Clinical pharmacology of ertapenem in the treatment of multidrug-resistant tuberculosis

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CHAPTER 1

General Introduction

General Introduction

Tuberculosis (TB) is caused by *Mycobacterium tuberculosis*. TB is the deadliest infectious disease worldwide. It mostly affects the lungs, but can also attack other organs; together, these forms of TB are referred to as extrapulmonary. Lymph nodes, the pleural and peritoneal space; the axial skeleton; the gut; the urogenital system; and the most lethal form: the central nervous system can all be affected**.** In 2017, 10 million new cases were reported, and approximately 1.6 million people died of TB [1]. An estimated 1 million children fell ill with TB, a quarter of whom died. TB has a global impact, however over 95% of TB deaths occur in low- and middle-income countries, with 61% of all new cases reported in Asia and 26% of new cases in Africa, with six countries accounting for 60% of this total. An increase in refugee flow, increased travelling and globalization, social inequality and poverty, lack of safe water, poor sanitation and poor hygiene services are important risk factors for TB. As immigration and international travel is common in affluent regions, TB ought to be a concern for high income countries [1-3].

Tuberculosis is transmitted via respiratory droplets, microbes carried in droplets or aerosols loaded with *M. tuberculosis* from an infected person via close personal contact, coughing, sneezing and laughing. Only a small minority – an estimated 5-10% of all infected individuals - will ever develop active TB with symptoms such as productive cough with purulent sputum that may contain blood, weight loss, fatigue and night sweats; besides, depending on the site of disease manifestation, people may experience chest pain, back pain, abdominal pain, headache, and seizures. The immune response of most people successfully fights off TB bacilli that may be killed by activated macrophage immune cells, with TB bacilli in the phagosome-lysosome. TB bacilli may however also survive within the phagosome of these macrophages, and in the latter case, these people are said to be latently infected. Their bacilli survive in a hibernating mode, controlled by a genetic system referred to as *dos*R regulon [4]. This is a genetic program controlled by a set of 48 genes that allows the tubercle bacilli to survive under stress conditions; during active immune suppression, hibernating organisms are called 'latent' while during drug treatment, these organisms are called 'persistent'. Latently infected individuals typically have only limited numbers of living bacterial cells that slowly replicate and are hardly metabolically active. People with latent TB infection feel well, have no symptoms and are not contagious [5].

M. tuberculosis belongs to a small group of highly pathogenic bacteria (*M. tuberculosis* complex) in the very large family of mycobacteria, characterized by a thick cell wall, consisting of several different specific lipid molecules including lipoarabinomannan and mycolic acids. The majority of crosslinks in the peptidoglycan layer are formed differently compared to gram positive bacteria, making mycobacteria more resistant to chemical damage and hydrophilic antibiotics. As the replication rate of *M. tuberculosis* – even if actively replicating and metabolically active - is very slow (≈20 h) compared to other bacteria (≈20 min), TB requires more specific antibiotics and prolonged treatment, as most antibiotics only work on actively replicating bacteria [5]. Typically, rapidly dividing metabolically active bacilli can be reduced rapidly within weeks; several highly active agents have bactericidal properties. Due to the slow replication rate of *M. tuberculosis*, especially of difficult to eradicate persister phenotype bacteria. TB treatment needs to last long to obtain a sterilising effect. The World Health Organisation (WHO) therefore advises to threat TB with a standard first-line treatment consisting of isoniazid (H), Rifampicin (R), pyrazinamide (Z) and ethambutol (E) – HRZE – And thereby intensively decrease the bacterial load (intensive phase). Followed by the intensive phase, a four-month continuation with isoniazid and rifampicin is needed to provide the opportunity to eliminate the last TB bacteria which are in a persistent state of being capsulated by macrophages.

MDR TB - Antimicrobial resistance of TB

Unfortunately, our world is facing a public health crisis and security threat due to the treatment of TB becoming increasingly challenging with the emergence of resistance to first-line drugs. Multidrug resistant (MDR)-TB is defined as an infectious disease caused by *M. tuberculosis* that is resistant to at least isoniazid and rifampicin, which are the cornerstone drugs of drug-susceptible TB treatment. Extensively drug resistant (XDR)-TB is defined as MDR-TB with additional resistance to at least one of the fluoroquinolones and to at least one of the injectable second line drugs [6]. WHO estimates that there were 558.000 new cases with resistance to rifampicin, the most effective first line drug [2].

Development of new drugs is slow and expensive due to the obligatory market access regulations such as randomized clinical trials. Bedaquiline and Delamanid, both with a novel mechanism of action, were included in the WHO guidelines on MDR-TB, after approval by

Federal Drug Administration (FDA) and European Medicines Agency (EMA) almost five years ago. An individual patient data meta-analysis revealed that of all drugs used to treat MDR-TB, the added value of the injectable agents' kanamycin and capreomycin was actually associated with poor outcome. Bedaquiline; the fluoroquinolones levofloxacin, gatifloxacin and moxifloxacin; and linezolid were associated with beneficial outcome [7]. Based on this meta-analysis, WHO issued a rapid communication updating the provisional guidelines for MDR-TB treatment [8]. Bedaquiline has now obtained a position in Group A; together with Fluoroquinolones and Linezolid, this drug is now considered among the most powerful agents to fight MDR-TB. Unfortunately, resistance to these novel agents has already been detected [9]. Obviously, the costs of these highly effective novel agents are a constraint for use in low-resourced settings.

The challenges to eradicate TB by 2030 are vast; many of the second line drugs are also associated with toxicity and adverse effects; and there is therefore a desire for additional drugs with low inherent toxicity. Apart from developing additional new drugs, the repurposing of drugs that are already available for other indications would be an asset to improve and extend current treatment options, by developing more active – sterilizing- anti TB drugs [10-11]. Multiple partnerships have been initiated with the joint goal of eradicating resistance by developing and producing new drugs and rediscovering old drugs.

Rediscovery of old drugs

One particularly effective strategy is rediscovery of old drugs as new agents for treatment against multidrug resistant tuberculosis. Linezolid and moxifloxacin already have been explored as new agents against MDR-TB [12-15]. Benefits of repurposing old antibiotics is that these drugs are commonly cheap and clinical experience is substantial resulting in a well-established drug safety profile. To unlock their potential as new TB agents and obtain market approval, efficacy, safety and toxicity profile needs to be established. Therefore, a pharmacokinetic and pharmacodynamics profile needs to be established and dose-finding studies are needed to establish required dose in TB patients.

Nowadays, beta-lactam antimicrobial drugs are widely used drugs for the treatment of a range of infections [16]. Beginning with the discovery of penicillin by Alexander Fleming in the late 1920s, antibiotics changed treatment of bacterial infections, saving millions of lives. By

mid-1940 it became clear that penicillin was not effective at killing M. tuberculosis. In the 1960s it became clear that M. tuberculosis produced an enzyme, called beta-lactamase (BLaC), which rapidly hydrolysis's the beta-lactam ring. Carbapenem activity have therefore long been considered to be of limited use. However, more than a decade ago, researchers showed that clavulanate irreversibly blocked beta-lactamase enzyme of M. tuberculosis [17]. Recent studies suggest that beta-lactams, using clavulanate/clavulanic acid, show more activity against M. tuberculosis and could be beneficial in the treatment of TB [18-22].

Carbapenems are earmarked as potentially active drugs for the treatment of *M. tuberculosis*. Imipenem-cilastatin and meropenem have been listed as add-on drugs in the updated WHO treatment guidelines. Ertapenem, approved in 2001 by the FDA, an old drug widely used against gram positive and negative bacteria has shown to be active in MDR-TB [23-25]. In general, ertapenem appears to be favourable and a highly promising drug for the treatment of MDR-TB that warrants further investigation.

Aim of the thesis

To better understand the potential role of ertapenem for the treatment of M/XDR-TB, the aim of this thesis was to evaluate current literature, in vitro activity, and pharmacokinetics and safety in TB patients.

OUTLINE OF THE THESIS

In this thesis, we plan to evaluate the pharmacology of ertapenem in the treatment of multidrug resistant tuberculosis.

in **chapter 2**, We plan to study literature to evaluate current knowledge on in vitro, in vivo and human activity of carbapenems

In **chapter 3** we aim to develop a simple validated LC-MS/MS for the validation and quantification of ertapenem required for future pharmacokinetic studies.

In **chapter 4** we plan to evaluate pharmacokinetics and safety of ertapenem used to complete a treatment regimen for MDR TB patients

In **chapter 5** we aim to develop a suitable experiment to evaluate the susceptibility of *M. tuberculosis* for ertapenem as the currently used assays are not suitable because ertapenem degrades fast under standard conditions (37C)

In **chapter 6**, we intend to study the sterilizing effect of ertapenem-clavulanate in a hollow fiber model of tuberculosis to select a dose for future clinical studies

In **chapter 7,** we propose to develop a pharmacokinetic model and a limited sampling strategy which could be used for a future phase II study

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CHAPTER 2

Evaluation of Carbapenems for Treatment of Multi- and Extensively Drug-Resistant *Mycobacterium Tuberculosis*

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Abstract

M/XDR-TB has become an increasing threat in high burden countries but also in affluent regions due to increased international travel and globalization. Carbapenems are earmarked as potentially active drugs for the treatment of *M. tuberculosis*. To better understand the potential of carbapenems for the treatment of M/XDR-TB, the aim of this review was to evaluate the literature on currently available *in vitro*, *in vivo* and clinical data on carbapenems in the treatment of *M. tuberculosis* and detection of knowledge gaps, in order to target future research. In February 2018, a systematic literature search of PubMed and Web of Science was performed. Overall the results of the studies identified in this review, which used a variety of carbapenem susceptibility tests on clinical and lab strains of *M. tuberculosis*, are consistent. *In vitro* the activity of carbapenems against *M. tuberculosis* is increased when used in combination with clavulanate, a BLaC inhibitor. However, clavulanate is not commercially available alone, and therefore is it practically impossible to prescribe carbapenems in combination with clavulanate at this time. Few *in vivo* studies have been performed, one prospective, two observational and seven retrospective clinical studies to assess effectiveness, safety and tolerability of three different carbapenems (imipenem, meropenem and ertapenem). Presently we found no clear evidence to select one particular carbapenem among the different candidate compounds, to design an effective M/XDR-TB regimen. Therefore, more clinical evidence and dose optimization substantiated by hollow fiber infection studies are needed to support repurposing carbapenems for the treatment of M/XDR-TB.

Introduction

Treatment of tuberculosis (TB), a disease caused by *Mycobacterium tuberculosis*, has become more challenging with the emergence of multidrug resistant (MDR)-TB and extensively drug resistant (XDR)-TB among previously and newly detected cases (1). M/XDR-TB has become an increasing threat in high burden countries but also in affluent regions due to increased international travel and globalization.

MDR-TB is defined as an infectious disease caused by *M. tuberculosis* that is resistant to at least isoniazid and rifampicin. XDR-TB is defined as MDR-TB with additional resistance to at least one of the fluoroquinolones and to at least one of the injectable second line drugs (amikacin, capreomycin or kanamycin). New TB drugs, with a novel mechanism of action, include bedaquiline and delamanid that have recently been approved and included in the World Health Organization guidelines on MDR-TB as add-on agents (2). Unfortunately, resistance to these agents has already been detected (3). Exploration of currently available drugs for their potential effect against TB, may be an additional source for potential candidates to be used in case of extensive resistance to try to compose a treatment regimen (4-5).

Beta-lactam antimicrobial drugs are widely used drugs for the treatment of a range of infections. Also, imipenem-cilastatin and meropenem have been listed as add-on drugs in the updated WHO treatment guidelines (6). Carbapenem activity has long been considered to be of limited use, due to rapid hydrolysis of the beta -lactam ring by broad-spectrum mycobacterial class A beta-lactamases (BLaC). The addition of the BLaC inhibitor clavulanate suggests that beta-lactams combined with BLaC inhibitors could be beneficial in the treatment of TB (7). Recent studies suggest that beta-lactams, using clavulanate/clavulanic acid, show more activity against *M. tuberculosis* (7-14).

The bacterial activity of beta-lactams is due to the inactivation of bacterial transpeptidases, commonly known as penicillin binding proteins (PBP), which inhibit the biosynthesis of the peptidoglycan layer of the cell wall of bacteria (8,15). Polymerizations of the peptidoglycan layer in most bacteria are predominantly cross-linked by D,D-transpeptidases (DDT), the

enzymes inhibited by beta-lactams (8,16). The majority of crosslinks in peptidoglycan appear to be formed by the non-classical L,D-transpeptidases (LDT) in *M. tuberculosis (*17-23). Several studies revealed the structural basis and the inactivation mechanism of LDT and the active role of carbapenems, providing a basis for the potential use of carbapenems in inhibiting *M. tuberculosis* (24-28).

Beta-lactams show time-dependent activity, carbapenems have been shown to have bactericidal activity when the free drug plasma concentration exceeds the MIC for at least 40 % of the time in non-TB bacterial species (29-30).

Carbapenems are earmarked as potentially active drugs for the treatment of *M. tuberculosis*. To better understand the potential of carbapenems for the treatment of M/XDR-TB, the aim of this review was to evaluate the literature on currently available *in vitro*, *in vivo* and clinical data on carbapenems in the treatment of *M. tuberculosis* and detection of knowledge gaps, in order to target future research.

Methods

Prisma

This systematic review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement.

Search

In February 2018, a systematic literature search of PubMed and Web of Science, without restrictions with respect to publication date was employed using the key words (´Carbapenem' OR 'Carbapenems' OR 'Imipenem' OR 'Meropenem' OR 'Ertapenem' OR 'Doripenem' OR 'Faropenem' OR 'Biapenem' OR 'Panipenem' OR 'Tebipenem') AND ('Tuberculosis' OR TB OR *Mycobacterium tuberculosis*) as MeSh Terms. Retrieved studies and abstracts from both PubMed and Web of Science were pooled and duplicates were removed. Titles and abstracts of retrieved articles were screened. Reviews, case-reports or studies on other species than TB or studies on other drugs than carbapenems were excluded. Studies were screened for eligibility. If eligible, the full-text was read by a researcher (SvR). A second

researcher (MZ) independently repeated the article search and selection. Discrepancies were resolved by discussion, or a third researcher was consulted (JWA). Full text papers were subdivided into three sections; *in vitro, in vivo* and clinical data. Full text papers for *in vitro* data were eligible for inclusion if an *M. tuberculosis* strain was studied and minimum inhibitory concentrations were reported. Full text papers for *in vivo* data were eligible for inclusion if treatment of *M. tuberculosis* infections with carbapenems were studied in animal models, and if colony forming units and/or survival data were reported. Full text papers for clinical data were eligible for inclusion if pharmacokinetics of carbapenems or safety or response to treatment measured as surrogate end points (sputum conversion) or clinical end points were studied and reported. References of all included articles were screened by hand. The same systematic search was performed using clinicaltrials.gov to find ongoing studies investigating carbapenems in TB patients (Feb 2018).

Data extraction

A researcher (SvR) performed data extraction first by using a structured data collection form. A second researcher (MZ) verified the data extraction independently. Data were subdivided into three sections; *in vitro*, *in vivo* and clinical data. Variables in the section '*in vitro*' included; *M. tuberculosis* strain, experimental methods, drug of interest. Minimal inhibitory concentration, minimal inhibitory concentration with clavulanic acid, minimal bactericidal concentration and colony forming units (CFU) were extracted from the included articles. For the section '*in vivo*' the following data were included; *M. tuberculosis* strain, mice, route of infection, drug of interest with or without clavulanic acid, dose, and treatment, colony forming units and survival rate, were retrieved from the included articles. For the clinical section, we extracted data from the included articles on type of study population, number of subjects, study design, drug of interest, and dosage. Sputum smear, sputum culture, treatment success, adverse events and interruption due to adverse events were noted as outcomes. AUC, Peak drug concentration (C_{max}), half-life ($t_{1/2}$), Distribution volume (Vd), and clearance were extracted. Possibility of pooling data from included data was assessed on data presentation.

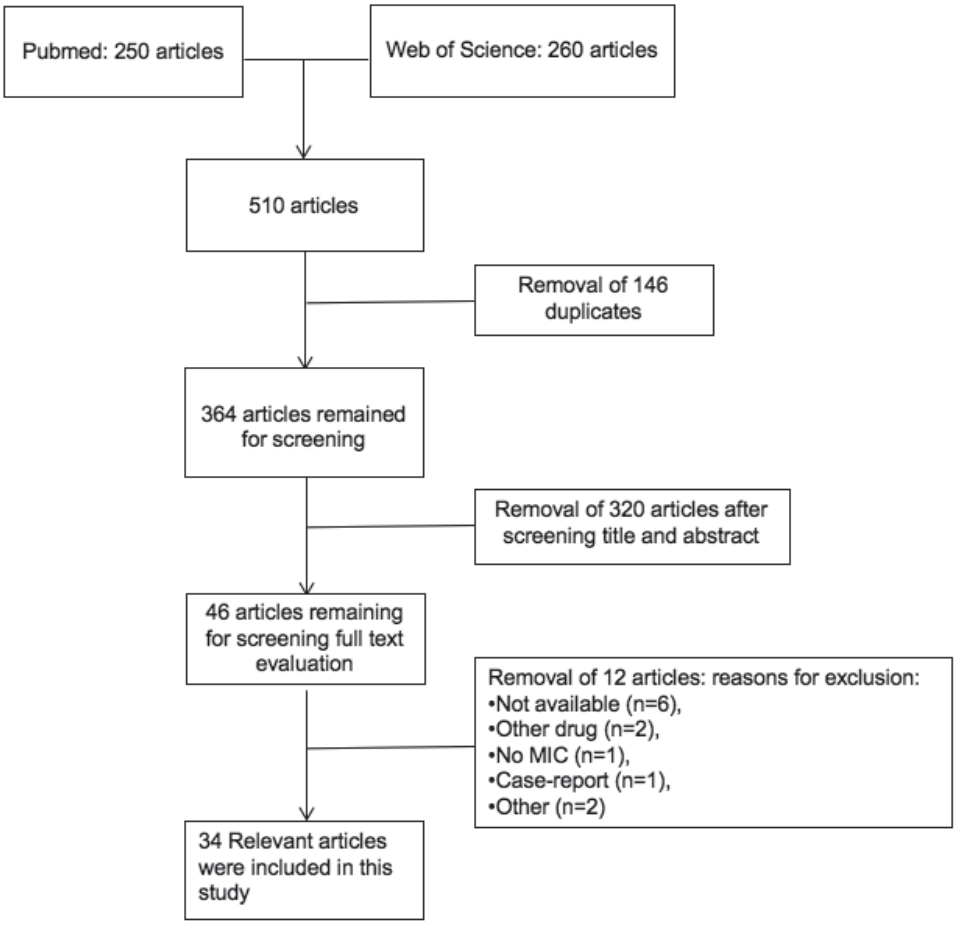
Data quality

No validated tool for risk of bias assessment for *in vitro* studies, *in vivo* studies and pharmacokinetic studies was available. To be able to assess the quality of each study, we verified if each study reported on key-elements required for adequate data interpretation. If studies reported adequately on the key-elements, risk of bias was considered to be low. If studies had missing data or if procedures were not clear or not mentioned, risk of bias was considered to be high. The following key-elements were identified for *in vitro* studies; description of lab or clinical strains, minimal sample size of >10 strains, >3 concentrations tested per drug, MIC/CFU determined using the proportion method, evaluation endpoint of minimal inhibitory concentration (MIC 50 or MIC 90), evaluation of endpoint of minimal bactericidal concentration (MBC99) and CFU reduction, for *in vivo* studies; description of laboratory or clinical strains, type of mice, route of administration of the drug, dose and treatment duration, MIC/CFU determined using the proportion method, evaluation of endpoint of CFU and survival rate and for clinical studies; for *human* studies; study design, patient population (TB/MDR-TB; HIV co-infection), number of study participants, endpoints tested, defined as sputum smear conversion, sputum culture conversion, treatment success, adverse events. The following components were checked for pharmacokinetic studies: sample size, type of patients, type of assay, number of plasma samples drawn per patient, sample handling, use of validated analytical methods and method of AUC calculation.

Results

Based on the selection criteria, 250 articles were retrieved in PubMed and 260 in Web of Science. After removal of 146 duplicates, 364 articles remained for screening. After screening of the title and abstract, 46 articles remained for full text evaluation. Reasons for exclusion included; not available (n=6), other drugs (n=2), no MIC (n=1), case-report (n=1), other (n=1). After this process, 35 relevant articles were included in this study (Flow chart; Fig 1). Due to low number and high diversity of strains, analytical methods and study designs, presence of biochemical instability of the drugs of interest, the short half-life of drugs of interest in mice and the diversity in MIC determination, we did not have enough data to perform a meta-analysis. Risk of bias of the included studies is shown in table S1. Studies on clinicaltrials.gov are shown in S2.

Figure 1. Flow chart



In vitro

Results of the *in vitro* studies reporting on carbapenems are presented in table 1.

Imipenem

Susceptibility testing of imipenem, using various analytical methods against strain H37Rv, H37Ra, Erdman and clinical isolates of *M. tuberculosis* showed a range of MIC's between 2 – 32 mg/ L without clavulanic acid and a range of MIC's between 0.16 – 32 with clavulanic acid. (8,32-37). When Imipenem was combined with clavulanate it showed a 4-16-fold lower MIC against the *M. tuberculosis* H37Rv reference strain (8,33-35).

Table 1. Results of the *in vitro* studies reporting on carbapenems.

						Value(s) [mg/liter; median (range)] for:							
						Carbapenem			Carbapenem with CLV (2.5 mg/L)				
First author (ref).	**Strain**	**N**	**Method**	**Carbapenem(s)**	**β-Lactamase inhibitor(s)**	MIC	MIC_{50}	MIC_{90}	MIC	MIC_{50}	MIC_{90}	MBC 99	Δ log CFU reduction
Chambers et al (32)	H37Ra, H37Rv, clinical isolates	7	Bactec TB system	Imipenem	None	(2–4)							
Cohen et al (38)	H37Rv, Clinical isolates	91	Microplate alamar Blue assay	Meropenem	Clavulanate	22 (2–32)			5.4 (0. 5–32)				
Cavanaugh et al (39)	Clinical isolates	153	Resazurin microdilution assay	Meropenem	Clavulanate				(<0. 12–>16)	1	8		
Deshpande et al (47)	H37Ra, THP 1 monocytes	1	Resazurin microdilution assay, CFU counts	Faropenem	None	1							2.71 log
Dhar et al (49)	H37Rv, Erdman	2	96 Well flat-bottom polystyrene microtiter plate	Faropenem, meropenem, imipenem	Clavulanate	1.3; 2.5; 2.5			1.3; 0.3; 0.5				
England et al (40)	H37Rv, macrophages	1	CFU counts	Meropenem	Clavulanate								2 log
Forsman et al (41)	H37Rv, Clinical isolates	69	Broth microdilution	Meropenem	Clavulanate				(0.125–32)		1		2 log
Gonzalo et al (42)	H37Rv, Clinical isolates	28	960 MGIT system	Meropenem	None	Resistant at 5 mg/L			(1.28–2.56)				
Gurumurthy et al (48)	H37Rv	1	96 Wells plate	Faropenem	Clavulanate, avibactam [a]			(5–10)				20	0 log
Horita et al (43)	H37Rv, Clinical isolates	42	Broth microdilution	Meropenem Biapenem Tebipenem	Clavulanate	(1–32), (1–32), (0.25–8)	16, 16, 4	32, 32, 8	(0.063–8), (0.25–8), (0.063–8)	2, 2, 1	4, 4, 1		

Hugonnet et al (8)	Erdman, H37Rv, Clinical isolates	15	Broth microdilution	Imipenem Meropenem	Clavulanate				0.16, (0.23–1.25)				
Kaushik et al (33)	H37Rv, Clinical isolates	1	Broth microdilution	Imipenem, meropenem, ertapenem, doripenem, biapenem, faropenem, tebipenem, panipenem	None		(40–80), (5–10), (10–20), (2.5–5), (2.5–5), (2.5–5), (1.25–2.5) >80			(20–40), (2.5–5), (5–10), (1.25–2.5), (0.6–1.2), (2.5–5), (0.31–0.62), ND	ND 80 ND 20 20 20 10 ND	
Kaushik et al (51)	H37Rv, strain 115R, strain 124R	3	Broth microdilution	Biapenem	Clavulanate	(2–16)							
Sala et al (44)	18b cells	1	Serial dilutions, CFU counts	Meropenem	Clavulanate								
Solapure et al (34)	H37Rv, 18b cells	1	Resazurin microdilution assay, CFU counts	Imipenem, meropenem, faropenem	Clavulanate	4, 8, 4			0.5, 1, 2			4 2 4	2 log
Srivastava et al (45)	H37Ra	1	Resazurin microdilution assay	Ertapenem	Clavulanate	0.6							
Veziris et al (35)	H37Rv	1	Broth microdilution	Imipenem, meropenem, ertapenem			16, 8, 16			1, 1, 4			2.38 log10

a. MIC values for carbapenems with avibactam are not shown in this table.

