic frequently used to treat serious infections in intensive care unit patients. The main objective was to develop and validate a sensitive and specific ultra high performance liquid chromatography method with photodiode array detection for the quantitation of meropenem in human plasma. The applicability of the method for meropenem monitoring was also examined.

Design and methods: The validation of the method was performed following the FDA's guidelines for bioanalytical methods. In parallel, the method was applied for monitoring meropenem in forty plasma samples from ten critically ill patients treated intravenously at a total dose of 1 g. Drug levels were measured in each patient at 0 h, 2 h, 4 h and 8 h after meropenem infusion.

Results: With this method, intraday and day-to-day variation was below 10%; intraday and day-to-day accuracy was between 94% and 114%; the limit of quantification was 0.5 μg/mL and recovery was above 70%. The method was successfully applied to quantitate meropenem concentrations and the results showed significant pharmacokinetic interindividual variability. Of special interest is that 50% of treated patients had meropenem plasma levels below the minimum inhibitory concentration at 8 h after the start of infusion, which was strongly related to creatinine clearance >60 mL/min.

Conclusions: The method meets the requirements to be applied for meropenem concentration measurements in pharmacokinetics studies and clinical routine. The results suggest the need for therapeutic drug monitoring of meropenem in treated critically-ill patients.

Introduction

Meropenem is a β-lactam antibiotic of the carbapenem family with a broad spectrum of activity against Gram-negative and Gram-positive organisms. It is indicated for the treatment of a broad range of infections and is an important option for the empirical treatment of serious bacterial infections in hospitalized patients [1].

Multicenter studies have shown the clinical efficacy of meropenem in the treatment of complicated infections in hospitalized patients [2,3]. Data from clinical trials in different patient groups indicate that meropenem is a well tolerated antibiotic whose adverse events profile is similar to that of other antibiotics used in the same clinical situations [1,4–6]. Because its bactericidal activity is time-dependent, several investigations in animal models of infection have suggested that meropenem concentrations should be maintained above the minimum inhibitory concentration (MIC) for a minimum of 40% of the dosing interval (f%T > MIC ≥ 40%; 40% of the time or more the free drug should remain above the MIC of the organism) [7] to achieve maximal bactericidal activity.

However, there is evidence of wide interindividual variability in the elimination kinetics of the drug [7] and that standard meropenem dosing regimens may not be adequate in special patient populations such as critically-ill and hemato-oncological neutropenic septic patients due to altered meropenem pharmacokinetics [8,9] and the need for a more stringent pharmacodynamic target (f%T > MIC = 80%–100%) to achieve maximal clinical efficacy [10,11]. These patients may benefit from therapeutic monitoring of meropenem concentrations, which could serve as a guide to individualizing dose regimens by adjusting the dose to achieve and maintain target concentrations. Importantly, clinical response to carbapenems may be unnoticeable before 48 h of

* Corresponding author at: Pharmacology and Toxicology, Hospital Clínic Universitari, Villarroel 170, Barcelona 08036, Spain. Fax: +34 93 22775697.
E-mail address: casals@clinic.ub.es (G. Casals).

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therapy [12]. Therapeutic drug monitoring could allow clinical failure due to underdosing to be distinguished from lack of organism susceptibility in a timely fashion.

Various methods to quantify meropenem in human plasma have been developed and validated; these methods used high performance liquid chromatography with ultraviolet [13–24] or mass-spectrometry [15,25–27] detection and employed different extraction processes, including solid phase extraction [13,15,18,19,22,27], protein precipitation [16,17,21,23,24,26], ultrafiltration [14,24] and homogenization [19]. The main problems of HPLC-UV methods include the high plasma volume required and the difficulty of achieving adequate limits of detection. In contrast, mass spectrometric detection has the advantage of being faster and more specific and sensitive but is expensive and is not generally available in all laboratories. Ultra high performance chromatography (UHPLC) diode array detector is a less expensive methodology than mass spectrometry but allows faster chromatographic runs and improved resolution, throughput and sensitivity compared with conventional HPLC-UV.

Our aim was to validate an accurate UHPLC-diode array detector method for the determination of total concentrations of meropenem in human plasma, which could be useful to evaluate pharmacokinetic and pharmacodynamic relationships in critically-ill patients treated with this drug.

Material and methods

Chemical reagents

Meropenem trihydrate, ceftazidime, disodiumhydrogenphosphate dihydrate ($Na_2HPO_4 \cdot 2H_2O$) and 2-(N-Morpholino)ethanesulfonic acid (MES monohydrate) were obtained from Sigma (Steinheim, Germany). Acetonitrile and methanol (HPLC grade) were purchased from Panreac (Barcelona, Spain). Ortho-phosphoric acid (H_3PO_4, 85%, ACS grade) and sodium hydroxide (NaOH) were purchased from Merck (Darmstadt, Germany). Drug-free human plasma (citrate) used as a biological matrix for calibrators and quality controls was purchased from the *Banc de Sang i Teixits* (Barcelona, Spain).

