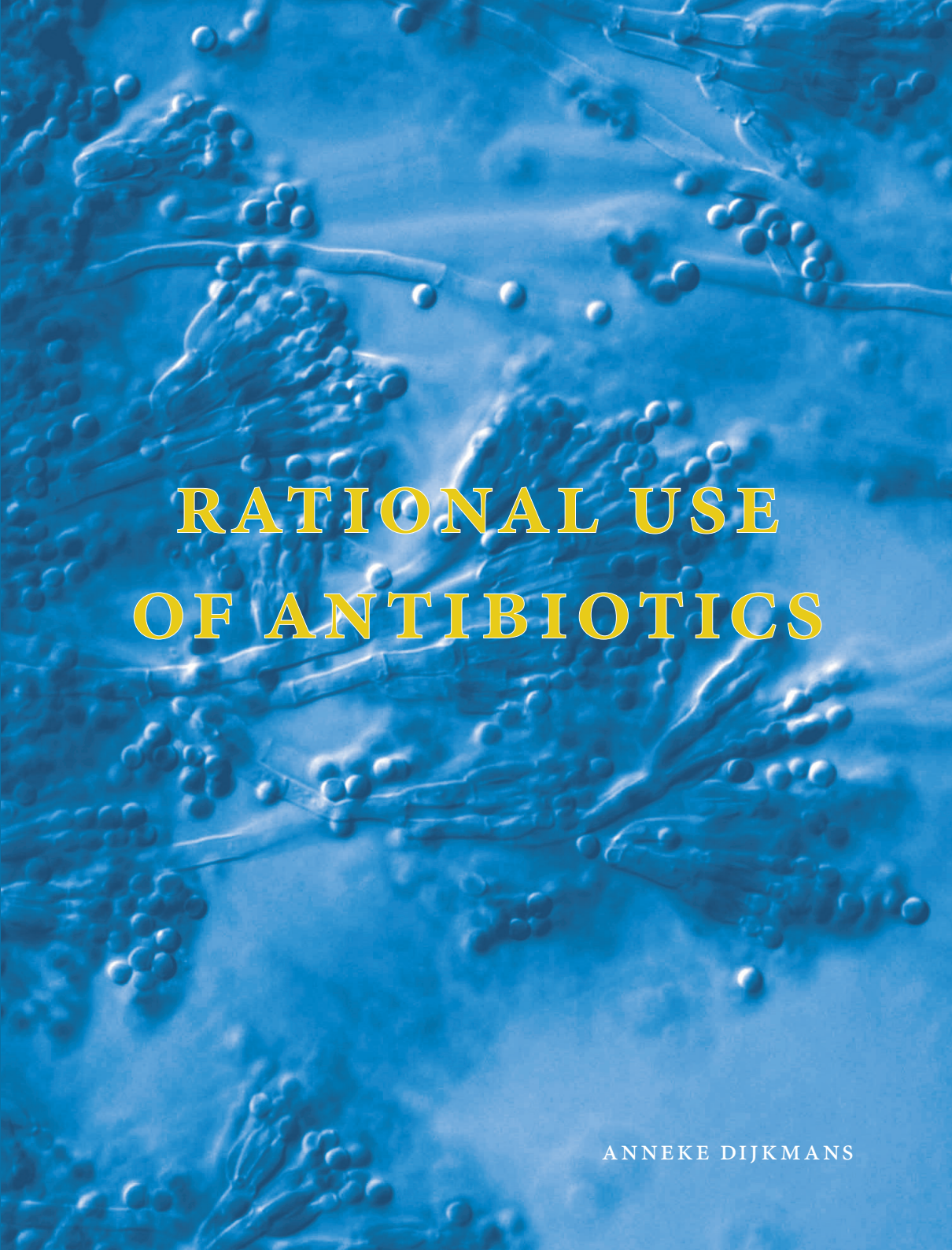

RATIONAL USE OF ANTIBIOTICS

ANNEKE DIJKMANS

RATIONAL USE OF ANTIBIOTICS

PROEFSCHRIFT
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CHAPTER 1

INTRODUCTION

INTRODUCTION

Infectious diseases are much older than mankind and when *Homo sapiens* arrived by evolutionary descent, they were vulnerable for infections. However, the existence, causes and mechanisms of diseases due to microscopically small organisms remained obscure for many centuries and it became clear only since the second part of the nineteenth century. The transport of 'contagious material' was suggested by Ignaz Semmelweis, who was convinced that transfer of the putrefying organic materials from a sick woman resulted in puerperal fever in a healthy woman,[1] without recognizing the relationship of microorganisms to disease. Although the existence of microorganisms had already been revealed as a result of van Leeuwenhoek's development of the microscope, it took two additional centuries to recognise that microorganisms could be the cause of diseases, that at least some of them were pathogenic to man. Experiments to proof that a microorganism could cause a specific disease were performed by Robert Koch in 1877 with his experiments with *Bacillus anthracis*.[2] The next step in Koch's work was to define the conditions in which a specific microorganism could cause a specific disease, i.e. Koch's postulates.[3] With the application of these postulates a connection was made between clinical manifestations of a disease and microorganisms observed under the microscope, establishing the causative role of specific microorganisms for specific diseases as tuberculosis, anthrax and cholera.

Antibacterial agents

HEAVY METALS

In the early sixteenth century heavy metals such as gold and mercury were considered as an elixir of life by Paracelsus. In 1908 Paul Ehrlich pioneered the use of arsenic and 25 years he discovered that arsenical compounds had activity against syphilis, and discovered that compound 606 (Salvarsan; 'beneficial arsenic') was amazingly active against such infection.

Koch reported the *in vitro* inhibition of tubercle bacilli growth by gold cyanide and in 1894 a mixture of gold and magnesium was used to treat tuberculosis. Many publications were written about chrysotherapy for tuberculosis. Believing that tuberculosis and rheumatoid arthritis (RA) had a common infectious aetiology, Forester applied gold for the treatment of RA.[4] Despite the fact that RA turned out not to be directly caused by microorganisms, chrysotherapy proved to be rather effective and became an established drug in the treatment of RA.

CHEMOTHERAPEUTICS

Based on the idea's and work of Paul Ehrlich, Mietzsch and Klarer had synthesized Prontosil in 1932. Prontosil was not active against bacteria *in vitro*, but was quite active in experimentally infected animals. The explanation for this discrepancy was that Prontosil is a pro-drug metabolized by the recipient to the active agent sulphanilamide. The sulpha drugs were the first chemical substances that made a real difference in the treatment of bacterial infections in particular infections caused by streptococcal species. The historical importance of sulpha drugs is great, and even to date have wide usage in the treatment of systemic or localized infections.

ANTIBIOTICS

During the time that sulphonamides were available another important development took place when Sir Alexander Fleming did his historical discovery of penicillin in 1928.[5] While studying a staphylococcal variant, he found one of the culture plates contaminated with a fungus which destroyed the surrounding staphylococcal colonies. This accidental rediscovery of the long-known ability of Penicillium fungi to suppress the growth of bacterial cultures spurred additional research. Fleming investigated the properties of 'mould broth filtrates' which he named penicillin for brevity. He described penicillin as an antiseptic 'more powerful than phenol', and the name 'penicillin' has since been applied to pure antibiotic substances. After this first historic observation it took a decade before penicillin could be sufficiently purified and produced on an industrial scale. Indeed, only after the successful purification and concentration by Chain and Florey in 1940, it became possible to demonstrate the importance of penicillin by a clinical trial in 1941. Thereafter, in the course of the 20th century numerous new antibiotics were discovered and developed and introduced in daily practice.

Antibiotics are unique among all other medical drugs. They are the only class of medicines, whose primary target is present in bacterial cells but absent in human cells, i.e. they are highly selective for bacterial cells. Although the value of hygiene measures cannot be underestimated, antibiotics have revolutionized human healthcare in a way that only a few other scientific discoveries have. Antibiotics have not only enabled us to overcome 'the captain of the men of death' by saving lives of patients with serious infections, these drugs have also played a pivotal role in major advances in medicine and surgery, a role which is less often highlighted and yet has paramount significance.[6]

On the other hand, antibiotics disturb natural ecological niches by exerting selective evolutionary pressure on bacteria present in the niches were they are applied, both in the human, the animal and agricultural domains of society as well as in the innate environment. As a consequence of exposure to antibiotics the less susceptible and fully resistant mutants of susceptible species survive as do the intrinsically resistant (see below) species; these surviving cells may subsequently greatly expand their niche. We currently face an era of serious threats to human and animal health due to the world wide emergence of multi-drug resistant bacteria. This threat was always on the horizon. As early as 1943, Sir Alexander Fleming noted that microbes can be 'educated to resist penicillin'.[7] Notwithstanding this early observation, only little has been done to prevent the emergence of drug resistance.[6,7]

ANTIBIOTIC RESISTANCE

Antibiotic resistance is recognised as one of the most serious threats to the treatment of infectious diseases globally. Already in 1993 Calvin M Kunin, an infectious diseases specialist in the USA, wrote a perspective in the Annals of Internal Medicine warning against a worldwide calamity due to the global emergence of antimicrobial resistance.[38] However, only in 2004 this emerging threat finally prompted the World Health Organisation (WHO) to issue a warning that antibiotic resistance would seriously impact the opportunities to treat infectious diseases in the future.[7] In 2015 the WHO published its Global Action Plan on Antimicrobial Resistance, outlining the way for all nations to combat the emergence of resistance, a worldwide effort that is currently being implemented in many countries. The awareness posed by the threat of emerging antimicrobial resistance is now greater than ever.

Methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin resistant enterococci (VRE), multiple resistant (MR) enteric gram negative bacilli (*Escherichia coli*, Klebsiella species, Enterobacter species) and penicillin-resistant *Streptococcus pneumoniae* (PRSP) are prime examples of common pathogenic microorganisms with increasing rates of resistance to commonly used antimicrobials. The degree of resistance varies worldwide and depends highly on the availability of antibiotics for the population and national policies regarding antibiotic use. Countries with easy access to antibiotics, even without prescription, have a higher degree of antibiotic resistance than countries with a stricter regimen. Nordic European countries including the Netherlands have a strict regimen and as a consequence have lower resistance compared to the South-European countries.[8] In low and middle income countries antimicrobial resistance is higher due to routine over the counter availability and misuse of antibiotics.

TYPES OF RESISTANCE

Resistance to antibiotics is commonly divided in two different classes: primary (intrinsic) and secondary (acquired) resistance. Primary resistance refers to resistance that a bacterium has naturally – i.e. without manipulations such as exposure to antibiotics – against the activity of an antibiotic compound. This resistance is considered to reflect genomic information of a bacterium. Secondary resistance is the acquired capacity of a bacterium to resist the activity of an antibiotic compound to which it was previously susceptible by acquisition of additional genomic information. This renders the bacterium resistant to an antibiotic compound to which it was naturally susceptible. It is particularly the increase in secondary resistances that causes world-wide concern. Bacteria have become more and more adapted due to the long-term massive usage of antibiotics in human, animal and agricultural sectors of society. The global resistance emergence is further aggravated by the fact that resistant organisms selected in one sector of society may contaminate the innate environment and spread to other sectors of society via the environment and via travel of people and animals, and through the transport of agricultural produce and other goods.

Biochemically, several types of resistance mechanisms are observed including antibiotic inactivation, target modification, altered permeability and 'bypass' metabolic pathways.[9] It can be stated that resistance emergence is due to a combination of two groups of factors:

1. Selection of resistant clones as a direct consequence of exposure to antibiotics favouring:
   - intrinsically resistant bacterial species
   - variants of originally susceptible bacteria that have acquired resistance traits by spontaneous mutations in their genome or by acquisitions of resistance genes through horizontal gene transfer from other bacteria in their vicinity
2. Expansion and the spread of resistant strains from their place of emergence to other sites, resulting in outbreaks, epidemics and pandemics of resistant clones.

Resistance thus seems an inevitable consequence of the evolution of microorganisms under antibiotic pressure and their ability to spread globally. Moreover, it is difficult to unravel the separate contribution of each of the factors leading to resistance problems. Although appropriate use of antibiotics essentially exerts the same selective effect favouring the emergence of resistance, it is the inappropriate use of antibiotics that can and should be targeted in the combat against the emergence of antibiotic resistance. Inappropriate usage is still highly prevalent and there are several types of inappropriate use of antibiotics (Table 1).[10,11]

The contribution of the different types of inappropriate use of antibiotics to acquired resistance is difficult to estimate. However, correlation between antibiotic prescriptions and resistance is well described.[12,13] In primary care antibiotic exposure is associated with a subsequent twofold risk of antibiotic resistance in respiratory and urinary bacteria.[14] In hospitals antibiotic prescribing selects for resistant bacteria both at patient level and at the level of institutions.[15] An almost twofold risk of increase in emergence of MRSA was observed in patients exposed to antibiotics.[16] The risk of mortality from infection in patients harbouring resistant microorganisms in prescribing ineffective antibiotics for patients harbouring resistant organisms was associated with an 1.6-fold increased risk.[17]

With regard to the section 'overuse' (Table 1) general practitioners are the most involved group of physicians. Apart from clinical experience to discriminate a bacterial infection from other infections modern techniques are helpful as shown in as the use of biomarkers such as C-reactive protein to guide antibiotic prescribing in COPD.[18,19] Continuous medical education is required in the awareness of overuse and underuse by general practitioners and hospital doctors. In 2015 poor knowledge and confidence amongst final year medical students in Australia were observed.[20] In 2004 in a survey amongst doctors in a teaching hospital in the USA it was seen that only 21% of doctors feel confident that they were using antibiotics optimally and 90% of doctors prefer more education about antibiotic use.[21]

A recent publication shed light on a separate problem, the (re)filling of an antibiotic prescription over time: the probability of filling an antibiotic prescription in 62 million enrolees in health insurance plans was 62% over 4 years.[22]

Next to this, a substantial component, perhaps the most influencing factor, for inappropriate use is the influence of the pharmaceutical industry. The publication of Podolsky gives impressive information about aggressive pharmaceutical marketing.[23]

Combating Resistance

Cornerstones of an effective strategy to respond to antibiotic resistance include refining stewardship of existing antimicrobials, re-introducing old antibiotics within the framework of antimicrobial stewardship, and introducing new agents.

ANTIBIOTIC STEWARDSHIP

It is likely that the real change in combatting antimicrobial resistance can only be achieved by rational and restricted or controlled use of antimicrobials. The change in attitude and behaviour towards antibiotics is the goal of so called antibiotic stewardship programs. Antibiotic stewardship is essential for the human, animal and agricultural sectors of society in a coordinated attempt to stop the emergence of antimicrobial resistance and even to redress the degree of multi resistant microorganisms.[24] Essential is that the rules of antibiotic stewardship hold for all antibiotics, the existing ones and also for the new and renewed ones.

Since the relationship between antibiotic prescription and resistance is recognised[12] measures for appropriate prescription have been taken, which are bundled as antibiotic stewardship.[31] Antibiotic stewardship has three primary goals:

- Ensure effective treatment and prevention of bacterial infection[32]
- Reduce unnecessary antibiotic use and costs
- Minimise collateral damage

At patient level, stewardship has been defined as 'the optimal selection, dosage and duration of antimicrobial treatment that results in the best clinical outcome for the treatment or prevention of infection, with minimal toxicity to the patient and minimal effect on subsequent resistance.[6] The Infectious Diseases Society of America together with the Society of Health Care Epidemiology of America was the first to publish an extensive guideline on Antibiotic Stewardship in 2007.[33] The purpose of these guidelines was to improve the use of antimicrobial agents in hospitals and to prevent antimicrobial resistance in hospitals. Next to this the guidelines were aimed to provide evidence-based recommendations for developing a program to enhance antimicrobial stewardship in the hospital setting to improve the quality of care. The first Dutch guideline on this topic by Stichting Werkgroep Antibioticabeleid (SWAB) was largely based on this example. Stewardship has led to interventions to monitor and direct antimicrobial use. For new drugs and old revisited drugs the antibiotic vigilance, as incorporated in guidelines for promoting antibiotic stewardship should be as strict or even stricter to avoid resistance against these last resort drugs.

In The Netherlands so called A-teams including at least an infectiologist, a clinical microbiologist and a pharmacist have been established. During their regular (weekly) meeting they discuss patients with infections. The use of last resort antibiotics is restricted and specific care is taken to switch as soon as possible from intravenous to oral therapy. Additionally, an Antibiotic Stewardship Committee is in charge of the standard antibiotic treatments and provides alternatives in case of an allergy or adverse effect.

Education in the hospital to medical doctors/pharmacists and for general practitioners plays a major role in restriction of antibiotics.

Re-introducing old antibiotics As one of the possible solutions for the low number of effective antibiotic compounds, it has been suggested to re-introduce antibiotics that have been used previously but currently are no longer in use in clinical setting. These older drugs were for some reason, deleted from antibiotic guidelines and formularies, and, therefore, disappeared from the standard of care. For this re-evaluation it is important to first focus on the reasons why these drugs were removed from the market. In this respect, the fate of colistin, a prototypical polymyxin, may serve as an example. In its handbook 'The use of antibiotics' (third edition, 1979) Kucers states that '"*The polymyxins" are not absorbed from the gastrointestinal tract and, therefore, are administered intramuscularly or intravenously for treatment of systemic infections*' (page 534). *'Patients with renal failure: the polymyxins accumulate in these patients, so a modified dosage schedule with serum level monitoring is necessary'* (page 535). *'The polymyxins frequently cause side effects', 'untoward effects were observed in 25.1 percent'* (page 539). Due to these warnings regarding adverse effects, and due the concurrent development of several aminoglycosides (without these side effects), colistin became a second choice antibiotic and finally disappeared from daily practice. However, the current situation with many strains of multi-drug resistant Gram-negative bacteria causing serious infections in health care setting across the globe prompted a re-evaluation of the polymyxin class of antimicrobial agents. Another antibiotic that was not used for decades, is fosfomycin which was already discovered in 1969.[30] It was not even mentioned in the 1982 edition of Kucers handbook, suggesting it was not clinically relevant at that time. A genuine interest in this antibiotic only arose in the first decade of the 21st century.