N: number of strains, MIC: Minimal inhibitory concentration (mg/L), MIC50: Minimal inhibitory concentration required to inhibit growth of 50% of the organisms, MIC90: Minimal inhibitory concentration required to inhibit growth of 90% of the organisms, CLV: clavulanate (mg/L), MBC99: minimal bactericidal concentration that kills 99% of replication culture (mg/L), CFU: colony forming units (Log/(CFU/ml))

Meropenem

Multiple studies reported that meropenem in presence of clavulanate is active *in vitro* against clinical and lab strains, H37Rv and H37Ra, of *M. tuberculosis*, showing MIC's ≤ 1 mg/L. *In vitro* studies reporting susceptibility of meropenem of *M. tuberculosis* reference strain and clinical isolates showed MIC values between 1 - 32 mg/L (8,33-44). Meropenem in combination with clavulanic acid was shown to have a MIC between 0.063 – 32 mg/L (33-35,38,43) Meropenem in combination with clavulanate killed the non-replicating ss18b strain of *M. tuberculosis* moderately and was shown to have a MIC of 0.125 – 2.56 mg/L against *M. tuberculosis* H37Rv strains (8,34-35,40). A decrease of 2 log10 CFUs over six days was reported in *M. tuberculosis*-infected murine macrophages (40).

Ertapenem

In clinical strains of *M. tuberculosis* the MIC of ertapenem, as single agent, was 16 mg/L and when combined with clavulanate 4 mg/L (33,35). Another study showed ertapenem was unstable degrading faster than the doubling time of *M. tuberculosis* in the growth media used, suggesting previous published MICs of ertapenem are likely to be falsely high (45). In a hollow fiber model with supplementation of ertapenem in a broth microdilution test, ertapenem showed a MIC of 0.6 ml/L (46). A 28-day exposure-response hollow fiber model of TB study tested 8 different doses of ertapenem in combination with clavulanate and identified the ertapenem exposure associated with optimal sterilizing effect for clinical use. Monte Carlo simulation with 10,000 MDR-TB patients identified a susceptibility breakpoint MIC of 2 mg/L for an intravenous dose of 2 g once a day that achieved or exceeded 40%T>MIC (46)

Faropenem

Faropenem showed a 4-fold reduction when combined with clavulanic acid (33,34), resulting in a MIC range between 1 - 5 mg/L (33-34, 47-49) In a hollow fiber model, the optimal target exposure was identified to be associated with optimal efficacy in children with disseminated TB using Monte Carlo simulations; the predicted optimal oral dose was 30 mg/kg of faropenem-medoxomil 3-4 times daily. The exposure target for faropenem-medoxomil was 60% T_{free}>MIC (50).

Other carbapenems

Other carbapenems, such as doripenem, biapenem and tebipenem showed at least a 2-fold reduction in MIC when combined with clavulanic acid (33,37,43,51).

In vivo

Results of the *in vivo* studies reporting on carbapenems are presented in table 2.

Imipenem

The bacterial burden in imipenem-treated CD-1 female mice (twice daily (BID) 100 mg/kg), infected with *M. tuberculosis* strain H37Rv, was reduced by 1.8 log10 in splenic tissue and 1.2 log10 in lung tissue after 28 days, showing an anti-mycobacterial effect as well as improved survival in this mouse model (52). In another study Swiss mice, infected with *M. tuberculosis* strain H37Rv, were treated with a subcutaneous administration of 100-mg/kg imipenem in combination with clavulanate once a day to simulate a human equivalent dose. The CFU count after 28 days of treatment increased compared to the CFU count at start of treatment. There only was a significant difference in the imipenem-clavulanate treated mice (35).

Meropenem

It has been reported that 300 mg/kg BID meropenem alone, and in combination with 50 mg/kg clavulanate both resulted in a significant, though modest reduction, in CFUs in lung and spleen tissues in C57BL/6 mice (40). Veziris *et al.* reported a CFU increase compared to start of the treatment of meropenem when given as mono-therapy or in combination with clavulanate in a dose of 100 mg/kg, on CFUs, spleen weights, or lung lesions in Swiss mice (35). Meropenem in a dose of 300 mg/kg in combination with clavulanate, 75 mg/kg thrice-daily given to BALB/c mice showed marginal reduction in CFU counts in the acute model and no reduction in the chronic model (34). Meropenem, given subcutaneously 300 mg/kg three times a day, showed a CFU count reduction of 1.7 log in the lungs of TF3157 DHP-1 deficient mice (53).

Table 2. Results of the *in vivo* studies reporting on carbapenems.

First author (ref).	Strain	Mice of mice of other animal model	Infection	Drug	Dose	Infection model	Treatment	End-point	Organs	CFU reduction	Survival rate	CFU/ clv
Chambers et al (52)	H37Rv	CD-1 Female mice	subcutaneously	Imipenem	Bid 100 mg/kg	ND	28 days	CFU count, Survival rate	Spleen, lungs	1.8 log	65%	ND
Dhar et al (49)	H37Rv	adult C57BL/6J mice	intratracheal	Faropenem	500 mg/kg	acute TB	8 days	CFU count	Lungs	reduction of CFU: 10^5 - 10^6	ND	ND
England et al (40)	H37Rv	C57BL/6 Mice	subcutaneously	Meropenem	bid 300 mg/kg	Chronic stage	2 weeks	CFU count	Spleen, lungs	1 log	ND	1 log
		New Zealand white rabbits	intravenous bolus	Meropenem	75 mg/kg 125 mg/kg	ND	ND	PK data	ND	ND	ND	ND
Kaushik et al (51)	H37Rv	BALB/c mice	Aerosol	Biapenem	200 mg/kg BID 300 mg/kg BID	Late phase acute TB rifampicin resistant TB	8 weeks 4 weeks	CFU count	Lungs	1 log ND	ND	ND
Rullas et al (53)	H37Rv	TF3157 DHP-I KO	subcutaneously	Meropenem Faropenem	TID 300 mg/kg TID 500 mg/kg	Acute TB model	21 days	CFU count	Lungs	1.7 log 2 log	ND ND	ND ND
Solapure et al (34)	H37Rv	BALB/c mice	Aerosol	Meropenem	TID 300 mg/kg	Acute and chronic model	4 weeks	CFU count	lungs	no reduction	ND	no reduction
Veziris et al (35)	H37Rv	Female Swiss mice	Intravenously	Imipenem Meropenem Ertapenem	100 mg/kg 100 mg/kg 100 mg/kg	preventive model	28 days	CFU count, Survival rate	Spleen, lungs	>1.2 log* >1.8 log* >1.7 log*	1 dead 3 dead 3 dead	>0.9 log* >1.4 log* >1.6 log*

* There was a CFU increase in the groups with and without clavulanate compared to the start of the treatment.

MIC: Minimal inhibitory concentration (mg/L), CLV: clavulanate (mg/L) , MBC: minimal bactericidal concentration (mg/L), CFU: colony forming units, od: once a day, bid: twice a day, tid: three times a day, qid: four times a day, ND: not described.

Ertapenem

In a murine TB model infected with H37Rv, a dose of 100 mg/kg once daily ertapenem as monotherapy or in combination with clavulanate had neither bactericidal nor bacteriostatic activity in lungs and spleens of TB-infected mice. Spleen weight and lung lesions remained similar compared to the untreated group of mice. There was an increase in CFUs compared to the CFUs at the start of the treatment (35).

Other Carbapenems

An oral dose of 500 mg/kg faropenem-medoxomil, given three times daily, gave a reduction of 2 log CFU count in the lungs of TF3157 DHP-1 deficient mice (53). Neither *in vivo* nor clinical studies for other carbapenems as part of a multi-drug regimen against TB were retrieved.

Clinical studies

Results of the clinical studies reporting on carbapenems are presented in table 3.

Imipenem

Ten patients were treated with imipenem in combination with two or more other antimicrobial agents. It was reported that it was likely that 1g of imipenem (BID) contributed to sputum culture conversion in these patients (52). A prospective study evaluated 1000 mg/day imipenem/clavulanate at a dose of once daily in 12 patients, 11 of these patients received linezolid-containing regimens. All patients showed sputum and culture conversion within 180 days. No adverse events were reported for imipenem/clavulanate (54). In a large observational study, the clinical outcomes of 84 patients, treated with 500 mg imipenem/clavulanate four times a day, were compared with results from 168 controls. The study showed that imipenem-containing regimens achieved comparable results compared to the imipenem sparing regimens, while success rates were similar to major international MDR-TB cohorts (55).

Meropenem

A regimen including meropenem-clavulanate given to 18 patients with severe pulmonary XDR-TB led to sputum culture conversion in 15 patients, of which 10 has successfully completed and five patients were considered cured according to WHO guidelines. Long-

Table 3. Results of the *in human* studies reporting on carbapenems.

First author (ref)	Year of publication	Country	Study population	Study design	Drug	Dosage	Patients	Paediatric	Sputum Smear	Sputum culture	Treatment succes	Adverse events	interruption due AE
Arbex et al (54)	2016	Brazil	2013 -2015	Observational, retrospective	Imipenem	1 g oc	12	No	12/12	12/12	7/12	0/12	0/12
Chambers et al (52)	2005	USA	ND	Prospective	Imipenem	1 g bid	10	No	ND	8/10	7/10	ND	ND
De Lorenzo et al (58)	2014	Italy, The Netherlands	2001-2012	Observational case-control	Meropenem	1 g tid	37	No	28/32	31/37	ND	5/37	2/5
Payen et al (57)	2018	Belgium	2009-2016	Retrospective case series	Meropenem	2 g tid (then bid)	18	No	16/18	16/18	15/18	0/18	0/18
Palmero et al (59)	2015	Argentina	2012-2013	Retrospective	Meropenem	2 g tid (then 1 g tid)	10	No	ND	8/10	3/6	0/10	ND
Van Rijn et al (10)	2016	The Netherlands	2010-2013	Retrospective	Ertapenem	1 g oc	18	yes	ND	15/18	15/18	2/18	3/18
Tiberi et al (61)	2016	Italy,	2008-2015	Retrospective, cohort	Ertapenem	1 g oc	5	No	3/5	3/5	4/5	0/5	0/5
Tiberi et al (60)	2016	Multicentred in 3 countries	2003-2015	Observational, retrospective, cohort	Meropenem	1 g tid (2g tid)	96	No	55/58	55/58	55/96	6/93	8/94
Tiberi et al (11, 55)	2016	Multicentred in 8 countries	2003-2015	Observational retrospective case-control	Imipenem	500 mg qid	84	No	51/64	51/64	34/57	3/56	4/55

od: once a day, bid: twice a day, tid: three times a dat, qid: four times a day. PK: pharmacokinetic, ND: not described

term safety was not a problem in this study as no adverse events were reported (56-57). The first study, that evaluated efficacy, safety and tolerability, was a case-control study in 37 patients, who received meropenem/clavulanate as part of a linezolid based multi-drug regimen. This is the first study that showed an added value of meropenem/clavulanate in a multi-drug regimen. The meropenem/clavulanate containing regimen showed a sputum microscopy conversion of 87.5 % and a sputum culture conversion of 83.8%, while the meropenem/clavulanate sparing regimen showed a sputum microscopy conversion of 56.3% and a sputum culture conversion of 62.5% after 90 days of treatment (58). In another study, 10 XDR and pre-XDR female patients were treated with multi-drug regimens and received meropenem/clavulanate for 6 months or more. Eight patients achieved sputum conversion after 6 months, while two patients died. (59). Pharmacokinetic parameters of 1 g meropenem/clavulanate given intravenously over 5 minutes showed a serum peak of 112 mg/ml and a concentration of 28.6 mg*h/L (39). In an observational retrospective cohort-study, efficacy and safety were evaluated in 96 patients treated with meropenem/clavulanate containing regimens and compared with 168 controls. Sputum smear and culture conversion rates were found to be similar (60) In an observational study comparing therapeutic contribution, such as sputum smear and culture conversion rates and success rates, of imipenem/clavulanate and meropenem/ clavulanate in a background regimen, suggested that meropenem/clavulanate can contribute to the efficacy of a regimen in treating M/XDR-TB patients (11).

Ertapenem

The first report on clinical experience with ertapenem presented data from five patients who were treated with an intravenous injection of 1 g ertapenem once daily in a multi-drug regimen. Three of these patients showed sputum smear and culture conversion; four of five patients had a successful treatment outcome. Two patients interrupted treatment due to an adverse event. These adverse events were considered unrelated to the study drug (61). In an observational study 18 patients were treated with 1 g ertapenem once daily; fifteen of these patients had a successful treatment outcome were cured. Three patients were lost due to follow-up. Three patients stopped ertapenem treatment due to ertapenem unrelated adverse events. Pharmacokinetic parameters were evaluated in 12 patients, showing a mean peak plasma of 127.5 (range 73.9 - 277.9) mg /L and an AUC of 544.9 (range 390 - 1130) mg*h/L.

Based on a MIC of 0.25 mg/ml 11/12 patients reached the target value of 40% T_{free}>MIC was exceeded (10). The pharmacokinetic model composed in this study was shown to adequately predict ertapenem exposure in MDR-TB patients. The Monte Carlo simulation, which had a time restriction of 0–6 h, showed that the best performing limited sampling strategy was at 1 and 5 h after intravenous injection. (62). In another pharmacokinetic model study using prospective data from 12 TB patients it was observed that 2 g ertapenem once daily resulted in a more than a dose-proportional increase in AUC compared to once daily 1 g ertapenem. Based on a MIC of 1.0 mg/L, 11 out of 12 patients reached the target value of 40% T_{free}>MIC (63).

Discussion

Hugonnet and colleagues first stated that carbapenems have antimycobacterial activity (7). Subsequently, studies addressing the inactivation mechanism of LDT provided the underlying evidence to support the hypothesis of activity of carbapenems against *M. tuberculosis* (14-28). In spite of this a series of *in vitro* studies have been carried out, some of which detected an effect and some of which did not (8,32-50). Only later, was it recognized that these confusing results are probably explained by the chemical instability of carbapenems, in culture media at the temperatures typically used in *in vitro* studies, and many previously published *in vitro* studies are likely to have reported falsely high MICs (45).

Overall the results of the studies identified in this review, which used a variety of experimental methods to test clinical and laboratory strains of *M. tuberculosis* for susceptibility to carbapenems, are consistent. Carbapenems are more active against *M. tuberculosis* if used in combination with clavulanate, a BLaC inhibitor. (8,32-50). In line with these *in vitro* studies the addition of clavulanate improved the survival rate in mice (35). As the European Medicines Agency (EMA) has accepted and qualified the *in vitro* hollow fiber system models as a methodology to define pharmacokinetic and pharmacodynamic (PK/PD) parameters, these modern *in vitro* studies can be used to avoid the problems associated with the chemical instability of these agents in standard agar-based MIC testing. Thus, hollow fiber systems have the potential for dose finding and regimen selection studies on the use of carbapenems in the treatment of TB (64-65).

Few *in vivo* studies have been performed due to the short half-life and lower serum concentrations of carbapenems in mice (35).

One prospective, two observational and seven retrospective clinical studies to assess effectiveness, safety and tolerability of three different carbapenems (imipenem, meropenem and ertapenem) have been performed. Adverse events due to carbapenems were mild, confirming what we know from other infectious diseases; but in contrast to other repurposed drugs like linezolid (55,58,60). To date, only two large retrospective studies with M/XDR-TB patients have been performed with imipenem (84 patients), and meropenem (96 patients) (11). Meropenem/ clavulanate was suggested to be more efficient in managing M/XDR-TB (11), however interpretational limitations were mentioned.

We found no clear evidence to select one particular carbapenem among the different candidate compounds, when designing an effective M/XDR-TB regimen. Both economical and clinical factors play a role. Whereas imipenem is the cheaper carbapenem, ertapenem has the potential advantage that it is only given once daily; and meropenem is by some authors believed to be the most effective in humans, but no head-to-head comparison studies have confirmed this to date. Therefore, more clinical evidence and dose optimization substantiated for example by hollow fiber infection studies are needed to support the repurposing carbapenems for the treatment of M/XDR-TB.

Clinical studies are hampered by the fact that currently no combination of a carbapenem with clavulanate is commercially available. Furthermore, clavulanate is not available alone so at present it is not practically possible to prescribe carbapenem with clavulanate. Therefore, amoxicillin – clavulanate is often co-administered along with a carbapenem in case the latter is preferred for treatment. Unfortunately, amoxicillin has gastrointestinal side effects potentially complicating prolonged treatment. Therefore, combined treatment amoxicillin–clavulanate with a carbapenem is only an option for TB treatment of complicated cases showing multi- or extensive drug resistance (42). Although, Gonzalo *et al.* reported a potential benefit that MIC values drop when amoxicillin is added to a combination of meropenem and clavulanate.

Due to different procedures, analytical methods and design, the biochemical instability of the drugs of interest, the short half-live of drugs of interest in mice, diversity in MIC determination and intolerance in addition to resistance, it was not possible to perform a meta-analysis. While the observational data are promising, carbapenems can only recommended in case of resistance to group A and group B drugs in M/XDR-TB treatment.

The ideal carbapenem would have the antimycobacterial activity of imipenem, the half-life of ertapenem and the oral bioavailability of tebipenem-pivoxil. Due to increasing resistance observed in XDR-TB isolates (66-67) and in MDR-TB patients with resistance to an aminoglycoside, carbapenems may be a valuable alternative to the current injectable second line drugs. Assessment of intracellular activity as well as activity against dormant *M. tuberculosis* by carbapenems is a critical step to further explore the potential of these repurposed drugs.

As successful treatment outcome for M/XDR-TB is still poor, ranging from 25-50% (1) an improvement of the current treatment is urgently needed. An individual data meta-analysis among 12,030 individual patients from 50 studies showed a significantly better treatment outcome for patients who received carbapenems compared to other drugs traditionally used for treatment of MDR-TB. (68). Since there is a need for new or repurposed drugs for the treatment of M/XDR-TB, phase II/ III clinical trials are urgently needed for carbapenems to further evaluate their potential. Long term safety and activity against *M. tuberculosis* are supported by observational data and several studies (41,50,69). A phase II prospective randomized controlled study evaluating a carbapenem plus a BLaC inhibitor on top of an optimized background regimen versus standard of care would be an appropriate strategy to test the potential benefits of carbapenems for M/XDR-TB treatment.

Conclusion

Now the variable results of *in vitro* studies have been explained and the activity of carbapenems in the presence of a BLaC inhibitor is established, these drugs should be further developed for the treatment of multi- and extensive drug resistant *M. tuberculosis*. Ultimately, a well-designed phase 2 study is needed to substantiate the claimed benefits of carbapenems in patients with drug-resistant TB.

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CHAPTER 3

Quantification and Validation of Ertapenem Using a Liquid Chromatography-Tandem Mass Spectrometry Method

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Abstract

Background: Ertapenem, a carbapenem, relies on time-dependent killing. Therapeutic drug monitoring (TDM) should be considered, when used in specific populations, to achieve optimal bactericidal activity and optimize drug-dosing regimens. No validated LC-MS/MS method has been reported using deuterated ertapenem as internal standard. A new simple and robust LC-MS/MS method using a quadruple mass spectrometer was developed for analysis of ertapenem in human plasma, using deuterated ertapenem as internal standard. The calibration curve was linear over a range of 0.1 (LLOQ) to 125 mg/L. The calculated accuracy ranged from -2.4 % to 10.3%. Within-run CV ranged from 2.7 % to 11.8% and between-run CV ranged from 0 % to 8.4%. Freeze-thaw stability biased between -3.3% and 0.1%. Storage of QC samples for 96h at 4°C differed -4.3 to 5.6%, storage at room temperature for 24h, biased from -10.7% to -14.8% and storage in the autosampler biased between -2.9% and -10.0%. A simple LC-MS/MS method to quantify ertapenem in human plasma using deuterated ertapenem as internal standard has been validated. This method can be used in pharmacokinetic studies and in clinical studies by performing TDM.

Introduction

Carbapenems belong to the Beta-lactam antibiotics and are widely used against a broad spectrum of aerobe and anaerobe gram-positive and gram-negative bacteria (1, 2). Ertapenem, approved by the FDA in 2001, is one of these carbapenems. Since ertapenem has an approximate half-life of 4h, it can be administered once daily. Therefore, ertapenem can be favored above other carbapenems (3). The pharmacodynamic (PD) parameter of ertapenem correlates with time dependent killing, which means that the plasma concentration of ertapenem has to exceed minimal inhibitory concentration (MIC) for a percentage of time of its dosing interval (4).

Pharmacokinetic data obtained in healthy volunteers are difficult to extrapolate to specific patient populations. Due to this high variability in pharmacokinetic parameters, exposure of ertapenem might be suboptimal in these specific populations (5-10). Since ertapenem is a time-dependent antibiotic, therapeutic drug monitoring (TDM) should therefore be considered, when used in specific populations, to achieve optimal bactericidal activity and optimize drug-dosing regimens. Yet more PK studies have to be performed to determine efficacy and safety of ertapenem in specific population (11, 12).

Ertapenem is being suggested for having potential use against M. tuberculosis (TB) (1). However, according to Caminero et al. carbapenems are used as fifth line drugs in the treatment of TB and can only be used in severe cases only, since carbapenems are administered intravenously, costs are high and clinical experience is restricted (13). Clinical studies assessing efficacy or safety profiles for carbapenems are scarce, but showed favorable preliminary results (14-16). Before clinical efficacy in TB can be determined a dose-finding study should be performed to evaluate pharmacokinetic parameters in this specific patient population. Therefore, an analytical method to measure ertapenem concentrations is mandatory.

