Pharmacokinetics of antimicrobial agents

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Pharmacokinetics of antimicrobial agents: optimization and treatment individualization

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Stellingen

behorende bij het proefschrift

Pharmacokinetics of antimicrobial agents: optimization and treatment individualization

1. Het moment voor de afname van een bloedmonster voor de schatting van de AUC met behulp van een populatie model voor TDM dient praktisch gekozen te worden. *(Dit proefschrift)*

2. Het effect van een interactie tussen geneesmiddelen kan tussen patiënten en gezonde vrijwilligers klinisch relevant verschillen. *(Dit proefschrift)*

3. Naar mate een patiënt zieker is, voldoet de dosering in de bijsluiter minder. *(Dit proefschrift)*

4. Een universele analyse methode is de hoeksteen van een laboratorium maar goed geschoolde analisten en ziekenhuisapothekers zijn het fundament voor een klinisch georiënteerde TDM service. *(Dit proefschrift)*

5. Het feit of een dosering hoog is hangt af van de bloedspiegel. *(Dit proefschrift)*

6. De activiteit van metabolieten van antibiotica kan niet gegeneraliseerd worden maar dient per micro-organisme bepaald te worden. *(Dit proefschrift)*

7. Als apothekers voor arts gaan spelen, wie runt dan de apotheek?

8. Als de indicatie van een geneesmiddel bij herhaalrecepten beter geëvalueerd zou worden, had polyfarmacie geen negatieve klank gehad.

9. Meten is weten, maar leidt nog niet noodzakelijkerwijs tot begrijpen.

10. Het resultaat van een vergadering is vaak omgekeerd evenredig met het aantal mensen die onvoorbereid aan de vergadering deelnemen.

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Voor E^3

Pharmacokinetics of antimicrobial agents:
- optimization and treatment individualization -

Chapter 1

General introduction, scope and outline of the thesis

INTRODUCTION

One size (dose) fits all has been the standard in the development of drugs prescriptions and drug registration. This principle is based on reducing costs in the development and marketing of drugs, because costs of the investment of bringing a drug to the market has to be earned back in a relatively short period of time, before patents are expired. Difficult dosing regimens to individualize treatment can not be easily sold, especially outside the hospital. Therefore most drugs are marketed in a dosage regimen that is effective for the majority of patients without too many side effects. However, in daily practice physicians and pharmacists encounter patients that do not fit within this general population of the pharmaceutical industry. The standard dose will result in the desired effect in these patients, especially when the therapeutic window is narrow. They are faced with the challenge to optimize treatment in these patients. Often these health care workers join forces into multidisciplinary teams to evaluate systematically remarkable findings observed in daily practice [1, 2]. The joint effort leads to investigator initiated studies investigating clinically relevant questions in the quest to optimize patient care.

Especially critically ill patients with severe infectious diseases differ the most from the general population and would therefore benefit most from individualised therapy [3]. Several studies have shown that critically ill patients tend to respond differently to standard drug dose. Altered organ function or changes in body composition may change pharmacokinetics in these individuals [4, 5]. In particular, patients suffering from invasive fungal infections (IFI) and mycobacterial infections are at risk of severe morbidity and mortality, have important co-morbidities, and receive multiple drugs. Such patients challenge health care providers to explore the variability in clinical pharmacokinetics of antimicrobial drugs to optimize dosing of these drugs to increase efficacy and limit toxicity. Here we first briefly introduce the concepts mode of action, pharmacokinetics and therapeutic drug monitoring, but also highlight the importance of individualised therapy in the treatment of IFI, TB and Buruli ulcer disease.

Antimicrobial drugs: mode of action

Antimicrobial drugs can be defined as compounds that kill or inhibit the growth of microorganisms in a concentration, which is not too toxic for man. These compounds can be derived from natural substances like Penicillium, modified chemically from original compounds or be designed and purely chemically synthesized. The mode of action of the compounds is diverse and targeted against the integrity, functioning or replication of the microorganisms by inhibition of protein synthesis, cell wall production, DNA or RNA synthesis or cell metabolism. By means of natural selection microorganisms that are not affected by used antimicrobial agents survive and procreate. This occurrence of resistance has increased the need for the development of new antibiotics [6]. To prevent further emerging of resistance antibiotics must be prescribed rationally [7, 8].

Pharmacokinetics of antimicrobial agents

Pharmacokinetics of antimicrobial agents is defined, like for any other drug, as the study of the absorption, distribution, metabolism and excretion of that drug. The following parameters are used to describe the pharmacokinetics of a drug: dose (D), bioavailability (F), volume of distribution (Vd), clearance (Cl). All these factors influence the total exposure, described by the area under the concentration time curve (AUC), which describes the drug exposure in one individual to one individual drug at one particular dosage. Compared to other drugs the pharmacodynamics of antibiotics can be defined as the effect of the drug on the pathogen instead of the effect on the human body. Pharmacokinetics (PK) and pharmacodynamics (PD) are combined to explain the action of the drug to the pathogen in the body

[9, 10]. PK/PD parameters to describe the action of the drug are Cmax/MIC ratio, AUC/MIC ratio and Time > MIC. Variability in pharmacokinetics, resulting in an altered exposure, can have a great impact on these PK/PD parameters [11]. This variability can result is reduced efficacy, especially when the more resistant pathogen has a higher MIC.

To be able to explain pharmacokinetic variability and its effect on the efficacy of the treatment one has to understand the influence of different parameters on the concentration of a drug over time in target tissues, or – if this is impossible – in body fluids such as serum that have equilibrium with target tissues. In drug development the first encounter with the pharmacokinetic variability is in animal models. However, these do not necessarily reflect the pharmacokinetic variability in humans. Therefore, in phase I studies pharmacokinetic variability in humans can be assessed for the first time. Dose escalation studies investigating safety and tolerability increase the knowledge of effects of a drug and further insight in the pharmacokinetics is established. In Phase II/III studies the efficacy of the drug is explored and often compared to placebo or competitor. In randomised Phase III studies larger number of selected patients are exposed to the drug, but most of the time the intensity of the pharmacokinetic evaluation of the drug is diminished to a few random drug concentrations. The potential to correlate the efficacy or toxicity to the variability in drug concentration is therefore limited. Once the drug has entered the market and is used for the labelled indication more patients are exposed to the drug. For a substantial part of these patients, the patients participating in the phase III studies are not representative [12]. Therefore post marketing surveillance has been instated. The limitations of post marketing surveillance is the high abstraction level of these surveys, often neglecting the individual patients.

In daily practice, pharmacokinetic variability is frequently observed by physicians and clinical pharmacists working in the field of infectious diseases. Therefore tools are needed to optimize treatment in patients in whom this variability is observed.

Therapeutic drug monitoring

In general, drugs are used without monitoring of drug concentrations in blood, as their dosage can be titrated based on the clinical response of the patient to that drug. Monitoring of concentrations of drugs with a narrow therapeutic window in blood or other bodily fluids to optimize treatment is defined as therapeutic drug monitoring [13]. In case of antimicrobial agents the focus is on preventing too low concentrations with relation to the pathogen and too high concentrations with relation to the host. Too low concentrations of the antimicrobial agent will result in treatment failure and drug resistance may occur. Too high concentrations will result in toxicity with potentially irreversible organ damage, but also in discontinuation of the antimicrobial agent resulting in ineffective treatment.

Invasive fungal infections

WHO has identified changes in morbidity and mortality 1, with a growing population of elderly; in many of these individuals, comorbidities and multiple disease conditions become increasingly important. Morbidity and mortality due to cancer, and transplant-related complications for instance are partly due to opportunistic infections. In this thesis we will focus on fungal filamentous infections, notably, by Aspergillus spp. Conidiae (spores) of Aspergillus are ubiquitous, and these spores are inhaled without causing damage to airways and lung tissues in normal hosts. In immuno-compromised individuals however, and especially so in neutropenic hosts, defence mechanisms fail, and potentially fatal invasive fungal infection may result. In these critically ill heterogeneous patient population individualised treatment seems mandatory to increase the poor results achieved by current available antifungal drugs [14].

Tuberculosis

Tuberculosis, caused by Mycobacterium tuberculosis (or other species of M. tuberculosis complex, notably, M. africanum and M. bovis) is the most deadly among mycobacterial infections. A recent report discussed advances in the combat against the three major infectious diseases: HIV/AIDS, malaria and tuberculosis. Clearly, much progress has been made in the fight against HIV/AIDS and malaria, but the fight against tuberculosis is less effective [15, 16]. One of the Millenium Development Goals (MDG 6) [17] aiming to half the number of deaths from tuberculosis by 2015 is unlikely to be met. This is at least partly due to the limited number of drugs available, with ever increasing numbers of patients harbouring drug-resistant organisms, and only very few new drugs in the pipeline for tuberculosis to be investigated in phase 2 and phase 3 trials [18]. Poor health recourses, lack of drugs and limited adherence due to a high pill burden or intravenous administration and co-infection with HIV/AIDS presents even greater challenges. As the number of drugs for the treatment of TB is limited, antibiotic agents developed for other infectious diseases are evaluated for efficacy against TB. These agents are evaluated in in vitro models, resulting in a selection of PK/PD parameters which are correlated the best to efficacy. However the dose established in vitro is not necessarily the dose that is marketed for the labelled indication of that specific drug. Therefore the current policy is to improve the use of currently available compounds in a prudent fashion, by optimizing their use, restrict available compounds to designated health care providers in dedicated institutions, and optimizing drug delivery and monitoring efficacy and toxicity [19, 20].

Buruli ulcers disease

WHO has also identified 14 Neglected Tropical Diseases (NTD) [21], with Buruli ulcer featuring among the two mycobacterial infections (along with leprosy) listed among the NTD. Buruli ulcer is caused by M. ulcerans, and according to WHO, it is a tool-deficient emerging infection. The purpose of antimycobacterial drugs is to kill and prevent replication of the bacilli, but also to halt the production of the toxin, mycolactone causes the tissue damage. Although many anti-mycobacterial drugs appeared effective in vitro and in animal models against M. ulcerans infection, clinical evidence for effectiveness of antimicrobial treatment is limited [22]. Therefore, an efficacious and safe oral treatment schedule is urgently needed to reduce the number complications from surgical interventions and intravenously administered aminoglycosides.

OBJECTIVES OF THE THESIS

The central theme of this thesis is to study the pharmacokinetics of antimicrobial agents to optimize individual treatment of critically ill patients suffering from IFI and mycobacterial infections.
The main objectives are to gain insight in:

- drug-drug interactions on the pharmacokinetics of both antimicrobial drugs
- alteration on drug absorption, distribution, metabolism and excretion of antimicrobial drugs
- influence of disease severity on these pharmacokinetics
- PK/PD parameters of antimicrobial drugs

Different research principles and methods were applied to attain these objectives: case reports, case series, review, and prospective pharmacokinetic studies.

OUTLINE OF THE THESIS

Chapter 1: Voriconazole

In the first chapter we investigated the pharmacokinetics of voriconazole, an antifungal agent. It is used as first line treatment for invasive aspergillosis [23]. This fungal infection is most common in patients with haematological malignancies and solid organ transplant recipients (e.g. lung transplant recipients) as these patients have a compromised immune system due to chemotherapy or immunosuppressive drugs. The drug of choice, voriconazole, is readily absorbed from the gastrointestinal tract and metabolized in the liver by the cytochrome P450 (CYP) 2C9, 2C19 and 3A4 [24]. This agent displays non-linear pharmacokinetics, which means that a small dosage increase can result in a disproportional increase in serum concentration.

- To be able to measure drugs in serum, a validated method of analysis using LC/MS/MS had to be developed with accuracy and reproducibility at the limit of quantification. These limits of quantification had to be wide enough to include both sub-therapeutic and toxic drug levels.
- The agent is administered in a loading dose, followed by a maintenance dose to achieve steady state levels. However, it seems plausible that when a loading dose in insufficient the maintenance dose is not enough to achieve steady state levels. Therefore critically ill patients that need the drug most, might be at risk for under-dosing [5]. To evaluate this hypothesis we investigated the correlation between drug exposure (AUC) and disease severity in an observational prospective pharmacokinetic study.
- In case of non-linear pharmacokinetics the possibility of a loading dose in excess of lower limits of toxicity should be considered. Saturation of the enzymatic metabolism of voriconazole can lead to toxic drug concentrations, which can result in adverse drug effects like visual disturbances and hepatotoxicity [25-27]. In patients with haematological malignancies we evaluated whether hepatic function influenced the metabolism of voriconazole and altered its pharmacokinetics in a retrospective study.
- To show that dosing of voriconazole in paediatric patients differs from dosing in adults a remarkable case-report is included [28].

Chapter 2: drug-drug interactions with azole antifungal agents

Azole antifungal agents are well known for their wide range of drug-drug interactions as these drugs are both substrate as inhibitor of cytochrome P450 enzymes and some are also inhibitors of P-glycoprotein drug transporter. The extent of the drug-drug interactions differs from one azole to the other, and therefore the physician is often unaware of the result of the drug-drug interaction.

- The extent of the drug-drug interaction is difficult to translate into a clinical advice to adapt treatment in daily practice [29]. Therefore we evaluated most published trials reporting on azole drug interactions and rated these on relevance for clinical practice. This review on the clinical impact of azole drug-drug interactions can serve the physician to improve the management of individual patients.
- The purpose of the letter to the editor was to make physicians aware of a drug-drug interaction not yet listed in the product leaflet, which had the significant effect on the serum concentrations of posaconazole, an azole antifungal drug.
- Based on daily observations a retrospective study was performed to reassess the advice in the product leaflet how to handle drug-drug interaction between phenytoin and voriconazole [30].

Chapter 3: Increasing the potential of a third line antituberculosis drug: Linezolid
Linezolid, labelled for MRSA, is also active in vitro against M. tuberculosis, but appeared very toxic when administered beyond the labelled period of 28 days. It is listed as third line anti-tuberculosis drug and should only be used based on susceptibility data and plasma concentration guided dosing to optimize treatment. As the MIC for linezolid is very low, a dosage reduction seems plausible [31]. However, a standard dose reduction neglects individual pharmacokinetic variability and does not account for more resistant strains of the tubercle bacillus.

- To be able to measure drugs in serum a validated method of analysis using LC/MS/MS had to be developed with accuracy and reproducibility at the limit of quantification.
- To evaluate the possibility to individualize linezolid treatment based on drug exposure and MIC we studied two dosage regimen in patients with MDR-TB in a prospective pharmacokinetic study.
- To guide treatment with linezolid in daily practice we developed a pharmacokinetic model using limited blood sampling that may simplify the monitoring of treatment.

Chapter 4: Establishing the optimal dose of Moxifloxacin for individual TB treatment
Moxifloxacin (MFX), one of the newer fluoroquinolones, labelled for the treatment of respiratory tract infections, appeared the most active of its class against M tuberculosis during in vitro and in vivo experiments [32]. Like for other fluoroquinolones, the AUC/MIC was the parameter that predicted efficacy best. However, this parameter was significantly influenced by variability in AUC, partly caused by drug-drug interaction by rifampicin, and MIC. Protein binding appeared to vary significantly. This influences the efficacy of the drug, as only the unbound drug reflects the active concentration that should excess the MIC.

- To be able to measure drugs in plasma and liquor a validated method of analysis had to be developed with accuracy and reproducibility at the limit of quantification.
- In a retrospective study we evaluated factors that influenced AUC/MIC ratio and treatment of MFX in TB patients in general.
- As treatment options in patients with tuberculous meningitis are limited we investigated the pharmacokinetics of MFX in two dosages in a prospective manner. MFX concentrations in plasma and liquor were evaluated to investigate the AUC/MIC ratio of MFX in the treatment of this complex form of TB.

Chapter 6: EMB, obese patients need to be dosed based on their IBW
The volume of distribution of a drug mainly depends on its lipophilicity. Lipophilic drugs have a large volume of distribution compared to hydrophilic drugs. In patients with an altered body composition this has to be taken into account [33]. However, the majority of TB patients tend to have a low BMI due to the disease and worsening clinical condition and therefore little attention has been paid to the obese. As obesity is increasing world-wide, more obese TB patients can be expected. Dosing of antituberculosis drugs in the obese has not been fully explored.

- We retrospectively investigated ethambutol, a very water soluble drug, which causes optic neuropathy in a dose dependent manner when overdosed. If ethambutol is given to obese patients and is dosed on total body weight the concentration in the water compartment may be high compared to the normally underweighted TB patients and therefore an increased prevalence of this adverse drug effect may be observed.

Chapter 7: Exploring the effect of a drug-drug interaction (rifampicin/clarithromycin) in relation to combined treatment in patients with Buruli ulcer.
As discussed earlier, drugs can exhibit significant drug-drug interactions especially if subjected to enzymatic metabolism by the CYP450 system. As drug-drug interactions can be so profound that one of the two drugs is decreased to sub-therapeutic concentrations, studies evaluating combination treatment should always evaluate the extent of the drug-drug interaction during the study. Otherwise the question whether the clinical efficacy of the combination treatment could be the result of mono therapy or actual combination treatment can not be answered. In the search for a new oral treatment regimen for M. ulcerans disease, the current WHO recommendation consisting of eight weeks of streptomycin and rifampicin was compared with 4 weeks of streptomycin and rifampicin followed by 4 weeks rifampicin plus clarithromycin in a randomised controlled trial in Ghana [NCT00321178].

- To be able to measure both drugs including potential active metabolites in plasma a validated method of analysis using LC/MS/MS had to be developed with accuracy and reproducibility at the limit of quantification.
- The extent of the drug-drug interaction of rifampicin and clarithromycin was yet unknown and therefore we studied the effect on drug concentrations of this drug-drug interaction in a subset of patients from this randomised controlled trial.

Chapter 8: General discussion and future perspectives
In the final chapter we will discuss the clinical impact of the thesis and evaluate the strength of individualised treatment by means of TDM guided dosing of antimicrobial drugs. The different research strategies used in this thesis will be reviewed and placed in perspective of the future research and development of optimization and individualisation of antimicrobial treatment.

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Chapter

2.1

Method for therapeutic drug monitoring of azole antifungal drugs in human serum using LC/MS/MS.

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ABSTRACT

Fungal infections occur in immunocompromised patients. Azole antifungal agents are used for the prophylaxis and treatment of these infections. The interest in therapeutic drug monitoring azole agents has increased over the last few years. Inter- and intra-patient variability of pharmacokinetics, drug-drug interactions, serum concentration related toxicity and success of therapy has stressed the need of frequently therapeutic drug monitoring of the drugs, belonging to the group of azoles. Therefore a simple, rapid and flexible method of analysis is required. This method is based on the precipitation of proteins in human serum with LC/MS/MS detection. Validation was performed according to the guidelines for bioanalytical method validation of the food and drug administration agency. The four most used azole drugs can be detected in human serum within the clinical relevant serum levels with good accuracy and reproducibility at the limit of quantification. Intra- and inter-day validation demonstrated good accuracy and reproducibility. A rapid, sensitive and flexible LC/MS/MS method has been developed and validated to measure voriconazole (VRZ), fluconazole (FLZ), itraconazole (ITZ) and posaconazole (PSZ) in human serum. This new method is suitable for clinical pharmacokinetic studies and routine monitoring in daily practice.

INTRODUCTION

Invasive fungal infections occur especially in immunocompromised patients[1] and azole antifungal compounds (e.g. fluconazole, itraconazole, posaconazole and voriconazole) have gained a solid position in the prophylaxis and treatment of these fungal infections. Fluconazole is used for treatment and prophylaxis of candidiasis while voriconazole is mainly used for the treatment of invasive aspergillosis[2] or infections with fluconazole resistant *Candida spp*[3]. Itraconazole and posaconazole are both active against *Candida* en *Aspergillus spp*. and have a place in the prophylaxis of fungal infections[4, 5]. Posaconazole is also recommended for the treatment of zygomycosis or as salvage therapy for invasive aspergillosis[6, 7].

The pharmacokinetics of azole antifungal agents show a large inter- and intraindividual variability which can be partly explained by non linear pharmacokinetics, differences in bioavailability, drug-drug interactions and cytochrome P450 polymorphisms. For many years the clinical impact of this variability was unknown until observational studies showed a correlation between plasma concentrations of azole agents and efficacy and toxicity [8-15]. Therapeutic drug monitoring of azole agents may therefore be warranted [10, 16-21]. In the last few years therapeutic drug monitoring of azole agents has evolved and has now reached the point of becoming daily practice dosing as the patients condition may change rapidly [22, 23]. For routine monitoring of plasma levels of azole antifungal agents an universal method of analysis for high through put of samples is required. Therefore our objective was to develop a simple, rapid and flexible LC-MS/MS method of analysis for the four different azoles in human serum preferably without solid phase extraction. This method was based on the precipitation of proteins in human serum with precipitation reagent containing the internal standard and subsequently HPLC analysis and MS/MS detection of the transition ions of the azole and the internal standard.

METHODS

Chemicals, materials and reagents

Fluconazole and voriconazole were kindly provided by Pfizer (New York, USA), posaconazole by Schering-Plough (New York, USA) and itraconazole and hydroxyitraconazole were received from Janssen-Cilag. The internal standard, cyanoimipramine, was supplied by Roche (Woerden, the Netherlands). Acetonitrile for LC/MS and water for LC/MS were provided by BioSolve BV (Valkenswaard, the Netherlands). Methanol Lichrosolv and the other used chemicals were of suitable analytical grade and purchased from VWR (Amsterdam, the Netherlands). The precipitation reagent consisted of 0.04 mg/L cyanoimipramine, internal standard, dissolved in a mixture of methanol and acetonitrile (4:21 v/v respectively). Pooled human serum samples were made available according to the guidelines of the University Medical Center Groningen.

LC/MS/MS instrumentation and conditions

All experiments were performed on a Thermo Fisher (San Jose, USA) triple quadrupole LC-MS/MS with a Finnigan™ Surveyor® LC pump and a Finnigan™ Surveyor® autosampler which was set at a temperature of 20 °C. After sample preparation, 5 µL of the clear upper layer were injected on a 50 x 2.1 mm C_{18}, 5-µm analytic column (HyPURITY AQUASTAR, Interscience Breda, the Netherlands) for chromatographic separation. The column temperature was set at 20 °C. The mobile phase had a flow of 0.3 mL/min and consisted of an aqueous buffer (containing ammonium acetate 10 g/L, acetic acid 35 mg/L and trifluoroacetic anhydride 2 mL/L water), water and acetonitrile. The following gradient was

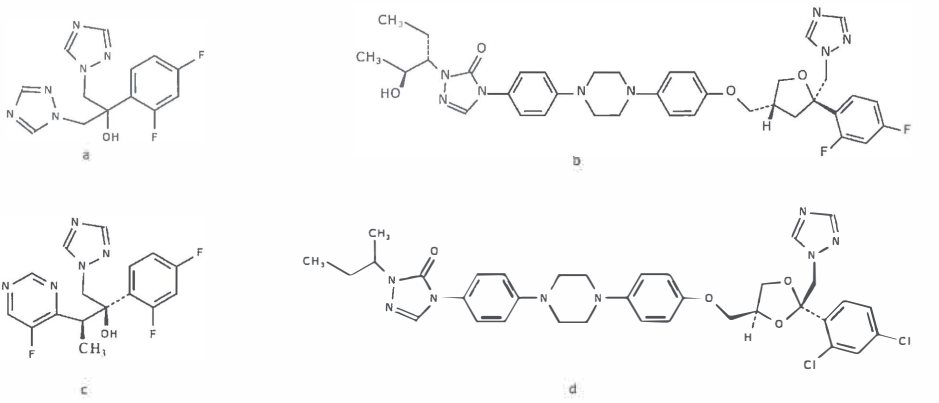

Figure 1: Chemical structures of azoles

Structure a = fluconazole; b = posaconazole; c = voriconazole; d = itraconazole.

run, time 0-2 minute: 5% buffer, 95%-0% water and 0%-95% acetonitrile, time 2-3 minutes: 5% buffer and 95% acetonitrile, time 3.0-3.1 minutes: 5% buffer, 0%-95% water and 95%-0% acetonitrile, time 3.1-3.6 minutes: 5% buffer and 95% water. The Finnigan™ TSQ® Quantum Discovery mass selective detector was operating in electrospray positive ionization mode and performed selected reaction monitoring (SRM).

The following mass parameters were used at a scan width of 0.5 *m/z*.: fluconazole *m/z* 307.2 > 219.9 (collision energy 20 eV), voriconazole *m/z* 350.0 > 281.1 (collision energy 17 eV), posaconazole *m/z* 701.3 > 683.2 (collision energy 32 eV), itraconazole *m/z* 705.2 > 392.0 (collision energy 36 eV), hydroxyitraconazole *m/z* 721.2 > 408.0 (collision energy 42 eV), and cyanoimipramine *m/z* 306.2 to *m/z* 218.0 (collision energy 39 eV). The ion source spray voltage was set at 3500 V, the sheath and auxiliary gas pressure at 35 Arbitrary units (Arb.) and 5 Arb., respectively and the capillary temperature at 350 °C. Xcalibur® software version 1.4 SR1 (Thermo Fisher, San Jose, USA) was used for peak height integration for all components.

Preparation of standard stocks and serum samples

For the preparation of the calibration standards and the quality control (QC) samples of each compound two stock solutions (A1 and B1 respectively) were prepared. Secondary working stock solutions (stock A2 and B2) were prepared by diluting stock A1 and B1 (see table 1). Stock A1 and A2 were used for preparing the calibration samples by diluting it with pooled human serum (see table 1). QC samples were prepared by spiking stock B1 and B2 to pooled human serum (see table 1).

The calibration standards and QC samples were prepared on day zero and stored at – 20 °C. The QC samples for determining the freeze/thaw stability were freshly prepared from stocks B1 and B2 on the first day of analysis.

Table 1: concentrations of stock solutions, calibration standards and QC samples

Compound	Stock solution (A1 and B1)	Working stock solutions (A2 and B2)	Calibration standards (mg/L serum)	QC samples (mg/L serum)			
				LLOQ	LOW	MED	HIGH
Fluconazole	4000 mg/L methanol	80 mg/L methanol	0.5; 1.5; 2.5; 5.0; 25; 50; 75; 100 and 200	0.5	5.0	40	80
Voriconazole	250 mg/L methanol	25 mg/L methanol	0.1; 0.25; 0.5; 1.0; 2.5; 5 and 10	0.1	0.25	2.5	5.0
Posaconazole	400 mg/L methanol:water (50:50 v/v)	40 mg/L methanol:water (50:50 v/v)	0.1; 0.2; 0.5; 1.0; 2.0; 4.0; 8.0 and 10.0	0.1	0.5	2.5	5.0
Itraconazole	200 mg/L methanol	80 mg/L methanol	0.1; 0.25; 0.5; 1.0; 2.0; 3.0; 4.0 and 5.0	0.1	0.5	1.0	2.0
Hydroxyitra-conazole	200 mg/L methanol	80 mg/L methanol	0.1; 0.25; 0.5; 1.0; 2.0; 3.0; 4.0 and 5.0	0.1	0.5	1.0	2.0

LLOQ, lower limit of quantification; MED, medium;

Procedure of sample preparation

In a 2.0 mL autosampler vial 100 µL serum sample ((blank serum, calibration standard, Quality Control sample or patient sample), 10 µL in case of fluconazole and voriconazole)) and 750 µL precipitation reagent containing the internal standard were vortexed for 1 minute, stored at -20 °C for 30 minutes to promote protein precipitation and subsequently centrifuged at 11,000 *g* for 5 minutes. From the clear upper layer 5 µL was injected onto the LC-MS/MS system.

Methodology for validation

In accordance with the Guidance for Industry Bioanalytical Method Validation of the Food and Drug Adminstration, method validation included selectivity, linearity, accuracy, precision, recovery and stability[24]. Therefore, on each analytical day, a single calibration curve was obtained and the QC samples were analyzed in fivefold. Furthermore six pools of blank human serum were analyzed in triple. The accuracy and precision were determined by analyzing QC samples on four levels in fivefold on three different days and analysed using one-way ANOVA.

Clinical practice

At the University Medical Center voriconazole serum levels are monitored three times a week. Special attention is paid to paediatric patients with invasive fungal infections as voriconazole concentrations display a large inter-individual variability in a greater extend than in adults [25, 26]. Itraconazole is applied as prophylaxis for *Aspergillus spp* in adults and children with haematological malignancies in our Hospital [5]. Routine monitoring of itraconazole concentrations is performed to evaluate absorption in those patients with mucositis [27]. In case of serum trough levels below 1 mg/L the dosage is increased and serum concentrations are evaluated. Absorption of posaconazole, which is used for (secondary) prophylaxis in specific high risk patients or as salvage therapy for invasive fungal infection in our Hospital is monitored as it needs to be administered in combination with a high fatty meal which is generally not well tolerated by critically ill patients.

Quality control program

Our laboratory participates in the international quality control program of antifungal drugs of the Association for Quality Assessment in Therapeutic Drug Monitoring and Clinical Toxicology (KKGT) [28]. The participation in the program contributes to optimization of the performance of our clinical service of therapeutic drug monitoring of azoles. Sources of error in the method of analysis, and dosage recommendations are externally evaluated[28].

RESULTS

Method development

Fluconazole and voriconazole cause of very high detector response and to avoid detector overload it was decided to take 10 µL serum sample, (calibration standard, Quality Control sample or patient sample) for the analysis of these two compounds.

Selectivity and interference

The selectivity of this method was evaluated by analyzing six lots of pooled human serum in comparison with LLOQ samples. There were no peaks observed in any of the pooled human serum samples at the retention time of the azoles respectively the internal standard. No ion suppression was observed by analyzing six lots of pooled human serum and simultaneously direct infusion of a stock solution containing fluconazole or voriconazole or posaconazole or itraconazole-hydroxyitraconazole and cyanoimipramine by a syringe pump.

Linearity, accuracy and precision

All calibration curves were linear by using a weight factor of 1/x over a range of 0.5 to 200 mg/L for fluconazole, 0.05 to 10 mg/L for voriconazole, 0.1 to 5 mg/L for itraconazole and hydroxyitraconazole and 0.1 to 10 mg/L for posaconazole. The results of accuracy and precision are listed in table 2. The interassay variability of the calibration curves is shown in table 3.

Recovery

The recovery was calculated by comparison the peak height of LOW, MED and HIGH control samples in serum (n=5) with spiked control samples prepared in a mixture of methanol and acetonitrile (4:21 v/v) corresponding to the same concentrations (n=5). The recovery for the LOW, MED and HIGH control samples and internal standard are shown in table 4.

Stability and dilution integrity

After 2-3 cycles of freeze-thaw the concentration of the azoles was measured as percentage of the mean concentration of the fresh prepared samples for QC-level LOW, MED and HIGH. In addition the stability of the azoles in human serum was measured after 36 h at + 4 °C, at room temperature and, after sample preparation, in the autosampler. The results are displayed in table 5.

Clinical practice

This method of analysis for therapeutic drug monitoring of the azole antifungal agents has been used for two years now in our hospital. We observed that patients with haematological malignancies presenting multiple increased liver enzyme values of three to five times the upper level of normal are 'at risk' of developing high voriconazole trough levels. It appeared that the voriconazole elimination half-

Table 2: Results of accuracy and precision

	QC levels of FLU				QC levels of VRZ				QC levels of PSZ				QC levels of ITZ (+ HITZ)			
	LLOQ	LOW	MED	HIGH	LLOQ	LOW	MED	HIGH	LLOQ	LOW	MED	HIGH	LLOQ	LOW	MED	HIGH
Mean concentration (mg/L)	0.53	5.05	42.2	83.3	0.10	0.26	2.52	5.05	0.11	0.53	2.58	4.98	0.11 (0.10)	0.53 (0.53)	1.07 (1.06)	2.06 (2.05)
Bias (%)	7.1	1.3	5.8	4.4	2.1	2.3	0.1	0.2	7.9	9.9	6.3	2.4	4.7 (2.8)	6.0 (5.1)	6.7 (5.8)	3.0 (2.2)
Within-run CV (%)	3.5	4.3	4.7	9.2	7.8	4.2	1.9	1.9	5.2	3.2	1.3	1.8	5.2 (6.1)	1.8 (2.0)	2.2 (2.2)	4.6 (3.7)
Between-run CV (%)	4.3	0.8	0	7.8	0.0	2.1	3.1	1.0	0.0	1.5	0.1	0.9	0.0 (0.0)	0.0 (0.4)	0.4 (0.0)	0.0 (0.0)

QC, quality control; FLU, fluconazole; VRZ, voriconazole; PSZ, posaconazole; ITZ, itraconazole; HITZ, hydroxy-itraconazole; LLOQ, lower limit of quantification; MED, medium

Table 3. Interassay variability of the calibration curves

Component	Slope±SD	Intercept±SD	Correlation coefficient
Fluconazole	0.0267±0.001	0.0031±0.006	0.9955
Voriconazole	0.000026±0.000000351	0.000216±0.000408	0.9980
Itraconazole	0.764±0.00792	0.0192±0.00742	0.9988
Hydroxyitraconazole	0.214±0.00150	0.00358±0.00142	0.9995
Posaconazole	0.422±0.00341	0.0110±0.00419	0.9992

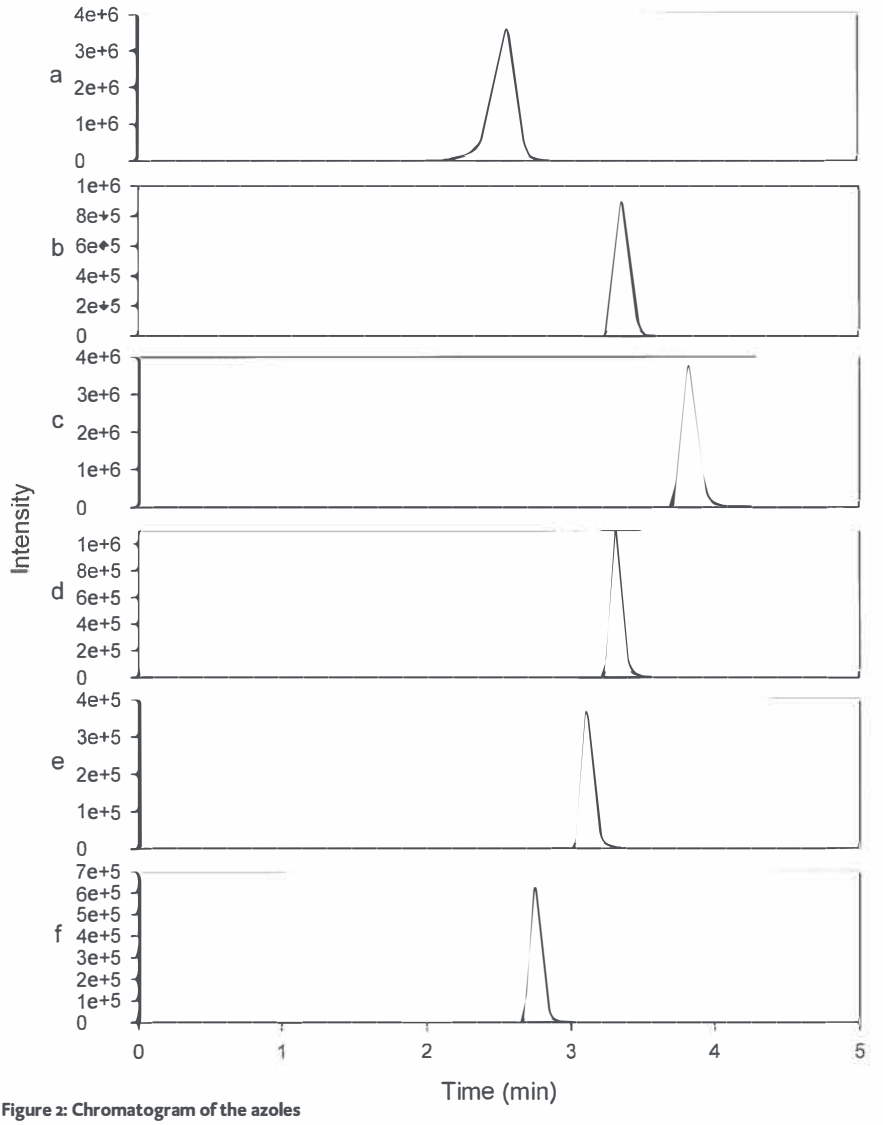

Figure 2: Chromatogram of the azoles

'Chromatogram' a = fluconazole; b = voriconazole; c = posaconazole; d = itraconazole; e = hydroxyitraconazole; f = cyanoimipramine

extreme prolonged, which suggest that routine monitoring of voriconazole levels after 3 days of therapy is necessary to prevent voriconazole adverse effects caused by toxic levels [29].

Drug-drug interactions with azole agents are frequently observed. Guidelines on the clinical management of these drug-drug interactions are helpful to guide the therapeutic drug monitoring of azole antifungal agents [30]. However, the extent of these interactions can be more profound in critically ill patients than in healthy volunteers. We observed that a dosage increase of voriconazole was not sufficient to compensate for phenytoin induced metabolism compared to healthy volunteers [31]. This resulted in the change of clinical management of patients with haematological maligancies needing prophylaxis for busulphan-induced seizures.

Table 4: Recovery (%)

	QC levels			Cyanoimipramine
	LOW	MED	HIGH	
Fluconazole	76,1	83,2	93,4	104,4
Voriconazole	81,5	82,5	82,9	104,4
Posaconazole	94,8	95,6	94,0	104,9
Itraconazole	88,1	109,4	91,1	105,8
Hydroxyitraconazole	90,9	99,1	82,7	105,8

QC, quality control; MED, medium

Serum level guided dosing of voriconazole in children was very useful as doses had to be increased to great magnitude in order to result in measurable serum levels [32].
Routine monitoring of posaconazole let to the observation that co-administration of omeprazole resulted in a significant decrease of posaconazole serum trough concentrations based on a reduced bioavailability due to increased pH of gastrointestinal fluids [33].

Quality control program

The participation in the quality control program in 2008 for all four azole compounds passed the requirements, as defined by the program regulations, for all analysed samples using the described methods of analysis. Samples with a low concentration had a median value of 101 % (interquartile range 94.8 - 101 %) of the theoretical value and samples with a high concentration had a median value of 98% (interquartile range 92.3 - 101.3%) of the theoretical value.

DISCUSSION

We developed a universal method of analysis for routine monitoring of azole antifungal drugs without time consuming sample preparation and purification or changes to the configuration of LC/MS/MS system. Our method was based on the precipitation of proteins in human serum with LC/MS/MS detection and validation was performed according to the guidelines for bioanalytical method validation of the food and drug administration agency. All compounds were detected in human serum with good accuracy and reproducibility at the limit of quantification. Intra- and inter-day validation demonstrated good accuracy and reproducibility. The method is suitable for clinical pharmacokinetic studies and routine monitoring in daily practice. To our knowledge this is the first described validation using one method of analysis for all azole compounds.

In the literature several methods of analysis of azoles in human serum have been described with conventional methods like HPLC-UV or HPLC-fluorescence using liquid-liquid extraction (LLE), solid phase extraction (SPE) but these often lack sensitivity and selectivity and sample preparation is time consuming. For high trough put analysis of large sample numbers a rapid and universal method of analysis for azole compounds is mandatory. Liquid chromatography combined with mass spectrometry has

become the most suitable apparatus to comply to these requirements. Several methods have been reported based on liquid chromatography combined with mass spectrometry but these have some limitations. Single mass spectrometry lacks sensitivity and specificity compared to a tandem mass spectrometry [34]. Some of the described methods still use SPE [35-37] or LLE [38-43], which is expensive and time consuming. On-line solid phase extraction has the advantage of an increased sensitivity [44] but lacks universal application for the different antifungal agents and is therefore less suitable for routine monitoring. Also optimization of the method of analysis by different columns or great differences in mobile phase can cause a significant delay in measuring the clinical samples as stabilization of the system takes time. Only a few methods were reported based on a protein precipitation without additional purification procedures. However, these reported methods did not cover the entire range of azoles and were limited by being only validated for measuring voriconazole in plasma of rodents [34, 45] or lacked a description of the sample preparation[46]. Our method, based on precipitation of proteins in human serum, has the advantage of a simple and rapid sample preparation without further workup with LLE and SPE and is therefore easily to implement. Having the same method of analysis for the four most used azole antifungal drugs enables routine monitoring of these compounds in daily practice without additional changes in configuration of columns or fluids. The impact of therapeutic drug monitoring can increase as samples can be easily processed and results be made available at the same day. This enables hospital pharmacists and physicians on ICU's to respond rapidly to subtherapeutic or toxic concentrations of azole agents to optimize treatment of critically ill patients.

CONCLUSION

In summary, a selective, linear, accurate and precise method, which can be used routinely for the determination of the four azoles in human serum, has been developed. This method is applied successfully in clinical practice. Due to the high throughput sample analysis a clinical relevant service can be offered to the physician.

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Chapter

2.2

Disease severity influences pharmacokinetics of voriconazole in critically ill patients

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ABSTRACT

Background

The large variability in pharmacokinetics of voriconazole has been not been fully elucidated. We studied the effect of disease severity on of pharmacokinetics of voriconazole.

Patients and Methods

In a prospective observational pharmacokinetic study patients suspected to have a fungal infection received voriconazole in a dose of 6mg/kg followed by 4mg/kg IV or 400 mg followed by 200 mg orally. Serum samples were drawn and analyzed by LC/MS/MS. Pharmacokinetic parameters were calculated with MW-Pharm 3.60. Disease severity was assessed by Simplified Acute Physiology Score (SAPSII) and Sequential Organ Failure Assessment (SOFA) scoring systems.

Results

Eighteen patients were included in this study, (ICU n= 8; ward n=10). On day two ICU patients had significantly lower drug exposure - median $AUC_{day\ 2}$ 30.1 (IQR 16.3 – 38.0) compared to the AUC of patients on the ward (median 51.5; IQR 42.3 – 63.9; P = 0.01). The correlation (R) between the two disease severity scores and the $AUC_{day\ 1}$ was non-significant for the SAPSII score and SOFA score. However, if voriconazole was administered intravenously (R = 0.6; P =0.077) or when the SAPSII score was ignored for the parameters *chronic diseases* and *type of admittance* (R = 0.4; p=0.1) there was a trend for correlation between $AUC_{day\ 1}$ and SAPSII. A significant correlation was observed between $C_{max\ 1}$ and SOFA score (R = 0.48; P = 0.044).

Conclusion

A correlation between disease severity and pharmacokinetics of voriconazole is suggested and warrants exploration of new dosing strategies of voriconazole in critically ill patients.

INTRODUCTION

Invasive fungal infections (IFI) in immunocompromised patients are predominantly caused by *Candida* and *Aspergillus species*. Voriconazole, one of the newer azole compounds, proved to be more efficacious and less toxic than amphotericin B [1]. Despite effective antifungal treatment with voriconazole, mortality is still high [1-6]. Clearly there is a need for optimizing the antifungal treatment. It has been suggested that therapeutic drug monitoring (TDM) could have an added value to the treatment with voriconazole [5, 7, 8]. Inter and intra patient pharmacokinetic variability [9, 10], a potential correlation between efficacy and plasma concentrations [5, 7, 8] and adverse effects [7, 11, 12] support the idea for TDM. Based on retrospective observational studies, a therapeutic window has been suggested with a lower level of 1 – 2 mg/L and a upper level of about 5 - 6 mg/l [13].

Treatment with voriconazole is started in a loading dose on day one to reach steady state concentrations [14], and continued in a maintenance dose. During sepsis, drug distribution may change due to an inflammatory response characterised by capillary leakage reduced, plasma albumin and altered protein binding. An increased volume of distribution has been observed in critically ill patients for a number of antimicrobial compounds [15, 16]. Therefore, based on pharmacokinetic variability, a standard loading dose may not be sufficient to reach steady state concentrations for all patients across a range of disease severities. As plasma concentrations are related to efficacy this variability may result in reduced efficacy when steady state concentrations are reached later in treatment [7, 8] or not at all. To study the influence of severity of illness on pharmacokinetics of voriconazole we hypothesized an inverse correlation between disease severity score and the area under the concentration time curve (AUC) of voriconazole.

METHODS

Study population

Patients aged 18 years and older, admitted at the University Medical Center Groningen suspected to have an IFI were eligible for inclusion. Diagnosis was based on clinical, radiological and microbiological findings.

Study design and procedures

Voriconazole was given in a loading dose of 6 mg/kg twice daily followed by a maintenance dose of 4 mg/kg twice daily intravenously or 400 mg twice daily followed by a maintenance dose of 200 mg orally. Voriconazole oral suspension was administered by enteral feeding tube in patients on the ICU. As these patients received predominantly enteral nutrition bioavailability would not have been compromised by little amounts of enteral nutrition, given to prevent gastroparesis [17]. Blood samples were collected on day one and two immediately before and at 1, 2, 4, 8 and 12 hours after drug administration. Serum was separated and analysed the same day, or frozen at -20°C, until processed. Disease severity was computed on day one by Simplified Acute Physiology Score (SAPSII) [18] and Sequential Organ Failure Assessment (SOFA) [19]. If after pharmacokinetic analysis the AUC on day one and two appeared significantly higher or lower than 30-45 mg*h/L [20], the attending physician could choose to adopt the dose after advice of the clinical pharmacist. On day 3, 5 and 8 the serum samples were collected at t = 0, 1 and 3 hours post dosage. Based on these serum concentrations therapeutic drug monitoring could be continued at the discretion of the attending physician.