NEW ANTIBIOTICS

When the number of effective antibiotics decreases over time, development of new antibiotics becomes crucial.[24] Indeed, several new compounds of known classes of antibiotics and a small number of new classes of agents have been developed and introduced into clinical practice to overcome existing and emerging antimicrobial resistances. However, the number of new antibiotics in the pipeline is currently low, with only two novel classes discovered in the last 20 years.[25-27] In part this is an economic issue since for many manufactures it has been and still is much more profitable to invest in medicines for prevalent chronic illnesses such

as cardiovascular and rheumatic diseases, rather than in medicines that are predominantly prescribed as short courses in selected individuals which actually lead to a rapid cure obviating the need for further treatment as is the case with most infections.[6] In 2012 the Infectious Diseases Society of America has proposed an alternative antibacterial drug approval pathway which would accept smaller and less expensive clinical trials.[28] As possible a result of this action, in 2019 studies with new antimicrobial drugs have been published.[24,29] The increased activity in the discovery and development of new drugs is shown by – for example – the next generation aminoglycoside,[29] plazomicin,. Although this development possibly marks the beginning of a new era, it can only be part of the solution to overcome resistance to antibiotics. In case these few new antimicrobials are used in the same way as antibiotics were used the past, resistance for these compounds will occur sooner or later. It is also hard to imagine that a new antibiotic compound (for example a second generation aminoglycoside) will drastically change the treatment landscape, as new antibiotics will not be introduced as first line agents in standard care. Rather, they will only be used as second line or as a last resort antimicrobial agents.

Scope of this thesis

The aim of this thesis was to stimulate rational and effective use of antimicrobials, by addressing the first two cornerstones: (1) refining stewardship of existing antimicrobials and (2) re-introducing old antibiotics within the framework of antimicrobial stewardship.

The overall aim is to contribute to antimicrobial stewardship and to explore the value of the re-introduction of old antibiotics that are currently scarcely used. The basic step is the *in vitro* relationship expressed as minimal inhibitory concentration (MIC) for a given bacteria for a given antibiotic. The next step is the *in vivo* situation. This thesis concentrates on the *in vivo* situation.

OUTLINE OF THIS THESIS

Refining stewardship of existing antimicrobials (chapters 2-5)

Chapter 2 and **chapter 3** describe studies on the proper use of the small spectrum oral antibiotic, flucloxacillin, which is known to have variable absorption. Flucloxacillin, first described in 1970,[34] is used to treat infections caused by *Staphylococcus (S.) aureus* strains. It is used empirically for presumed staphylococcal infection in countries, including the Netherlands, that have documented low rates of methicillin-resistant *S. aureus* (MRSA) strain.[35] In case of severe systemic staphylococcal infections flucloxacillin treatment is usually started intravenously, followed by prolonged oral administration. Since absorption of orally administered flucloxacillin is variable and unpredictable, absorption tests have been recommended. For this purpose we designed a simplified absorption test. This new test was compared with the original absorption test (**chapter 2**) and the next step was to validate the new test in a much larger patient population (**chapter 3**).

Chapter 4 describes studies on the absorption of orally administered penicillin, another small spectrum agent with a highly variable and unpredictable absorption profile.

Chapter 5 describes a new method for simplified therapeutic drug monitoring (TDM) for the long-term oral use of rifampin. Rifampin is notorious because it quickly develops resistance as a result of many factors, including underdosing. Universal testing to assess the absorption of oral rifampin seems to be necessary but remains elusive in many low to middle income settings where most new cases of tuberculosis occur. However, the simplified absorption test may be of value in such settings as well as in developed countries including the Netherlands.

Re-introducing old antibiotics within the framework of antimicrobial stewardship (chapters 6-10)

Chapter 6 presents a detailed review of the old polymyxin class drug colistin. In brief, colistin was isolated in 1949 from the *Bacillus polymyxa 'colistinus'* and became available for clinical use in 1959, but was largely replaced by other agents after only two decades. The increasing resistance to antibiotics led clinical investigators to reconsider the position of colistin, with emphasis on the impact of the reported adverse events and strategies against colistin resistance.[36-37]

Chapter 7 describes the results of a study on the chemical stability of colistin over time, which is relevant for long term infusion therapy at home, e.g., for cystic fibrosis patients with chronic pulmonal infections, especially with *Pseudomonas aeruginosa*.

Chapter 8 is a review about fosfomycin, which was discovered in 1969.[30] Fosfomycin has a broad spectrum of activity, including multi-drug resistant bacteria. In the Netherlands, fosfomycin has long been registered as an oral formulation prescribed for the treatment of uncomplicated urinary tract infection, while intravenous administration was only recently approved.

Chapter 9 evaluates different fosfomycin dosing regimens for the treatment of systemic infections. In this chapter a new pharmacokinetic model for dosing of fosfomycin is described.

Chapter 10 describes a study on the kinetics of fosfomycin after an oral and intravenous dose of 3 gram in patients suffering from urine tract infections with multi-drug resistant strains of the species *Escherichia coli*.

Chapter 11 provides a summary and general discussion of the findings and implications of the studies described in this thesis, as well as some suggestions for antibiotic stewardship and future research.

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TABLE 1 Examples of inappropriate use of antibiotics.*

Type	Example
Overuse	• Prescribing antibiotics for viral infections
	• Prescribing antibiotics for non-infectious processes (eg febrile patient with pancreatitis)
	• Treating minor bacterial infections that do not require antibiotics (eg small skin abscesses that resolve with incision and drainage)
	• Treating bacterial colonisation (eg positive catheter urine culture from an asymptomatic older patient)
	• Prescribing prolonged treatment courses (eg >24 hours for low-risk surgical prophylaxis)
Misuse	• Use of broad-spectrum antibiotics effective against multidrug-resistant organisms in a patient with a community-acquired infection
	• Failure to de-escalate broad-spectrum antibiotics according to culture results
	• Failure to adjust antibiotic prescription according to culture results when the isolated organism is resistant to initial therapy
Underuse	• Inadequate dosing of antibiotics
	• Premature discontinuation of antibiotics
	• Delay to prompt treatment of severe sepsis
	• Failure to prescribe an antibiotic regimen with an adequate spectrum of activity in a patient with a life-threatening infection
Abuse	• Prescribing antibiotics for financial incentive
	• Prescribing particular antibiotics as a result of pressure from a pharmaceutical industry representative

** Hand K., Antibiotic stewardship, Clin Med 2013, vol 13,499-503*

TABLE 2 Examples of hospital antibiotic stewardship interventions.*

Type of intervention	Example
Governance structures	• Organisational strategy for antibiotic stewardship • Antibiotic prescribing policy (statement of principles of responsible prescribing and expected quality standards), which may include: – 48-hour review – intravenous-to-oral switch – automatic stop orders (termination of prescriptions after a specified interval unless authorisation to continue obtained) – compulsory order forms (prescribers required to complete a form with clinical details to justify use of restricted antibiotics) – expert approval (prescriptions for restricted antibiotics authorised by infection specialist or head of department) – dedicated antibiotic prescription chart – removal by restriction (restrictive policy imposed in target wards, units or operating theatres – eg by removing restricted antibiotics from drug cupboards) – therapeutic substitution (pharmacists authorised to substitute alternative antibiotics) – antibiotic cycling and rotation policy – mixing, diversity and heterogeneous prescribing policy • Antibiotic stewardship committee (including medical microbiologist or infectious diseases physician, specialist pharmacist and information analyst)
Operational delivery	• Antibiotic formulary – may incorporate limited list of antibiotics subject to prescribing restrictions such as requirement for preauthorisation • Guidelines for initial treatment of common infections (evidence based, peer reviewed and informed by local resistance data where possible) • Guidelines for perioperative prophylaxis for common surgical procedures • Reminder systems (eg preprinted adhesive labels for medical case notes) • Computerised physician order entry (electronic prescribing) – may incorporate computerised decision-support systems • Mobile device software applications for point-of-care information and guidance
Risk management	• Guidelines for management of infection in patients with allergy to antibiotics • Information on safe administration of intravenous antibiotics • Guidelines for dosing and monitoring of serum levels of toxic antibiotics
Clinical microbiology/ infectious disease specialist and laboratory support	• Validation and interpretation of microscopy, culture and susceptibility results for laboratory reporting • Surveillance and reporting of trends in antibiotic resistance • Telephone consultation for advice on infection management • Bacteraemia follow-up service • Antibiotic stewardship ward rounds • Point-of-care rapid tests for bacterial infection • Advanced sepsis biomarkers (eg procalcitonin)
Controls and quality assurance	• Surveillance of antibiotic prescribing trends • Public reporting and benchmarking of antibiotic consumption data (eg World Health Organisation-defined daily doses) • Audit and feedback of adherence to prescribing policy • Audit and feedback of adherence to guidelines
Education and training	• Induction training on antibiotic stewardship • Revalidation training • Distribution of printed educational materials (eg pocket guidelines and patient information leaflet) • Educational meetings • Electronic learning • Antibiotic prescribing competency assessment • Academic detailing or educational outreach (one-on-one educational intervention) • Nominated clinical champions for antibiotic stewardship • Provision of patient information and counselling

** Hand K., Antibiotic stewardship, Clin Med 2013, vol 13, 499-503*

CHAPTER 2

A SIMPLIFIED ORAL FLUCLOXACILLIN ABSORPTION TEST FOR PATIENTS REQUIRING LONG-TERM TREATMENT

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ABSTRACT

BACKGROUND Patients with severe methicillin-sensitive *Staphylococcus aureus* infections are effectively treated with initial continuous intravenous (IV) flucloxacillin followed by oral maintenance therapy. As the absorption of oral flucloxacillin is variable, an oral absorption test (OAT) is used to ensure efficacious therapy. The classical OAT (test A) requires overnight fasting, interruption of IV therapy, and is laborious. We designed a simplified OAT (test B) in which IV therapy is continued and oral dosing is performed after a 1-hour fast.

METHODS In 43 hospitalized patients on IV flucloxacillin, either test A or test B was performed. In each variant, 1g of oral flucloxacillin was given, and blood samples were taken before and at 1 and 2 hours after dosing. Flucloxacillin concentration was determined by high-performance liquid chromatography. Adequate absorption was defined as a 10 mg/l increase in flucloxacillin concentration at 1 or 2 hours after dosing.

RESULTS In a population of 43 patients (18F/25M), test A was done in 19 patients and test B in 24 patients. The groups had similar baseline characteristics such as age, renal function, gender, diagnoses, or comedication. All the patients tolerated the test without problems. The absorption was highly variable between patients. The average (SD; range) maximal increase for test A was 22.3 (11.6; 7–50) mg/l and 26.5 (12.6; 8–53) mg/l for test B. There was no significant difference between the 2 tests (p=0.23), and 10% of the patients were poor absorbers (increase <10 mg/l). There was no influence of serum creatinine, age, or pretest flucloxacillin concentration. No clinical condition or drug use that may have impaired flucloxacillin absorption could be identified.

CONCLUSIONS We designed a simplified OAT that performs well and can be implemented easily. This test may be helpful to rationally and effectively treat patients with severe methicillin-sensitive *S. aureus* infections with an orally administered small-spectrum antibiotic.

INTRODUCTION

Staphylococcus aureus is a pathogen responsible for infections of different severity.[1] Severe infections are increasing worldwide, mostly due to the increasing use of indwelling catheters, vascular and orthopedic prostheses, and prosthetic heart valves. The treatment of choice for these severe infections is dependent on the susceptibility pattern of the pathogen, the country-specific prevalence of methicillin-resistant *S. aureus* (MRSA) strains and physician preference. In low endemic MRSA countries such as the Nordic countries, the Baltic states, and The Netherlands,[2] the preferred and efficacious treatment consists of initial continuous intravenous (IV) flucloxacillin followed by oral flucloxacillin for a variable period of time. The advantages of the latter treatment option are the bactericidal properties of the penicillin, its small-spectrum, the low costs, and the possibility to switch to oral dosing as soon as possible, allowing early discharge of the patient. The pharmacokinetics of oral flucloxacillin is characterized by a rapid absorption with maximal concentrations at approximately 1 hour, 90% protein binding, and an elimination half-life of 1 hour. However, the amount of flucloxacillin absorbed after oral administration is highly variable.[3] The reasons for the variability are largely unknown and likely related to factors such as gastrointestinal motility as there is no published data suggesting involvement of (genetic) variability in drug transporter expression or function. Thus, the preferred therapeutic approach ideally requires assessment of the efficacy of oral absorption of flucloxacillin. The criterion for adequate absorption can be derived from the full pharmacokinetic profile of flucloxacillin and the minimum inhibitory concentration (MIC) of the isolate. The breakpoint MIC for flucloxacillin-susceptible *S. aureus*, defined as the highest MIC value still to be interpreted as indicating susceptibility, is commonly <0.5 mg/l of free drug and translates into a total drug concentration of 5 mg/l. Therefore, we routinely accept flucloxacillin concentration of at least 10 mg/l. This results in free drug concentrations >1 mg/l, which is well above the MIC for most strains.

As the elimination half-life of flucloxacillin is 1 hour, it can be calculated that with a daily dose regimen of at least 5 times 1g of oral flucloxacillin, concentrations will be above the MIC for at least 60% of the dosing interval, which is the generally accepted exposure goal to achieve efficacious treatment of susceptible *S. aureus* strains by beta-lactam antibiotics.[4] Indeed, the oral absorption test (OAT) routinely performed in our institution shows that approximately 10% of the patients do not absorb well enough, for example, have a maximal concentration <10 mg/l. The disadvantages of the test in its current format are that it requires cessation of the continuous IV administration and that it is laborious. We hypothesized that a simpler test with continuation of the IV therapy would perform equally well and can be used routinely in clinical practice at low costs.

PATIENTS AND METHODS

This trial complied with institutional guidelines and Dutch law as the evaluation concerned daily routine practice that subsides under the law on the medical treatment agreement (WGBO; Wet op de Geneeskundige Behandelings Overeenkomst). Hence, separate medical ethical approval was not needed.

PATIENTS

The evaluation period included patients admitted in 2009 and 2010 to Leiden University Medical Center, Leiden, The Netherlands. Data were collected from 43 hospitalized patients with the only inclusion criteria that they received initial continuous IV flucloxacillin and were scheduled for maintenance treatment with oral flucloxacillin. No potentially eligible patients were excluded from the evaluation.

FLUCLOXACILLIN ORAL ABSORPTION TESTS

We evaluated 2 different test protocols to assess the oral absorption of flucloxacillin. The first test (test A) started with an overnight interruption of the continuous IV flucloxacillin for 8 hours during which period the patients also fast. Thereafter, an oral dose of 1g of flucloxacillin was given. Serum flucloxacillin was measured before, and at 1, and 2 hours after the oral dose. In the second test (test B), IV flucloxacillin was continued and the 1g oral dose was given after a fast of at least 1 hour. Measurement of flucloxacillin concentrations was at the same times as in test A. These sample times were chosen because the time of maximal concentration is, on average, at 1 hour after intake. However, the time to maximal concentrations cannot be predicted reliably for individual patients, and it was therefore decided to take samples at 1 and 2 hours as this would allow assessment of the absorption also in case of diminished gastrointestinal motility. Adequate absorption was defined as an increase in flucloxacillin concentration of at least 10 mg/l at either sampling time.

FLUCLOXACILLIN ASSAY

Flucloxacillin serum concentrations were determined using a validated high performance liquid chromatography (HPLC) method with ultraviolet detection (all apparatus from Dionex Corporation, Sunnyvale). In short, 10 microliter of a 1 mg/l of cloxacillin solution (Sigma) and 0.5 ml of acetonitril (Promochem) were added to 0.5 ml of thawed patient serum sample. The samples were then vortexed for 5 seconds and subsequently centrifuged for 5 minutes at 25,000g. Thereafter, 0.8 ml of the supernatant was transferred to a 10-ml polypropylene test tube, and 3.5 ml of chloroform (Merck) was added.

The samples were vortexed for 5 seconds and centrifuged for 3 minutes at 5,500g. Of the aqueous upper layer, 0.1 ml was mixed with 0.1 ml of acetate buffer (0.1 mole/l), and 20 microliter of 68 (39–87) 68 (37–179) this solution was assayed by HPLC. The chromatographic system consisted of an octadecylsilica Hypersil stationary phase (3 mm particle size, length 12.5 cm, id 4.6 mm), and a mixture of 1 mole/l acetate buffer solution (pH 6), water, and acetonitrile (40 + 710 + 250, vol/vol) as mobile phase. Flow rate was 1.0 ml/min, and detection took place at a wavelength of 210 nm. A flucloxacillin reference solution in serum was pretreated in the same manner as for the patient samples. This solution was used to determine the flucloxacillin/cloxacillin signal ratio in patient samples. From this ratio, the serum concentrations of flucloxacillin were calculated. The lower limit of quantification (inaccuracy and imprecision <15%) is 3 mg/l, and the assay shows linearity for flucloxacillin concentrations up to at least 100 mg/l. Accuracy of a quality control sample at 40 mg/l tested 15 times over a 1-month period was 111% and precision, expressed as the coefficient of variation, was 4.0%.

DATA ANALYSIS

The data are summarized as mean with SD and range or as median and range. The maximal concentrations reached in each test variant were compared using an unpaired Student *t*-test. Linear regression analysis was performed regarding the relationship between age, serum creatinine, and pretest concentration and the maximal concentrations.