There are a few methods published about LC-MS/MS quantification and validation of ertapenem in human plasma (17, 18). Since LC-MS/MS is easy to use frequently in daily routine, more pharmacokinetic studies are being performed to quantify drugs. It is therefore

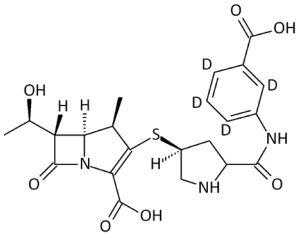
important to have established standards in order to compare results of PK studies between laboratories. Present validated LC-MS/MS methods for ertapenem are using extensive sample preparation, e.g. liquid-liquid extraction (LLE), solid phase extraction (SPE) and nitrogen gas drying (17, 18). These methods are time consuming and less cost-effective. The choice of an internal standard for LC-MS/MS is important as it corrects for extraction, injection and ionization variability. Particularly the latter one, ion suppression and ion enhancement, is a source of variability. Only a deuterated internal standard is suitable to compensate for this and assure a robust high throughput bioanalytical method (19). Since no LC-MS/MS method has been validated using a deuterated internal standard, the purpose of this study is to develop a new simple and robust LC-MS/MS method using a quadruple mass spectrometer without extensive sample processing, using deuterated ertapenem as internal standard to quantify concentrations of ertapenem in human plasma for pharmacokinetic studies.

Material and methods

Analysis

Ertapenem (Fig 1) and the internal standard used, ertapenem-D4, were purchased from Alsachim (Illkirch, Graffenstaden, France).

Figure 1. Chemical structure of ertapenem



Acetonitrile for LC-MS/MS was purchased from BioSolve (Valkenswaard, The Netherlands). The chemicals used, including methanol Lichrosolv and trifluoroacetic anhydride are of HPLC or analytical grade and were purchased from VWR (Amsterdam, The Netherlands). Purified water was obtained from a Milli-Q water purifying system (Millipore Corporation, Billerica, MA, USA). The precipitation reagent consisted of a mixture of methanol and acetonitrile

(4:21, v/v). Pooled human plasma samples with EDTA as anticoagulant and pooled human serum samples were made available according to the standard operating procedures of University Medical Center Groningen.

The calibration standards, blank and QC samples were fully thawed at room temperature. To 100 µL of each sample a volume of 500 µL of precipitation reagents and 10 µL of ertapenem-D4 (250 mg/L) were added in a vial. The samples were vortexed for 1 minute. To promote protein precipitation, the vials were stored at -20°C for 30 minutes. The vials were centrifuged for 5 minutes at 11000 rpm. Five µL of the upper layer were injected into the LC-MS/MS. QC samples and calibration standards were stored at -20°C.

The analysis was performed on a triple quadrupole LC-MS/MS (Thermo Scientific, San Jose, CA USA) with a MS pump Plus (Finnigan, surveyor) and autosampler (Finnigan, surveyor). The mass spectrometer was a TSQ Quantum Access Max mass spectrometer. The autosampler temperature was set at 10°C. Liquid chromatographic separation was performed by a HyPURITY C_{18}, analytical column, 50 * 2.1 mm, 3-µm (Thermo Scientific, Interscience, Breda, The Netherlands) and temperature was set at 20°C. The mobile phase had a flow of 300 µL/min and consisted of purified water, acetonitrile and an aqueous buffer (containing ammonium acetate 10 g/L, acetic acid 35 mg/L and trifluoroacetic anhydride 2 mg/L water. The method had a runtime of 4 minutes and the elution gradient is shown in Table 1.

Table 1. Eluent gradient

Time (minutes)	A (%)	B (%)	C (%)
0.00	5	95	0
0.50	5	35	60
1.30	5	35	60
1.40	5	0	95
2.80	5	0	95
3.00	5	95	0
4.00	5	95	0

A: aqueous buffer; B: purified water, C: acetonitrile

The MS was configured onto positive electrospray ionization mode and Selected Reaction Monitoring (SRM) with a spray voltage of 3500 V, a capillary temperature of 350°C and a sheath gas pressure and auxiliary pressure 35 and 5 arbitrary units respectively.
Mass transitions for ertapenem were 476.1 m/z → 432.1 m/z and for ertapenem-D4 480.1 m/z → 436.1 m/z, using a scan width of 0.5 m/z. Collision energy was determined on 10 eV for both transitions. Peak height integration for all components was calculated by Xcalibur software version 2.0.7 (Thermo Fisher, San Jose, CA, USA).

Analytical method validation

Validation of the method included selectivity and sensitivity, linearity, accuracy and precision, recovery and dilution integrity conform the guidance for Industry of the Food and Drug Administration. Since the FDA did not postulate a maximum CV requirement for stability, a maximum CV of 15 % was employed according to EMA guidelines (20, 21).
In some cases, for example in pharmacokinetic studies, human serum is being collected for the quantification of ertapenem. To check if there's a difference between the analysis of ertapenem in human plasma and in human serum, a matrix comparison has been performed.

The calibration curve of ertapenem consisted of 8 samples with concentrations of 0.1, 0.5, 2.0, 7.5, 20, 50, 90 and 125 mg/L. Quality Control (QC) samples with 4 different concentrations of ertapenem were used, whereas LLOQ is 0.1 mg/L, LOW is 2.5 mg/L, MED is 40 mg/L and HIGH is 120 mg/L. For selectivity 6 pooled human plasma samples were examined for interference and compared with response of the lower limit of quantification samples (LLOQ). During three days, each day a single calibration curve in plasma and in serum was analyzed and accuracy was measured by evaluation of five determinations per QC sample on three consecutive days. Precision is divided into within-run and between-run using the same method as accuracy. The coefficient of variation for LLOQ may not exceed 20% and for other QC levels not exceed 15%.

The recovery was determined on three levels (LOW, MED and HIGH) in fivefold. As protein precipitation is used as the only way of sample preparation in this method, relative recovery was measured by comparing the ratios of integrated peak heights of ertapenem and the internal standard of the processed QC samples with the average peak heights of the recovery

samples. Recovery samples (LOW, MED and HIGH) were post-extraction blank samples spiked at the same concentrations as the QC samples.

Stability of ertapenem was tested in different test conditions, including storage stability and freeze-thaw stability. Storage stability of ertapenem was examined by storing QC samples at room temperature (20°C - 25°C) in a refrigerator at 4°C and after sample preparation in the autosampler at 10°C. Stability was also tested using three freeze-thaw cycles at -20°C. All stability tests were done using three different QC levels (LOW, MED, HIGH) in five determinations per concentration. Stability is defined as a change in concentration and should be ≤ 15%. Since FDA guidelines have no requirements for the coefficient of variation of each QC sample (LOW, MED, HIGH), EMA guideline requirements are taken into account, stating that CV of each QC sample should not exceed 15% (20, 21).
To determine the dilution integrity, on three consecutive days, a sample with a concentration of 500 mg ertapenem/L plasma was diluted 10 times and then prepared in fivefold.

Statistics

Results were analyzed using one-way ANOVA and validated Excel sheets (Microsoft, Redmond, WA).

Results

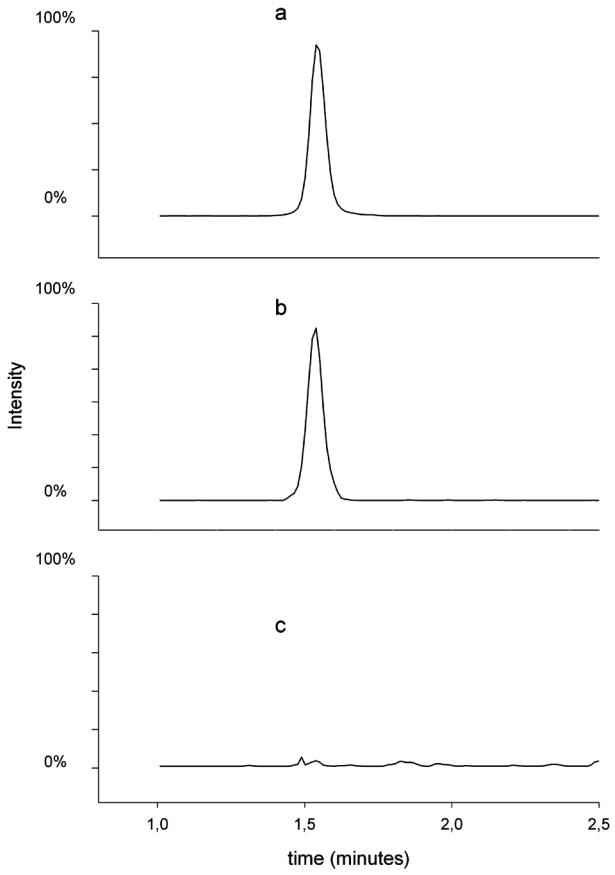
Mean retention times of ertapenem and ertapenem-D4 were equal to 1.5 minutes.
Examining the selectivity of this analytical method, there were no interfering peaks observed at the retention time of ertapenem or ertapenem-D4 in any of the six lots of pooled human plasma (Fig.2).

The calibration curve in plasma was linear over a range of 0.1 (LLOQ) to 125 mg/L and the correlation coefficient (R^2) was 0.9988. The calibration curve parameters are as follows; slope, 0.487 ± 0.00519 (average ± standard deviation); intercept, 0.0149 ± 0.0257; average regression coefficient, 0.99762; correlation coefficient, 0.9981.

For comparison of the analysis in plasma and in serum, the peak height ratios of ertapenem and the internal standard in plasma were compared to those in serum. Analyzing both data sets using Passing-Bablok regression, showed no statistically significant difference between the two matrices: y = 1.01(0.95 - 1.02)x + 0.00 (-0.01 - 0.04) at 95% confidence level.

Figure 2. Chromatogram

(a) Ertapenem D4 at LLQ, (b) ertapenem at LLQ and (c) blank plasma



Accuracy and precision, divided in within run and between run, were calculated using spiked samples for 5 determinations per concentration on 3 consecutive days. The calculated accuracy ranged from -2.4 %to 10.3%. Within-run precision ranged from 2.7 % to 11.8% and between-run precision ranged from 0 % to 8.4%. The results of accuracy and precision for all QC levels are shown in Table 2.

Table 2. Concentrations of calibration standards and QC samples.

QC samples	LLOQ	LOW	MED	HIGH
Concentration (mg/L)	0.1	2.5	40	120
Accuracy (% bias)	-2.4	9.3	7.3	10.3
Within-run precision (% CV)	11.8	5.6	3.1	2.7
Between-run precision (% CV)	8.4	0	1.5	1.6

QC samples LOW (2.5 mg/L), MED (40 mg/L) and HIGH (120 mg/L) were used to determine recovery; relative recovery was 101.7%, 97.9% and 95.1% for these three samples, respectively. Dilution integrity was determined in five-fold on 3 consecutive days. Accuracy was 1% and within-run and between-run precisions were 3.2% and 2.3%, respectively. Stability of ertapenem using different test conditions is shown in table 3.

Table 3. Stability testing results for ertapenem.

Test condition	LOW	MED	HIGH
Bench top, RT*, 24h (% bias)	-11.9	-10.7	-14.8
Refrigerator, 4°C, 96h (% bias)	5.6	-0.1	-4.3
Freeze-thaw, -20°C, 3 cycles (% bias)	-0.2	0.1	-3.3
Autosampler, 10°C, 24h (%bias)	-10.0	-2.9	-4.8

**RT room temperature*

Measured concentrations of QC samples (LOW, MED, HIGH) for the freeze-thaw stability biased between -3.3% and 0.1% and therefore comply with the guidelines. Stability was determined by measuring QC samples stored for 96h at 4°C and differed -4.3 to 5.6% from the nominal concentrations. After storage at room temperature for 24h, the concentration of ertapenem biased from -10.7% to -14.8%, compared to the initial concentrations. After sample preparation the concentration of ertapenem stored in the autosampler biased between -2.9% and -10.0% from the nominal concentrations.

Discussion

This is the first design and validation of a new, simple and rapid analysis method using a triple quadruple LC-MS/MS for the quantification of ertapenem in human plasma and deuterated ertapenem as internal standard.

This LC-MS/MS method was validated for accuracy and precision according to FDA guidelines, having biases <20% for LLOQ and <15% for other QC levels (20). The calibration curve was linear within a range of 0.1 (LLOQ) – 125 µg/mL, compared to other studies, which were validated up to 50 µg/mL and had a LLOQ of respectively 0.5 and 1.0 µg/mL (17, 18) This method used deuterated ertapenem as internal standard, resulting in better inter-day, intra-day variation and accuracy, compared to methods of Pickering et al. and Koal et al, which used Beta-lactam monologues as internal standard (17, 18).

Matrix comparison showed no difference between the analysis of ertapenem in human plasma and in human serum. However, because of the poor stability of ertapenem at room temperature it's recommended to draw whole blood (with EDTA as the anticoagulant) as it can be placed on ice for a short time during transport from the nursing ward to the analyzing laboratory.

As mentioned in the introduction a major advantage of this LC-MS/MS method is that a simple protein precipitation is used instead of LLE, SPE or nitrogen gas drying, resulting in a less time consuming and a less expensive method, compared to other LC-MS/MS methods (17, 18). The run time is very short since the retention time of ertapenem is 1.5 minutes. This facilitates a high sample trough put. This is a great advantage for laboratories having only one LCMSMS to support their clinical TDM service.

Ertapenem in plasma stored at room temperature decreases within a short period time with 10 to 15%. Therefore, it is crucial to store samples in the freezer until analysis and to process samples containing ertapenem within the validated time frame of stability to assure accurate and precise results. Reinjection of processed samples stored at 10°C in the autosampler is tolerated within 24 hours.

Since ertapenem is a time dependent antibiotic, it is necessary that plasma concentration exceeds MIC at least 40% of its dosing interval. To attain this target in patients suspected to have altered pharmacokinetics due to renal function, high variability in plasma proteins ertapenem concentration measuring may be of help, especially if more resistant pathogens are targeted with higher MIC values. This method meets the criteria for TDM but is also suitable for clinical pharmacokinetic studies or clinical trials to further investigate the use of ertapenem in other infectious diseases or other specific patient populations.

Conclusion

A simple LC-MS/MS method to quantify ertapenem in human plasma using deuterated ertapenem as internal standard has been developed and validated. This method can be used in pharmacokinetic studies and in clinical studies by performing TDM.

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CHAPTER 4

Pharmacokinetics of Ertapenem in Patients with Multidrug-Resistant Tuberculosis

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Abstract

Treatment of multidrug resistant (MDR) and extensively drug resistant (XDR) tuberculosis (TB) is becoming more challenging because of increased level of drug resistance against second line tuberculosis drugs. One promising group of antimicrobial drugs are carbapenems. Ertapenem is an attractive carbapenem for the treatment of MDR and XDR-TB because its relative long half-life enables once daily dosing. A retrospective study was performed for all MDR-TB suspected patients at the Tuberculosis Center Beatrixoord of University Medical Center Groningen (Haren, The Netherlands) who received ertapenem as part of their treatment regimen between the first of December 2010 and the first of March 2013. Safety and pharmacokinetics were evaluated. Eighteen patients were treated with 1000 mg ertapenem for a mean of 77 days (range 5-210). Sputum smear and culture were converted in all patients. Drug exposure was evaluated in 12 patients. The mean AUC0-24 was 544,9 (range 309 – 1130) mg*h/L. The mean Cmax was 127.5 (73.9 – 277.9) mg/L. In general, ertapenem treatment was well tolerated during MDR-TB treatment and showed a favourable PK/PD profile in MDR-TB patients. We conclude that ertapenem is a highly promising drug for the treatment of MDR-TB that warrants further investigation.

Introduction

Treatment of multidrug resistant (MDR) and extensively drug resistant (XDR) tuberculosis (TB) is becoming more challenging because of increased level of drug resistance against second line tuberculosis drugs. New drugs are being evaluated in clinical trials, but only bedaquiline and delamanid have entered the market to date. Therefore, antimicrobial drugs, which have been developed and labeled for other bacterial infections may be of potential use in the treatment of MDR-TB.

One promising group of antimicrobial drugs are carbapenems [1, 2]. An early *in vitro* experiment showed that imipenem and meropenem were active against *M. tuberculosis* [3]. Chambers and co-workers showed that imipenem has anti-mycobacterial activity in mice and humans [4]. Imipenem and meropenem are currently listed as group 5 drugs for the treatment of MDR-TB [5]. More recently, clinical experience of carbapenems in MDR-TB patients showed promising results [6-7].

Carbapenems are poor substrates for beta lactamase C (BLaC) due to rapid acylation and slow deacylation. Therefore, unlike beta-lactams, they are not rapidly hydrolyzed by BLaC and therefore maintain their potential activity against *M. tuberculosis* [8]. The binding of carbapenems to the LD transpeptidases results in inhibition of the peptidoglycan polymerization of the cell wall [9]. Combined with a beta lactamase inhibitor, such as clavulanate, activity against *M. tuberculosis* is higher [10].

Efficacy of carbapenems is correlated with the percentage of time the free plasma drug concentration transcends the MIC (T_{free}>MIC). Maximal bactericidal activity is reached if the time above MIC is at least 40% of dosing interval [11,12]. To reach this target for gram positive, gram negative and anaerobic bacterial infections ertapenem is given intravenously in a dose of 1000 mg once daily [13]. Ertapenem has the advantage over other carbapenems because of a long half-life of 4 h enabling once daily dosing [12], which is attractive for MDR-TB treatment. Another advantage is that ertapenem is not affected by drug-drug interactions as it is neither metabolized by cytochrome P450 nor a substrate for P-glycoprotein [14].

To include ertapenem among the other carbapenems as a group 5 drug for the treatment of MDR-TB additional pharmacokinetic and safety data are urgently needed [15]. Therefore, the

objective of this study was to evaluate pharmacokinetics and safety in patients that received ertapenem as part of their treatment MDR-TB regimen.

Patients and methods

Patients

All patients suspected to MDR-TB at the Tuberculosis Center Beatrixoord of the University Medical Center Groningen (Haren, The Netherlands) who received ertapenem as part of their treatment regimen between first of December 2010 and the first of March 2013 were included in this retrospective study. The study was evaluated by the Medical Ethical Review Board of the University Medical Center Groningen (metc 2013-492). The need for written informed consent was waived for the retrospective collection and analysis of anonymous data because it was not required under Dutch Law (WMO). For each MDR-TB suspect, age, gender, weight, length, ethnicity, drug susceptibility pattern, localization of tuberculosis, antiretroviral therapy, sputum conversion, adverse effects induced by ertapenem, dose, total exposure to ertapenem, and duration of treatment were collected.

Drug susceptibility to Ertapenem

Drug susceptibility testing (DST) of ertapenem was performed with and without clavulanic acid using the middlebrook 7H10 agar dilution method at the Dutch National Tuberculosis Reference Laboratory (National Institute for Public Health and the Environment RIVM), Bilthoven, The Netherlands) [16].

Pharmacokinetics and pharmacodynamics

All patients received ertapenem in a dosage of 1000 mg once daily, given as intravenous infusion in 30 min. In all MDR-TB patients routine plasma concentrations were collected at steady state to assess drug exposure to enable individualized dosing. For plasma sampling a peripheral intravenous catheter was inserted. Patency of the peripheral catheter was maintained by a saline drip. Before a blood sample was taken, the drip was stopped and the first 4 mls of blood were discarded. The samples were collected before administration and at t = 1, 2, 3, 4, 5, 6, 8, 12, hrs post-dosage and stored at –80 °C until analysis. Plasma concentrations were assessed and validated using a validated liquid chromatography-tandem

mass spectrometry (LC-MS/MS) in the laboratory of Clinical toxicology and Drugs Analysis of the department of Clinical Pharmacy and Pharmacology at the University Medical Center Groningen [17]. Population pharmacokinetic parameters were calculated using the KinPOP module. Both KinFIT and KinPOP were part of the software package MWPharm 3.82 (Mediware, The Netherlands). The T_{free}>MIC was calculated as this has been proposed as the best pharmacokinetic/pharmacodynamic parameter to predict *in vivo* efficacy of carbapenems [10]. Free drug concentration was assumed to be 5% [12,18]. Eucast minimal inhibitory concentrations for ertapenem (non-species related) of 0.5 and 1.0 mg/L were used to calculate T_{free}>MIC.

Safety and tolerability

Reported adverse effects (AE) in medical charts were used to evaluate the safety of ertapenem. Specific attention was paid to AE's mentioned in earlier studies: i.e. diarrhea and vomiting. The Naranjo algorithm was used to evaluate for causality between adverse effects that occurred and ertapenem [19].

Statistics and pharmacokinetic evaluation

SPSS 20 was used as statistical software (SPSS, Virgina, IL). Correlation between pharmacokinetic parameters and patient characteristics were analyzed using the Spearman correlation coefficient. MIC data were statistical analyzed using a methodology for censored MIC data [20].

Results

Patients

Eighteen patients treated with ertapenem, mean age 29 (range 13 - 66 years), were retrieved. Ertapenem was part of the treatment regimen because of suspected extensive drug resistance, intolerance to second line drugs or combination of both. Based on the results of the drug susceptibility test ertapenem was discontinued in three patients who appeared to have drug susceptible TB. Gender was unequally distributed between patients as 8 were male (44.4%) and 10 patients were female (55.5%). The mean body mass index was 21.3 (range

13.7-32.6) kg/m2. Patients originated predominantly from Africa (11/18) and Europe (5/18). Patients were primarily diagnosed with pulmonary TB (13/18), extra pulmonary sites were involved in 7 patients.

Prescribed dosage of ertapenem was 1000 mg once daily in all patients. Mean total treatment duration of ertapenem was 77 days (range 5-210 days). Drug resistance pattern of the patients to anti-tuberculosis agents are shown in table 1. Most prescribed anti-TB drugs were: moxifloxacin (17/18), injectable (16/18), linezolid (15/18), clofazimine (8/18), clarithromycin (6/18) and pyrazinamide (5/18).

In total 15 patients completed treatment and were cured. Three patients were lost to follow up. All patients with positive sputum-smear converted within a mean period of 17 days (range 0-97 days). Cultures remained negative after culture conversion and no relapse of MDR-TB was observed.