Determination of voriconazole concentrations in serum

Serum concentrations (total and unbound) of voriconazole were determined at the Laboratory for Clinical Toxicology and Drugs Analysis of the Department of Hospital and Clinical Pharmacy of the University Medical Center Groningen using a validated liquid chromatography tandem mass spectrometry (LC/MS/MS) assay [21]. To obtain an ultrafiltrate volume of 500 µL human serum was transported into a Centrifree® Ultrafiltration device with an Ultracel® YM-T membrane (Millipore, Germany). The devices were capped afterwards and placed in a 35 degree fixed angel rotor (Hettich EBA 21) and spun at 1,640 *g* for 20 minutes at room temperature.

Pharmacokinetic analysis

Pharmacokinetic parameters were calculated using KINFIT (MWPharm 3.60; Mediware, The Netherlands) [22]. C_{max} was defined as the highest observed serum concentration with T_{max} as corresponding time. C_{min} was the serum concentration at 12 h after intake of the dose. The $AUC_{0-12\ h}$ was calculated using the log-linear trapezoidal rule from 0 up to 12 hours. The elimination half-life ($t_{1/2}$) was calculated by $0.693/k_e$. The apparent clearance of the drug (Cl) was calculated by *dose*/AUC_{0-12h} and the volume of distribution (V_d) was calculated by *dose*/concentration at steady state (C_{ss}).

Co-medication

As voriconazole is a substrate of CYP2C9, CYP2C19 and CYP3A4, drugs co-administered with voriconazole that could influence the pharmacokinetics of voriconazole were retrieved from the patients medical charts [23].

Determination of CYP2C19 and CYP2C9 genotypes

The CYP2C19 and 2C9 genotype were determined by real time polymerase chain reaction (PCR)-restriction fragment length polymorphism CYP2C9*2 and *3, and CYP2C19*2 and *3 were investigated. Patients were defined as poor metabolizer (PM) if they were homozygous and as intermediate metabolizer (IM) if they were heterozygous for noncoding alleles. All other patients were classified as extended metabolizers (EM)[24-26].

Statistical analysis

Data are presented as median values with interquartile range (IQR). Differences in age, body weight, disease severity scores, and pharmacokinetic parameters of voriconazole between the patients admitted on the ICU and the patients admitted on the ward were assessed with the Wilcoxon rank sum (Mann-Whitney *U*) test for unpaired data. The primary outcome measure, i.e. the correlation between AUC and disease severity was assessed by the Spearman correlation coefficient. To diminish the chance of a type two error because of small sample size two independent scoring systems were chosen to evaluate the correlation. A sample size of 18 was estimated to detect a correlation of 60% between AUC and disease severity with a statistical power of 80% and a significance level of 5%. Secondary outcome measures were correlation between C_{max} and C_{min} and disease severity.

Ethics

If the attending physician was planning to start antifungal treatment with voriconazole, the patients or their first-degree relatives were informed about the study to allow appropriate time for consideration. Prior to blood draws for the study written informed consent was obtained from all patients or their next of kin if patients were unable to give consent because of impaired consciousness, or sedation in the ICU. The study was approved by the Ethics Review Committee of the University Medical Center

Groningen, Groningen, The Netherlands, and was conducted at the departments of Internal Medicine, Surgery and Hospital and Clinical Pharmacy in accordance with the Declaration of Helsinki (1996).

RESULTS

Eighteen patients were included in this study. Patients' characteristics at baseline are presented in Table 1. All patients were treated for a suspected IFI. The median loading dose of voriconazole was 5.7 (5.3 – 6.0) mg/kg twice daily and continued in a median dose of 3.5 (2.8 – 4.0) mg/kg twice daily for a median treatment duration of 15 days (IQR 9 - 29). These doses were somewhat lower than the intended loading and maintenance doses of 6 and 4 mg/kg, respectively, due to the fixed, not weight adapted oral doses.

Table 1: Patient characteristics (n=18)

Parameter	
Gender (M/F)	13/5
Age (years)	55 (38 - 60)
Weight (kg)	70 (62 - 79)
Diagnostic group	
Hematological malignancies	8 (AML 4; Lymphoma 2; ALL 1; Multiple myeloma 1)
Organ transplant recipient	4 (LTx 2; HTx 2)
Miscellaneous	6 (Trauma 2; HIV 1; Endothelioma 1; mediastinitis 1; pneumothorax following aspergilloma1)
Co-morbidities	
Cardiac	3 (HT 1; PHT 1; PTCA 1)
DMII	1
COPD	2
Miscellaneous	5 (gout 1; osteoporosis 1; arthritis 1; M Wegener 1; hyperthyroid 1)
Isolated pathogen	
Aspergillus fumigatus	10
Candida albicans/other spp	3
Empirical treatment	5
Type of admission (ICU/ward)	8/10

M, Male; F, female; ICU, intensive care unit; AML acute myeloid leukemia; ALL acute lymphocytic leukemia; LTx lung transplantation; HTx liver transplantation; HIV human immunodeficiency virus; DMII, diabetes type II; COPD, chronic pulmonary obstructive disease; HT hypertension; PHT pulmonary hypertension; PTCA percutaneous transluminal coronary angioplasty. Data are presented as median with IQR.

The route of administration was intravenously in ten patients and orally in eight patients, and two patients switched from intravenous to oral therapy. After one month, 13 patients were alive and five were dead; two of these deaths were attributable to the IFI and three to the underlying malignancy and organ failure. Voriconazole was well tolerated and adverse effects were mild. In two out of three patients, presenting with a trough level above 5 mg/L (10.4 mg/L and 11.3 mg/L) visual disturbances and hallucinations were reported. In none of the patients a significant increase of liver enzymes leading to discontinuation of voriconazole treatment was observed.

The median pharmacokinetic parameter values along with their interquartile ranges (IQR) are shown in table 2. The concentration time curve is shown in Figure 1. As expected the C_{max} and C_{min} showed a significant correlation with the corresponding AUC on day one (R = 0.8; P <0.005 and R = 0.9; P <0.001 respectively) and day two (R = 0.97; P < 0.001 and R = 0.96; P < 0.001 respectively). The linear correlation between C_{min} and AUC (R^2 = 0.94) is shown in Figure 2.

Table 2: Pharmacokinetic parameters of voriconazole on day 1, 2, 3, 5 and 8

Parameter	Treatment				
	Day 1	Day 2	Day 3	Day 5	Day 8
AUC_{0-12h} (mg*h/liter)	28.9 (15.2 – 33.9)	42.5 (30.8 – 52.0)			
C_{max} (mg/liter)	4.1 (2.4 – 4.5)	4.7 (3.9 – 5.6)	3.9 (2.9 - 6.7)	4.1 (2.7 – 5.9)	4.3 (2.5 – 4.9)
C_{min} (mg/liter)	1.3 (0.6 – 2.1)	3.0 (2.3 – 4.1)	3.4 (1.8 - 3.8)	2.2 (0.9 – 4.2)	2.1 (1.1 – 5.0)
T_{max} (h)	1.2 (1.0 – 1.5)	1.1 (1.0 – 1.4)			
$t_{1/2}$ (h)	9.7 (5.9 – 28.0)	20.7 (15.5 – 45.5)			
CL (liter/h)	8.0 (5.3 – 15.4)	1.8 (1.0 – 2.2)			
V_d (liters)	157 (93.0 – 248)	60.7 (42.0 – 94.8)			

Median values with IQR of the following parameters are shown in the table: AUC0–12h, area under the concentration–time curve from 0 to 12 h; Cmax, maximum observed concentration of drug in serum; Cmin, minimum observed concentration of drug in serum; Tmax, time to maximum concentration of drug in serum; t1/2, half-life; CL, total clearance; Vd, volume of distribution.

The median albumin level was 24 (IQR 20 – 27) g/L, which is low compared to the reference value of 35 – 50 g/L. Patients on the ICU had a significant lower albumin level compared to patients on the ward (P = 0.02). Median protein binding of voriconazole was 56 (IQR 48 – 58) %. Voriconazole protein binding was significant lower compared to patients on the ward (P = 0.04). The linear correlation (R^2 = 0.97) between total voriconazole concentration and unbound voriconazole concentration is shown in figure 3.

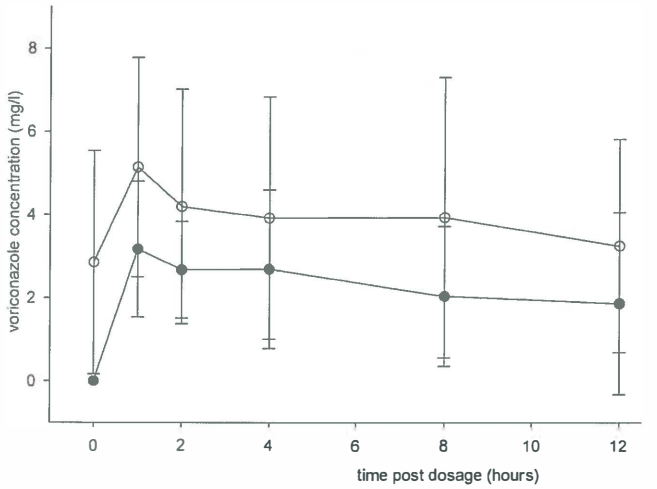

Figure 1: Concentration time curve of voriconazole on day one and two of treatment

Mean (± SD) serum concentration-time curves of voriconazole in a loading dose (solid circles) and maintenance dose (open circles) for all patients (n = 18).

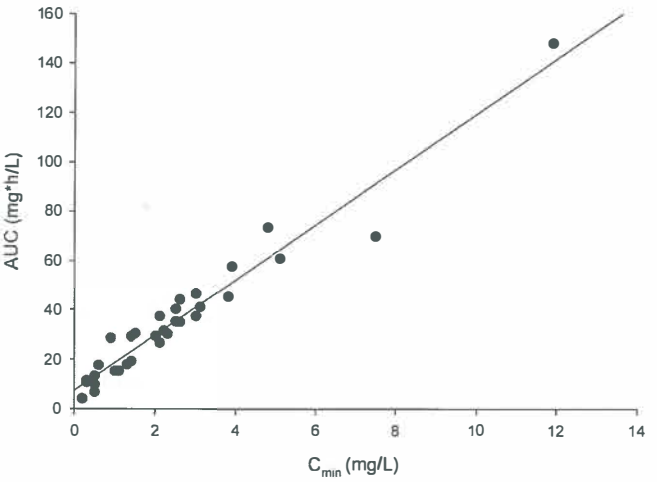

Figure 2: C_{min} vs AUC

Correlation (R^2 = 0.94) between C_{min} and AUC for all patients (n = 18).

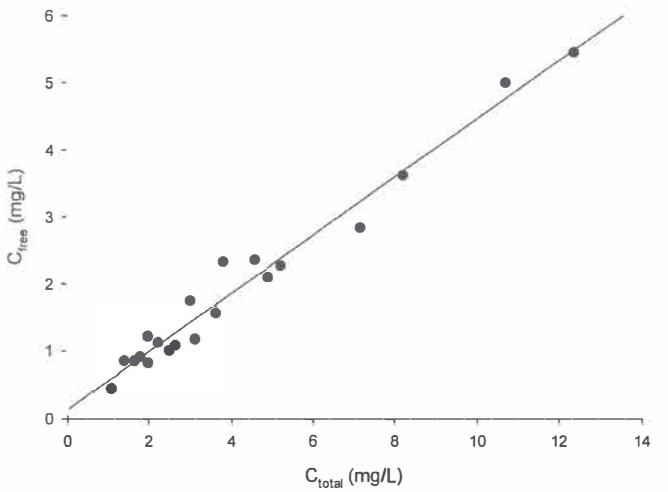

Figure 3: C_{free} vs C_{total}
Correlation (R^2 = 0.97) between C_{free} and C_{total}.

Evaluation of the co-administered drugs showed that seven patients received omeprazole or esomeprazole 40mg once daily, and one patient received pantoprazole 40 mg once daily. No other drugs that could have influenced the pharmacokinetics of voriconazole were co-administered. No difference was observed in AUC of voriconazole compared to patients that did not receive proton pump inhibitors (P = 0.6).

The determination of CYP genotypes revealed three patients with a polymorphism for CYP2C9 in one and CYP2C19 in two of them leading to IM phenotype. The observed median AUC of 10.7 (IQR; 8.8 – 13.0) mg*h/L in these two patients was lower compared to the median value of 29.2 (IQR; 17.8 – 36.2) mg*h/L of the other patients (P = 0.03).

The weight of the patients showed significant correlations with the C_{max1} and AUC_{day1} (R = 0.6; P = 0.01) and a near significant correlation with C_{max2} and AUC_{day2} (R = 0.38, P = 0.14; R = 0.46, P = 0.078).

On day one, voriconazole drug exposure tended to be less for patients in the ICU while on day two, this difference was highly significant: the median AUC_{day1} was 15.3 (10.3 – 22.2) mg*h/l of patients in the ICU, compared to 29.3 (27.1 – 37.4) mg*h/l of patients in the ward (P = 0.07); the median AUC_{day2} was 30.1 (16.3 – 38.0) of ICU patients compared to 51.5 (42.3 – 63.9) of patients admitted on the ward (P = 0.01). The median C_{min2} was significantly lower (P = 0.01) for patients on the ICU [2.3 (IQR 0.95 – 2.8) mg/L vs 3.9 (3.0 – 4.8) mg/L) on the ward] but C_{min1}, C_{max1} and C_{max2} did not differ significantly.

The median SAPSII and SOFA scores were 44 (IQR 20 – 52) and 5 (IQR 2 - 7). The median SAPSII score of 42 (35 – 50) of ICU patients was not significantly different from the median SAPSII score of 45 (16 – 52) of patients admitted at the ward (P = 0.83). However, the median SOFA score of 7 (7 – 11) was significantly higher in ICU patients compared with patients admitted at the ward [2.5 (0.25 – 4); P = 0.001].

The correlations between the AUC_{day1} of voriconazole and the SAPSII and SOFA score are presented in Figure 4a and Figure 4b. No correlation with the AUC_{day1} was observed (R = 0.1; P 0.7) for the SAPSII score, and only a weak correlation (R = 0.36; P = 0.14) was found for the SOFA value. For AUC_{day2} no

correlation was found with SAPSII (R = 0.0; P 1.0) and only a weak correlation (R = 0.33; P = 0.22) was found for the SOFA score.
A significant correlation was observed between $C_{max\ 1}$ and SOFA score (R = 0.48; P = 0.044) but not with SAPSII. No correlation was observed between $C_{max\ 2}$, $C_{min\ 1}$, $C_{min\ 2}$ and either of the two severity scores.

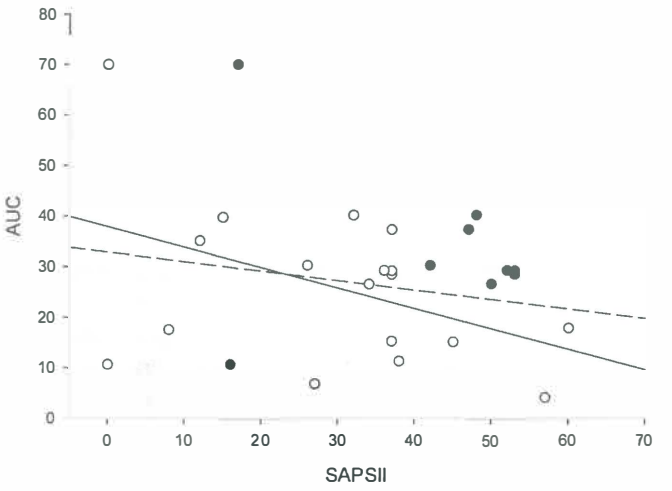

Figure 4a: Correlation between AUC and SAPSII score

Correlation (R = 0.1) between SAPSII score and AUC for each patient (solid circle). Correlation (R = 0.4) between SAPSII (parameters type of admission and chronic diseases are ignored) and AUC is displayed by open circles.

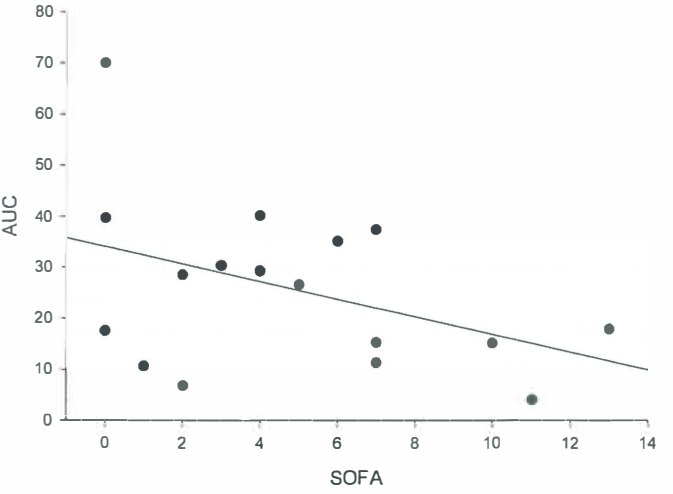

Figure 4b: Correlation between AUC and SOFA score

Correlation (R = 0.36) between SOFA score and AUC for each patient (solid circle).

When the patients were analysed separately by route of administration of voriconazole the correlation between $AUC_{day\,1}$ of intravenously administered voriconazole and SAPSII approached significance (R = 0.6; P = 0.077) but not for SOFA nor for orally administered voriconazole. For patients on the ward the correlation between $AUC_{day\,1}$ and SOFA score (R = 0.5, P = 0.11) was better than with SAPS (R = 0.02; P = 0.97). For patients at the ICU both SAPS and SOFA showed no trend towards significance for the relation with AUC.

In the SAPSII scoring system the parameters *chronic diseases* and *type of admittance* contribute to a significant degree to the total score. However, these factors are not expected to contribute to the change of volume of distribution of voriconazole. When these parameters were ignored the observed correlation showed a trend towards significance between the $AUC_{day\,1}$ and SAPSII score value (R = 0.4; P = 0.1).

DISCUSSION

This is the first study trying to explain the variability in pharmacokinetics of voriconazole by disease severity. For this purpose the SAPSII and SOFA disease severity scores were used. These scoring systems were validated in organ transplant recipients and patients with haematological malignancies. Although these scoring systems were developed to predict mortality and not variability in pharmacokinetics based on disease severity, they were used successfully to explain pharmacokinetic variability [27-30]. In this study with a small sample size, we showed that these disease severity scoring systems partly explain the differences in pharmacokinetics of voriconazole, most evident from the significant correlation between AUC_{day1}, C_{max1}, and C_{min2} and SOFA. When some less relevant parameters such as chronic state of the disease and type of admittance were ignored the correlation with SAPSII scoring system also increased towards significance. The evaluation of these or other scores can be the first step in developing an algorithm of dosing voriconazole in critically ill patients based on their clinical condition. Our data suggest that with appropriate adjustments, e.g., a modified SAPSII scoring system, the variability in pharmacokinetics of voriconazole may be explained, although we realize that more work should be done to capture most of the variance with a clinical scoring system.

We chose to measure the AUC of the loading dose as this reflects the initial volume of distribution best. It was hypothesized that the volume of distribution was increased in critically ill patients by increased vascular permeability caused by inflammation. Also, the voriconazole concentrations would probably lack the influence of its nonlinear accumulation as the concentration curve of the first dose is measured.

A limitation in our study was that we included patients that either initially received voriconazole orally, or intravenously. If voriconazole was administered intravenously, a significant correlation was observed between C_{max} at day one and the SOFA score. However, the AUC at day one showed no significant correlation with SAPS or SOFA score. This may be partly caused by the fact that voriconazole dosing is based on body weight when administered intravenously, but not when administered orally. In addition, intravenous dosing not unexpectedly seems to produce more rapid therapeutic serum levels than oral dosing although voriconazole is almost completely absorbed. Nevertheless, by oral dosing the patients can be underdosed or overdosed compared to intravenously administered voriconazole when the body weight deviates from the targeted body weight of 66.7 kg (400 mg divided by 6 mg/kg). In our study body weight significantly correlated with the $AUC_{day\,1}$. It could therefore be expected that

if all patients had received the same dosage based on mg/kg the variability in AUC could be reduced and therefore the correlation between disease severity and AUC would increase. On the ICU patients are not weighted daily and the actual weight can differ from the earlier recorded weight. Correcting the AUC for dose per kg body weight afterwards and recalculating the correlation between disease severity and AUC is not possible as voriconazole displays non-linear pharmacokinetics.

The median protein binding of 58 % observed in our patients was consistent with earlier published data and irrespective of the voriconazole concentration [31]. However, protein binding was lower (44%) in ICU patients, which can be explained by the critical condition of our patients having lower protein blood levels. The lower protein binding of voriconazole appeared consistent with the lower albumin levels in ICU patients. Despite a lower total exposure to voriconazole, critically ill patients have a relatively high unbound concentration of voriconazole in their blood. As the efficacy of azoles is characterised by free AUC/MIC ratio lower protein binding may compensate partly for lower total concentration levels.

Although in most patients, the loading dose appeared to be sufficient to reach voriconazole levels within the therapeutic window [13], the maintenance dose was too low to maintain these levels in five patients. In three patients, the trough levels increased on the maintenance dose to toxic levels [i.e. > 5 mg/l [13]] during maintenance dosing. Eight patients had received proton pump inhibitors, but in only one of these patients a toxic voriconazole concentration was observed. In healthy volunteers, the AUC of voriconazole increased with 41% (90% CI 29 – 55%) after co-administration with omeprazole 40 mg once daily [32]. In our study no difference was observed between patients receiving a proton pump inhibitor and patients that did not. However, our study was not designed to detect differences of voriconazole AUC caused by co-administered drugs.

We observed three patients that were IM for CYP2C9 or CYP2C19. The effect of CYP2C9 would have been limited as it contributes to a much lesser extent to the metabolism of voriconazole compared to CYP2C19 and CYP3A4[25]. In our study the observed polymorphisms did not result in increased voriconazole concentrations compared to patients without mutations in CYP2C9 or 2C19. Based on our results these polymorphisms contribute to a lesser extent to the variability of voriconazole pharmacokinetics. However, our study was not designed to detect differences in PK based on CYP polymorphisms. Furthermore, we were unable to evaluate the recently recognised CYP2C19*17 polymorphism which resulted in significantly lower exposure compared to wild type CYP2C19 based on single dose of voriconazole [33, 34], but we think it is unlikely this would markedly affect te voriconazole concentrations in our study population as the median dosage 3.5 mg/kg twice daily administered during voriconazole treatment resulted in serum trough concentrations within the therapeutic window of 2-5 mg/L.

In eight of the 18 patients the voriconazole dosage was adapted to reach therapeutic levels. This study underlines that routine monitoring in this specific patient population is required to maintain therapeutic levels and confirms observations of other studies [7, 8, 10, 35]. A voriconazole trough level can be recommended for therapeutic drug monitoring (TDM) as a strong linear correlation between C_{min} and AUC was observed. The authors hypothesized about the clinical benefit of TDM, but the clinical outcome of TDM should be investigated in a prospective randomised clinical trial comparing the standard dosage of voriconazole to serum concentration-guided dosing. The endpoint of this trial should be comprised of both efficacy and toxicity as both are influenced by TDM.

CONCLUSION

In conclusion, a correlation between disease severity and pharmacokinetics of voriconazole seems plausible. Additional studies are needed to further investigate new weight based dosing strategies of voriconazole based on disease severity scoring systems to optimize initial treatment in critically ill patients.

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CONFLICT OF INTEREST: none to declare

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Chapter

2.3

High voriconazole trough levels in relation to hepatic function: how to adjust the dosage?

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Voriconazole has become the drug of first choice to treat invasive fungal infections (IFI) [1]. Standard intravenous dosing of voriconazole consists of an infusion of a dose of 6 mg/kg b.i.d on day one followed by a maintenance dose of 4 mg/kg. Voriconazole is metabolised by the liver, by CYP2C19, CYP2C9 and CYP3A4 enzymes, with renal excretion of the metabolites. The elimination half life of voriconazole is approximately 6 hours. As the capacity of the CYP isoenzymes is limited the metabolism of voriconazole can be saturated [2]. The observed large inter- and intra-patient variation of its pharmacokinetics[3], the possible relationship between therapeutic failure and low serum levels [3-5], and the relation of high serum levels with adverse effects [6, 7] has resulted in the advice to monitor serum levels. How to adjust the dosage to improve therapy is unfortunately not described.

We report our experience with therapeutic monitoring of voriconazole in 20 patients with hematological malignancies receiving voriconazole for the treatment of proven or suspected IFI. In most of these patients multiple samples (trough levels) were drawn for pharmacokinetic evaluation of voriconazole concentrations over time. It has been recommended that trough levels should be above 1-2mg/l and not exceed 5mg/l [8]. In 11 of the 20 patients (55 %), a mean trough concentration of 2.7 mg/l (range 1.6 - 4.1 mg/l) was observed, i.e. within the desired range. In 3 of the 20 patients (15%) a mean trough concentration below the therapeutic range was observed (mean 0.6 mg/l, range 0.2 – 0.9 mg/l). In 6 of the 20 patients (30%), however, standard intravenous dosing led to trough concentrations >5 mg/l (mean 7.2 mg/l; range 5.4-8.8 mg/l), which is the presumed toxic range. High voriconazole concentrations occurred within two to three days after the start of therapy. As voriconazole is mainly metabolized by the liver, we focused our evaluation on possibility of voriconazole metabolism influencing factors like CYP isoenzymes and concomitant medication. The hepatic function in relation to medication, chemotherapy, and parenteral nutrition were evaluated from one week before the start of until two weeks after stopping voriconazole therapy. In 5 of the 6 patients with a trough level >5mg/l, mild preexisting liver dysfunction was present at start of voriconazole. Mild hepatic dysfunction was characterized by a bilirubin level of 5 times > upper level of normal (ULN) and at least 2 of the following alkaline phosphatase (ALP), aspartate aminotransferase (ASAT), Alanine transaminase (ALAT), gamma glutamyl transpeptidase with a level of 3 times > ULN. In these cases, the high voriconazole level seemed to result from, rather than being the cause of, the liver dysfunction. Only in one patient an acute deterioration of hepatic liver tests was observed, probably caused by voriconazole since the Naranjo score was 8 (where 5-8 is probable, and >9 is definite) [9]. The hepatic dysfunction displayed a characteristic pattern of a strong rise in ASAT/ALAT level combined with a smaller and enduring increase in ALP level pointing to acute drug-induced liver toxicity [10].

In 5 of these 6 patients, CYP2C19 and CYP2C9 isoenzymes were determined, but in only one patient an intermediate CYP2C19 isoenzyme for reduced metabolism was detected. In none of the 6 patients no drug-drug interaction could be noted between co-medication and voriconazole. In patients presenting with a trough level of >5mg/l, voriconazole was stopped. Strikingly, an exponentially increased voriconazole elimination half-life of 77.6 hours (range 46.8-99.4 hours) was calculated [11] in patients presenting with a voriconazole trough level of >5 mg/l, compared to the commonly observed half-life of 6-8 hours [12]. Our results show that voriconazole could be stopped for at least two consecutive days. Meanwhile serum monitoring continued and voriconazole levels slowly decreased to levels within the therapeutic window (fig. 1). Voriconazole was than restarted successfully using a lower dosage and monitoring continued in those patients who needed continued therapy. Eventually the dosage had to be increased stepwise to achieve levels within the therapeutic window. The non-linear pharmacokinetics of voriconazole combined with a decreased hepatic function is probably the most logic explanation of these high serum

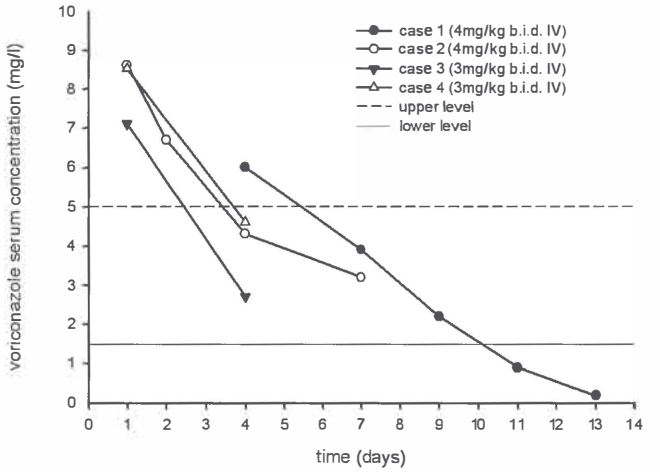

Figure 1: voriconazole concentration over time after stop of voriconazole. Dosage voriconazol at time of stopping is displayed.

Case 1 was a 55-year old man with diffuse large B-cell lymphoma received empiric stepping-up after not responding to antibacterial drugs for febrile neutropenia and recovered uneventful. Case 2 was a 61-year old woman with relapse AML who was suspected of having fungal pneumonia during a consolidation course. Case 3 was a 55-year old woman with AML who developed febrile neutropenia and pulmonary infiltrates on X-rays and a positive galactomannan. Case 4 was a 42-year old man developed fungal pneumonia during aplasia, initially responding to antifungal therapy, but succumbed later on due to pulmonary bleeding.

voriconazole levels and increased elimitation half-lifes. It seems logical that voriconazole metabolism is reduced above a certain serum threshold level (limited capacity of hepatic clearance), compatible with a saturation mechanism for enzymatic degradation[13]. In our experience patients presenting with multiple liver enzyme values of 3-5 times > ULN are "at risk" of developing high voriconazole trough levels. The observations suggest that routine monitoring of voriconazole levels in patients with hematological malignancies after 3 days of therapy is necessary to voriconazole adverse effects caused by toxic levels.

ACKNOWLEDGEMENT: The authors would like to thank Pfizer (USA) for kindly providing the purified voriconazole powder.

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Chapter

2.4

High dose voriconazole in a critically ill paediatric patient with neuroblastoma

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Pediatr Infect Dis J. 2008 Feb;27(2):189-90.

Voriconazole has gained a solid position in the treatment of children with invasive fungal infections [1, 2]. Optimal dosing of voriconazole in children is still subject of debate. The approved dosage is 7mg/kg twice daily [3], but suggestions have been made that a larger dosage may be needed [4] to reach therapeutic serum concentrations (2-5mg/L) [5].

A 3 years old boy, diagnosed 6 months before with Neuroblastoma stage 4, was admitted to the pediatric intensive care unit (PICU), unable to be extubated after a CT-scan of his thorax. As part of his consolidation therapy, he had received melfalan 70 mg/m²/day for 3 days; carboplatin 425 mg/m²/day and etoposide 338 mg/m²/day for 4 days.

Because of persisting fever, while receiving vancomycin 30mg/kg and meropenem 20mg/kg both trice daily, with norepinephrin and fentanyl as co-medication and a persisting granulocytopenia, voriconazole was started empirically. The mortality risk, as predicted by PRISM was moderate (5-15%). Voriconazole serum samples were taken to estimate steady state values (fig. 1). On the second day *Candida parapsilosis* was cultured from his sputum, and from the broncho-alveolar lavage fluid.

Voriconazole (intravenous infusion) was started in a dose of 7mg/kg twice daily and followed by 5mg/kg twice daily. After four days the dosage was increased to 6mg/kg twice daily, which resulted in a serum trough value of 9 mg/L. The dosage was reduced and steady state trough value was measured (fig 1).
After cessation of vancomycine, meropenem and norepinephrin the voriconazole serum trough value had dropped to 0.3mg/L. The voriconazole dosage was increased every two days from 5mg/kg to 13mg/kg twice daily, all resulting in serum trough concentrations below 0.1mg/L. At this time the boy was edematous and his body weight had increased from 14 to 17 kilogram in a few days. His body temperature was 40°C. The dosage was increased to 21mg/kg twice daily. The resulting serum values are displayed in figure 1.

After being admitted for 26 days on the PICU the patient was transferred back to the general oncology ward. He became clinically well and, after recovering from the granulocytopenia and with all cultures remaining sterile, voriconazole was stopped.

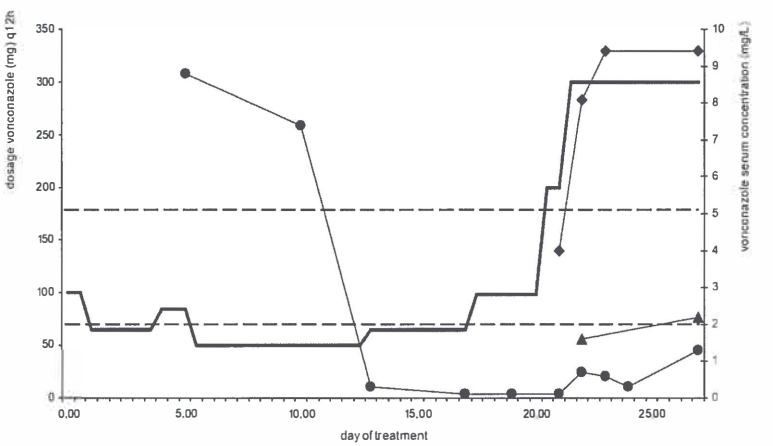

Figure 1: Voriconazole serum concentrations.

(□) = serum trough value; (□) serum peak concentration; (□) serum mid value;

(----) therapeutic window; (□) dosage in mg

This case clearly shows that although standard dosage appeared effective to reach target serum concentrations at the onset of therapy, it failed to maintain these concentrations[2]. Disease stage marked by an increased vascular permeability, possibly aggravated by cessation of norepinephrin, could have lead to an increased volume of distribution, resulting in low serum values. Co-medication would not have decreased voriconazole serum values as no drug-drug interactions were detected [3].

Increasing the dosage resulted in slightly increased trough concentrations but not in expected high peak values. Because AUC/MIC is the pharmacodynamic marker for efficacy of voriconazole, additional concentrations were measured. The AUC was more than adequate (60h*mg/L) in comparison with previous published data (40h*mg/L)[2]. This leads to the important conclusion that measuring only trough levels can lead to the wrong assumption that serum values are inadequate.

Monitoring voriconazole serum values seems necessary in critically ill pediatric patients, who have altered and fluctuating pharmacokinetics in order to maintain therapeutic serum concentrations.

ACKNOWLEDGEMENT: Authors thank Pfizer for supplying the voriconazole reference substance used for the validation of the method of analysis.

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Chapter

3.1

Clinical relevance of pharmacokinetic drug interactions with antifungal azole drugs

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ABSTRACT

There are currently a number of licensed azole antifungal drugs, however only four are used frequently in a clinical setting for prophylaxis or treatment of systemic fungal infections, namely, fluconazole, itraconazole, posaconazole and voriconazole. This article reviews the pharmacokinetic drug-drug interactions of these azole antifungals.

The focus of this article is the definition of the (two-way) interactions with azole antifungal drugs, the metabolic pathway or supposed mechanism involved in these interactions, and their extent. The interaction table provides an overview of all published interactions in humans (either healthy volunteers or patients) and, based on these findings, we have developed recommendations for dealing with the specific interactions.

INTRODUCTION

Azole antifungals exhibit a wide range and variety of drug-drug interactions. Azole drugs are a substrate for and inhibitors of cytochrome P450 (CYP450) enzymes as well as being inhibitors of membrane transporters such as P-glycoprotein (P-gP). Inhibition or induction of CYP450 enzymes may alter the pharmacokinetic profile of the drugs involved and can thus affect both interacting agents. The interaction, should be avoided whenever possible, as this can lead to either over-dosing or under-dosing of both drugs, leading to toxicity or a loss of efficacy, respectively. The risk of interactions between azole antifungal drug and other drug classes can differ substantially between individual drugs, even within the same class of drugs.

METHODS

A Pubmed search of peer-reviewed journals and review articles was performed using the keywords 'antifungal', 'pharmacokinetics', 'metabolism', 'drug interactions' and the names of the individual antifungal drugs. Only drug interactions studied clinically are dealt with in detail in this review since the clinical importance of theoretical interactions cannot be properly assessed. The drug interactions were tabulated according to risk category with the most relevant category mentioned first. Within each category the interacting agent is the primary determinant. Advice on how to cope with specific interactions has been provided as clearly as possible.
The prediction of metabolic drug interactions in humans from *in-vitro* investigations or studies in laboratory animals is not straightforward [1, 2]. Only human studies were considered for review as, with respect to animals, substrate specificities and inhibitor potencies for the enzymes mediating drug biotransformation are not conserved between species.

PHARMACOKINETICS OF AZOLE ANTIFUNGAL DRUGS

Fluconazole

After oral administration, fluconazole is rapidly and fully (>90%) absorbed with a maximum absorption (T_{max}) 0.5-1.5 hours after intake [3]. Tablets are bio-equivalent to oral suspension as well as rectal suppositories [3, 4]. Fluconazole has a protein binding of around 11-12%, but this can increase up to 23% in patients with chronic renal failure [5]. The volume of distribution in adults is 0.56 to 0.82 L/kg [3, 6]. Elimination is primarily by renal excretion, with 80% of the drug being excreted unchanged and approximately 10% as metabolites [7, 8]. The elimination half life for adults is around 30 hours but is markedly reduced in children (15-25 hours) [3]. In patients with renal failure the maintenance dose has to be reduced to 50%[3]. In patients requiring hemodialysis, one full dose (100%) should be administered after every dialysis session [3].

Itraconazole

The oral formulation of itraconazole consists of capsules and an oral solution. Capsules have a less favourable pharmacokinetic profile compared to the oral solution. [9]. Systemic bioavailability of itraconazole oral solution is optimized under fasting conditions [10] and is approximately 55% [11]. Itraconazole is highly protein bound (>99%) [12], penetrates extensively into human tissue [13] but has limited penetration into the cerebrospinal fluid [14]. Itraconazole is extensively metabolised by the

liver, predominantly by the CYP3A4 isoenzyme system, and is known to undergo enterohepatic recirculation [15]. Hydroxy-itraconazole is the major metabolite and shows an equal antifungal activity to the parent compound. Metabolites of itraconazole are excreted into the urine (40%) and bile (55%). Itraconazole is both a substrate to and inhibitor of CYP3A4, and an inhibitor of P-gP [10, 16].

Voriconazole

Voriconazole, given orally, is rapidly and almost fully absorbed (oral bioavailability > 90%) with a maximum plasma concentration (C_{max}) being achieved about 2 hours after administration in a fasting state [17]. The volume of distribution of voriconazole is estimated to be around 4.6 L/kg with a plasma protein binding of approximately 58% [18]. Voriconazole is extensively distributed into tissues and penetrates well into cerebrospinal fluid [19, 20] and into vitreous and aqueous humours [21-23]. The steady state plasma concentrations of voriconazole in healthy volunteers are reached after 5-7 days of treatment but can be advanced to 24 hours by giving a loading dose [24-26]. Voriconazole has non-linear pharmacokinetics with C_{max} and Area Under the concentration vs. time Curve (AUC) values that increase more than proportionally to the increase in dose, possibly due to saturation of the hepatic metabolism of the drug. The major redundant metabolic pathway is through CYP2C19, with CYP2C9 and CYP3A4 being involved to a much lesser extent. The major metabolite of voriconazole possesses no antifungal activity. Voriconazole is an inhibitor of CYP2C9, CYP2C19 and CYP3A4. The drug's metabolites are primarily excreted in the urine [18]. The apparent serum half life of voriconazole is approximately 6 hours and increases with higher dosages [27].

Posaconazole

Posaconazole is only available as an oral formulation. Like itraconazole, posaconazole is slowly absorbed with a median T_{max} of 5 hours and is also strongly bound to plasma proteins (>98%). The volume of distribution varies considerably and steady state is reached after a period of 7-10 days [28-31]. Administration of 50 to 800 mg to healthy volunteers resulted in a linear pharmacokinetic profile [29], and doses over 800 mg showed no marked increase in total exposure. Administration of posaconazole in 2 or 4 divided doses leads to a 2-fold or 3-fold increase in exposure to once daily administration respectively. Posaconazole is metabolised through a phase II reaction (UDP-glucuronosyltransferase 1A4 enzyme system) and converted to a non-active metabolite [32, 33]. About 78% of the drug is recovered in faeces [33] and most of the metabolites are excreted into the urine with an elimination half-life of the parent compound of around 35 hours (range 20-66 hr) [29]. Posaconazole is a substrate for P-gP *in vitro*.

MECHANISM OF DRUG INTERACTIONS

Generally, pharmacokinetic interactions occur at the level of drug absorption, distribution, excretion, and metabolism, with the frequent involvement of the CYP450 metabolising enzyme system and drug transporters such as P-gP (Figure 1). The results of these interactions can be a decrease or an increase in exposure of both interacting drugs, which can in turn lead to reduced efficacy or increased toxicity, respectively. The mechanisms involved in pharmacokinetic drug interactions are outlined in the following sections.

Pharmacokinetic Interactions

Drug Absorption

All four antifungal drugs discussed in this review can be given orally and require absorption through the mucous membranes of the gastrointestinal tract, so a change in plasma concentrations can be the result of incomplete drug absorption. Drug absorption, and thus the pharmacokinetic profile of a drug, can be substantially influenced by gastric pH or by the presence of food.

Effect of food on the absorption of azoles

The pharmacokinetics and bioavailability of fluconazole are not affected by food. The mean bioavailability of itraconazole oral solution under steady state conditions was 43% higher under fasting conditions compared to fed conditions [34]. Single and multiple oral administration of voriconazole with food lowered the bioavailability by approximately 22% and delayed absorption compared to a fasting state [35]. Administration of voriconazole with a high fat meal reduced mean C_{max} and AUC by 34% and 24% respectively [18]. For this reason oral administration is recommended either 1 hour before or 1 hour after meals. Posaconazole absorption is strongly affected by the presence and composition of food. The mean increases in AUC and C_{max} values were about 400% when administered with a high-fat meal compared with the fasting state [36]. Administration of posaconazole with a non-fat meal enhanced exposure, resulting in a 2.6 and 3.0 fold increase in AUC and C_{max} relative to the fasting state [37, 38]. Co-administration of posaconazole with a nutritional supplement increased the C_{max} and AUC of posaconazole 3.4 and 2. 6-fold compared to those for the fasting state [39]. The daily dose of posaconazole should be divided for patients suffering from malnutrition, e.g. 200 mg, four times daily.

Effect of gastric pH and Acid reducing agents (ARA) on the absorption of azoles

The absorption of fluconazole is not affected by drugs that increase gastric pH [40]. The concomitant administration of itraconazole and proton pump-inhibitors (e.g. omeprazole) or H_2-receptor antagonists (e.g. famotidine, ranitidine) leads to impaired absorption of itraconazole, resulting in a decrease in exposure [10]. The absorption of voriconazole is not markedly influenced by antacids or proton-pump-inhibitors (PPI), although the mean C_{max} and AUC of voriconazole are increased by 15% and 41 % by omeprazole due to inhibition of plasma clearance of voriconazole [41]. Posaconazole C_{max} and AUC were reduced by 39 % when co-administered with cimetidine (400 mg twice daily) possibly as a result of decreased gastric acid production. Effects of proton pump inhibitors (e.g. esomeprazole) that may suppress gastric acidity for several hours have only been presented in abstract form [36]. Concomitant administration of posaconazole with esomeprazole resulted in a 33% decrease in posaconazole exposure [28]. Not only the gastric pH but also the integrity of the gastro-intestinal tract may be important for the absorption of antifungal drugs. For instance, posaconazole absorption appeared to be reduced in patients with grade 1 to 2 mucositis compared to patients without mucositis (AUC 4.54 mg*h/L versus 8.85 mg*h/L for twice daily 400 mg posaconazole) [42].

Metabolism

Drug metabolism mainly occurs in the liver, where two types of reactions occur. Metabolism of most drugs occurs via phase I reactions involving oxidation, reduction and hydrolysis principally involving the CYP450 enzymes. In contrast, phase II reactions are not mediated by CYP enzymes but involve conjugation of the drugs. Drugs can be substrates, inducers, or inhibitors. Substrates are moieties that

undergo metabolism by one or more enzyme. Enzyme induction can lead to both increased drug exposure (in case of a prodrug) as well as decreased drug exposure, with subsequent decreased effect. It may take from a few days to up to 2–3 weeks for enzyme induction to reach its full extent. Enzyme inhibition on the other hand is instantaneous and dose dependent.

Effect of azole antifungals on co-medication

All azoles inhibit the CYP3A4 isoenzyme (Figure 1). In humans, CYP3A comprises the largest fraction of the total CYP content and is responsible for the metabolism of a broad range of drugs. Furthermore, CYP3A4 is involved in pre-systemic metabolism in the gastro-intestinal tract, influencing the absorption of CYP3A substrates. The inhibitory potential of the azole antifungals vary greatly, with itraconazole and posaconazole being more potent inhibitors of CYP3A4 than fluconazole or voriconazole. In addition to CYP3A4, fluconazole and voriconazole are also strong non-competitive or mixed-type inhibitors of CYP2C9 and CYP2C19 [27, 43, 44].

The clinical relevance depends on the CYP isoform that metabolises the co-administered drug and the potency of the antifungal agent as an inhibitor of that isoform.