RESULTS

The study population consisted of 43 patients (18 female, 25 male) treated with IV flucloxacillin with an individualized dose ranging from 6–12g/d. The OATs were performed in groups with similar baseline characteristics (*table 1*). The majority of the patients had treated comorbidities such as hypertension (46%), type 2 diabetes mellitus (21%), or hypercholesterolemia (40%) or combinations thereof and were in addition treated with other drugs including antacids (46%), psychoactive drugs (21%; mainly benzodiazepines), or analgesics or antithrombotics (23%, mainly heparins and occasional antiplatelet agents). There was no difference in age, renal function, gender, diagnoses, or comedication. Test A was performed in 19 patients and test B in 24 patients. The maximally observed increase was highly variable between patients (*figure 1*). The average (SD; range) maximal increase for test A was 21.7 (11.3; 7–50) and 26.1 (12.8; 8–53) mg/l for test B. There was no significant difference in the maximal increase in flucloxacillin concentration between the test variants (p=0.23). Also, there was no relationship between age (r^2=0.06), serum creatinine (r^2=0.01), or pretest flucloxacillin concentration (r^2=0.01) and the observed maximal concentration.

In 4 of the 43 patients (9%), the maximal increase in flucloxacillin concentration did not reach the predefined value of 10 mg/l. This was found in 1 patient using test variant A and 3 patients using test variant B. This difference does, however, not mean that one test variant is more sensitive than the other but reflects the relative small sample size and the inherent variability in absorption. Indeed, if a cut-off value of 12.5 mg/l had been used, both test variants would have identified 16%–17% of the population as poor absorbers. The majority of the concomitant medications used by

patients and gastrointestinal conditions present in the patients who absorbed well and patients who absorbed poorly were alike. Therefore, there were no reasons to a priori suspect impaired flucloxacillin absorption in any of these patients. Specifically, 2 patients who showed poor absorption were treated for preexisting diabetes, but this was also present in another 18 patients who absorbed well. Also, retrospectively, no remarkable clinical condition or drug use that may have impaired flucloxacillin absorption could be identified.

DISCUSSION

Patients with severe methicillin-sensitive *S. aureus* infections are effectively treated with initial continuous IV flucloxacillin for at least 2 weeks, which, if effective is often followed by an additional 2–4 weeks of oral maintenance therapy. There are many advantages to start oral therapy as soon as possible including, but not limited to, ease for the patient and the possibility to treat on an outpatient basis. However, the reliability of an early switch to oral dosing may be complicated by the highly variable oral flucloxacillin absorption, which may jeopardize treatment outcome. Treatment failure due to insufficient absorption can be avoided by performing an OAT, and this is commonly practiced in our institution.

We were used to employ test A, which starts with interruption of the continuous IV flucloxacillin for 8 hours during which period the patients also fast. Thereafter, an oral dose of 1g of flucloxacillin is given. Serum flucloxacillin is measured before, and at 1 and 2 hours after the oral dose. However, this test variant is cumbersome and possibly even less safe as the flucloxacillin levels are below the MIC for several hours due to interruption of the IV therapy. Therefore, we explored if equal results could be obtained with a test variant in which IV dosing is continued while the oral test dose is given. This approach was deemed feasible as it has been shown that flucloxacillin has a wide therapeutic ratio and thus (short term) exposure to higher concentrations is safe and well accepted. In addition, with this approach the practical disadvantages of the earlier test variant such as the need to timely stop and restart the IV pump are avoided. Thus, the new approach is easier executed by the nursing staff and likely reduces mistakes and need for retesting.

We confirm the large interindividual variability in oral flucloxacillin absorption,[3,5] and this was similarly detected with both test variants. We chose to apply a cut-off value of 10 mg/ L, because the MIC of the isolated cultures was <1 mg/l of (free) flucloxacillin. With this criterion, our results indicate that a significant proportion (10%) of the hospitalized patients show insufficient oral absorption. Obviously, the cut-off value can be adapted for individual patients based on MIC of the isolated strain for flucloxacillin, but this is rarely necessary. Also, the findings can assist in determining the dose regimen for oral flucloxacillin that will be prescribed. Although this study did not specifically address intrapatient variability, our experience is that patients who absorb well generally do consistently so in repeat tests, while in patients who are identified as poor absorbers more variable results are obtained during retesting. A potential weakness of our findings is that we did not study test B in a sufficient number of patients with severe renal dysfunction which can affect flucloxacillin disposition. However, based on pharmacokinetic principles and our clinical experience with test variant A, we argue that test B ought to perform adequately in those patients, but this should be verified. Taken together, we provide a simpler and robust, test variant with lesser burden for physicians, nursing and laboratory personnel. In addition, the HPLC-based assay for flucloxacillin is easy to perform and can be implemented in hospital pharmacies at low equipment or staff costs. With the adaptations that we have done, we introduce a simple test that may be considered in applicable cases to guide transition from IV to oral maintenance therapy of flucloxacillin.

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FIGURE 1 **Box and whisker plots for the maximal increase in flucloxacillin at either 1 or 2 hours after an oral dose of 1g using test variants A and B (interruption or continuation of the IV flucloxacillin, respectively). The individual data are indicated by the symbols, the horizontal line across the box shows the median, the boxes indicate the 25th to 75th percentile of the data and the whiskers from the edge of the box are the 5th and 95th percentiles. The dashed line indicates the cut-off value at 10 mg/l.**

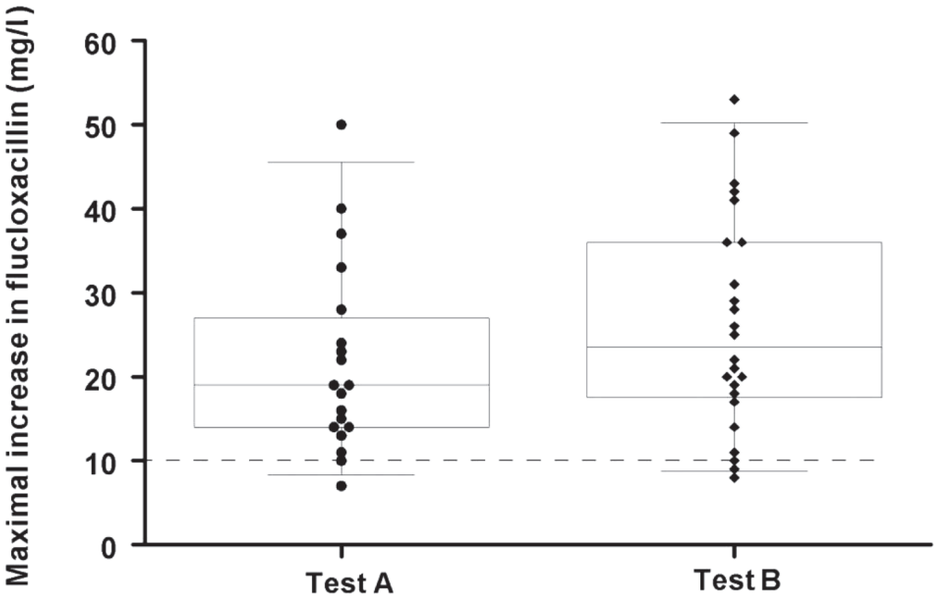

TABLE 1 **Population Characteristics.**

	Test A		Test B	
Number of patients	19		24	
Gender	8 F	11 M	10 F	14 M
Age (yrs)*	63 (18–83)	58 (39–77)	68 (26–85)	64 (20–88)
Creatinine in serum (µmole/l)*	51 (34–89)	70 (39–120)	68 (39–87)	68 (37–179)

**Median (range)*

CHAPTER 3

THE SIMPLIFIED ORAL FLUCLOXACILLIN ABSORPTION TEST: AN ACCURATE METHOD TO IDENTIFY PATIENTS WITH INADEQUATE ORAL FLUCLOXACILLIN ABSORPTION

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ABSTRACT

BACKGROUND The preferred treatment for severe methicillin-sensitive *Staphylococcus aureus* infections is flucloxacillin, a small-spectrum antibiotic administered intravenously (IV) and orally. However, clinicians switch to the less preferred broad-spectrum antibiotics because of the variable absorption after oral administration of flucloxacillin. A classical oral absorption test (OAT) requires overnight fasting and interruption of IV therapy, and is laborious. In the current study, we investigated whether a simplified OAT can be utilized in a clinical setting to guide antibiotic treatment in patients with severe *S. aureus* infections. For this, OAT IV therapy is continued and oral dosing is performed after a one-hour fast and implemented after a small study

METHODS In 196 patients receiving IV flucloxacillin by continuous infusion, a classical OAT (test A) or simplified version of the OAT (test B) was performed. In both tests, 1g oral flucloxacillin was given and serum samples were taken prior to intake and at one and two hours after administration. Flucloxacillin concentrations were determined by high-performance liquid chromatography. Adequate absorption was defined as an increase of flucloxacillin concentration of at least 10 mg/l after one or two hours compared to baseline.

RESULTS In a sample of 196 patients (85 F/111 M), test A was performed in 28 patients, and test B in 168 patients. Age, gender, and baseline values of creatinine and albumin were similar in both groups. The maximal increase of flucloxacillin absorption was highly variable between patients. In 26 (13%) of the 196 patients, the flucloxacillin increase did not reach the value of 10 mg/l. The median (interquartile range, IQR) maximal increase of flucloxacillin absorption was 22.0 (15-31.25) mg/l for test A and 21.5 (13-32.25) mg/l for test B. There was no significant difference in maximal increase of flucloxacillin absorption between test A and B (p=0.74), nor between males and females (p=0.95). Age, creatinine, and albumin were not correlated with flucloxacillin levels.

CONCLUSIONS The simplified version of the OAT is useful to identify patients with adequate oral flucloxacillin absorption, and to ensure the effective continuation of an oral small-spectrum treatment.

INTRODUCTION

Despite advances in antibacterial therapy and stewardship, the effective treatment of severe *Staphylococcus aureus* infections remains an important clinical challenge. Globally, the incidence of severe staphylococcal infections remains high[1,2], which is partly due to the increasing use of indwelling catheters, vascular and orthopaedic prostheses, and prosthetic heart valves.[3] Severe *S. aureus* infections are associated with a high mortality rate and with associated complications (including infective endocarditis) being more prevalent compared to other bacterial infections.[4] It is clear that the effective management and treatment of severe *S. aureus* infections is essential.

The treatment of choice for severe *S. aureus* infections depends on concomitant variables including pathogen antibiotic susceptibility, patient factors (including underlying co-morbidities and concurrent medication), and physician preference. In countries with low endemic methicillin-resistant *S. aureus* (MRSA) rates, such as the Netherlands[5,6], intravenous (IV) flucloxacillin is the preferred choice of treatment because of its bactericidal activity and narrow-spectrum of activity. Continuous IV infusion of flucloxacillin is followed by a course of oral flucloxacillin, which allows for earlier discharge of the patient from the clinic and has a reduced risk of catheter-associated complications.

Flucloxacillin is rapidly absorbed with maximal serum concentrations observed at approximately one hour after intake. It has a high degree of protein binding (approximately 90%) and an elimination half-life of one hour.[7] Of note, previous studies have demonstrated a high degree of variability of flucloxacillin absorption following oral administration,[7-9] and the mechanisms underlying the observed variability remain unclear. It is of clinical importance to ensure that oral flucloxacillin is adequately absorbed in patients and that therapeutic serum levels are maintained, in favour of effective treatment of the underlying bacterial infection.

Knowledge of the full pharmacokinetic profile of flucloxacillin in serum and the minimum inhibitory concentration (MIC) of the isolate are indicators of adequate oral dosing. The breakpoint MIC for flucloxacillin-susceptible *S. aureus*, defined as the highest MIC value indicating susceptibility, is commonly defined as <0.5 mg/l of free drug. Given its high binding capacity, this will translate into a total serum drug concentration of 5 mg/l. Therefore, we routinely accept serum flucloxacillin concentrations of at least 10 mg/l as therapeutic levels, as these are associated with protein-free drug concentrations of >1 mg/l, which is well above the MIC.[10,11] This level of exposure was therefore commonly accepted and associated with the efficacious treatment of susceptible *S. aureus* strains by beta-lactam antibiotics.[11]

The oral absorption test (OAT) is used to ensure an efficacious switch from intravenous to oral therapy and has been routinely performed at our institution (Leiden University Medical Centre, the Netherlands). Results have shown that in approximately 10% of patients, the absorption of oral flucloxacillin was insufficient to reach therapeutic levels (i.e., a maximal serum concentration increase of <10 mg/l).[9]

The OAT format was laborious and error-sensitive, as it required the cessation of the continuous IV flucloxacillin administration eight hours prior to oral intake of the test dose. Recently, we demonstrated that a simplified version of the OAT performed similarly to the classical OAT, was easy to administer and could be implemented in hospital pharmacies at low equipment and staff costs.[9] However, our key concern was the small patient sample (43 patients) and retrospective design of that study. In the current study, we aimed to confirm our previous findings in a larger patient population, to investigate whether a simplified OAT can be utilized in a clinical setting to guide antibiotic treatment in patients with severe *S. aureus* infections, and to screen for factors associated with the previously observed inter-individual variability in oral flucloxacillin absorption.

PATIENTS AND METHODS

This study complied with institutional guidelines and Dutch law, as the evaluation concerned daily routine practice that adheres to the law on the medical treatment agreement (WGBO; Wet op de Geneeskundige Behandelings Overeenkomst). Hence, separate medical ethical approval was not needed.

PATIENTS

The evaluation period included adult patients admitted between 2011 and 2017 to Leiden University Medical Centre, Leiden, the Netherlands. Data were retrospectively collected from 196 hospitalised patients receiving continuous IV flucloxacillin and scheduled for oral flucloxacillin treatment. No potentially eligible patients were excluded from the evaluation.

FLUCLOXACILLIN ORAL ABSORPTION TESTS

We evaluated two separate test protocols to assess the oral absorption of flucloxacillin (*figure 1*). The simplified version of OAT (test B) was already implemented. The classical OAT (test A) was occasionally performed due to ingrained habits. Test A commenced with an overnight fast and interruption of continuous IV infusion of flucloxacillin for eight hours. Thereafter, an oral test dose of flucloxacillin (1g) was given. Blood samples for serum flucloxacillin concentrations were taken at baseline, and at one hour after oral dosing, in line with the expected C_{max} of flucloxacillin. Because of the expected inter-patient variability in C_{max} levels, it was decided to add a measurement at two hours. This also allowed for a better estimation of flucloxacillin absorption in case of diminished gastrointestinal motility. Test B required the continuation of IV infusion of flucloxacillin. An oral test dose of flucloxacillin (1g) was given after a one-hour fast. Measurement of serum flucloxacillin concentrations were performed at similar time-points as in test A (*figure 1*).

Adequate absorption was defined as an increase of serum flucloxacillin concentration of at least 10 mg/l from baseline at either sampling time. Because of the high level of protein binding and renal excretion of flucloxacillin, albumin and serum creatinine were also assessed in patients prior to oral flucloxacillin dosing.

FLUCLOXACILLIN ASSAY

Flucloxacillin serum concentrations were determined using a validated high-performance liquid chromatography (HPLC) method with ultraviolet detection (all equipment from Dionex Corporation, Sunnyvale, CA, USA). We added 10 µl of a 1 mg/l of cloxacillin solution (Sigma) and 0.5 ml of acetonitrile (Promochem) to 0.5 ml of a thawed patient serum sample. The samples were then vortexed and subsequently centrifuged for five minutes at 25,000g. Thereafter, 0.8 ml of the supernatant was transferred to a 10 ml polypropylene test tube, and 3.5 ml of chloroform (Merck) was added. The samples were vortexed and centrifuged for three minutes at 5,500g. We mixed 0.1 ml of the aqueous upper layer with 0.1 ml of acetate buffer (0.1 mole/l), and 20 ml of this solution was assayed by HPLC.

The chromatographic system consisted of an octadecylsilica Hypersil stationary phase (3 mm particle size, length 12.5 cm, id 4.6 mm), and a mixture of 1 mole/l acetate buffer solution (pH 6), water, and acetonitrile (40 + 710 + 250, vol/vol) as mobile phase. Flow rate was 1.0 ml/min, and detection took place at a wavelength of 210 nm. A flucloxacillin reference solution in serum was pre-treated using similar methodology as in the patient samples. This solution was used to determine the flucloxacillin/cloxacillin signal ratio in patient samples. From this ratio, serum concentrations of flucloxacillin were calculated. The lower limit of quantification (inaccuracy and imprecision, 15%) was 3 mg/l, and the assay showed linearity for flucloxacillin concentrations up to at least 100 mg/l. For a quality control sample of a predefined concentration (40 mg/l) we found a mean concentration of 44.4 mg/l (111%) after 15 tests over a one-month period, with a coefficient of variation of 4.0%.

DATA ANALYSIS

Demographics of the study population were summarised. Baseline flucloxacillin levels, age, serum creatinine concentration and serum albumin concentration in relation with the maximal increase of flucloxacillin concentrations were visually explored. The change in maximal increase of flucloxacillin levels between test A and test B, and between males and females was tested using an unpaired Student's *t*-test. A p of 0.05 was considered statistically significant.

RESULTS

Of the 196 patients (85 females and 111 males) who were treated with IV flucloxacillin, the individualised dose of continuous infusion ranged from 6–12g/d. Baseline characteristics of patients were comparable between OATs A and B (*table 1*). Two measurements at two hours after dosing were removed from the analysis due to unrealistic outliers (>200 mg/l), probably because samples were taken erroneously

from the flucloxacillin catheter. There was a difference in maximal increase of flucloxacillin absorption from baseline (*figure 2*). The median (IQR) maximal increase was 22.0 (15-31.25) mg/l for test A and 21.5 (13-32.25) mg/l for test B (*figure 2*). There was no significant difference in maximal increase of serum flucloxacillin levels between tests A and B (p=0.744). No relationship could be identified between any of the covariates and the maximal increase of serum flucloxacillin concentrations (*figure 3*). The inter-subject variation in flucloxacillin seemed to increase with increasing age. No statistical tests were performed due to insufficient data.