Table 1. Resistance pattern

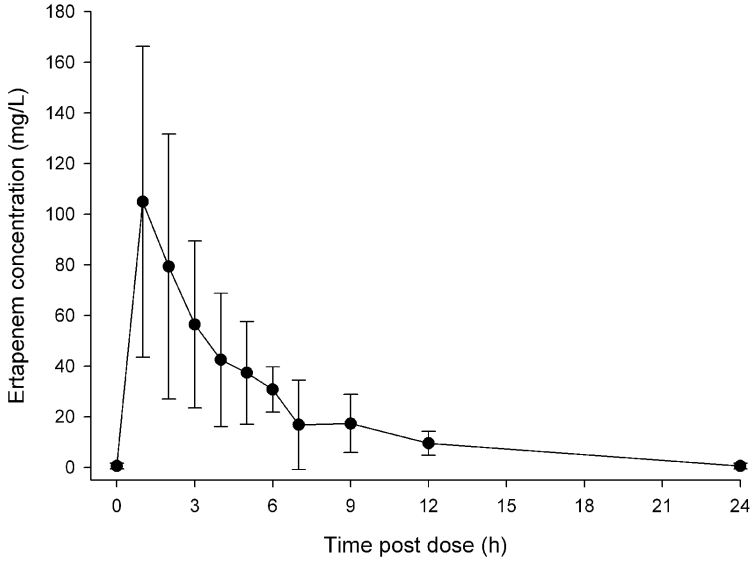
WHO			**Resistant N (%)**	**Sensitive N (%)**
Group 1	First-line oral drugs	Isoniazid	17 (94,4)	1 (5,55)
		Rifampicin	15(83,3)	3 (16,6)
		Pyrazinamide	8(44,4)	8 (44,4)
		Ethambutol	11(61,1)	6 (33,3)
		Rifabutin	13 (72,2)	4 (22,2)
Group 2	Injectable agents	streptomycin	14 (77,7)	4 (22,2)
		Amikacin	3(16,6)	14 (77,7)
		kanamycin	3(16,6)	14 (77,7)
		capreomycin	5 (27,7)	12 (66,6)
Group 3	Fluorquinolones	Moxifloxacin	2 (11,1)	15 (83,3)
Group 4	Oral bacteriostatic Second-line agents	protionamide	7 (38,8)	10(55,5)
Group 5	Agents with unclear	Linezolid		17 (94,4)
	Role in treatment of	Clarithromycin	3 (16,6)	7(38,8)
	drug resistant- TB	Clofamizine		8(44,4)
Other		Ertapenem		18 (100)

Data are presented as N (%), N=18

Drug susceptibility of *M. tuberculosis* to ertapenem

All the *M. tuberculosis* strains appeared susceptible to ertapenem. However, actual determination of the MIC was complicated by the fact that ertapenem itself appeared an instable compound at 37°C [17]. This was confirmed by the fact that after 7 days MIC values were lower than after 14 days. In addition, freshly prepared plates showed lower MIC's compared to plates stored at 4°C. The refrigerator-stored plates showed lower MIC's than plates stored at room temperature. If ertapenem was combined with clavulanic acid all MIC's were even lower.

Figure 1. Ertapenem plasma concentration-time curves in 12 patients



Pharmacokinetics and pharmacodynamics

The plasma concentration time curves were obtained in 12 patients with MDR-TB. In the remaining 6 patients routine plasma concentrations were collected at a time point at which they did not yet receive ertapenem or this drug was no longer administered. Three patients had multiple plasma concentration time curves and these were consistent. The mean curve is shown in figure 1. The mean AUC0-24 was 544,9 (range 309 – 1130) mg*h/L. The steady state pharmacokinetic parameters are shown in table 2.

Based on a MIC of 0.25 mg/L, 11 out of 12 patients exceeded a minimum of 40%-time above MIC. In 9 patients the MDR-TB remained susceptible with a MIC of 0.5 mg/L. Except for 2 patients, none exceeded a minimum of 40%-time interval with a MIC of 1 mg/L. The pharmacokinetic population model (KinPOP) of ertapenem showed a clearance of 2.26 (range 0.86-3,19) L/h/1.73m2 and a volume of distribution of 8.79 (range 4.76-13,57) L.

Table 2. pharmacokinetic parameters of Ertapenem

Study	AUC 0-24 (h*mg/L)	Cmax (mg/L)	T1/2 (h)	Vd (L)	CL (L/h)
1g IV in MDR-TB patients	544.9 (309 – 1130)	127.5 (73.9 – 277.9)	2.4 (2.047 – 3.528)	7.3 (2.612 – 11.1)	2.1 (0.0884 – 3.231)
1g IV in healthy volunteers [16]	572.1 (572 – 672)	154.9 (145 – 175)	4 (3.8-4.4)	8.2	1.8

Data are presented as mean (range). AUC 0-24: Area under the curve-time curve up to 24h. Cmax: highest observed plasma concentration. Cl: clearance. T1/2: half-life. Vd: volume of distribution.

Safety and tolerability

In general, ertapenem was tolerated very well. In three patients, treatment with ertapenem was stopped. One of these patients suffered from Crohn's disease and developed MDR-TB after multiple dosages of infliximab, a TNF alpha-blocker [21-22]. This patient experienced allergic fever, shortly after administration of ertapenem and ethambutol. After reintroduction this happened again (Naranjo score= 4). Both adverse events subsided after withdrawal of the offending drug. In the second patient ertapenem was stopped after an increase in liver enzymes (ASAT: 109 / ALAT: 255) after 13 days of treatment with ertapenem. However, after two months, while this patient was still on treatment without ertapenem, liver enzymes remained elevated (Naranjo score = 1). In one patient kanamycin, linezolid and ertapenem were stopped due to line sepsis. This was considered not-to-be related to ertapenem. After removal of the venous access port, the patient recovered. Ertapenem and a new venous access port were not reintroduced, since it was not indicated anymore, due to low bacillary load at that time and these IV drugs could be substituted with oral antimycobacterial drugs. None of the patients experienced diarrhea, vomiting or dizziness.

Discussion

This is the first study, following a clinical report of ertapenem [6], presenting pharmacokinetic and safety data in patients with MDR-TB. In comparison with healthy volunteers, MDR-TB patients showed lower AUC0-24 values. Mean values of volume of distribution and clearance of MDR-TB patients were higher compared to healthy volunteers. Our observation is consistent with other studies that showed a lower drug exposure of ertapenem in patients with infectious diseases [23-25]. More surprising was the inter-variability in AUC between patients with MDR-TB. Other antimycobacterial drugs also show highly variability and lower drug exposure in TB patients as well [2, 26-27]. It is not yet completely clear why drug exposure is lower in TB patients. Apparently, stage of disease and altered body composition may potentially help to explain this observation.

Since ertapenem belongs to the class of beta-lactams, ertapenem has a time-dependent bactericidal activity. The Tfree>MIC is therefore important to evaluate the efficacy of ertapenem against *M. tuberculosis*. Nicolau and colleagues indicated that in case meropenem showed 40 %T>MIC bactericidal activity is observed whereas 20 %T>MIC appeared to have bacteriostatic activity. Other studies have mentioned this T>MIC as well [11-12,14]. Protein binding of ertapenem was assumed to be 5%, since ertapenem shows concentration-dependent plasma protein binding. Healthy volunteers, whom average a peak plasma concentration of 150 mg/L after the end of infusion of 1 g of ertapenem, have a percentage of 8 % unbound protein. When total drug plasma concentration declines below 50 mg/L peak plasma concentration, the percentage of unbound ertapenem is circa 5% [18]. It is very promising to notice that the non-species related breakpoint of ertapenem of 0.5 mg/L was exceeded for more than 40% of the day in the majority of patients assuming that patients have a protein binding of 5%. At a higher MIC value of 1 mg/L bacteriostatic activity could be expected. Therefore, ertapenem seems a very attractive drug for MDR-TB treatment. It seems warranted that doses of ertapenem higher than 1g/day should be used in the treatment of MDR-TB. However, *in vitro* experiments evaluating PK/PD targets for ertapenem against *M. tuberculosis* have yet to be performed. The hollow fiber infection model is suitable for PK/PD experiments and has already been used successfully before [28].

Besides pharmacokinetics of ertapenem in patients with MDR-TB, additional safety data are described for the first time as well. Only one patient, with Crohn's disease, experienced AE, which might be potentially related to ertapenem. One can speculate this may be related to an infusion related AE, due to a developed immune disorder and eventually a consequence of an infliximab treatment. Drug induced fever is a common AE of infliximab in the treatment of Crohn's Disease [29]. AE's of other carbapenems, such as diarrhea, nausea, vomiting, headache and rash are well documented and found to be mild [1, 30]. According to the product leaflet, ertapenem is given for a maximum of two consecutive weeks. Previous studies explored the safety and tolerability of ertapenem for this period of time and concluded that adverse side effects were mild to moderate [13]. In our study we showed that AE did not increase during prolonged treatment.

The measurement of actual MIC values was complicated by the fact that ertapenem is an instable compound at 37°C. As drug susceptibility testing for *M. tuberculosis* takes at least two weeks at 37°C, it is highly likely that the initial drug concentration decreases rapidly in time. Unfortunately, with the current drug susceptibility systems, e.g. MGIT or plate, this problem cannot be overcome, as the drug concentration in the medium cannot be corrected for a decrease in concentration due to degradation of the drug. Recently the hollow fiber infection model solved this problem as drug concentrations can be increased to correct for degradation [31]. As systems are expensive and difficult to manage its not likely that routine DST will be performed using hollow fiber systems. Another alternative may be the use of E-tests [32]. This is much cheaper and easier to employ but it is unclear if it can help to overcome the instability of ertapenem.

The most important limitation of our study is the retrospective character and its limited sample size, and absence of control group, thereby preventing a meaningful conclusion on efficacy of ertapenem. Secondly the inability to define MIC for ertapenem in clinical isolates is another limitation. Nevertheless, all patients were cured and no relapse was noticed after being treated with combination regimen including ertapenem. Likely the combination of drugs contributed to sputum culture conversion and favorable treatment outcome. This is in line with recently published data on tolerability and outcomes in 5 patients receiving ertapenem [6].

A recent editorial proposed a new classification of anti-tuberculosis drugs. It marked the potential of carbapenems as group 5 drugs, however carbapenems are still in need of proper evaluation and clinical evidence [33]. Before ertapenem can be labeled as a group 5 drug and used as part of MDR-TB treatment, a valid procedure to test drug susceptibility has to be made available. Ideally, the use of ertapenem would be supported by the results of a clinical trial.

In conclusion this study provides new knowledge on the use of ertapenem in patients with MDR-TB, presenting pharmacokinetic and additional safety data.

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CHAPTER 5

Susceptibility Testing of Antibiotics That Degrade Faster than the Doubling Time of Slow-Growing Mycobacteria: Ertapenem Sterilizing Effect Versus *Mycobacterium Tuberculosis*

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Abstract

Drug susceptibility tests (DST) for *Mycobacterium tuberculosis* require at least 7 days of incubation. For unstable at 37°C drugs such as ertapenem, it is likely that this drug could be degraded before killing or inhibiting slow growing bacteria. This would alter the minimum inhibitory concentrations (MICs) of the ertapenem, leading to falsely high MICs. Here, we developed a new strategy to perform DST and MICs for such unstable compounds.

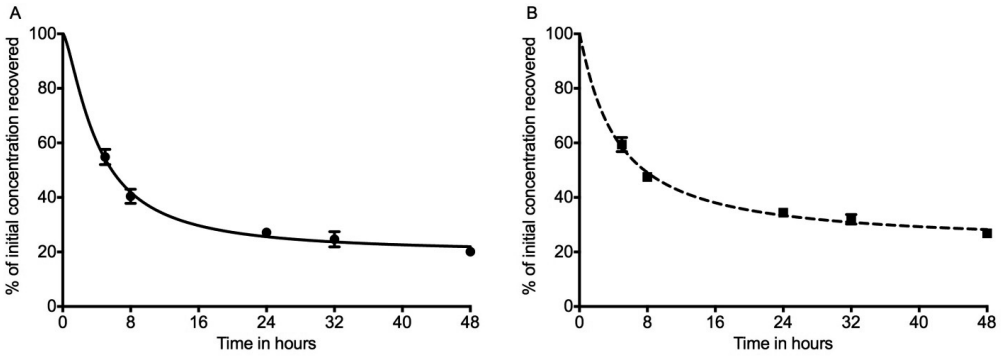
Ertapenem, a β-lactam agent of the carbapenem class, has shown promising clinical results and favourable pharmacokinetics against *Mycobacterium tuberculosis* (*Mtb*) (1,2). The scourge of multidrug-resistant tuberculosis (MDR-TB) and extensively drug-resistant tuberculosis (XDR-TB), a global problem, has increased the urgency for the use of carbapenems such as ertapenem, meropenem, and faropenem (2-4). Recently the first phase II study (NCT02349841) evaluating early bactericidal activity of meropenem and faropenem has been completed and results are expected soon.

Carbapenems inhibit the peptidase domain of penicillin binding proteins, leading to autolysis and peptidoglycan weakening of the cell wall (5). Degradation of ertapenem on storage following reconstitution and dilution is temperature dependent and the proposed in-use shelf-life is 6 hr at room temperature or 24 hours at 2 to 8°C (6). *Mtb* has a doubling time of at least 24 hours under the best of circumstances (7,8). *Mtb* cell division is particularly slow; FtsZ, a protein responsible for initiating cell division and recruiting proteins for formation of new cell wall is known to have a rate of polymerization that is at least 20 times slower in *Mtb* compared to *Escherichia coli* for example (9). In *Mtb* at low pH, the replication rate is up to10-20 times slower, kill of such semi dormant bacteria is defined as sterilizing effect (7,8,10). Thus, microbial kill and inhibition of growth by the most effective of antibiotics is slow and takes place over several days, especially by β-lactams that depend on cell wall turnover.

Drug susceptibility tests (DST) for *Mtb* using Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) approved methods require at least 7 days of incubation (11). For drugs such as ertapenem, already appearing unstable at 37°C (2), it is highly likely that this drug could be degraded before killing or inhibiting slow growing bacteria, especially semi dormant *Mtb*. This would be expected to alter the minimum inhibitory concentrations (MICs) of ertapenem, leading to falsely high MICs and false resistance. Here, we show the rapid decline of ertapenem during DST and thereby we developed a new strategy to perform DST and MICs for such unstable compounds.

Ertapenem (purchased from SIGMA) was first dissolved in purified water and subsequently diluted in Middlebrook 7H9 broth to the desired drug concentrations of 5.0 and 50 mg/L respectively. The two solutions were incubated at 37ºC. After 0, 5, 8, 24, 32 and 48 hours, three samples were collected from each solution and immediately stored at -80ºC until further analysis. All samples were then fully thawed at room temperature and analysed in duplicate using a fully validated assay (12). The calibration curve of ertapenem was linear over a range of 0.1 to 125 mg/L and the correlation coefficient was 0.999. The % coefficient of variation between the replicates for each concentration at each time point was 2.7-11.2%. Figure 1 shows the decrease in ertapenem concentration in the solution at 37°C. After 5 hours of incubation, ertapenem concentration was reduced by 45.3% and 40.7% in comparison with the initial concentrations of 5 and 50 mg/L, respectively. After 48 hours, the concentrations were 20.1% and 26.8% of the time zero concentrations.

Figure 1. LC-MS/MS analysis of ertapenem to determine the rate of degradation at 37^0C.

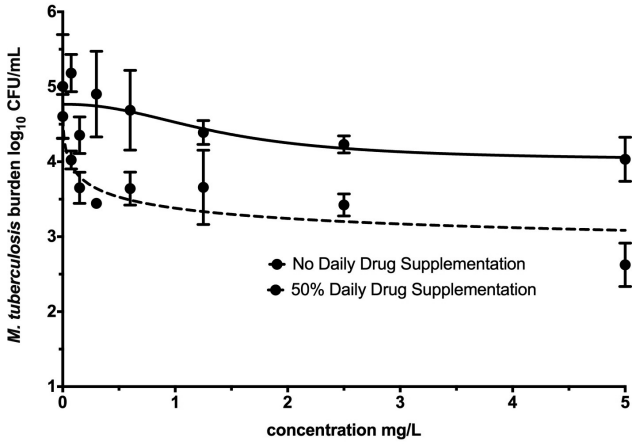


Panel A and B, 5 and 50 mg/L initial concentration, respectively show the percentage of ertapenem measured after incubation at 37^0C. *M. tuberculosis* grows "in slow motion" compared to these other bacteria (72 times slower than *E. coli* when in log-phase growth and 720 times slower for semi dormant *M. tuberculosis*) (5). Whereas, as shown in the figure, longer the incubation period more is the degradation of ertapenem. This could eventually lead to falsely high ertapenem MIC.

Mtb H37Ra (ATCC 25177) was used in the MIC and dose-response experiments. For each experiment, one stock vial was thawed and bacteria grown to logarithmic growth phase (log phase growth) in Middlebrook 7H9 broth enriched with 10% OADC for 4 days at 37**°**C under shaking conditions and 5% CO_2. For semi dormant bacteria under acidic conditions, the day 4 culture was inoculated in Middlebrook 7H9 acidified to pH of 5.8 by means of citric acid as described earlier (7). Sterilizing effect MICs were identified using both the broth dilution and resazurin colorimetric assay (11,13). *Mtb* in acidified Middlebrook 7H9 broth was exposed to the following ertapenem concentrations, in triplicate: 0, 0.075, 0.15, 0.3, 0.6, 1, 1.25, 2, 2.5, 4, 5, 8, 16, 32, and 64 mg/L. The cultures were incubated at 37^0C with 5% CO_2 for 7 days. In one set, each replicate received a daily 50% supplementation of ertapenem concentration at volumes of <1%. For the resazurin assay, an aliquot of the day 7 cultures had resazurin (0.001% v/v) added to the samples and plates, which were then further incubated for 24 hours at 37°C under 5% CO_2. The ertapenem MIC without daily ertapenem supplementation was 64 mg/L, while that with supplementation was 0.6 mg/L.

Day 7 cultures described above that were not used for resazurin assays were washed twice in normal saline to prevent drug carry over, and were subsequently spread on Middlebrook 7H10 agar, and incubated for 3 weeks at 37°C for enumeration of colony forming units (CFU) counts. Inhibitory sigmoid E_{max} curves for concentration versus CFU/mL under sterilizing effect conditions are shown in Figure 2. Comparison of the two regressions, with the null hypothesis that the maximal kill (E_{max}) or efficacy and concentration mediating 50% of E_{max} (EC_{50}) or potency revealed a ratio of probabilities of 7.46 and a difference in corrected Akaike Information criteria scores of 4.02, which means that the efficacy and potency differed with supplementation. The EC_{50} was 1.41 mg/L without ertapenem supplementation compared to 0.19 mg/L with daily supplementation. The efficacy was 0.751 $\log_{10}$ CFU/mL without daily supplementation versus 2.38 $\log_{10}$CFU/mL with supplementation. Thus, ertapenem displays potential for sterilizing effect that would otherwise be masked by not accounting for the degradation.

Figure 2. Ertapenem dose response against slowly replicating *M. tuberculosis*.



As shown in the figure, when the degradation rate was taken in to account by supplementing the drug daily, there was better kill of semi-dormant *M. tuberculosis* by ertapenem.

Here, first we show that ertapenem degrades considerably, at rates >20-fold the doubling times of *Mtb* under acidic conditions. This effect is striking when one compared the MICs with and without supplementation. The MICs in the absence of supplementation would be considered in the resistance range by EUCAST breakpoints (14). Second, we show that ertapenem supplementation brings it well within the susceptibility range. This suggests that most of the published MICs for this drug are likely falsely high and rates of resistance are likely falsely elevated. In addition, since many people have used MICs to choose which of the carbapenems would be better suited for treatment of XDR-TB, good drugs may have been discarded because of the artefactual manner in which current MICs are performed for unstable molecules. Third, we show that ertapenem is likely to have good sterilizing effect in tuberculosis. Follow up hollow fiber studies for sterilizing effect have now been completed in order to identify the ertapenem dose, which can be used against both drug resistant and sensitive *Mtb* (manuscript in preparation).

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POTENTIAL CONFLICT OF INTEREST

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CHAPTER 6

Sterilizing Effect of Ertapenem-Clavulanate in a Hollow-Fiber Model of Tuberculosis and Implications on Clinical Dosing

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Abstract

Carbapenems are now being explored for treatment of multi-drug resistant tuberculosis (MDR-TB), especially in conjunction with clavulanate. Clinical use is constrained by the need for multiple parenteral doses per day, and lack of knowledge of the optimal dose for sterilizing effect. Our objective was to identify the ertapenem exposure associated with optimal sterilizing effect and then design a once a day dose for clinical use. We utilized the hollow fiber system model of tuberculosis in a 28-day exposure-response study of 8 different ertapenem doses in combination with clavulanate. The systems were sampled at predetermined time-points to verify the concentration-time profile and identify the total bacterial burden. Inhibitory sigmoid E_{max} modelling was used to identify the relationship between total bacterial burden and the drug exposure, and identify optimal exposures. Contrary to the literature, ertapenem-clavulanate combination demonstrated good microbial kill and sterilizing effect. In a dose-fractionation hollow fiber study, efficacy was linked to percentage of the 24-hour dosing interval of ertapenem concentration persisting above MIC ($\%T_{MIC}$). We performed 10,000 MDR-TB patient computer-aided clinical trial simulations, based on Monte Carlo methods, to identify the doses and schedule that would achieve or exceed $\%T_{MIC} \geq 40\%$. We identified an intravenous dose of 2 grams once per day as achieving the target in 96% of patients. An ertapenem susceptibility breakpoint MIC 2 mg/L was identified for that dose. An ertapenem dose of 2g once daily is the most suitable to be tested in a phase II study of sterilizing effect in MDR-TB patients.

Introduction

The emergence of drug resistant tuberculosis (TB), especially multidrug-resistant TB (MDR-TB), extensively drug resistant (XDR-TB), and virtually incurable TB (termed totally drug-resistant TB by some), is a global emergency that threatens to undermine many gains of chemotherapy (1-4). As a result, there is currently a four pronged effort; (i) identification of new small molecules to kill drug resistant *Mycobacterium tuberculosis*, (ii) repurposing of antimicrobial drugs not currently used to treat TB into TB therapeutics, (iii) host-directed therapy, and (iv) use of pharmacokinetic/pharmacodynamics science to optimize efficacy while suppressing emergence of acquired drug resistance (5-8). Carbapenems, extensively used to treat Gram-negative bacteria over the last 30 years, have also been shown to be effective against *M. tuberculosis* in *vitro* and *in vivo* when in the presence of a β-lactamase inhibitor (6, 9).

Several initiatives are ongoing to explore the added value of carbapenems given as part of a multidrug regimen for M/XDR-TB (10, 11). In murine TB, efficacy has been demonstrated for meropenem and imipenem with clavulanate; however ertapenem was no better than non-treated controls (9). In addition, ertapenem demonstrated high MICs, suggesting possible natural resistance. However, ertapenem degrades rapidly in *in vitro* growth media at incubation temperatures used to measure MICs with conventional methods (12). We have since developed a MIC assay that corrects for this degradation, which has demonstrated much lower MICs (12). The main advantage of ertapenem to patients could be its half-life of 4 hours, which could allow a once a day schedule, as opposed to 0.6-0.7 hrs for meropenem and imipenem which necessitates multiple and prolonged intravenous infusions per day (13). The multiple infusions per day with meropenem and imipenem make it rather difficult to administer long duration therapy in M/XDR-TB. Recently the first TB clinical data with ertapenem showed that it was well tolerated as part of a salvage regimen for MDR-TB patients (13, 14). Unfortunately, the efficacy of the drug could not be assessed as it was used in a multidrug regimen; moreover, its sterilizing effect is unknown.

The hollow fiber system model of TB (HFS-TB) has been used to examine the sterilizing effect of anti-TB agents, defined as the ability to kill either semi-dormant *M. tuberculosis* under

acidic conditions or of non-replicating persisters under hypoxia (15-17). It was qualified by the European Medicines Agency and editorially endorsed by the US Food and Drug Administration (http://www.ema.europa.eu/docs/en_GB/document_library/Regulatory_and_procedural_guideline/2015/02/WC50018199.pdf). The HFS-TB in tandem with computer-aided clinical trial simulations was found to have a forecasting accuracy of >94% of observed optimal exposures and doses in TB patients in the clinic (18-20). This makes this model ideal to identify optimal doses for treatment of M/XDR-TB, which can directly be translated into clinical use. Our objective was to use these models to identify the optimal sterilizing effect dose of ertapenem for treatment of MDR-TB.