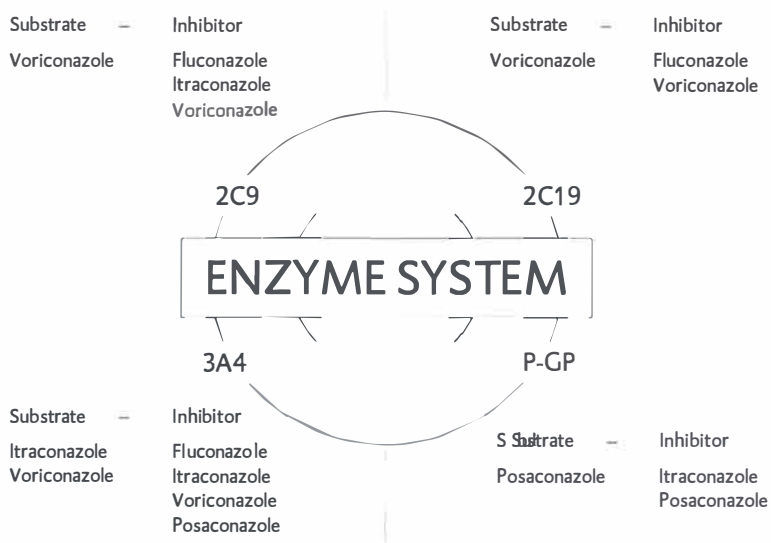

Figure 1: Involvement of Cytochrome P450 enzymes and P-gP in the metabolism of azole antifungals

Effect of co-medication on azole antifungals

Itraconazole and voriconazole are metabolised by the liver via phase I reactions. Voriconazole is substrate of CYP2C19 and, to a lesser extent, CYP2C9 and CYP3A4 [45, 46]. Itraconazole is predominantly metabolised by CYP3A4 and is the only azole antifungal with an active metabolite [13, 15]. Fluconazole is mainly excreted unchanged into the urine, and hepatic metabolism through CYP3A4 accounts for only 11% of total drug excreted [6]. Interactions influencing the pharmacokinetic profile of fluconazole through this system are therefore unlikely. Posaconazole metabolism involves phase II reactions (glucuronidation of the drug) [32]. Drugs influencing phase II enzyme systems such as lopinavir / ritonavir [47] can exert a change in the pharmacokinetic profile of the azole drug involved, leading to an increased or decreased exposure.

Genetic Polymorphism of Cytochrome P450 enzyme systems

All enzymes involved in the metabolism of azole antifungals are known to have multiple polymorphisms that divide the population into poor metabolisers (PM) and extensive metabolisers (EM). Patients that are homozygous or heterozygous PMs have a limited enzymatic capacity of the isoenzyme, which leads to a lower metabolic turnover of the drug involved and thus higher exposure. Polymorphisms of CYP2C9 and CYP2C19 may play a clinically relevant role while polymorphisms of CYP3A4 are not considered clinically relevant. In homozygous PMs of CYP2C19, the C_{max} and AUC of voriconazole are approximately 2-5 times higher than those of EMs [48]. The CYP2C9 genotypic variation does not significantly influence the exposure to voriconazole, as only a small fraction of the drug is metabolised through this enzymatic pathway [49]. The prevalence of variations in the gene sequence differs by race, with 20-30% of the Asian population being heterozygous PM for CYP2C19 and 2-3 % of Caucasians [50]. Determining the CYP2C19 genotype upfront may predict possible toxicity; Asian patients might especially benefit from this approach. A cost-benefit analysis should be performed before bringing this to general practice. We do not recommend determining genotype before initiation of therapy, as monitoring of plasma concentrations and clinical signs provide a better basis for management.

Drug transporters

Active transporters such as P-gP, Organic Anion-Transporting Polypeptides (OATP), and Breast Cancer Resistant Protein (BCRP) play an important role in drug interactions by regulating access of drugs to the drug-metabolising enzymes and by controlling drug concentrations in enterocytes and hepatocytes. Hence the contribution of efflux transporters in drug–drug interactions cannot be excluded [16, 51] however there is a lack of convincing data on the clinical relevance of drug transporters.

P-Glycoprotein (P-gP)

P-gP acts as an energy-dependent efflux pump that exports substrates out of the cell and is an important molecular determinant of oral bioavailability, brain penetration, and treatment resistance to several therapeutically used drugs. Modulation of P-gP function may play a significant role in drug interactions. Two azole antifungals, itraconazole and posaconazole, are substrates and inhibitors of the MDR-1 gene product P-gP [16, 28]. *In vivo*, no concrete relations between azole antifungal drug and P-gP have been established [51].

Other transporter systems

The organic anion-transporting polypeptide 1B1 (OATP1B1) is a multi-specific carrier capable of bidirectional transport across the sinusoidal liver membrane [52]. For instance, atorvastatin is subject to cellular membrane transport by OATP 1B1 and P-gP. It is suggested that itraconazole might block transport of atorvastatin due to inhibition of the OATP 1B1 enzyme system [53]. The exact role of OATP however has not been established and thus its specific role in drug-interactions with selected substrates and inhibitors such as itraconazole remains unclear and requires further investigation

The human breast cancer resistance protein (BRCP) belongs to the ATP-binding cassette transporter family. BCRP does not seem to be inhibited by fluconazole and voriconazole. A simulation model has demonstrated that it is highly likely that BCRP is inhibited by itraconazole [54]. *In vivo* no relations between azole antifungal drug and BCRP have been established.

RENAL EXCRETION

Drug interactions based on alterations in renal elimination mainly involve changes in tubular secretion or changes in kidney function. Drugs that use the same active transport system in the kidney tubules can compete for this excretory system. The two drugs excreted by the kidneys are fluconazole and hydroxy-itraconazole. There have been no reports as yet that impaired renal function caused by nephrotoxic drugs such as cyclosporine or gentamicin has led to increased toxicity of fluconazole or hydroxyl-itraconazole as a result of increased exposure.

PREDICTION OF DRUG INTERACTIONS

Drug interactions can cause many clinical problems. Ideally, comprehensive information should be available before a new drug completes the registration process. As we aim for the maximum attainable therapeutic effect when treating invasive fungal disease it is important to be aware of the mechanisms, whether theoretical or proven, behind drug-interactions. Nowadays, software tools are available which facilitate rapid monitoring for interactions thereby assisting clinical decision making.

Therapeutic Drug Monitoring

A drug-drug interaction is never straightforward, in a way that every single patient shows a similar degree of the effect when experiencing a drug-drug interaction. Therapeutic drug monitoring (TDM) is an important tool in identifying the extent of the interaction and help resolve actual and potential problems. TDM can be used to guide dosing and to optimize therapy to prevent sub therapeutic effects or toxicity [55].

Practical issues for use of the interaction table

The interaction table (Table 1) provides an overview of drug-drug interactions published in peer-reviewed journals. The table has a risk ranking order (adapted from Up to Date/ Lexi Interact Tool [56]): Avoid combination (X), Consider therapy modification (D), Monitor therapy (C), No action needed (B) and No known interaction (A). The interactions that are most severe are listed first. This type of grading system helps the clinician to judge which drug combination to avoid or which combinations can be safely used. Futhermore, we have defined nine areas (see Table 1 legend) which we consider necessary to be able to make a judgement of the drug-drug interaction. In the advice section, the authors have provided recommendations on how to deal with the specific interaction. Both the risk ranking and the advice reflect the opinion of the authors of this article, and is based on interpretation from (multiple) studies and gathered information. The advice may therefore differ slightly from the opinion in the referred article.

CONSIDERATIONS

We aimed to provide a comprehensive review of drug-drug interactions with the azole antifungals in current use for treating invasive fungal diseases. New information is emerging rapidly and thus this review is by its very nature incomplete. Awareness of the mechanisms involved in these interactions is pivotal for the optimization of treatment of patients requiring antifungal therapy.
For the clinical interpretation, it should be kept in mind that much of the data presented are from stud-

ies in healthy volunteers or a limited number of patients and the clinical setting may therefore differ from the controlled setting of an interaction study.

Drug interactions do not only occur when therapy is initiated but can also become evident only after the drug is stopped, particularly if the agent is question is an enzyme inducer as this might lead to toxic concentrations.

TDM is a valuable tool in assessing the effect of a drug-drug interaction for both antifungal azole drugs and, if possible, the co-administered drug. To perform TDM, a validated analytical method has to be available to determine whole blood or plasma concentrations. Assays have to be validated to ensure accuracy and precision. Measurement of samples can be done in-house if facilities are available or sent to reference laboratories with validated assays. The shorter the turnaround time, the better (for instance, within 48 hours would be preferable for prompt patient management). Interpretation of the results can be carried out by a clinical pharmacist/pharmacologist or another healthcare professional familiar with TDM.

Finally, addressing the problem of drug-drug interactions is a multidisciplinary task, with the goal of minimizing unwanted side effects, while optimizing patient care.

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CATEGORY X

AVOID COMBINATION

Data demonstrate that the specified agents may interact with each other in a clinically significant manner. The risks associated with concomitant use of these agents usually outweigh the benefits. These agents are generally considered to be contraindicated.

Co-administered agent	Antifungal agent	Type of study	Subjects involved	Nr of patients
Antacids	ITZ	Open label randomized, cross-over	Healthy volunteers	12
Carbamazepine and / or Phenobarbital	ITZ	Case report	Patients	3
Cimetidine	PSZ	Not available	Not available	Not available
Didanosine chewable tablet	ITZ	Open label, randomized, cross-over	Healthy volunteers	7
Fluticasone	ITZ	Open label, non-randomized, parallel	Lung TX Patients	20
Grapefruit juice	ITZ tablet	Open label randomized, cross-over	Healthy volunteers	11
Lovastatin	ITZ	Double-blind, randomized, cross-over	Healthy volunteers	12
Lovastatin	ITZ	Double-blind, randomized, cross-over	Healthy volunteers	10
Omeprazole	ITZ	Open label, non-randomized	Healthy volunteers	11
Phenytoin	ITZ	Open label, randomized, parallel	Healthy volunteers	28
Phenytoin	PSZ	Open label, randomized, parallel	Healthy volunteers	36
Rifabutin	PSZ	Open label, non-randomized,parallel	Healthy volunteers	24
Rifampin	ITZ	Open label, non-randomized, parallel, cross-over	Combination of healthy volunteers, and AIDS Patients	9
Sirolimus	PSZ	Healthy volunteers	Not available	Not available
Sirolimus	VRZ	Retrospective	Patients	11
St John's Wort	VRZ	Controlled open label	Healthy volunteers	16
Terfenadine	ITZ	Open label, non-randomized, cross-over	Healthy volunteers	6
Triazolam	ITZ	Open label, randomized cross-over	Healthy volunteers	10
Triazolam	ITZ	Double-blind, randomized, cross-over	Healthy volunteers	9
Vincristine	ITZ	Case reports	Patients	Multiple patients multiple reports

Interaction mechanism	Effect of interaction	Advice	References
Stomach pH	ITZ AUC↓↓ and Cmax↓	Avoid combination	[57]
Induction of CYP3A4	ITZ Cmin↓	Avoid combination	[58]
?	PSZ AUC ↓ and Cmax↓	Avoid combination. If combined: TDM of PSZ and increase dose of PSZ if necessary	[28]
?	ITZ AUC↓↓ and Cmax↓↓	Use didanosine enteric coated capsules (Videx EC) not chewable tablet (these contain antacids)	[59]
nhibition of CYP3A4	Fluticasone Css↑↑	Avoid combination, monitor side effects	[60]
nhibition of intestinal CYP3A4	ITZ AUC↓ and Cmax↓, HITZ AUC↓ and Cmax↓	Avoid combination, use ITZ oral solution	[61]
nhibition of CYP3A4	Lovastin AUC↑↑ and Cmax↑↑	Avoid combination	[62]
nhibition of CYP3A4	Lovastin AUC↑↑ and Cmax↑↑	Avoid combination	[63]
Stomach pH increase	ITZ Cmax↓↓ and AUC↓↓	Avoid combination with ITZ capsules, use oral solution ITZ	[64]
nduction of CYP3A4	ITZ AUC↓↓ and Cmax↓↓; Phenytoin AUC↑	Avoid combination; TDM of ITZ	[65]
	PSZ AUC↓↓ and Cmax↓	Avoid combination; If no alternative: increase dose of PSZ and TDM of PSZ; Monitor for toxicity of phenytoin, TDM of phenytoin	[66]
	PSZ AUC ↓ and Cmax↓; rifabutin AUC↑ and Cmax↑	Avoid combination; otherwise increase PSZ dose, TDM of PSZ; Monitor for toxicity of rifabutin	[67]
nduction of CYP-P450	ITZ AUC↓↓ and Cmax↓↓	Avoid combination	[68]
	Sirolimus AUC↑↑ and Cmax↑↑	Avoid combination	[28]
nhibition of CYP3A4	Sirolimus AUC↑↑	Avoid combination	[69]
nduction of CYP-P450	Short term increase in exposure followed by a reduced exposure of VRZ	Avoid combination	[70]
nhibition of CYP3A4	Terfenadine AUC↑; QT prolongation	Avoid combination	[71]
nhibition of CYP3A4	Triazolam AUC↑↑ and Cmax↑	Avoid combination; monitor for toxicity of triazolam	[72]
nhibition of CYP3A4	Triazolam AUC↑↑ and Cmax↑↑	Avoid combination; monitor for toxicity of triazolam	[73]
nhibition of CYP3A4, P-gP	Unknown	Avoid combination. Monitor for toxicity of vincristine;	[74-82]

CATEGORY D

CONSIDER THERAPY MODIFICATION

Data demonstrate that the two medications may interact with each other in a clinically significant manner. A patient-specific assessment must be conducted to determine whether the benefits of concomitant therapy outweigh the risks. Specific actions must be taken in order to realize the benefits and/or minimize the toxicity resulting from concomitant use of the agents. These actions may include aggressive monitoring, empiric dosage changes, choosing alternative agents.

Co-administered agent	Antifungal agent	Type of study	Subjects	Nr of patients	Interaction mechanism
Alfentanil	FLZ	Double-blind, randomized, cross-over	Healthy volunteers	9	Inhibition of CY
Alfentanil	VRZ	Open randomised cross-over	Healthy volunteers	12	Inhibition of CY
Amitriptyline	FLZ	Case report	AIDS and ESRD Patients	3	Inhibition of CY
Atazanavir (either with or without ritonavir)	PSZ	Not available	Patients	Not available	?
Atorvastatin	ITZ	Double-blind, randomized, cross-over	Healthy volunteers	10	Inhibition of CY
Budesonide	ITZ	Double-blind, randomized, cross-over	Healthy volunteers	10	Inhibition of CY
Budesonide	ITZ	Open label, non-randomized, with retrospective matched controls.	CF and CGD Patients	67	Inhibition of CY
Cabergoline	ITZ	Case reports	PD Patients	2	Inhibition of CY
Celiprolol	ITZ	Open label, randomized cross-over	Healthy volunteers	12	P-gP
Ciclosporine	FLZ	Double-blind, randomized	Renal TX Patients	16	Inhibition of CY
Ciclosporine	FLZ	Open label, cross-over	Renal TX Patients	6	Inhibition of CY
Ciclosporine	FLZ	Open label, non-randomized	Patients	6	Inhibition of gu metabolism,
Ciclosporine	ITZ	Open label, non-randomized	Renal TX Patients	8	Inhibition of CY
Ciclosporine	ITZ	Open label, non-randomized, cross-over	SCT Patients	8	Inhibition of CY
Ciclosporine	PSZ	Open label, non-randomized	Heart TX Patients	4	Inhibition of CY
Ciclosporine	VRZ	Randomized, placebo controlled, double-blind, cross-over	Renal TX Patients	7	Inhibition of CY
Cimetidine	ITZ	Open label, non-randomized, cross-over	Healthy volunteers	8	P-gP
Diclofenac	VRZ	Two-way, open label, cross-over	Healthy volunteers	10	Inhibition of CY and CYP3A4 ar CYP2C19

ect of interaction	Advice	References
entanil AUC↑	Monitor for toxicity of alfentanyl and adjust dose of alfentanyl if necessary	[83]
entanil AUC↑↑	Monitor for toxicity of alfentanyl and adjust dose of alfentanil if necessary	[84]
itriptyline AUC↑	Monitor for toxicity of amitryptiline	[85]
zanavir AUC↑↑ and Cmax↑↑	Monitor for toxicity of atazanavir, TDM of atazanavir	[28]
rvastatin AUC↑↑, Cmax↑; ve metabolites↑↑	Monitor for toxicity of atorvastatin and adjust dose if necessary. Upon initiation of therapy, start with low dose of atorvastatin	[86]
esonide AUC↑↑ and Cmax↑	Increased risk of adverse effects	[87]
available	Monitor adrenal function	[88]
ergoline Css↑↑	Reduce dose of cabergoline	[89]
prolol AUC↑ and Cmax↑	Monitor for toxicity of celiprolol and reduce dose if necessary	[90]
osporine AUC ↑	Monitor for toxicity of ciclosporine and adjust dose if necessary. TDM of ciclosporine	[91]
osporine AUC↑↑ and Cmax↑	Monitor for toxicity of ciclosporine and adjust dose if necessary. TDM of ciclosporine	[92]
osporine AUC↑ and Cmax↑	Monitor for toxicity of ciclosporine and adjust dose if necessary. TDM of ciclosporine	[93]
osporine AUC↑ and Cmax↑	Monitor for toxicity of ciclosporine; TDM of ciclosporine; Upon initiation of ciclosporine, start with a 50% reduced dose of ciclosporine; a dose reduction of 50% of ciclosporine upon initiation of azole seems warranted.	[94]
osporine Css↑	Monitor for toxicity of ciclosporine; TDM of ciclosporine; Upon initiation of ciclosporine, start with a 50% reduced dose of ciclosporine; a dose reduction of 50 % of ciclosporine upon initiation of azole seems warranted.	[95]
osporine ↑	Monitor for toxicity of ciclosporine; TDM of ciclosporine; Upon initiation of ciclosporine, start with a 75% reduced dose of ciclosporine; a dose reduction of 75% of ciclosporine upon initiation of azole seems warranted.	[96]
osporine AUC↑↑	Monitor for toxicity of ciclosporine; TDM of ciclosporine; Upon initiation of ciclosporine, start with a 66% reduced dose of ciclosporine; a dose reduction of 66% of ciclosporine upon initiation of azole seems warranted.	[97]
etidine AUC↑	Monitor for toxicity of cimetidine	[98]
ofenac AUC↑↑ and Cmax↑↑	Clinical relevance unknown	[99]

Digoxin	ITZ	Double-blind, randomized, cross-over	Healthy volunteers	10	P-gP
Efavirenz	PSZ	Not available	Not available	Not available	?
Efavirenz	VRZ	Randomized, placebo-controlled	Healthy volunteers	34	Induction of CY and CYP2C9 by efavirenz, inhib CYP3A4 by VRZ
Efavirenz	VRZ	Open label, non-randomized	Healthy volunteers	16	Induction of CY and CYP2C9 by efavirenz, inhib CYP3A4 by VRZ
Ethinyl estradiol and norethindrone	VRZ	Open label, non randomized	Healthy volunteers	16	Inhibition of CY by ethinyl estra inhibition of CY by VRZ
Felodipine	ITZ	Double-blind, randomized, cross-over	Healthy volunteers	9	Inhibition of CY
Fluvastatin	FLZ	Double-blind, randomised, cross-over	Healthy volunteers	12	Inhibition of CY
Glimepiride	FLZ	Double-blind, randomized, cross-over	Healthy volunteers	12	Inhibition of CY
Methadone	VRZ	Randomized, placebo controlled, double-blind, parallel	Patients on methadone therapy	23	Inhibition of CY CYP2C9 and /o CYP2C19
Midazolam	FLZ	Open label, randomized, cross-over	Healthy volunteers	9	Inhibition of CY
Midazolam	FLZ	Open label, parallel	ICU Patients	10	Inhibition of CY
Midazolam	FLZ	Double-blind, randomized, cross-over	Healthy volunteers	12	Inhibition of CY
Midazolam	FLZ	Open label, parallel	Healthy volunteers		Inhibition of CY
Midazolam	ITZ	Double-blind, randomized, cross-over	Healthy volunteers	12	Inhibition of CY
Midazolam	ITZ	Open label, non-randomized, cross-over	Healthy volunteers	9	Inhibition of CY
Midazolam	ITZ	Double-blind, randomized cross-over	Healthy volunteers	9	Inhibition of CY
Midazolam	ITZ	Double-blind, randomized cross-over	Healthy volunteers	12	Inhibition of CY
Midazolam	PSZ	Open label, randomized, cross-over	Healthy volunteers	13	Inhibition of CY
Midazolam	VRZ	Open, randomized, cross-over	Healthy volunteers	10	Inhibition of CY
Nelfinavir	FLZ	Case report	HIV Patients	3	Inhibition of CY and CYP3A4
Nevirapine	ITZ	Open label, randomized, cross-over	Healthy volunteers	12	Induction of CY by nevirapine
Omeprazole	FLZ	Open label, non-randomized, cross-over	Healthy volunteers	18	Inhibition of CY and CYP3A4
Omeprazole	VRZ	Open, randomized, placebo controlled, two way cross-over	Healthy volunteers	18	Inhibition of CY

;oxin AUC↑ and Cmax↑	Monitor digoxin plasma level	[100]
Z AUC↓ and Cmax↓	TDM of PSZ, increase dose of PSZ if necessary	[28]
virenz AUC↑ and Cmax↑; VRZ C↓↓ and Cmax↓↓	Use 300 mg efavirenz once daily, use 400 mg VRZ twice daily; TDM of efavirenz and VRZ	[101]
virenz AUC↑ and Cmax↑; VRZ C↓↓ and Cmax↓	Use 300 mg efavirenz once daily, use 400 mg VRZ twice daily; TDM of efavirenz and VRZ	[102]
Z AUC↑ and Cmax↑; ethinyl adiol AUC↑ and Cmax↑; ethindrone AUC↑ and Cmax↑;	Monitor for toxicity of voriconazole and oral contraceptive	[103]
odipine Cmax↑↑ and AUC↑↑	Monitor for felodipine toxicity, adjust dose of felodipine if necessary	[104]
vastatin AUC↑ and Cmax↑	Monitor for toxicity of fluvastatin and adjust dose if necessary. Upon initiation of therapy, start with low dose of fluvastatin	[105]
nepiride AUC↑↑ and Cmax↑	Monitor for glimepiride toxicity and adjust dose if necessary	[106]
thadone AUC↑↑	Monitor for toxicity of methadone and adjust dose if necessary	[107]
lazolam AUC ↑↑ and Cmax↑	Monitor for toxicity of midazolam and adjust dose if necessary	[108]
lazolam AUC↑↑	Monitor for toxicity of midazolam and adjust dose if necessary	[109]
lazolam AUC↑↑ and Cmax↑	Monitor for toxicity of midazolam and adjust dose if necessary	[110]
lazolam AUC↑	Monitor for toxicity of midazolam and adjust dose if necessary	[111]
azolam AUC↑↑ and Cmax↑↑	Monitor for toxicity of midazolam and adjust dose if necessary	[112]
lazolam AUC↑↑ and Cmax↑↑	Monitor for toxicity of midazolam and adjust dose if necessary	[113]
azolam AUC↑↑ and Cmax↑↑	Monitor for toxicity of midazolam and adjust dose if necessary	[114]
azolam AUC↑↑ and Cmax↑↑	Monitor for toxicity of midazolam and adjust dose if necessary	[110]
azolam AUC↑	Monitor for toxicity of midazolam and adjust dose if necessary	[115]
azolam AUC↑↑ and Cmax↑↑	Monitor for toxicity of midazolam and adjust dose if necessary	[116]
inavir AUC↑	TDM of nelfinavir and adjust dose if necessary if boosted with ritonavir	[117]
Cmax↓ and AUC↓↓	TDM of ITZ and increase dose of ITZ	[118]
eprazole AUC↑↑ and Cmax↑	Monitor for toxicity of omeprazole and adjust dose if necessary. Upon initiation of therapy, start with low dose of omeprazole	[119]
AUC↑; omeprazole AUC↑↑ Cmax↑	No clinical relevant effect on VRZ; Monitor for toxicity of omeprazole and adjust dose if necessary. Upon initiation of therapy, start with low dose of omeprazole	[41]

Phenytoin	FLZ	Open label, randomized, parallel	Healthy volunteers	20	Inhibition of CY
Phenytoin	VRZ	Study A: open label Study B: double-blind, randomized	Healthy volunteers	Study A: 21 Study B: 15	Induction of CY and CYP2C9 by phenytoin; inhi of CYP2C9 by V
Ritonavir	VRZ	Study A: Randomized , double-blind, placebo controlled, parallel Study B: Randomized	Healthy volunteers	Study A: 34 Study B: 17	Induction of CY and CYP2C9
Ritonavir	VRZ	Randomized placebo controlled cross over	Healthy volunteers	20	Inhibition of CY
Saquinavir	ITZ	Open label, randomized, parallel	HIV Patients	17	Inhibition of CY and P-gP
Simvastatine	ITZ	Double-blind randomized, cross-over	Healthy volunteers	10	Inhibition of CY
Tacrolimus	FLZ	Open label, non-randomized	Patients	15	Inhibition of gu metabolism
Tacrolimus	FLZ	Open label, non-randomized	Kidney, liver and heart transplant patients	16	Inhibition of CY
Tacrolimus	ITZ	Open label, non-randomized, cross-over	HSCT Patients	9	Inhibition of CY
Tacrolimus	ITZ	Retrospective	Lung TX Patients	40	Inhibition of CY
Tacrolimus	PSZ	Open label, non-randomized	Healthy volunteers	34	Inhibition of CY
Zolpidem	VRZ	Open, randomized, two phase, cross over	Healthy volunteers	10	Inhibition of CY and CYP3A4

enytoin AUC↑	Monitor for toxicity of phenytoin and adjust dose if necessary. TDM of phenytoin	[120]
Z AUC↓↓ and Cmax↓↓; enytoin AUC↑↑ and Cmax↑↑	Increase VRZ maintenance dose to 400 mg bid, TDM ofVRZ; monitor for toxicity of phenytoin, TDM of phenytoin	[45]
ZAUC↓↓ and Cmax↓↓	Consider alternative therapy or monitor VRZ	[121]
Z AUC↑ and Cmax↑	Consider alternative therapy or monitor VRZ	[122]
quinavir AUC↑	Monitor for toxicity of saquinavir and adjust dose if necessary.	[123]
mvastatin AUC↑↑ and Cmax↑↑	Monitor for toxicity of simvastatin and adjust dose if necessary. Upon initiation of therapy, start with low dose of simvastatin	[124]
rolimus AUC↑ and Cmax↑	Monitor for toxicity of tacrolimus; TDM of tacrolimus; Upon initiation of tacrolimus, start with a 50% reduced dose of tacrolimus; a dose reduction of 50 % of tacrolimus upon initiation of azole seems warranted.	[93]
rolimus Cmin↑↑	Monitor for toxicity of tacrolimus; TDM of tacrolimus; Upon initiation of tacrolimus, start with a 50% reduced dose of tacrolimus; a dose reduction of 50 % of tacrolimus upon initiation of azole seems warranted.	[125]
rolimus Css↑	Monitor for toxicity of tacrolimus; TDM of tacrolimus; Upon initiation of tacrolimus, start with a 50% reduced dose of tacrolimus; a dose reduction of 50 % of tacrolimus upon initiation of azole seems warranted.	[95]
rolimus Cmin↑↑	Monitor for toxicity of tacrolimus; TDM of tacrolimus; Upon initiation of tacrolimus, start with a 50% reduced dose of tacrolimus; a dose reduction of 50 % of tacrolimus upon initiation of azole seems warranted.	[126]
rolimus AUC↑↑ and Cmax↑↑	Monitor for toxicity of tacrolimus; TDM of tacrolimus; Upon initiation of tacrolimus, start with a 50% reduced dose of tacrolimus; a dose reduction of 50 % of tacrolimus upon initiation of azole seems warranted.	[96]
pidem AUC↑	Monitor for toxicity of zolpidem and adjust dose if necessary	[127]

CATEGORY C

MONITOR THERAPY

Data demonstrate that the specified agents may interact with each other in a clinically significant manner. The benefits of concomitant use of these two medications usually outweigh the risks. An appropriate monitoring plan should be implemented to identify potential negative effects. Dosage adjustments of one or both agents may be needed in a minority of patients.

Co-administered agent	Antifungal agent	Type of study	Subjects	Nr of pati
Alprazolam	ITZ	Double-blind randomized cross-over	Healthy volunteers	10
Bromperidol	ITZ	Open label, non-randomized, cross-over	Healthy volunteers	8
Cimetidine	VRZ	Open label, randomized, placebo controlled, cross-over	Healthy volunteers	12
Clarithromycin	ITZ	Case report	MAI Patients	3
Cyclophosphamide	FLZ	Open label, randomized,	SCT Patients	104
Cyclophosphamide	ITZ	Open label, randomized	SCT Patients	105
Dexamethasone	ITZ	Double-blind, randomized, cross-over	Healthy volunteers	8
Diazepam	FLZ	Open label, randomized, cross-over	Healthy volunteers	12
Diazepam	ITZ	double-blind, randomized, cross-over	Healthy volunteers	10
Diazepam	VRZ	Open label, randomized, cross-over	Healthy volunteers	12
Etizolam	ITZ	Double-blind, randomized, cross-over	Healthy volunteers	12
Fentanyl	FLZ	Open label, randomized, cross-over	Healthy volunteers	12
Fentanyl	ITZ	Double-blind randomized cross-over	Healthy volunteers	10
Fentanyl	VRZ	Open label, randomized, cross-over	Healthy volunteers	12
Haloperidol	ITZ	Double-blind randomized cross-over	Healthy volunteers	19
Ibuprofen	FLZ	Open label, randomized, parallel	Healthy volunteers	12
Ibuprofen	VRZ	Open label, randomised cross-over	Healthy volunteers	12
Loperamide	ITZ	Open, randomized cross-over	Healthy volunteers	12
Losartan	FLZ	Open, randomized, parallel	Healthy volunteers	16
Losartan	FLZ	Double-blind randomised, cross-over	Healthy volunteers	11
Methylprednisolone	ITZ	Double-blind, randomized, cross-over	Healthy volunteers	10
Perispirone	ITZ	Open label, non-randomized, cross-over	Healthy volunteers	9
Quinidine	ITZ	Open label, non-randomized, cross-over	Healthy volunteers	6

Interaction mechanism	Effect of interaction	Advice	References
nhibition of CYP3A4	Alprazolam AUC↑	Monitor for toxicity of alprazolam	[128]
nhibition of CYP3A4	Bromperidol Css↑	Monitor for side effects of bromperidol	[129]
nhibition of CYP450 by :imetidine	VRZ AUC↑ and Cmax↑	Monitor for toxicity of voricconazole	[130]
nhibition of CYP3A4	Clarithromycin AUC↑	Clinical relevance unknown; Monitor for toxicity of clarithromycin	[131]
nhibition of CYP2C9	Alternative metabolic pathway	Monitor for toxicity of cyclophosphamide	[132]
nhibition of CYP3A4	AUC of metabolites↑	Monitor for toxicity of cyclophosphamide	[132]
nhibition of CYP3A4	Dexamethasone AUC↑↑ and Cmax↑	Monitor for toxicity of dexamethasone	[133]
nhibition of CYP2C19 ınd CYP3A4	Diazepam AUC↑	Monitor for toxicity of diazepam and adjust dose if necessary	[134]
nhibition of CYP3A4	Diazepam AUC↑	Monitor for toxicity of diazepam	[135]
nhibition of CYP2C19 ınd CYP3A4	Diazepam AUC↑	Monitor for toxicity of diazepam and adjust dose if necessary	[134]
nhibition of CYP3A4	Etizolam AUC↑	Monitor for toxicity of etizolam	[136]
nhibition of CYP3A4	Fentanyl AUC↑	Monitor for toxicity of fentanyl	[137]
nhibition of CYP3A4	No effect	No clinical relevance, no action	[138]
nhibition of CYP3A4	Fentanyl AUC↑	Monitor for toxicity of fentanyl	[137]
nhibition of CYP3A4	Haloperidol AUC↑ and Cmax↑	Monitor for toxicity of haloperidol	[139]
ıhibition of CYP2C9	Ibuprofen AUC↑	Monitor for toxicity of ibuprofen	[140]
ıhibition of CYP2C9	Ibuprofen AUC↑	Monitor for toxicity of ibuprofen	[140]
ıhibition of CYP3A4, -gP	Loperamide AUC↑↑ and Cmax↑↑	Monitor for toxicity of loperamide	[141]
ıhibition of CYP2C9 nd CYP3A4	Losartan AUC↑ and Cmax↑	Effect unclear ; Monitor for toxicity of Losartan	[142]
ıhibition of CYP2C9	Losartan AUC↑; Active metabolite AUC↓	Effect unclear; Monitor for toxicity of Losartan	[143]
ıhibition of CYP3A4	Methylprednisolone AUC↑↑	Monitor for toxicity of methylprednisolone	[144]
ıhibition of CYP3A4	Perispirone AUC↑↑ and Cmax↑↑	Monitor for toxicity of perispirone.	[145]
ıhibition of CYP3A4, -gP	Quinidine Cmax↑	Monitor for toxicity of quinidine	[146]

Quinidine	ITZ	Double-blind, randomized, cross-over	Healthy volunteers	9
Repaglinide	ITZ	Open label, randomized cross-over	Healthy volunteers	12
Rifabutin	FLZ	Open label, non-randomized, cross-over	HIV Patients	12
Rifabutin	FLZ	Open label, randomized	HIV Patients	10
Rifampin	FLZ	Open label, non-randomized	AIDS Patients	11
Rifampin	FLZ	Open label, parallel	AIDS Patients	24
Rifampin	FLZ	Case series, parallel controlled	IC Patients	2
Risperidone	ITZ	Open-label, non-randomized, cross-over	Schizophrenic patients	19
Ropivacaine	ITZ	Double-blind, randomised, cross-over	Healthy volunteers	8
Triazolam	FLZ	Double-blind, randomized, cross-over	Healthy volunteers	
Venlafaxin	VRZ	?	Healthy volunteers	12
Warfarin	FLZ	Open-label, non-randomized	Healthy volunteers	6
Warfarin	VRZ	Double-blind, placebo controlled, cross over	Healthy volunteers	17

Inhibition of CYP3A4, P-gP	Quinidine AUC↑↑ and Cmax↑	Monitor for toxicity of quinidine	[147]
Inhibition of CYP3A4	Repaglinide AUC↑ and Cmax↑	Monitor for toxicity of repaglinide	[148]
Inhibition of CYP3A4	Rifabutin AUC↑ and rifabutin metabolite AUC↑↑	Monitor for toxicity of rifabutin	[149]
Inhibition of CYP3A4	Rifabutin AUC↑ and Cmax↑	Monitor for toxicity of rifabutin	[150]
Induction of CYP-P450	No effect	Monitor efficacy of FLZ; TDM of FLZ	[151]
Induction of CYP-P450	FLZAUC ↓ and Cmax↓	Monitor efficacy of FLZ; TDM of FLZ	[152]
Induction of CYP-P450	FLZAUC↓	Monitor efficacy of FLZ; TDM of FLZ	[153]
Inhibition of CYP3A4	Risperidone Css↑	Monitor for toxicity of risperidone	[154]
Inhibition of CYP3A4	Ropivacaine AUC↑ and Cmax↓	Monitor for toxicity of ropivacaine	[155]
Inhibition of CYP3A4	Triazolam AUC↑ and Cmax↑	Monitor for toxicity of triazolam	[156, 157]
Inhibition of CYP3A4, CYP2C9, CYP2C19	Venlafaxin AUC↑	Monitor for toxicity of venlafaxin	[158]
Inhibition of CYP3A4 and CYP2C9	Warfarin AUC↑	Monitor for toxicity of warfarin	[43]
Inhibition of CYP2C9	Warfarin induced increase of AUEc and maximum of prothrombin time	Monitor for toxicity of warfarin	[159]

CATEGORY B

NO ACTION NEEDED

Data demonstrate that the specified agents may interact with each other, but there is little to no evidence of clinical concern resulting from their concomitant use

Co-administered agent	Antifungal agent	Type of study	Subjects	Nr of pati
Aripiprazole	ITZ	Open-label, non-randomized, cross-over	Healthy volunteers	24
Atenolol	ITZ	Open-label, randomized, cross-over	Healthy volunteers	10
Azithromycin	VRZ	Open, randomized, parallel	Healthy volunteers	30
Bupivacaine	ITZ	double-blind randomized cross-over	Healthy volunteers	7
Busulfan	FLZ	Open label, non-randomized, parallel	SCT Patients	52
Cotrimoxazole	FLZ	Open label, randomized, parallel	HIV Patients	Part A: 9 Part B: 12
Didanosine	ITZ	Open label, randomized, cross-over	Healthy volunteers	27
Erythromycin	VRZ	Open label, randomized, parallel	Healthy volunteers	30
Ethinyl estradiol	FLZ	Open label, randomized, cross-over	Healthy volunteers	20
Ethinyl estradiol and norethindrone	FLZ	Double-blind, randomized, cross-over	Healthy volunteers	21
Everolimus	FLZ	Case report	Renal TX Patient	16
Fexofenadine	ITZ	Open label, randomized, cross-over	Healthy volunteers	10
Fexofenadine	ITZ	Open label, non-randomized	Healthy volunteers	14
Fexofenadine	ITZ	Open label, randomized cross-over	Healthy volunteers	11
Gefitinib	ITZ	Open label, randomized, cross-over	Healthy volunteers	48
Grapefruit juice	ITZoral solution	Open label randomized, cross-over	Healthy volunteers	20
Lasofoxifene	FLZ	Open label, randomized, parallel	Healthy volunteers	45
Lignocaine IV	ITZ	Double-blind, randomized, cross-over	Healthy volunteers	9
Lignocaine oral	ITZ	Double-blind, randomized, cross-over	Healthy volunteers	9
Lumiracoxib	FLZ	Open label, randomized cross-over	Healthy volunteers	13
Methadone	FLZ	double-blind, randomized,	Patients on methadone therapy	25
Omeprazole	ITZ	Open label, randomized, cross-over	Healthy volunteers	15
Oxybutinin	ITZ	double-blind, randomized, cross-over	Healthy volunteers	10
Prednisolone	ITZ	Double-blind, randomised, cross-over	Healthy volunteers	10

Interaction mechanism	Effect of Interaction	Advice	References
Inhibition of CYP3A4	Aripiprazole AUC↑ and Cmax↑	No clinical relevance, no action	[160]
P-gP	No effect	No clinical relevance, no action	[161]
?	VRZ AUC↑ and Cmax↑	No clinical relevance, no action	[162]
?	Bupivacaine Css↑	No clinical relevance, no action	[163]
?	Busulfan AUC↑	No clinical relevance, no action	[164]
CYP2C9, CYP3A4	AUC of hydroxylamine↓	Clinical relevance unknown	[165]
pH	No effect	Use didanosine enteric coated capsules (Videx EC) not chewable tablet (these contain antacids)	[166]
Inhibition of CYP3A by erythromycin	VRZ Cmax↑	No clinical relevance, no action	[162]
Inhibition of CYP3A4	Ethinyl estradiol AUC↑ and Cmax↑	Clinical relevance unknown	[167]
?	Ethinyl estradiol AUC↑ and Cmax↑; norethindrone AUC↑ and Cmax↑	No threat of contraceptive failure, no action	[168]
Inhibition of CYP3A4	No effect	No clinical relevance, no action	[169]
P-gP	Fexofenadine AUC↑↑ and Cmax↑↑	No clinical relevance, no action	[170]
P-gP (MDR1 - G2677T or C3435T haplotype)	Fexofenadine AUC↑↑	No clinical relevance, no action	[171]
P-gP	Fexofenadine AUC↑↑ and Cmax↑↑	No clinical relevance, no action	[172]
Inhibition of CYP3A4	Gefitinib AUC↑ and Cmax↑	No clinical relevance, no action	[173]
Inhibition of intestinal CYP3A4	ITZ AUC↑	No clinical relevance, no action	[174]
Inhibition of CYP2C9	No effect	No action	[175]
Inhibition of CYP3A4	Lignocaine AUC↑ and Cmax↑	No clinical relevance, no action	[176]
Inhibition of CYP3A4	Lignocaine AUC↑ and Cmax↑	No clinical relevance, no action	[177]
Inhibition of CYP2C9	Lumiracoxib AUC↑,	No clinical relevance, no action	[178]
Inhibition of CYP3A4	Methadone AUC↑ and Cmax↑	No clinical relevance, no action	[107]
Stomach pH increase	No effect on ITZ	No action in case of ITZ oral solution	[179]
Inhibition of CYP3A4	Oxybutinin AUC↑ and Cmax↑	No clinical relevance, no action	[180]
Inhibition of CYP3A4	Prednisolone AUC↑	No clinical relevance, no action	[181]

Ranitidine	VRZ	Open label, randomized, placebo controlled, cross-over	Healthy volunteers	12
Ritonavir	FLZ	Open label, randomized, cross-over	Healthy volunteers	8
Ritonavir	FLZ	Open label, parallel	HIV Patients	3
Rosuvastatin	FLZ	Double-blind, randomized, cross-over	Healthy volunteers	14
Rosuvastatin	ITZ	double-blind, randomized, cross-over	Healthy volunteers	Trial A: 12 Trial B: 14
Saquinavir	FLZ	Open label, parallel	HIV Patients	5
Temazepam	ITZ	Double-blind, randomized, cross-over	Healthy volunteers	10
Terfenadine	FLZ	Open label, non-randomized	Healthy volunteers	6
Zidovudine	FLZ	Open label, non-randomized, parallel	HIV Patients	20
Zolpidem	ITZ	Open label, randomized, cross-over	Healthy volunteers	10
Zopiclone	ITZ	double-blind, randomized, cross-over	Healthy volunteers	10

Inhibition of CYP450	VRZ AUC_τ and $Cmax_\tau$	No clinical relevance, no action	[130]
Inhibition of CYP3A4	Ritonavir AUC_τ and $Cmax_\tau$	No clinical relevance, no action	[182]
Inhibition of CYP3A4	No effect	No clinical relevance, no action	[183]
Inhibition of CYP2C9 and CYP2C19	Rosuvastatin AUC_τ	No clinical relevance, no action	[184]
Inhibition of CYP3A4	Rosuvastatin AUC_τ and $Cmax_\tau$	No clinical relevance, no action	[185]
Inhibition of CYP3A4	Saquinavir AUC_τ and $Cmax_\tau$	No clinical relevance, no action	[183]
unknown	Temazepam AUC_τ	No clinical relevance, no action	[186]
Inhibition of CYP3A4	Terfenadine AUC_τ	No clinical relevance, no action	[187]
Inhibition of CYP2C9	Zidovudine AUC_τ	No clinical relevance	[188]
Inhibition of CYP3A4	Zolpidem AUC_τ	No clinical relevance	[189]
Inhibition of CYP3A4	Zopiclone AUC_τ and $Cmax_\tau$	No clinical relevance	[190]

CATEGORY A

NO KNOWN INTERACTION

Data have not demonstrated either pharmacodynamic or pharmacokinetic interactions between the specified agents

Co-adminstered agent	Antifungal agent	Type of study	Subjects
Bromazepam	FLZ	Double-blind, randomized, cross-over	Healthy volunteers
Clozapine	ITZ	double-blind randomized	Psychiatric Patients
Delavirdine	FLZ	Open label, randomized, parallel	HIV Patients
Didanosine	FLZ	Open label, non-randomized	HIV Patients
Didanosine (Videx EC)	FLZ	Open label, randomized, cross-over	Healthy volunteers
Digoxin	VRZ	double-blind, randomized, placebo-controlled, parallel-group	Healthy volunteers
Eprosartan	FLZ	Open label, randomized, parallel	Healthy volunteers
Estazolam	ITZ	double-blind randomized cross-over	Healthy volunteers
Fluvastatin	ITZ	Open label, randomized, cross-over	Healthy volunteers
Indinavir	FLZ	Double-blind, randomized, cross-over	HIV Patients
Indinavir	VRZ	Study A: Open label randomized, placebo-controlled Study B: double-blind, randomized, two-way cross-over	Healthy volunteers
Lidocaine inhalation	ITZ	Double-blind, randomized, cross-over	Healthy volunteers
Mexiteline	FLZ	Open label, non-randomized, cross-over	Healthy volunteers
Omeprazole	FLZ	Randomized, cross-over	Healthy volunteers
Pioglitazone	ITZ	double-blind, randomized, cross-over	Healthy volunteers
Pravastatine	FLZ	Double-blind, randomized, cross-over,	Healthy volunteers
Pravastatine	ITZ	double-blind, randomized, cross-over	Healthy volunteers

Table 1. Interaction table of azole antifungal drugs with co-administered agents.

FLZ = fluconazole; ITZ = itraconazole; hITZ = hydroxy-itraconazole; PSZ = posaconazole ; VRZ = voriconazole; ? = no interaction mechanism given in article

ALL = acute lymphoblastic leukaemia
AML = acute myeloid leukaemia
BMT = bone marrow transplantation
CF = cystic fibrosis
CGD = chronic granulomatous disease
ESRD = end stage renal disease
HSCT = haematopoetic stem cell transplant
IC = Intensive Care
MAI = Mycobacteria avium infection
PD = Parkinson disease

Nr of patients	Interaction mechanism	Effect of interaction	Advice	References
12	?	No effect	No action	[191]
7	Inhibition of CYP3A4	No effect	No clinical relevance, no action	[192]
13	Inhibition of CYP3A	No effect	No clinical relevance, no action	[193]
12	?	No effect	No clinical relevance, no action	[194]
14	?	No effect	No clinical relevance, no action	[166]
25	Unknown, possibly P-gP related	No effect	No clinical relevance, no action	[195]
16	?	No effect	No action	[142]
10	Inhibition of CYP3A4	No effect	No action	[196]
10	Inhibition of CYP3A4	No effect	No action	[63]
13	?	No effect	No action	[197]
Study A: 18 Study B: 14	Inhibition of CYP3A4	Study A: No effect of indinavir on VRZ	No clinical relevance, no action	[198]
10	Inhibition of CYP3A4	No effect	No action	[199]
6	?	No effect	No action	[200]
12	Stomach pH increase	No effect on FLZ	No action	[201]
12	Inhibition of CYP3A4	No effect	No action	[202]
12	?	No effect	No action	[105]
10	Inhibition of CYP3A4	No effect	No action	[124]

All patient participating in a study protocol are included in the number of participants. In case of controlled study the control group is included in the total amount of participants. In case of two research arms both arms are included.