In 26 (13.27%) of the 196 patients, the maximal increase of flucloxacillin concentration did not reach the predefined target of 10 mg/l. This was found in 10.7% patients using test A and 13.7% patients using test B.

There was no significant difference in maximal increase of serum flucloxacillin concentration between male and female subjects for test A (p= 0.80) and test B (p=0.95). Additionally, there was no relationship between both serum creatinine and serum albumin levels and the observed maximal increase of serum flucloxacillin concentration (*figure 2*). Serum creatinine (n=143) and albumin (n=48) samples were not available for all included subjects (*figure 3C* and *D*).

Most of the maximal flucloxacillin concentrations were reached at two hours after dosing (54.6%). In tests A and B, 45.4% of the apparent maximum concentrations were achieved one hour post dose and 54.6%, two hours post dose.

DISCUSSION

In the current study, we confirmed the finding of our previous study, wherein we demonstrated that a simplified version of the OAT was easy to perform and could be implemented in hospital pharmacies at low equipment or staff costs, and performed similarly to the classical OAT.[9] In the current study, we observed that there was no significant difference in maximal increase in serum flucloxacillin levels from baseline between tests A and B, the classical and simplified version of the OAT. As flucloxacillin has a wide therapeutic window, it was deemed that the short-term exposure to higher levels of flucloxacillin in the simplified OAT was safe. As such, the simplified OAT is not only a safe, viable alternative, but also has practical advantages compared to the classical OAT; for example, nursing staff is no longer required to stop and start IV pumps, which saves approximately 20 minutes of nursing staff time per test.

A large inter-subject variability in oral flucloxacillin absorption was observed in our patient sample. It should be noted that outliers were seen in the simplified OAT group, which adds an extra level of variability in absorption. We believe that this is mainly caused by the larger sample size in the simplified OAT group. Median and IQR values for both tests were comparable.

Our study highlighted that a significant proportion of all patients (13%) demonstrate insufficient drug absorption of flucloxacillin. This finding is in agreement with the results of our previous study[9], and of previously published pharmacokinetic data.[7,8,10] Our results further showed that there was no correlation between serum creatinine and serum albumin levels and the observed maximal increase of serum flucloxacillin concentration. In tests A and B, 45.4% of the apparent maximum concentrations were achieved one hour post dose and 54.6%, two hours post dose. This suggest that, the C_{max} likely occurred sometime in between the sampling times. Future research may focus on optimising the sampling times to identify sufficient absorption to reduce the required samples from two to one. However, as covariates and high variability may impact the absorption process[12], two samples are recommended to perform the absorption test at this stage. Since C_{max} mainly depends on the extent and rate of drug absorption, minor or no effects could be expected from renal function or serum albumin. Knowing that flucloxacillin is predominantly excreted by the kidneys and is associated with a high degree of protein binding (approximately 90%), the observed lack of correlation suggests that both renal function and variation in serum albumin are not responsible for the observed inter-individual variability in oral flucloxacillin absorption in our patient sample.

The results of both simplified and classical OAT show that 45.4% of the maximum flucloxacillin concentrations were achieved one hour after administration and 54.6%, two hours after administration. This confirms the expected inter-patient variability in C_{max} levels, and justifies the use of two sampling time points in our study.

It should be noted that a substantial proportion of patients (14%) in the simplified and classical OAT groups did not absorb well. This could be explained by genetic variation in drug transporter enzymes or enzymes involved in the first-pass metabolism of flucloxacillin; however, the mechanism underlying the observed inter-individual variability in oral flucloxacillin absorption remains undetermined. Based on our personal experience and expertise, we believe that patients with an adequate level of oral absorption will generally demonstrate consistent levels of absorption in repeated tests. On the other hand, patients who are identified as poor absorbers, generally display variable levels of absorption during retesting. The reason for this variability remains unclear. Factors such as gastric emptying and intestinal motility could play a role; for example, late absorption of rifampin was associated with delayed gastric emptying such as diabetes mellitus.[13] Polymorphisms in the gut could also result in a reduced absorption of flucloxacillin and drug transporter polymorphisms like P-glycoprotein, or first-pass enzymes could further complicate the pharmacokinetic profile of variable flucloxacillin absorption. Another explanation could be a pharmacogenomic mechanism, since a substantial proportion of the patient sample in our study showed levels of inadequate absorption. Currently, there is no evidence for the involvement of genetic variability leading to a differentiated expression or function of metabolic enzymes such as cytochrome P450 (CYP) or drug transporters, while un-identified polymorphisms in CYP gene expression and/or enzyme activity could indeed play a role in the increased hepatic breakdown of flucloxacillin. Similarly, genetic variation in P-glycoprotein polymorphisms or hepatic enzymes may influence the absorption and first-pass effect of flucloxacillin, resulting in

inter-individual differences in flucloxacillin absorption and, hence, peak levels. We do know that a high dose of IV flucloxacillin for a minimum of two weeks prior to oral dosing might be responsible for the induction of increases in gene expression of drug transporters and/or CYP enzymes, as previous studies have reported the induction of hepatic CYP 3A4 and P-glycoprotein by flucloxacillin.[14-16] Therefore, there is a need for novel studies exploring how pharmacogenomics affect the pharmacokinetic profile of flucloxacillin.

Limitations of our study were the retrospective design and the absence of clinical assessments.

In summary, we have designed and confirmed that a simplified OAT can be utilized in a clinical setting to guide antibiotic treatment in patients with severe *S. aureus* infections. The HPLC/ultraviolet flucloxacillin assay can be easily performed by most labs, and can be implemented in hospital pharmacies with limited equipment and staff costs. We have demonstrated that this adapted OAT can be safe, less expensive, less time consuming, and less error-sensitive, compared to the classical OAT, and can adequately identify patients with insufficient oral flucloxacillin absorption without interruption of IV therapy; this is advantageous since serum levels will not drop down below therapeutic levels. As the mechanism(s) underlying the observed inter-individual variability in oral flucloxacillin absorption remain elusive, it is vital to develop a quantitative test to clinically assess the efficacy of oral absorption of flucloxacillin to ensure patient safety and the efficacious treatment of underlying bacterial infections. For optimal management of patients with severe *S. aureus* infections, we strongly encourage fellow clinicians to adopt and implement our simplified OAT in clinical practice.

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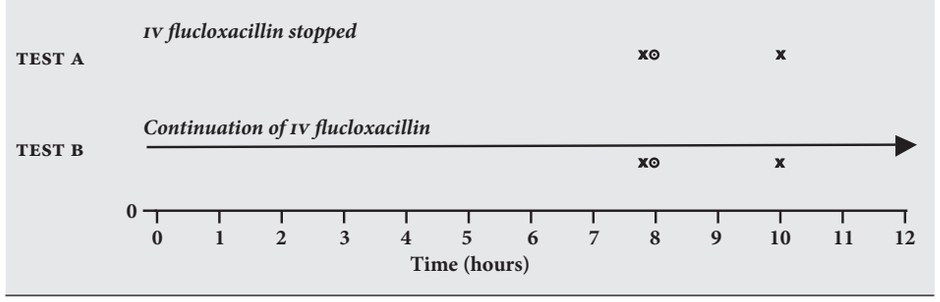
TABLE 1 **Population Characteristics. Median (interquartile range) [range].**

	TEST A		TEST B	
Number of patients	28		168	
Gender	10 F	18 M	75 F	93 M
Age (yrs)	68.5 (64-75) [51-94]	54 (46-67) [24-77]	61 (49 - 70) [20-94]	66 (49-72) [21-91]
Serum Creatinine concentration (µmol/l)	61(54-67) [33-79]	59 (51-77) [48-90]	80.5 (61 – 134) [44-1012]	90 (67- 139) [29-807]
Serum Albumin concentration (g/l)	39 (33-40) [27-41]	42 (39-43) [27-44]	32 (28-38) [19-44]	27.5 (26 – 35) [20-55]

M=male; F=female

FIGURE 1 **Diagram to show test A and test B OAT protocols.**



o = OAT; × = blood sample taken for serum flucloxacillin concentration

FIGURE 2 **Box and whisker plots for the maximal increase in flucloxacillin at either 1 or 2 hours after an oral flucloxacillin dose of 1g using test variants A and B (interruption or continuation of IV flucloxacillin, respectively). The horizontal bold line across the box shows the median, the under and upperline indicate the 25th to 75th percentile of the data and the whiskers depict 1.5x the IQR. Data outside this range is indicates with black circles. The dashed line indicates the cut-off value at 10 mg/l.**

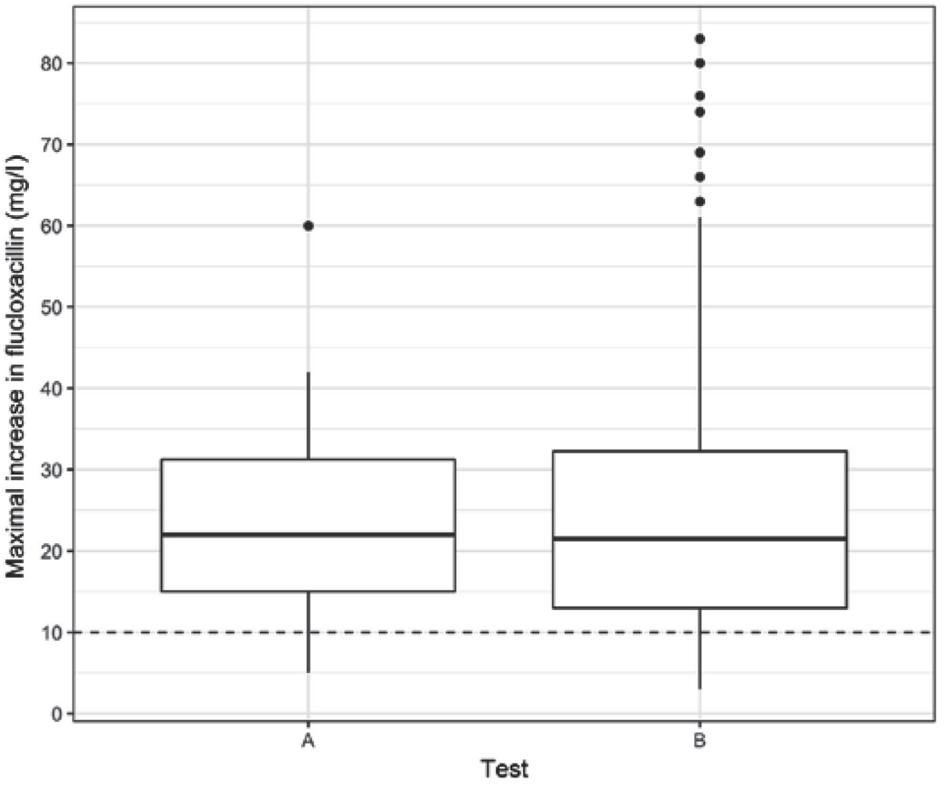
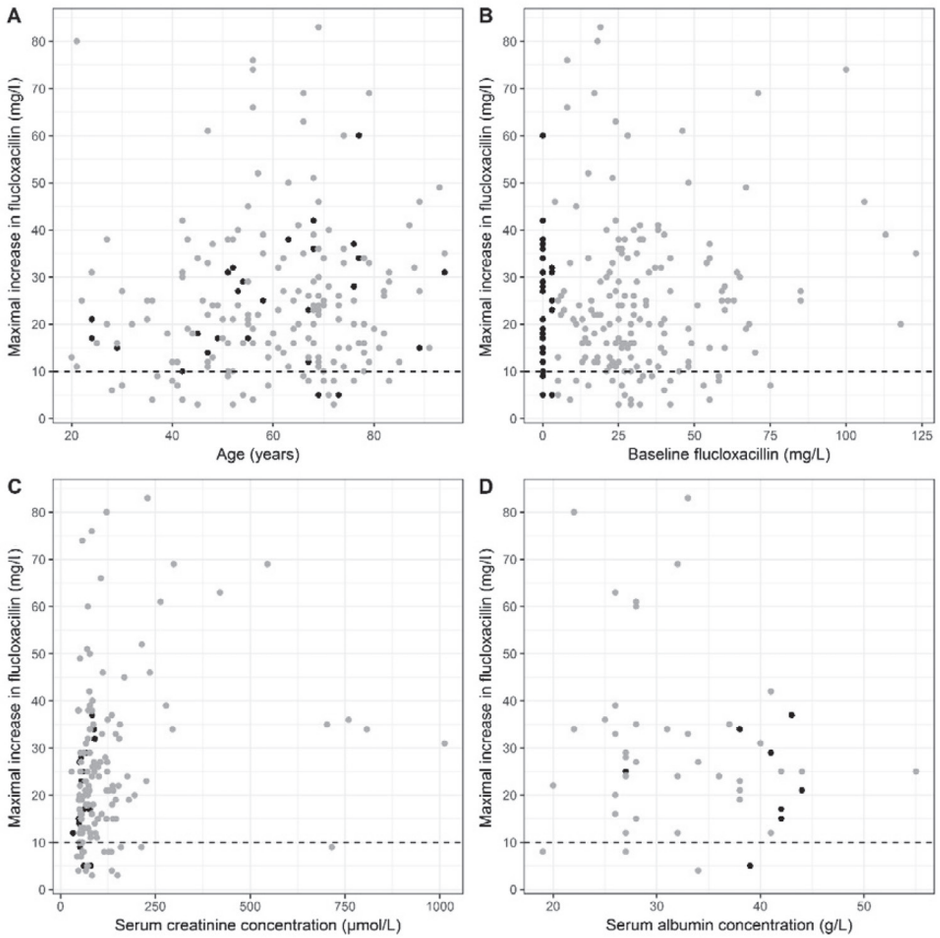


FIGURE 3 **Maximal increase in flucloxacillin after oral absorption test A (black circles) or B (grey circles) versus age (A), baseline flucloxacillin (B), serum creatinine (C) (n=143), and albumin (n=48) (D). Dashed line indicates cut-off for inadequate oral absorption of 10 mg/l.**



CHAPTER 4

THE ORAL PHENETICILLIN ABSORPTION TEST: AN ACCURATE METHOD TO IDENTIFY PATIENTS WITH INADEQUATE ORAL PHENETICILLIN ABSORPTION

Antibiotics (Basel) 2019; 8(3)

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ABSTRACT

Severe streptococcal infections are commonly treated with intravenous followed by oral penicillin (pheneticillin) therapy. However, switching from IV to oral therapy is complicated by the variability in oral pheneticillin absorption. We employed an Oral Absorption Test (OAT) for pheneticillin to identify patients in whom oral pheneticillin absorption is poor. Out of 84 patients 30 patients (36%) were identified as insufficient absorbers. Treatment failure due to pheneticillin malabsorption can be avoided by performing an OAT, and these patients should be treated by another antibiotic, which is known to be absorbed well.

BACKGROUND

In the Netherlands, patients with severe streptococcal infections are effectively treated with narrow-spectrum antibiotics, most frequently with initial intravenous (IV) penicillin G followed by oral maintenance therapy, usually with pheneticillin. The advantages of this treatment approach are the bactericidal activity of penicillin, the low costs, the possibility to switch to an oral antibiotic as soon as possible, and the lower risk of introducing resistance associated with narrow-spectrum antibiotics. However, the disadvantage is the highly variable absorption of pheneticillin,[1] and patients in whom adequate dosing is critical should undergo an oral pheneticillin absorption test before switching to oral pheneticillin maintenance therapy. The purpose of this report is to describe our findings with the oral absorption test (OAT) implemented in our medical center.

PATIENTS AND METHODS

The gathering of the data for this investigation complies with Dutch law as the evaluation concerned with daily routine practice; the law on the medical treatment agreement (WGBO; Wet op de Geneeskundige Behandelings Overeenkomst). Hence, separate medical ethical approval was not needed.

The evaluation included patients admitted to Leiden University Medical Centre, Leiden, the Netherlands from 2005 to 2013. Data were collected from hospitalized patients with the only inclusion criterion that they received initial penicillin G and were scheduled for maintenance treatment with oral pheneticillin.

PHENETICILLIN ORAL ABSORPTION TESTS

Like other beta-lactam antibiotics, treatment efficacy of pheneticillin is defined by the time the plasma concentration is above minimum inhibitory concentration (MIC). As a proxy for this, we used pheneticillin peak concentrations as absorption is the limiting factor to reach bactericidal effects after oral administration. While on IV penicillin G therapy, patients received an oral dose of 1g of pheneticillin in the fasted state. Blood samples for serum pheneticillin concentration were taken at baseline and at 1 and 2 h after the oral dose. These sample times were chosen because of the expected time of maximal concentration at 1 h after intake.[1] However, because the time to maximal concentrations cannot be predicted reliably for individual patients, it was decided to have a relatively wider window and take samples at 1 and 2 h as this would allow assessment of the absorption also in case of diminished gastrointestinal motility. Adequate absorption was defined as an increase of ≥10 mg/l pheneticillin relative to trough concentration (t=0) either 1 or 2 h after dosing. The reason for choosing this concentration is that the highest MIC value indicating susceptibility is defined as <0.5 mg/l of free drug (e.g., the breakpoint MIC for most microorganisms for phenoxypenicillins by EUCAST[2]). Given the high protein binding (80%)[1]

of pheneticillin, total serum drug concentration should be at least 5 mg/l. We used a safety margin and considered serum pheneticillin concentrations above 10 mg/l as therapeutic levels. The maximal pheneticillin level during the absorption test (at either 1 or 2 h after dosing) was taken forward in the analysis to decide on adequate absorption of a patient. An unpaired *t*-test was applied to explore sex differences in oral absorption of pheneticillin. Correlations and the adjusted R2 of linear regression were calculated to identify any trends of oral absorption over age. The effect of diabetes mellitus or use of gastric acid inhibitors on pheneticillin absorption was also investigated.