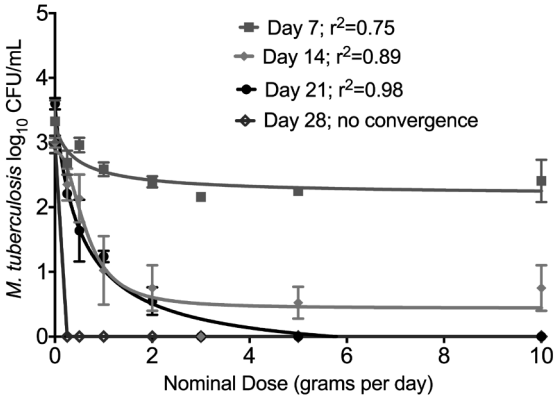
Results

Dose-effect HFS-TB study for sterilizing effect

In the first HFS-TB, which was mainly a dose ranging study, we cultured *M. tuberculosis* H37Ra under acidic conditions to a semi dormant state, and the inoculated into HFS-TB units with circulating Middlebrook 7H9 acidified to a pH of 5.8, as described in the past (15, 21). Different ertapenem exposures, based on human equivalent doses of 0.25, 0.5, 1.0, 2.0, 3.0, 5.0, and 10.0 grams were administered into the central compartment of duplicate HFS-TB via a computer-controlled syringe pump over 30 minutes, as in patients; drug concentrations achieved in each of the 16 HFS-TB were measured at 8 different time points over the first 24 hours. Clavulanate was also dispensed via syringe pump to achieve a peak of 3 mg/L at the end of 30 minutes infusion. Pharmacokinetic modelling of the measured drug concentrations, revealed that the lowest Akaike Information Criteria scores (22) were for a one compartment model. The ertapenem total clearance (± standard deviation) was 4.11±1.83L, and the volume of 22.55±4.0 L, which translates to a half-life of 3.80 hours. The regression for observed concentrations versus pharmacokinetic model predicted concentrations had an r^2=0.997 and the slope was 0.996± 0.006, which is close to unity. Thus, the one compartment model described the data well, with no bias.

Figure 1 shows that ertapenem achieved good sterilizing effect. The bacterial burden at the start of therapy was 4.0 $\log_{10}$ CFU/mL. The data are presented as inhibitory sigmoid E_{max} models between “nominal” human equivalent dose and microbial burden.

Figure 1. Ertapenem-clavulanate dose-effect sterilizing effect in the hollow fiber model.



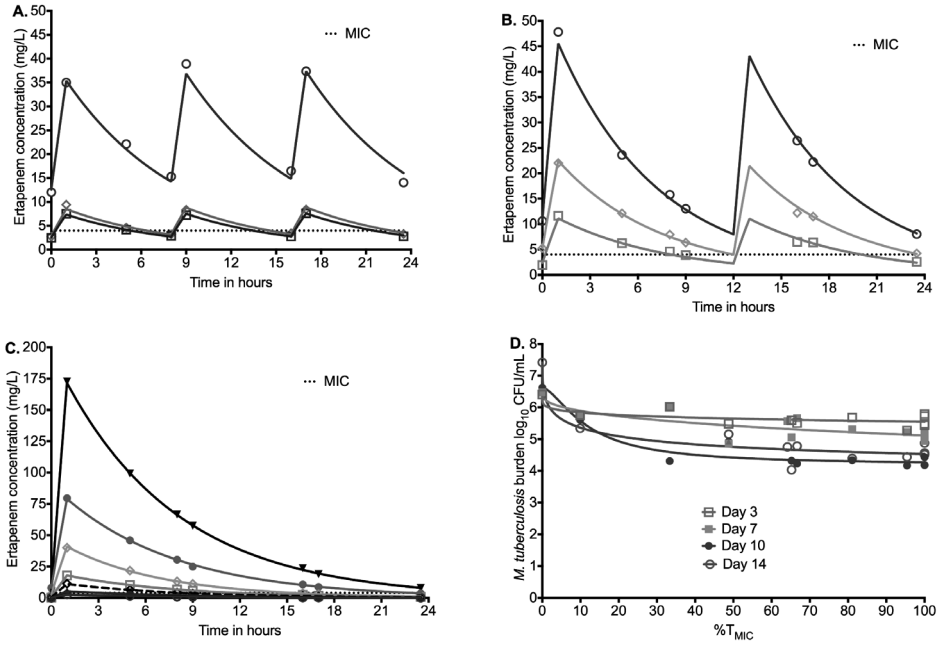
Drug treatments are depicted as "nominal" human equivalent doses. On day 3, inhibitory sigmoid E_{max} modelling demonstrated no model converge, and there was very little kill, thus regressions for day 3 were left out of the figure. However, by day 7 there was already good microbial kill, characterized by maximal kill (E_{max}) of 1.13±0.34 $\log_{10}$ CFU/mL. By day 28, all ertapenem treated HFS-TB completely sterilized the bacteria.

In Figure 1, there was no model convergence on day 3, while on day 28, at the end of the experiment, all ertapenem treated systems now had bacterial burden below limits of detection. All systems achieved % of time above MIC ($\%T_{MIC}$) for 100% of the dosing interval; the trough at 23.5hrs was >4 mg/L in all systems, and all achieved the same microbial kill on day 28.

Ertapenem dose-fractionation study in the HFS-TB

Next, we performed a new HFS-TB, this time using *M. tuberculosis* H37Rv and a dose-fractionation design, for a treatment duration of 14 days. On measurement of ertapenem concentrations, similar to the first study, the concentrations were also best described using a one compartment model; the observed versus predicted concentrations revealed a slope of 0.995±0.002 (r^2>0.999). The concentration-time profiles achieved with each dose are shown by dosing schedule in Figures 2A-C., together with the ertapenem (plus clavulanate 2.5 mg/L) MIC of 4 mg/L. Inhibitory sigmoid E_{max} model fitting by exposure expressed as either C_{max}/MIC or $AUC_{0\text{-}24}$/MIC or $\%T_{MIC}$ revealed Akaike Information Criteria scores shown in Table 1.

Figure 2. Dose fractionation study to PK/PD index linked to ertapenem efficacy



The concentration time profiles are shown relative to the MIC. Symbols indicate measured concentrations, and the lines modeled profile. **A.** Concentration-time profiles of ertapenem identified in the HFS-TB with every 8hr dosing schedule**. B.** Concentration-time profiles of ertapenem identified in the HFS-TB with every 12hr dosing schedule**. C.** Concentration-time profiles of ertapenem identified in the HFS-TB with once a day dosing schedule. Given the concentration range, the scale obscures the time that concentrations persisted above MIC for some of doses. For the blue open circles, the lowest concentration, the time above MIC was 0hrs. For the dose shown by cayenne trinagles, the time was 3hr, for the black open diamonds it was 8.32hr, while for the open magenta squares it was 11.7hr. The rest can be read off the graph. **D.** Inhibitory sigmoid E_{max} model for $\%T_{MIC}$ versus bacterial burden. On day 7, the maximal kill (E_{max}) was 1.14 $\log_{10}$ CFU/mL, consistent with findings in the first HFS-TB dose-effect study. The study was for only 14 days. Examination of the curves on each day shows that 80% of maximal kill occurs around a $\%T_{MIC}$ of 40% on all sampling days, except day 3 when it occurs with lower exposures.

Table 1. Akaike Information Criteria Scores for PK/PD index versus ertapenem sterilizing effect.

	Day 3	**Day 7**	**Day 10**	**Day 14**
AUC_{0-24}/MIC	-30.26	-18.99	-31.41	-5.112
C_{max}/MIC	-30.63	-17.19	-31.55	-2.144
$\%T_{MIC}$	-60.39	-62.96	-49.39	-45.06

The lowest scores were for $\%T_{MIC}$, which means this is the PK/PD index linked to microbial kill. Figure 2D shows the inhibitory sigmoid E_{max} MIC curves for each sampling day based on $\%T_{MIC}$. Based on day 10, which had the highest r^2 of 0.94, the relationship between $\%T_{MIC}$ and bacterial burden was:

$$\log_{10} \text{CFU/mL} = 5.68 - \%T_{MIC}^{2.56} / [23.52^{2.56} + \%T_{MIC}^{2.56}]$$

From this relationship, we calculated the EC_{80} as a $\%T_{MIC}$ of 40.41% of the dosing interval. Indeed, this can be read off Figure 2D as well, which shows that one gets the same exposure for optimal kill whichever sampling day is examined.

Monte Carlo simulations to identify optimal ertapenem dose

In TB patients, pharmacokinetic variability is one of the most important drivers of sterilizing effect (23-29). Therefore, in order to identify the optimal ertapenem dose for pulmonary TB, we performed Monte Carlo simulations of 10,000 patients with pulmonary TB, using the pharmacokinetic parameter estimates and between-patient variability indices shown in Table 2 based on Burkhardt *et al* (30-32). We also accounted for the ertapenem penetration into epithelial lining fluid (ELF) of 7.48±8.17% (which mirrors the non-protein bound concentration of 5-15%), and that in lung tissue of 23.6±12.3% (33). We performed simulations to determine how much 1.0g once a day, or 1.0g twice a day, or 2.0g once a day, or 2.0g twice a day, or 3.0g once a day would achieve or exceed the target exposure, which is $\%T_{MIC}$ of 40.41% associated with optimal sterilizing effect in ELF of patients. For internal validation, we compared the pharmacokinetic parameters in the 10,000 simulated patients to those of Burkhardt *et* al in Table 2, which shows that the simulations faithfully recapitulated the pharmacokinetic parameters and variability. As an extra external validation step, we compared the pharmacokinetic parameters in the simulations to those we actually observed in our MDR-TB patients in the Netherlands, as shown in Table 2 (13). Table 2 shows that the pharmacokinetic parameters and variance in our simulations were virtually identical to those we observed in patients. Therefore, the simulations were accurate in reproducing what is identified in the clinic.

Table 2. Comparison of pharmacokinetic parameter and concentration estimates and ranges in 10,000 simulated patients to those actually observed in patients treated with 1G.

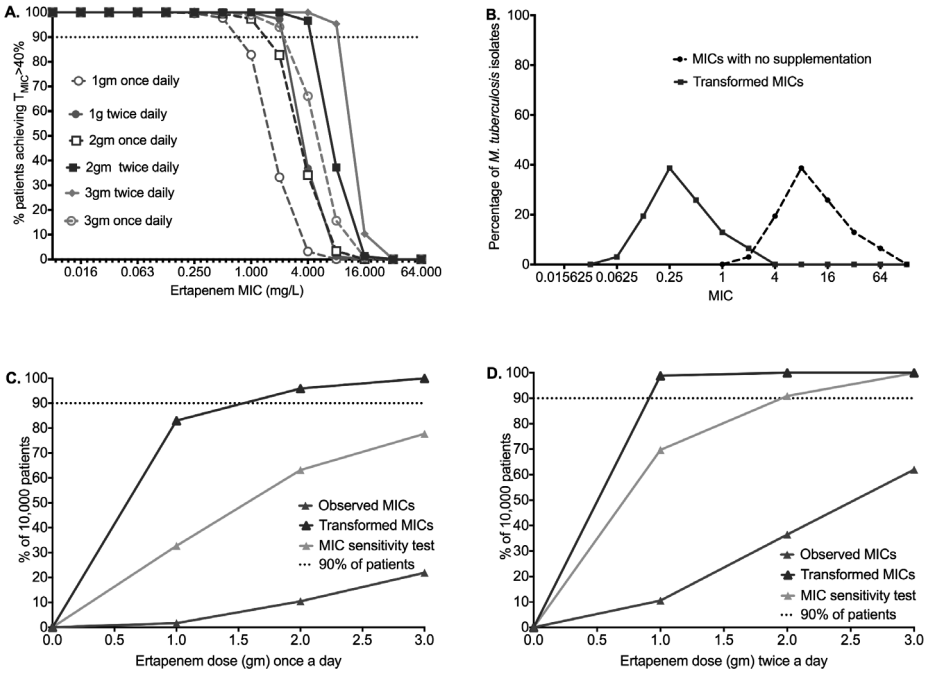
	Subroutine PRIOR based on literature (± SD)	**10,000 simulated TB patients**	**Observed in MDR-TB patients**
Total clearance in L/hr	2.63±0.83	2.6 (0.02-6.00)	2.1 (0.09 – 3.23)
Volume in L	10.6±2.51	11 (1.2-19)	7.3 (2.61 – 11.10)
Half-life in hours	-	2.8 (2.20-3.70)	2.4 (2.05 – 3.53)
$AUC_{0\text{-}24}$ in mg*h/L	-	448 (166-4255)	545 (309 – 1130)

Figure 3A shows the target attainment probability (TAP) for each dose and dosing schedule as the MIC changes. On one extreme, the 1gm once a day dose had a TAP less than 90% once the MIC was 1 mg/L, while the dose of 3gm twice a day achieved a high TAP until 8 mg/L, then fell precipitously at 16 mg/L. For 2gm a day, the TAP fell at an MIC of 2 mg/L. This means that the susceptibility breakpoint for ertapenem plus clavulanate will fall between MIC of 1 and 16 mg/L, and will depend on the final dose chosen.

Since MIC variability is also an important determinant of therapy response in TB patients (25, 34-36), we also took into account the MIC distribution. Figure 3B shows the ertapenem MIC distribution from 33 MDR-TB patients isolates in the Netherlands, in the presence of clavulanate. Figure 3B shows that all isolates would have MICs between greater than 1 mg/L and below 128 mg/L. However, in the past we have shown that ertapenem degrades during the MIC testing, and if once accounts for the degradation, there is a 4-tube dilution decrease in MICs; if clavulanate is added and ertapenem is supplemented there is a 7-tube dilution difference (12). Thus, we transformed the MICs for the 33 clinical isolates down by 4-tube dilutions as well, as shown in Figure 3B. In that scenario, only 6.5% of isolates had an MIC greater than 1 mg/L.

Summation of all TAPs to account for distribution of MICs gives the proportion of 10,000 TB patients who would achieve the argett exposure of $\%T_{MIC}$ of 40%, termed the cumulative fraction of response (CFR). Figure 3C shows the CFRs for the once a day dosing schedule for both observed MICs and transformed MICs. For the transformed MICs the dose of 2gm a day had a CFR of 96%.

Figure 3. Target attainment probability and cumulative fraction of response for various ertapenem doses.



A. Target attainment probability for $\%T_{MIC}$ of 40% as *M. tuberculosis* MIC changes. No dose or dosing schedule is effective once MICs are 16 mg/L. **B.** Ertapenem MIC distribution in isolates from the Netherlands, with and without transformation to account for ertapenem degradation. **C.** Proportion of 10,000 patients who achieved or exceeded $\%T_{MIC}$ of 40% with once a day dosing. The proportion is highly sensitive to the MIC, and fell on sensitivity analysis, a worst-case scenario. **D.** Proportion of 10,000 patients who achieved or exceeded $\%T_{MIC}$ of 40% with twice a day dosing. The twice a day dosing schedule achieved the target in higher proportions of patients, even on sensitivity analysis. However, given the hardship of twice a day administration of therapy in TB, we chose the 2gm once a day dose as being most practical.

We also performed ertapenem plus clavalunate MICs in 4 clinical isolates incubated at 4°C versus 37°C to try and slow down drug degradation: MICs were lower at 4°C by 4, 2, 3, and 2-tube dilutions. Therefore, we performed sensitivity testing by examining CFR is MIC transformation was only 2-tube dilution (worst case scenario). Figure 3C shows that the dose of 2gm once a day would not achieve the target in 90% of patients; nevertheless it would achieve this in 63% of patients, which is still reasonable. Figure 3D shows the results of twice a week dosing schedule; as would be expected from a $\%T_{MIC}$ driven drug, this dosing schedule

performed better. The dose of 1.0gm twice a day would achieve target exposure in 99% of patients, and on sensitivity testing would still achieve this in 70% of patients. The dose of 2gm twice a day would achieve >90% even on sensitivity testing.

Discussion

This is the first study that showed the ertapenem-clavulanate efficacy and sterilizing effect, unlike findings in the murine model, likely because the HFS-TB mimicked the half-life of 4hrs encountered in patients unlike that of 1.0 hours in mice. We were able to recapitulate ertapenem's pharmacokinetics, and its half-life of 4hrs, as encountered in TB patients, which likely explains better efficacy in this model compared to that encountered in mice in which ertapenem half-life is 1 hour. Moreover, dosages simulated in the model were in a range that would likely be tolerable in patients. This study showed the advantage of the hollow fiber system, namely a better recapitulation of human like pharmacokinetics, and of microbial sterilizing effect conditions. The Monte Carlo simulations then introduced the variability that would be encountered for pharmacokinetic parameters between patients and MICs between *M. tuberculosis* strains. Our two-step external validation approach in the simulations ensured that our simulations reflected clinical reality; sensitivity testing accounted for any uncertainty in MIC distribution. This allowed us to perform dose-effect studies that take into account the exposure-effect relationship as described in the hollow fiber model, the essential aspects of drug behaviour in patients such as pharmacokinetic variability and the drug penetration ratios to lungs that are important in determining efficacy, and susceptibility of *M. tuberculosis* isolates encountered in hospitals. This approach, in many experiments based on the same *M. tuberculosis* isolate we used in the current study, has been found to be >94% accurate in identifying clinical doses that are optimal in TB patients based on recent presentation for regulatory approval (19).

Ertapenem–clavulanate may play an important role in the intensive phase of TB treatment due to its sterilizing effect. In addition, the intravenous administration may be more suitable for the intensive phase in which M/XDR-TB patient are likely to be administered in a TB clinic. As carbapenems are already part of the WHO list of TB drugs for M/XDR-TB, the next step is to explore the use of ertapenem–clavulanate in patients, using the 2g once a day dose we

identified. Recently, it has been shown that meropenem–clavulanate has promising activity against MDR-TB *in vitro* (37, 38). Indeed, imipenem-clavulanate and meropenem-clavulanate were associated with a treatment success of >57% and culture conversion >60%, in a recent systemic analysis of five studies (39). However, since clavulanate is administered as oral amoxicillin-clavulanate, gastrointestinal side effects may become a problem if this formulation is administered for a prolonged duration multiple times a day with meropenem or imipenem, which would compromise absorption of other oral drugs. Unfortunately, the current suppliers of carbapenems, are not interested in developing an infused combination of carbapenem and clavulanate. The main advantage of ertapenem is its long half-life enabling once daily dosing, which would also allow a once a day clavulanate dose, potentially reducing side-effects. This may even facilitate dosing in an outpatient setting. Patients may present at the clinic once a day for their drug administration as part of direct observed treatment, or could receive treatment as a once a day infusion at home when sputum culture negative in those countries where the drug is already part of home care for treatment of other chronic infections. Ertapenem has a labelled infusion time of only 30 minutes, which will facilitate a relatively short stay at the outpatient clinic. Even more rapid infusion have been explored and showed similar drug exposure and tolerability (40). We show that a dose of 2 g given once daily could contribute to an effective regimen. Ertapenem up to a dose of 3 g has been administered to healthy volunteers (41). Moreover, doses up to 2 g have been administrated in 30 min without any additional complications (42). However, there is a need for a prospective phase II study exploring safety and efficacy of 2g ertapenem with clavulanate once a day in MDR-TB patients.

On the other hand, the amount of time clavulanate has to be around to keep potentiating the ertapenem is still unclear. Thus, the target concentration to aim with dosing is unclear. Clavulanate has a shorter half-life compared to ertapenem. However, penetration into bronchial mucosa is 118%, and its protein binding is minimal at 20%, and likely an effective concentration remains at site of effect even when dosed once. Since clavulanate is renally eliminated, between-patient variability in systemic clearance, which is about 58%, is driven mainly by renal function: the lower the creatinine clearance, the less it is cleared (43). Separate dose-effect studies on clavulanate role will need to be studied, after which simulations similar to the current ones can be performed.

Finally, pharmacokinetic/pharmacodynamics-based susceptibility breakpoints in TB, mostly derived from hollow fiber model monotherapy studies, have been shown to be highly accurate in delineating TB patients who fail or respond to combination therapy (25, 34, 35). The 2 mg/L ertapenem susceptibility breakpoint we identified for the dose of 2gm a day should be thus used by clinicians as decision-making tool to determine if a patient will respond to ertapenem therapy. This breakpoint will differ from the epidemiological cut-off value, which may be more useful for epidemiological tracking of acquired ertapenem resistance, as opposed to clinical decision-making.

There are some limitations to our study. First, we used two isolates *M. tuberculosis* for the sterilizing effect experiments. Inclusion of a larger number of isolates could change that final target exposure associated with optimal efficacy. However, hollow fiber studies in the past when we used these strains were found to be predictive of the optimal exposure targets in patients for sterilizing effect (15, 24, 25, 44-46). A second limitation is that we used pharmacokinetic data from critically ill patients as prior data for our Monte Carlo simulations. Type of disease that a patient has can alter the pharmacokinetic parameters, so that TB patients could have different pharmacokinetics. However, as shown in Table 2, the pharmacokinetic parameter estimates in simulated patients, and the $AUC_{0\text{-}24}$ achieved with 1g doses, were virtually identical to those we have identified in TB patients in the Netherlands, as part of therapeutic drug monitoring. This validates that simulated patients had pharmacokinetic parameters and concentrations similar to those encountered in TB patients.

In conclusion, we have shown by simulation of human drug exposure of different dosage in an *in vitro* infection model of *M. tuberculosis* that ertapenem–clavulanate may be a valuable asset to TB treatment. Based on available pharmacokinetic data, we have identified that the dose of ertapenem most suitable to be tested in a phase II study is 2 g once daily. An MIC 2 mg/L should be used to define resistance to this drug.

Materials and methods

We used *M. tuberculosis* H37Ra (ATCC #25177) and H37Rv (ATCC #27294) for our experiments, with growth and storage conditions described before (15). These isolates have been used in the HFS-TB before, with good forecasting accuracy of the clinic. Ertapenem was purchased from Merck Sharp & Dohme. Clavulanate was purchased from Sigma-Aldrich. Drugs were dissolved in sterile water, and syringe filtered for further use. Hollow fiber cartridges were purchased from FiberCell (Frederick, MD, USA).

The hollow fiber system model of TB

Construction of the HFS-TB to measure sterilizing effect has been described in detail in the past (15). The system recapitulates concentration-time profiles of drugs encountered in patients, taking into account the penetration into lungs. In the sterilizing effect studies, semi-dormant *M. tuberculosis* growing in the Middlebrook 7H9 broth acidified using acetic acid to a pH of 5.8 was used, which grow at a rate 8-10-fold slower than log-phase growth *M. tuberculosis (47)*. The HFS-TB in this model use acidified Middlebrook 7H9 broth without oleic acid, albumin or catalase but with 20% dextrose. The peripheral compartment of each of 16 HFS-TB units with circulating acidified Middlebrook 7H9 broth was inoculated with *M. tuberculosis*. All HFS-TB were incubated at 37°C under 5% CO_2 for the entirety of the study. Different ertapenem exposures, based on human equivalent doses of 0.25, 0.5, 1.0, 2.0, 3.0, 5.0, and 10.0 grams were administered into the central compartment via a computer-controlled syringe pump over 30 minutes, as in patients. The concentrations achieved with the doses were an $AUC_{0\text{-}24}$ of 0, 25, 50, 100, 200, 250, 500, 1000 mg*h/L. There were two replicates hollow fiber systems for each dose or $AUC_{0\text{-}24}$. Clavulanate was also dispensed via syringe pump to achieve a peak of 3 mg/L at the end of 30 minutes infusion. Media inflow and outflow were set to mimic the ertapenem half-life of 4 hours encountered in patients; we took into account the degradation rate of the drug that we have identified in the past. We recapitulated pharmacokinetics as described in the INVANZ® ertapenem for injection) for intravenous (IV) or intramuscular (IM), package insert.