1. The first column 'Co-administered agent' is presented in alphabetical order, either for the individual drug or the drug class .
2. The second column 'Antifungal agent' describes which antifungal agent is involved in the drug-drug interaction. For one co-medicated agent, more than one row can be presented. This occurs when information on a drug interaction with an antifungal agents is presented with supposed different effects.
3. The third column describes 'Type of study'. The setup of the study is reviewed in this column. Randomized or non-randomized, Open label or blinded, cross-over or case series reports are summarized.
4. This column indicates whether healthy volunteers or patients are recruited to judge the extend of the interaction. Also the kind of patient is mentioned in this column, for instance HIV patient, renal transplant patients etc.
5. The number of patients or healthy volunteers involved in the study is reviewed. In case of multiple drugs being investigated, only the number of the patients involved in an azole interaction (including control group) is mentioned.
6. In the section 'Interaction mechanism', the most plausible mechanism is given. Some drug interactions are based on theoretical considerations, e.g. because it is known that the drugs are metabolized by the same CYP isoenzymes. When a question mark is given, no certain mechanism can be provided.
7. The column 'Effect of interaction' includes the effect of the increase or decrease in plasma concentration of the antifungal drug and/or co-medication. The name of the drug influenced is stated first followed by the pharmacokinetic parameter and a single or double arrow. A single arrow indicates an increase ranging from 0- 100% or a decrease from 0-50% in AUC or C_{max}. A double arrow indicates an increase of more than 100% or a decrease to less than 50%.
8. The section 'Advice' suggests how to deal with the interaction.
   The advice as mentioned is judged by the authors of this review. It might reflect the opinion of the authors of the published article but may also differ slightly based on interpretation from multiple studies and gathered information. Therapeutic drug monitoring (TDM) of selected azole antifungals is currently considered as an additional clinical tool since relationships have been described between plasma concentrations and efficacy and/or toxicity [203-206]. A validated assay needs to be used to determine adequate plasma concentrations and interpretation of the result should be performed by a qualified person (e.g. clinical pharmacist / clinical pharmacologist).
9. The last column ('Reference') provides the literature source that describes the drug interaction. It could be that a presented drug interaction is not supported by a literature reference. In this case, an interaction can be based on another drug interaction with a co-medicated agent that is structurally similar. In addition, the drug interaction can be based on knowledge of the metabolic pathway of the drugs involved and/or the capacity to inhibit or induce this metabolic pathway by one of the implicated drugs.

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Chapter

3.2

Omeprazole significantly reduces posaconazole serum trough level

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Cimitidine has been shown to reduce the bioavailability of posaconazole [1], and this interactive effect has also been predicted for proton pump inhibitors [2]. In our center we monitor posaconazole serum trough levels regularly in patients who receive posaconazole as salvage treatment. Serum samples are measured by liquid chromatography tandem mass spectrometry with a validated method of analysis. In a 58 years old male patient, treated for invasive aspergillosis, a significant drop of the posaconazole serum trough level was observed (figure 1).

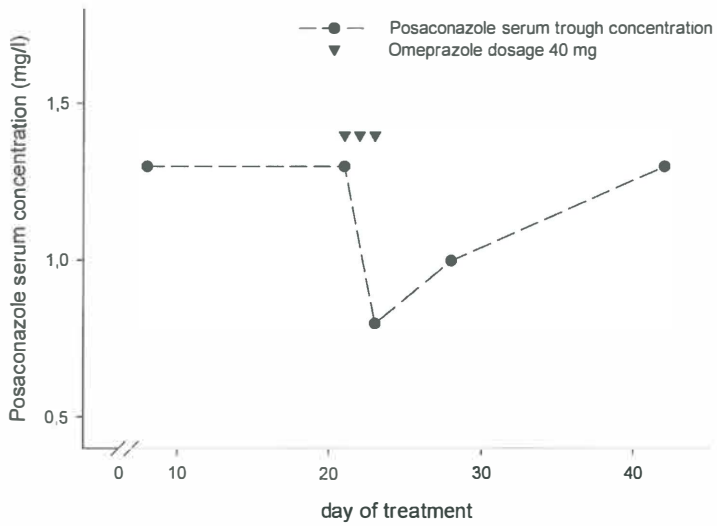

Figure 1: Posaconazole serum concentration during treatment

It appeared that omeprazole 40 mg once daily was started routinely to prevent corticosteroid induced gastrointestinal haemorrhage. Later on, because the patient had no additional risk factors for gastrointestinal haemorrhage, the omeprazole was discontinued. During continued monitoring of posaconazole concentrations the serum trough concentration rose to baseline levels again (figure 1). As the proton pump inhibitor was administered for only three days the clinical impact in this patient was likely to be limited; posaconazole tissue levels tend to be much higher than in serum and therefore adequate tissue levels may have been maintained. However, with prolonged proton pump inhibition at a higher dosage, the effect could be profound, with an inherent risk of therapeutic failure. If combination with a proton pump inhibitor can not be avoided, serum levels should be monitored at all times to evaluate posaconazole absorption, or antifungal therapy should be switched to an alternative compound.

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Chapter

3.3

Phenytoin-induced reduction of voriconazole serum concentration is not compensated by doubling the dosage

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Phenytoin is a commonly used antiepileptic agent that has also a place in the prophylaxis of seizures in patients with haematological malignancies. It is co-administered in patients receiving busulfan and in patients with meningeal involvement of haematological malignancies. As patients with these disorders encounter prolonged chemotherapy-induced neutropenia, they are at risk for bacterial and fungal infections. For the treatment of invasive pulmonary aspergillosis (IPA), one of the predominant fungal infections in these patients, voriconazole has been recommended as the first line agent [1]. However, voriconazole is extensively metabolised by CYP2C9 and CYP2C19 and, to a lesser extend, by CYP3A4 and at the same time inhibits CYP2C9 and CYP3A4. Phenytoin is widely known for inducing CYP3A4 as well as CYP2C9 and CYP2C19 activity. Besides this, it is a substrate of CYP2C9 and CYP2C19. This drug–drug interaction was recognised earlier and therefore evaluated in a prospective study in healthy volunteers [2]. This study showed that phenytoin in a dose of 300 mg once daily reduced the area under the concentration-time curve (AUC) of voriconazole by 69% given in a dose of 200 mg twice daily. From the same study appeared that a dose increase of voriconazole to 400 mg twice daily, while receiving phenytoin 300mg once daily, resulted in an AUC increase by 39%. The authors concluded that increasing the dose of voriconazole from 200 mg twice daily to 400 mg twice daily compensated for the effect of phenytoin on voriconazole AUC and this statement was incorporated in the product information [3].

In our institute, we monitor voriconazole serum trough levels regularly in patients with an invasive fungal infection. Serum samples are measured by liquid chromatography tandem mass spectrometry with a validated method of analysis. In case phenytoin is co-administrated, the dosage of voriconazole is increased to 400 mg twice daily according to the manufacturer's advice. From the hospital database (2007-2009) we retrieved three Caucasian patients (49- 57 yrs; 2 female, 1 male) with a diagnosis of malignant lymphoma who received co-medication with both agents and in whom the voriconazole and phenytoin serum concentrations were evaluated. Despite the increased dose, very low voriconazole serum concentrations (0.1 – 0.2 mg/l) were observed at steady state in all three patients. The patients had received voriconazole as empiric treatment for IPA in a dose of 400 mg twice daily and phenytoin in a dose of 300 mg daily for prophylaxis of busulfan- induced seizures in one patient, or brain metastasis in two patients. The phenytoin steady state serum concentration was 21.7 mg/l and 21.4 mg/l in two patients and 5.5 mg/l in the third patient (therapeutic range 8 - 20 mg/l). The patients received no other co-medication that could have influenced voriconazole metabolism. Evaluation of the voriconazole concentration resulted in switching phenytoin to levetiracetam in one patient, discontinuation of phenytoin because prophylaxis for busulfan-induced seizures was no longer indicated in the second, and discontinuation of voriconazole because there was no suspicion anymore for an invasive fungal infection in the third patient. CYP genotyping was performed in one patient and did not show any mutation in CYP2C9 or CYP2C19.

These three cases show that, compared to healthy volunteers, the effect of the drug-drug interaction for voriconazole and phenytoin seems to be more profound in heavily treated patients. This can possibly be explained by the large variability of pharmacokinetics of voriconazole observed in patients [4, 5] which might be caused by several mechanisms including genotypic heterogeneity for CYP2C19 that distinguishes rapid from slow metabolizers. The exposure to voriconazole in patients already having low voriconazole serum concentrations could be further reduced to almost undetectable levels by phenytoin as observed in our three patients. A similar case, describing a patient with low voriconazole levels during phenytoin treatment, has been reported before and is consitent with our observations [6].

Recently it was shown that CYP2C19*17 polymorphism resulted in significantly lower exposure after a single dose of 200 mg or 400 mg [7, 8]. Although voriconazole tends to accumulate considerably in time, the rapid metabolism of voriconazole is expected to result in significant lower voriconazole exposure. However, the extend of the effect on voriconazole steady state serum concentrations is unknown, needs to be determined at steady state. The prevalence of CYP2C19 polymorphism varies considerable [7, 8] but the difference between our patients and the healthy volunteers can not be explained solely based on CYP2C19 polymorphisms as in one patient no polymorphism was observed. Besides, in two patients high phenytoin concentrations were observed, which contradicts rapid metabolism.

In our opinion, the combination of voriconazole and phenytoin should be avoided. Levetiracetam, a newer antiepileptic agent, could be a good alternative for phenytoin in the prophylaxis of busulfan-induced seizures [9] and additionally prevents the drug-drug interaction between busulfan and phenytoin [10]. In case pre-existing epilepsy is treated with phenytoin, antifungal treatment should preferably be started with a lipid formulation of amphotericin B or anidulafungin as both are not metabolised by CYP450 enzymes. If combination of phenytoin and voriconazole can not be avoided, serum levels should be monitored at all times to evaluate voriconazole serum concentrations and adapt the voriconazol dose.

From these cases it can be concluded that a drug-drug interaction between voriconazole and phenytoin in severely ill patients can be more profound and less predictable than in healthy volunteers. Monitoring of voriconazole and phenytoin is needed, but the drug-drug interaction should be avoided whenever possible by using other combinations of antiepileptic and antifungal drugs.

COMPETING INTEREST: JWCA has received a speaking fee from Pfizer. Other authors none to declare.

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Chapter

4.1

A rapid and simple liquid chromatography-tandem mass spectrometry method for the determination of linezolid in human serum

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Abstract

Background

Linezolid is an antibiotic that is reserved as the last therapeutic line of defence when resistant micro-organisms are present.

Methods

A sensitive, robust, simple and rapid method was developed for the analysis of linezolid in serum. The method was based on the precipitation of proteins in human serum using a precipitation reagent containing an internal standard (cyanoimipramine) and subsequently, using high-performance liquid chromatography (HPLC) analysis and tandem mass spectrometry (MS/MS) detection in an electron positive mode. The method validation included selectivity, linearity, accuracy, precision and stability.

Results

The calibration curve of linezolid was linear in a range of 0.05 mg/L to 40.0 mg/L, with within-run coefficients of variation (CVs) and between-run CVs in the range of 0% to 7.1%. Linezolid was stable in human serum after three freeze-thaw cycles and for 36 hours at 4°C, at room temperature and, after sample preparation, in the autosampler without loss of product.

Conclusion

The developed method is a linear, selective, accurate and precise liquid chromatography-tandem mass spectrometry (LC-MS/MS) method and can be applied to therapeutic drug monitoring of linezolid in patients with resistant micro-organisms. The serum samples from the patient are in the range of the linearity of the method.

INTRODUCTION

Linezolid is from the oxazolidinone class of antibiotics and has shown good activity against Gram-positive micro-organisms and mycobacteria [1-3]. Its molecular structure is shown in Figure 1. It inhibits the formation of 70S subunits in bacterial ribosomes, which are essential components in the translation procedure [1]. Because of this unique mechanism of action, linezolid can be used for the treatment of resistant micro-organisms, including meticillin-resistant Staphylococcus aureus (MRSA), vancomycin-resistant enterococci (VRE) and multidrug resistant Mycobacterium tuberculosis [2-4].

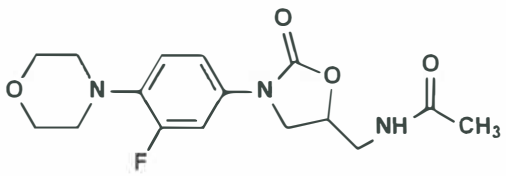

Figure 1: Chemical structure of linezolid

Worldwide resistance to first-line antibiotics is an increasing problem. Therapeutic drug monitoring (TDM) can be used for the prevention of resistance to antibiotics which are reserved as the last line of defense, such as linezolid [3, 5]. Preferably, in the Netherlands, linezolid is only used after microbiological examination or identification of the minimal inhibition concentration (MIC) of the micro-organism. An area under the concentration-time curve (AUC), over 24 hours in steady state, divided by the MIC (AUC/MIC ratio) above 100 and percentage time above the MIC (% of time >MIC) above 85 are good predictors of the development of resistance to linezolid [5, 6]. Inter-patient differences in pharmacokinetics and the frequently associated toxicity of linezolid after prolonged use makes TDM strongly recommended [5, 7]. Therefore a good analytical method for linezolid in serum is necessary.

LC-MS/MS is a commonly used method in laboratories nowadays for the analysis of many different compounds, and can also be used for the determination of linezolid in human serum.
Several determinations of linezolid in human fluids using a HPLC-UV method have been published [8-11]. In one paper, a LC-MS/MS method was described. That method required solid phase extraction cartridges for extraction and two calibration curves (from 0.1–5.0 µg/mL and 5–20 µg/mL, respectively)[10]. Solid phase extraction is an expensive method and takes a lot of time.

The objective of this study was to develop a simple, rapid and validated LC-MS/MS analysing method for an easily manageable analysis of linezolid, in human serum, without solid phase extraction. Our method was based on the precipitation of proteins in human serum with the precipitation reagent containing the internal standard (cyanoimipramine), with subsequent HPLC analysis and MS/MS detection of the fragment ions of linezolid and the internal standard.

MATERIALS AND METHODS

Materials

Linezolid was obtained from Pfizer (New York, US). The generally used internal standard, cyanoimipramine, was supplied by Roche (Woerden, the Netherlands).
Acetonitrile for LC/MS and water for LC/MS were provided by BioSolve BV (Valkenswaard, the Neth-

erlands). Methanol Lichrosolv and the other chemicals used were of HPLC or analytical grade and purchased from VWR (Amsterdam, the Netherlands).
The precipitation reagent consisted of 0.04 mg/L cyanoimipramine, the internal standard, dissolved in a mixture of methanol and acetonitrile (4:21 respectively).
Pooled human serum samples were made available according to the protocols of the University Medical Center, Groningen.

Sample preparation

In a 2.0 mL autosampler vial, 10 µL serum sample (blank serum, calibration standard, quality control sample or patient sample) and 750 µL precipitation reagent containing the internal standard were vortexed for one minute, stored at -20°C for 30 minutes to promote protein precipitation, and subsequently centrifuged at 11,000 *g* for five minutes. From the clear upper layer 5 µL was injected into the LC-MS/MS system.

LC-MS/MS analyses

All experiments were performed on a Thermo Fisher (San Jose, US) triple quadruple LC-MS/MS with a Finnigan Surveyor LC pump and a Finnigan Surveyor autosampler, which was set at a temperature of 20°C. After sample preparation, 5 µL of the clear upper layer were injected into a 100 x 2.1 mm C_{18}, 5-µm analytical column (HyPurity Aquastar, Interscience Breda, the Netherlands) for chromatographic separation. The column temperature was set at 20°C. The mobile phase had a flow of 0.2 mL/minute and consisted of an aqueous buffer (containing ammonium acetate 10 g/L, acetic acid 35 mg/L and trifluoroacetic anhydride 2 mL/L water), water and acetonitrile. The mobile phase operated as follows: 0–1 minute: buffer 5%, water 95% and acetonitrile 0%; 1–4 minutes: buffer 5%, water 95% at one minute to 0% at four minutes, and acetonitrile 0% at one minute to 95 % at four minutes; 4–6 minutes: buffer 5%, water 0% and acetonitrile 95%.
The Finnigan TSQ Quantum Discovery mass selective detector operated in electrospray positive ionization mode and performed selected reaction monitoring as scanning mode. The following mass parameters were used at a scan width of 0.5 m/z.: linezolid m/z 338.1 to m/z 296.1 (collision energy 19 eV) and cyanoimipramine m/z 306.2 to m/z 218.0 (collision energy 39 eV). The ion source spray voltage was set at 3,500 V, the sheath and auxiliary gas pressure at 35 arbitrary units (arb) and five arb respectively, and the capillary temperature at 350°C.
Xcalibur software version 1.4 SR1 (Thermo Fisher, San Jose, US) was used for peak height integration for all components.

Method validation

In accordance with the US Food and Drug Administration's *Guidance for Industry Bioanalytical Method Validation*, such validation includes the five criteria: selectivity, linearity, accuracy, precision and stability[18]. Therefore, on each analytical day, a single calibration curve was obtained and the quality control (QC) samples were analysed for all five criteria. Furthermore, six pools of blank human serum were analysed in triplicate.
For the preparation of the calibration standards and the QC samples, two stock solutions of linezolid (stock A1 and B1) were prepared by dissolving linezolid in water (1,000 mg/L). Secondary working stock solutions (stock A2 and B2) were prepared by diluting stock A1 and B1 with water.
Stock A1 and A2 were used for preparing the calibration samples by diluting them with pooled human serum to the concentrations: 0.05, 0.25, 0.50, 2.50, 10.0, 15.0, 25.0 and 40.0 mg/L, respectively.
For QC samples, concentrations of 0.05 mg/L (lower limits of quantification LLOQ)), 0.50 mg/L (low), 15.0 mg/L (medium) and 30.0 mg/L (high) were prepared by spiking stock B1 and B2 with pooled human serum. The calibration standards and QC samples were prepared on day zero and stored at – 20 °C. The QC

linezolid were 91.5%, 87.7% and 95.5% of the concentration at t = 0, respectively. For QC-level high the concentrations of linezolid were 93. 6%, 97.2% and 91.2% respectively.

Pharmacokinetic profile

The measured serum samples from a patient with a linezolid-sensitive nocardia infection are shown in Figure 3. The concentrations of linezolid are between 4.58 mg/L and 17.2 mg/L with a t_{max} of two hours, the AUC was 109.1 The MIC of the nocardia was 0.5 mg/L. The AUC/MIC ratio was 436.4.

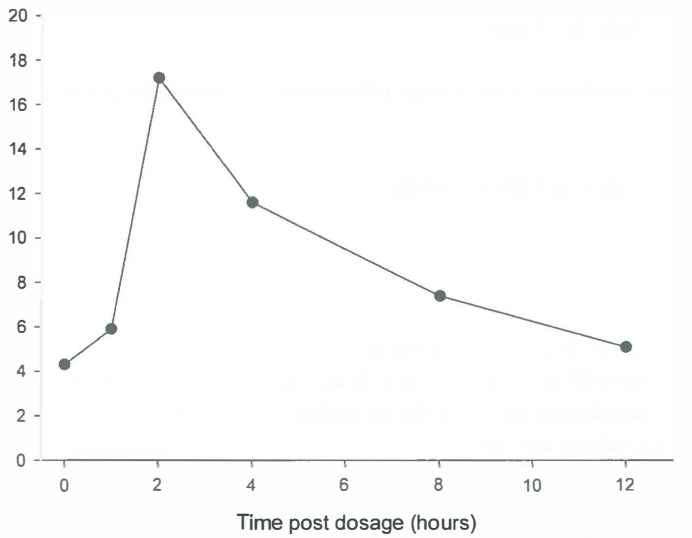

Figure 3: Pharmacokinetic profile after four doses of linezolid 600 mg twice daily by mouth

DISCUSSION

The aim of this study was to develop a simple and rapid method in which only one calibration curve and no solid phase extraction needed be used [10]. The results of the validation demonstrated that the method was selective and linear in the range of 0.05 mg/L to 40.0 mg/L. Our results were consistent with the US Food and Drug Administration's Guidance for Industry Bioanalytical Method Validation, which states that all values for accuracy and precision should be less than 15%, except for LLOQ, in which case a value of 20% can be accepted [11]. The tests for determining the stability of linezolid in human serum demonstrated that the linezolid samples are stable for at least three freeze-thaw cycles, for 36 hours at the bench top and in the refrigerator at + 4°C and, after sample preparation, in the autosampler.

Because of the possibility of a large concentration range in samples taken from patients, there is a risk of carryover when a low concentration is measured after measuring a sample with a high concentration. Carryover is a problem which cannot be totally eliminated, but it should be reduced to an acceptable range [12]. The reinjection procedure is acceptable, because of the expectation that most of the samples contain a concentration higher than 0.50 mg/L. This was confirmed by the measured serum concentrations from the patient with a linezolid sensitive nocardia infection.

During the validation, cyanoimipramine was used as internal standard. Cyanoimipramine has the advantage that it is never used as a drug in patients. Nevertheless, other suitable internal standards may also be used for the same method, after complete validation.

CONCLUSION

In summary, a selective, linear, accurate and precise method, which can be used for the determination of linezolid in human serum, has been developed.

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Conflict of interest: Authors declare no conflict of interest.

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Chapter 4.2

Comparison of pharmacokinetics and tolerability of two dosage regimens of linezolid in MDR/XDR –TB patients

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Clinical Pharmacokinetics. Accepted

ABSTRACT

Background and objectives

For the treatment of multi – and extensively drug resistant tuberculosis (MDR-TB and XDR-TB), new potent drugs are urgently needed. Linezolid (ZYVOXID®) is a promising drug, but its use limited by toxicity using 600 mg twice daily at prolonged administration. In order to reduce its toxicity and maintain efficacy, we investigated whether linezolid in a reduced dosage resulted in drug serum concentrations exceeding the *in vitro* MIC and AUC_{0-24h}/MIC ratio > 100.

Methods and Patients

This open-label prospective pharmacokinetic study evaluated two dosages (300mg and 600mg) of linezolid in the treatment of MDR-TB patients, who received linezolid as part of their treatment. They received 300 mg of linezolid bid, 3 days later followed by 600 mg bid. Serum samples taken at predefined intervals were processed by a validated LC-MS/MS procedure. At steady state of each dosage an 12-h pharmacokinetic study for linezolid was performed. Pharmacokinetic parameters were evaluated using the KINFIT (MWPharm 3.60; Mediware, the Netherlands). The AUC_{0-24h}/MIC ratio was used as a predictive model for efficacy. Toxicity of linezolid was evaluated by clinical and laboratory assessments including peripheral neuropathy.

Results

Eight patients were included in this study. The median treatment duration with linezolid was 56 days (range 34 – 100 days) with a median cumulative dose of 51,000 mg (range 32, 400 – 75,600 mg). The median AUC_{0-12h} was 57.6 mg*h/l (IQR 38.5 – 64.2 mg*h/l) with 300 mg, and 145.8 mg*h/l (range 101.2 – 160.9 mg*h/l) with 600 mg dosage. The AUC_{0-24h}/MIC ratios were 450 (IQR, 343 – 514) with 300 mg, and 1151 (range: 656 – 1500) with the 600 mg dosage. Linezolid was well tolerated.

Conclusion

As the AUC_{0-24h}/MIC ratio was at least 100, the dose of linezolid of 300 mg twice daily was effective in all patients. Larger numbers of patients should be studied, to confirm the efficacy of the 300 mg twice daily in MDR-or XDR-TB treatment.

INTRODUCTION

Multi-drug resistant tuberculosis (MDR-TB) is a major global health threat [1]. Resistance is caused by consecutive periods of inadvertent mono therapy, resulting from inconsistent or partial treatment, non-compliance, wrong treatment regimens, malabsorption, unreliable drug supply, or primary transmission of resistant bacteria. With decreasing numbers of drugs to which the causative micro-organisms appear susceptible, the treatment of MDR-TB becomes less and less effective, resulting in an increased chance to fail on drug treatment. Moreover, the morbidity increases with prolonged exposure to toxic second- and third-line drug regimens. Because of the extension of the treatment period, often accompanied by hospitalization, also the financial costs increase significantly [2]. The treatment of MDR-TB can be optimized if it is tailored to the *in vitro* susceptibility profile of causative bacteria [3-6]. This becomes increasingly important, since no new drugs have been registered for use in TB treatment since the early eighties. Linezolid, though not registered for TB may be a promising drug for the treatment of MDR-TB as it has a high antibacterial activity (0.125 – 0.5 mg/l) against *M. tuberculosis* (including first-line drug resistant isolates)[7-9]. Linezolid 600mg b.i.d. has been evaluated as initial treatment in a limited number of MDR-TB or XDR-TB patients in the USA (New York, n=7, 2004-2007), China (Hong Kong, n=5, 2005-2008), Spain (Madrid, n=5, 1999-2004), Norway (Oslo , n=10, 1998-2002), Korea (Seoul, n=24, 2007-2008) and the Netherlands (Haren, n=12, 2002-2004) [10-15]. This drug may be an important asset in the treatment of MDR-TB combined with other agents. However, severe adverse effects like anaemia, thrombocytopenia and peripheral neuropathy limit its use. The mechanism of toxicity has not been completely elucidated, but it is likely that linezolid induces an immune-mediated platelet destruction, as this condition responds favourably to intravenously administered immunoglobulin [16]. In addition, the toxic neuropathy may be related to interference with mitochondrial function. This effect might be similar to the toxic effect of chloramphenicol [17]. Therefore, monitoring of toxicity has been proposed as a safeguard in the use of linezolid in TB treatment. Indicators in this surveillance should be: cessation of medications interacting with serotonin prior to start of linezolid, twice weekly hematological and liver function monitoring, assessment of serum lactate, and routine ophthalmologic and neurological assessment if the treatment would be continued beyond 28 days [18]. As the MIC of drugs against *Mycobacterium tuberculosis* and other *Mycobacterium* spp. is often low, dose reduction might therefore be justified to reduce toxicity in these patients [10]. However, the results of small series with linezolid 600 mg once daily instead of twice daily were not consistent in preventing adverse effects [10, 19]. In an earlier study total drug exposure expressed as AUC was found the most relevant pharmacokinetic (PK) variable, besides T > MIC, to assess efficacy of linezolid treatment against different types of bacteria, including *M tuberculosis* [20-25]. Linezolid showed excellent activity at an AUC/MPC_{90} ratio of 116[25]. As PK parameters may vary among different patient groups, intensive monitoring of PK in MDR-TB is essential to optimize treatment in this patient group. In this study we tested the hypothesis that linezolid in a reduced dosage of 300 mg twice daily results in drug concentrations exceeding the MIC and AUC_{0-24h}/MIC ratio > 100.

PATIENTS, MATERIALS AND METHODS

This open-label prospective pharmacokinetic study evaluated two dosages (300mg and 600mg) of linezolid in the treatment of MDR-TB patients. A full pharmacokinetic curve was recorded at steady state after a minimum of three days of consecutive treatment with the same dose. This study was conducted at the Tuberculosis Centre Beatrixoord (University Medical Center Groningen, University of Groningen, Haren, The Netherlands). The study protocol was approved by the local institutional ethics committee. Written informed consent was obtained from the patients.

Study subjects

Patients ≥18 years of age admitted with MDR-(and XDR-)TB were eligible for inclusion if their attending physician had decided to start linezolid treatment. Exclusion criteria were allergy for linezolid or any of the excipients, or concomitant treatment with MAO-A or MAO-B inhibitors [26].

Bacteriologic assessment

The isolates were subjected to laboratory diagnosis at the Dutch National Mycobacteria Reference Laboratory (National institute for Public Health and the Environment; RIVM). The Middlebrook 7H10 agar dilution method was applied for drug susceptibility testing of the isolate(s)[27].

Drug administration

All patients enrolled in the study received linezolid tablets in combination with other antituberculosis drugs. None of the patients had received linezolid prior to the study. The dosage of linezolid administered to each patient was 300 mg twice daily for a minimum of 3 consecutive days at 08:00 and 20:00 h in order to reach steady state serum concentrations ($t_{1/2}$ = 5-7 h) [26]. After obtaining the blood samples for assessing the AUC_{0-12h} of the 300 mg dosage, the dosage was increased to 600 mg twice daily. As food does not influence the AUC the participants were allowed to take a light breakfast after drug ingestion [28]. Based on the AUC_{0-24h}/MIC ratio > 100 the dosage was reduced to 300 mg twice daily. Adherence to the treatment regimen was 100% as the patients were in a directly observed treatment in-patient program.

Pharmacokinetic assessment

Blood samples (4 ml) were drawn into vacutainer tubes (ref 369032, Becton and Dickinson, Plymouth, UK) before a dose of linezolid was administered (t = 0) and 1, 2, 4, 8, 12 h after administration of the dose. All samples were obtained at steady state which is 3 days after the initiation of linezolid treatment [29]. Serum was separated and frozen at -20°C until processed.

Analytical methods

Serum concentrations of linezolid were determined at the Laboratory for Clinical Toxicology and Drugs Analysis of the department of Hospital and Clinical Pharmacy of the University Medical Center Groningen using a validated liquid chromatography tandem mass spectrometry (LC-MS/MS) assay [30].

Pharmacokinetic analysis

Pharmacokinetic parameters were evaluated using the KINFIT (MWPharm 3.60; Mediware, the Netherlands) [31]. C_{max} was defined as the highest observed serum concentration with T_{max} as corresponding time. C_{min} was the serum concentration at 12 h after intake of the dose. The $AUC_{0-12\ h}$ was calculated using the trapezoidal rule from 0 up to 12 hours. The elimination half-life ($t_{1/2}$) was calculated by 0.693/k_e. The apparent clearance of the drug (Cl) was calculated by dose/AUC_{0-12h} and the volume of distribution (V_d) was calculated by Cl/ke. As the AUC_{0-12h} is determined at steady state, we estimated that the AUC_{0-24h} was calculated by doubling the AUC_{0-12h}. The AUC_{0-24h}/MIC ratio was calculated for each dose enabling to calculate an adequate dose for treatment continuation.

Toxicity

Toxicity of linezolid was assessed by evaluation of peripheral neuropathy at baseline and once a weekly during linezolid treatment. The Vibrameter (Medic, Norway) was used to measure elevated thresholds for detection of stimuli as these may be the first sign of drug induced peripheral neuropathy. Perception threshold and disappearance threshold were measured by one physical therapist

[32] in threefold at the metacarpal bone of the index finger, the flat surface of the proximal part of the tibia and the lateral malleolus. The patients were also questioned for numbness, burning, involuntary muscle contractions (cramp) and weakness in the legs when walking. At baseline and after one month a full ophthalmologic assessment was performed. The assessment included the best corrected visual acuity (Snellen acuity Chart), a colour vision test (Hardy-Rand-Rittler), examination of the media and fundus in mydriasis. All of the tests were performed for each eye separately, and under the same conditions. In case of bad performance or deterioration compared to baseline measurements, there was the opportunity to perform visual field testing (Static automated Humphrey perimetry) and an extended colour test (Farnsworth D-15-Hue). Haemoglobin level, renal and hepatic function were evaluated at least once a week. Renal toxicity was defined as an increase in serum creatinine of 25% from baseline. Hepatic dysfunction was defined as an increase in liver enzymes three times above the upper level of normal (ULN).

RESULTS

Study subjects

Eight patients were included in this study. Patient characteristics at baseline are presented in table 1. All patients were referred to our Center for treatment of either MDR-TB or XDR-TB. M. tuberculosis culture were positive in all of them. By definition, all patient isolates were resistant to at least isoniazid and rifampicin. Besides, resistance to second line drugs (injectables – streptomycin / amikacin / capreomycin - or one of the quinolones) was observed in 4 of the 8 patients; one patient had XDR-TB. The median age of the 8 patients was 28 years (IQR, 26 – 38 years). The median weight of the patients was 58.3 kg (IQR, 52.7 - 62.8 kg) measured at the time of the pharmacokinetic assessment (+/- 3 days).

Table 1. Baseline demographics

Parameter	(n = 8)
Age (yrs) median (IQR)	28 (26 - 38)
Gender (male/female)	4/4
Weight (kg) median (IQR)	58.3 (52.7 – 62.8)
BMI (kg/m2) median (IQR)	20.8 (19.8 – 24.3)
Co-morbidity	
DMII	1/8
Alcohol abuse	1/8
Resistant to first line drugs	8/8
Resistant to second line drugs	6/8
Resistant to number of drugs mean (range)	5 (3 - 8)

BMI, body mass index; DMII, diabetes mellitus

Pharmacokinetic study

The linezolid concentration-time curves of both regimens are displayed in figure 1. The pharmacokinetic parameters of the two dosage schedules of linezolid are summarised in table 2. None of the patients had received co-medication (e.g. rifampicin) that could have influenced the pharmacokinetics of linezolid[33, 34]. The median $AUC_{0\text{-}12h}$ of linezolid was 57.6 mg*h/l (IQR, 38.5 – 64.2 mg*h/l) for the 300 mg schedule and 145.8 mg*h/l (IQR, 101.2 – 160.9 mg*h/l) for the 600 mg schedule respectively. The geometric mean ratio of the $AUC_{0\text{-}12h}$ of linezolid in a dosage 600 mg and 300 mg is 2.6 (range 1.9 – 4.2).

Table 2: Steady-state pharmacokinetics of linezolid

Parameter	Linezolid dose			
	300 mg		600 mg	
AUC0–12h (mg*h/liter)	57.6	(38.5 – 64.2)	145.8	(101.2 – 160.9)
Cmax (mg/liter)	9.5	(7.7 -10.1)	20.4	(16.3 - 21.9)
Cmin (mg/liter)	1.9	(0.6 - 2.2)	5.8	(2.7– 6.8)
Tmax (h)	1.2	(0.5 – 1.2)	1.4	(0.8 – 1.4)
t1/2 (h)	5.6	(3.0 – 6.4)	5.8	(4.7 – 6.0)
CL (liter/h)	6.2	(3.2 – 7.0)	4.2	(2.7 – 4.6)
Vd (liters)	33.6	(26.8 – 36.1)	29.4	(23.3 – 31.7)

Median values (IQR) of the following parameters are shown in the table: AUC0–12h, area under the concentration–time curve from 0 to 12 h; Cmax, maximum concentration of drug in serum; Tmax, time to maximum concentration of drug in serum; t1/2, half-life; CL, total clearance; Vd, volume of distribution.

The values for AUC_{0-24h}/MIC are shown in table 3. The median value (IQR) for the AUC_{0-24h}/MIC of the 300 mg dosage is 452 (IQR, 343 - 513) and 1151 (IQR, 656 - 1500) for 600 mg. The linezolid serum concentration was always above the MIC when given in either a dose of 300 or 600 mg twice daily.

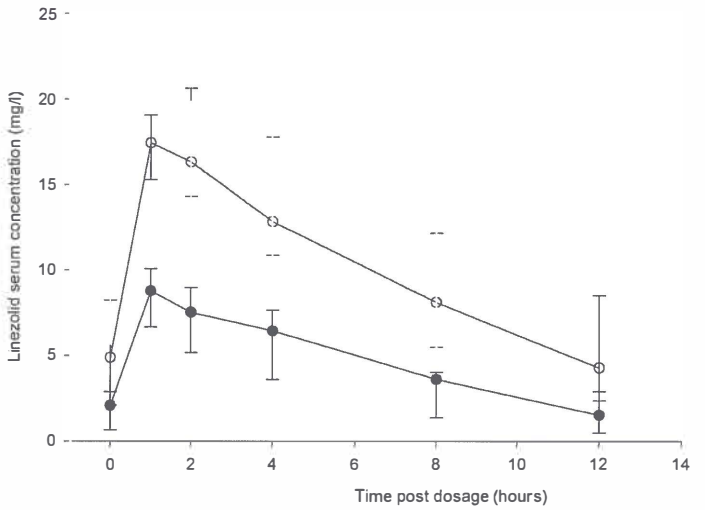

Figure 1: Pharmacokinetic curve of linezolid 300mg and 600mg twice daily.

Median (IQR) serum concentration-time curves of linezolid in a dosage of 300mg twice daily (open circles) and 600mg twice daily (solid circles) for all study subjects (n=8).

Table 3: AUC0-24h/MIC ratio of linezolid at 300 mg and 600 mg

		Linezolid dose					
		300mg			600mg		
Patient	MIC90 (mg/l)	AUC0-12h (mg*h/L)	AUC0-24h/MIC	T > MIC (%)	AUC0-12h (mg*h/L)	AUC0-24h/MIC	T > MIC (%)
1	0.25	83.4	667	100	155.9	1247	100
2	0.5	41.8	167	100	86.6	347	100
3	0.25	50.2	402	100	94.7	757	100
4	0.25	63.6	509	100	181.0	1448	100
5	0,25	65.9	527	100	131.9	1055	100
6	0.125	27.8	445	100	115.6	1850	100
7	<0.125	28.7	460	100	103.4	1654	100
8	1.0	46.2	92	100	176.0	352	100

MIC_{90}, Minimum Inhibitory Concentration required to inhibit the growth of 90% of organisms; AUC_{0-12h}, Area under the concentration curve 0-12h post dosage; AUC_{0-24h}/MIC, Area under the concentration curve 0-24h post dosage over the MIC; T > MIC, time exceeding the MIC.

Based on the AUC_{0-24h}/MIC, a dose reduction was performed in all patients. The median treatment duration with linezolid was 56 days (range 34 – 100 days) with a median cumulative dose of 51,000 mg (range 32,400 – 75,600 mg). The cumulative dose was given in a dose of 300 mg and 600 mg twice daily for a median period of 35 days (range 14 – 70 days) and 12 days (range 6 – 31 days) respectively.

Toxicity

Linezolid was well tolerated. None of the patients had signs or symptoms suggesting significant adverse effects attributable to linezolid. Neither an increase of serum creatinine of 25% from baseline, nor a significant increase of liver enzymes was observed during treatment. The median haemoglobin level after one month of treatment with linezolid was 7.8 (IQR, 7.5 – 8.9), which was not significantly lower (P = 0,34) compared to baseline (median value of 8.3; IQR, 7.0 – 9.0).

The results of the vibration sense threshold measured at the metacarpal bone, tibia and malleolus are displayed in figure 2. No increase in thresholds for detection of stimuli was observed. No numbness, muscle contractions (cramp) and weakness in the legs when walking were mentioned by the subject. Two patients experienced a burning sensation, which was already present at baseline in one patient.

All patients had a normal visual acuity (Snellen 1.0-1.5) and perfect colour vision in both eyes. There were no signs of optic neuropathy. None of the patients had to be referred for visual field testing, or a D-15 Hue colour test. One patient showed some background diabetic retinopathy, which was stable. One patient developed a transient hyperemic conjunctiva in the left eye, which did not seem to be related to the MDR-TB or any medication. Hypromellose 0.3% eye drops three times daily were prescribed successfully.

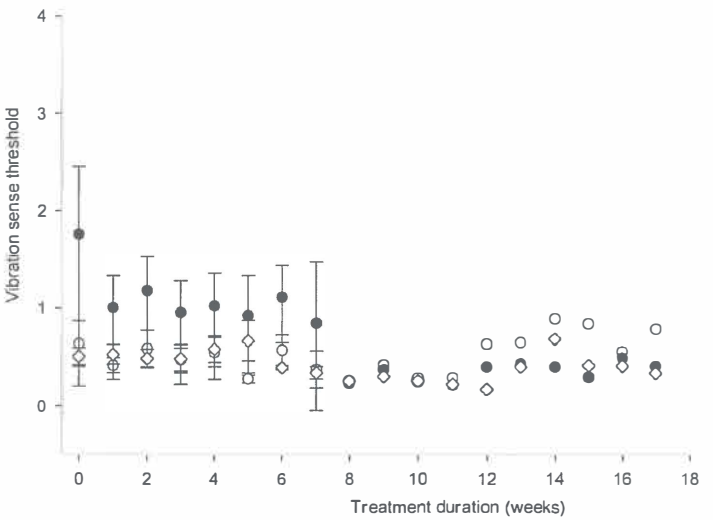

Figure 2: Vibration sense threshold during linezolid treatment

The mean (± S.E.) values of the vibration threshold are shown in figure 2 for the metacarpal hand bone (open circles), the tibia (solid circles) and the lateral malleolus (open diamantes) for all the subjects (n=8). Only one subject was treated for more than 7 weeks and therefore no error bars are available.

DISCUSSION

This is the first study in MDR-TB (and one with XDR-TB) patients to show that dose limitation of linezolid to 300 mg bid results in drug concentrations well above the MIC, with AUC_{0-24h}/MIC value exceeding 100. This finding suggests that dose reduction may be used to prolong treatment with linezolid with sustained efficacy and with limitation of toxicity.

The pharmacokinetic parameters of anti-tuberculosis drugs are known to be influenced by various patient-specific parameters, as well as drug-drug interactions. It is therefore important to study pharmacokinetics when new drugs are evaluated in the treatment of tuberculosis. To our knowledge no studies have addressed pharmacokinetic parameters of different dosage schemes of linezolid in MDR-TB patients in any systematic way.

In a recent study, linezolid in a dose of 600 mg once or twice daily was evaluated for early bactericidal activity [35]. The pharmacokinetics of the 600 mg twice daily showed similar results for AUC_{0-12h} with a value of 116.4 mg*h/l (range 50.4-197.2 mg*h/l) compared to the findings in our study of 145.8 mg*h/l (range 86.6 – 181.0 mg*h/l). Comparing the AUC_{0-24h} of 96.9 mg*h/l (range 47.8- 143.7) of linezolid 600 mg once daily to the AUC_{0-24h} of 115.2 mg*h/l (range 55.6 – 166.8 mg*h/l) of linezolid 300 mg twice daily a trend towards higher AUC values can be observed. This is in accordance with a value more than two times higher AUC_{0-24h} values of linezolid 600 mg twice daily than once daily [35]. Apparently, the clearance of linezolid is concentration dependent. In a case series

Compared to a recent study, evaluating the C_{max} (mean 11.6 mg/L; range 1.5–15.9 mg/L) and C_{min} (mean 2.1 mg/L; range 0.4–4.5 mg/L) of 300 mg once daily, the variability of these pharmacokinetics parameters in our study of 300 mg twice daily was smaller (table 2) but values were comparable[15]. This confirms that pharmacokinetic values vary among patients, which urges the need for individualized dosing of this toxic drug.

In vitro linezolid appeared to have a short post antibacterial effect of four hours against *M. tuberculosis* [36]. How this observation has to be interpreted in relation to total drug exposure expressed as AUC being the most relevant PK variable to assess efficacy of linezolid treatment is unclear. Although not powered, no difference was observed in early bactericidal activity between 600mg once or twice daily [35]. Due to a limited sample size and lack of prospective studies evaluating efficacy of linezolid treatment the question how to dose linezolid in an optimal manner should be addressed in future studies [13]. In any case, the fact that linezolid is given together with other anti-tuberculosis drugs complicates the evaluation of the efficacy of individual drugs.

In the limited sample series, once daily dosing did not result in reduced toxicity compared to twice daily dosing of 600 mg of linezolid [19]. However, this study was not powered to detect differences in toxicity and the treatment duration is extremely prolonged beyond its licensed use of 28 days. In our study linezolid was given in a dose of 300 mg twice daily. The cumulative dose is the same compared to 600 mg once daily, but high peak levels are avoided. The toxicity in our study was very low. This could be explained by a relatively low cumulative dose compared to the previously published case series. Our hypothesis that toxicity could be related to peak levels can therefore not be evaluated. However, linezolid in a dosage of 300 mg once daily for a longer period showed a lower toxicity compared to 600 mg once daily[15]. In future studies on the toxicity of linezolid, the large inter patient variability of AUC values and cumulative dose should be taken into account when toxicity is evaluated.

The benefit of linezolid should be weighed against the potential risks of adverse effects [37]. Appropriate frequent monitoring of adverse effects is a conceivable option. Observational data on different dosage regimes of linezolid are necessary to further evaluate the relation between dose and adverse effects.

CONCLUSION

In conclusion, our study shows that the dose of linezolid in 300 mg twice daily results in AUC_{0-24h}/MIC ratios > 100 and the concentration is always above MIC. This dosage scheme can be used in a randomised prospective study evaluation both efficacy and toxicity of linezolid in a reduced dose.

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CONFLICT OF INTEREST: none to declare

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Chapter

4.3

Limited sampling strategies for therapeutic drug monitoring of linezolid in MDR-TB patients

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Therapeutic Drug Monitoring. Accepted

ABSTRACT

Background

Linezolid is a potential drug for the treatment of multidrug resistant tuberculosis but its use is limited because of severe adverse effects like anaemia, thrombocytopenia and peripheral neuropathy. This study aimed to develop a model for the prediction of linezolid area under the plasma concentration-time curve from 0 to 12 hours (AUC_{0-12h}) by limited sampling strategy to enable individualised dosing.

Patients and Methods

Fourteen MDR-TB patients received linezolid twice daily as part of their anti-tuberculosis treatment. Linezolid concentrations were determined at steady state by high-performance liquid chromatography tandem mass spectrometry before and at 1, 2, 4, 8 and 12 hours after dosing. Linezolid AUC_{0-12h}, population model and limited sampling models were calculated with MWPharm software. The correlation between predicted linezolid AUC_{0-12h} and observed linezolid AUC_{0-12h} was investigated by Bland-Altman analysis.

Results

A total of 26 pharmacokinetic profiles were obtained. The median AUC_{0-12h} was 51.8 (IQR 41.8 – 65.9) mg*h/l at 300 mg, and 123.8 (IQR 100.9 – 152.5) mg*h/l at 600 mg both twice daily. The clinically most relevant model for prediction of linezolid AUC_{0-12h} was by using a linezolid trough level ($r = 0.91$, prediction bias = -2.9 % and root mean square error (RMSE) = 15 %).

