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Determination of free ertapenem in plasma and bronchoalveolar lavage by high-performance liquid chromatography with ultraviolet detection

Jean-Baptiste Gordien [a], Emmanuel Boselli [b], Catherine Fleureau [c], Bernard Allaouchiche [b], Gérard Janvier [c], Olivier Lalaude [d], Marie-Claude Saux [a], Dominique Breilh [a,*]

[a] *Clinical Pharmacokinetic Department, Victor Segalen Bordeaux 2 University and Clinical Pharmacy Haut-Lévêque Hospital, Bordeaux, France*
[b] *Anesthesiology and Intensive Care Department, Edouard Herriot Hospital, Lyon, France*
[c] *Anesthesiology and Intensive Care Department, Haut-Lévêque Hospital, Bordeaux, France*
[d] *Clinical Department, MSD Laboratory, Paris, France*

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Abstract

A sensitive assay for the determination of unbound ertapenem in human plasma and bronchoalveolar lavage (BAL) was developed using ultrafiltration of plasma and BAL samples. A rapid HPLC method was used with ultraviolet detection set at a wavelength of 305 nm and a separation on a Prontosil AQ C18 column, with imipenem used as internal standard. This assay was linear over the concentration range of 0.5–100 μg/mL and 0.25–50 μg/mL in plasma and BAL, respectively. Limits of detection and quantitation were respectively 0.05 and 0.25 μg/mL. Validation data for accuracy and precision were CV < 2.48 and 8.25%, accuracy in the range 98.1–104.2% and 102.2–108.4%, respectively, for intra and inter-day.

Keywords: Free ertapenem in plasma and BAL; Ultrafiltration; HPLC UV detection; Pharmacokinetics

1. Introduction

Ertapenem (Ivanz®, MK-0826, Merck & Co) is a new once-a-day parenteral β-lactam antibiotic effective for the treatment of community-acquired and mixed infections caused by Gram-positive and Gram-negative aerobic and anaerobic bacteria [1].

Ertapenem differs from imipenem by a unique anionic side-chain and a 1-β methyl group (Fig. 1). It is resistant to human renal dihydropeptidase-I (DHP-I) probably due to the presence of the 1-β methyl and the anionic side-chain presumably increases the binding to plasmatic proteins. Those characteristics give to ertapenem a longer plasma half-life than imipenem, about 4.9 h versus 1 h for imipenem, which allows for a once-daily administration [2–4]. Ertapenem inhibits bacterial cell wall synthesis by binding to penicillin binding proteins (PBPs).

Ertapenem is especially effective against *Bacteroides fragilis* and other anaerobic bacteria, also against Gram-negative aerobic bacilli. It has little effect on *Pseudomonas aeruginosa* and *Acinetobacter baumanii*. The major indication is community-acquired infections, particularly intra-abdominal or pelvic infection caused by aerobic or anaerobic Gram-negative bacteria, and community-acquired pneumonias; with other indications such as skin and skin structure infections and complicated urinary infections. Ertapenem can be also used in deep anaerobic infections of bone tissue in diabetic patients [1,2]. In plasma, some of the drug binds to plasma proteins or blood cells, or diffuses into the blood cells. Some drug also remains unbound in plasma and can move freely in the body. A similar scenario also occurs in the tissue. Most infections occur in the tissues of the body rather than in the blood so that it is accepted today that appropriate antibiotic therapy requires achievement of significant concentrations of antibiotics at the sites of infection. Several studies determined ertapenem concentrations in plasma [4–6], urine [5,7], cerebrospinal fluid [8], but no data on BAL concentrations is available. Determination of ertapenem plasma and BAL concentrations in critically ill patients on mechanical ventilation with community-acquired pneumonia is necessary to assess its pharmacokinetic parameters and to ensure its effi-

* Corresponding author. Tel.: +33 557656495; fax: +33 557656459.
E-mail address: dominique.breilh@chu-bordeaux.fr (D. Breilh).

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cacy in this particular subset of patients who often present some pathophysiological conditions that may alter the pharmacokinetic behavior of this agent. The difference between the total plasma concentrations and the free plasma or tissue concentrations can be quite significant when the protein binding of the antibiotics is high. Therefore, the free plasma concentration is an interesting parameter for the rational dosing of antibiotics and the unbound drug concentration at the infection site should be preferred. Moreover, it is important to realize that in vitro MIC values are determined in the presence of the free antibiotic concentrations and the protein binding of the antibiotic is frequently not taken account.