The central compartments of each HFS-TB were sampled six times during the first 24hrs, and ertapenem concentrations measured using a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method as described in the past (48) in order to verify that human-

like pharmacokinetics had been achieved. Ertapenem concentrations were modelled using a one compartment pharmacokinetic model with first order input and elimination, using ADAPT 5 software, as described in the past (15, 24, 25, 44-46). These actual exposures achieved in the HFS-TB were subsequently used in the PK/PD analyses. In order to enumerate the *M. tuberculosis* burden as CFU/mL, the peripheral compartment of each HFS-TB was sampled on days 0, 3, 7, 14, 21, and 28. Samples were washed and processed as described in the past (15) and spread on Middlebrook 7H10 agar supplemented with 10% Oleic acid-dextrose-catalase. The cultures were incubated for 21 days at 37^0C with 5% CO_2 before the colonies were counted.

Identification of optimal ertapenem dose using computer-aided clinical trial simulations.
For the domain of input, we utilized the pharmacokinetic parameter estimates and between-patient variability indices identified by Burkhardt *et al* (31). We performed simulations to determine how much 1.0g once a day, or 1.0g twice a day, or 2.0g once a day, or 2.0g twice a day, or 3.0g once a day or 3.0gm twice a day would achieve or exceed the target exposure, which is $\%T_{MIC}$ associated with optimal sterilizing effect in lung tissue of patients.

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Transparency declaration

TG is a consultant for Lumina Care solutions, and founded Jacaranda Biomed, Inc. JWA reports personal fees from Pfizer, Astellas, MSD, Gilead, grants from Pfizer, Astellas, MSD, all outside the submitted work. All other authors have no conflict of interest.

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CHAPTER 7

Pharmacokinetic Modelling and Limited Sampling Strategies Based on Healthy Volunteers for Monitoring of Ertapenem in Patients with Multidrug-resistant Tuberculosis

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Abstract

Ertapenem is a broad spectrum carbapenem antibiotic and is being explored against *Mycobacterium tuberculosis*. Carbapenems have anti-bacterial activity when the plasma concentration exceeds the minimal inhibitory concentration at least 40% of the time (40%T>MIC). To assess 40%T>MIC in multidrug-resistant *tuberculosi*s (MDR-TB) patients, a limited sampling strategy was developed using a population pharmacokinetic model based on healthy volunteers. A two-compartment population pharmacokinetic model was developed from data in forty-two healthy volunteers, using an iterative two-stage Bayesian method. External validation was performed by Bayesian fitting of the model developed in volunteers to the individual data of MDR-TB patients ($AUC_{0\text{-}24h,fit}$) using the developed population model for volunteers as a prior. A Monte Carlo simulation (n=1000) was used to evaluate limited sampling strategies. Additionally, the *free ertapenem fraction (f)* 40%T>MIC for MDR-TB patients was estimated with the population pharmacokinetic model. The developed population pharmacokinetic model was shown to estimate the $AUC_{0\text{-}24h}$ in MDR-TB patients with an overestimation of 6.8 (range: -17.2 – 30.7) %. The best performing limited sampling strategy, with a time-restriction of 0-6h, was found to be sampling at 1 and 5h (R^2 = 0.78, mean prediction error = -0.33% and a root mean square error = 5.5%). Drug exposure was overestimated by a mean percentage of 4.2 (-15.2 – 23.6) %. Considering a free fraction of 5% and the MIC set at 0.5 mg/L, 9 out of 12 patients would have exceeded a minimum of *f* 40% T>MIC. A population pharmacokinetic model and limited sampling strategy, developed using data from healthy volunteers, showed to be adequate to predict ertapenem exposure in MDR-TB patients.

Introduction

Ertapenem is a broad spectrum carbapenem antibiotic, used against a range of infectious diseases (1). Like for all other beta-lactam antimicrobial products, the efficacy of ertapenem is characterized by time-dependent killing. Carbapenems have anti-bacterial activity when the plasma concentration exceeds the minimal inhibitory concentration at least 40% of the time (40%T>MIC) (1,2). Although not yet studied in tuberculosis (TB) patients, *free* 40%T>MIC is expected to be an important pharmacodynamic parameter (3). Carbapenems in combination with clavulanic acid has created interest since activity was shown in a murine model of TB (3). Additionally, a recent study showed that carbapenems efficiently inactivated peptidoglycan cross-linking in *M. tuberculosis* (3,4) and a recent meropenem amoxicillin/clavulanic acid EBA study showed activity of carbapenems in TB (5). Recently a new susceptibility testing method to estimate the MIC of ertapenem has been introduced (6) showing that ertapenem might be more potent *in vitro* than previously thought because its chemical degradation had never been considered (7). To date only a limited number of multidrug-resistant *tuberculosi*s (MDR-TB) patients have been treated with ertapenem as part of a multidrug regimen. Based on this data, the drug appeared well tolerated during prolonged treatment (8,9). However, ertapenem is not yet added to the World Health Organization (WHO) list of anti-TB drugs, in contrast to imipenem and meropenem.

Pharmacokinetics of ertapenem have typically been studied in healthy volunteers (10), people with obesity (11,12), patients with renal failure (13-15) and critically ill patients with various pathologies (16-18). Lower drug exposure was observed in obese individuals (12), and an increase in dose interval was needed in patients with renal insufficiency with an estimated glomerular filtration rate (eGFR) below 30 mL/min/1,73m^2 (14), suggesting that the optimal dose of ertapenem is different for various health conditions. A recent study on pharmacokinetics of MDR-TB patients suggested that there was substantial pharmacokinetic variability in these patients (8).

For studies exploring the use of ertapenem against *M. tuberculosis* it would be valuable to assess *f* 40% T>MIC in patients. To be able to calculate the *f* 40% T>MIC, a good indication of the plasma concentration profile is mandatory. However, measurement of the plasma concentration over the entire 24h dosing interval is time consuming, expensive and burdening

to patients. A limited sampling strategy through a population pharmacokinetic model can be used to predict this plasma concentration profile as has been done for other anti-TB drugs (19-22).

The aim of this study was to develop a population pharmacokinetic model and a limited sampling strategy based on data from healthy volunteers, in order to estimate drug exposure of ertapenem in MDR-TB patients.

Materials and methods

This study was based on two data sets. The first set was comprised of forty-two healthy volunteers from five clinical studies receiving 0.25 to 2 g i.v. doses of ertapenem (10). For population pharmacokinetic model comparison with MDR-TB patients we only used the data of healthy volunteers receiving 1g. The second set was comprised of a retrospective dataset of patients with MDR-TB, receiving 1g ertapenem administered once daily via a 30-minute infusion at the Tuberculosis Center Beatrixoord, University Medical Center Groningen, The Netherlands between December 1, 2010 and March 1, 2013 (8). Plasma concentrations of ertapenem were collected at steady state before administration and at 1, 2, 3, 4, 5, 6, 8 and 12 hours after administration. Ertapenem plasma concentrations were analyzed by a validated liquid chromatography-tandem mass-spectrometry (LC-MS/MS) method (23). Both data sets included demographic and medical data, such as age at start of treatment, height and body weight and serum creatinine at the time of pharmacokinetic assessment.

Population pharmacokinetic model

All pharmacokinetic calculations were performed using MW\Pharm 3.82 (Mediware, Zuidhorn, The Netherlands). Based on previous reports and recent pharmacokinetic studies on ertapenem (11-18, 24-26), concentration time-curves were evaluated in a one-compartmental and two-compartmental model. The final model was selected based on the Akaike information criterion (AIC) (27). The drug plasma-concentrations of the forty-two healthy volunteers were used to develop a two-compartmental population pharmacokinetic model using an iterative two-stage Bayesian (ITSB) procedure (module KinPop of MW\Pharm) (28). The clearance was calculated using CL = CL_m (metabolic clearance (L/h/1.85m^2)) * BSA (body surface area (m^2)) /1.85 + f_r (drug clearance – creatinine clearance ratio) * CL_{cr} (creatinine clearance (L/h)) (24). Pharmacokinetic parameters were assumed to be log-

normally distributed and the residual error was assumed to be normally distributed with SD = 0.1 + 0.1*C, in which C is the observed plasma concentration of ertapenem. Nonparametric 95% confidence intervals of the population parameters and their inter-individual standard deviation were estimated using a bootstrap analysis (n = 1000). The area under the plasma concentration-time curve from 0 up to 24 hours ($AUC_{0\text{-}24h,ref}$), used as a reference value, was calculated using the log trapezoidal rule (KinFit module of MW\Pharm).

Internal validation was performed by leaving three healthy volunteers out of the pharmacokinetic model development, creating fourteen n-3 sub models, obtained by randomization using Microsoft Excel 2010. The estimated $AUC_{0\text{-}24h}$, ($AUC_{0\text{-}24h,n\text{-}3}$) was obtained by Bayesian fitting using the data of the three left out volunteers in the corresponding n-3 sub models. Agreement between $AUC_{0\text{-}24h,n\text{-}3}$ and $AUC_{0\text{-}24h,ref}$ was assessed by Bland-Altman analysis and Passing and Bablok regression and subsequent residual plot. External validation was performed by Bayesian fitting of the model developed in volunteers to the individual data of MDR-TB patients ($AUC_{0\text{-}24h,fit}$), using the developed population model for volunteers as a prior. For comparison of the pharmacokinetics between MDR-TB patients and volunteers, a similar analysis was performed with the data of 18 volunteers who received 1 g of ertapenem. Bland-Altman analysis was also used to assess the agreement between $AUC_{0\text{-}24h,fit}$ and the $AUC_{0\text{-}24h,ref}$ of MDR-TB patients.

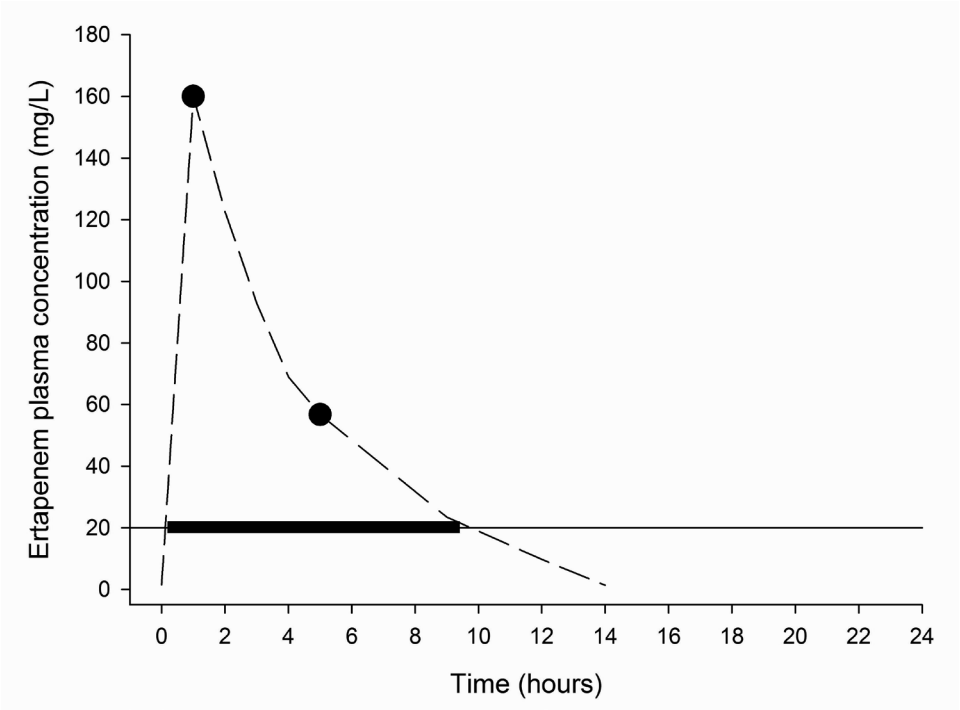
Limited sampling strategies

A Monte Carlo simulation was used to calculate limited sampling strategies for estimation of AUC ($AUC_{0\text{-}24h,LSS}$), as implemented in MW\Pharm. This stochastic simulation consisted of 1000 random patients drawn from the population pharmacokinetic model. For each patient limited sampling strategies were calculated at 1 to 3 sampling time points by Bayesian MAP procedure. We evaluated limited sampling strategies based on separate calculations with time restrictions of 0-6h, 0-12h, and 0-24h. Performance was considered suitable for application in prospective studies if the adjusted r squared (R^2) was > 0.95, the root mean square error (RMSE) was < 15%, and the mean prediction error (MPE) was <5%. The prediction errors were calculated as ($AUC_{0\text{-}24h,LSS}$ - $AUC_{0\text{-}24h,ref}$)/$AUC_{0\text{-}24h,ref}$ ·100%.

Prediction of *free* 40%T>MIC

The ertapenem concentration-time curve of each patient was used to establish if the *f* 40%T>MIC was reached. For this cause the time that the concentration-time curve was above the MIC was assessed in MW/Pharm. The percentage protein unbound ertapenem used for the assessment was 5 (4,7). European Committee on Antimicrobial Susceptibility Testing (EUCAST) MIC value for ertapenem (non-species related) of 0.5 $mg \cdot L^{-1}$ was used to calculate *f* 40%T>MIC (6,7). Exposure was considered adequate if the concentration was 40% of time above MIC, which corresponds with 9.6 h of each 24 h interval as shown in figure 1.

Figure 1. Ertapenem plasma concentration time curve. 40% of time is corresponding with 9.6h of each 24h interval.



Statistics

Differences between the population characteristics and pharmacokinetic parameters of healthy volunteers and TB patients were calculated using the Mann Whitney U test. All statistics were calculated with Analyse-it™ for Microsoft Excel (version 2.30).

Results

Data set

Data of forty-two healthy volunteers was used to develop the population pharmacokinetic model. Since blood samples from MDR-TB patients were collected for another purpose, no data was available between 5 and 8 hours. The baseline characteristics of healthy volunteers and MDR-TB patients were shown to differ significantly ($P<0.05$), except for age (table 1). The median age of the volunteers was 31 (23 - 38) years and body mass index was 24.5 (23.6 – 26.2) kg/ m^2.

Table 1. Baseline characteristics of healthy volunteers versus multidrug-resistant tuberculosis patients

	Healthy volunteers	MDR-TB patients	p-value
	n=42	n=12	
Sex [n (%)]			
Male	36 (86%)	5 (42%)	0.0083[a]
Female	6 (14%)	7 (58%)	
Age (years; median, IQR)	31 (23-38)	25 (18-30)	0.064[b]
Weight (kg; median, IQR)	78.9 (72.2-83.8)	55.5 (47.3-70.3)	0.000[b]
Height (cm; median, IQR)	178 (172-183)	167 (164-174)	0.004[b]
Body mass Index (kg/m^2; median, IQR)	24.5 (23.6-26.2)	19.2 (17.9-23.7)	0.002[b]
Ethnicity			
Black (%)	5 (12%)	7 (58%)	0.000[c]
Caucasian (%)	36 (86%)	3 (25%)	
Asian (%)	1 (2%)	1 (8%)	
Other (%)	0 (0%)	1 (8%)	
Serum Creatinine (mg/dl; median, IQR) *	0.9 (0.8-1.1) **	0.5 (0.4-0.7)	0.000[b]
Creatinine clearance (ml/min/1.73m^2; median, IQR)	91 (81 – 97)	181 (122 – 248)	<0.0001 [b]
Dose/weight (mg/kg; median; IQR)	18.0 (14.2-21.1) **	12.9 (6.0-20.0)	0.044[b]
Samples per patient (median;IQR)	28 (16-48)	7 (6-8)	

IQR = Interquartile range

*On the day the plasma concentrations were determined

** Some patients received more than one dose with subsequent serum creatinine

a. Fisher exact test, b. Mann-Whitney U test, c. Pearson Chi-Squared test

Pharmacokinetic model

Development of pharmacokinetic model in volunteers

The selection of the two-compartmental model was based on the AIC values for the one- (AIC = 1280) and two- (AIC = -1073) compartment models (28). Final population pharmacokinetic model parameters developed from data of healthy volunteers (N=42) are shown in table 2. $AUC_{0\text{-}24h,n\text{-}3}$ values estimated in the internal validation were underestimated by a mean value of 0.3% (range: -8.1 – 7.6), when compared with $AUC_{0\text{-}24h,ref}$ shown in Figure 2. The observed $AUC_{0\text{-}24h,ref}$ and model calculated ertapenem $AUC_{0\text{-}24h,n=3}$ were assessed for agreement, using Passing and Bablok regression in figure 3.

Figure 2. Internal validation of the population pharmacokinetic model. Bland-Altman plot showing the agreement between the area under the concentration-time curve for 24h of healthy volunteers estimated with the population pharmacokinetic model ($AUC_{0\text{-}24h,n=3}$) and the $AUC_{0\text{-}24h,ref}$

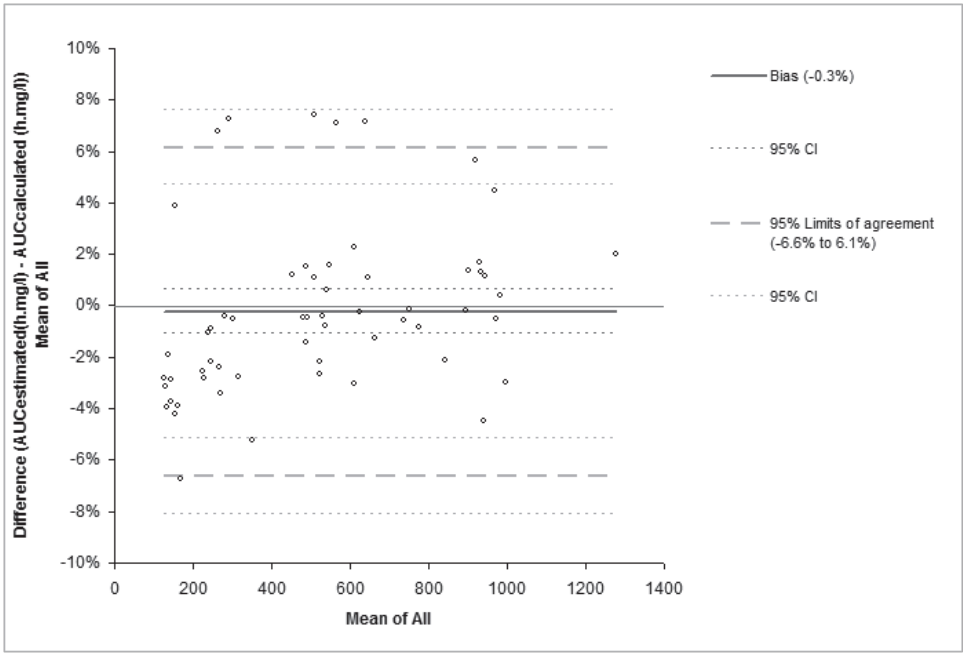


Table 2. Pharmacokinetic population model developed from data in volunteers.

Parameter	Mean (95% CI)	SD (95% CI)
CL_m (L/h/1.85m^2)	1.06 (0.54 – 1.34)	0.16 (0.09 – 0.23)
f_r	0.130 (0.073 – 0.237)	0.039 (0.016 – 0.063)
V_1 (L/kg)	0.0824 (0.0789 – 0.0860)	0.0095 (0.0073 – 0.0119)
CL_{12} (L/h/1.85m^2)	2.56 (2.34 – 2.85)	0.14 (0.11 – 0.28)
V_2 (L/kg)	0.0543 (0.0527 – 0.0559)	0.0016 (0.0013– 0.0021)

CL_m = metabolic clearance; f_r =ertapenem clearance/creatinine clearance ratio; V_1 = volume of distribution of the central compartment; CL_{12} = inter-compartmental clearance; V_2 = volume of distribution in the peripheral compartment; CI = nonparametric confidence interval from bootstrap analysis

Population PK parameters TB patients compared to healthy volunteers

Pharmacokinetic parameters of 18 healthy volunteers, who received 1 g of ertapenem, compared to TB patients are shown in table 3. $AUC_{0\text{-}24h,fit}$ values of the MDR-TB patient data were underestimated by mean 6.8% (range: -17.2 – 30.7), when compared with $AUC_{0\text{-}24h,ref}$. $AUC_{0\text{-}24h,fit}$ correlated well with $AUC_{0\text{-}24h,ref}$, as determined with a Bland-Altman analysis - shown in Figure 4.

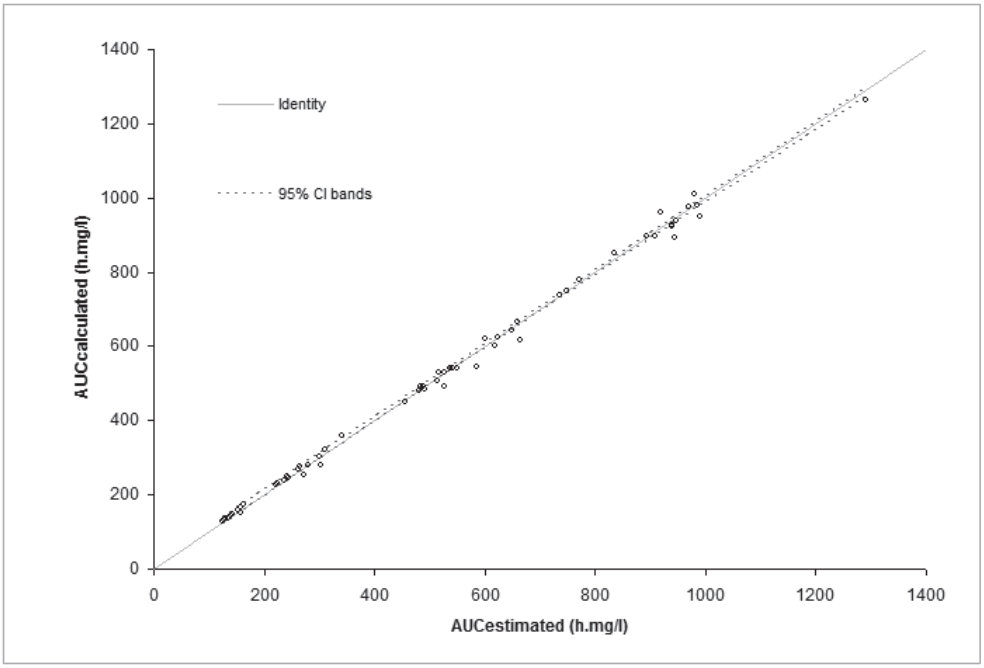
Table 3. Pharmacokinetic parameters in volunteers who received a 1 g dose and MDR-TB patients.

	Healthy volunteers (n=18)	Tuberculosis patients (n=12)	p-value*
CL_m (L/h/1.85m^2)	1.06 ± 0.08	0.944 ± 0.132	0.0023
f_r	0.158 ± 0.051	0.104 ± 0.039	0.0017
V_1 (L/kg)	0.0846 ± 0.0094	0.0876 ± 0.0166	0.4717
CL_{12} (L/h/1.85m^2)	2.52 ± 0.03	2.55 ± 0.04	0.0023
V_2 (L/kg)	0.0543 ± 0.0005	0.0549 ± 0.0008	0.2885

* Mann-Whitney U test

Cl_m = metabolic clearance; f_r =ertapenem clearance/creatinine clearance ratio; V_1 = volume of distribution of the central compartment; CL_{12} = inter-compartmental clearance; V_2 = volume of distribution in the peripheral compartment

Figure 3. Passing and Bablok regression. Plot showing the agreement between the area under the concentration-time curve for 24h ($AUC_{0\text{-}24h,ref}$) and the $AUC_{0\text{-}24h,n=3}$ estimated with the population pharmacokinetic model (dotted lines: 95% confidence interval (CI)).