Discussion

The difference between choosing a trough concentration and two to three samples increased the correlation from 0.90 to 0.95, but appeared not clinically relevant as it did not result in a different dosage advice.

Conclusion

This study showed that linezolid AUC_{0-12h} in MDR-TB patients could be predicted accurately by minimal sampling strategy and can be used to individualize the dose.

INTRODUCTION

Multi-drug resistant tuberculosis (MDR-TB) is a major concern for global health [1] and its treatment is less effective compared to first line regimens for susceptible TB. There is a paucity of new highly active anti-TB agents, and now with the emerging threat of failing second class agents as well (extensively drug resistant, or XDR-TB) there is a dire need to explore the optimal use of agents that have already reached the market.[2-4]

Therefore optimization of MDR-TB treatment, based on *in vitro* susceptibility profile of causative bacteria, is crucial.[5-8] Linezolid has a high in-vitro antibacterial activity (MIC 0.125 – 0.5 mg/L) against *M. tuberculosis*[9, 10] and has been evaluated as initial treatment in a limited number of MDR-TB patients.[11-15] The potential benefit of this drug is limited because of severe adverse effects like anaemia, thrombocytopenia and peripheral neuropathy. In a large German survey evaluating outcome of MDR-TB patients, treatment with linezolid in a dose of 600 mg twice daily was interrupted in 19 of 25 patients and not reintroduced in 11 of the 19 patients.[16] As the MIC of linezolid against *M. tuberculosis* is often low and total drug exposure expressed as AUC to MIC ratio was found the most relevant pharmacokinetic-pharmacodynamic parameter[17-21], dose reduction might be justified to reduce toxicity in these patients.[11, 22] Prospective evaluation of linezolid dose based on serum concentration in relation to the MIC of *M. tuberculosis* isolated in these patients is warranted to fully explore the potential benefits and risks of this agent. Since obtaining a full pharmacokinetic curve is time consuming, expensive, and represents yet another burden to these already harassed patients, limited sampling seems warranted to enable AUC/MIC ratio based dosing. Therefore the aim of this study was to develop a model best fitted for the prediction of linezolid area under the plasma concentration-time curve from 0 to 12 hours by limited sampling strategy.

PATIENTS AND METHODS

Pharmacokinetic data from MDR-TB patients, who received linezolid 600 mg twice daily as part of their treatment at the Tuberculosis Center Beatrixoord (University Medical Center Groningen (UMCG), University of Groningen, Haren, The Netherlands) were used for this study. This open-label prospective pharmacokinetic study was approved by the Medical Ethical Committee of the UMCG and all patients gave informed consent.

Linezolid (ZYVOXID®, Pfizer, USA, NY) blood samples were collected at steady state, which was at least three days after initiation of treatment [23]. Blood samples were taken immediately before administration and at 1, 2, 4, 8, and 12 hours after the morning dose of linezolid. Patients were allowed to take a light breakfast after drug ingestion as food does not influence the AUC[24]. Linezolid serum concentrations were measured by a validated high-performance liquid chromatography tandem mass spectrometry method.[25] The AUC values from 0 to 12 hours after linezolid intake (trapezoidal rule), the apparent clearance of the drug (Cl), volume of distribution (Vd) and elimination half life ($t^{1/2}$) were calculated using the 'KINFIT module' using the software package MWPharm version 3.60 (Mediware, Groningen, The Netherlands). C_{max} was defined as the highest observed serum concentration with T_{max} as corresponding time. C_{min} was the serum concentration at 12 h after intake of the dose. The minimum AUC_{0-24h}/MIC ratio for effective treatment was defined to be 100.[17-21] Figure 1 shows AUC/MIC ratios between a range of AUC and MIC values. This figure may be used to assess whether a dosage reduction or escalation is feasible to achieve an AUC/MIC ratio of 100.

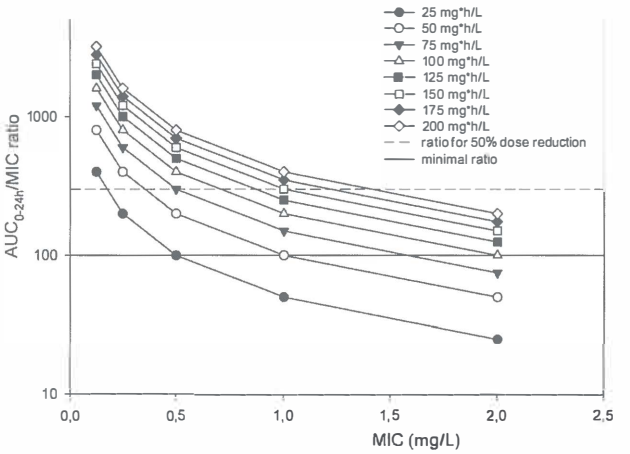

Figure 1: AUC0-24h/MIC ratio's at different AUC0-12h and MIC's.
Symbols in the legend represent AUC0-12h values

Limited Sampling Models

The 'KINPOP module' of MWPharm was used to develop a population one-compartment model (POP-PK) with first-order absorption pharmacokinetics without lag time and was generated from the linezolid dosing of 600 mg twice daily, the body surface area of the patients, and following linezolid serum concentration using an iterative two-stage Bayesian procedure.[26] Pharmacokinetic parameters were assumed to be distributed log-normally, and as the patients received linezolid only orally the bioavailability was fixed at 1, which was adapted from an earlier study.[27] Limited sampling models (LSM) were calculated using Monte Carlo data simulation. Single, double, triple, quadruple blood sampling time points ranging from 0 to 12 hours with a 30 minute interval were evaluated. We considered models, using time points with a maximum time span between the first and the last sample to be four hours, suitable for daily practice. These samples can be obtained before and/or after administration of linezolid during a regular visit at an outpatient clinic. LSM were considered reliable for estimation of the AUC as the predictive bias defined by the percentage mean prediction error was < 5% and the precision defined by percentage root mean square error (RMSE) was < 15%.

Statistics

Differences between the pharmacokinetic parameters of the two different dosages were calculated with the Mann Whitney *U* test. The correlation between the AUC_{0-12h} based on LSM and observed linezolid AUC_{0-12h} was tested by means of a Bland and Altman analysis. The POP-PK model was cross validated by developing a POP-PK model based on n-1 and by predicting the AUC of the subject left out during the model development. The predictive performance of the POP-PK model for the AUC_{0-12h} of linezolid in a dosage of 300mg twice daily was tested using the pharmacokinetic data obtained from 14 patients receiving 300 mg twice daily.[28] The AUC calculated by the population model and measured AUC were compared with the Spearman correlation coefficient. All statistical procedures were performed using SPSS 16.1 (SPSS, Chicago, Ill, USA).

RESULTS

Fourteen MDR-TB patients were referred to our Center for treatment. *M. tuberculosis* culture was positive in all of them, with a median MIC for linezolid of 0.25 (range 0.125 - 1) mg/L. Their median age was 28 (IQR 24 – 36) years and body mass index was 20.1 (IQR 18.5 – 20.9) kg/m^2. After obtaining the blood samples at 600 mg twice daily (steady state) treatment was continued with 300 mg twice daily. Linezolid was well tolerated and no serious adverse events occurred.

From these patients 12 linezolid concentration time curve were obtained at 600 mg twice daily and 14 at 300 mg twice daily (figure 2). The median AUC_{0-12h} was 50.2 (IQR 41.8 – 65.9) mg*h/l at 300 mg twice daily, and 123.8 (IQR 100.9 – 152.5) mg*h/l at 600 mg twice daily. The steady state pharmacokinetic parameters are shown in table 1. The geometric mean ratio of the AUC_{0-12h} of linezolid in a dosage 600 mg and 300 mg was 2.3 (range 1.6 – 4.1). In a dosage of 600 mg of linezolid twice daily, drug clearance was slightly but non-significantly lower (P = 0.25) compared to a dosage of 300 mg. Also, half-life of linezolid in a dosage of 600 mg twice daily (5.5 (range 4.7 – 6.6) h) was slightly but non-significantly longer (P = 0.25) compared to linezolid in a dose of 300 mg twice daily (4.4 (3.0 – 5.4) h).

Table 1: Steady-state pharmacokinetics of linezolid

Parameter	Linezolid dose	
	n = 12	n = 14
	600 mg	300 mg
AUC_{0-12h} (mg*h/L)	123.8 (100.9 – 152.5)	51.8 (41.8 – 65.9)
C_{max} (mg/L)	17.8 (15.9 – 21.9)	8.7 (7.1 – 10.1)
C_{min} (mg/L)	4.4 (2.7 – 7.5)	1.7 (0.9 – 2.5)
T_{max} (h)	1.2 (0.8 – 2.5)	0.9 (0.8 – 4.0)
$t_{1/2}$ (h)	5.5 (4.7 – 6.6)	4.4 (3.0 – 5.4)
CL/F (L/h)	3.7 (2.5 – 4.8)	5.1 (3.3 – 6.4)
V_d/F (L)	27.6 (23.3 – 31.4)	29.4 (23.4 – 37.7)

Median values (IQR) of the following parameters are shown in the table

AUC_{0-12h}, area under the concentration–time curve from 0 to 12 h; C_{max}, maximum concentration of drug in serum; T_{max}, time to maximum concentration of drug in serum; $t_{1/2}$, half-life; CL, total clearance; V_d, volume of distribution.

Population pharmacokinetic model parameters based on the 600 mg concentration time curves are shown in table 2.

Table 2. Population pharmacokinetic model parameters

Parameter	Mean (± SD)
CL (L/h/1.85 m2)	6.10 ± 1.87
Vd (L/kg LBMc)	0.65 ± 0.025
ka (/h)	0.939 ± 0.419
F	1 (fixed)

CL, apparent clearance; Vd, volume of distribution; Ka, absorption rate constant; F, oral bioavailability

The cross validation showed that the median values of the POP-PK models (n-1) were CL 6.20 (IQR 5.82 – 6.25) L/h/1.85 m^2, V_d 0.668 (IQR 0.655 – 0.675) L/kg LBMc, Ka-po 1.049 (IQR 0.999 – 1.213) /h, which was not different from the POP-PK model. The AUC values of the subject left out in each run of the analysis were underestimated with a median percentage of 0.85 (range 0.09 – 4.0) and RMSE = 3.5%. The AUC values of linezolid in a 300 mg dosage based on the concentration time curves were overestimated with a median percentage of 4.6 (range 1.7 – 10.0) and RMSE = 3.5 %. Limited sampling models that were suitable for daily practice are shown in table 3. As the pharmacokinetic parameters were obtained at steady state the concentration immediately before intake was equal to the concentration 12 hours after dose administration. In figure 3, the Bland-Altman analysis, the agreement between predicted linezolid $AUC_{0\text{-}12h}$ with the POP-PK model and observed linezolid $AUC_{0\text{-}12h}$ is shown.

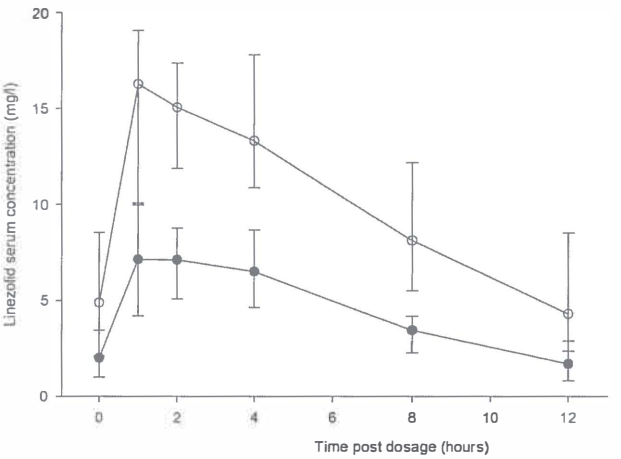

Figure 2: Pharmacokinetic curve of linezolid 300mg and 600mg twice daily.

Median (IQR) serum concentration-time curves of linezolid in a dosage of 300mg twice daily (solid circles; n = 14) and 600mg twice daily (open circles; n = 12) for all patients.

Table 3: Limited sampling models

Time point of sampling	r	Prediction bias (percentage)	RMSE (percentage)
0 h	0.91	-2.9	15
0 h, 11 h	0.92	-4.4	14
0 h, 11.5 h	0.92	-4.4	14
0 h, 12 h	0.92	-4.5	14
0 h, 11 h 11.5 h	0.93	-5.1	13
0 h, 11 h, 12 h	0.93	-5.2	14
0 h, 8 h, 11.5 h	0.93	-5.7	14
0 h, 11 h, 11.5 h, 12 h	0.94	-5.7	13
0 h, 8 h, 11 h, 11.5 h	0.94	-6.0	13
0 h, 9 h, 11 h, 11.5 h	0.94	-6.0	13

Based on the population model, the AUC of the 300mg dose could be predicted using the linezolid concentrations obtained from the 300 mg concentration time profiles. When the concentrations of linezolid immediately before oral intake of a 300 mg dose are used in the POP-PK model, the individual AUC values at a dose of 300 mg were overestimated with a median percentage of 11.4 (IQR 2.1 – 22.0) % and RMSE = 8.9 %. The geometric mean AUC/MIC ratios were 720 (range 347 – 2880) at 600 mg dose and 235 (range 92 – 829) at 300 mg dose. The geometric mean predicted AUC/MIC ratio at the 300 mg dose was 227 (range 121 - 714), which was not significantly different from the calculated ratio (P = 0.92).

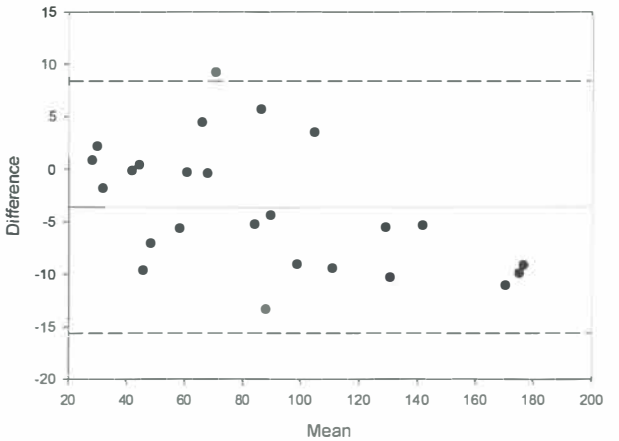

Fig. 3. Bland-Altman plot of calculated linezolid AUC0-12h vs predicted linezolid AUC0-12h.
The solid line represents the mean difference; the dashed lines represent the 'limits of agreement' (mean difference ± 2 SD difference)

DISCUSSION

This study demonstrated that individualized dosing of linezolid based on limited sampling and using a population pharmacokinetic model with acceptable precision and bias is feasible. This model can be used in a prospective pharmacokinetic study to evaluate an altered linezolid dosing scheme to minimize the risk of toxicity while maintaining efficacy. The time points used for limited sampling are convenient for out-patients clinics and are also suitable for monitoring other antituberculous drugs that can be determined in the same samples.[29, 30] There was a trend to prolonged elimination half life and lower clearance of linezolid at a dosage of 600 mg twice daily compared to linezolid at a dosage of 300 mg twice daily. Although this was not statistically significant, the trend we found is consistent with earlier observations that linezolid inhibits its own metabolism.[31] Therefore the $AUC_{0\text{-}12h}$ of linezolid in a dosage of 300mg twice daily is slightly overestimated using the POP-PK model based on the 600 mg dosage. Compared to earlier data obtained from 9 TB patients our data showed similar median values for pharmacokinetic parameters but less variability in C_{max} and $AUC_{0\text{-}12h}$.[32] Despite the observed variability, dose individualisation can be performed using limited sampling in combination with a POP-PK model.

The POP-PK model was first developed with lag-time. However, this resulted in a mean lag-time of 0.999 ± 0.00007 hour and a mean k_a of 878 ± 726 /h, which is identical to an intravenous administration. Leaving out the lag-time resulted in a more realistic model.
We used cross validation for our POP-PK model instead of prospective validation with additional patients because linezolid is a third line antituberculous drug and is not frequently used because of its toxicity and costs. Although we used the second best method of validation for our POP-PK model the results are consistent and RMSE is below 5%.
Because linezolid is a third line anti-tuberculosis agent, treatment should be based on *in vitro* susceptibility of the isolated bacteria. Generally, *in vitro* susceptibility data are available within two to three weeks. Linezolid could therefore be started in a dosage of 600 mg twice daily and the $AUC_{0\text{-}12h}$ could be estimated using limited sampling. When the MIC becomes available the $AUC_{0\text{-}24h}$/MIC ratio can be calculated and a favourable dose estimated with the population model. The RMSE of the POP-PK model that we developed is 15% when using only the trough level. A trough level is very useful for therapeutic drug monitoring as it can be easily obtained without burden for the patient.
This implicates that the 95% confidence interval ranges from 0.7 to 1.3 of the actual AUC value. The $AUC_{0\text{-}24h}$/MIC ratio is therefore influenced by this variation. However, the regular dose of 600 mg twice daily will only be changed per approximately the half or quarter of a tablet. Smaller changes in the dosage require the pharmacy to prepare capsules from the 600 mg tablets available from the manufacturer. To prevent underdosing, the dosage should be only changed if the $AUC_{0\text{-}24h}$/MIC ratio after dosage adjustment is over 130. The smallest dosage reduction being a quarter of a tablet would imply that a dosage reduction can be performed in case of an $AUC_{0\text{-}24h}$/MIC ratio of at least 175. Taking into account the non-proportional decrease in AUC of 2.3 between 600 and 300 mg the ratio should be at least 200. Treatment can subsequently be continued with a more appropriate dose. In figure 1, a potential 50% dose reduction can be observed if the $AUC_{0\text{-}24h}$/MIC ratio is above the solid line (300).

With our population model we showed that the AUC/MIC ratio, using the estimated AUC based on a trough level, did not differ significantly from the calculated ratio. We showed that the difference between choosing a trough concentration and two to three samples is not clinically relevant resulting in an increase of correlation increased from 0.90 to 0.95, but not leading to a different dosage advice. Therefore, this model can be used in a prospective randomised study evaluating the efficacy and toxicity of linezolid, comparing individualised treatment of linezolid to linezolid in a dose of 600 mg twice daily. It can also be used to guide treatment in individual patients experiencing linezolid toxicity without the risk of subtherapeutic drug concentrations.

CONCLUSION

This study showed that linezolid $AUC_{0\text{-}12h}$ in MDR-TB patients could be predicted accurately using limited sampling and by the developed POP-PK model. The predicted $AUC_{0\text{-}12h}$, based on a trough level, can be used to individualize treatment.

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Chapter

5.1

Determination of moxifloxacin in human plasma, plasma ultrafiltrate and cerebro spinal fluid by a rapid and simple liquid chromatography-tandem mass spectrometry method

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ABSTRACT

Moxifloxacin (MFX) is a useful agent in the treatment of multi-drug-resistant tuberculosis (MDR-TB). In Tuberculosis Centre Beatrixoord, a referral centre for TB in the Netherlands approximately 36 percent of the patients received MFX. Based on the variability of MFX AUC, the variability in *in vitro* susceptibility to MFX of *M. tuberculosis* and the variability of penetration in to sanctuary sites, measuring the concentration of MFX in plasma and CSF could be recommended. Therefore a rapid and FDA-validated liquid chromatography-tandem mass spectrometry analysing method with a simple pre-treatment procedure is developed for therapeutic drug monitoring of MFX in human plasma and CSF. Because of the potential influence of protein binding on efficacy, we decided to determine both bound and unbound (ultrafiltrate) MFX to plasma protein. The calibration curves were linear in the therapeutic range of 0.05-5.0 mg/L plasma and CSF, with variation coefficients (CV) in the range of -5.4%-9.3%. MFX ultrafiltrate samples could be determined with the same method setup for analysis of MFX in CSF. The LC/MS/MS method developed in this study is fully validated according to the FDA guidelines and suitable for monitoring of MFX in human plasma, plasma ultrafiltrate and CSF.

INTRODUCTION

Moxifloxacin (MFX; figure 1), one of the newer fluoroquinolones, has a broad spectrum of antimicrobial activity and is used for treating bacterial infections of the respiratory tract and soft tissues [1]. From *in vitro* and *in vivo* experiments appeared bactericidal activity of MFX against *Mycobacterium tuberculosis* [1-3], which was high in comparison with other fluoroquinolones and is equal to or greater than isoniazid [1-3]. Based on these results MFX is more frequently introduced, as second-line agent, in the treatment of tuberculosis (TB) in case of resistance or intolerance to first line agents like rifampicin, isoniazid, pyrazinamide and ethambutol [4-5]. In Tuberculosis Centre Beatrixoord, a referral centre for TB in the Netherlands 36 percent of the patients received MFX, which accounts for approximately 33 patients each year (2006 – 2008). For the treatment of TB in sanctuary sites, like Tuberculosis meningitis, MFX seems also promising. Data from *in vivo* studies and humans suggest that MFX penetrates in cerebrospinal fluid (CSF) [4;6-9], but an inter- and intra individual variable CSF penetration of MFX was observed [7-9].

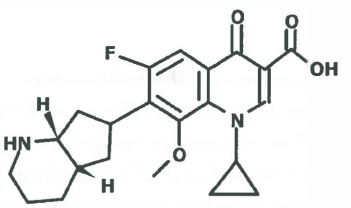

Figure 1. Chemical structure of moxifloxacin

Like other fluoroquinolones, the most predictive parameter for efficacy of treatment with MFX is the ratio of the area under the concentration curve (AUC) to the minimal inhibitory concentration (MIC) [10]. This ratio is based on a study with an aerosol infection model of tuberculosis in BALB/c mice and can be used to estimate the adequate dose for treatment continuation or therapeutic drug monitoring (TDM) [10]. Both the AUC and the MIC value can be variable. Because the AUC of MFX is subjected to variability caused by drug-drug interaction of MFX with rifampicin (RIF) the AUC/MIC ratio can be influenced significantly [11, 12]. Measuring plasma concentrations can therefore be warranted in patients co-medicated with RIF. Despite the high activity of MFX against *M. tuberculosis*, based on the methoxy group at the C-8 position [13, 14], increased *in vitro* MIC's have been observed [15]. An higher MIC has a major effect on the AUC/MIC ratio, limiting the use of MFX in a dosage of 400 mg once daily. Therefore a higher dosage might be necessary in the treatment of multi drug resistant TB (MDR-TB). A daily dose of 600-800 mg is desirable to kill *M.tuberculosis* and prevent resistance [16]. However, experience with long-term clinical use of this dose is, still not available [16, 17]. As the efficacy of an antimicrobial agent is based on the unbound concentration of that drug, and therefore the variability in protein binding can influence the $AUC_{unbound}$/MIC ratio. Human data regarding this subject is lacking but animal data showed intra individual concentration depended variability in protein binding [10] and *in vitro* data showed that antimicrobial activity is influenced by plasma protein binding [18].

Considering the variability in MFX AUC, variability in *in vitro* susceptibility to MFX of *M. tuberculosis*, potential influence of protein binding on efficacy and variability of penetration into sanctuary sites, a prospective observational pharmacokinetic study is mandatory to explore the effect on the treatment of TB with MFX as part of an anti-tuberculosis regimen. For this purpose, a simple and rapid method of analysis has to be developed for the determination of MFX (bound and unbound) in plasma, in CSF, complying with ICH-guidelines.

The most commonly used method of analysis for the determination of MFX in human plasma or CSF uses high performance liquid chromatography followed by fluorescence or ultraviolet detection [19-22]. However, these methods require liquid-liquid extraction and are therefore time consuming. To our knowledge there is a paucity of data on methods of analysis using LC/MS/MS for the determination of MFX in human plasma or CSF. Only one method is described using Solid Phase Extraction (SPE) as sample pre-treatment procedure for the determination of MFX in plasma [23]. As SPE is expensive and time consuming, the objective of this study was, to setup a simple, rapid and validated LC/MS/MS method of analysis for the determination of MFX in human plasma, plasma ultrafiltrate and CSF.

EXPERIMENTAL

Chemicals and reagents

MFX HCl was kindly provided by Bayer (Bay-12-8039). The internal control, cyanoimipramine, was supplied by Roche (Woerden, The Netherlands).

Acetonitrile and water for LC/MS were purchased from BioSolve (Valkenswaard, The Netherlands). The chemicals used, including methanol Lichrosolve and trifluoroacetic anhydride, are of HPLC or analytical grade and were obtained from VWR (Amsterdam, the Netherlands). The precipitation reagent consisted of 0.04 mg/L cyanoimipramine dissolved in a mixture of methanol and acetonitril (4:21, v/v). Pooled human plasma (ultrafiltrate) samples with EDTA as anticoagulant and pooled human CSF samples were made available according to the standard operating procedures of the University Medical Center Groningen.

Preparation of stock solutions

Different stocks were prepared for the calibration standards and the quality control (QC) samples by dissolving MFX HCl in water. The stock solutions were diluted with the same solvent to obtain stock solutions for the validation of MFX in plasma and CSF at concentrations of 100 mg/L (calibration standards; Stock C and E) and 200 mg/L (QC samples; Stock D and F), respectively. The original stock solutions were diluted to working stock solutions at concentrations of 5 mg/L (calibration standards; Stock C1 and E1) and 10 mg/L (QC samples; Stock D1 and F1), respectively. For the QC samples of MFX in plasma ultrafiltrate, the same stock solutions were used as applied for the QC samples in plasma (Stock D and D1).

Sample pre-treatment procedure for plasma ultrafiltrate

A volume of 500 µL controlled human plasma was transported into a Centrifree® Ultrafiltration device with an Ultracel® YM-T membrane for volumes up to 1.0 ml (Millipore, Germany). The devices were capped afterwards. The membrane had a hold up volume of 10 µL and an active surface area equal to 0.92 m^2. The devices were placed in a 35 degree fixed angel rotor (Hettich EBA 21) and spun at 1,640 *g* for 20 minutes at room temperature.

Preparation of calibration standards and quality control samples

The calibration standards and QC samples were prepared by spiking controlled human edetate plasma and human CSF with the appropriate amount of one of the eight made stock solutions. In the same way QC samples of MFX in controlled human plasma ultrafiltrate were prepared. The theoretical concentrations are displayed in table 1. A maximum of five percent of the final volume consisted of the added stock solution. The calibration standards and QC samples were prepared at day one of validation and stored at -20°C.

Sample preparation

The samples were thawed until reaching room temperature. A volume of 750 µL precipitation reagents, to promote protein precipitation, was added to 100 µL of each sample (blank sample, calibration standard, QC sample) and vortexed for 1 minute. The samples were then stored at -20 °C for at least 15 minutes to promote protein precipitation and finally centrifuged at 11,000 *g* for five minutes. From the clear upper layer, 5 µL was injected in the LC/MS/MS system.

LC/ESI-MS/MS conditions

All experiments were performed on a Thermo Fisher (San Jose, US) triple quadruple LC/MS/MS with a Finnigan Surveyor LC pump and an autosampler, which was set at a temperature of 20 °C. After sample preparation, 5 µL of the clear upper layer was injected on a Thermo Electron 50 x 2.1 mm HyPurity C_{18}, 5-µm analytical column (Interscience Breda, The Netherlands) for chromatographic separation. The column 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 trifluoracetic anhydride 2 mL/L water), which is maintained constant at 5% during the gradient (table 2).

The Finnigan TSQ Quantum Discovery mass selective detector was utilized for detection of the positive ions (ESI) because of the presentation of amine and ketone groups in the MFX structure. These groups could be easily protonated [23]. Selected reaction monitoring (SRM) was used as scanning mode. With a scan width of 0.5 m/z the following mass parameters were measured: MFX m/z 402.0 to m/z 358.2 (collision energy 19 eV) and cyanoimipramine m/z 306.0 to m/z 218.0 (collision energy 39 eV).
The ion spray voltage, sheath gas pressure, auxiliary gas pressure and capillary temperature were set at 3,500 V, 35 arb (arbitrary units), 5 arb and 350 °C, respectively.
Peak height integration of all components was achieved by Xcalibur software version 1.4. SRI (Thermo Fisher, San Jose, US).

Method validation

The developed method was fully validated in accordance with the US Food and Drug Administration's *Guidance for Industry Bioanalytical Method Validation*. Selectivity, linearity, accuracy, precision, sensitivity, recovery and stability are the seven criteria included in this guidance. Precision was subdivided into within run and between run [24].

During three days, each day a single calibration curve was analysed. The calibration curves were constructed using a 1/x weighted linear regression of the ratios of the observed peak heights of MFX and the internal control cyanoimipramine against the spiked concentrations of the standards. The concentration of a sample of unknown concentration (patient samples) will then be determined based on one-point-calibration (highest calibration point). Quality control samples were analyzed on three single days in fivefold. Selectivity was evaluated by processing and analyzing six different pools of controlled human plasma and human CSF. Sensitivity will be examined by comparison the response of respectively six lots of pooled human plasma and six lots of human CSF with the response of Lower Limit of Quantitation (LLQ) samples. To evaluate interaction of MFX with other anti tuberculosis drugs, which can be co-medicated with MFX, a simultaneous determination of these agents with MFX will be done by using the developed method. A blank sample spiked with concentrations in the middle of the therapeutic range of ethambutol (5 mg/L), linezolid (10 mg/L), rifampicin (10 mg/L) and isoniazid (5 mg/L) will be used.

During three days respectively a sample with a concentration of 10 mg MFX/L plasma and a sample with a concentration of 10 mg MFX/ L CSF was diluted ten times and then prepared in fivefold. Finally, each day, the five freshly prepared samples were measured.

Recovery was determined on three levels (LOW, MED, HIGH) in fivefold and measured by comparing the average peak height of the processed QC samples to the average peak height of the recovery samples. Recovery samples (LOW, MED, HIGH) were spiked in water instead of controlled human plasma or CSF (QC samples). A plasma or CSF sample after the precipitation procedure is comparable to water. The recovery also was processed for the internal control, using the average peak height of the internal control.

Stability tests included three freeze-thaw cycles and storage stability. Storage stability consists of storage in the refrigerator (4 °C), at room temperature (20-25 °C) and after sample preparation in the autosampler (20 °C). Stability at room temperature was measured with and without the influence of light, because of the known light-sensitiveness of MFX [25]. Autosampler stability is included in connection with the opportunity to re-inject earlier measured samples. All stability tests were performed on two levels (LOW and HIGH) in fivefold, during three days. Stability is defined as a change of concentration and should be ≤15%.

To investigate ion suppression or ion enhancement, simultaneously post-column infusion of a solution of MFX and cyanoimipramine and first injection of five samples precipitation reagents without internal control followed by five samples controlled human plasma (prepared with precipitation reagent without internal control), five samples controlled human plasma (prepared with precipitation reagent with internal control cyanoimipramine), five samples controlled human plasma (prepared with precipitation reagent with internal control cyanoimipramine) and two samples of the highest calibration point (5 mg/L), respectively. The same experiment is done with human CSF instead of plasma.

RESULTS

Sample Carry Over

When high concentrations were analysed during method development, sample carry over was observed. Sample carry over is defined as a residue of a previous sample in the current measurement [26-28] . Carry over was greater than 0.05-0.1% by samples with a concentration higher then 0.50 mg/L. It was decided to re-inject patient samples with a measured concentration below 0.50 mg/L. Prior to this re-injection, blank samples were injected (precipitation reagent) until no peak of MFX was observed. Five blank samples appeared to be enough to prevent sample carry over.

Internal control

As in our sample preparation no extraction is preformed, an internal control in stead of an internal standard is suitable. Cyanoimipramine is used as an internal control by our laboratory for all validation procedures of many drugs for TDM. As long as the results of our method validation comply with ICH criteria for method validation this compound can be used. The advantage of using a single agent in the laboratory is that only one precipitation reagent is available and therefore mistakes are prevented. Moreover, cyanoimipramine has never been used as drug in patients. The fact remains, other suitable

internal controls, like structural analogue lomefloxacin, may also be used for the same method, after complete validation.

Ion suppression and ion enhancement

During post-column infusion no ion suppression or ion enhancement was observed. There was no variation of baseline height between precipitation reagent (blank) and pooled human plasma with and without internal control, respectively.

Chromatography

The mean retention times of MFX and cyanoimipramine were 1.58 and 1.81 minutes, respectively. An example of the chromatographic results is shown in figure 2.

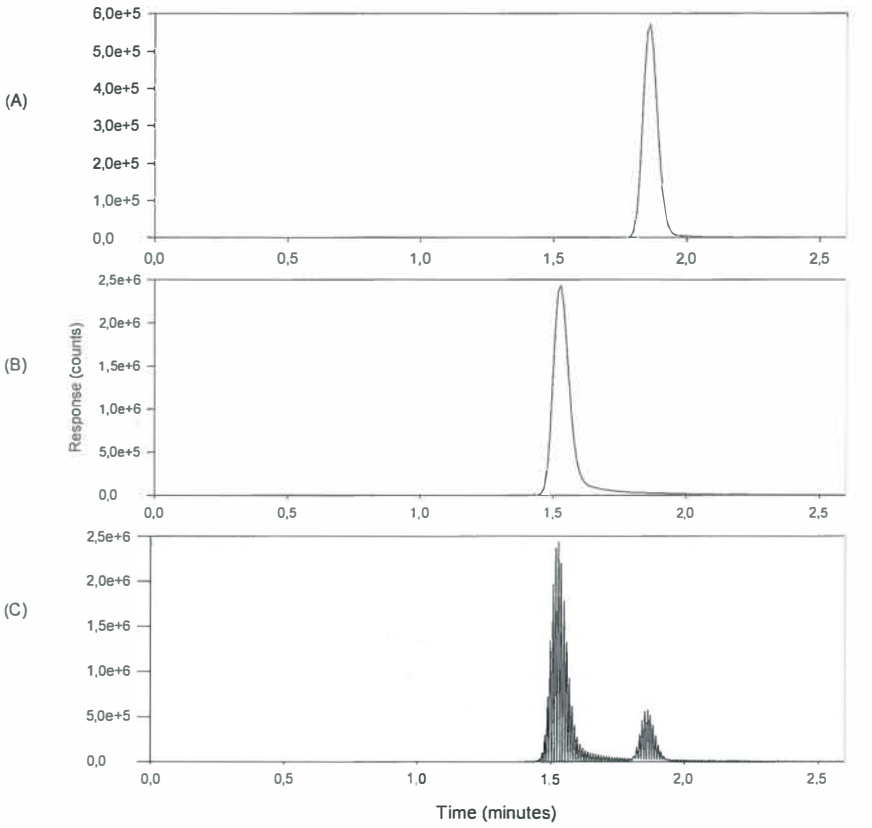

Figure 2. Chromatogram of cyanoimipramine (internal control)(a + c) and moxifloxacin (b+c)

Selectivity and sensitivity

There were no interfering peaks, peaks at the retention time of MFX or cyanoimipramine, observed in six lots of human plasma or CSF, respectively. There also were no interfering peaks observed by simultaneous determination of ethambutol, linezolid, rifampicin and isoniazid. The analyte response at the LLOQ is greater than five times the blank responses.

Linear regression

The calibration curves were linear over a range of 0.05 to 5.00 mg/L MFX, encompassing the therapeutic range of MFX in plasma and CSF by MDR-tuberculosis [16]. Using a weight factor of 1/x, the mean equations of the plasma (Eq.1) and CSF (Eq.1) standards were equal to:

(Eq.1); $y = 0.7623\ x + 0.00062$

The mean correlation- and determination coefficient were greater than 0.990; 0.999 and 0.998 respectively.

(Eq.2); $y = 0.4558\ x - 0.00158$

The mean correlation- and determination coefficients were greater than 0.990; 0.997 and 0.993 respectively. Variation coefficients of plasma and CSF calibration standards were measured in range of -5.4 to 9.3%.

Concentrations above the calibration curve

The mean concentration of the diluted plasma samples was equal to 1.0 mg/L (undiluted: 10 mg/L) with a bias of 4%. The within run and between run CV were 2.9% and 3.7%, respectively. The mean concentration of the diluted CSF samples was equal to 1.0 mg/L (undiluted: 10 mg/L) with a bias of -2%. The within run and between run CV were 3.0% and 2.2%, respectively.

Accuracy and precision

The responses of the analysed QC samples (LLOQ, LOW, MED and HIGH) were statistically compared and calculated by using one-way ANOVA for each single concentration level. Results of these statistical calculations are shown in table 3. All coefficients of variation correspond to the rules of the FDA, namely CV and bias < 20 percent for the LLOQ; CV and bias < 15 percent for concentration levels LOW, MED and HIGH [24].

For this method, the mean LLOQ of the plasma samples was equal to 0.05 mg/L with an accuracy of 7.1%, and an overall Coefficient of Variation of 4.8%. The mean LLOQ of the CSF samples was equal to 0.05 mg/L with an accuracy of 2.8%, a within-run CV of 6.9% and a between-run CV of 3.2%. In some cases an F-test gave no significant difference in between run and within run precision. Then the overall coefficients of variance need no tot to be subdivided into between and within run precision. In these cases only the overall coefficient of variation is noted.

Determination of the QC plasma ultrafiltrate samples with the method setup for determination of MFX in CSF seems possible according the coefficients of variation, which al correspond to the earlier mentioned rules of the FDA.

Recovery

The recoveries ranged from 114.2% till 124.8%, depending on the concentration MFX and the matrix type (table 4). The internal control had a recovery of 96.5% and 100.2% for the plasma en CSF samples, respectively. Al coefficients of variation were less then fifteen percent.

Stability

The results of the stability test are summarized in table 5. The concentration of MFX will be viewed in comparison to the mean concentration of freshly prepared QC samples on two levels (LOW and HIGH).

After three freeze-thaw cycles, the stability of the QC samples (LOW and HIGH) was not affected. For 120 hours storage at refrigerator (4 °C), at room temperature and after sample preparation in the autosampler (20 °C), stability of QC samples (LOW and HIGH) is also not affected. Exposing to or protection from light made no significant difference on stability of the QC samples, during 120 hours.

Clinical application

This method was implemented in routine monitoring of MFX in TB patients suspected to have low AUC/MIC ratio and in patients with TB meningitis. In figure 3 a MFX concentration time curve is shown of a patient with TB meningitis receiving MFX in a dose of 400 mg once daily as a part of the anti tuberculosis regimen. The MIC for MFX of the isolate was 0.125 mg/L. The AUC was 36.1 mg*h/L in plasma and 21.9 mg*h/L in CSF following a dose of MFX of 400 mg once daily. The AUC/MIC ratio was 289 for plasma and 175 for CSF. The fraction unbound was equal to 0.72 and as a result the estimated $AUC_{unbound}$/MIC was 208. Treatment was continued and the patient was cured after 9 months of treatment.

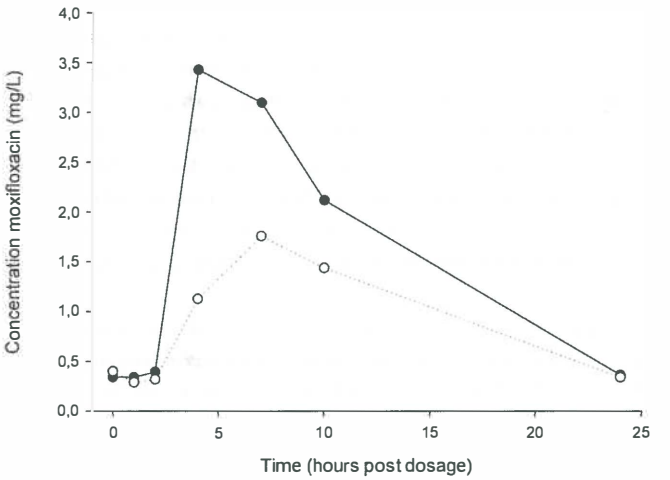

Figure 3. Typical patient curve in plasma and CSF

MFX plasma concentrations are represented by solid circles and CSF concentrations by open circles.

DISCUSSION

The objective of this study was, to setup a simple, rapid and validated LC/MS/MS analysing method for TDM of MFX in human plasma and CSF without time consuming and expensive solid phase extraction. The developed method is fully validated based on the rules of the US Food and Drug Administration's Guidance for Industry Bioanalytical Method Validation [24].

The slope of the calibration curve of MFX for plasma was different from that of CSF. In both matrixes, there was no ion-enhancement or -suppression observed during post-column infusion. Without using an internal control for calculating the slopes an equal difference between them was found. The sensitivity of the LC/MS/MS is more or less variable, which is acceptable as long as quality control samples are analysed in the same run.

The observed sample carry over, which is a common problem on LC/MS/MS [26-28], could not be entirely eliminated. By re-injection of a calculated amount < 0.05 mg/L, this problem can be reduced to an acceptable level.
However, in daily practice concentration levels are expected to be higher and re-injection will not have to apply frequently. As no interference was observed with other anti tuberculosis drugs the method of analysis was suitable for TDM in daily practice.

Because a plasma or CSF sample after the precipitation procedure is comparable with water, comparing of the average peak height of the QC samples in plasma or CSF with the average peak height of the QC samples in water corresponds to recovery in our developed LC/MS/MS method. However, a recovery of MFX of 114.2%-124.8% from plasma and CSF is remarkable. The authors don't have an explanation for this phenomenon. However, according to the rules of the FDA, the recovery of the analyte needs not to be 100%. The recovery of the analyte and the internal control should be consistent, precise and reproducible [24]. In this case the coefficients of variations comply with the FDA guidelines.

Variability in protein binding has potential influence on efficacy of an antimicrobial agent, as the unbound fraction of the drug can pass membrane and interact with the bacteria. For exploring the unbound drug AUC/MIC ratio, determination of MFX in plasma ultrafiltrate seems necessary. Setup and validation of an individual method for the determination of MFX in plasma ultrafiltrate is very time consuming, based on the collection of ultrafiltrate. A matrix comparison showed that the full validated method for determination of MFX in CSF was suitable to determine MFX in plasma ultrafiltrate. However, one has to keep in mind that the collection of ultrafiltrate has to be reproducible. For example, pH [29] and temperature control [29, 30] during ultrafiltration influences percentage (un)bound drug.

The developed method of analysis is suitable for measuring MFX in plasma, CSF and plasma ultrafiltrate as is shown by the presented patient concentration time curve. The LLOQ of 0.05 mg/L is low enough to measure the unbound fraction of a MFX plasma sample obtained before the dose.

Recently, MFX was compared with the anti tuberculosis drug ethambutol in the initial treatment of tuberculosis in a randomised controlled trial [31]. As MFX resulted in earlier sputum compared to ethambutol, the position of this drug will probably expand. Considering the variability in MFX AUC, variability in *in vitro* susceptibility to MFX of *M. tuberculosis*, potential influence of protein binding on efficacy and variability of penetration in to sanctuary sites a simple, rapid and validated LC/MS/MS analysing method for analysis is mandatory to evaluate MFX in prospective studies and perform therapeutic drug monitoring.

CONCLUSION

A simple, rapid and validated LC/MS/MS analysing method for the analysis of MFX in human plasma and CSF was developed. As no analytical interaction with other co medication was observed, this method can be used for prospective pharmacokinetic studies and TDM of MFX in patients with Tuberculosis.

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Chapter

5.2

Evaluation of Moxifloxacin for the treatment of Tuberculosis: 3 years of experience from a TB referral centre in The Netherlands

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In Preparation

ABSTRACT

Background

Moxifloxacin (MFX) is a useful agent in treatment of tuberculosis. However, optimal dose has not yet been established and long term safety data are limited.

Methods

To evaluate safety and pharmacokinetic/pharmacodynamics parameters, we reviewed the medical charts of tuberculosis patients treated at Tuberculosis Centre Beatrixoord, The Netherlands between the 1st of January 2006 and the 31st of December 2008, receiving MFX 400 mg once daily as a part of their TB treatment. Hepatic and renal function, gastrointestinal side effects and QT prolongation were evaluated to assess the safety of MFX. Efficacy was predicted based on the AUC_{0-24h}/MIC ratio.

Results

Ninety two patients met the inclusion criteria and received a median dose of 6.9 (interquartile range (IQR) 6.0-8.2) mg MFX/kg for 76.5 (IQR; 29 - 197.3) days. Geometric mean total and unbound MFX AUC_{0-24h} were evaluated in a subset of patients (n=18) and were 26.7 (range 8.5 – 72.4) mg*h/L and 22.3 (range 12.3 – 64.4) mg*h/L. Geometric mean AUC/MIC ratio's were 163 (range 13 - 579) for bound and 120 (range 10 - 515) for unbound MFX. Rifampicin (RIF) was administered in 75% of the patients. MFX was very well tolerated. Adverse effects that occurred during treatment were vomiting (n=2), diarrhoea (n=6) and *Clostridium difficile* (n=2) but let to discontinuation in only 2 cases (vomiting and diarrhoea). Upon evaluation for risk factors for QT prolongation, thirty five patients had at least one additional risk factor, nineteen patients had two additional risk factors, one patient had three risk factors and one patient had four risk factors. Despite these risk factors for QT prolongation, it was not observed.

Conclusions

These data show that MFX in a dose of 400 mg once daily was well tolerated for a prolonged period. Considering the variability in unbound AUC_{0-24h}/MIC ratio, as a result of variability in protein binding, AUC_{0-24h}, MIC and influence of co-medication with RIF, therapeutic drug monitoring could be recommended. Additional safety measures regarding hepatic and renal function and QT prolongation do not seem mandatory.