PHENETICILLIN ASSAY

Pheneticillin concentration was determined with high performance liquid chromatography[3]. This method allowed for the simultaneous detection and quantification of pheneticillin, benzylpenicillin, and flucloxacillin in a single sample. The assay shows linearity for pheneticillin concentrations up to 50 mg/l with a lower limit of quantification of 3 mg/l. The accuracy and reproducibility of the method (determined by repeated measurement of a quality control sample) were 103.3 and 5.6%, respectively.[3]

RESULTS

Eighty-four (84) hospitalized patients (59 males, 25 females), mean age ± standard deviation (range)=58.3 ± 15.4 (19–90 years), were included in the analysis. A total of 30 patients (36%) were identified as insufficient absorbers. The median increase from baseline was 11.6 mg/l (inter-quartile range=8.5–16.1 mg/l, range=3.8–27.7 mg/l) as depicted in *figure 1*. The majority of maximal concentrations were reached at 1 h after dosing (80%).

No linear correlation between the maximal increase from baseline and age was detected (correlation 0.22, adjusted R2=0.04) (*figure 2A*). No significant difference between gender in maximal absorption was identified (p=0.66). A wide scatter in the maximal absorption between patients having diabetes mellitus and/or on gastric acid inhibitors was observed (*figure 2B*); no formal statistical analysis was performed due to the low sample sizes.

CONCLUSION AND DISCUSSION

There is little doubt that the use of narrow-spectrum antibiotics is effective to not further aggravate the increasing problem associated with of multi-resistant bacterial resistance. Severe streptococcal infections are commonly adequately treated with intravenous penicillin followed by oral therapy. Indeed, there are many advantages to start oral therapy as soon as possible. However, the early switch from penicillin G to pheneticillin is complicated by the variability in oral pheneticillin absorption, which may jeopardize treatment outcome, especially in cases in which adequate dosing is pivotal. Future research should investigate additional variables that explain the observed variability in order to optimize dose selection in this heterogeneous patient population. Treatment failure due to pheneticillin malabsorption can be simply avoided by performing an OAT. In case it is not feasible to perform an OAT, knowing the high rate of non-absorbers, we advise not to switch to oral pheneticillin and choose for another antibiotic, such as – if feasible – amoxicillin per os, which is known to be absorbed well.[1,4,5]

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FIGURE 1 Distribution of maximal increase from baseline in pheneticillin showing the median, interquartile range, and outliers. Horizontal dashed line indicates the cut-off for adequate absorption (10 mg/l).

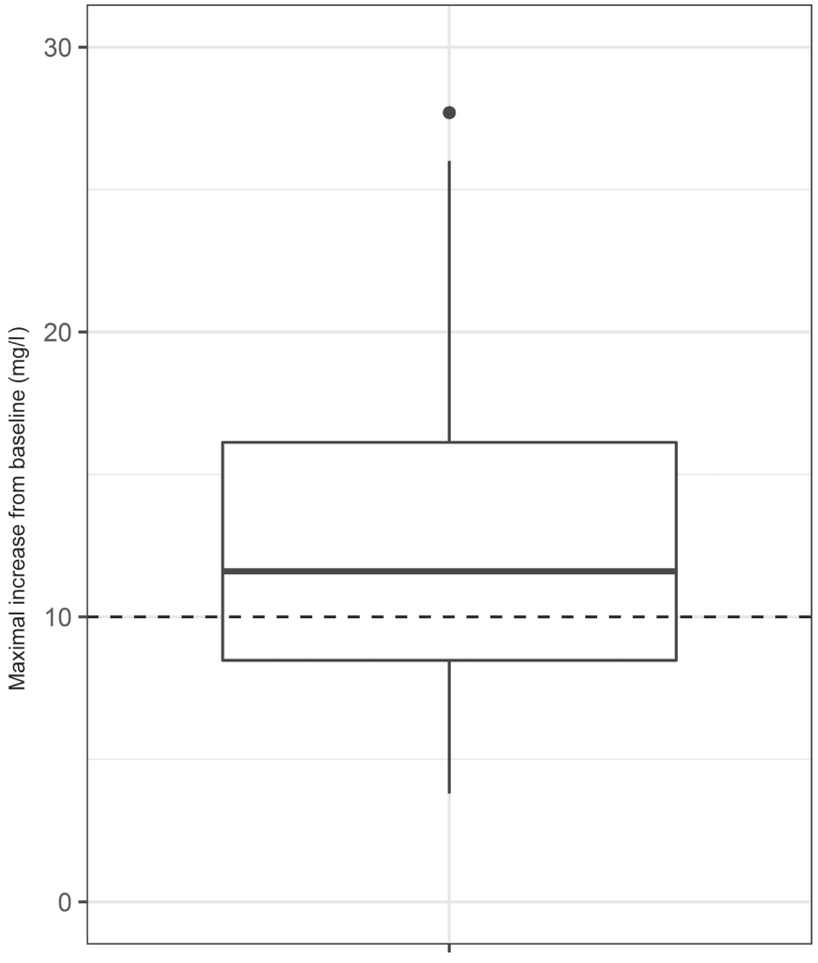
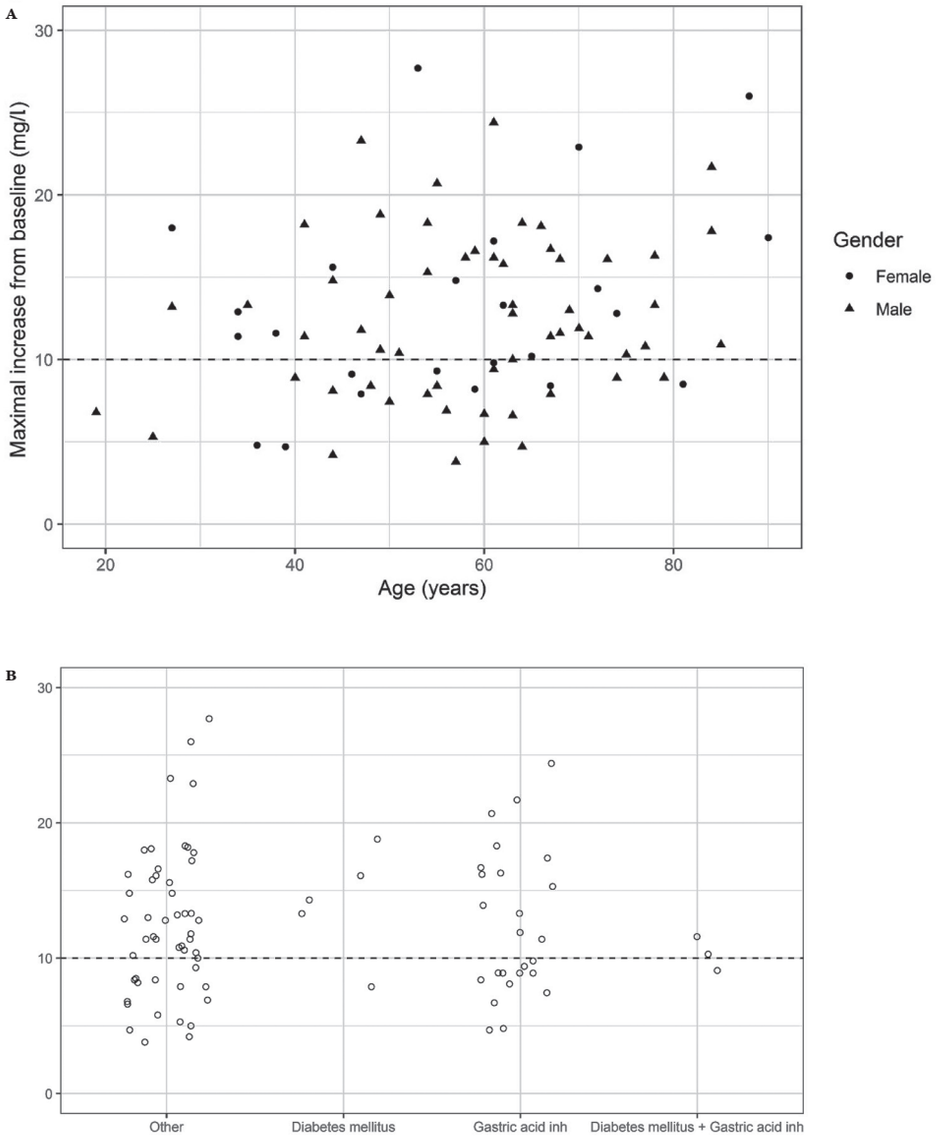


FIGURE 2 (A) Maximal increase from baseline versus age for men and women. (B) Maximal increase from baseline for diabetes mellitus patients and/or patients on gastric acid inhibitors. Horizontal dashed line indicates the cut-off for adequate absorption (10 mg/l).



CHAPTER 5

RIFAMPIN LEVELS IN DAILY PRACTICE: THE ACCURACY OF A SINGLE MEASUREMENT

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ABSTRACT

BACKGROUND Measurement of rifampin levels is not part of routine practice. However, low levels are associated with failure of tuberculosis treatment. The clinical relevance of serum levels in daily practice is unclear. The objective was to evaluate rifampin serum concentrations and factors associated with insufficient concentrations.

METHODS Patients with at least one rifampin concentration drawn 3 hours after intake (C_3) between 2005 and 2014 were included. Data on demographic and clinical characteristics were collected, including side effects and dose adjustments. Two different criteria were used to define adequate concentrations (criterion 1: C_3 and C_6 ≥ 3 mg/l; criterion 2: C_3 or C_6 ≥ 5 mg/l).

RESULTS Of 63 patients, 66% and 76% had a sufficient level according to criterion 1 or 2, respectively. C_3 exceeded C_6 in most patients, while a late maximum was significantly associated with diabetes mellitus (p=0.003). A dose adjustment was made in 19% of cases, more frequently in patients with insufficient levels (p=0.02) or with ≥ 2 side effects (p=0.03).

CONCLUSION Rifampin levels varied but were mostly adequate and a single measurement at 3 hours after intake provided the required information in most cases, indicating that full AUC_{0-24} measurements could be limited to specific situations.

BACKGROUND

Tuberculosis (TB) remains one of the world's most important infectious threats, reflected by 1.8 million deaths in 2015, of which 0.4 million deaths among people living with HIV.[1] Hence, adequate treatment is paramount. Rifampin is a key drug in the first-line treatment of active or latent TB, due to its high activity against *Mycobacterium tuberculosis* with an MIC_{90} of ≤ 0.25 µg/ml.

The treatment success rate, especially in new cases, is improving although treatment failure occurs in up to 14% of patients.[5] While multiple factors, including poor treatment adherence, bacterial resistance and even drug quality, may contribute to treatment failure, drug dosage and insufficient concentrations are relevant in this regard. In a previous study, the risk of failure of long-term treatment was almost 9-fold higher in patients with low drug exposure, expressed as 24-hour area under the concentration time curve (AUC_{0-24}) for pyrazinamide, rifampin and/or isoniazid.[6]

That study and other data showed that insufficient serum concentrations may even result in development of drug resistance.[6,7] Apart from the prescribed dose, drug exposure may be influenced by factors such as comorbidities, food intake and inter-individual differences in pharmacokinetics.[7-12] Therapeutic drug monitoring (TDM) of rifampin is not routinely performed and there is no consensus on adequate levels. In previous studies, rifampin serum concentrations at 2 hours (C_2) and at 6 hours (C_6) after intake have been used to approximate the peak level.[13-15] A recent study found that the rifampin AUC_{0-24} in TB patients was predicted optimally using sampling at time points 1, 3, and 8 hours,[16] which would be impractical for most outpatients or require availability of alternative methods such as dry blood spot analysis. During the past decades, a rifampin absorption test at our centre has consisted of measurement of serum concentrations at 0, 3 and 6 hours after intake, and only at the physician's request. The aim of the present study was to retrospectively evaluate the results of these absorption tests of rifampin regarding adequate levels, and factors associated with out of range serum concentrations.

STUDY POPULATION AND METHODS

Study population

The study population consisted of patients in whom one or more rifampin serum concentrations had been measured at Leiden University Medical Centre (LUMC), a tertiary care hospital, between October 2005 and May 2014. Demographic and clinical characteristics were collected from the medical charts, including age, sex, weight, country of origin, clinical diagnosis, comorbidity (HIV infection, present or past malignancy, liver disease, diabetes mellitus, chronic kidney failure, autoimmune disease(s) or other), pregnancy, concomitant medication, rifampin dose at the time of TDM, kidney and liver function, indication for TDM and side effects. Serum

concentrations of rifampin at 0, 3 and 6 hours after intake, time of blood sampling, possible dose change and results of possible repeated TDM were collected. Patients were excluded if only a trough level was available or if the clinical data could not be retrieved. The protocol of this retrospective study with anonymised data collection was evaluated by the Medical Ethics Committee of the LUMC and waived from the requirement of informed consent (protocol G16.017).

Criteria for interpretation of serum concentrations

As there are no uniform criteria for adequate rifampin levels, we used two different criteria. According to the original protocol used at our institution for several decades, the source of which could not be retrieved, serum levels of the sum of rifampin and desacetyl-rifampin ≥ 3 mg/l at 3 hours (C_3) and 6 hours (C_6) after intake were defined as adequate (criterion 1: C_3 and $C_6 \geq 3$) and clinical decisions therefore were only based on this criterion. As an alternative criterion, adequate absorption was defined as a single measurement of the sum of rifampin and desacetyl-rifampin ≥ 5 mg/l (criterion 2: C_3 or $C_6 \geq 5$) as is nowadays implemented in several institutions. The data were analysed according to both criteria.

Method of measurement of rifampin concentrations

Serum concentrations of rifampin and desacetylrifampin were measured by high performance liquid chromatography according to the method published by Chandi et al.[17] The method was linear in a concentration range of 0.5 mg/l up to at least 15 mg/l rifampin and/or desacetyl-rifampin. Accuracy was >98.8% and imprecision <5.7%.

Statistics

Descriptive statistical parameters were used. To compare proportions or continuous values between two groups, two-way chi square tests (or Fisher's exact probability test in case of comparison of proportions including numbers <5), and ANOVA tests were used, respectively. Differences using two-sided testing were considered significant at $p < 0.05$. Statistical analysis was performed using IBM SPSS Statistics version 23.

RESULTS

Study population

Of 90 patients in whom rifampin levels had been determined, 63 met the inclusion criteria (15 were excluded because only a trough level had been measured and 12 because clinical data were unavailable). Patient characteristics are shown in *table 1*. The majority (42/63, 67%) were immigrants from TB endemic regions. Most patients had one or more comorbidity, with autoimmune disease, chronic liver disease and malignancy being most frequent. The most frequent reason for TDM was control of compliance (52%), followed by suspected high (29%) or low concentration (6%). More than half of the patients had received rifampin for active TB and one-third for latent TB.

Serum rifampin concentrations

In 63 patients, a total of 138 rifampin concentrations (at 0, 3 and/or 6 hours) were available. Rifampin levels were not always available for all three time points (*table 2*). C_3 was available for all 63 patients, C_0 was available for 34/63 patients (54%) and C_6 for 41/63 patients (61%). According to the guidelines for TB treatment the standard dose of rifampin is 10 mg/kg, with a maximum of 600 mg. Most patients (45/63, 71.4%) were treated with a dose of 600 mg (*table 2*). The dose was 600 mg in 42/46 (91.3%) patients with a body weight ≥ 55 kg. The mean ± SD dose per weight was 11.2 ± 3.9 mg/kg. Maximal rifampin levels did not differ according to dose per weight (data not shown). Maximal levels did not vary by any demographic or clinical parameter (*table 1*).

Trough levels were <2 mg/l in 31/34 patients (91.2%) and were 3.2 mg/l, 5.6 mg/l and 9.9 mg/l respectively in the remaining three patients. In the last of these three patients (patient 41 in *figure 1*), C_0 exceeded C_3 and C_6 and thus had most likely been measured after intake of rifampin. The average individual maximal concentration, which could be either at 3 or at 6 hours, was 8.9 mg/l (range 0.0 mg/l to 26.7 mg/l). With regard to criterion 1: C_3 and $C_6 \geq 3$, 41 patients could be evaluated. Criterion 1 was met in 27/41 (65.9%). Criterion 2: C_3 or $C_6 \geq 5$ was met in 48/63 patients (76.2%). There was no significant relation between age, sex, comorbidities, co-medication or indication for rifampin comorbidities and meeting the criteria or not. Levels in immigrant patients more frequently met criterion 2 than did those from native Dutch patients (86.4% vs 52.6%, p=0.004). *Figure 1* shows all individual rifampin concentrations, ranked by the value of C_3 which was available for all 63 patients. C_3 exceeded C_6 in all but 8 patients (case 2, 9, 12, 17, 18, 24, 46 and 53 in *figure 1*). C_6 was ≥ 5 mg/l and often even much higher in all of these eight patients with late maximal concentrations. In 7/8 patients criterion 1: C_3 and $C_6 \geq 3$ was also met. Of the eight patients with late maximal levels, four (50%) had diabetes mellitus and one additional patient suffered from systemic sclerosis. In the remaining three patients no factors associated with delayed absorption could be identified. The proportion of patients with diabetes in those with late maximal levels (4/8 patients with $C_6 > C_3$) was significantly different from that in patients with early maximal levels (1/33 patients with $C_3 > C_6$; Fisher's exact probability test p=0.003). In 12 patients (19%) rifampin measurements including at least C_3 were later repeated after a median interval of 11 days (range 1-50 days, and one outlier at 248 days) because of out of range first levels, newly experienced side effects and/or after adjustment of the dose based on initial levels. The results of paired individual maximal serum concentrations are shown in *figure 2*.

Side effects

At least one side effect was reported in 27/63 patients (42.8%). Side effects varied from mild to very severe, ranging from minor nausea to drug-induced hepatitis (*table 2*). The maximal rifampin level in patients experiencing side effects was not significantly different from that in patients without side effects. In the six patients with serum transaminases >100 IU/l, the maximal level was not different from that in patients without liver function disturbances.