Limited sampling strategies

Using the population pharmacokinetic model, limited sampling strategies were evaluated for 0-6h, 0-12h and 0-24h restriction of the dosing intervals. The R^2, bias and RMSE were subsequently determined. For each dosing interval and for one, two or three sampling time points, the limited sampling strategies with the best performance of RMSE and bias are shown in Table 4.

Figure 4. External validation of the population pharmacokinetic model. Bland Altman plot showing the agreement of the area under the concentration-time curve of multidrug-resistant tuberculosis patients ($AUC_{0\text{-}24h,ref}$) and the $AUC_{0\text{-}24h,fit}$ estimated with the population pharmacokinetic model. Mean of All is the mean AUC of both $AUC_{0\text{-}24h,\ fit}$ and $AUC_{0\text{-}24h,ref}$.

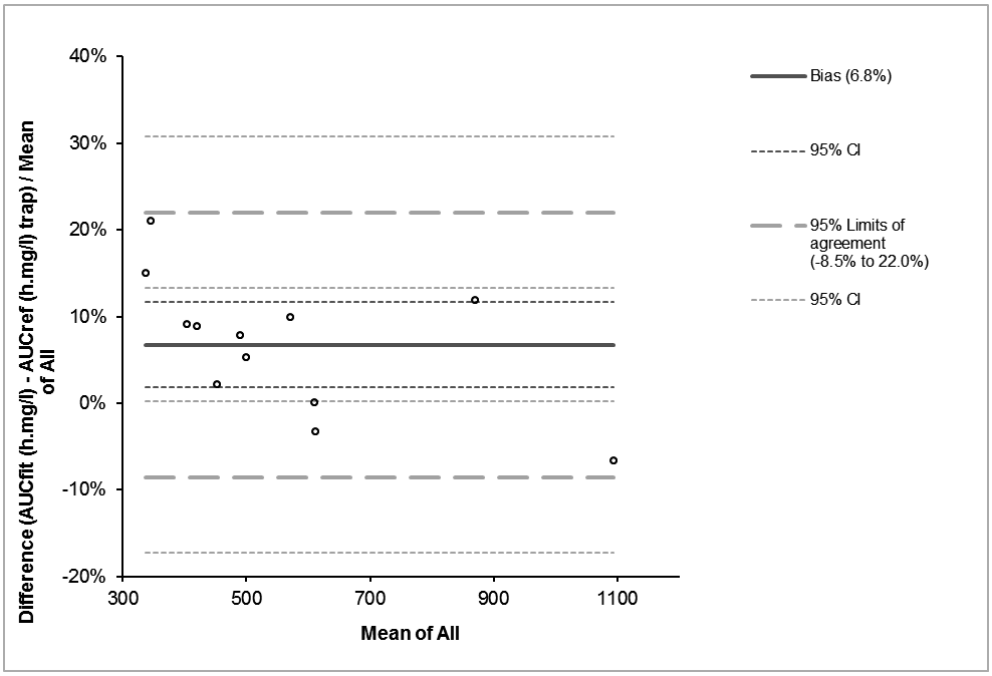
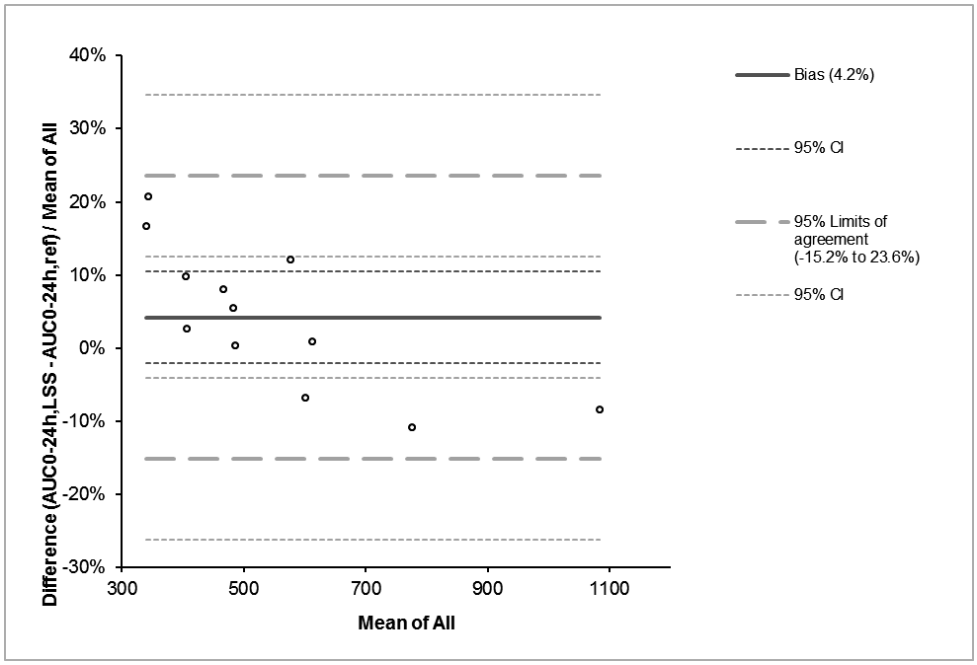


Table 4. Best-performing limited sampling strategies

	First sampling time point (h)	Second sampling time point (h)	Third sampling time point (h)	Coefficient of determination (r^2)	Mean prediction error (%bias)	% RMSE[a]
0-6h	5			0.57	-0.09	7.4
0-6h	1	5		0.78	-0.33	5.5
0-6h	1	3	5	0.83	-0.39	4.9
0-12h	8			0.72	-0.84	7.1
0-12h	1	11		0.83	-0.83	5.6
0-12h	1	4	8	0.87	-0.89	4.9
0-24h	14			0.82	-0.19	6.9
0-24h	1	14		0.88	-0.44	5.5
0-24h	1	9	14	0.92	-0.46	4.7

a. Root mean square error

Figure 5. Validation of the limited sampling strategy. The Bland-Altman plot shows the agreement between the area under the concentration-time curve for 24h from multidrug-resistant tuberculosis patients obtained from the population pharmacokinetic model applying the limited sampling strategy of 1 and 5h ($AUC_{0\text{-}24h,LSS}$) and the $AUC_{0\text{-}24h,ref}$ Mean of All is the mean AUC of both $AUC_{0\text{-}24h,\ fit}$ and $AUC_{0\text{-}24h,ref}$.



All limited sampling strategies met the bias and RMSE criteria. Three sampling time points, at 1, 4 and 9h, enabled the best prediction of ertapenem exposure reflected by $AUC_{0\text{-}24h,LSS,}$ considering bias, RMSE and R^2 (R^2 = 0.92, MPE = —0,46% and RMSE = 4.7%). However, due to the lack of data within these time intervals and clinical relevance of time samplings within a certain amount of time, it would be preferred to use 1, 3 and 5 h (R^2 = 0.83, RMSE = 4.7% and MPE = -0.39%). Based on clinical suitability, using two sampling time-points with a time-restriction of 0-6h , a limited sampling strategy at 1 and 5h, showed the lowest RMSE (5.5%) and low MPE (-0.33%). The $AUC_{0\text{-}24h,LSS}$, estimated by applying this two sampling time-point limited sampling strategy was compared with the $AUC_{0\text{-}24h,ref}$ using Bland-Altman analysis, showing a bias in $AUC_{0\text{-}24h,LSS,}$of 4.2% (-15.2 – 23.6) (figure 5).

%T>MIC

Considering a free fraction of 5% and the MIC set at 0.5 mg/L, 9 out of 12 patients would have exceeded a minimum of *f* 40% T>MIC (range 6.8h – 19.7h) thereby having sufficient therapeutic effect in MDR-TB Patients with once daily dosing.

Discussion

This is the first study showing that a population pharmacokinetic model of ertapenem based on data of healthy volunteers can predict pharmacokinetics of ertapenem in patients with MDR-TB, even though the baseline characteristics of both healthy volunteers and MDR-TB patients differed significantly (table 1). We showed that the $AUC_{0\text{-}24h}$ of MDR-TB patients could be estimated with this population pharmacokinetic model with a mean overestimation of 6.8 (range: -17.2 – 30.7%).

The robustness of this population pharmacokinetic model was validated using a n-3 cross-validation, showing an underestimation of 0.3%. The limited sampling strategy that we present here can be used to assess individual drug exposure of ertapenem in TB patients with limited treatment options. Moreover, the model and limited sampling strategy can be used to evaluate drug exposure of ertapenem in phase II studies studying early bactericidal activity of ertapenem in TB patients. Such a study is urgently needed to provide data on efficacy of the potential attractive TB drug.

In the population pharmacokinetic model, multiple doses were treated as single doses on day one to avoid duplication, as an earlier study had found that there was no accumulation of ertapenem following dosing over eight days and mean plasma concentrations were found to be very similar on day one as well as on day eight (9).

Pharmacokinetic modeling of ertapenem has been performed in previous studies (11-18, 24-26), but it has never been performed for application in MDR-TB treatment. Comparing healthy volunteers and the TB patients, we found that there is a small pharmacokinetic variability between the two groups in contrast to other antimicrobial drugs, which show high variability in pharmacokinetic parameters (19,20). This might be explained by the parenteral route of

administration of ertapenem thereby having no loss of ertapenem due to absorption. Several studies looking at the exposure of ertapenem in patients have shown that the exposure in patients varies greatly (14,15). There is little pharmacokinetic variability in the MDR-TB patients (table 3).

A limited sampling strategy using two sampling time points is favored as it would give the least burden to patients as it is minimally invasive and least time-consuming. Additionally, less time between sampling time points is more feasible in clinical practice, since it is less prone to sampling mistakes. Moreover, limitation of this study is that after 6h sparse data was available, therefore results after 6h is less substantiated with clinical data.

As there are limited options for treating MDR-TB and resistance of antibiotics is an emerging problem, we think it is time to start assessing efficacy of ertapenem in MDR-B patients in Phase II clinical trial testing the early bactericidal activity. The developed limited sampling strategy can be used to evaluate drug exposure and thereby reduce costs and burden for the study subjects.

Conclusion

A pharmacokinetic model and limited sampling strategy bases on data from healthy volunteers was able to predict the $AUC_{0\text{-}24h}$ and *f 40*%T>MIC in MDR-TB patients. This model can be used in phase II studies.

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CHAPTER 8

General Discussion and Future Perspectives

General Discussion

The landscape of Multi- and Extensive drug resistant (M/XDR) treatment of TB is continuously changing. M/XDR-TB must be treated with multiple drugs to achieve efficacy and to prevent development of further resistance [1]. Multiple trials for different regimens with minimal resistance are ongoing to assess shorter, affordable, fully oral and optimized TB treatment. Recently, an individual patient data meta-analysis was performed of observational and experimental studies, published between January 2009 and April 2016, reporting end of treatment outcomes (completion, failure or relapse) of 12,030 patients from 25 countries in 50 studies. Treatment success of MDR-TB was positively associated with the use of bedaquiline, later generation fluoroquinolones, linezolid, clofazimine and carbapenems. This meta-analysis of predominantly observational studies had a large impact on setting new standards of care for M/XDR treatment. In the new guidelines issued by WHO [2], the role of 2nd line injectable agents changed entirely. These compounds were no longer considered primordial to obtain successful outcomes; two of these agents - kanamycin and capreomycin - were even associated with a poor outcome, and are no longer recommended in the revised treatment [2]. For the initial phase of MDR-TB treatment, at least four effective drugs; and in the continuation phase, at least five agents are recommended. Bedaquilline, moxifloxacin and linezolid have been listed as backbone and standard of care in longer MDR-TB regimens [1-2]. Imipenem-cilastatin and meropenem have been listed as Group C drugs for use in longer MDR-TB regimens guidelines to complete regimens, when other medicines cannot be used [2]. While ertapenem has not yet been included in the WHO regimen, it has a favourable advantage over the other carbapenems due to its once-daily dosing its activity in MDR-TB has been demonstrated. This thesis provides additional data on ertapenem as potentially advantageous carbapenem compound in treatment of MDR-TB.

Drug Susceptibility Testing

Effective management of M/XDR-TB relies on rapid diagnosis and treatment. Currently, phenotypic Drug Susceptibility Testing (DST) is the gold standard for drug resistance detection [3]. This DST methodology has been in use in medical microbiology but has inherent flaws when it comes to predicting what happens in vivo. The drug that is to be tested is added to the culture medium at a fixed concentration; in animal models and in humans treated for TB, however, drug concentrations typically follow a pharmacokinetic concentration-time curve.

Moreover, with every new drug dosage every day, in vivo systems are not vulnerable to chemical degradation which is an inherent flaw in the in vitro standard DST assay. One way to overcome these methodological flaws would be to use the hollow fiber infection model (HF). HF is an in vitro system that uses a pump system with culture media with fluctuating drug concentrations over time, mimicking much more closely the in vivo exposure to drug concentrations over time, correcting for chemical degradation of the compound under study as well as many other inherent flaws in the classical DST assay.

In Chapter 2, we discuss in vitro studies reporting MICs of imipenem and meropenem alone or combined with clavulanic acid at a range between 0.16 – 32 mg/L [4]. This high variability of results between in vitro studies might be best explained by the chemical instability of carbapenems in growth media at temperatures typically used in in vitro studies. Carbapenems are heat-sensitive and as *M. tuberculosis* growth needs an incubation at 37°C for 15 days in liquid media and 4 weeks in solid media, carbapenems have to withstand a high temperature for a long period of time. Ertapenem, that is unstable at 37°C, is likely to be degraded before killing or inhibiting slow-growing *M. tuberculosis,* suggesting that erroneously high MICs for Mtb might have been reported for carbapenems [5]. It is comprehensible that this stability issue of ertapenem has likely not occurred before, since ertapenem has only been prescribed for a wide range of bacterial infections, consisting mostly of a wide range of gram-positive, gram-negative and anaerobic bacteria with a typical generation time of 20 min. DST for these micro-organisms are available within days (maximum of 48h), compared to slow growing *M. tuberculosis* with generation time around 24h - resulting in DST reporting time in weeks.

In Chapter 5, we demonstrate a new strategy that shows how perform DSTs and measure MICs for such unstable compounds [6]. We first show that ertapenem degrades considerably and secondly, we show that ertapenem supplementation - to compensate for loss due to chemical degradation - brings it well within the EUCAST susceptibility range of 0.5 – 1 mg/L [6]. However, only one reference strain, *M. tuberculosis* H37Ra, was used in our MIC experiment. Today antimicrobial DST is based on critical concentration - also referred to as the breakpoint - defined as 'the lowest concentration of drug that will inhibit 95% of wild strains of *M. tuberculosis* that have never been exposed to drugs, while at the same time not inhibiting clinical strains of *M. tuberculosis* that are considered to be resistant' [3]. A next step

should be to study the wild type population distribution MIC of carbapenems with a minimum range of at least 15 putative wild-type isolates and with an overall total of at least 100 isolates, preferably using the HF model. Subsequently, the critical concentration and the epidemiological cut off (ECOFF) can then be defined [7].

Treatment of MDR-TB

In Chapter 2, we show that there is a paucity of studies on the use of carbapenems in M/XDR TB. Few in vivo studies have been carried out to date, and only two large retrospective studies with M/XDR TB patients using imipenem and meropenem have been performed; we found no clear evidence to select one particular carbapenem [4]. No evidence is available to label ertapenem as potential Group C drug among the carbapenems in the treatment of M/XDR TB. Our focus of this thesis was therefore, to explore ertapenem treatment in order to perform a phase II study for ertapenem to substantiate its potential as repurposed drug.

For studies exploring the clinical efficacy of ertapenem, a pharmacokinetic profile of ertapenem needs to be established and we therefore need a procedure to quantify and validate ertapenem in human plasma and human serum. As mentioned above, stability of ertapenem is an issue, therefore ertapenem needs to be tested in different test conditions, including storage stability and at different storage temperatures as samples need to be moved from clinical trial sites to laboratories. We have therefore developed and validated a liquid chromatography tandem-mass spectrometry (LS-MS/MS) method to quantify ertapenem in human plasma, described in Chapter 3 of this thesis [8].

In chapter 4, we performed a retrospective study to evaluate safety and pharmacokinetics for all patients with suspected MDR-TB who received 1 g ertapenem on a daily basis as part of their treatment regimen [9]. It is interesting to note that patients were given ertapenem for a prolonged time, up to 9 months; this is in contrast with the treatment of Gram-negative or Gram-positive bacterial infections where the on-label use typically lasts two weeks. In a study on safety and efficacy of long-term outpatient ertapenem treatment, ertapenem was given consecutively for a prolonged period of time, 8 to 16 weeks, for consecutively skin and soft tissue infections, intra-abdominal infections and osteomyelitis [10].

In general, ertapenem treatment appears well tolerated in long-term therapy in a broad range of bacterial infections. Ertapenem also showed a favorable pharmacokinetic/ pharmacodynamic profile, without any constraints in MDR-TB patients [9].

Like all beta-lactam antimicrobials, ertapenem exhibits time-dependant killing. Assessment of drug exposure of ertapenem should aim at assessing cumulative time above the MIC. Obviously, and ideally, the assessment should address the full drug combination used to treat MDR-TB [11]. Using limited sampling strategies might facilitate trials in less affluent settings. In chapter 7 of this thesis, we describe a two-compartment pharmacokinetic model and a limited sampling strategy that we developed to support therapeutic drug monitoring for an ertapenem dosage of 1 g daily. We show that two well-timed samples - at 1 and 5h - was adequate to predict ertapenem exposure in MDR-TB patients to reduce the burden imposed on patients and the health system by more extensive monitoring [12]. Typically, two or three samples are needed to estimate exposure. Other limited sampling strategies with one to three sampling time points post-dose have already been developed for other second-line anti-TB drugs, such as linezolid, levofloxacin and amikacin. Results showed that sampling points at 1 and 5 h post-dosing would be sufficient for amikacin, 0 and 5 h for levofloxacin and at 0 and 12 h for linezolid. As only best limited sampling strategies were shown for these TB drugs individually, it would be important as a next step to develop one limited sampling strategy for all anti-TB drugs, used as backbone therapy in the treatment of MDR-TB [13-15].

Dose finding

Correct dosing is a critical first step during clinical development. Though our patients were treated with a dosage of 1 g, this dose was not clinically explored in a prospective phase 2 (dose-finding) study. Pharmacokinetics/pharmacodynamic modelling is essential to optimise dosing. Dose fractionation is deemed necessary to determine which PK/PD parameter is most important for clinical efficacy. The optimal therapeutic dose range can be selected in a dose-finding study using a HF infection model study to mimic pharmacokinetic concentration-time curves of antibiotics observed in TB patients. Results of this HF system can be used in a Monte Carlo simulation to identify the optimal dose of ertapenem and the susceptibility breakpoint based on MIC above which therapy by ertapenem will fail [16-17].

In chapter 6, we present a HF infection model for dose finding on the use of ertapenem. We tested different ertapenem exposures, based on human equivalent doses in a range of 0.25 – 10 g ertapenem. Dose fractionation showed that ertapenem was linked to the percentage of the 24 h dosing interval of ertapenem concentration persisting above MIC (%T/MIC). An intravenous dosage of 2 g once per day was identified as most effective for sterilizing effect. This dosage can be used as a once-a-day dose for the treatment of MDR-TB. An ertapenem susceptibility breakpoint MIC of 2 mg/l was identified for that dose [18].

The best possible way to establish efficacy in a proof-of-principle study would be to test ertapenem in an early bactericidal activity (EBA) study of 2-weeks duration in treatment-naive patients with drug-susceptible TB. There are two techniques that can be used to measure EBA; in liquid and solid media. In solid media, the EBA is measured to the fall in $\log_{10}$CFU of *Mycobacterium tuberculosis* per ml sputum per day over the first 14 days of treatment. In liquid media the EBA is determined by measuring the daily prolongation of time to positivity (TTP) from baseline. For TB drugs, an EBA study has been established as the best way to establish efficacy [19-20]. Data in this thesis can be used as a starting point for a well-designed prospective phase 2 EBA study to substantiate efficacy and safety of 2 g ertapenem in combination with clavulanic acid on top of an optimized background regimen versus standard of care in patients with drug-resistant TB. A population model and limited sampling strategy was designed to support therapeutic drug monitoring for 2 g ertapenem [21].

Future perspectives

Current standard DST systems cannot overcome rapid decrease of initial drug concentration over time due to chemical instability of ertapenem in standard-agar based MIC assays. Although conceptually superior, HF infection models are expensive for routine DST. Genotypic testing might be an option to monitor for resistance to carbapenems. In other bacteria unrelated to *M. tuberculosis,* it has already been shown that changes in membrane permeability and presence of efflux pumps might lead to resistance to beta lactams [22]. Recently, a single nucleotide polymorphism (SNP) in de Rv2421c-Rv2422 intergenic region was found to be common among *M. tuberculosis* mutants, named as carbapenem resistance factor A (CrfA) [22]. Whole-genome sequencing was compelling to attribute carbapenem resistance to this mutation. In contrast to time-consuming MIC testing, utilizing genotypic

testing for these mutations would accelerate knowledge on carbapenem resistance, thereby preventing poor treatment outcomes. Whole genome sequencing (WGS) has the potential to rapidly enable insight into resistance profiles of *Mycobacterium tuberculosis* strains and improve individualized treatment on a large scale [23]. Therefore, further genetic studies and exploration of this carbapenem resistance factor are essential to understand its potential for detection of carbapenem resistance to *M. tuberculosis*.

A high-quality individual patient data meta-analysis reported a lack of benefit of commonly prescribed second-line injectable (kanamycin, capreomycin) and oral drugs (ethionamide); indeed, their use was associated with poor outcomes [1]. It was communicated that it is not expedient or desirable to give these second-line injectables. Moreover, FDA black box warning for aminoglycosides appeared on the package insert due to side effects such as hearing loss and kidney damage [24]. We hypothesize that carbapenems might be preferred above these injectable drugs. After efficacy and safety of ertapenem has been shown in a phase 2 EBA study, a phase 3 study could help for evaluating the different TB injectable classes. Amikacin could be compared with the carbapenem that shows best efficacy in a comparative EBA study; such phase 3 trial might help to establish which injectable should be preferred in MDR-TB treatment.

Beta-Lactamase C (BLaC) inhibitors, for example clavulanate, tazobactam and sulbactam, are currently not available on the market as single agent or in combination with a carbapenem. Clinical studies are confined since carbapenems need combined treatment in combination with amoxicillin/clavulanate. Unfortunately, gastrointestinal side effects are common, complicating prolonged treatment. Addition of amoxicillin in combination showed a synergistic effect in vitro, but its use was associated with significantly less success and great mortality [1,25]. Therefore, amoxicillin is not recommended as a separate agent against MDR-TB [2]. It would bring added value when figuring out how many patients would benefit from a combination of a carbapenem with a beta-lactamase inhibitor to create a business case for generic pharmaceutical companies. Choosing a combination of a carbapenem with a beta-lactamase inhibitor, the best partner beta-lactam antibiotic needs to be identified. Clavulanic acid, tazobactam and sulbactam all have a half-life of 1h, however pharmacokinetic properties as in vitro activity in combination with carbapenems are considerably different

between these compounds [26]. Sulbactam was shown to have the most potent activity against MDR-TB isolates [27]. Meanwhile, ceftazidime-avibactam, a cephalosporin is available on the market in combination with a BLaC inhibitor as parental agent and showed potent sterilizing activity against drug-resistant TB [28].