INTRODUCTION

Moxifloxacin (MFX) is a fluoroquinolone with an *in vivo* and *in vitro* bactericidal activity against *Mycobacterium tuberculosis*, which is comparable to or greater than that of isoniazid[1-3]. Besides for pulmonary tuberculosis (TB) it seems also a useful drug in the treatment of TB in sanctuary sites, including osteomyelitis and meningitis[4-6]. Like for other fluoroquinolones, the area under the concentration time curve (AUC) to the minimal inhibitory concentration (MIC) has proven to be the best parameter to predict *in vivo* efficacy against gram-negative bacteria and *M.tuberculosis*[7-9]. Based on theoretical calculations, a daily dose of 600-800 mg MFX should be recommended for optimal killing of the isolate and suppression of drug resistance [10], which is higher than the currently approved daily dose of 400 mg once daily. As the efficacy of the treatment is influenced by plasma protein binding, the exposure to unbound MFX should be taken in to account[8, 11].
Due to its high activity against *M tuberculosis*, MFX is more frequently prescribed for TB, but one should keep in mind that the drug is labelled for that indication. Therefore special attention should be paid to drug-drug interactions and safety. The most important drug interaction is the interaction of MFX with rifampicine (RIF), resulting in a decrease of MFX exposure of 27-31%[12, 13]. Some mineral supplements or antacids decrease the bioavailability of MFX[14-16], but a daily dose of 400 mg MFX could be administered safely with food and calcium supplements, without dosage adjustments[17, 18]. The first evaluation of prolonged treatment with MFX in a daily dose of 400 mg MFX in thirty eight patients with complicated TB appeared well tolerated [19]. In our opinion a larger study is needed to confirm long term safety. Safety data to support switching to the suggested higher dose is scarce[4, 20, 21].

The major concern for prolonged treatment is that adverse effects will result in decreased compliance resulting in drug resistance.The adverse effects of MFX, like vomiting, diarrhoea and *Clostridium difficile* infection[22], could influence the tolerability of prolonged treatment. A dangerous adverse effect of MFX is the potency to aggravate QT prolongation[20, 23, 24]. Therefore ECG monitoring is recommended in patients treated with MFX, with a normal heart rate and other pre co-existing risk factors for QT prolongation[23].

To evaluate safety and pharmacokinetic/pharmacodynamic parameters of MFX in TB treatment we reviewed the medical charts of TB patients.

MATERIALS AND METHODS

A retrospective chart view was performed for all patients receiving MFX as part of their TB treatment for at least five days (steady state) [25] between 1st of January 2006 and 31st of December 2008 at the Tuberculosis Centre Beatrixoord, University Medical Centre Groningen, The Netherlands. Demographic data was collected from the medical chart, including age, sex, weight, length, ethnicity, co-morbidity, type of diagnosis, localisation of TB, MIC, resistance pattern, medical history, dose and duration of MFX treatment, dose and duration of (tuberculosis) co-medication, MFX-induced adverse effects, dose and days of treatment..

Pharmacokinetics/pharmacodynamics

When available, MFX concentrations in plasma, plasma ultrafiltrate and CSF samples were determined by a validated LC/MS/MS method. Samples were eligible for evaluation when obtained at steady state, which was after at least five days of treatment [25]. The area under the concentration-time curve up to 24 hours post dosage ($AUC_{0\text{-}24h}$) for plasma and CSF was determined with a standard non-compartmental pharmacokinetic method using the KINFIT module of MW Pharm 3.60 (Mediware, The Netherlands). The $AUC_{0\text{-}24h}$ was calculated according the log-linear trapezoidal rule. For a low (<1.0 mg/L) and a high MFX concentration (>1.0 mg/L) of each plasma curve in, the concentration of MFX in plasma ultrafiltrate was determined. The mean unbound concentration was used to assess the unbound concentration-time curve ($AUC_{0\text{-}24h\ unbound}$). Finally, both bound and unbound AUC/MIC ratio could be calculated. The drug susceptibility test of the available *M. tuberculosis* isolates was performed on the Middlebrook 7H10 agar dilution method [26] at the Dutch National Mycobacterium Reference Laboratory (National Institute for Public Health and the Environment, RIVM).

Safety

To evaluate the safety of MFX treatment, the following adverse effects were retrieved from the medical chart, including diarrhoea, vomiting, *Clostridium dificille* infection and QT prolongation. MFX is contra indicated by patients with transaminase values five times the upper level of normal[27]. MFX induced hepatic injury was characterized by an increase to five times the upper level of normal value of at least one of the following: aspartate aminotransferase (ASAT; > 200 U/L), alanine transaminase (ALAT; >225 U/L), gamma glutamyl transpeptidase (GGT; >200-275 U/L), bilirubine, compared to baseline. Any MFX induced renal injury was defined as a least 25% increase of the serum creatinine level compared to baseline level. The upper level of normal was defined at a serum creatinine value of > 112.5 μmol/L (female) or 137.5 μmol/L (male). A QT period of more than 500 milliseconds is associated with a higher risk of cardiac events [28]. To estimate the risk of QT prolongation by long term MFX treatment, we have appointed risk factors, which can together result in or aggravate QT prolongation. Following risk factors are included: female gender, hepatic dysfunction, pro aritmic conditions, hypokalemia (<3.5 mmol/L serum), hypomagnesaemia (< 0.7 – 1.0 mmol/L blood) and simultaneous treatment with anti-dysrhytmics class IA en III, antipsychotics, tricyclic antidepressants or the antihistaminic drug terfenadine[27, 29, 30].

Special attention was paid to discontinuation of MFX. Reasons were categorized into five classes; (1) MFX was started based on expected drug resistance (country of origin, medical historoy) and discontinued after drug susceptibility pattern became available and showed an isolate susceptible to first line agents. (2) MFX was started because of intolerance to first line agents and discontinued after the adverse effects had resolved (3) Completion of MFX treatment; (4) MFX-induced adverse events and (5) other reasons. To determine causality between adverse effect and MFX treatment, the Naranjo algorithm was used (0 to 9 points, of which 9 represents the highest likelihood) [31].

Drug-drug interactions

To assess the occurrence of potential efficacy influencing drug-drug interactions by interaction with MFX absorption, metabolism or excretion we evaluated co-medication for the following drugs: RIF, antacids, mucosal protectants, minerals (zinc, iron) and didanosine[12, 14-16, 18, 32].

Statistics

When not normally distributed, data was statistical analysed with a non-parametric tests, i.e. Mann-Whitney *U* test for unpaired data and Wilcoxon rank sum test for paired data. Chi square tests was used to compare clinical data based on efficacy or safety, between subgroups.

RESULTS

Ninety two patients with a median age of 35.5 (27-47.3) years met the inclusion criteria; thirty five (38%) patients were female and fifty seven (62%) were male. One patient (trans gender) was excluded, because of the unknown influence of administered hormones on several important clinical parameters. Pulmonary TB was the most common diagnosis, found in 69 patients (75%). In 32 (34. 8%) patients MFX was started because of expected resistance, from which 30 patients suspected to have MDR-TB. Patients received a median dose of 6.9 (6.0-8.2) mg/kg for a period of 76.5 (29 - 197.3) days. Two patients died during MFX treatment, which was attributable to a clinical worsening condition due to AIDS and tuberculosis, respectively. The other patients recovered well from TB. An overview of the baseline patient characteristics and anti-tuberculosis drugs is shown in Table 1 and Table 2, respectively.

Table 1: Patient characteristics at baseline (total n = 92)

parameters	
Female (%)	35 (38)
Age (yr)	35.5 (27-47.3)
Weight (kg)	58.2 (49.4-66.5)
Length (cm)	169.5 (162-175)
BMI (kg/m²)	20.1 (17.7-23.0)
Ethnicity	
-caucasian	31 (33.7)
-asian	18 (19.6)
-afro-american	41 (44.6)
-other	2 (2.2)
Duration of hospital stay	63 (35-112.5)
Tuberculosis	
Localisation	
Pulmonary (%)	69 (75.0)
Extra pulmonary(%)	23 (25.0)
Miliary (%)	7 (7.6)
Other (%)	5 (5.4)
Diagnosis	
Sputum (%)	62 (67.4)
Biopt(%)	9 (9.8)
Thorax (%)	1 (1.1)
Other (%)	21(22.8)
Resistance pattern	
Normal (%)	55 (59.8)
MDR (%)	20 (21.7)
INH resistant (%)	3 (3.3)
INH and ethambutol resistant (%)	1 (1.1)
Unknown (%)	13 (14.1)
Comorbidity	
Hepatic failure (%)	6 (6.5)
Renal failure (%)	1 (1.1)
Epilepsy	1 (1.1)
Diabetes Mellitus (%)	10 (10.9)
HIV positive (%)	10 (10.9)
Alcohol abuse (%)	8 (8.7)

Results are presented as median with interquartile range between brackets or as number of patients (n) with the percentage between brackets (%). BMI = body mass index; MDR = Multi Drug Resistant; HIV =human immunodeficiency virus

Table 2: Antituberculous medication (total n=92)

Medication	
First line agents	
Ethambutol	68 (73.9)
Rifampicin	71 (77.2)
Isoniazid	72 (78.3)
Pyrazinamid	72 (78.3)
Second line agents	
Amikacin	24 (26.1)
Kanamycin	16 (17.4)
Linezolid	22 (23.9)
Protionamid	17 (18.5)
Clofazimin	17 (18.5)
Cycloserine	4 (4.3)
Ofloxacine	1 (1.1)
Rifabutine	2 (2.2)
Claritromycine	2 (2.2)
Thioazeton	3 (3.3)
Azitromycine	3 (3.3)

Results are presented as number of patients (n) with the percentage between brackets (%)

Pharmacokinetics/pharmacodynamics

From 17 patients a full pharmacokinetic curve in plasma, CSF and/or plasma ultrafiltrate was available. The median plasma concentration time curve is shown in figure 1. Steady state pharmacokinetic parameters of MFX are shown in Table 3. Geometric mean $AUC_{0\text{-}24h}$ in plasma and CSF of MFX 400 mg once daily were 26.7 (range 8.5 - 72.4) mg*h/L and 14.1 (range 7.6 - 18.0) mg*h/L, respectively. The median MIC of MFX was 0.125 (interquartile range (IQR) 0.125-0.25) mg/L. The geometric mean $AUC_{0\text{-}24h}$/MIC ratios were respectively 163 (range 13 - 579) and 91 (range 50 - 144) for MFX in plasma and CSF. In plasma, fifteen of eighteen patients (88.3%) had an $AUC_{0\text{-}24h}$/MIC ratio above 100 and two (16.7%) had an $AUC_{0\text{-}24h}$ below 100. In CSF, three patients had an $AUC_{0\text{-}24h}$/MIC above 60. In one patient the $AUC_{0\text{-}24h}$/MIC ratio was below 60.

In this study population we observed a plasma protein binding ranged from 11.0 to 41.7%. No significant difference (P = 0.274) was observed between the percentage plasma protein binding of 26.3 (IQR 18.7 - 34.0) % and 30.3 (IQR 24.0 - 37.8) % of respectively high MFX concentrations of 2.6 (IQR 2.3 – 2.8) mg/L and low MFX concentrations of 0.3 (IQR 0.25 -0.33) mg/L in plasma. The geometric mean unbound $AUC_{0\text{-}24h}$ and unbound $AUC_{0\text{-}24h}$/MIC were equal to 22.3 (range 12.3 – 64.4) mg*h/L and 120 (range 10 - 515) respectively.

Table 3: Steady state pharmacokinetic parameters of MFX

Parameter	(n = 18)
AUC_{0-24} plasma (mg*h/L)	27.5 (20.9-34.8)
C_{max} (mg/L)	2.70 (2.47-3.69)
T_{max} (h)	3 (2-5)
$T_{1/2}$ (h)	7.7 (6.6-10.2)
Fraction unbound #	0.74 (0.63-0.79)
$AUC_{0-24\ unbound}$ (mg*h/L) #	20.2 (16.0-24.3)
AUC_{0-24} CSF (mg*h/L) $	17.0 (14.3-17.7)
C_{max} (mg/L) $	1.28 (1.00-1.46)
T_{max} (h) $	4.0 (4.0-5.5)
$T_{1/2}$ $	6.2 (5.8-7.0)

= n=10; $=n=4. Data were presented as median with inter quartile range.

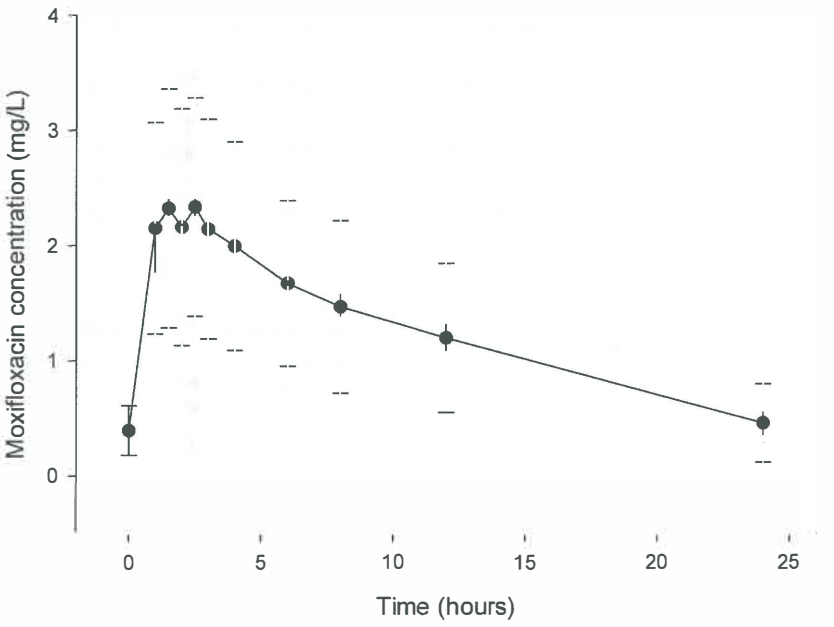

Figure 1: Mean MFX concentration-time curve in plasma (n=17)

Mean MFX plasma concentrations are represented by solid circles. Standard deviations were presented as error bars.

Safety

MFX was tolerated very well; it was only discontinued in two (2.2 %) patients because of adverse effects, including both vomiting and diarrhoea (n=1), diarrhoea alone (n=1). An overview of adverse effects is shown in Table 4. No significant difference between liver enzyme and renal values at baseline and during treatment was observed.

However, in one patient GGT values increased to more then five time the upper level of normal (Naranjo score = 3). In four patients renal function decreased during treatment but serum creatinine values stated within normal values. Vomiting, diarrhoea and a *Clostridium difficile* infection were observed during MFX treatment in respectively, two (2,2%; Naranjo score = 3), six (6,5%; Naranjo score = 3 or 4) and two (2,2%; Naranjo score = 2) patients. Upon evaluation, thirty five patients had at least one additional risk factor for QT prolongation, nineteen patients had two additional risk factors, one patient had three risk factors and one patient had four risk factors. Despite these risk factors no QT prolongation was not observed.

Table 4: Adverse effects of MFX treatment (total n=92)

Parameter	*baseline*	*During treatment*	*P*
Hepatic function			
ASAT (U/L)	34.5 (22.8-41.8)	24.5 (19.0-36.0)	0.057
ALAT (U/L)	23.0 (15.0-47.0)	19.5 (11.3-38.0)	0.175
GGT (U/L)	73.0 (50.5-111.0)	55.0 (25.0-91.0)	0.141
Direct bilirubin (µmol/L)	2.0 (1.0-3.8)	2.0 (1.0-4.0)	0.662
Total bilirubin (µmol/L)	7.0 (5.0-9.0)	7.0 (5.0-9.0)	0.774
Unknown values (%)	48 (52.2)	14 (15.2)	
Hepatic failure (%)	6 (6.5)	7 (7.6)	
Renal function			
Creatinine (µmol/L)	62 (54.0-70.5)	61.0 (49.0-74.0)	0.688
Ureum (mmol/L)	4.0 (3.1-5.4)	4.3 (3.4-5.4)	0.486
Unknown values (%)	49 (53.3)	23 (25.0)	
Renal failure (%)	1 (1.1)	1 (1.1)	
Adverse events			
Super infection (%)		2 (2.2)	
Diarrhoea (%)		6 (6.5)	
Vomiting (%)		2 (2.2)	
QT prolongation (%)		0 (0.0)	
Hepatic injury (%)		1(1.1)	
Renal injury (%)		4 (4.3)	
Other(%)		2 (2.2)	
Reason to stop MFX treatment			
MFX until resistance pattern is known (%)		32 (34.8)	
Standard regimen of TB medication was temporary not tolerated. Until toleration of the standard regimen, MFX was used (%)		16 (17.4)	
Completion of treatment (%)		38 (41.3)	
Adverse events of MFX (%)		3 (3.3)	
Other(%)		3 (3.3)	

Results are presented as median with interquartile range between brackets or as number of patients (n) with the percentage between brackets (%). MFX=moxifloxacin; ASAT= aspartate aminotransferase; ALAT= alanine transaminase; GGT= gamma glutamyl transpeptidase.

Drug-drug interactions

RIF was frequently co-administered with MFX. In 69. 6, 9.8, 1.1 and 1.1 percent of the patients MFX was combined with RIF in a dose of 600, 450, 300 and 150 mg, respectively.
Full Pharmacokinetic concentration curves were available of six patients who received MFX alone and of twelve patients received RIF and MFX. Co-medication with RIF did not result in a significant lower geometric mean plasma $AUC_{0\text{-}24h}$ value (32.3 (12.8 - 50.3) vs 24.2 (14.9 - 72.4) mg*h/L; P = 0.134). No significant difference between MFX dose in mg/kg was observed between patients with or without RIF concomitant treatment of MFX (P=0.482).In our population MFX was not simultaneously administered with antacids, mucosal protectants, minerals (zinc, iron), or didanosine.

DISCUSSION

The $AUC_{0\text{-}24h}$/MIC ratio is the pharmacokinetic parameter to predict efficacy of moxifloxacine best and it is generally accepted that the value of 100 needs to be exceeded [33]. To prevent occurrence of resistance it might be necessary to aim at higher $AUC_{0\text{-}24h}$/MIC ratios[34]. Based on a median $AUC_{0\text{-}24h}$ of 27.5 mg*h/L, a standard dose of MFX of 400 mg once daily can be used in the treatment of isolates with a maximum MIC of 0.28 mg/L. As higher MIC are observed the standard dose is not sufficient for all patients. Before increasing the standard dose the $AUC_{0\text{-}24h}$/MIC ratio can be assessed in an individual patient by measuring both $AUC_{0\text{-}24h}$ and MIC. In two of the eighteen patients $AUC_{0\text{-}24h}$/MIC ratio was below 100. By increasing the dose to 600 mg once daily, the $AUC_{0\text{-}24h}$ would increase with a factor of about 1,5 [20] resulting in an $AUC_{0\text{-}24h}$/MIC ratio of at least 100.

However, only the unbound fraction of MFX is active against *M. tuberculosis.* From the total $AUC_{0\text{-}24h}$/MIC ratio of 100 can be derived that the unbound $AUC_{0\text{-}24h}$/MIC ratio should be at least 60 using the most described value of protein binding of approximately 40% for MFX [35]. As in our study the protein binding showed a large variability and ranged from 11.0 to 41.7%, possible as a result of malnutrition, it seems logical to determine the unbound MFX concentration in each individual. As the fraction of unbound MFX appeared not concentration depended, unlike earlier results[8], a single random blood sample can be used to assess plasma protein binding. Measuring unbound plasma concentration could result in no dosage adjustment if the unbound $AUC_{0\text{-}24h}$/MIC ratio is over 60, while the total $AUC_{0\text{-}24h}$/MIC ratio is below 100. As in CSF the protein level will be nearly zero, the target $AUC_{0\text{-}24h}$/MIC ratio of 100 can be decreased to 60. In case of a ratio below 60 it can be recommended to increase the dose [4].

Of all patients, 69 (75%) patients received RIF simultaneously with MFX. However, in contrast with earlier published results[12, 13], concomitant treatment of RIF and MFX did not cause a significant decrease of MFX exposure. A difference in MFX dose based on mg/kg could not have compensated for RIF reduced MFX exposure as both groups received the same dose. Possibly the inter patient variability is larger than the AUC reduction caused by RIF. Besides this our study was not designed nor powered to detect a difference in AUC as a result of concomitant use of RIF compared to a previous study[12].
Therefore our results do not rule out the significance of the drug-drug interactions between RIF and MFX, especially as a trend towards significance was observed.

Adverse effects were observed infrequently during MFX treatment and resulted in only two cases in discontinuation of MFX. Based on the low Naranjo score the observed adverse effect were possibly a result of MFX treatment but other medication then MFX could also induce these adverse effects. As

MFX is used in patients intolerant to first line drugs and is subsequently tolerated well [36] our results confirm these findings. Despite several additional risk factors, besides MFX it selves, no QT prolongation was observed. Based on the results of our study population and of a recent published clinical trial in 74 patients [37], ECG monitoring doesn't seem mandatory in case of a standard dose of 400 mg once daily. In patients receiving a higher dose, especially in those having a high AUC, ECG monitoring could recommended [4, 20, 21, 27].

In earlier published work, MFX (400 mg) was well tolerated in thirty eight tuberculosis patients for a median period of about 174 days [19] and in seventy four patients for a period of 56 days [37]. Our study of ninety two patients with median treatment period of 75 days adds important safety information as our patient population was not pre selected like patients included in clinical trials.

MFX is a very promising drug in the treatment of tuberculosis. However, drug-drug interactions and plasma protein binding and MIC values need to be taken into account to assure optimal treatment. Based on our results it can be recommended to measure at least a peak level of MFX and determine plasma protein binding along with drug susceptibility testing to guide treatment. To evaluate the true potency of this strategy it should be evaluated in a randomised clinical trial comparing a standard dose of MFX of 400 mg with an individualised dose of MFX. Patients with MDR-TB may potentially benefit the most as the MIC for MFX is higher in these patients. Further investigation should evaluate the safety of MFX in a dose of 600 to 800 mg which may sufficient to kill M tubercolis isolates with a MIC of 1 mg/L. These dose regimen might also be sufficient to reach adequate $AUC_{0\text{-}24h}$/MPC ratios.

CONCLUSION

MFX treatment was well tolerated in ninety two patients, receiving a median dose of 6.9 mg/kg for a median duration of 76.5 days. Evaluation of unbound $AUC_{0\text{-}24h}$/MIC ratio is needed to evaluate the optimal dose in each patient.

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Chapter

5.3

Pharmacokinetics of moxifloxacin in cerebrospinal fluid and plasma in patients with tuberculous meningitis

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ABSTRACT

Moxifloxacin cerebrospinal fluid (CSF) penetration was evaluated by obtaining full plasma and CSF time concentration curves from four patients with tuberculous meningitis. Geometric mean AUCCSF/AUCPlasma ratios were 0.82 (range 0.70 – 0.94) at 400 mg and 0.71 (0.58 – 0.84) at 800 mg, both given once daily.

INTRODUCTION

The outcome of tuberculous meningitis (TBM) is dominated by diagnostic delay and limited therapeutic options, because of adverse effects or resistance to first-line drugs, as well as poor cerebrospinal fluid (CSF) penetration of some of the first- and second-line drugs. The combination of isoniazid (INH), rifampicin (RIF), pyrazinamide and ethambutol or streptomycin is recommended for the treatment of TBM by the WHO. However, this schedule is not based on strong scientific evidence; no randomised controlled trials, nor extensive pharmacokinetic studies have supported these recommendations.

Moxifloxacin (MFX), which shows the highest *in vitro* activity against *M. tuberculosis* compared to other quinolones[1], has emerged as most promising second-line agent in TB treatment, when adverse effects or drug resistance limit the use of first-line agents. Although MFX is not approved for TB treatment it appeared to be well tolerated in a dose of 400 mg during long-term treatment of pulmonary tuberculosis[2]. This drug has shown the potential to shorten TB treatment in the murine model and is being evaluated in clinical trials as first line agent that may enable treatment shortening. Based on *in vitro* and *in vivo* studies, a dosage of 800mg of MFX once daily has been suggested to suppress resistance [3], but no safety data are available to support this dose. MFX can't be recommended yet for standard use in the treatment of TBM as there is a paucity of data on the CSF penetration of MFX in humans[4, 5] and animal data showed variability in the CSF penetration of MFX[6, 7]. Besides, plasma drug concentrations are lowered by 30% by co-administration of RIF[8]. Therefore additional studies in humans are needed to measure plasma and CSF MFX concentrations over time to establish the optimal dose for treatment of TBM.

MATERIALS AND METHODS

MFX was started in an oral dosage of 400 mg once daily for at least five days followed by 800 mg once daily. Plasma and CSF concentrations were evaluated twice, after each fifth dose (steady-state)[9]. The local ethics committee of the University Medical Centre Groningen approved the treatment protocol and informed consent of all included patients was obtained.
Blood and CSF samples were collected before MFX administration and at t = 1, 2, 4, 8, 12 and 24 hours post-dosage. For plasma and CSF sampling a peripheral intravenous catheter and an external lumbar drain were 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 ml of blood were discarded. Total (protein bound plus unbound) concentrations were assessed in plasma and CSF samples using a validated method on HPLC with fluorescence detection[8] and unbound concentrations (which represent the drug that can cross biological barriers and interact with the bacterium) were assessed following ultrafiltration (Centrifree® Ultrafiltration device, Millipore, Germany). Pharmacokinetic (PK) parameters were assessed with standard non-compartmental pharmacokinetic methods using the software package MWPharm 3.60 (Mediware, The Netherlands). Ratios of the area under the concentration-time curves up to 24 hours post dose ($AUC_{0\text{-}24h}$) in CSF versus plasma were calculated to express the penetration of MFX in CSF. The patients' *Mycobacterium tuberculosis* isolates were subjected to drug susceptibility testing on basis of the Middlebrook 7H10 agar dilution method[10]. The ratio of the $AUC_{0\text{-}24h}$ and the minimal inhibitory concentration (MIC) was calculated as this $AUC_{0\text{-}24h}$/MIC ratio has been proposed as the best pharmacokinetic/pharmacodynamic parameter to predict *in vivo* efficacy of fluoroquinolones against fast-growing gram-negative bacteria [11] as well as *M. tuberculosis*[12]. In

order to assess the AUC_{0-24h}/MIC more accurately, these values were also calculated for unbound drug concentrations [11]. To assess the safety of MFX at a higher dosage, serum glucose level, liver and renal function tests were measured. ECG monitoring was performed to assess possible QTc prolongation. All results are presented as median with interquartile range between brackets. As data were not normally distributed, non-parametric tests–i.e, Wilcoxon signed-rank test and the Spearman correlation test - were used in the statistical analysis.

RESULTS

Four male patients were referred to our centre for treatment of TBM in the period of 2007 to 2008; the diagnosis was confirmed by lepto-meningeal biopsy or CSF analysis; *M. tuberculosis* culture was positive in all of them. Their median age was 47 (IQR; 30 – 65) years and body mass index was 21.1 (IQR; 16.7 – 21.7) kg/m². All patients had received RIF 600 mg and MFX 400 mg, both once daily. One patient received additional INH. During PK studies, the CSF was colourless and clear; median total protein value amounted 0.6 g/L (IQR; 0.5 – 0.7, normal value; 0.3-0.7), the median glucose level was 2.5 mmol/L (IQR; 2.4 – 2.8; normal value; 2.2 -4.4)and a median mononuclear cell count of 21 $*10^6$ /L (IQR; 15 – 25; normal value; 0 - 3) was recorded. MIC values of *M. tuberculosis* to MFX ranged from 0.125 – 0.25 mg/l. Pharmacokinetic and pharmacodynamic parameters are shown in table 1.

The geometric mean AUC_{CSF}/AUC_{Plasma} ratio for total (bound plus unbound) concentrations was 0.82 (range 0.70 – 0.94) at 400 mg once daily, which was not significantly different from the geometric mean AUC_{CSF}/AUC_{Plasma} ratio of 0.71 (range 0.58 – 0.84) at 800 mg once daily (P = 0.3). Unbound concentrations could be measured in two patients. The unbound fractions of MFX were 40 and 60% in serum and 90 and 95% in CSF, both at doses of 400 mg and 800 mg. In these patients, the unbound AUC_{CSF}/AUC_{Plasma} ratios were 1.31 and 1.75 at a dose of 400 mg and 0.85 and 1.16 at a dose of 800 mg.

After administration of 400 mg and 800 mg MFX, the AUC_{CSF} was significantly related to the peak concentration in CSF (R 0.8; P < 0.001). The time to reach the maximum concentration in CSF was significantly longer compared to the interval needed for plasma (P = 0.002).
Two patients continued therapy with MFX in a dose of 600 mg for two months and eight months, one patient continued therapy with 400 mg for 9 months and one patient continued treatment with 800 once daily for four months. During treatment with MFX no adverse effects were reported that could be attributed to MFX, based on evaluation of ECG, serum glucose level and blood sampling of renal and liver function.

DISCUSSION

We describe a combined CSF and plasma MFX concentration measurement over time in four patients treated for TBM during steady-state conditions. We show that MFX penetrates readily in CSF. This confirms earlier data of CSF penetration, based only on a single CSF sample, in patients undergoing a scheduled urological operation[5]. The data in our TBM patients show complete CSF concentration-time curves, which is the gold standard to assess CSF/plasma ratios and this adds important information to the current knowledge and enables pharmacokinetic/pharmacodynamic parameter calculations. Based on unbound AUC_{CSF}/AUC_{plasma} ratios an accumulation of MFX in CSF could be observed.

Table 1: Pharmacokinetic parameters of moxifloxacin based on total (bound plus unbound) concentrations (n=4)

Parameter	Plasma		CSF	
	400mg	800mg	400mg	800mg
AUC_{0-24h} (mg.h/l)	24.4 (20.5 – 24.5)	38.3 (32.2 – 42.4)	18.1 (14.8 – 19.8)	28.4 (24.9 – 28.9)
C_{max} (mg/l)	2.57 (1.79 – 3.47)	3.65 (3.31 – 4.09)	1.29 (1.04 – 1.50)	2.22 (1.72 – 2.63)
T_{max} (h)	1.0 (1.0 – 1.3)	2.0 (2.0 – 2.5)	4.0 (3.8 – 5.5)	4.0 (3.8 – 5.5)
C_{min} (mg/l)	0.21 (0.17 – 0.26)	0.37 (0.24 – 0.48)	0.19 (0.14 – 0.24)	0.33 (0.25 – 0.38)
$T_{1/2}$ (h)	6.7 (6.0 – 7.5)	5.8 (5.0 – 10.0)	6.0 (5.7 – 7.3)	7.0 (6.4 – 7.4)
Cl/F (L/h)	14.9 (12.6 – 30.6)	19.2 (15.3 – 24.62)	18.8 (17.5 – 34.6)	25.7 (24.2 – 31.2)
V_d (L)	159 (143 – 161)	140 (139 – 142)	NA	NA
AUC/MIC	103 (60 – 196)	186 (132 – 322)	84 (56-154)	132 (95 – 226)
Unbound AUC/MIC (n=2)	#1:39; #2: 98	#1: 118; #2: 193	NA	NA

Median values (IQR) of the following parameters are shown in the table: AUC_{0-24h}, area under the concentration–time curve from 0 to 24 h; C_{max}, maximum concentration of drug; C_{min}, trough concentration of drug; T_{max}, time to maximum concentration of drug in serum; $t_{1/2}$, terminal elimination half-life; CL, total clearance; V_d, volume of distribution; NA, not applicable. Geometric mean (range) of the AUC/MIC ratio (n=4) and unbound AUC/MIC ratio (n=2) of patient number (#) 1 and 2 are shown in the table.

Assuming that the AUC/MIC ratio, based on total drug concentration, is the best predictive model for efficacy for fluoroquinolones, the value of 100 has to be exceeded [13], but protein binding differs among the range of fluoroquinolones - and only the unbound drug fraction is antimicrobially active. In our two patients in whom we measured unbound drug, we observed a considerable difference in the unbound concentration of MFX that could not be explained by MFX concentration dependent protein binding[12]. To interpret the value of the AUC/MIC in patients to guide individual dosing is therefore difficult. As all patients received RIF, which generally results in a nearly 30% reduction in AUC[8] of MFX, it might be considered to increase the dosage of MXF to achieve MXF concentrations similar to those without RIF. As a MFX peak level has a good predictive value for the AUC, a CSF sample could be taken at t = 4 hours after ingestion of the drug as a means to predict and verify the resulting CSF exposure. Although, MFX induced QTc prolongation should be assessed when a dosage is increased, significant prolongation was observed at higher AUC's of approximately 86 mg*h/L, which is two times higher than in our study [14]. As a next step we would propose to design a prospective study to evaluate the efficacy and safety of dosing of MFX in TB patients based on unbound AUC_{plasma}/MIC ratios.

CONCLUSION

Adequate MFX concentrations were achieved in CSF as well as in plasma using a dosage of 400 mg to 800 mg once daily. Monitoring of MFX concentration levels may be warranted in patients with interacting co-medication or a high *in vitro* MIC. These data suggest that MFX is an important addition in the treatment of TBM.

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Chapter 6

Ethambutol-induced optical neuropathy: risk of overdosing in obese subjects

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ABSTRACT

Background

Ethambutol (EMB) is one of the first-line drugs in the treatment against tuberculosis (TB). Side-effects are infrequent but its main adverse effect, optical neuropathy, has long been recognized. The mechanisms of action and predisposing factors have not been fully understood.

Method

We conducted a retrospective study (1992-2007) in an attempt to find predisposing factors for optical neuropathy.

Results

In 1.3% of the 760 patients treated with EMB, visual disturbance was reported, of which 0.8% were EMB-related. We presented the six cases; four were clearly overdosed but in two obese patients was correctly calculated for total body weight.

Conclusion

Analysis of the case histories and previous reports suggest that optical neuropathy may at least partly caused by EMB overdosing due to daily dosing based on total body weight instead of dosing on lean body mass.

INTRODUCTION

Ethambutol (EMB; dextro-2,2'-ethylenedi-imino-di-butanol dihydrochloride) has been used in the treatment of tuberculosis since the beginning of the 1960's. Today its role in therapy, as the fourth antituberculosis drug, is the prevention of rifampicin (RIF) resistance in isoniazid (INH) resistant strains [1]. Also due to the possible severe adverse effects of thioacetazone in HIV-positive patients, EMB has made a comeback.
The exact mechanism of action of EMB is unknown although several mechanisms have been proposed. One comprises the chelating effects of the drug on zinc or copper, thereby disrupting essential metal-containing enzymes in mycobacteria [2]. Other studies have shown that EMB inhibits synthesis of arabinogalactan, thereby inhibiting the formation of the mycobacterial cell wall, increasing cell wall permeability for other intracellularly working antituberculosis drugs such as RIF [3]. Inhibition of RNA-synthesis in mycobacteria might be a third potential mechanism of action [4].

Since the introduction of EMB in the treatment of tuberculosis its main possible adverse effect, optical neuropathy, has been well recognized [5]. The ocular toxicity can clinically present in two forms. The most common form is toxicity to the central fibers of the optic nerve, causing a decreased visual acuity, a central scotoma and dyschromatopsia. The majority of cases is reversible on drug cessation, although there are several reports of irreversible visual defects [6]. Occasionally defects in peripheral isopters of the visual field occur, without disturbed color vision and visual acuity. In the latter case the peripheral fibers of the optic nerve are affected.
The incidence of optical neuropathy appears to be dose- and time-related [7], although a relationship with serum levels of the drug has never been established. Studies report an incidence of 18% in subjects treated for more than two months with more than 35 mg/kg/day, 5-6% with 25 mg/kg/day, 3% with 20 mg/kg/day and less than 1% with 15 mg/kg/day.

The World Health Organization recommends a daily EMB dose of 15-20 mg/kg, but does not set a maximum dosage in their guidelines for treatment of tuberculosis. In their joint statement [8], the American Thoracic Society, the Centers for Disease Control and Prevention and Infectious Disease Society of America suggest dosages for EMB based on estimated lean body weight with a maximum daily dose of 1600 mg, which is markedly lower than the generally recommended maximum dose of 2500 mg. The Tuberculosis and Chest Service of the Department of Health of Hong Kong Special Administrative Region gives recommendations [9] concerning EMB-dosages based on ideal body weight (IBW). As most patients with tuberculosis lose weight resulting in a low BMI, dosing on total body weight (TBW) usually equals that of IBW. As obesity is now increasingly common in the Western World, dosing on TBW has become a point of concern.
The most recent formula for the calculation of IBW is given by Hammond: men 48 kg for 150 cm + 1.1 kg per extra centimeter (cm). Women: 45 kg for 150 cm + 0.9 kg per extra cm [10]. This calculation correlates with approximately a BMI of 21.3 and 20.0, for men and woman respectively.

The average time between start of therapy and manifestation of ocular side effects is generally reported to be 3 to 5 months [11-13], although much shorter [14] and longer [6] intervals have been reported. Renal dysfunction [15], diabetes, hypertension, optical neuritis by alcohol or tobacco abuse [16] and increased age [17] have been reported as risk factors for developing optical neuropathy.

As EMB has gained an important role in TB treatment, analysis of risk factors for developing side effects and toxicity is important. We retrospectively studied the records of hospitalized tuberculosis patients treated with EMB to identify risk factors for EMB induced optical neuropathy. We hypothesize that toxicity may partly result from inadequate dosing, and that such toxicity may be avoided by dosing based on IBW.

MATERIALS AND METHODS

In an attempt to find predisposing clinical factors among subjects developing optical neuropathy during treatment with EMB, we retrospectively analyzed the records of all hospitalized 760 patients, treated with EMB, between 1992 and 2007 at the Tuberculosis Centre Beatrixoord, University Medical Center Groningen, The Netherlands. This TB Center has been a tertiary referral center for most Dutch TB patients. Diagnosis, previous medical history (where available), dose and treatment duration, co-morbidity, hepatic and renal functions, co-medication (dose and duration), age, sex, weight, height, other than EMB-induced adverse events, ophthalmologic examinations and time to manifestation of ocular adverse events were investigated in the patients developing EMB-induced optical neuropathy for possible correlations. A control group, obese -matched for BMI values-, treated with ethambutol with no ocular symptoms was selected from the same database. IBW can be used as a good and safe estimate for drug dosing [10] and is less inaccurate than lean body mass, which is dependent on wide generalisations. The Naranjo algorithm was used for causality assessment [18].

RESULTS

Of 760 patients treated with EMB, 10 (1.3%) reported a disturbance in vision. In six patients (0.8%), no other explanation was found by an ophthalmologist than EMB-use. The mean age of the evaluated patients with presumed EMB toxicity was 39.5 ± 18.1 years. These subjects received a mean daily EMB dose of 26.4 ± 11.0 mg/kg (Four of six patients receiving 24 mg/kg/day or less). At that time (1992) a higher dosage of EMB was prescribed regularly. The average time to manifestation of optical neuropathy was 3.3 ± 2.8 months (range, 0.4 - 6.1 months). The patients in the control group were treated with a mean daily dose of 13.6 ± 1.2 mg/kg and a mean treatment duration of 3.2 ± 2.7 months (range, 0.6 - 6 months).

No clinical factors, such as age, sex, co-morbidity, serum albumin, renal or hepatic function, seemed to correlate with the development of optical neuropathy. For an overview of patient characteristics, who experienced EMB-induced optical adverse effects, and the matched control group (for patient 1 and 6) see table 1.

Patient 1 and 6 are described in more detail below as illustration for a possible cause of EMB-induced neuropathy.

CASE REPORTS

Patient 1

A 57-year-old obese (BMI=37.3 kg/m^2) woman, with no previous medical history, started treatment for lymph node tuberculosis with INH 300 mg/day, RIF 600 mg/day and 1000 mg pyrazinamide (PZA) twice a

day, orally. Renal and hepatic functions were normal. When it became known that the *Mycobacteria* were insensitive to RIF, it was replaced by EMB 2000 mg (18.3 mg/kg/day). After185 days the patient reported a disturbance in color vision and decreased visual acuity. EMB was stopped a week later. The Ishihara color vision-test, which was carried out flawlessly before treatment, was disturbed on all but two plates. Ophthalmologic examination was repeated one month later and showed a visual acuity of 1/60 in the right eye and 1/20 in the left. The patient's vision slowly improved and 3.5 months after discontinuation of EMB, her visual acuity was 20/100 bilaterally. After 18 months vision was still impaired, however the extent of this impairment could not be accurately measured, because the patient also developed cataract.

Patient 6

A 43-year-old obese (BMI=30.6 kg/m^2) woman was given therapy for her recurring tuberculosis spondylodiscitis with INH 300 mg/day, RIF 600 mg/day, PZA 2000 mg/day, levofloxacin 500 mg/day, KM 400 mg/day and EMB 1400 mg/day (21.9 mg/kg), orally. Renal and hepatic functions were normal. After 75 days the EMB dose was lowered to 1200 mg/day (18.8 mg/kg).

After 109 days, the patient complained about decreased visual acuity. Ten days later, two days before the planned end of treatment, all anti-tubercular drugs were stopped.

Before therapy was initiated her visual acuity was 20/20 bilaterally. Upon ophthalmologic examination, two months after cessation of the drug, a decreased visual acuity of 20/60 in the right and 20/50 in the left eye was found. Also bitemporal defects in the peripheral visual field were found. After 3.5 months visual acuity had improved to 20/25 bilaterally, but the bitemporal visual field defects remained, although they were slightly improved.

Table 1: Characteristics of patients who experienced EMB-induced optical adverse effects and control group

Pt	Sex	Age (yr)	BMI (kg/m^2)	Dose (mg / kg TBW)	Dose (mg / kg IBW)	Time to onset (mo)	Diagnosis	Co-morbidity	Naranjo score
1	F	57	37,3	18, 3	31.3	6,1	Lymph node TB	None	7
2	M	23	18,1	31,0	22.5	6,1	Pulmonary TB	None	7
3	F	30	17,9	23,3	20.2	0,4	Spinal TB	None	6
4	F	57	19,1	21,3	19.5	2,6	Spinal TB	High blood pressure, DM II	7
5	M	21	15,4	35,6	22.5	2,4	Miliary TB	HIV+, liver function disturbances	7
6	F	43	30,6	18,8	33.3	5,4	Spinal TB	None	7
									7
C1*	M	60	32,5	12,5	16.0	#NA	Pulmonary TB	Alcohol abuse	7
C2*	M	61	29,7	14,9	17.8	#NA	Pulmonary TB	DM II, alcohol abuse	7
C3*	M	75	36,6	13,3	19.8	#NA	Renal TB	Heart failure, alcohol abuse	7

* = subject from control group

NA = not applicable, control group did not experience EMB side effects.

DISCUSSION

Six patients out of the cohort of 760 hospitalized patients were detected with optical neuropathy induced by EMB. Patient 2-5 developed ocular nerve damage, which can be solely explained by the high dosage EMB/kg. Patient 1 and 6 developed severe but partly reversible ocular nerve damage and were described in more detail. Both patients were treated with dosages that have been presumed to be safe and associated with a low incidence of toxic effects like optical neuropathy, although no safe dose of EMB has been established [19].
In both cases none of the classical risk factors, such as hypertension, pre-existing ocular diseases, diabetes, renal function disturbances or alcohol or tobacco abuse were present. At first glance the only similarities were obesity and sex. However, sex is unlikely to be a predisposing factor, since in previously conducted studies no link between sex and the occurrence of ocular side effects was detected [19, 20]. The causality assessment revealed a 'probable' association between the optical neuropathy and EMB. A higher Naranja score could have been obtained by readministrating EMB but this is in case of a established side effect of EMB like optical neuropathy not ethical.

Unfortunately, despite the large cohort, it has not been possible to further match the control group in regard to sex, ethnic background, co-morbidity, renal and hepatic function as TB patients tend to be mostly underweight.
Obesity can alter the distribution and elimination of various drugs [22]. The volume of distribution is increased and the elimination half-life is prolonged in obese subjects for highly lipophilic substances as i.e. benzodiazepines. Other drugs, e.g., vecuronium, being less lipohilic, are mainly distributed in lean tissues [23] and therefore pharmacokinetic parameters are much less influenced. According to Place et al. [24] it is unlikely that EMB accumulates (or distributes) in other tissues, i.e. fat. Dosing on TBW could therefore result in serious overdosing.

From our findings we suspect a correlation between EMB-induced optical neuropathy and possible overdosing due to dosing on total body weight in obese patients. A similar suggestion was made in a case report by Geiseler et al. already more than twenty years ago [26]. Therein they show that serum levels of anti-tubercular drugs like EMB and RIF are within expected ranges, when dosing on IBW instead of actually measured body weight in morbidly obese patients.
If we correct the dosages of our presented cases based on IBW:
Patient 1 received a dosage of 31.3 mg/kg/day and patient 6 received 38.9 mg/kg/day for two months and 33.3 mg/kg/day for the following 4 months. These dosages, in combination with the treatment duration, are associated with a significant elevated risk of developing optical neuropathy.
The replacement of RIF with EMB and stopping INH in patient 1 seems an unusual step considering their different modes of action, but this patient was treated in 1992 with a regimen we would not use nowadays. In patient 6 treatment was started elsewhere, the diagnosis tuberculosis was established but cultures had failed. The woman came from a country with a high rate of resistance for first line tb-drugs and because of the recurrence second line drugs were added. Cultures failed to show growth but AFB were demonstrated by immunofluorescent staining.
Upon further investigation, the database revealed three other obese patients (C1-C3, table 1), who did not experience ocular adverse events after treatment with EMB. Overall these patients were treated with a lower dose (mean daily dose 13.3 mg/kg TBW; 17.9 mg/kg IBW) and had a shorter treatment duration (mean duration 3.2 months). Recently dosing of EMB in children was discussed by Thee et al [26]. Dosing on body surface, rather than on body weight is mandatory to reach therapeutic drug

levels in children. This study suggests that certain patient populations need specific dosing to optimize EMB therapy.

Guidelines regarding the optimal dosing methods of EMB in obese patients have not been established to date. Although we studied EMB toxicity retrospectively, and no serum concentrations were measured, the two cases presented in detail have probably been overdosed. Based on these findings we recommend dosing in adults patient on ideal body weight. In addition, pharmacokinetic studies on EMB in obese patients are necessary to optimize EMB treatment in obese individuals.