Dose adjustments

Twelve out of 63 patients (19.0%) had a dose adjustment. Six of 15 patients (40%) who did not meet criterion 2 had a dose increase. Six of 48 patients (12.5%) meeting criterion 2 had a dose reduction. This difference in proportion with a dose adjustment was significant (p=0.02).

A dose adjustment was made in 5/13 patients who experienced ≥ 2 side effects, in 3/14 patients with one side effect and in 4/36 patients without side effects (p=0.03 for comparison of patients with ≥ 2 to those without side effects).

Of 12 patients who had a second measurement of the rifampin level, dose changes were reported in five (*figure 2*). In four of these, the maximal levels were adequate after a dose increase (n=3) or reduction (n=1).

Follow-up

None of the patients with active TB had treatment failure and none of the patients treated for latent TB infection and who later received immunosuppressive drugs had a TB reactivation during a follow-up time between two and ten years.

DISCUSSION

In the present study we retrospectively evaluated rifampin levels which had been determined in routine practice in a mixed population consisting mainly of patients treated for active or latent TB. The data showed considerable inter-individual variation but in the majority of patients serum levels were adequate as based on two different criteria, one of which had been in use for decades at our institution and an alternative criterion based on a single peak level of at least 5 mg/l, which is nowadays implemented in several Dutch institutions. Nevertheless, the dose was adjusted in 20% of patients because of either too low or very high levels. In most patients in whom both C_3 and C_6 were available, C_3 was highest and therefore most informative. Maximal serum levels were not affected by demographic parameters, the presence of comorbidities or use of co-medication.

Despite the recognition that adequate rifampin concentrations are crucial for treatment success, TDM is not common practice. In addition, there are no clear criteria for the interpretation of concentrations. Studies in animals showed that the $AUC_{0\text{-}24}$ in steady state divided by the MIC was the best predictive parameter for efficacy of rifampin.[18,19] In humans, treatment failure has been associated with low $AUC_{0\text{-}24}$, and with development of bacterial resistance.[6,7] In a population pharmacokinetic model in patients with active TB, the rifampin $AUC_{0\text{-}24}$ could be predicted with high precision using sampling at 0, 1, 3, and 8 hours after intake.[16] However, such timing is not practical for most outpatients and the investment of the patient's time and the costs must be weighed against the value of the information thus obtained. In a previous study a single measurement of rifampin at four hours after intake gave the best estimate for $AUC_{0\text{-}24}$.[20] While precise $AUC_{0\text{-}24}$ of rifampin is generally not needed, there are specific situations in which such information can be essential, such as in patients with extensive TB and a high bacillary load, or in patients with TB meningitis because of limited penetration. In general practice there may also be reasons to measure rifampin levels, however without the need for a precise $AUC_{0\text{-}24}$, e.g. if treatment adherence is doubted, if poor absorption is suspected or because of suspected high levels. In these situations it may suffice to measure the concentration at the time of expected peak concentration. Because there is a large inter-individual variation in pharmacokinetics the peak value can be missed if just one sample is used. However, the results of the present study showed that C_3 almost always exceeded C_6. This is in agreement with a peak between 1 and 3 hours (occasionally 4 hours) after intake in studies in which multiple time points were used, the peak being closer to 2 hours if the drug was taken without food and closer to 3 hours if taken with a light meal.[16,21] Thus, if full $AUC_{0\text{-}24}$ is not required a single measurement at 2 to 3 hours after intake may provide sufficient information. In the limited number of patients in the present study in whom C_6 exceeded C_3, more than half had a disorder associated with delayed gastric emptying such as diabetes mellitus, and including a later time point should thus be considered in that setting. In accordance with our finding, in a previous study in Indonesian patients the $AUC_{0\text{-}6}$ was about 50% lower in patients with diabetes compared with nondiabetic[22] TB patients. Trough levels were not informative and our data suggest that these could be omitted.

Combining data from the literature with those from the present study, we designed a simple and practical algorithm for the selection of time points for measurement of rifampin concentrations (*figure 3*). We think that testing rifampin concentrations at just one time point in most patients, and more frequently only on indication, could save time and money without loss of quality of care. In the LUMC, based on this study the single measurement is now implemented for routine practice, while $AUC_{0\text{-}24}$ is available if needed. Regarding the standard rifampin dose of 600 mg it has been argued that the 600 mg dose is at the lower end of the dose-response curve.[23] An update of the TDM in the treatment of tuberculosis of rifampin suggests higher doses to be more effective.[24] The pharmacokinetic profile of rifampin

is nonlinear and a dose increase will result in a greater than proportional increase in AUC. Previous studies using a higher rifampin dose of 13 mg/kg or 20 mg/kg did not observe increased hepatotoxicity or other adverse events.[23,25-29] In a recent study even a 1200 mg dose was well tolerated,[30] indicating that a higher dose can probably be given without increasing the risk of side effects. Higher rifampin doses were evaluated in large clinical trials targeting C_{max} values ≥ 8 mg/l. Higher doses were associated with a better outcome and/or no increase of toxicity.[31-33] *Boeree et al.* even described a possibility of a shorter regimen of tuberculosis treatment with a higher dose (up to 35 mg/kg) of rifampin.[32] A limitation of our study was the retrospective nature and the probable selection bias because rifampin levels were not routinely measured.

CONCLUSIONS

The results of this study show that in most cases a single rifampin level measured at 3 hours after intake provided sufficient information regarding adequacy of treatment. In the presence of risk factors for delayed absorption sampling at a later time point had added value. We think that a complete AUC_{0-24} measurement can be limited to specific situations. Our findings could contribute to a cost-effective, rapid and patient-friendly approach to TDM of rifampin and to effective treatment. However, further studies in different populations and settings are needed to assess the generalisability of our findings.

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TABLE 1 Clinical characteristic and rifampin levels in 63 patients.

Characteristic	Categories	No. (%)	Maximal rifampin level (average ± SD) in mg/l	P value
Sex	Men	37 (58.7)	8.6 ± 4.9	0.5
	Women	26 (41.3)	9.5 ± 6.0	
Age (range in years)	0-15	11 (17.5)	9.2 ± 5.0	0.6
	16-30	13 (20.6)	9.5 ± 4.9	
	31-45	12 (19.0)	9.0 ± 5.9	
	46-60	14 (22.2)	7.8 ± 4.3	
	61-75	11 (17.5)	10.4 ± 6.9	
	>75	2 (3.2)	3.5 ± 4.9	
Immigration	No	19 (30.2)	7.65 ± 6.4	0.2
	Yes	44 (69.8)	9.5 ± 4.8	
Region of origin	Western Europe	19 (30.2)	7.6 ± 6.4	0.4
	Eastern Europe/Russia	4 (6.3)	5.6 ± 2.7	
	Africa	19 (30.2)	9.8 ± 5.6	
	Middle East	7 (11.1)	10.9 ± 2.2	
	Asia (other than Middle East)	11 (17.5)	10.5 ± 4.7	
	North and Central America	2 (4.5)	4.4 ± 2.8	
	South America	1 (2.3)	9.9	
Comorbidities	None	8 (12.7)	7.4 ± 3.5	0.4
	≥ 1	55 (87.3)	9.2 ± 5.5	
	HIV	4 (6.3)[a]	4.5 ± 1.8	
	Malignancy	13 (20.6)	11.4 ± 6.4	
	Chronic liver disease	10 (15.9)	9.4 ± 5.4	
	Diabetes mellitus	6 (9.5)	7.5 ± 2.2	
	Pregnancy	4 (6.3)	9.6 ± 7.0	
	Chronic kidney failure	3 (4.8)	9.3 ± 2.3	
	Autoimmune disease	20 (31.7)	8.5 ± 4.8	
	Other	29 (46.0)	9.6 ± 6.4	
No. of comorbidities[b]	0	16 (25.4)	8.6 ± 5.2	1.0
	1	35 (55.6)	9.1 ± 5.8	
	2	11 (17.5)	9.2 ± 4.5	
	3	1 (1.6)	6.9	
Indication for rifampin	Active tuberculosis	35 (55.6)	9.3 ±6.0	0.9
	Latent tuberculosis	20 (31.7) I	8.3 ± 4.5	
	IV catheter-related infection	6 (9.5)	8.8 ± 5.6	
	Other	2 (3.2)	9.1 ± 0.6	

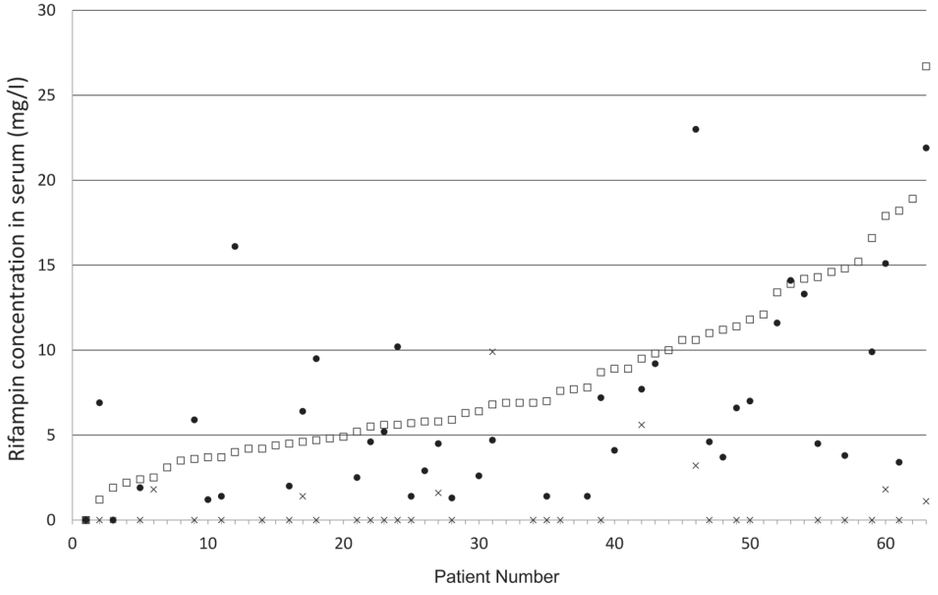
HIV=human immunodeficiency virus; IV=intravascular. a: The sum of the comorbidities exceeds 63 (100%) as patients could have more than one comorbidity; b: based on the reported seven specific comorbidities as listed in this table, thus excluding the category of other comorbidities.

TABLE 2 Dose, side effect, available concentrations, interpretation and dose adjustments.

Parameter	Category	No. (%)[a]
Dose (mg)	600	44 (70.1)
	450	6 (9.7)
	300	3 (4.8)
	Other	9 (14.5)
Side effects	≥ 1 side effect	27 (42.8)
	≥ 2 side effects	13 (20.6)
	General symptoms	19 (30.2)[b]
	Gastrointestinal complaints	7 (11.1)
	Drug induced hepatitis	6 (9.5)
	Skin involvement	5 (7.9)
	Headache	2 (3.2)
	Neurological symptoms	1 (1.6)
	Other	6 (9.5)
Available rifampin levels	Only C_3	18 (28.6)
	Only C_3 and C_6	11 (17.5)
	Only C_0 and C_3	4 (6.3)
	C_0, C_3 and C_6	30 (47.6)
Criterion	Yes	27/41 (65.9)
C_3 and C_6	→ dose change	2/27 (7.4)
≥3 mg/l[c]	No	14/41 (34.1)
	→ dose change	3/14 (21.4) *p*=n.s.
Criterion	Yes	48 (76.2)
C_3 or C_6	→ dose change	6/48 (12.5)
≥5 mg/l	No	15 (23.8)
	→ dose change	6/15 (40.0) *p*=0.02

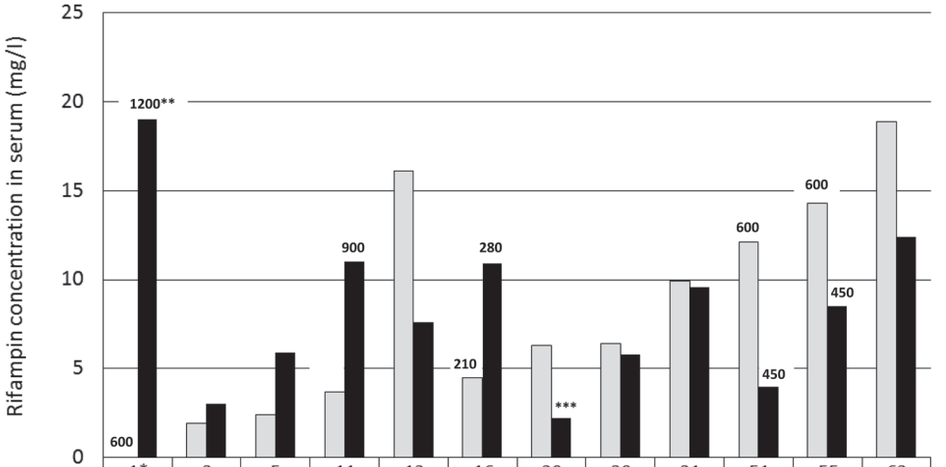
a. Denominator was 63 unless otherwise specified
b. the sum of the side effects exceeds 27 as patients could have more than one side effect;
c. this criterion could only be tested for 41 patients for whom at least C_3 and C_6 were available

FIGURE 1 Distribution of rifampin levels in 63 patients, ranked by the concentration at 3 hours after intake.



Trough value is indicated by ×; C_3 (concentration 3 hours after intake) is indicated by □; C_6 is indicated by •

FIGURE 2 Maximal rifampin levels in 12 patients in whom rifampin concentrations were measured twice.



	1*	3	5	11	12	16	29	30	31	51	55	62
Conc. 1	0	1.9	2.4	3.7	16.1	4.5	6.3	6.4	9.9	12.1	14.3	18.9
Conc. 2	19	3	5.9	11	7.6	10.9	2.2	5.8	9.6	4	8.5	12.4

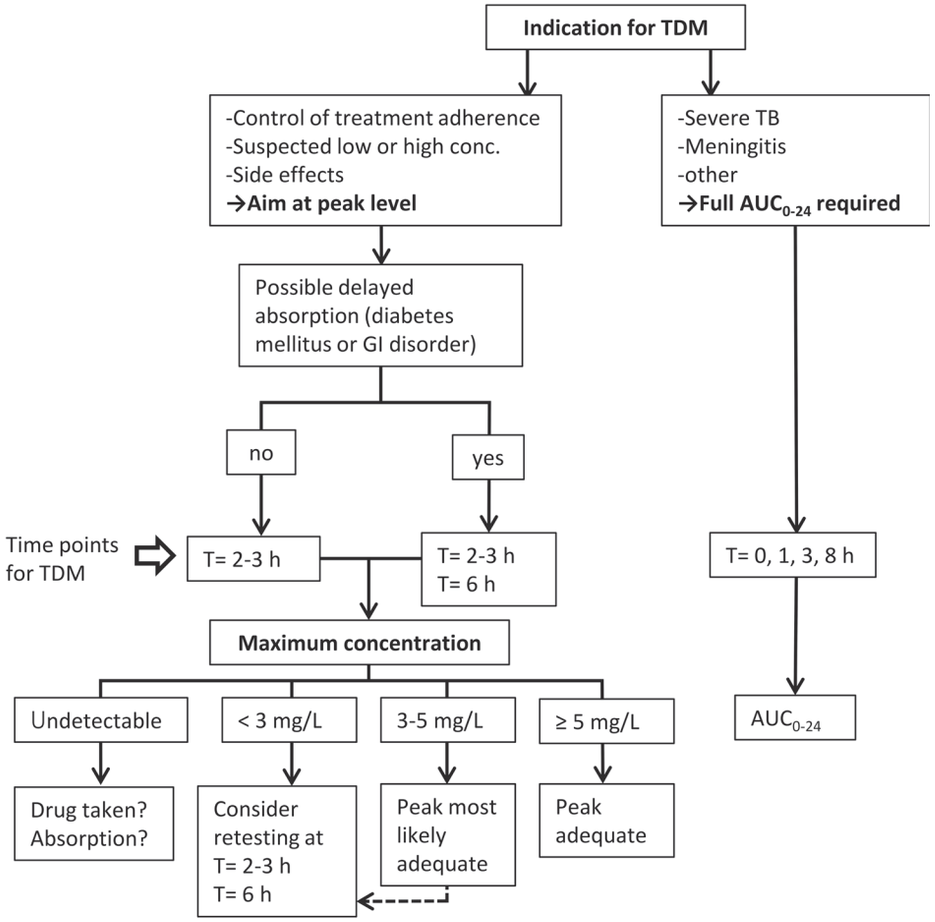
Dose changes are indicated above the bars as dose in mg.

* *The top row indicates the patient numbers corresponding to those used in figure 1.*

** *In patient 1 with initial undetectable rifampin concentrations, the maximal concentration was very high after doubling the dose, which suggested that rifampin may not have been taken at the time of first* TDM.

*** *In patient 29 the dose was increased from 500 mg to 600 mg based on the results of the repeated level.*

FIGURE 3 **The dotted line reflects the authors' opinion that retesting is generally not necessary if the clinical course is favourable but can be considered depending on the specific clinical situation.**



CHAPTER 6

COLISTIN: REVIVAL OF AN OLD POLYMYXIN ANTIBIOTIC

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ABSTRACT

Colistin (polymyxin E) is a positively charged decapeptide antibiotic that disrupts the integrity of the outer membrane of the cell wall of gram-negative bacteria by binding to the lipid A moiety of lipopolysaccharides, resulting in cell death. The endotoxic activity of lipopolysaccharides is simultaneously inhibited. Colistin is increasingly being prescribed as rescue treatment for infections with multidrug-resistant bacilli. Nephrotoxicity and, to a lesser degree, neurotoxicity occur often during systemic colistin therapy, and have severely limited its application in the past. However, these side effects are largely reversible and can be managed through close monitoring. The prodrug colistimethate sodium (CMS) is less toxic and is, therefore, the preferred formulation for parenteral administration. Importantly, resistance to colistin seems to emerge often unless it is combined with another antibiotic, but further studies into this phenomenon are necessary. Pharmacokinetic and pharmacodynamic properties have received little attention, partly because of the physicochemical peculiarities of polymyxin antibiotics, especially their propensity to stick to other molecules and surfaces. The ratio between the area under the curve of free colistin and the pathogen's Minimal Inhibitory Concentration (MIC) best predicts microbiological and clinical responses, but more studies are needed in this area. Likewise, further standardization is needed in production and labeling of colistin formulations, and in the way the susceptibility of bacteria to colistin is determined.