The World Health Organization has now ranked TB medication with a preference for oral agents over injectable drugs [2]. Therefore, we anticipate that TB programs will start using injectables less frequently. In order for ertapenem to have a place in an oral treatment regimen, a next step would be to alter the physical-chemical properties of ertapenem in such a matter that it can be designed as an oral drug. As of today, two carbapenems, faropenem-medoxomil and tebipenem-pivoxil, are already available as oral pro-drugs, but both have yet to be approved in Europe (EMA) and the United states (FDA). Tebipenem-pivoxil is now in clinical development as first oral carbapenem for treatment in bacterial infections in adult patients [29]. Like ertapenem a better understanding of safety, tolerability, pharmacokinetics and a dose-finding study for both drugs in MDR-TB patients should be performed to explore the feasibility as anti-TB oral agents.

Recently, inhaled antibiotic therapy has attracted increased attention and is becoming a promising alternative for parental administration. A formulation of colistin improved the aerolization of meropenem and showed synergistic bacterial killing against multi-drug resistant gram-negative pathogens [30]. Some of the potential benefits of inhalation antibiotics are that the inhaled drug doses are delivered directly to the target areas in pulmonary TB; and that topical delivery might reduce systemic side effects. Therefore, dry powder inhalation antibiotics in low-cost generic inhalers, which can deliver multiple high-dose dry powder capsules in resource-low settings might be the lifesaver everyone is waiting for.

Conclusion

In conclusion, ertapenem should be further developed and studied to explore its potential as a valuable asset in the treatment of MDR-TB. It is time to study its merits in a phase 2 EBA study and next, label ertapenem as group C drug amongst the other carbapenems.

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CHAPTER 9

Summary

Summary

Tuberculosis (TB) is caused by *Mycobacterium tuberculosis* and is the deadliest infectious disease worldwide. Our world is facing a public health crisis as treatment of TB has become more challenging with the emergence of resistance to first-line drugs, making it difficult to eradicate TB by 2030. Therefore, it is urgent to focus on improving current treatment by developing more active – sterilizing- anti TB drugs. One particularly effective strategy is rediscovery of old drugs as new agents for treatment against in multidrug resistant tuberculosis. Beta-lactam antimicrobial drugs are widely used drugs for the treatment of a range of infections. Ertapenem, approved in 2001 by the FDA and widely used against gram positive and negative bacteria, has shown to be active in MDR TB. To better understand the potential role of ertapenem for the treatment of M/XDR-TB, the aim of this thesis was to evaluate current literature, in vitro activity, and pharmacokinetics and safety in TB patients.

Carbapenems are earmarked as potentially active drugs for the treatment of M. tuberculosis. **In chapter 2** we evaluated the potential of carbapenems for the treatment of M/XDR-TB. The aim of this review was to evaluate the literature on currently available in vitro, in vivo and clinical data on carbapenems in the treatment of M. tuberculosis and detection of knowledge gaps, in order to target future research. Overall the results of the studies identified in this review are consistent. Carbapenems in combination with clavulanate showed increased activity *in vitro.* Few in *vivo* studies have been performed. Ten clinical studies to assess effectiveness, safety and tolerability of three different carbapenems (imipenem, meropenem and ertapenem) were performed. No clear evidence was found to select one particular carbapenem to design an effective M/XDR-TB regimen. More clinical evidence is needed to support repurposing carbapenems for the treatment of M/XDR-TB.

In chapter 3 a new simple and robust LC-MS/MS method using a quadruple mass spectrometer was developed for analysis of ertapenem in human plasma, using deuterated ertapenem as internal standard. The calibration curve was linear over a range of 0.1 (LLOQ) to 125 mg/L. The calculated accuracy ranged from -2.4 % to 10.3%. Within-run CV ranged from 2.7 % to 11.8% and between-run CV ranged from 0 % to 8.4%. Freeze-thaw stability biased between -3.3% and 0.1%. Storage of QC samples for 96h at 4°C differed -4.3 to 5.6%,

storage at room temperature for 24h, biased from -10.7% to -14.8% and storage in the autosampler biased between -2.9% and -10.0%.

In chapter 4, a retrospective study was performed for all MDR-TB suspected patients at the Tuberculosis Center Beatrixoord of University Medical Center Groningen (Haren, The Netherlands) who received ertapenem as part of their treatment regimen between the first of December 2010 and the first of March 2013. Safety and pharmacokinetics were evaluated. Eighteen patients were treated with 1000 mg ertapenem for a mean of 77 days (range 5-210). Sputum smear and culture were converted in all patients. Drug exposure was evaluated in 12 patients. The mean AUC0-24 was 544,9 (range 309 – 1130) mg*h/L. The mean Cmax was 127.5 (73.9 – 277.9) mg/L. In general, ertapenem treatment was well tolerated during MDR-TB treatment and showed a favourable PK/PD profile in MDR-TB patients.

In chapter 5 we presented the rapid decline of ertapenem during DST and we have thereby developed a new strategy to perform DST and MICs for such unstable compounds. We have shown that ertapenem supplementation brings it well within the susceptibility range and is likely to have good sterilizing effect in tuberculosis. This suggests that most of the published MICs for this drug are likely falsely high and rates of resistance are likely falsely elevated.

In chapter 6 our objective was to identify the ertapenem exposure associated with optimal sterilizing effect and then design a once a day dose for clinical use. We utilized the hollow fiber system model of tuberculosis in a 28-day exposure-response study of 8 different ertapenem doses in combination with clavulanate. The systems were sampled at predetermined time-points to verify the concentration-time profile and identify the total bacterial burden. Ertapenem-clavulanate combination demonstrated good microbial kill and sterilizing effect. In a dose-fractionation hollow fiber study, efficacy was linked to percentage of the 24-hour dosing interval of ertapenem concentration persisting above MIC. We identified an intravenous dose of 2 grams once per day as achieving the target in 96% of patients.

In chapter 7 a limited sampling strategy was developed using a population pharmacokinetic model based, using an iterative two-stage Bayesian method, on healthy volunteers and showed to be adequate to predict ertapenem exposure in MDR-TB patients.
External validation was performed by Bayesian fitting of the model developed in volunteers to the individual data of MDR-TB patients using the developed population model for volunteers as a prior. A Monte Carlo simulation (n=1000) was used to evaluate limited sampling strategies. The best performing limited sampling strategy, with a time-restriction of 0-6h, was found to be sampling at 1 and 5h (R2 = 0.78, mean prediction error = -0.33% and a root mean square error = 5.5%). Drug exposure was overestimated by a mean percentage of 4.2 (-15.2 – 23.6%). Considering a free fraction of 5% and the MIC set at 0.5 mg/L, 9 out of 12 patients would have exceeded a minimum of f 40% T>MIC.

In the **general discussion** we discussed insight, tools and understanding of ertapenem as potential in treatment of multidrug-resistant Tuberculosis. We discussed drug susceptibility testing of carbapenems and that the high variability of results of in vitro studies might be best explained by the chemical instability of carbapenems in growth media at temperatures typically used in in vitro studies. We noticed that ertapenem treatment seems well tolerated in long-term therapy in broad range of bacterial infections as it was shown retrospectively for MDR-TB treatment. As patients were treated with a dosage of 1 gram, it was not clinically substantiated in a prospective phase 2 study. Therefore, we discussed that a dose fractionation was deemed necessary to determine which PK/PD parameter is most important for clinical efficacy. To attain the optimal therapeutic dose range, a hollow fiber study was performed to mimic pharmacokinetic concentration profiles of ertapenem in TB patients. As 2 g once per day was identified as most effective for sterilizing effect, we suggest that data in this thesis can be used as starting point for the design of a well-designed prospective phase 2 study. This phase 2 study can substantiate efficacy and safety of 2 g ertapenem combined with clavulanic acid on top of an optimized background regimen versus standard of care in patients with MDR-TB.

In the **future perspectives** we elaborate on genotypic testing as option to anticipate resistance to carbapenems as hollow fiber models are expensive and drug susceptibility is complicated due to chemical instability of ertapenem. We hypothesize that carbapenems

might be preferred above these injectable drugs as an individual patient data meta-analysis, reported a lack of benefit of commonly prescribed second-line injectable drugs. Furthermore, we anticipate that injectables will be used less frequently by TB programs upcoming years. In order for ertapenem to have a place in an oral treatment regimen, next step would be to alter the physical-chemical properties of ertapenem in such a matter that it can be designed as an oral drug or a dry powder inhalation antibiotic.

We **conclude** that that 2 g ertapenem in combination with clavulanic acid might be a valuable asset in the treatment of multidrug resistant TB.

CHAPTER 10

Samenvatting
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Samenvatting

Tuberculose (tbc) wordt veroorzaakt door *Mycobacterium tuberculosis* en is wereldwijd de dodelijkste infectieziekte. Onze wereld wordt geconfronteerd met een crisis voor de volksgezondheid doordat de behandeling van tuberculose een grotere uitdaging is geworden met de opkomst van resistentie tegen eerstelijnsgeneesmiddelen. Het is noodzakelijk om de huidige behandeling te verbeteren door meer actieve - steriliserende - tbc-geneesmiddelen te ontwikkelen. Een bijzonder effectieve strategie is herontdekking van oude geneesmiddelen als nieuwe middelen voor de behandeling van multiresistente tuberculose (MDR-tbc). Bèta-lactam antimicrobiële geneesmiddelen zijn veel gebruikte geneesmiddelen voor de behandeling van een reeks infecties. Ertapenem is goedgekeurd in 2001 door de FDA en wordt veel gebruikt tegen grampositieve en gramnegatieve bacteriën. Ertapenem lijkt actief te zijn tegen MDR-tbc. Om de potentiële rol van ertapenem voor de behandeling van M/ XDR-tbc beter te begrijpen, was het doel van dit proefschrift om de huidige literatuur, in vitro activiteit, farmacokinetiek en veiligheid bij tbc-patiënten te evalueren.

Carbapenems zijn geoormerkt als potentieel actieve geneesmiddelen voor de behandeling van M. tuberculosis. **In hoofdstuk 2** hebben we het potentieel van carbapenems voor de behandeling van M/ XDR-tbc onderzocht. Het doel van deze review was om de literatuur te evalueren over de momenteel beschikbare in vitro, in vivo en klinische gegevens over carbapenems bij de behandeling van M. tuberculosis en het opsporen van kennislacunes, om zich te richten op toekomstig onderzoek. Over het algemeen zijn de resultaten van de onderzoeken die in deze beoordeling zijn geïdentificeerd consistent. Carbapenems in combinatie met clavulanaat vertoonden verhoogde activiteit in vitro. Er zijn weinig in vivo studies uitgevoerd. Er zijn tien klinische onderzoeken uitgevoerd om de werkzaamheid, veiligheid en verdraagbaarheid van drie verschillende carbapenems (imipenem, meropenem en ertapenem) te beoordelen. Er werd geen duidelijk bewijs gevonden om een bepaald carbapenem te selecteren om een effectief M/ XDR-tbc-regime te ontwerpen. Meer klinisch bewijs is nodig ter ondersteuning van herbestemming van carbapenems voor de behandeling van M/ XDR-tbc.

In **hoofdstuk 3** werd een nieuwe eenvoudige en robuuste LC-MS/MS-methode ontwikkeld met behulp van een viervoudige massaspectrometer voor de analyse van ertapenem in menselijk plasma, waarbij gedeutereerde ertapenem als interne standaard werd gebruikt. De kalibratiecurve was lineair over een bereik van 0,1 (LLOQ) tot 125 mg/ L. De berekende nauwkeurigheid varieerde van -2,4% tot 10,3%. De CV binnen het bedrijf varieerde van 2,7% tot 11,8% en de CV tussen de runs varieerde van 0% tot 8,4%. Bevries-dooi-stabiliteit vooroordeel tussen -3,3% en 0,1%. Opslag van QC-monsters gedurende 96 uur bij 4 ° C verschilde -4,3 tot 5,6%, opslag bij kamertemperatuur gedurende 24 uur, vooringesteld van -10,7% tot -14,8% en opslag in de autosampler vooringesteld tussen -2,9% en -10,0%.

In **hoofdstuk 4** werd een retrospectieve studie uitgevoerd voor alle vermoedelijke MDR-tbc-patiënten in het tuberculosecentrum Beatrixoord van het Universitair Medisch Centrum Groningen (Haren, Nederland) die ertapenem kregen als onderdeel van hun behandelingsregime tussen 1 december 2010 en 1 maart 2013. Veiligheid en farmacokinetiek werden geëvalueerd. Achttien patiënten werden behandeld met 1000 mg ertapenem gedurende een gemiddelde van 77 dagen (bereik 5-210). Sputumuitstrijkje en -cultuur werden bij alle patiënten omgezet. Blootstelling aan geneesmiddelen werd geëvalueerd bij 12 patiënten. De gemiddelde AUC0-24 was 544,9 (bereik 309 - 1130) mg * h/ L. De gemiddelde Cmax was 127,5 (73,9 - 277,9) mg/ L. Over het algemeen werd de behandeling met ertapenem goed verdragen tijdens MDR-tbc-behandeling en vertoonde ertapenem een gunstig PK/ PD-profiel bij MDR-tbc-patiënten.

In **hoofdstuk 5** hebben we de snelle afname van ertapenem tijdens een geneesmiddel gevoeligheidstest (DST) laten zien. Hiervoor hebben we een nieuwe strategie ontwikkeld om DST en MIC's uit te voeren voor dergelijke onstabiele verbindingen. We hebben aangetoond dat ertapenem-suppletie het goed binnen het gevoeligheidsbereik brengt en waarschijnlijk een goed steriliserend effect bij tuberculose heeft. Dit suggereert dat de meeste gepubliceerde MIC's voor dit medicijn waarschijnlijk onjuist hoog zijn en dat de resistentiepercentages waarschijnlijk lager zijn dan wij nu vermoeden.

In **hoofdstuk 6** was ons doel het identificeren van de ertapenem-blootstelling geassocieerd met optimaal sterilisatie-effect en vervolgens een eenmaal daagse dosis te ontwerpen voor

klinisch gebruik. We gebruikten het Hollow-Fiber-systeemmodel van tuberculose in een 28-dagen blootstellingsreactie onderzoek van 8 verschillende ertapenem-doses in combinatie met clavulanaat. De systemen werden bemonsterd op vooraf bepaalde tijdstippen om het concentratie-tijdprofiel te verifiëren en de totale bacteriële last te identificeren. Ertapenem-clavulanaat-combinatie vertoonde een goed microbieel dodings- en steriliserend effect. In een dosis-fractionering Hollow-Fiber onderzoek was de werkzaamheid gekoppeld aan het percentage van het 24-uurs doseringsinterval van ertapenem concentratie dat aanhoudt boven MIC. We identificeerden een intraveneuze dosis van 2 gram eenmaal per dag om het doelwit te bereiken bij 96% van de patiënten.

In **hoofdstuk 7** werd een Limited sampling strategie ontwikkeld met behulp van een populatie farmacokinetisch model op gezonde vrijwilligers en toonde voldoende te zijn om ertapenem blootstelling bij MDR-tbc-patiënten te voorspellen. Externe validatie werd uitgevoerd door Bayesiaanse aanpassing van het model aan de individuele gegevens van MDR-tbc-patiënten met behulp van het ontwikkelde populatiemodel voor vrijwilligers als een voorafgaande. Een Monte Carlo-simulatie (n = 1000) werd gebruikt om limited sampling strategieën te evalueren. De best presterende strategie voor limited sampling, met een tijdbeperking van 0-6 uur, bleek te zijn op 1 en 5 uur (R2 = 0,78, gemiddelde voorspellingsfout = -0,33% en een RSME = 5,5%). De blootstelling aan geneesmiddelen werd overschat met een gemiddeld percentage van 4,2 (-15,2 - 23,6%). Als we een vrije fractie van 5% en de MIC ingesteld op 0,5 mg/ L beschouwen, zouden 9 van de 12 patiënten een minimum van f 40% T> MIC hebben overschreden.

In de **algemene discussie** bespraken we het inzicht, hulpmiddelen en het begrip van ertapenem als potentieel in de behandeling van multiresistente tuberculose. We bespraken het testen van de gevoeligheid voor geneesmiddelen van carbapenems en dat de hoge variabiliteit van de resultaten van in-vitro-onderzoeken het best kan worden verklaard door de chemische instabiliteit van carbapenems in groeimedia met temperaturen die doorgaans worden gebruikt in in-vitro-onderzoeken. We hebben gemerkt dat ertapenem-behandeling, net zoals retrospectief werd aangetoond voor MDR-tbc-behandeling, goed verdragen lijkt te worden gedurende lange termijn therapie bij een breed scala aan bacteriële infecties. Patiënten werden behandeld met een dosering van 1 gram, echter is dit niet klinisch

onderbouwd in een prospectief fase 2-onderzoek. Daarom hebben we bediscussieerd dat een dosis-fractionering noodzakelijk werd geacht om te bepalen welke PK/ PD-parameter het belangrijkst is voor klinische werkzaamheid. Om het optimale therapeutische dosisbereik te bereiken, werd een Hollow-Fiber studie uitgevoerd om de farmacokinetische concentratieprofielen van ertapenem bij tbc-patiënten na te bootsen. Omdat 2 g eenmaal per dag werd geïdentificeerd als het meest effectief voor het steriliserende effect, stellen we voor dat de gegevens in dit proefschrift kunnen worden gebruikt als startpunt voor het ontwerp van een goed ontworpen prospectief fase 2-onderzoek. Deze fase 2-studie kan de werkzaamheid en veiligheid van 2 g ertapenem in combinatie met clavulaanzuur bevestigen bovenop een geoptimaliseerd achtergrondregime versus standaardbehandeling bij patiënten met MDR-tbc.

In de **toekomstperspectieven** zijn we ingegaan op genotypische testen als optie om te anticiperen op resistentie tegen carbapenems, omdat Hollow-Fiber modellen duur zijn en de gevoeligheid van geneesmiddelen gecompliceerd is als gevolg van chemische instabiliteit van ertapenem. Sinds een individuele patiëntgegevens-meta-analyse een gebrek aan voordeel van algemeen voorgeschreven tweedelijns injecteerbare geneesmiddelen gemeld heeft, veronderstellen we dat carbapenems mogelijk de voorkeur hebben boven deze injecteerbare geneesmiddelen. Verder verwachten we dat injecteerbare geneesmiddelen in de komende jaren minder vaak zullen worden gebruikt door tbc-programma's. Om ertapenem een plaats te geven in een regime voor orale behandeling, zou de volgende stap erin bestaan de fysisch-chemische eigenschappen van ertapenem te veranderen in een dergelijke zaak dat het kan worden ontworpen als een oraal geneesmiddel of een antibacterieel middel voor het inhaleren van droog poeder.

We **concluderen** dat 2 g ertapenem in combinatie met clavulaanzuur een waardevolle aanwinst kan zijn bij de behandeling van multiresistente tbc.

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About the Author

Sander Pascal van Rijn was born on February 12th, 1988 in Bochum (BRD) as the elder of an identical twin. After attending high School at Johan-de-Witt gymnasium in Dordrecht, he began studying Pharmacy at the University of Groningen in 2006. In 2008, he was Commissioner of General Affairs of the Royal Dutch Pharmaceutical Students' Association (KNPSV) and Liaison Secretary and Dutch representative for the European Pharmaceutical Students' Association (EPSA). Throughout the second halve of his master's programme, he started his research on clinical pharmacology of ertapenem under supervision of Jan-Willem Alffenaar, finally resulting in a PharmD/PhD program at the University Medical Center Groningen. During this program, he had the honour to visit Dallas, Texas, United States of America and to evaluate ertapenem in a Hollow-Fiber Infection model against M. Tuberculosis. At the end of 2014, he earned his Master's degree of Pharmacy, and jumpstarted his career directly as Global Management Trainee at Fagron. After 2 years and several assignments both at home and abroad, he switched to an insurance company to start as 'beleidsadviseur intramurale geneesmiddelen'. During his stay at Zilveren Kruis, he was responsible for the affordability, accessibility and quality of expensive medicines within inpatient hospital care. In addition, he helped designing the 'gezamenlijke inkoop dure geneesmiddelen voor zorgverzekeraars Nederland'. In 2018, he started as consultant at Ter Welle & Associés (TW&A), a consultancy that specializes in innovative market access and healthcare pathways. In addition, he started as relations manager for PharmIntel, an independent big data firm, providing management information and insights in the (appropriate) use and outcome of expensive medicine for most hospitals in the Netherlands. His future perspective is to create value by helping hospitals and pharmaceutical companies to bring affordable and accessible medicines to patients in the Netherlands and the rest of the world.

Publication list

1. Sander P. van Rijn, Marlanka A. Zuur, Richard Anthony, Bob Wilffert, Richard van Altena, Onno W. Akkerman, Wiel C.M. de Lange , Tjip S. van der Werf , Jos G.W. Kosterink, Jan-Willem C. Alffenaar. **2019.** Evaluation of carbapenems for treatment of multi- and extensively drug-resistant *Mycobacterium tuberculosis*. Antimicrob. Agents Chemother.
2. Van Rijn SP, Zuur MA, van Altena R, Akkerman OW, Proost JH, de Lange WCM, Kerstjens HAM, Touw DJ, van der Werf TS, Kosterink JGW, Alffenaar JWC. **2017.** Pharmacokinetic modeling and limited sampling strategies based on healthy volunteers for monitoring of ertapenem in patients with multidrug-resistant tuberculosis. Antimicrob Agents Chemother 61:e01783-16. Doi:10.1128/AAC.01783-16
3. Van Rijn SP, Srivastava S, Wessels MA, van Soolingen D, Alffenaar JW, Gumbo T**. 2017.** The sterilizing effect of ertapenem-clavulanate in a hollow fiber model of tuberculosis and implications on clinical dosing. Antimicrob Agents Chemother doi:10.1128/AAC.02039-16
4. Srivastava S, van Rijn SP, Wessels AMA, Alffenaar JWC, Gumbo T. **2016.** Susceptibility testing of antibiotics that degrade faster than the doubling time of slow-growing mycobacteria: Ertapenem sterilizing effect of Mycobacterium tuberculosis. Antimicrob Agents Chemother 60:3193-3195. Doi:10.1128/AAC.02924-15.
5. Van Rijn SP, van Altena R, Akkerman, OW, van Soolingen D, van der Laan T, de Lange WCM, Kosterink JGW, van der Werf TS, Alffenaar JWC. **2016.** Pharmacokinetics of ertapenem in patients with multidrug-resistant tuberculosis. Eur Respir J. 47:1229-1234. Doi:10.1183/13993003.01654-2015.
6. van Rijn SP, Wessels AM, Greijdanus B, Touw DJ, Alffenaar JWC. **2014**. Quantification and validation of ertapenem using a liquid chromatography-tandem mass spectrometry method. Antimicrob Agents Chemother; 58: 3481–3484.