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Chapter

7.1

Simultaneous determination of clarithromycin, rifampicin and their main metabolites in human plasma by liquid chromatography-tandem mass spectrometry

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ABSTRACT

The drug combination rifampicin and clarithromycin is used in regimens for infections caused by Mycobacteria. Rifampicin is a CYP3A4 inducer while clarithromycin is known to inhibit CYP3A4. During combined therapy rifampicin concentrations may increase and clarithromycin concentrations may decrease. Therefore a simple, rapid and easy method for the measurement of the blood concentrations of these drugs and their main metabolites (14-hydroxyclarithromycin and 25-desacetylrifampicin) is developed to evaluate the effect of the drug interaction. The method is based on the precipitation of proteins in human serum with precipitation reagent containing the internal standard (cyanoimipramine) and subsequently high-performance liquid chromatography (HPLC) analysis and tandem mass spectrometry (MS/MS) detection in an electron positive mode. The method validation included selectivity, linearity, accuracy, precision, dilution integrity, recovery and stability according to the "Guidance for Industry – Bioanalytical Method Validation" of the FDA.

The calibration curves were linear in the range of 0.10-10.0 mg/L for clarithromycin and 14-hydroxyclarithromycin and 0.20-5.0 mg/L for rifampicin and 25-desacetylrifampicin, with within-run and between-run precisions (CVs) in the range of 0% to -10%. The components in human plasma are stable after freeze-thaw (three cycles), in the autosampler (three days), in the refrigerator (three days) and at room temperature (clarithromycin and 14-hydroxyclarithromycin: three days; rifampicin and 25-desacetylrifampicin: one day).

The developed rapid and fully validated liquid chromatography-tandem mass spectrometry (LC/MS/MS) method is suitable for the determination of clarithromycin, 14-hydroxyclarithromycin, rifampicin and 25-desacetylrifampicin in human plasma.

INTRODUCTION

Rifampicin (rifampin) is combined with clarithromycin to improve efficacy and prevent resistance in drug regimens for the treatment of Mycobacterial infections [1].
Clarithromycin is primarily metabolised by cytochrome P450 (CYP) 3A iso-enzymes to an active 14-hydroxy metabolite[2] and rifampicin to the active metabolite 25-desacetylrifampicin[3]. Rifampicin is known to induce several metabolic liver enzymes, including CYP P450 iso-enzymes (e.g. CYP1A2, 2C9, 2C19 and 3A4)[4], while clarithromycin is an inhibitor of CYP3A4.[2]
Several small studies have suggested that rifampicin may decrease the clarithromycin serum concentrations without influencing the 14-hydroxyclarithromycin levels.[5, 6] The effect of clarithromycin on the metabolism of rifampicin and its plasma levels is unknown.
In a randomised controlled trial in Ghana [BURULICO; NCT00321178] the standard treatment for Mycobacterium Ulcerans infection (streptomycin and rifampicin for 8 weeks) is compared with streptomycin and rifampicin for 4 weeks followed by clarithromycin and rifampicin for 4 weeks. The pharmacokinetics of clarithromycin and rifampicin are studied in a subset of patients to evaluate the effect of the drug interaction on the plasma concentration of the drugs.
Therefore a simple, rapid and easy method for the measurement of the plasma concentrations of these drugs and their metabolites had to be set up.
Several HPLC-based methods for the detection of the individual drugs have been developed, but no previous method with a simultaneous determination of the two drugs and their main metabolites has been described. Chromatographic methods with fluorescent, ultraviolet, electrochemical or amperometric detection of the individual drugs[7-15] are time consuming, mostly require large sample volumes and are less sensitive than methods with mass spectrometric detection. The reported chromatographic methods for clarithromycin with tandem mass spectrometric detection (LC/MS/MS)[16-19] achieve sufficient sensitivities in short run times, but suffered from lacking the active 14-hydroxymetabolite. In one LC/MS/MS method the 14-hydroxyclarithromycin concentration was only estimated.[20] Two previous LC/MS/MS methods for the determination of rifampicin and the 25-desacetylmetabolite have been reported. The first method[21] determined the components in plasma and used a normal phase column. The other method[22], determining the components in serum, was limited by methodological flaws (i.e. the purity of the components and the linearity of the calibration curves). The two published LC/MS methods determined in plasma[23] or tissue[24] only rifampicin without measuring the metabolite.

For therapeutic drug monitoring, many drugs are measured in plasma or serum and are analyzed with a reversed phase column. Standardization of these chromatographic and mass spectrometric characteristics improves the efficiency, flexibility and cost effectiveness of therapeutic drug monitoring.
This paper describes a simple, rapid and fully validated LC/MS/MS method for the determination of clarithromycin, rifampicin and their main metabolites in human plasma, using a reversed phase column and electrospray positive ionization.

EXPERIMENTAL

Reagents

Clarithromycin ($C_{38}H_{69}NO_{13}$) and 14-hydroxyclarithromycin ($C_{38}H_{69}NO_{14}$) were provided by Abbott (Illinois, USA). Rifampicin ($C_{43}H_{58}N_4O_{12}$) and 25-desacetylrifampicin ($C_{41}H_{56}N_4O_{11}$) were provided by Sanofi-

Aventis (Frankfurt, Germany). The chemical structures of these components are shown in Figure 1. The internal standard, cyanoimipramine, was supplied by Roche (Woerden, the Netherlands). Acetonitrile for LC/MS and water for LC/MS were purchased from BioSolve BV (Valkenswaard, the Netherlands). Methanol Lichrosolv and the other used chemicals were of HPLC or analytical grade and were obtained from VWR (Amsterdam, the Netherlands). The precipitation reagent consisted of 0.04 mg/L cyanoimipramine dissolved in a mixture of methanol and acetonitrile (4:21, v/v). Pooled human plasma samples with EDTA as anticoagulant were put available according to the rules of the University Medical Center Groningen.

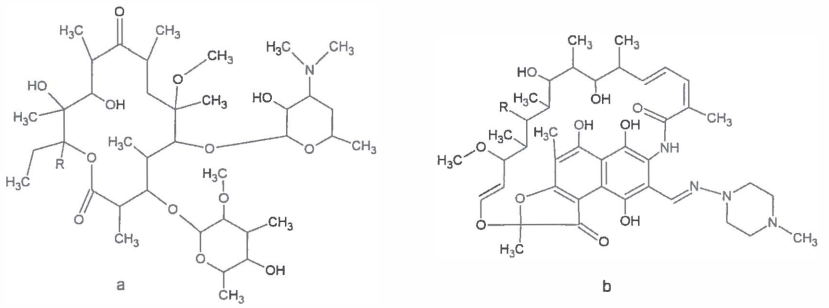

Figure 1 (a). Chemical structure of clarithromyin (R = H) and 14-hydroxy-clarithromycin (R = OH). (b). Chemical structure of rifampicin (R = OH) and 25-desacetyl-rifampicin (R = $COOCH_3$)

Calibration standards and quality control samples

Separate stock solutions for the calibration standards and QC samples were prepared by dissolving the components in methanol/water (1:1, v/v). These stock solutions were diluted with methanol/water (1:1, v/v) to prepare working stock solutions. The calibration standards and QC samples were prepared by diluting the stock or working stock solution with controlled pooled human plasma. The amount of the methanol solutions added to the plasma was less than 5% of the final volume. The calibration standards and QC samples were prepared on day zero and stored at – 20 °C. The concentrations of the stock solutions, calibration standards and QC samples are listed in Table 1.

Sample preparation

Frozen samples were thawed at room temperature. Of each sample (blank plasma, calibration standard or QC sample) 10 µL and 750 µL precipitation reagent containing the internal standard were vortexed for 1 minute, stored at -20 °C for about 30 minutes to promote protein precipitation and thereafter centrifuged at 11,000 *g* for 5 minutes. From the clear upper layer 5 µL was injected onto the LC/MS/MS system.

LC/MS/MS conditions

All experiments were performed on a Thermo Fisher (San Jose, USA) triple quadrupole LC/MS/MS with a Finnigan™ Surveyor® LC pump and a Finnigan™ Surveyor® autosampler which was set at a temperature of 20 °C. After sample preparation, 5 µL of the clear upper layer were injected on a 50 x 2.1 mm C_{18}, 5-µm analytic column (HyPurity Aquastar, Interscience Breda, The Netherlands) for chromatographic separation. The column temperature was set at 20 °C. The mobile phase had a flow of 0.3 mL/min and consisted of an aqueous buffer (containing ammonium acetate 10 g/L, acetic acid 35 mg/L and trifluoroacetic anhydride 2 mL/L water), water and acetonitrile. The mobile phase operated in the following gradient: 0 – 2 minute: 5% buffer, 95% at 0 minute to 0% at 2 minutes water and 0% at 0

minute to 95% at 2 minutes acetonitrile; 2 – 3 minutes: 5% buffer, 0% water and 95% acetonitrile; 3 – 3.1 minutes: 5% buffer, 0% at 3 minute to 95% at 3.1 minutes water and 95% at 3 minute to 0% at 3.1 minutes acetonitrile; 3.1 – 3.6 minutes: 5% buffer, 95% water and 0% acetonitrile.

The Finnigan™ TSQ® Quantum Discovery mass selective detector was operating in electrospray positive ionization mode and performed selected reaction monitoring (SRM) as scanning mode. The mass parameters were used at a scan width of 0.5 *m/z* and are listed in Table 2. The ion source spray voltage was set at 3500 V, the sheath and auxiliary gas pressure at 35 Arbitrary units (Arb.) and 5 Arb., respectively and the capillary temperature at 350 °C. Xcalibur® software version 1.4 SR1 was used for peak height integration for all components.

Table 2. LC/MS/MS conditions

Component	Parent $[M-H]^+$ (m/z)	Product $[M-H]^+$ (m/z)	Collision energy (eV)
clarithromycin	748.5	590.2	18
14-hydroxyclarithromycin	764.4	606.2	20
rifampicin	823.3	791.2	17
25-desacetylrifampicin	781.4	749.2	14
cyanoimipramine	306.2	218.0	39

Method validation

In accordance with the "Guidance for Industry - Bioanalytical Method Validation" of the FDA[25], method validation included selectivity, linearity, accuracy, precision and stability. Therefore, during three days, on each day a single calibration curve was obtained and the quality control samples were analyzed in fivefold. Linear regression was used to obtain calibration curves of the standards. Selectivity was evaluated by processing and analyzing six pools of blank human plasma.

Matrix effects (suppression or enhancement of ionization) were determined by continuous post-column infusion of a solution with clarithromycin, 14-hydroxyclarithromycin, rifampicin, 25-desacetylrifampicin and cyanoimipramine in methanol/water (1:1, v/v) and injection onto the column of first, the precipitation reagent without the internal standard, and second, processed blank plasma samples (precipitated with the precipitation reagent without the internal standard). Suppression or enhancement of the LC/MS/MS responses was investigated by comparing the responses of the blank precipitation reagent with the corresponding responses of the processed blank plasma samples.

The dilution integrity was investigated by diluting an over-the-curve QC (clarithromycin, 14-hydroxyclarithromycin: 25 mg/L; rifampicin, 25-desacetylrifampicin: 30 mg/L). Five over-the-curve QC's in three days were processed after a 10-fold dilution with blank human plasma.

Recoveries of the two drugs and the two metabolites were determined on three levels (LOW, MED, HIGH) by comparing the average peak height of the components in the processed QC's in plasma with the average peak height of the components in the processed standard solutions in 50% methanol, representing 100% recovery. The recovery of the internal standard was calculated by dividing the average peak height of the internal standard in 20 processed plasma QC's by the average peak height of the internal standard in 20 blank precipitation reagent samples and multiplying by 100.

Stability tests including three freeze-thaw cycles, storage in the refrigerator (5 °C), storage at room temperature (20-25 °C) and storage in the autosampler (20 °C, re-injection stability of processed samples) were evaluated by QC samples on two levels (LOW, HIGH) in five-fold.

All calibration standards and QC samples were stored with protection to light, because rifampicin is known to be light-sensitive[23]. Besides, QC samples on two levels (LOW, HIGH) in five-fold were stored at room temperature without protection to light, to compare the results with the correspond-

ing light-protected QC samples.
All samples (plasma samples and solutions in 50% methanol) were processed as described in the sample preparation.

RESULTS

Chromatography

Cyanoimipramine, clarithromycin, 14-hydroxyclarithromycin, rifampicin and 25-desacetylrifampicin had a mean retention time of 2.50 min, 2.55 min, 2.25 min, 2.45 min and 2.30 min, respectively. An example of the chromatographic results is shown in Figure 2.

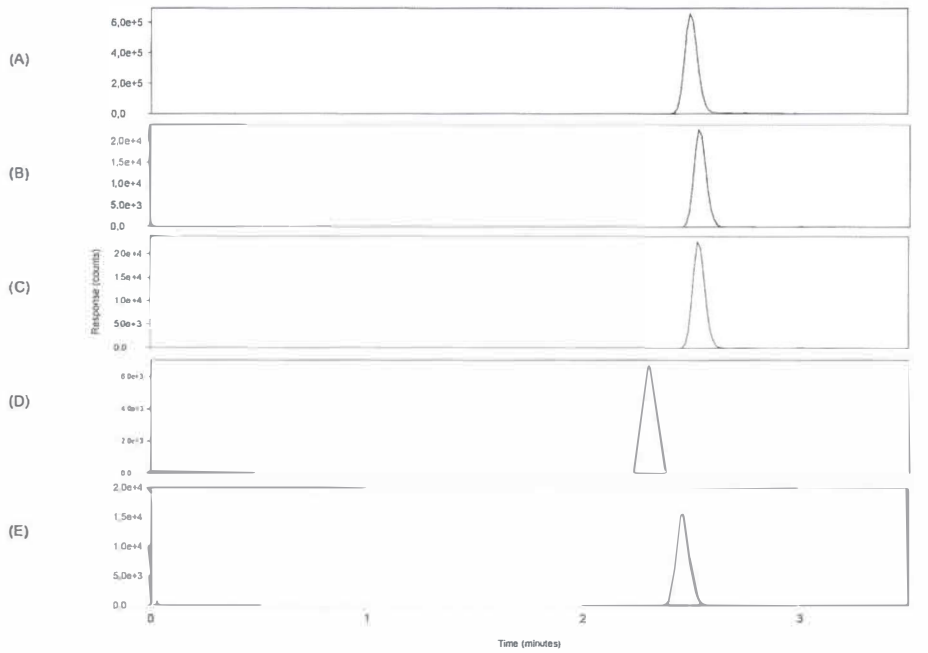

Figure 2a. Chromatograms of a LLOQ sample of cyanoimipramine (A), clarithromycin (B), 14-hydroxyclarithromycin (C), 25-desacetylrifampicin (D) and rifampicin (E)

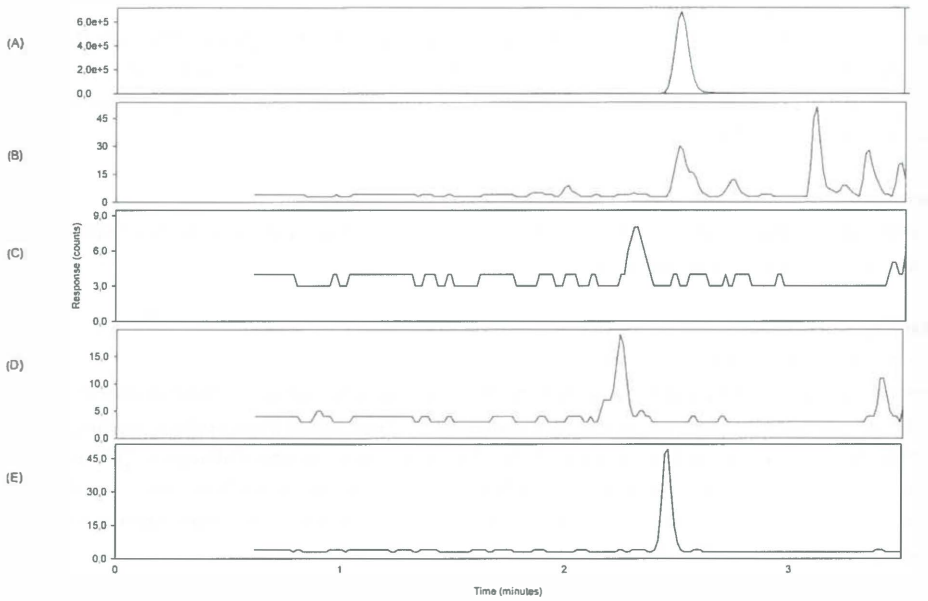

Figure 2b. Chromatograms of a blank human plasma sample of cyanoimipramine (A), clarithromycin (B), 14-hydroxyclarithromycin (C), 25-desacetylrifampicin (D) and rifampicin (E)

Selectivity

The selectivity of this method was evaluated by analyzing six lots of pooled human plasma in comparison with LLOQ samples. There were no peaks observed in any of the pooled human plasma samples at the retention times of clarithromycin, 14-hydroxyclarithromycin, rifampicin, 25-desacetylrifampicin and cyanoimipramine.

Matrix effects

No suppression or enhancement was observed during the continuous post-column infusion.

Linearity

Over a range of 0.10 – 10.0 mg/L (clarithromycin, 14-hydroxyclarithromycin) the calibration curves were linear by using a weight factor of 1/x. Over a range of 0.20 – 5.0 mg/L (rifampicin, 25-desacetylrifampicin) the calibration curves were linear (without using a weight factor). The interassay variability of the calibration curves is shown in Table 3.

Table 3. Interassay variability of the calibration curves

Component	Slope±SD	Intercept±SD	Correlation coefficient
clarithromycin	0.283±0.00236	0.000173±0.00310	0.99934
14-hydroxyclarithromycin	0.267±0.00526	0.00142±0.00703	0.99634
rifampicin	0.137±0.00442	0.00143±0.0122	0.99027
25-desacetylrifampicin	0.0532±0.00147	-0.000191±0.00409	0.99283

Accuracy, precision and dilution integrity

The accuracy, within-run precision and between-run precision of the four QC's and the over-the-curve QC met the acceptance criteria suggested by the FDA (for LLOQ: <20% CV and <20% bias; for the other levels: <15% CV and <15% bias). The results were statistical analyzed by using one-way ANOVA. The data are listed in Table 4.

Recovery

The recoveries ranged from 100.1-116.9%, depending on the drug type and concentration (Table 4). The recovery of the internal standard is 94.7%.

Stability

The results of the stability experiments are summarized in Table 5.
The autosampler stability test showed that the processed samples can be re-injected after three days in the autosampler. The QC samples were not affected by three freeze-thaw cycles and storage in the refrigerator (5 °C) for three days. Storage of the rifampicin and 25-desacetylrifampicin QC samples at room temperature (on the desktop without light protection) for one day had little effect, but after two days the concentration already declined for about 25-30%. The clarithromycin and 14-hydroxyclarithromycin QC samples could be stored for three days at room temperature.

Table 6 shows the influence of light-protecting the rifampicin and 25-desacetylrifampicin QC samples stored at room temperature. The decline of the concentration of the QC samples exposed to light for three days was larger compared to the QC samples protected from light for three days. The QC samples exposed to light had a significant lower concentration than the QC samples protected from light (Mann-Whitney test, $p<0.001$).
Clarithromycin and 14-hydroxyclarithromycin were not affected by either light exposure or light protection. Therefore the results of the clarithromycin and 14-hydroxyclarithromycin QC samples are not shown in Table 6.

Table 5. Results of stability experiments (%bias).

	CLR		14-OH-CLR		RIF		25-DA-RIF	
	LOW	HIGH	LOW	HIGH	LOW	HIGH	LOW	HIGH
Concentration (mg/L)	0.2	8.0	0.2	8.0	1.0	4.5	1.0	4.5
Autosampler (re-injection): 3 days	2.0	0.0	-0.4	3.0	-2.4	-12	-0.9	-4.0
Freeze-thaw: 3 cycles	1.3	-1.0	-2.7	2.0	-4.3	-6.0	-0.8	-1.0
Refrigerator: 3 days	0.9	-2.0	-3.0	2.0	-5.8	-7.0	-1.0	-2.0
Room temperature: 1 day	-2.8	-1.8	-3.0	0.6	-7.6	-9.1	-6.3	-9.8
Room temperature: 2 days	4.1	0.1	2.0	1.6	-26.9	-25.4	-29.8	-28.1
Room temperature:3 days	10.7	-1.5	-4.9	-8.7	-38.3	-35.3	-41.9	-38.1

[CLR: clarithromycin, 14-OH-CLR: 14-hydroxyclarithromycin, RIF: rifampicin, 25-DA-RIF: 25-desacetylrifampicin]

Carryover

At concentrations above 40.0 mg/L of rifampicin and 25-desacetylrifampicin carryover was observed. Several flush and wash solutions (used in the injection device in the autosampler) were tried, but they did not have the desired effect. Therefore 5.0 mg/L was chosen as the upper value of the calibration

Table 1. Concentrations (mg/L) of stock solutions, calibration standards and QC samples

Component	Stock solution	Working stock solution	Calibration standards	QC samples			
				LLOQ	LOW	MED	HIGH
Clarithromycin	500	50	0.10, 0.50, 1.0, 2.0, 4.0, 7.0, 10.0	0.10	0.20	5.0	8.0
14-hydroxyclarithromycin	500	50	0.10, 0.50, 1.0, 2.0, 4.0, 7.0, 10.0	0.10	0.20	5.0	8.0
Rifampicin	250	50	0.20, 0.50, 1.2, 2.0, 3.0, 4.0, 5.0	0.20	1.0	2.5	4.5
25-desacetylrifampicin	250	50	0.20, 0.50, 1.2, 2.0, 3.0, 4.0, 5.0	0.20	1.0	2.5	4.5

Table 4. Results of accuracy, precision and dilution integrity.

	clarithromycin					14-hydroxyclarithromycin					rifampicin					25-desacetylrifampicin					
	LLOQ	LOW	MED	HIGH	OTC	LLOQ	LOW	MED	HIGH	OTC	LLOQ	LOW	MED	HIGH	OTC	LLOQ	LOW	MED	HIGH	OTC
Concentration (mg/L)	0.1	0.2	5.0	8.0	25.0	0.1	0.2	5.0	8.0	25.0	0.2	1.0	2.5	4.5	30.0	0.2	1.0	2.5	4.5	30.0
Accuracy (%bias)	-5.4	0.2	-2.8	-0.4	0.0	-9.5	-0.3	1.5	3.3	5.0	-0.5	-3.5	-8.9	-9.8	-10.0	-6.3	-2.9	-4.5	-4.4	-6.0
Within-run precision (%CV)	7.7	3.9	2.5	3.0	2.0	5.3	6.3	2.5	2.5	3.1	5.8	2.5	4.4	2.8	4.7	9.3	5.7	5.2	4.0	7.2
Between-run precision (%CV)	4.5	2.9	0.3	0.0	0.8	6.7	0.0	0.9	3.1	0.0	0.0	1.9	3.5	1.1	3.1	5.4	0.0	2.5	3.3	6.4
Recovery (%)		111.4	100.1	105.9			116.8	101.3	108.3			109.1	109.4	101.3			116.9	106.0	104.1	

[OTC: Over-The-Curve]

Table 6. Influence of light exposure on the concentration of rifampicin and 25-desacetylrifampicin at room temperature

		rifampicin		25-desacetylrifampicin	
		LOW	HIGH	LOW	HIGH
[illegible]	mean concentration±SD [range] (mg/L)	0.967 ± 0.014 [0.948 - 0.981]	4.534 ± 0.188 [4.34 - 4.83]	1.013 ± 0.052 [0.953 - 1.09]	4.948 ± 0.217 [4.74 - 5.28]
	mean precision (%bias)	-2.4	1.7	3.2	12.1
	mean concentration±SD [range] (mg/L)	0.612 ± 0.025 [0.569 - 0.629]	2.884 ± 0.105 [2.77 - 3.00]	0.570 ± 0.019 [0.537 - 0.583]	2.730 ± 0.087 [5.59 - 2.82]
	mean precision (%bias)	-38.3	-35.3	-41.9	-38.1
[illegible]	mean concentration±SD [range] (mg/L)	0.724 ± 0.028 [0.683 - 0.753]	3.312 ± 0.051 [3.25 - 3.39]	0.704 ± 0.044 [0.649 - 0.767]	3.228 ± 0.129 [3.09 - 3.43]
	mean precision (%bias)	-26.9	-25.8	-28.2	-26.9

curve. To minimize the carryover further, five blanks before the run and five blanks before the patient samples were placed in the run.
No carryover was observed for clarithromycin and 14-hydroxyclarithromycin at the maximum concentrations of the calibration curve.

Internal Standards
The structure analogues of rifampicin and clarithromycin (roxithromycin and rifamycin SV) were intended to use as internal standards. However, the response of the components declined in time during validation tests and the chromatograms of rifamycin SV showed a degradation peak from which instability was concluded.
Therefore cyanoimipramine, a commonly used internal standard at the laboratory of the hospital pharmacy in the University Medical Center Groningen, was used. An autosampler stability test of five days showed an interday variability of 97-111% of the nominal response.

DISCUSSION

This LC/MS/MS method for the determination of clarithromycin, 14-hydroxyclarithromycin, rifampicin and 25-desacetylrifampicin in human plasma is fully validated for selectivity, linearity, precision, accuracy, dilution integrity, matrix effects, recovery and stability. The QC samples are stable after three freeze-thaw cycles, three days in the autosampler and three days in the refrigerator. At room temperature clarithromycin and 14-hydroxyclarithromycin QC samples are stable for three days, while rifampicin and 25-desacetylrifampicin are stable for only one day. Plasma samples containing rifampicin and 25-desacetylrifampicin showed accelerated degradation in exposure to light. Instability of rifampicin and 25-desacetylrifampicin due to light and heat is described earlier [26, 27] but the magnitude of the instability seems to be dependent of the local laboratory circumstances. Stability experiments have therefore to be performed in the local setting during method development. Samples should be protected from light and be analysed directly or frozen at -20°C.
Although, ranges of the calibration curves are sometimes wider [17, 23] , in our study these were based on the expected plasma concentrations of the components (depending on the dose and individual variation) and on the MIC of Mycobacterium ulcerans. Much lower LLOQ's do not contribute significantly to the area under the concentration time curve and require liquid-liquid extraction LLE[17]. Due to observed carryover of rifampicin the calibration range had to be narrowed compared to the method of analysis of Hartkorn and co-workers [23]. However, the influence of carryover was minimized after scaling down the rifampicin and 25-desacetylrifampicin calibration curve to 0.20 – 5.0 mg/L and placing five blanks before the run and five blanks before the patient samples. Peak concentrations above 5.0 mg/L rifampicin and 25-desacetylrifampicin can be expected in the routine analysis. All patient samples should be diluted a 10-fold with matrix before processing, which makes it possible to analyse all samples in one sequence. When the measured concentration of rifampicin and 25-desacetylrifampicin in the diluted sample is below 0.2 mg/L (similar to an undiluted concentration of 2.0 mg/L), the sample will be analyzed undiluted.
Cyanoimipramine is used to observe flaws in critical steps during sample preparation and chromatography. As in protein precipitation no classical extraction is performed, an structure analogue of the analyte is not mandatory to correct for extractions like LLE or solid phase extraction (SPE). The advantage of using a single agent as cyanoimipramine is that only one precipitation reagent is available

in the laboratory and therefore mistakes are prevented. Moreover, cyanoimipramine has never been used as drug in patients. As long as the result of the method validation comply with the guidelines of FDA for method analysis this is acceptable.

CONCLUSION

A rapid and fully validated LC/MS/MS method was developed for the determination of clarithromycin, 14-hydroxyclarithromycin, rifampicin and 25-desacetylrifampicin in human plasma. The method is value for 0.10-10.0 mg/L clarithromycin and 14-hydroxyclarithromycin and 0.20-30.0 mg/L rifampicin and 25-desacetylrifampicin. In case of high concentrations of rifampicin and 25-desacetylrifampicin (above 5.0 mg/L) the samples should be diluted a 10-fold before processing due to a carryover risk. This method is suitable for the evaluation of the expected pharmacokinetic drug interaction between clarithromycin and rifampicin.

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Chapter

7.2

Pharmacokinetics of rifampicin and clarithromycin in patients treated for Mycobacterium ulcerans infection

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ABSTRACT

In a randomised controlled trial in Ghana, Streptomycin (SM) / Rifampicin (RIF) for 8 weeks was compared with 4 weeks SM/RIF followed by 4 weeks RIF/Clarithromycin (CLA) for the treatment of *Mycobacterium ulcerans* infection. The effect of the drug-drug interaction of combined RIF and CLA on the pharmacokinetics of the two compounds was studied in a subset of patients. Co-medication with CLA resulted in a nonsignificant increase of AUC of RIF of 25.8 (21.7 – 31.5) mg*h/L compared to the AUC of RIF of 15.2 (15.0 – 17.5) mg*h/L in patients co-medicated with SM (P=0.13). The median AUC of CLA and 14-hydroxyclarithromycin (14OH-CLA) were 2.9 (1.5 – 3.8) mg*h/L and 8.0 (6.7 – 8.6) mg*h/L. Sum concentrations of CLA and 14OH-CLA above MIC of *M. ulcerans* were reached. Combination of RIF and CLA resulted in antibiotic exposure considered sufficient for the treatment of *M. ulcerans* infection.

INTRODUCTION

Although many anti-mycobacterial agents appeared effective in vitro and in animal models [1-4] against *Mycobacterium ulcerans* infection, clinical evidence for effectiveness of antimicrobial treatment was predominantly based on a small study in patients in Ghana [5]. Conceivably, using antimycobacterial agents not only results in preventing bacilli to replicate, and in killing of microorganisms, but also in halting the production of the toxin, mycolactone[6] that is produced by the enzymes encoded in the pMUM001 plasmid[7], that causes the tissue damage. Current WHO recommendations suggest 8 or more weeks of rifampicin plus streptomycin for treatment of all clinical forms of active Buruli ulcer Disease (BUD). Daily injections with streptomycin are problematic as most patients live in remote areas with limited health care facilities. Hygiene with these injections is insufficient, and intrinsic oto- and renal toxicity are a concern. Therefore, an oral treatment schedule is urgently needed to reduce the number of injections to improve the tolerability and safety of the proposed regimen; and pregnant women might also benefit from treatment without aminoglycosides. This problem was addressed by comparing 8 weeks (8SR-arm) of streptomycin (SM; 15 mg/kg) and rifampicin (RIF; 10 mg/kg) versus 4 weeks of streptomycin and rifampicin followed by 4 weeks (4SR/4CR-arm) of RIF plus clarithromycin (CLA; 7.5 mg/kg) in a randomised controlled trial (ClinicalTrials.gov Identifier: NCT00321178). CLA was chosen in the treatment regimen because of in vitro data suggesting that this drug is active against *M. ulcerans* having a minimal inhibition concentration of <0.125 to 2.0 mg/l[4]. For the majority of the strains the MIC was well below average CLA plasma concentration obtained at recommended dosage.

Clinical effectiveness of macrolides is only partly explained by pharmacokinetics, because these drugs typically accumulate in inflammatory cells at the site of infection, especially, in macrophages[8]. Although *M. ulcerans* infection has long been regarded as a predominantly extracellular infection[9], recently evidence has emerged from animal models that *M. ulcerans* infection has an intracellular stage with multiplication inside macrophages[10, 11]. These data taken together suggest that intra-macrophage CLA drug concentration might add to the beneficial effect of the drug to fight *M. ulcerans* infection, and that an effect of CLA might be exerted inside these immune cells without reaching inhibitory drug concentrations in the blood stream. On the other hand if plasma CLA drug concentrations would not reach inhibiting or mutant inhibiting concentrations, inadvertent monotherapy with RIF alone would result, at least in the extracellular space where bacilli are present in later stages of the disease. In mycobacterial infections, monotherapy has invariably resulted in failure of treatment as drug-resistant pathogens within the microbial load in the patient might escape and re-populate the diseased lesions in the host [12].
RIF is known to induce cytochrome P450 iso-enzymes, e.g., CYP 3A4 involved in the elimination of CLA, while CLA is also known to inhibit enzyme activity of CYP 3A4[13, 14]. The P-glycoprotein efflux transporter (Pgp) is also effected, as RIF induces and is substrate of Pgp and CLA inhibits Pgp[15, 16]. The purpose of this study was to assess the influence of this drug--drug interaction on the AUC of the plasma concentration – time curves of CLA and RIF in the 4SR/4CR study arm compared to AUC of rifampicin in patients who are in the 8SR study arm. Besides, the average time that plasma drug concentrations are maintained in excess of MIC was studied.

PATIENTS, MATERIALS AND METHODS

This open-label prospective pharmacokinetic study evaluated the pharmacokinetics of RIF and RIF combined with CLA in the treatment of *Mycobacterium ulcerans* disease in patients already enrolled in the BURULICO trial. The study was conducted at the Nkawie-Toaso Hospital, Nkawie and at the Agogo hospital, Agogo both in the Ashanti Region, Ghana.

Study subjects

Patients ≥ 10 years of age (male and female) were eligible for inclusion in this side study to assess pharmacokinetics of clarithromycin and rifampicin when allocated to the 4SR/4CR arm) and pharmacokinetics of rifampicin when allocated to the 8SR arm. Exclusion criteria were: treatment with macrolide or quinolone antibiotics, or antituberculous medication, or immunomodulatory drugs including corticosteroids within the previous one month; current treatment with any drugs likely to interact with the study medication, e.g, anticoagulants, cyclosporin, phenytoin, oral contraceptive, and phenobarbitone; history of hypersensitivity to rifampicin, streptomycin and or clarithromycin; inability to take oral medication or having gastrointestinal disease likely to interfere with drug absorption.

Drug administration

Patients received clarithromycin in a dose of 7.5 mg/kg once daily and rounded to the nearest 125 mg. Rifampicin was administered in a dose of 10 mg/kg and rounded to the nearest 150 mg. The drugs were administered on an empty stomach, but the participants were allowed to take a light standardized breakfast after drug ingestion. Although food intake does not influence the AUC of CLA[17], it influences the AUC of RIF[18]. By offering a standardized light breakfast approximately 30 minutes after drug ingestion, this effect on drug absorption would be minimized and equally distributed over both groups. The adherence to the treatment regimen was 100% as the patients were in a guided patient program.

Pharmacokinetic assessment

At the day of this side study, a full pharmacokinetic curve was recorded at steady state after a minimum of seven days of consecutive treatment with the same dose. Blood samples (2 ml, edetaat) were obtained before a dose of CLA/RIF or RIF was administered (t = 0) and at 0.5,1, 1.5, 2, 2.5, 3, 5, 8, 10 h after administration of the dose. Plasma was separated and frozen at -20°C until processed.

Analytical methods

Plasma concentrations of rifampicin and 25-desacetylrifampicin (25DA-RIF) and clarithromycin and 14-OH-clarithromycin were determined at the Laboratory for Clinical Toxicology and Drugs Analysis of the department of Hospital and Clinical Pharmacy of the University Medical Center Groningen, Groningen, The Netherlands, using a validated liquid chromatography tandem mass spectrometry (LC-MS/MS) assay[20].

Pharmacokinetic analysis

Pharmacokinetic parameters were calculated using the KINFIT (MWPharm 3.60; Mediware, the Netherlands) [21]. C_{max} was defined as the highest observed serum concentration with T_{max} as corresponding time. C_{min} was the serum concentration before intake of the dose. The $AUC_{0-10\ h}$ was calculated using the log-linear trapezoidal rule from 0 up to 10 hours. The elimination half-life ($t_{1/2}$) was calculated by $0.693/k_e$. The apparent clearance of the drug (Cl) was calculated by $dose/AUC_{0-10h}$ and the volume of distribution (V_d) was calculated by $dose/C_{ss}$.

Statistical methods

Data are presented as median values with interquartile range between brackets. Differences in age, body mass index and in the pharmacokinetic parameters between the patients groups were assessed with the Wilcoxon rank sum (Mann-Whitney *U*) test for unpaired data. A sample size of 10 was estimated to detect clarithromycin peak drug concentrations > 0.5 mg/L in 80% of the patients with a statistical power of 80% and a significance level of 5%. A sample size of 5 was estimated to detect a 20% increase in rifampicin drug concentrations in the presence of clarithromycin compared to rifampicin drug concentrations in the group with streptomycin with a statistical power of 80% and a significance level of 5%.

Ethics

This study was a side study of a randomised trial (ClinicalTrials.gov Identifier: NCT00321178). As for the main study protocol written and verbal informed consent was obtained from patients of 10 years and over, and of their custodians if they are below 18 years of age. The present study protocol was also approved by the local institutional ethics committee in the Kwame Nkrumah University of Science & Technology, and Komfo Anokye Teaching Hospital. In this side study, only individuals aged over 10 were eligible. All study participants gave informed consent after appropriate time to consider participation; a small incentive in cash and kind was offered; and in participants aged between 10 and 18 yrs, also their parents or care takers gave written informed consent.

RESULTS

Study subjects

Thirteen patients, twelve females and one male were included in this study, eight from the SR4/CR4 arm and five from the SR 8 arm. Baseline characteristics were similar for the patients from the 4SR/4CR study arm and the patients from the 8SR study arm for age, body weight, body mass index. Patient characteristics are presented in table 1.

Table 1: baseline characteristics

	Treatment arm		
	4SR/4CR (n=8)	8 SR (n=5)	
Age (years)	26.0 (13.5-41.0)	26.5 (19.5-36.3)	P = 0.07
Gender (M/F)	-/8	1/4	P = 0.4
Weight (kg)	53.5 (51.5 – 56.5)	45.0 (35.0 – 60.0)	P = 0.4
BMI (kg/m2)	18.7 (15.2-22.0) kg/m2	20.5 (19.0-22.9) kg/m2	P = 0.17

M, Male; F, female; BMI; bodymass index

Pharmacokinetic study

In both study arms the patients received the same dose of RIF per kg body weight. The patients in the 8SR study arm received RIF in a dose of 8. 8 (8.6-10.0) mg/kg once daily which was not significantly different from the dose of 8. 8 (8.4-10.8) mg/kg administered in the 4SR/4CR study arm (P = 0.70). The patients had received the study medication for a median duration of 50 (29 – 55) days before blood samples were drawn. The rifampicin concentration-time curves of both arms are displayed in figure 1.

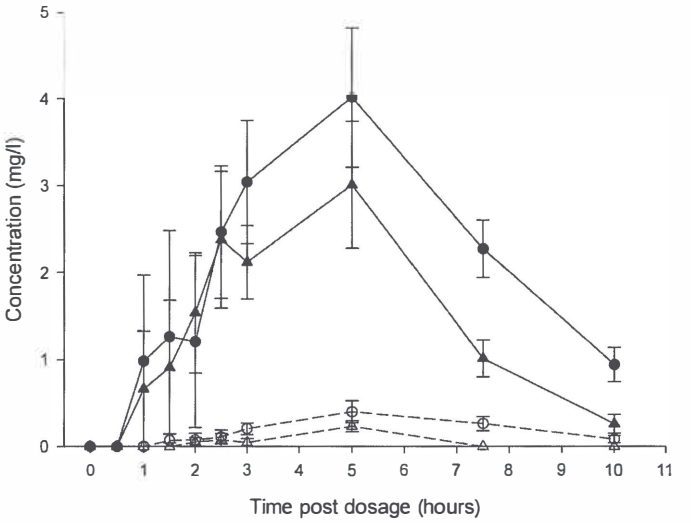

Figure 1: RIF/25DA-RIF concentration-time curve

Mean (SE) plasma concentration-time curves of RIF(solid) and 25DA-RIF (open) of SR4/CR4 arm (circles) and SR8 arm (triangles).

The clarithromycin concentration-time curve is displayed in figure 2. The pharmacokinetic parameters of the two treatment arms are summarised in table 2. The median $AUC_{0\text{-}10h}$ value of RIF of 25.8 (21.7 – 31.5) mg*h/L of patients from the 4SR/4CR arm, which was not significant different (P = 0.13) compared to the median $AUC_{0\text{-}10h}$ value of RIF of 15.2 (15.0 – 17.5) mg*h/L of patients from the 8SR arm. The elimination half-life of RIF was significantly longer in the 4SR/4CR group compared to the 8SR group (P = 0.045). Only in 5/13 patients 25DA-RIF levels could be detected. The mean time above the theoretical MIC of 0.5 mg/L was 101 (range 0 – 200) minutes for CLA, 344 (range 175 – 440) minutes for 14OH-CLA and 406 (range 295 - 483) minutes for the combined concentration of CLA and 14OH-CLA. The median AUC/MIC ratio of RIF was 52 (43 – 63) of patients from the 4SR/4CR arm, which was not significant different (P = 0.13) compared to the median AUC/MIC ratio of 30 (30 – 35) of patients from the 8SR arm.

DISCUSSION

This is the first study to investigate the drug-drug interaction between RIF and CLA in patients infected with *M. ulcerans*, as part of a prospective randomised trial comparing two drug regimen. We observed that the pharmacokinetic parameters of RIF were not significantly influenced by CLA. However, a significantly prolonged elimination half-life of RIF was observed in the patients also receiving CLA compared to patients receiving only RIF. This may be explained by the inhibitory effect of CLA on Pgp mediated efflux of RIF and not by the inhibitory effect of CLA on CYP3A4 as RIF is not a CYP3A4 substrate. The influence of the drug-drug interaction of CLA on Pgp on total drug exposure of RIF is limited as the AUC was not significantly increased. The effect of RIF on CLA is more difficult to explain as this study lacked a study arm without RIF. We observed that the exposure to the 14OH-CLA was significantly higher compared to CLA. The median 14OH-CLA concentration time curve (figure 2) is higher than the

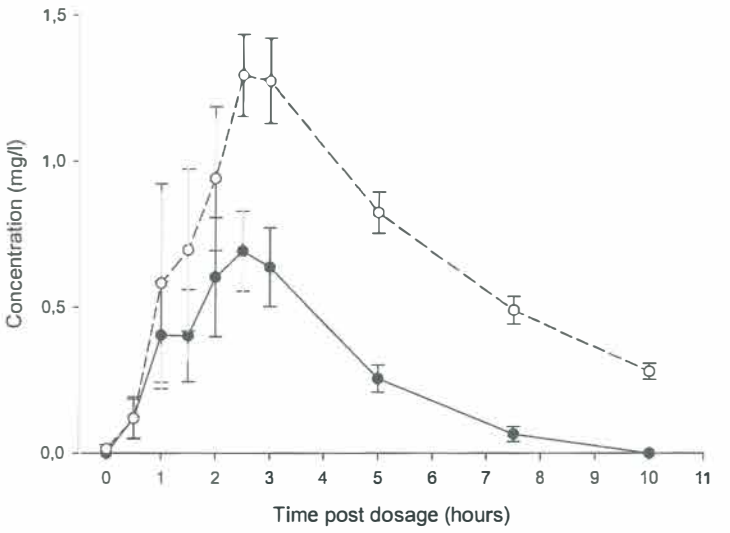

Figure 2: CLA/14-OH-CLA concentration-time curve

Mean (SE) plasma concentration-time curves of CLA (solid circles) and 14-OH-CLA (open circles) of SR4/CR4 arm.

CLA concentration time curve for the observed time period of 0-10h. This indicates that the metabolism of CLA to 14OH-CLA is induced by RIF as in healthy volunteers receiving only CLA, the 14OH-CLA concentration time curve is lower compared to the CLA concentration time curve [22].

The results of our study are in line with the observations of the drug-drug interaction of CLA and rifabutin (RIB), which has been studied in volunteers infected with HIV. Rifabutin, like rifampicin, induces Pgp and the CYP450 system, but less profound[23]. In this study the CLA-RIB drug-drug interaction resulted in a significantly decreased AUC of CLA compared to before introduction of RIB, but 14OH-CLA increased significantly along with a significant increase in RIB drug concentrations [24].

The major concern in case of these drug-drug interactions is that the resulting antibiotic exposure is not efficacious due to the altered plasma concentrations. The decrease in CLA levels has therefore to be compensated by the increase of 14OH-CLA and can be expressed as CLA/14OH-CLA ratio. Whether the observed ratio is sufficient for an efficacious treatment depends on the in vitro susceptibility of the targeted micro-organism for both CLA and 14OH-CLA. In our study we observed that the concentration of CLA and sum concentrations of CLA and 14OH-CLA above MIC of 0.5 mg/L of *M. ulcerans* were reached in plasma. We assume that that CLA and 14OH-CLA contribute equally to the bactericidal activity of the agent but in vitro susceptibility testing of 14OH-CLA, which needs to be carried out, have to confirm this assumption. The lower plasma concentration of CLA does not necessarily reflect its intracellular bactericidal efficacy as it depends on the distribution of the antibiotic into the tissue and cells[24]. Although interstitial fluid concentration in the skin might be lower compared to plasma[25], intracellular concentrations of CLA are expected to be higher than in plasma[22]. However, both were not determined. Despite these limitations, both CLA and RIF exposures are considered sufficient for the treatment of *M. ulcerans* infection.