INTRODUCTION

After the discovery of antibiotics[1] and their introduction into clinical practice, it was generally believed that infectious diseases would become history. However, this idea was proven to be wrong, and resistance to antibiotics has become an enormous global burden. Especially, gram-negative bacteria, including *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and *Klebsiella pneumoniae*, are becoming more and more resistant to an increasing number of antibiotics. As a consequence, mortality due to untreatable bacterial infections is increasing.

Physicians have to be aware of the risks of prescribing colistin because inappropriate use of antibiotics is the most important reason for emergence of resistance.[2] Lack of proper infection control has allowed resistant clones to spread further, sometimes even worldwide. In addition, there is an almost empty pipeline of new antibiotics. Pharmaceutical industry has shown little interest in marketing new antibiotics because of high development costs, relatively small return on investments, and the limited size of this niche in the pharmaceutical market.[3] New antibiotics tend to be categorized as 'reserve antibiotic,' resulting in further delay in reaping financial benefit for the pharmaceutical industry.

As there are presently no new antibiotics available for some multiresistant bacteria, an old molecule, colistin, is increasingly being applied over the last years. Colistin (polymyxin E) is an antimicrobial agent that has hardly been used since the reporting of its side effects decades ago. Consequently, there is a lack of relevant knowledge for its clinical use nowadays. Colistin consists of a mixture of colistin A and B. Two chemical formulations are used therapeutically: colistin sulfate and colistimethate sodium (CMS). The latter is a prodrug and is, both in vivo and *in vitro*, converted into a complex mixture of partially sulfamethylated derivatives and partly into active antibiotic colistin. CMS is less toxic than colistin sulfate when used intravenously or by inhalation.[4,5] Unfortunately, there is no unanimously accepted model for the pharmacokinetic and pharmacodynamic properties of colistin. In addition, the exact mechanisms of colistin resistance have not been fully elucidated yet.

Colistin's first life

Colistin was isolated in 1949[6] from the *Bacillus polymyxa* 'Colistinus' and became available for clinical use in 1959.[7] Colistin is a cyclic deca-peptide antibiotic containing 10 linked amino acids (*figure 1*).

Soon after its discovery, it turned out that colistin was identical to polymyxin E.[8] In the 60s, much was learned about the different types of polymyxins (A-E), including colistin, the latter being a mixture of polypeptides. Although most of the mechanism-of-action studies have focused on polymyxin B, the mechanism of action of other polymyxins, including colistin, is thought to be the same.[8] The purpose of this article is to review this antibiotic, in light of its increasing use in the era of emerging drug resistance.

METHODS

Systematic search strategy

The MEDLINE/PUBMED and OVID/EMBASE databases were searched systematically in April 2014 to identify relevant articles on colistin. The search terms concentrated on synonyms of colistin in article titles, to be as specific as possible. The following MEDLINE/PUBMED and OVID/EMBASE searches strategies were performed.

MEDLINE/PUBMED

('Colistin'[Major] OR colistin*[ti] OR 'Polymyxin E'[ti] OR 'Polymyxin E1'[ti] OR 'Polymyxin E2'[ti] OR 'Polymyxins'[ti] OR 'colistinmethanesulfonic acid' [Supplementary Concept] OR Colistimetha*[ti] OR colisticin*[ti] OR Colimycin[ti] OR 'Coly-Mycin'[ti] OR 'Coly Mycin'[ti] OR Totazina[ti] OR Colifin[ti] OR Colobreathe[ti] OR Tadim[ti] OR Colomycin[ti]) AND (eng[la] OR dut[la]).

OVID/EMBASE

(colistin*.ti. or Polymyxin E.ti. or Polymyxin E1.ti. or Polymyxin E2.ti. or Polymyxins. ti. or Colistimetha*.ti.or colisticin*.ti. or Colimycin.ti. or Coly-Mycin.ti. or Coly Mycin. ti. or Totazina.ti. or Colifin.ti. or Colobreathe.ti. or Tadim.ti. or Colomycin. ti.) AND (dutch or english).lg.

The search strategies were designed with specialist librarians and were restricted to English and Dutch. There were no publication or date restrictions.

A comprehensive database of the retrieved articles was built and electronically checked for removing any duplicates. The abstracts of all publications identified were then independently reviewed by the authors. All articles that focused on multidrug resistance gram negatives (such as *A. baumannii*, *P. aeruginosa*, Enterobacteriacea), pharmacokinetics, pharmacodynamics, critically ill patients, treatment outcome, or mode of action were included for full-text review.

To search for potential additional relevant references, the reference lists of included articles were screened as well as relevant guidelines and references from the product information. A final check to update the systematic search was repeated just before the article submission (November 2014) to include any new contribution on this issue.

RESULTS

The combined search in the databases PUBMED/ MEDLINE and OVID/EMBASE retrieved 2569 records, 880 of which were excluded being duplicates (*figure 2*). Of the remaining 1689 records screened by title and abstract, 1617 were excluded as they were judged not pertinent to the topic, whereas 55 references were examined in full text. Thirty-three references were found through handsearching (also including guidelines and product information).

Mechanisms of action

Polymyxins preferentially bind to the lipopolysaccharide (LPS) component of the outer membrane of gram-negative bacteria and damage it as a consequence.[9] Gram-positive bacteria do not contain LPS in their cell wall and, as a consequence, are not susceptible to polymyxins.

With the binding of polymyxins to the lipid A, a key component of the LPS, the 3-dimensional structure of the LPS is altered. This leads to an increased permeability of the cell envelope, which results in leakage of the cell contents and finally in cell death.[10-12] This process is called osmotic destruction. It has been described that polymyxins – in addition to osmotic destruction – are able to kill bacteria by penetrating into the cytoplasm where they interact with nuclear material and ribosomes.[13]

Because the activity of polymyxins involves a displacement of the divalent cations calcium and magnesium that normally stabilize the LPS, the bactericidal activity of polymyxins can be antagonized by physiological concentrations of calcium and magnesium at the level of the cell wall.[14,15] Furthermore, it is known that the endotoxic activity of LPS is significantly reduced because of the binding of polymyxin to the lipid A component of LPS. In this way, polymyxins inhibit LPS-induced activation of leukocytes, and as a consequence, the production of tumor necrosis factor–a and other interleukins.[16] Although antiendotoxin effects of colistin have been proven and validated in animal models,[17] this feature of colistin has not yet been evaluated in clinical practice.

Susceptibility testing

The routine disc diffusion test is not a reliable method to determine colistin susceptibility, because of the fact that the test fails to detect low-level resistance.[18,19] The European Committee on Antimicrobial Susceptibility Testing and the Clinical and Laboratory Standards Institute recommend in their current guidelines colistin susceptibility testing by measuring the MIC. Therefore, broth microdilution is the standard method in most parts of the world. Colistin has, at neutral pH, a polycationic nature, causing it to adhere to many materials, which results in unreliable results.[20] For that reason, polysorbate 80, a dispersing agent, has been added to the media, but this did not result in a gold standard.[21,22] In our opinion, the appropriateness of polysorbate 80 supplementation of the media during MIC testing remains to be proven. In conclusion, further work needs to be performed to gather information to change the current ISO 20776-1 standard.[23]

Colistin resistance

As with all other antibiotics, resistance against colistin has emerged.[24-26] Recently, the European Medicines Agency warned in their report[27] about the increasing resistance to

colistin, especially in southern European countries where colistin has been frequently used in agriculture. The mechanism of resistance against colistin is likely to be the target modification, that is, changes in the lipid A component of the LPS structure.[28] Among enteric pathogens and *P. aeruginosa*, the addition of aminoarabinose and/or phosphoethanolamine residues to the lipid A moiety of LPS removes its negative charge, and, thus, abolishes the affinity of the positively charged colistin for the bacterial cell wall, resulting in resistance. In *A. baumannii*, a complete loss of the LPS structure has been observed as the basis for colistin resistance.[29] Mutations in several genes (parRS, pmrAB, phoPQ, and cprRS) involved in the regulation and synthesis of LPS have been identified in clinical and laboratory-induced colistin-resistant strains.[2,30-32] Importantly, aminoglycosides are also cationic antimicrobials that may simultaneously become less effective by the LPS modifications described above.

Toxicity

In 1970, Koch-Weser et al[25] reported significant side effects of colistin. Nephrotoxicity – consisting of proteinuria, hematuria, and appearance of cylinders in the urine, as well as an increase in the blood levels of urea and creatinine – occurred in 20% of the patients treated with CMS. Apart from nephrotoxicity, neurotoxicity in the form of paresthesias, muscle weakness, peripheral neuropathy, and neuromuscular blockade resulting in respiratory paralysis was described.[25]

These effects and the emergence of new antibacterial agents with fewer side effects led to the disappearance of colistin from clinical practice in the 1970s. A more recent analysis of risks showed colistin dose, the presence of hypoalbuminemia, and the concomitant use of nonsteroidal anti-inflammatory drugs to be independent risk factors for nephrotoxicity.[33] A high body mass index has also been associated with colistin nephrotoxicity, possible because of overdosing based on actual body weight.[34] Importantly, the trough colistin level has been shown to be an independent risk factor for nephrotoxicity.[35]

Recent studies show that, although nephrotoxicity often develops, the level of toxicity might be acceptable. In healthy Japanese volunteers, transient signs of nephrotoxicity or neurotoxicity were observed only after repeated dosing of 75,000 IU/kg body weight CMS.[36]

In a series of intensive care patients treated with a 9-MIU loading dose and 4.5 MIU twice daily as maintenance dose, signs of acute kidney injury were observed in 18% of patients, but they were limited in severity and did not lead to interruption of CMS administration. Also, kidney function recovered after cessation of CMS.[37] In 2 other studies, signs of nephrotoxicity were observed in 14.3% and 18.6% of the patients treated with 3 MIU 3 times daily.[38,39] In a study in which intensive care patients received an average of 4.5 MIU of CMS daily during an average period of 21 days, nephrotoxicity was noted in 8% of patients.[40] Depending on the dose, formulation of CMS, duration, patient category and definitions of nephrotoxicity and (reversible) nephrotoxicity were reported in 10%–50% of patients exposed to this agent.[41,42]

Age, disease status, duration, dose, and concomitant administration of other potentially nephrotoxic drugs (ie, vancomycin and nonsteroidal anti-inflammatory drugs) were risk factors for the development of nephrotoxicity. In a nonrandomized study, 21 intensive care patients with ventilatorassociated pneumonia treated with CMS were compared with 14 similar patients treated with imipenem. Imipenem had twice the risk of developing nephrotoxicity (42% for imipenem versus 19% for colistin, no statistically significant difference).[43]

It can be concluded that kidney dysfunction regularly occurs among patients using CMS and that kidney function should be closely monitored. However, kidney dysfunction is usually not severe, and it is reversible upon discontinuation of CMS. Compared with the early experience with CMS, we now have better facilities for monitoring renal function, better management of renal dysfunction, and awareness of the additional risk when combining CMS with other potentially nephrotoxic drugs. Also, the formulations and the level of hydrolyzed free colistin present in intravenous (IV) solutions prepared at that time could have played a role in the development of renal toxicity.[44]

Practical information on colistin

PHARMACOKINETICS

CMS, as a prodrug, is hydrolyzed into active colistin.[2,45,46] CMS can be hydrolyzed *in vitro* during storage or sample handling, leading to overestimation of colistin when measured in biological samples.[2,45,46] Before 1997, the CMS concentration was either determined by microbiological assays or not measured at all.[46] Since the introduction of novel methods including HPLC and liquid chromatography-tandem mass spectrometry,[47,48] the plasma concentration of CMS and colistin can be quantified separately, allowing the development of better pharmacokinetic models.[49]

After parenteral administration of CMS, only a small fraction is hydrolyzed to colistin, whereas most of the dose is cleared by renal mechanisms. The distribution in the body is best described by a 2-compartment model for CMS and a 1-compartment model for colistin.[50,51] In healthy volunteers, after a single IV dose, CMS has an average half-life of 0.7– 2.0 hours, whereas colistin has an average half-life of 3.0–4.0 hours. However, with repeated dosing, the half-lives of CMS and colistin change to 0.5 and 5.0 hours, respectively.[36,52,53]

In contrast, critically ill patients with normal renal function show an increased half-life, up to 4.6 hours for CMS and from 9.1 up to 18 hours for colistin.[50,51,54] In case of a poor renal function, CMS clearance is reduced, and hence more CMS is available for conversion into the active colistin, increasing the risk of overdose and toxicity. Therefore, renal function and increase in volume of distribution should always be taken in account when dosing critically ill patients (eg, intensive care patients).[49,50] In healthy volunteers, the volume of distribution of colistin is around 0.171–1.443 l/kg with a single IV dose,[36,52] which can be increased in critically ill patients.

After IV administration of CMS to healthy subjects, the maximum concentration of colistin is reached after 2 hours[36]; whereas in critically ill patients, the time to reach the maximum concentration can increase up to 7 hours. Recent pharmacokinetic studies in intensive care patients (patients with burns) have revealed that plasma concentration of colistin increases slowly, requiring between 2 and 3 days to reach the steady-state concentration of colistin.[51] Therefore, a loading dose of CMS has been proposed in patients in whom an increased volume of distribution can be expected and implemented in several studies to achieve the desired target concentration sooner and use less frequent administrations.[37,50,51,54]

Regarding protein binding, colistin has been found to bind both to albumin and alpha1-acid glycoproteins. The fraction unbound (f_u) ranges from 26%–41% in the concentration range of 0.01–2.5 mg/l.[54] Binding, however, varies widely in critically ill patients with reported levels between 6% and 72%.[55] The free concentration or unbound concentration (fu) is important because it is responsible for the antibacterial activity,[27] and this should be taken into account when measuring levels of colistin. The activity of colistin can be best predicted when the free concentration of colistin is related to the MIC, especially the area under the curve (AUC), which has a better predictive value than the C_{max} or T. MIC.[4,56,57] The parameter that correlates best with the effectiveness is the AUC of the free concentration divided by the MIC (fAUC/MIC).

TISSUE PENETRATION

Colistin hardly crosses the blood–brain barrier.[58] Conflicting results on the penetration through the lung tissue are available.[59,60] However, in clinical practice, pneumonia is successfully treated with colistin, with IV administration and by inhalation administration.[2]

Little is known about intraocular penetration of colistin. However, in an animal study, it was demonstrated that colistin penetrates the aqueous humor of the eyes, when administered topically, intramuscularly, or by subconjunctival injection.[61]

After oral administration colistin is poorly absorbed, while after administration by inhalation, the rate of absorption will depend on the condition of the lung and the type of nebulizer used. Pharmacokinetics of CMS after inhalation has been studied in both cystic fibrosis (CF) and in mechanically ventilated patients.[62-64] In general, colistin concentrations found in sputum or epithelial lining fluid were well above the MIC of 1 mg/l for up to 12 hours, with concentrations ranging from 1 to 21 mg/l found after nebulization of 1–4 MIU of CMS. Because very high CMS concentrations were also measured in sputum – around 50–500 mg/l after 1 hour of nebulization – it has beenhypothesized that inhaled CMS provides a reservoir that allows prolonged delivery of colistin from the lung.[62-64]

THERAPEUTIC DRUG MONITORING OF COLISTIN

The use of therapeutic drug monitoring (TDM) to optimize colistin dosing and minimize toxicity has not been well established yet. On the basis of pharmacokinetic studies, substantial dose adaptations have been made in specific patient populations such as patients with burns and patients in the intensive care unit and those on continuous venovenous hemofiltration.[65-67] Trials to assess the value of TDM are ongoing. The value of TDM to prevent overdosing seems most appropriate in patients with decreased renal function and in patients who are obese to avoid toxicity. Trough levels above 3 mg/l at steady state at day 7 of treatment have been associated with an increased risk of nephrotoxicity.[35]

However, TDM can aid to avoid underdosing in patients with good renal function and in infections with microorganisms in which it is questionable whether the standard dosing level of 3 times 2 MIU is sufficient. In performing TDM, the free (unbound) concentration of colistin is preferably be measured since the fraction unbound is ranging from 26%–41% in the concentration range of 0.01– 2.5 mg/l.[54] Because AUC/MIC gave the best relationship with bacterial killing, this parameter is preferably used in TDM.[64] Alternatively, a trough concentration could be used to estimate the AUC value when a pharmacokinetic model is available. In patients with reduced renal function and/or an increased volume of distribution, the concentration of colistin seems rather constant at steady state and one could argue to use C_{min} to relate to the MIC.[54,66,68]

For example, for *P. aeruginosa*, a concentration of 4 times the MIC proved to result in successful bacterial killing at a MIC value of, 1 mcg/ml.[69] An AUC/MIC of 24 mg×hours/ml · 4/1 mcg/ml=96 hours could be used as a target for total colistin. Taken f_u into account (26%– 41%), an AUC between 25–40 hours could be aimed at for unbound colistin. This value is in line with animal experiments as reported by Bergen et al.[27] They reported target fAUC/MIC values of 15–45 depending on the log-killing and localization of the infection. If this level of exposure of colistin is not feasible, combination therapy has to be instigated. For other microorganisms, similar estimations of target AUC can be made.