The present paper describes the development and validation of a high-performance liquid chromatographic method (HPLC) coupled with ultraviolet (UV) detection for determination of free ertapenem in human plasma and BAL. This method will be used and adapted for patients in intensive care unit (ICU), to determine free ertapenem plasma and BAL concentrations in order to know the pharmacokinetic profiles of ertapenem in ICU infected patients. The new plasma pharmacokinetic parameters calculated could be used to optimize the design of administration of ertapenem by comparing them with pharmacodynamic parameters such as minimum inhibitory concentration (MIC). Finally, this specific assay might contribute to a better understanding of the distribution of ertapenem in different tissues such as BAL to quantify the actual concentrations met in respiratory tract.

2. Experimental

2.1. Chemicals

Ertapenem and imipenem, the internal standard, were obtained from Merck & Co (USA) (Fig. 1).

Acetonitrile HPLC quality was purchased from Scharlau (Barcelona, Spain). Di-natrium hydrogen phosphate Na_2HPO_4 and concentrated orthophosphoric acid were from Prolabo (Nogent sur Marne, France).

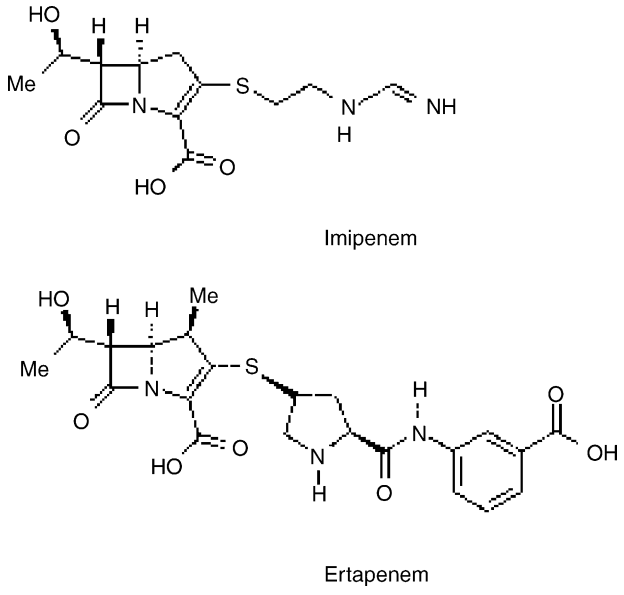

Fig. 1. Chemical structures of imipenem and ertapenem.

2.2. Equipment

The HPLC system consisted of Agilent 1100 series (Agilent technologies, Weldbroon, Germany): a model G1311A quaternary pump, a model G1315B DAD UV detector and an Agilent Chemstation for LC systems.

Ultracentrifugation was performed in a Sigma 2 MK centrifuge (Bioblock Scientific, Strasbourg, France).

2.3. Ultrafiltration

All the samples (calibration standards, quality controls or clinical) were thawed at room temperature. In a haemolysis tube, 200 μL of sample were mixed with 350 μL of 40 mM phosphate buffer containing 25 μg/mL imipenem pH 4.0. The solution of imipenem was prepared just before analysis; it was stable for 12 h at room temperature.

A 400 μL aliquot was transferred to a Microcon^{TM} YM10 centrifugal device with a molecular cut-off of 10,000 Da (Millipore, Bedford, USA), which was centrifuged at 15,000 × *g* at room temperature for 20 min in the sigma model 2 MK centrifuge. Then, 125 μL of the filtrate were transferred in an auto-sampler vial and 40 μL were injected into the chromatographic system.

All those operations were performed at room temperature.

2.4. Chromatography

The mobile phase consisted of 10 mM phosphate buffer adjusted to pH 6.5 with concentrated orthophosphoric acid (phase A), and mixed with acetonitrile (phase B); a gradient elution was performed with the two pumps: the gradient began with 94 and 6%, phase A and B, respectively, from 0 to 2 min, became 82 and 18%, phase A and B, respectively, from 2 to 7 min and came back to original conditions between 7 and 14 min. The mobile phase was filtered through a 0.45 μm filter from Millipore (Saint Quentin en Yvelines, France); the flow rate was set at 1 mL/min. The analytical column was a Prontosil 120 AQ+ C18 (4.6 mm × 150 mm, 5 μm) from Bischoff chromatography (Leonberg, Germany). The sample injection volume was 40 μL. The UV absorbance detection was set at 305 nm with a bandwidth of 4. The chromatographic run time was 14 min.

2.5. Preparation of calibration standards and quality controls

2.5.1. Plasma calibration and quality controls

A working stock solution of 1 mg/mL of ertapenem was prepared by appropriate dilution into pH 6.5, 10 mM phosphate buffer. It was diluted into free human plasma to obtain a concentration range from a 0.5 to 100 μg/mL of ertapenem for calibration. For quality controls, concentrations were different