Table 2 pharmacokinetics of rifampicin and clarithromycin in both treatment arms

parameter	Treatment arm						
			4SR/4CR (n=8)		8SR (n=5)		Diff in RIF
	CLA	14-OH-CLA	RIF	25DA-RIF#	RIF	25DA-RIF‡	P
AUC0–10h (mg*h/L)	2.9 (1.5 – 3.8)	8.0 (6.7 – 8.6)	25.8 (21.7 – 31.5)	4.2 (3.2 – 5.1)	15.2 (15.0 – 17.5)	1.1	0.13
Cmax (mg/L)	1.0 (0.5 – 1.3)	1.5 (1.2 – 2.1)	4.9 (3.3 – 6.4)	0.6 (0.4 – 0.6)	4.2 (4.0 – 4.3)	0.4	0.35
Tmax (h)	2.3 (1.6 – 2.9)	2.9 (2.4 – 3.5)	3.6 (3.2 – 4.4)	4.1 (3.3 – 4.4)	3.5 (2.5 – 3.9)	0.6	0.62
t1/2 (h)	1.3 (1.0 – 1.4)	2.6 (2.3 – 2.9)	2.0 (1.7 – 2.5)	2.2 (1.3 – 3.1)	1.5 (1.4 – 1.6)	0.1	0.045*
CL (L/h)	89.2 (68.8 – 51.2)	27.7 (25.0 – 36.6)	17.2 (15.8 – 23.5)	132 (109 – 156)	33.5 (23.9 – 36.6)	89.7	0.13
Vd (L)	174 (144 – 214)	107 (96.1 – 125)	60.7 50.1 – 67 .5)	425 (243 – 632)	58.4 (57.0 – 67.5)	13.5	0.83

Median values (IQR) of the following parameters are shown in the table: AUC_{0-10h}, area under the concentration–time curve from 0 to 10 h; C_{max}, maximum concentration of drug in serum; T_{max}, time to maximum concentration of drug in serum; $t_{1/2}$, elimination half-life; CL, total clearance; V_d, volume of distribution. Significant if $P < 0.05$. (#) 25DA-RIF n=4; (‡) 25DA-RIF n=1

CONCLUSION

Combination of RIF and CLA resulted in a non-significant increase in plasma levels of RIF, compared to RIF and SM. A decrease in CLA levels and increase in 14OH-CLA plasma levels was observed, but sum concentrations of CLA and 14OH-CLA above MIC of *M. ulcerans* were reached. Combination of RIF and CLA resulted in antibiotic exposure considered sufficient for the treatment of *M ulcerans* infection.

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Chapter 8

General discussion and future perspectives

The central theme of this thesis was to study the pharmacokinetics of antimicrobial agents to optimize individual treatment of critically ill patients suffering from invasive fungal infections and mycobacterial infections. We studied drug-drug interactions of antifungal and anti-mycobacterial drugs. We evaluated known drug-drug interactions and recommended how to manage patients by adjusting dosage. Further, we used drug level monitoring to describe new or reassess currently known interactions.

We have shown that the extent of drug-drug interactions may be more profound in critically ill patients than in healthy volunteers. We showed that in heavily treated patients with haematological malignancies the drug-drug interaction between voriconazole and phenytoin was more profound and less predictable than in healthy volunteers, which let to another recommendation than listed in the product leaflet [1]. Also metabolites should be evaluated along the parent compound in prospective drug-drug interaction studies as these metabolites may be active against the targeted pathogen and could achieve therapeutic concentrations [2]. Mechanistic studies using *in vitro* and *in vivo* models can improve the understanding of specific drug-drug interactions. Subsequently the translation has to be made to daily practice. Therefore new prospective studies should evaluate the effect of drug-drug interactions on the pharmacokinetics of antimicrobial drugs along a range of disease severity. Especially, pharmacokinetic studies only performed in healthy volunteers should be evaluated in critically ill patients. By necessity, such studies can only have an observational design, as withholding drugs in a cross-over design is unethical. Extensive monitoring of plasma or serum levels is feasible as these patients typically have central venous and arterial access, and only small volumes as 100 µl using LC/MS/MS are needed to measure drug concentrations. More challenging but equally important is the study of drug penetration in sanctuary sites such as CSF during the continuation phase of leptomeningitis. Obviously such studies are only possible in a close collaborative effort.
To easily detect these patients an computerised physician order entry system (CPOE) is essential as drug-drug interactions are presented and function as trigger for eligibility. The physician in consultation with a hospital pharmacist can take blood samples based on a treatment protocol. The evaluation of the drug-concentration can be used to optimize the treatment of that particular patient but also to evaluate the extent of the drug-drug interaction subjected to investigation. The drug-drug interaction between posaconazole and omeprazole [3] could have been prevented if it had been incorporated in the CPOE. However, it takes time before new drug-drug-interactions are incorporated because these systems are periodically updated. The CPOE enables the Clinical Pharmacist to retrieve patients with combinations of medications at risk for therapeutic failure by introduction of clinical rules. In case of the described drug interactions the clinical rule could have been: show all patients receiving [posaconazole] AND [any proton pump inhibitor]. The clinical pharmacist could subsequently contact the attending physician and propose alternative drugs. After incorporation of the new drug-drug interaction in the CPOE the clinical rule can be discontinued.

Population pharmacokinetics of critically ill patients has been described for several drugs, but the influence of disease severity on pharmacokinetics has not yet been extensively debated. A full range of disease severity needs to be captured in order to detect the correlation between disease severity score and PK parameters. The sample size of 18 patients of our study was unfortunately not sufficient to show a significant relation between the two parameters, but analysis of the results showed trends towards significance on subgroup analysis [4]. Nonetheless, this strategy might be the first step to developing initial dosing algorithms for antimicrobial agents in critically ill patients. In future studies a more homogenous patient population should be selected to be able to fully appreciate the potential of this approach. New target populations should include transplant recipients. Different stages of ill-

ness can be defined within these more homogenous patient populations. Selection criteria for such pharmacokinetic studies could be stratified for the different stages post-transplant, e.g., early post-operatively; 1-3 months after transplant; and later during follow-up. In the early post-transplant period, the whole spectrum of disease severity is likely to be represented, and correlations can be detected based on a sample size of about 20 patients.

In treatment individualisation of antimicrobial drugs, dose target values are very important. These target values should be based on PK/PD parameters of the antimicrobial drugs. The PK/PD parameters have to be evaluated in *in vitro* and *in vivo* animal models before being tested in man. After phase II and phase III studies antimicrobial drugs are registered and subsequently marketed with a recommended dose for a labelled indication. However, in this thesis we showed that the dose as indicated on the leaflet by the drug company may not be the optimal dose for every patient [4-7]. The first step towards individualised dosing can be a simple observational study to gather data on variability in PK values (including protein binding) and variation in treatment outcome on efficacy and safety in daily practice [8-10]. If a significant variation is observed, a randomised trial can be designed to compare the standard dosage of the drug with an individually tailored dose. Pharmacokinetic modelling can assist the clinical pharmacist in developing dose algorithms and enable individualised dosing in daily practice in a more time efficient manner [11]. These models can be used in prospective randomised trials to define the individually tailored dose in the intervention arm.

Superiority of the individually tailored dose has to be proven before this strategy can be implemented in clinical guidelines. Based on our prospective pharmacokinetic voriconazole study a prospective, clustered group randomised controlled trial was designed to evaluate the efficacy and safety of therapeutic drug monitoring of voriconazole in patients with haematological malignancies (NCT00893555). This study is the joint effort of thirteen hospitals and is financially supported by ZONMW. Study groups, like this one, are essential, as a single centre would be unable to enrol the number of study participants required to meet the calculated sample size.

More complicated is treatment individualisation in case of off-label prescription for TB. As phase I, II and III studies are lacking, the leaflet-indicated dose is often used for the off label indication. The susceptibility of different micro-organisms to that drug and PK/PD models [12] is the first step to assess its usefulness for other than the labelled indication. The next phase in the evaluation of the drug is often the review of case reports describing the success of that specific drug in critically ill patients without any treatment options. Case-series and retrospective evaluation of treatment results will increase the knowledge on how to dose the drug for the off label indication [8-10]. Eventually, prospective pharmacokinetic and safety studies will proceed on phase III studies for the off label indication. For these phase III studies financial support is mandatory and collaboration of different hospitals essential. Today, alliances like the TB alliance have been formed to develop drugs for this global infectious disease. Unfortunately, the involvement of pharmaceutical industry is still limited today as TB is not an attractive field for drug development. However, an increasing trend of participation in alliances is observed.

In an ageing population, suffering from multiple co-mordities, along with occurrence of drug resistance treatment individualisation by therapeutic drug monitoring (TDM) is an important tool to increase the efficacy and minimize toxicity in this frail population. New methods for sampling have to be developed to increase the patients comfort compared to traditional vena puncture. Dried blood spot technique [13] or measuring drug concentrations in saliva [14, 15] are very promising and can be used for pharma-

cokinetic studies and TDM. Apart from the frail elderly, children present an important target population that is poorly studied for pharmacokinetics. Studying these groups is even more challenging, both from ethical and from logistic points of view, but we believe these groups should benefit from individualised targeted treatment as well.

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Chapter 9

Summary

The central theme of this thesis was to study the pharmacokinetics of antimicrobial agents to optimize individual treatment of critically ill patients suffering from severe infectious diseases. The focus of the studies was on invasive fungal infections, tuberculosis and Buruli ulcer disease. Patients with tuberculosis and invasive fungal disease are at risk of severe morbidity and mortality, with important co-morbidities. While patients with invasive fungal disease are immuno-compromised, either due to underlying hemato-oncological conditions or the various treatments for these conditions, or have organ transplants for which they receive immunosuppressive agents, tuberculosis patients typically receive multiple drugs, especially if they have drug-resistant tuberculosis; and many suffer from co-morbid conditions including HIV/AIDS, or psychiatric co-morbidities including substance addiction with a myriad of potential drug interactions. Because these patient groups receive multiple drugs, they do not fit into a the definition of a *general population* of patients that can be assumed to be easily managed by standard fixed-dose treatment regimens, and we hypothesized therefore that these patient groups would benefit from individualised therapy to increase efficacy and limit toxicity. Buruli ulcer is a condition caused by a micro-organism sharing common phylogenetic ancestry with *M. tuberculosis*. We studied pharmacokinetics in patients receiving combination drug therapy, addressing questions derived from a randomized study in which these patients participated. We aimed to test whether we truly provided multiple drug therapy to prevent inadvertent monotherapy, and effective drug dosing, considering a complex array of possible drug-drug interactions resulting in potential inadvertent inappropriate drug dosing.
The main objectives are to gain insight in influence of disease severity, drug-drug interactions and ADME on pharmacokinetics of antimycobacterial and antifungal drugs and impact on PK/PD parameters.

Invasive fungal infections

In immuno-compromised individuals, suffering from invasive fungal infections, individualised treatment seems mandatory to increase the poor results achieved by current available antifungal drugs.

In **Chapter 2** we investigated in a prospective observational pharmacokinetic study the large variability in pharmacokinetics of voriconazole in patients suspected to have a fungal infection. We hypothesized that disease severity influenced on the pharmacokinetics of voriconazole. Serum samples were collected in patients who received voriconazole in a standard dose and analyzed by LC/MS/MS, which was developed for this study. Pharmacokinetic parameters were subsequently calculated and correlated with disease severity, which was assessed by Simplified Acute Physiology Score (SAPSII) and Sequential Organ Failure Assessment (SOFA) scoring systems. Eighteen patients were included in this study, (ICU n= 8; ward n=10). On day two ICU patients had significantly lower drug exposure compared to the AUC of patients on the ward. The correlation between the two disease severity scores and the $AUC_{day\,1}$ was non-significant for the SAPSII score and SOFA score. However, if voriconazole was administered intravenously or when the SAPSII score was ignored for the parameters *chronic diseases* and *type of admittance* there was a trend for correlation between $AUC_{day\,1}$ and SAPSII. A significant correlation was observed between C_{max1} and SOFA score. From this study we concluded that a correlation between disease severity and pharmacokinetics of voriconazole seems plausible. Unfortunately, due to the heterogeneous study population, the study lacked power to draw definitive conclusions.
Further efforts should be made to explore the relation between disease severity to support new dosing strategies of voriconazole in critically ill patients.

We addressed the question how to adjust the dosage in patients with high voriconazole serum concentrations, to reach the therapeutic window and prevent adverse effects, as no adjustment algorithms have been published to date. We retrospectively investigated patients presenting with a voriconazole trough level of > 5mg/L in whom the drug was stopped and monitoring of trough levels was continued. We showed that voriconazole levels slowly decreased to levels within the therapeutic window. Strikingly, an exponentially increased voriconazole elimination median half-life of 77.6 h was observed compared with the commonly observed half-life of 6–8 h. Our results showed that voriconazole could be stopped for at least two consecutive days. In our experience patients presenting with elevated liver enzyme values >3-5 times ULN are 'at risk' of developing high voriconazole trough levels. The observations suggest that routine monitoring of voriconazole levels in patients with haematological malignancies after 3 days of therapy is necessary to prevent voriconazole adverse effects caused by toxic levels.

Optimal dosing of voriconazole in children is still subject of debate. The approved dosage is 7 mg/kg twice daily, but suggestions have been made that a larger dosage may be needed to reach therapeutic serum concentrations. In a critically ill child we showed that although the standard intravenous dosage appeared effective to reach target serum concentrations at the onset of therapy, it failed to maintain these concentrations. Disease stage marked by an increased vascular permeability, possibly aggravated by cessation of norepinephrin, could have resulted in an increased volume of distribution with low serum values. Increasing the dosage resulted in slightly increased trough concentrations and in unexpectedly high peak values. This led to the important conclusion that measuring only trough levels could have led to the wrong assumption that serum values are inadequate. Monitoring not only trough levels but also peak and mid levels of intravenously administered voriconazole seems necessary in critically ill pediatric patients, to assure adequate exposure.

In **Chapter 3** we focused on drug-drug interactions with azole antifungal drugs. A systematic review was performed to identify drug-drug interactions with azole drugs, which resulted in an overview of all published drug-drug interactions in humans. The extent to which metabolic pathways and/or other supposed mechanisms are involved in these interactions were provided. We developed recommendations for managing the specific interactions based on a risk rating system, which ranged from "no known interaction / no action needed" to "monitor therapy / avoid combination". These recommendations are helpful for physicians and pharmacists to optimize the treatment in individual patients.

In a letter to the editor we showed that routine monitoring resulted in the recognition of a previously not described drug-drug interaction between posaconazole and omeprazole. Because of the increased pH of the stomach as a result of the proton pump inhibitor omeprazole the absorption of posaconazole was significantly decreased, which could result in therapeutic failure. We recommended that if combination with a proton pump inhibitor could not be avoided, serum levels should be monitored at all times to evaluate posaconazole absorption, or antifungal therapy should be switched to an alternative compound.

Based on daily observations a retrospective study was performed to reassess the advice in the product leaflet how to handle the drug-drug interaction between phenytoin and voriconazole. This drug–drug interaction was recognised earlier and therefore evaluated in a prospective study in healthy volunteers in whom increasing the dose of voriconazole from 200 mg twice daily to 400 mg twice daily compensated for the effect of phenytoin on voriconazole AUC. We showed that in heavily treated patients

with haematological malignancies the drug-drug interaction between voriconazole and phenytoin was more profound and less predictable than in healthy volunteers. Our recommendation was therefore different from the product leaflet and that the combination of phenytoin and voriconazole should be avoided whenever possible by using other antiepileptic (e.g. levetiracetam) and antifungal drugs (e.g. an echinocandin or lipid formulation of amphotericin B) or when not avoided, serum levels should be monitored at all times to evaluate voriconazole serum concentrations and adapt the voriconazol dose.

In **Chapter 4** we investigated linezolid, which is a promising drug for the treatment of multi – and extensively drug resistant tuberculosis (MDR-TB and XDR-TB). However, its use limited by toxicity (i.e. neuropathy and anaemia) using 600 mg twice daily at prolonged administration. In order to reduce its toxicity and maintain efficacy, we investigated whether linezolid in a reduced dosage resulted in drug serum concentrations exceeding the *in vitro* MIC. We developed a linear, selective, accurate and precise liquid chromatography-tandem mass spectrometry (LC-MS/MS) method to measure linezolid in serum. In a prospective pharmacokinetic study we investigated the pharmacokinetics of linezolid in two dosage regimens. Eight patients with either MDR-(or XDR) TB received linezolid as part of their treatment. They received 300 mg of linezolid bid, 3 days later followed by 600 mg bid. At steady state of each dosage a 12-h linezolid concentration time curve was obtained. MIC values of the TB bacilli were determined by the RIVM. We used $AUC_{0\text{-}24h}$/MIC ratio as a predictive model for efficacy. At dose of 300 mg the AUC/MIC ratio was at least 100 and sufficient to continue treatment. Linezolid in a dose of 300 mg was well tolerated. This study showed that a dose reduction was feasible, but larger numbers of patients should be studied, to confirm the efficacy of the 300 mg twice daily in MDR-or XDR-TB treatment.

To support individualised dosing a model for the prediction of linezolid area under the plasma concentration-time curve from 0 to 12 hours ($AUC_{0\text{-}12h}$) using limited sampling was developed. From fourteen MDR-TB patients, who received linezolid twice daily as part of their anti-tuberculosis treatment, serum concentration-time curves were determined at steady state by LC/MS/MS spec. Using the software package MWpharm 3.60 (Mediware, The Netherlands) we developed and validated an population pharmacokinetic model (POP-PK) and calculated limited sampling models. The clinically most relevant model for prediction of linezolid $AUC_{0\text{-}12h}$ was by using a linezolid trough level. The correlation between predicted linezolid $AUC_{0\text{-}12h}$ and observed linezolid $AUC_{0\text{-}12h}$ was investigated by Bland-Altman analysis and showed that linezolid $AUC_{0\text{-}12h}$ in MDR-TB patients could be predicted accurately. These two studies paved the road for a prospective randomised study evaluating the efficacy and toxicity of linezolid in the treatment of MDR-TB.

In **Chapter 5** we focused on moxifloxacin, a broad spectrum of antimicrobial drug, which is used for treating bacterial infections of the respiratory tract and soft tissues. From *in vitro* and *in vivo* experiments appeared bactericidal activity of MFX against *Mycobacterium tuberculosis*, which was high in comparison with other fluoroquinolones and is equal to or greater than that of isoniazid. Therefore this drug is more frequently used for the treatment of TB. MFX seems also promising for the treatment of TB in sanctuary sites, like tuberculosis meningitis. Like other fluoroquinolones, the most predictive parameter for efficacy of treatment with MFX is the AUC/MIC ratio. Both the AUC and the MIC value can be variable and drug-drug interactions can influence MFX exposure. As MFX is not licensed for the treatment of TB measuring plasma concentrations can therefore be useful to determine the optimal dose. We therefore developed a method of analysis using LC/MS/MS for the determination of MFX in

plasma, CSF. As the efficacy of an antimicrobial drugs is based on the unbound concentration of that drug method validation was also performed for MFX in plasma ultrafiltrate. To evaluate the current dose of MFX of 400 mg once daily we designed a prospective treatment protocol for TB meningitis and a retrospective study to evaluate the treatment with MFX.
Moxifloxacin cerebrospinal fluid (CSF) penetration was evaluated by obtaining full plasma and CSF time concentration curves from four patients with tuberculous meningitis. Pharmacokinetic (PK) parameters were assessed with standard non-compartmental pharmacokinetic methods using the software package MWPharm 3.60 (Mediware, The Netherlands). Ratios of the AUC_{0-24h} in CSF versus plasma were calculated and showed that adequate exposure was achieved in most, but not in all patients. Unbound concentrations could be measured in two patients and were 40 and 60% respectively. In the patients with low AUC/MIC ratio's the dosage was increased to continue treatment.

We retrospectively evaluated safety and pharmacokinetic/pharmacodynamics parameters in 92 tuberculosis patients receiving MFX 400 mg once daily as a part of their TB treatment. MFX was very well tolerated. Adverse effects that occurred during treatment were vomiting (n=2), diarrhoea (n=6) and *Clostridium difficile* (n=2) but let to discontinuation in only 2 cases (vomiting and diarrhoea). No QT prolongation was observed. They received a median dose of MFX of 6.9 (IQR) 6.0-8.2) mg /kg for median period 76.5 (IQR; 29 - 197.3) days. Geometric mean total and unbound MFX AUC_{0-24h} were evaluated in a subset of patients (n=18) and were 26.7 (range 8.5 –72.4) mg*h/L and 22.3 (range 12.3 – 64.4) mg*h/L. Geometric mean AUC/MIC ratio's were 163 (range 13 - 579) for bound and 120 (range 10 - 515) for unbound MFX. These data show that MFX in a dose of 400 mg once daily was well tolerated for a prolonged period. Considering the variability in unbound AUC_{0-24h}/MIC ratio, as a result of variability in protein binding, AUC_{0-24h}, MIC and influence of co-medication with RIF, therapeutic drug monitoring could be recommended in selected patients (i.e. MIC > 0.25 mg/L and RIF; MIC > 0.5 mg/L; diarrhoea or vomiting). Additional safety measures regarding hepatic and renal function and QT prolongation do not seem mandatory.

In **Chapter 6** we retrospectively investigated ethambutol in 760 TB patients (1992-2007) in an attempt to find predisposing factors for optical neuropathy, an infrequent and dose dependent side effect. Ethambutol, is very water soluble and if ethambutol is given to obese patients and is dosed on total body weight the concentration in the water compartment may be high compared to the normally underweighted TB patients and therefore an increased prevalence of these adverse drug effect may be observed. EMB related visual disturbance were reported in 0.8% of the patients and four of the six patients were clearly overdosed due to daily dosing based on total body weight instead of dosing on lean body mass. Based on these findings we recommend dosing in adults patient on ideal body weight.

In **Chapter 7** we explored the effect of a drug-drug interaction between rifampicin and clarithromycin in relation to combined treatment in patients with Buruli ulcer. To be able to measure both drugs including potentially active metabolites we developed a rapid and fully validated liquid chromatography-tandem mass spectrometry (LC/MS/MS) method which was suitable for the determination of clarithromycin, 14-hydroxyclarithromycin, rifampicin and 25-desacetylrifampicin in human plasma with accuracy and reproducibility at the limit of quantification. We performed a prospective pharmacokinetic study in a subset of patients from a randomised controlled trial comparing streptomycin (SM) and rifampicin (RIF) for 8 weeks with 4 weeks SM/RIF followed by 4 weeks RIF/clarithromycin (CLA) for the treatment of *Mycobacterium ulcerans* infection to evaluate the effect of the drug-drug inter-

action of combined RIF and CLA on the pharmacokinetics of the two compounds. Patients received clarithromycin in a dose of 7.5 mg/kg once daily and rounded to the nearest 125 mg. Rifampicin was administered in a dose of 10 mg/kg and rounded to the nearest 150 mg. At the day of this side study, a full pharmacokinetic curve was recorded at steady state and pharmacokinetic parameters were calculated using MWPharm 3.60. Co-medication with CLA resulted in a nonsignificant increase of AUC of RIF compared to the AUC of RIF in patients co-medicated with SM (P=0.13). As sum concentrations of CLA and 14OH-CLA above MIC of *M. ulcerans* were reached the combination of RIF and CLA resulted in antibiotic exposure considered sufficient for the treatment of *M. ulcerans* infection.

In the final chapter (**chapter** 8) we discussed the clinical impact of the thesis and showed the relevance of treatment individualisation by therapeutic drug monitoring and how provide supportive evidence based. We highlighted the strategy how to optimize the use of drugs, which are not labelled for the treatment of tuberculosis. In conclusion we pointed out that specific patients groups, like frail elderly or young children, besides the studied critically ill patients should benefit from individualised targeted treatment as well.

Samenvatting

In **hoofdstuk 1** worden de opzet en doelstellingen van dit proefschrift beschreven. Het doel was de individuele medicamenteuze behandeling van infectieziekten bij ernstig zieke patiënten te optimaliseren. Binnen het gebied van infectieziekten waren de studies gericht op invasieve schimmelinfecties, tuberculose en Buruli ulcus. Invasieve schimmel infecties komen voornamelijk voor bij patiënten met een verminderde afweer als gevolg van behandeling voor hematologische maligniteit of behandeling met immuno-suppressiva vanwege orgaan transplantatie. De medicamenteuze behandeling van tuberculose bestaat uit een combinatie behandeling van groot aantal geneesmiddelen. In het geval van resistente vormen van tuberculose, co-infectie met HIV/AIDS, andere co-morbiditeiten en bij gebruik van verslavende middelen wordt de behandeling complexer als gevolg van interacties en bijwerkingen. Aangezien deze patiënten niet gerekend kunnen worden tot de "algemene populatie" is het aannemelijk dat behandeling met een vaste dosering niet het optimale behandelresultaat zal opleveren. Onze hypothese was dat een op de patiënt toegespitste behandeling de effectiviteit van de behandeling kan verbeteren en bijwerkingen verminderd kunnen worden.

Buruli ulcus is een aandoening veroorzaakt door een aan de tuberculose bacterie verwant micro-organisme. Uit een gerandomiseerde studie naar de behandeling van Buruli ulcus met rifampicine (RIF) en claritromycine (CLA) ontstonden een aantal vragen omdat het onduidelijk of het effect van de interactie tussen RIF en CLA resulteerde in onbedoelde mono-therapie of de beoogde combinatie therapie.

Het primaire doel van dit proefschrift is inzicht te verwerven in de invloed van ziekte-ernst, geneesmiddelen interacties en absorptie, distributie, metabolisme en excretie op de farmacokinetiek van antimycobacteriële en antifungale geneesmiddelen en effect op de farmacokinetisch/farmacodynamische (PK/PD) parameters.

In **hoofdstuk 2** beschreven we in een prospectieve observationele farmacokinetiek studie de variabiliteit van voriconazol bij patiënten bij wie een invasieve schimmelinfectie werd vermoed. De hypothese van deze studie was dat ziekte-ernst van invloed zou zijn op de farmacokinetiek van voriconazol. Bloedmonsters werden afgenomen bij patiënten die behandeld werden met voriconazol. De concentratie voriconazol werd bepaald met behulp van een door ons ontwikkelde analysemethode met vloeistof chromatografie met massa spectrometrie (LC/MS/MS). De farmacokinetische parameters zijn op basis van de concentraties in bloed berekend en gecorreleerd met ziekte-ernst. De ziekte-ernst werd in kaart gebracht met de "Simplified Acute Physiology Score" (SAPSII) en "Sequential Organ Failure Assessment" (SOFA) score systemen. Achttien patiënten deden mee aan deze studie, van wie acht op de intensive care (IC) en tien op een verpleegafdeling waren opgenomen. Op dag twee van de behandeling hadden patiënten op de IC een lagere geneesmiddel serum concentratie (berekend als AUC) dan patiënten op de verpleegafdeling. De correlatie tussen de twee ziekte score systemen en de AUC op dag 1 was niet significant voor de SAPSII score en SOFA score. Een trend naar significantie werd gezien als patiënten werden geselecteerd die voriconazole als intraveneus infuus kregen toegediend. Indien van de SAPSII score de parameters chronic diseases en type of admittance werden genegeerd werd ook een bij significante trend naar correlatie tussen AUC op dag 1 and SAPSII gezien. Er werd een significante correlatie tussen C_{max} op dag 1en SOFA score gevonden. Op basis van deze studie lijkt een correlatie tussen ziekte-ernst en farmacokinetiek van voriconazol waarschijnlijk. Helaas kunnen door de heterogene samenstelling van de studiepopulatie en de beperkte aantallen nu nog geen definitieve conclusies worden getrokken.

Uit de literatuur is nog niet duidelijk hoe er met hoge (potentieel toxische) serum voriconazol concentraties dient te worden omgegaan. Praktische adviezen op welke manier de dosering aangepast dient te

worden om het therapeutisch gebied te bereiken en bijwerkingen te voorkomen zijn nog onbekend. In een retrospectief onderzoek hebben we daarom gekeken naar patiënten met een voriconazol dalspiegel groter dan 5 mg/L. Bij een deel van deze patiënten is het monitoren van de voriconazol spiegel gecontinueerd na staken van de behandeling. Het bleek dat bij deze patiënten de toxische voriconazol spiegels langzaam daalden tot in het therapeutisch gebied waarbij mediane eliminatie halfwaardetijd exponentieel was toegenomen van 6-8 tot 78 uur. Een mogelijke verklaring hiervoor is een verzadiging van het metabolisme. De lange eliminatie halfwaardetijd toond aan dat voriconazol veilig gestopt kan worden voor ten minste twee opeenvolgende dagen. Naar onze mening lopen patiënten met 3-5 keer verhoogde leverenzym waarden een groter risico op hoge voriconazol dalspiegels met mogelijke bijwerkingen als gevolg. Deze bevindingen suggereren dat routinematig afnemen van een dalspiegel 3 dagen na start van de behandeling bij patiënten met een hematologische maligniteit noodzakelijk is om bijwerkingen te voorkomen.

De juiste dosering van voriconazol voor kinderen is nog steeds onderwerp van discussie. De vastgestelde dosering is tweemaal daags 7 mg/kg, maar er zijn aanwijzingen dat hogere doseringen noodzakelijk zijn om spiegels in het therapeutische gebied te krijgen. Bij een ernstig ziek kind bleek dat de standaard dosering resulteerde in een therapeutische bloedspiegel. Echter, gedurende de behandeling bleven de spiegels niet stabiel maar daalden deze. De klinische situatie van het kind, met een toegenomen vasculaire permeabiliteit, resulteerde in een toegenomen verdelingsvolume met als resultaat lagere voriconazol dalspiegels. Het verhogen van de dosering had slechts een geringe toename van de dalspiegel als gevolg, maar zorgde wel voor sterk toegenomen topspiegels. Hieruit meenden wij dat geconcludeerd kon worden dat het meten van alleen een dalspiegel in dit geval tot de verkeerde aanname geleid zou hebben dat de blootstelling aan voriconazol onvoldoende zou zijn. Het lijkt daarom noodzakelijk om bij ernstig zieke kinderen, naast een dalspiegel, ook top en midspiegels te meten indien voriconazol als infuus wordt gegeven om ervoor te zorgen dat de blootstelling adequaat is.

Hoofdstuk 3 was gericht op geneesmiddelinteracties met antifungale geneesmiddelen van het azole type. Een systematisch review van de literatuur werd uitgevoerd naar geneesmiddelinteracties met azolen. Er werd nagegaan wat het onderliggende mechanisme van de interactie was. Op basis van een risico-inschatting werden aanbevelingen opgesteld bij gevonden interacties. Voor het weergeven van de aanbevelingen werd gebruik gemaakt van een gebruikelijke classificatie ("geen interactie / geen interventie noodzakelijk" tot "monitor de behandeling / vermijd de combinatie"). Deze aanbevelingen zijn zinvol voor artsen en apothekers om de behandeling bij individuele patiënten te optimaliseren.

Door het routinematig monitoren van posaconazol werd een belangrijke interactie met omeprazol ontdekt. Omeprazol remt de maagzuurproductie metals gevolg dat de pH van de maag stijgt. Aangezien de absorptie van posaconazol zuurafhankelijk is werd een sterke daling in de serumconcentratie van posaconazol waargenomen. Door de verminderde absorptie kan de behandeling uiteindelijk falen. Indien een protonpompremmer niet vermeden kan worden dan dient te allen tijde de serumconcentratie van posaconazol gevolgd te worden om absorptie te beoordelen of er dient een alternatieve antifungale behandeling gekozen te worden.

Een retrospectieve studie werd opgezet naar de interactie tussen fenytoïne en voriconazol aangezien er aanwijzingen waren dat het doseringsadvies uit de bijsluiter onvoldoende was. Dit advies was gebaseerd op een interactiestudie bij gezonde vrijwilligers. Het bleek dat de dosering voriconazol verhoogd diende te worden van tweemaal daags 200 mg naar tweemaal daags 400 mg om het effect van fenytoïne op de

serumspiegel van voriconazol te compenseren. In hematologische patiënten bleek dat deze geneesmiddelinteractie een groter effect had en minder voorspelbaar was dan bij gezonde vrijwilligers. Onze aanbeveling werd daarom - anders dan in de bijsluiter - dat deze geneesmiddelcombinatie vermeden diende te worden en een ander anti-epilecticum (bv. levetiracetam) of ander antifungaal middel (bv. echinocandine of lipide formulering van amfotericine B) gekozen diende te worden. Indien dit niet mogelijk is dan is het monitoren van de voriconazol-spiegel vereist om de dosering te verhogen en voriconazol-spiegels in het therapeutisch gebied te bereiken.

In **hoofdstuk 4** is het onderzoek naar linezolid beschreven, een veel belovend geneesmiddel voor de behandeling van "Multi – en eXtensively Drug Resistant Tuberculosis" (MDR-TB en XDR-TB). Echter, bij een dagelijkse dosering van tweemaal daags 600 mg gedurende langere tijd treden ernstige bijwerkingen als neuropathie en anemie op. De clinicus staat dus voor de lastige keuze: het optreden van ernstige toxiciteit maar wel een effectieve behandeling versus het niet behandelen met dit middel en het in isolatie houden van een patiënt. Om bijwerkingen te verminderen en effectiviteit te behouden hebben we onderzocht of serumconcentraties van linezolid, gegeven in een lagere dosering, hoger waren dan de in vitro Minimaal Inhiberende Concentratie (MIC). Daarvoor hebben we een analysemethode op basis van LC/MS/MS ontwikkeld die lineair, selectief, accuraat en precies linezolid in serum kan meten. In een prospectieve studie werd de farmacokinetiek van linezolid in twee doseringen onderzocht. Acht patiënten met MDR-(of XDR) TB kregen linezolid als onderdeel van hun behandeling. Er werd gestart met linezolid in een dosering van tweemaal daags 300 mg gedurende ten minste 3 dagen, gevolgd door tweemaal daags 600 mg. Tijdens steady state bij elke dosering werd een 12-uurs concentratie tijdscurve opgenomen. De MIC waarden van de tuberkelbacillen werden bepaald op de Afdeling Mycobacteriële diagnostiek van het RIVM, Bilthoven (hoofd: Prof Dr D van Soolingen). We hebben gebruik gemaakt van de $AUC_{0\text{-}24h}$/MIC ratio als voorspeller voor de effectiviteit van de behandeling. Als de AUC/MIC ratio ≥100 was dan werd de behandeling voortgezet met een dosering van twee maal daags 300 mg. Linezolid werd in de lage dosering van 300 mg goed door de patiënten verdragen. Deze studie toonde aan dat een dosisreductie haalbaar was.

Om geïndividualiseerd doseren mogelijk maken werd een model ontwikkeld dat de blootstelling aan linezolid kan voorspellen met behulp van een minimal aantal bloed-afnames. De AUC van linezolid op steady state werd bepaald bij 14 MDR-TB patiënten. Voor de ontwikkeling van een populatiemodel en het berekenen van de minimale bloedafname modellen werd gebruik gemaakt van de software MWpharm 3.60 (Mediware, Nederland). Het meest klinisch relevante model voor de voorspelling de AUC maakte gebruik van de linezolid dalspiegel. De correlatie tussen de voorspelde en gemeten AUC werd geanalyseerd met de Bland-Altman analyse. Daarmee werd aangetoond dat deze met voldoende betrouwbaarheid voorspeld kon worden.

In **hoofdstuk 5** werd moxifloxacine, een breed spectrum chinolon antibioticum voor onder andere de behandeling van luchtweginfecties, onderzocht. Uit *in vitro* en *in vivo* experimenten – en recent ook in klinische studies – was al gebleken dat moxifloxacine een hoge activiteit tegen *Mycobacterium tuberculosis* had in vergelijking met andere fluoroquinolones. Deze was gelijk dan wel hoger dan die van isoniazide. Moxifloxacine wordt om die reden ook vaker gebruikt voor de behandeling van TB en kan ingezet worden bij TB op lastig te bereiken delen in het lichaam als tuberculose meningitis. Net zoals bij andere fluoro-chinolonen, heeft de AUC/MIC ratio de grootste voorspellende waarde voor de effectiviteit van dit geneesmiddel. Zowel de AUC als de MIC waarde is variabel en geneesmiddelinteracties kunnen de blootstelling aan moxifloxacine beïnvloeden. Moxifloxacine is echter niet geregistreerd voor de behan-

deling van TB. Het meten van de moxifloxacine spiegels zou een toegevoegde waarde hebben voor het bepalen van de optimale dosering. Daarom hebben we een analyse methode ontwikkeld met behulp van de LC/MS/MS voor moxifloxacine in plasma en in liquor cerebrospinalis. Aangezien de effectiviteit van een antibioticum afhangt van de niet eiwitgebonden fractie werd de ook de concentratie van moxifloxacine in plasma ultrafiltraat bepaald. Om de huidige gebruikelijke dosering van één maal daags 400 mg te evalueren werd een prospectief behandelprotocol voor TB meningitis en een retrospectieve studie opgesteld. Uit de retrospectieve evaluatie van 92 patiënten bleek dat moxifloxacine goed werd verdragen. Slechts bij twee patiënten zorgden bijwerkingen (overgeven en ernstige diarree) ervoor dat de behandeling gestaakt moest worden. Klinisch relevante QT verlenging werd niet gezien. Onze bevindingen tonen aan dat moxifloxacine in een dosering van 400 mg goed verdragen wordt gedurende een langere periode. Gezien de variabiliteit in ongebonden $AUC_{0\text{-}24h}$/MIC ratio, veroorzaakt door variabiliteit in eiwitbinding, $AUC_{0\text{-}24h}$, MIC en invloed van co-medicatie als rifampicine, kan therapeutic drug monitoring geadviseerd worden bij bepaalde patiënten (MIC > 0.25 mg/L en rifampicine; MIC > 0.5 mg/L; diarree of overgeven). Bij deze dosering lijken bewaking van lever, nier en hartfunctie (QT verlenging) niet noodzakelijk.

Van vier patiënten met tuberculeuze meningitis werd de penetratie van moxifloxacine in liquor geëvalueerd door een volledige farmacokinetische curve op te nemen in plasma en liquor. Met behulp van niet-compartimentele analyse met MWPharm 3.60 werden de farmacokinetische parameters bepaald. De ratio van $AUC_{0\text{-}24h}$ in liquor en plasma werd berekend en toonde dat er in de drie van de vier patiënten een voldoende blootstelling werd bereikt. De vrije fractie was 40 en 60% (n = 2). In de patiënten met een lage AUC/MIC ratio werd de dosering verhoogd gedurende de resterende tijd van de behandeling.

Hoofdstuk 6 beschrijft een retrospectieve studie bij 760 TB patiënten (1992-2007) waarin geprobeerd werd om voorspellende factoren te vinden voor ethambutol geïnduceerde nervus opticus neuropathie. Ethambutol is zeer goed wateroplosbaar geneesmiddel. Als het gegeven wordt aan obese patiënten in een dosering gebaseerd op totaal lichaamsgewicht kan de concentratie ethambutol in bloed toxische waarden bereiken. De meeste TB patiënten hebben ondergewicht en bij hen kan wel veilig en effectief op basis van lichaamsgewicht gedoseerd worden. Ethambutol gerelateerde visuele stoornissen werden gevonden bij 0.8% van de patiënten en vier van deze zes patiënten waren duidelijk overgedoseerd aangezien ze gedoseerd waren op totaal lichaamsgewicht in plaats van op ideaal lichaamsgewicht. Op basis van deze gegevens adviseren we om ethambutol in volwassenen te doseren op basis van ideaal lichaamsgewicht.

In **hoofdstuk 7** hebben we de interactie tussen rifampicine (RIF) en claritromycine (CLA) onderzocht in het kader van de combinatiebehandeling bij Buruli ulcer. Om beide geneesmiddelen en de potentiële actieve metabolieten (14-hydroxyclaritromycine en 25-desacetylrifampicine) te meten hebben we een analysemethode met behulp van LC/MS/MS ontwikkeld. In een prospectieve studie werd het effect van de interactie op de farmacokinetiek van beide geneesmiddelen onderzocht door middel van een 10 uurs concentratie-tijdscurve. De studie werd uitgevoerd bij een deel van de patiënten van een gerandomiseerde studie waarin streptomycine (SM) en RIF gedurende 8 weken met 4 weken SM/RIF gevolgd door 4 weken RIF/CLA voor de behandeling van *Mycobacterium ulcerans* infectie werd gegeven. De patiënten kregen CLA in een dosering van eenmaal daags 7.5 mg/kg afgerond op 125 mg en RIF in een dosering van 10 mg/kg afgerond op 150 mg. Het samen toedienen van CLA naast RIF zorgde voor een niet-significante toename in de AUC van RIF in vergelijking met de AUC van RIF in patiënten die streptomycine als co-medicatie kregen (P=0.13). De concentratie van CLA en 14OH-CLA bereikte gedurende een gedeelte

van het 10 uur durende interval een concentratie groter dan de MIC van M. *ulcerans* - voldoende om gedurende meerdere uren per dag een therapeutische concentratie tegen M. *ulcerans* te bereiken en de infectie dus effectief te behandelen.

In het laatste hoofdstuk (**hoofdstuk** 8) bespreken we de impact van dit proefschrift voor de klinische praktijk en de mogelijkheden van TDM om behandeling met antibiotica toe te spitsen op het individu. De ontwikkelde farmacokinetische modellen en klinische beslisregels ondersteunen deze gedachte. Naar onze mening kan alleen een multidisciplinair team, ondersteund door een goed uitgerust laboratorium met hoog opgeleid personeel TDM in een klinische setting goed uitvoeren. We hebben aangetoond dat op relatief eenvoudige wijze met behulp van retrospectieve studies en kleine prospectieve studies klinisch relevante gegevens verzameld kunnen worden. Onder andere de correlatie tussen ziekte-ernst en farmacokinetiek en doseren op basis van AUC/MIC kunnen als onderwerp dienen voor grote gerandomiseerde multicentrische studies waarin zowel effectiviteit als veiligheid van antibiotica worden onderzocht.

Dankwoord

De finale is bereikt en het boekje is klaar. Met veel plezier heb ik naar dit punt toegewerkt. Wat kalm en rustig is begonnen eindigde in een ware stroomversnelling.

Mijn eerste schreden in de arena van het onderzoek waren in 2005 bij het vormen van de ideeën over een registratieonderzoek behorende bij de opleiding tot ziekenhuisapotheker. Ernstig zieke patiënten, intensive care, betekenis hebben in de individuele behandeling van een patiënt, bloedspiegelbepalingen en infectieziekten waren de componenten die vertegenwoordigd dienden te zijn. Dit heeft geleid tot zoals later bekend "De voriconazol studie". Tijdens het uitvoeren van deze studie heb ik alles meegemaakt: heroïsche momenten en frustratie. Dit bleek de ideale leerschool te zijn voor mij als onderzoeker in spé.

In de daarop volgende jaren zagen nieuwe studies het licht en werden laboratoriumformulieren met felgekleurde nummertjes een begrip op diverse afdelingen in het ziekhuis en in het lab van de apotheek. Het aantal collega's binnen en buiten de apotheek die betrokken raakte bij het onderzoek groeide gestaag. Nu het boekje klaar is, is het moment daar om bij hun betrokkenheid, medewerking, adviezen en support stil te staan. Zonder hen was dit proefschrift er niet geweest.

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Beste Mireille, als analist op het lab van de apotheek was jij aan mijn onderzoek verbonden. Alle nieuwe analyse methoden zijn door jou of onder onze gemeenschappelijke begeleiding door bijvakstudenten ontwikkeld. Zonder analysemethode kun je geen geneesmiddelconcentratie in serum bepalen en hypotheses niet toetsen. Niet alle analysemethodes waren even eenvoudig op te zetten en regelmatig hebben we moeten brainstormen over hoe we de analytische hobbels konden overwinnen. Door jouw inzet is het mogelijk geweest in vrij korte periode invulling te geven aan nieuw onderzoek. Hiervoor ben ik je veel dank verschuldigd.

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About the author

Johannes Willem Cornelis (Jan-Willem) Alffenaar was born on the 2nd of August 1977 in the city of Arnhem. He lived here for fifteen years and than moved to the city of Doetinchem. He started the study of pharmacy in 1995 at the University of Groningen. He received his Master of Science in 2000 after finishing his research subject "Synthesis of indole and indoline based ligands for the 5HT7 receptor" at the Department of Medicinal Chemistry of the School of Pharmacy. In 2002 he graduated as pharmacist.

In 2002 he started as a pharmacist in the Hospital Pharmacy of the Academic Hospital Groningen and at Proeftuin Farmacie Groningen. Thereafter he qualified in 2005 for a position as hospital pharmacist in residence, supervised by Jos GW Kosterink PhD PharmD. During his residency and specialization at the laboratory of the Hospital Pharmacy he became interested in therapeutic drug monitoring, clinical toxicology and critical care medicine. Prof Donald RA Uges PhD PharmD supervised the development of his skills during this period. His first clinical study focused on the pharmacokinetics of voriconazole in critically ill patients suspected to have invasive fungal infections. After that new studies were setup in TB patients and patients having Buruli ulcer disease. Prof Tjip S van der Werf PhD MD supervised on the clinical and medical aspects of this research. Together these studies resulted in this thesis.

His appreciation of pharmacokinetics in specific patient populations resulted in a residency in clinical pharmacology, supervised by Prof Donald RA Uges PhD PharmD and supported by the Dutch Association of Clinical Pharmacology and Biopharmaceutics by means of an unrestricted grant from MSD. Together with other hospitals in The Netherlands he succeeded to set up a randomised controlled trial into therapeutic drug monitoring of voriconazole, which was financially supported by a ZonMW grant. In 2009 he finished his residency and became hospital pharmacist with pharmaceutical care at the University Medical Center Groningen - Centre for Rehabilitation and further development of his research into pharmacokinetics of antifungal and anti-TB drugs as main topics of interest.

He is a member of the committee of TDM of antifungal drugs of the International Association of Therapeutic Drug Monitoring and Clinical Toxicology and co-chairs the of quality control program of antifungal and anti-TB drugs of the Association for Quality Assessment in Therapeutic Drug Monitoring and Clinical Toxicology. Jan-Willem is married to Emie van der Werf. He enjoys gardening and tropical aquarium pet fish, and appreciates the Greek hospitality, cuisine, culture and beautiful beaches.