Instrumentation

The UHPLC chromatographic system consisted of a Shimadzu Nexera system (Kyoto, Japan) comprising a quaternary pump (LC-30AD), a degasser (DGU-20A), an autosampler (SIL-30 AC), a thermostated column compartment (CTO-20AC) and a photodiode array detector (SPD-M20A). The analytical column was a Shimadzu Shimpack XR-ODS III (2.0 mm id × 75 mm, 1.6 μm particle size). Data acquisition and data processing were achieved with LabSolutions Software (Shimadzu, Japan).

Chromatographic conditions

The mobile phase for the chromatographic separation was mixed from buffer solution (87%) and methanol (13%). The buffer solution consisted of 0.1 M $Na_2HPO_4 \cdot 2H_2O$ adjusted to pH = 7 using H_3PO_4 85%. The flow rate was constant at 0.25 mL/min and the column temperature was set to 30 °C. The column effluent was monitored by a diode array detector in the range of 270–320 nm. For quantification, peak areas of meropenem and ceftazidime at 295 nm were evaluated.

Preparation of stock solutions, working solutions, calibrators and quality control samples

Stock solutions of meropenem and ceftazidime were prepared at a concentration of 1 g/L and stored at −70 °C. Working solutions were prepared by diluting the stock solutions in MES buffer (MES 1 M adjusted to pH = 6 using NaOH 5 M) to final concentrations of 100 μg/mL for meropenem and of 20 μg/mL for ceftazidime. Seven-point calibration curves (0.5, 2, 5, 10, 25, 50, and 100 μg/mL) were prepared for meropenem calibration by diluting meropenem working solution (higher calibrator, 100 μg/mL) in MES buffer. These diluted solutions (100 μL) were mixed with 100 μL of drug-free human plasma. For quality controls, three concentrations were prepared (6 μg/mL, 30 μg/mL and 60 μg/mL) by serially diluting meropenem working solution in MES buffer. These diluted solutions (100 μL) were mixed with 100 μL of drug-free human plasma.

Sample preparation

For calibrators and quality controls, 100 μL of the internal standard working solution (20 μg/mL of ceftazidime) was added to the 200-μL mixture of calibrators or quality controls in MES buffer (100 μL) with 100 μL of drug-free human plasma. Similarly, for patient samples, 100 μL of the internal standard working solution (20 μg/mL) was added to a 200-μL mixture of MES buffer (100 μL) with 100 μL of the plasma sample. Then, 500 μL of acetonitrile was added and after centrifugation (13,000 *g* for 5 min) the supernatant was transferred to a conical glass vial and evaporated to dryness under nitrogen at 37 °C. The residue was reconstituted in 200 μL of MES buffer and centrifuged at 1000 *g* for 10 min. The supernatant was transferred to an autosampler glass vial and 7 μL was injected in the chromatographic system.

Method validation

Calibration curve

The response function of the calibration curve was evaluated in runs of seven calibrators over a range between 0.5 and 100 μg/mL. Complete calibration curves were analyzed on 5 separate days and the target back-calculated concentrations of the calibration standards were calculated.

Accuracy and precision

Back-calculated results of multiple analysis of the three quality controls, the lower limit of quantification (LLoQ), and the upper limit of quantification (ULoQ) were used to guarantee the accuracy and precision of the analysis method. The LLoQ and ULoQ were set at the lowest (0.5 μg/mL) and highest (100 μg/mL) calibration standard values, respectively. In the case of intra-day accuracy and precision, five replicates were performed for each concentration on the same day. Inter-day accuracy and precision were calculated on 5 different days. To pass the accuracy test, the mean values had to be within 100 ± 15% of the theoretical value. Accuracy was determined as the difference between calculated meropenem concentrations with theoretical concentrations expressed in percent. Precision at each concentration level was expressed as relative standard deviation (%RSD) for each quality control and could not exceed 15%. For the LLoQ, accuracy had to be within 100 ± 20% and precision below 20%.

Recovery

The recovery ratio of meropenem was evaluated at the concentration levels corresponding to the three quality control values. This ratio was determined by comparing the peak areas of meropenem for the three levels of quality control samples after extraction with the peak areas of meropenem in three solutions at the same concentration that were not extracted.

Selectivity and specificity

Selectivity was investigated by analyzing five different blank plasma samples and was indicated by the absence of any endogenous interference at retention times of peaks of meropenem and ceftazidime in the chromatograms. Specificity was evaluated with respect to drugs commonly used in patients with severe infection in intensive care units. In addition, five different lithium heparin blank plasma samples

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