Current use

Aerosolized CMS is used in patients with CF to eradicate an initial pulmonary infection, to reduce the pulmonary colonization, and to prevent exacerbations of lung infections with *P. aeruginosa*. In severe pulmonary infections, inhalation is often combined with IV therapy. Colistin sulfate is also used as part of selective intestinal decontamination regimens.[70] Now that colistin is increasingly used as a rescue therapeutic antibiotic several experts advocate that an alternative agent should probably be included in the selective intestinal decontamination regimens. Otitis externa due to gram-negative bacteria, especially *P. aeruginosa*, represents another indication for the topical use of colistin sulfate often in combination with hydrocortisone.

Dosing and administration of CMS

INHALATION

Intrapulmonary delivery of colistin can be achieved either by means of nebulization of a solution of CMS or by dry powder inhalation. For the treatment of chronic pulmonary colonization with *P. aeruginosa*, adult CF patients usually inhale 1–2 MIU of CMS twice daily using a nebulizer. In children aged above 2 years, 1 MIU is inhaled twice daily. When exacerbations of infection with *P. aeruginosa* occur, 1–2 MIU of CMS is used 3 times per day by inhalation for a period of 3 months (*table 1*).

Dry powder inhalation is performed with a dose of 1.66 MIU of CMS twice daily in adults and children aged above 6 years. Safety for younger children has not been established.

IV ADMINISTRATION

For individuals. 60 kg body weight, 1–2 MIU CMS is given every 8 hours. In adults and children, 60 kg body weight, 16,000–25,000 IU/kg body weight is administered every 8 hours. For the US product Coly-Mycin M, a maximal daily dose of 5 mg/kg colistin base activity is advised (equivalent to 62,500 IU/kg and 13.3 mg/kg CMS). In patients with reduced renal clearance, the dose has to be adapted according to a schedule presented in *table 2*.

In intensive care patients with serious infection, higher doses have been recommended to compensate for the increased volume of distribution; a loading dose of 9 MIU and a maintenance dose of 4.5 MIU twice daily have been proposed.[54,68] This regimen has to be adapted to renal function. When the estimated creatinine clearance is between 20–50 ml/ min, 4.5 MIU can be dosed once daily and when clearance is, 20 ml/min, 4.5 MIU can be dosed once every 48 hours.[54,68] CMS can be administered intravenously by a bolus infusion of the required dose diluted in 50–100 ml saline in 30 minutes. Administration of 2 MIU of CMS dissolved in 10 ml water for injection is also feasible and can be used when administered through an implanted infusion device.

It is important to realize that, for safety reasons, only freshly prepared CMS solution should be used, both for nebulization and for IV infusion. After dissolving CMS, hydrolyzation of this product starts and free colistin, associated with a higher level of toxicity, will be formed. In the past, repeated inhalation of a CMS solution that had been prepared days in advance has led to fatal pulmonary damage.[74] The SmPC of Coly-Mycin M parenteral indicates that infusion solutions must be used within 24 hours and the reconstituted solution can be used 7 days if stored at 2–88˚C (SmPC Coly-Mycin M). Based on the report of McCoy (2007) and a Food and Drug Administration alert on pulmonary toxicity of Colistimethate premixed solutions, most guidelines prescribe that even in home treatment, CMS has to be dissolved just before infusion or inhalation.[62]

In patients dialyzed by continuous venovenous hemofiltration, substantial clearance of both colistin and CMS occurs and adaptation of the dose seems not necessary,[50,75,76] but further research enrolling larger numbers of patients is needed. In patients on peritoneal dialysis, CMS and colistin are poorly cleared and, therefore, the dose should be adapted as for patients with a creatinine clearance ≤ 20 ml/min.[77]

Return of colistin in clinical practice

Since the use of colistin is increasing, questions about its safety have reemerged. It seems that the side effects observed in the past are possibly less severe than was thought. In addition, more side effects may be accepted for patients with serious infections due to multiresistant organisms without other antibiotic treatment options. In *table 3*, a summary of CMS characteristics and doses are given. In the following paragraphs, we summarize information that should be taken into account when reintroducing systemic CMS in clinical practice.

Synergy with other antibiotics

Since colistin has been used as rescue treatment for multiresistant gram-negative infections, its use is associated with poor outcome.[8,78] Therefore, given the setting of multiresistant bacterial infections, colistin will often be combined with other antibiotics. However, little is known about the clinical efficacy of combined antibiotic therapy with colistin. *In vitro* data suggest that there is synergistic activity of colistin, particularly when combined with carbapenems[79] or rifampin.[80] These data are promising and are arguments for conducting randomized clinical trials on this topic. So far, in vivo studies have not been able to show a clinical benefit of colistin combination therapy, but it has to be emphasized that such studies are very difficult to conduct.[81,82]

Antimicrobial strategies against colistin resistance

One of the hypotheses is that colistin resistance develops because of underdosing.[83] At higher concentrations of colistin, there is significantly less chance of selecting colistin-resistant mutants compared with exposure to lower concentrations of colistin.[84] Thus, the dosing schedule is an important determinant of the development of colistin resistance.[29]

Monotherapy with colistin invariably leads to selection of colistin-resistant variants. The chance of emergence of resistant subpopulations is reduced when colistin is administered in combination with other antimicrobial agents,[29] including carbapenems, tigecycline, rifampin, amikacin, fosfomycin, azithromycin, vancomycin, and teicoplanin.[29] Interestingly, agents that by themselves are only effective against grampositive bacteria can, in the presence of colistin, become active against gram-negative bacteria because colistin increases their permeation of the cell wall.

Summary and suggestions for a safe and prudent use of colistin

Colistin is currently considered as rescue treatment for critically ill patients with infections caused by multidrug-resistant bacteria. Nephrotoxicity can be expected in a sizable proportion of patients receiving this agent, but this side effect seems to be less severe than initially thought[38,85] and is generally reversible.[36] However, patients on colistin should be closely monitored.

We recommend adjusting the dose on renal function and measuring markers for (early) kidney damage[84] whenever colistin is administered. Regarding the prevention of the development of resistance, colistin should only be given in combination with other antimicrobial agents.

The registered dose of 2 MIU 3 times daily may not be optimal for intensive care patients with a severe infection with multiresistant microorganisms. For those patients, a loading dose of 9 MIU followed by 2 times daily 4.5 MIU could safely be given and a steady-state serum concentration of colistin was reached faster. However, these data were based on limited research.[37,86] To avoid underdosing, free colistin levels should be measured. A FAUC/MIC ratio of at least 25–40 seems optimal,[4] but there is still no consensus on this topic. Prolonged colistin trough levels. 3 mg/l should be avoided.

To redeploy the use of colistin safely and effectively, more clinical and basic research is required. There is need for additional clinical pharmacological studies in healthy volunteers that enable us to predict pharmacokinetic behavior in critically ill patients. With proper translation, safe IV doses for different groups of patients can be identified. Finally, research is needed into which antimicrobial agent(s) that can best be combined with colistin to reduce the risk of the emergence of colistin resistance.

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TABLE 1 Pharmaceutical Products Containing CMS.

Brand Name	Product	CMS, MIU	CMS, mg	Colistin base, mg	Manufacturer
ColiFin Pari	Powder for solution for inhalation	1 MIU*	80[71-73]	33.3	Pari Pharma GMBH, Germany
		2 MIU*	160	66.6	
Colistin	Powder for solution for injection or Infusion	1 MIU*	80	33.3	Forest Laboratories Ltd, UK
Colobreathe	Powder for inhalation (dry powder)	1.66 MIU*	125	55.5	Forest Laboratories Ltd, UK
Tadim	Powder for solution for injection,infusion, or inhalation	1 MIU*	80	33.3	Profile Pharma Ltd, UK
Colomycin	Powder for solution for injection, infusion, or inhalation	1 MIU*	80	33.3	Forest Laboratories Ltd, UK
		2 MIU*	160	66.6	
Coly-Mycin M parenteral	Powder for solution for injection or Infusion	5 MIU	400	150*	JHP Pharmaceuticals, USA

**Labeled amount. Apart from the listed products, in some countries other brands and generic products are available.*

TABLE 2 Suggested colistin dose and frequency adapted to renal clearance.[72,73]

Creatinine clearance (% of Normal)	Dose in MIU (SmPC Colomycin)	Dose in mg colistin base activity (SmPC Coly-Mycin M)	Frequency/ Day	Daily dose in MIU (SmPC Colomycin)	Daily dose in mg colistin base activity (SmPC Coly-Mycin M)
76%–100%	1–2	100–150	3	4–6	300–450
40%–75%	1–1.5	75–115	2	2–3	150–230
25%–40%	1	66–150	1 or 2	1–2	133–150
<25%	1–1.5	100–150	1/36 h	0.6–1	100

TABLE 3 Summary of Colistimethate Sodium dosing and product characteristics.[87]

For injection formulation	
Bottle with 1 million units	=80 mg colistimethate sodium
Bottle with 2 million units	=160 mg colistimethate sodium
For infusion	Dissolve required dose in 50 ml 0.9% NaCl and administer over 30 min
Contraindications	
Hypersensitivity for polymyxins, myasthenia gravis	
Dose (children)	
Normal	3 times daily 16,000–25,000 units/kg
Dose	
Normal	3 times daily 1–2 million units
Dose adjustment in cases of renal failure after normal loading dose Creatinine clearance (% of normal)	
<76	No adjustment
40–75	2 times daily 1–1.5 million units
25–40	1–2 times daily 1 million units
<25	1–1.5 million units every 36 h
Dose in critically ill (ICU) patients[48,49]	
Maximum dose	Loading dose 9 million units, followed by 2 times daily 4.5 million units
Dose adjustment in critically ill (ICU) patients with renal failure Creatinine clearance (ml/min)	
<50	No adjustment
20–50	Once daily 4.5 million units
<20	4.5 million units every 48 h
Dose with patient with renal replacement therapy	
CVVHD	Dose as with normal renal function[38,63,64]
Peritoneal dialysis	1–1.5 million units every 36 h[65]
Adverse reactions	
Nephrotoxicity	≈10%–50% dose dependent, reversible
Neurotoxicity	≈7% dose dependent, reversible paresthesias, dizziness, ataxia
Hypersensitivity	≈2%

CVVHD, continuous venovenous hemofiltration.

FIGURE 1 **Two-dimensional chemical structure of colistin A.**

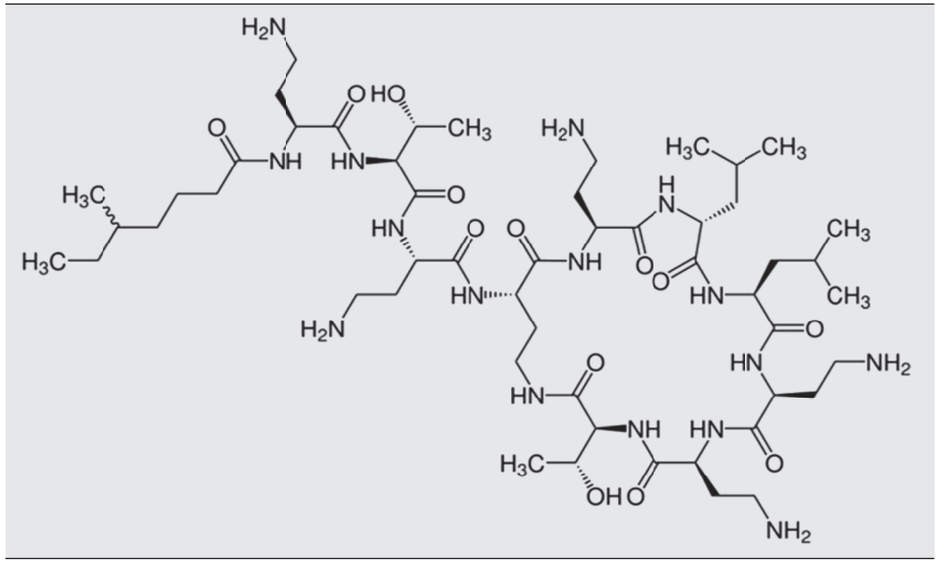
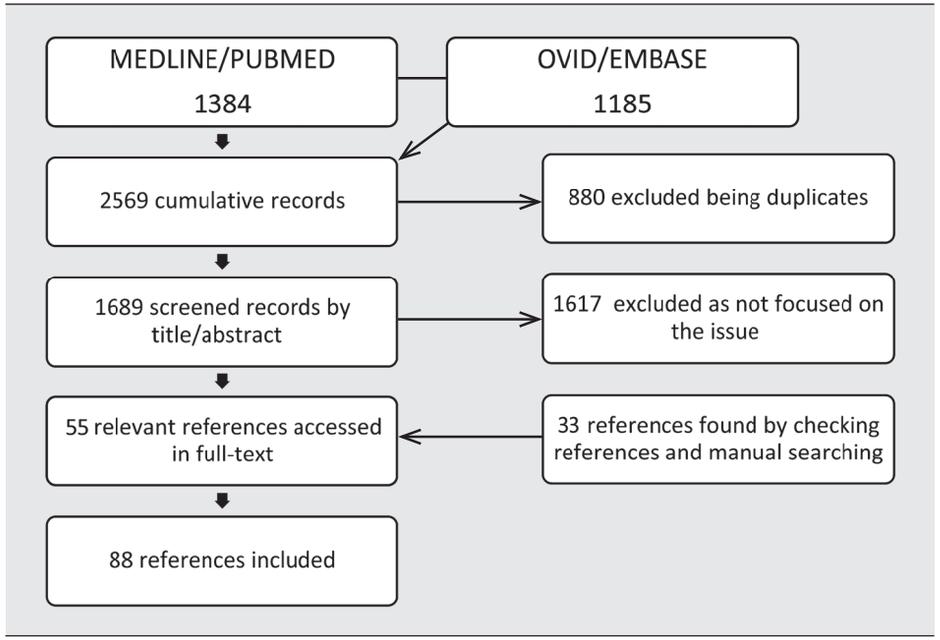


FIGURE 2 **Systematic search process and references included.**



CHAPTER 7

COLISTIN METHANESULFONATE INFUSION SOLUTIONS ARE STABLE OVER TIME AND SUITABLE FOR HOME ADMINISTRATION

Eur J Hosp Pharm 2018; 25(6): 337-9

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ABSTRACT

The stability of colistin methanesulfonate (CMS) was determined in quadruplicate in elastomeric home infusion pumps containing 1, 2 or 3 MU CMS and in infusion bags with 2 MU CMS all in 100 ml normal saline. Infusions were stored at room temperature (20°C–24°C) with or without exposure to natural light or refrigerated (4°C–8°C) and protected from light up to 2 weeks. In the initial solution of 2 MU CMS in 100 ml saline sampled immediately after reconstitution and dilution, 1.5% of CMS was hydrolysed to colistin. When stored at room temperature and exposed to natural light, colistin concentration in elastomeric infusion pumps increased to 2.6% in 8 days and to 2.1% when stored at 4°C. CMS stability increases at lower temperatures and higher concentrations. Based on the current data, chemical stability of CMS infusion solution is sufficient for a shelf life of 7 days refrigerated plus 1 day at room temperature.

INTRODUCTION

Colistin is an antibiotic used for the treatment of chronic infection with Gram-negative bacteria such as *Pseudomonas aeruginosa* which is the most common pathogen in cystic fibrosis (CF) lung disease. Colistin use by inhalation is widely practised for maintenance treatment. Due to increasing resistance of *P. aeruginosa* against other antibiotics, intravenous colistin can be incorporated in the treatment of exacerbations of CF lung disease.[1] Colistin is a multicomponent antibiotic, composed mainly of colistin A and colistin B.[2] For intravenous administration, colistin is marketed in the form of its inactive pro-drug colistin methanesulfonate (CMS), which hydrolyses *in vitro* and *in vivo* to active colistin.[3] Following intravenous administration of CMS, only a fraction is hydrolysed to colistin; preclinical and clinical studies have estimated that between 7% and 30% of CMS is converted to colistin.[4-6] The high variability regarding the extent of conversion might depend on differences in physiological processes and on factors related to storage conditions and administration of CMS infusions. In-depth knowledge of these factors is of importance as colistin has the potential to be used as prolonged maintenance (home) treatment, and colistin is known to be more toxic and causes more bronchial irritation compared with CMS.[3] A fatal complication with a CMS inhalation solution led to discussion about safety of in-advance prepared solutions of CMS.[7] Because of this, uncertainty has arisen about the stability and duration of storage of CMS solutions. Several studies have focused on the *in vitro* stability of colistin and CMS under different conditions, but these do not provide complete results.[8-11] Particularly, the stability of CMS in infusion bags was only studied for a period of 48 hours, which is a practical limitation for colistin if to be used in home treatment. In addition, CMS stability at room temperature and the influence of light have not been addressed. Therefore, extended *in vitro* stability testing of CMS is warranted. We tested stability during 14 days to cover a proposed shelf life of 8 days with a margin of more than 50%. A CMS concentration within the limits of 90%–110% at time of administration was used as the chemical stability specification. This paper reports stability data and the influence of temperature, concentration CMS and light on the *in vitro* stability of a standard CMS solution.

METHODS

In the current *in vitro* study, the stability of CMS was determined in infusion bags and elastomeric home infusion pumps at different concentrations and stored under different conditions, which were chosen to reflect clinically relevant conditions. Vials containing 1 MU CMS powder for infusion, corresponding to 80 mg CMS and 33.3 mg colistin, were used (Tadim, Profile Pharma, Chester, UK). These vials were reconstituted with 0.9% saline according to the summary of product characteristics. CMS was further diluted in 0.9% saline in infusion bags (100 ml, Baxter Viaflo) or elastomeric home infusion pumps (Intermate SV 200, Baxter) to achieve final concentrations of 800–2400 mg/l CMS. Infusions were stored at room temperature

(20°C–24°C) and exposed to natural light and protected from light, or refrigerated (4°C–8°C) and protected from light up to 2 weeks. Aliquots of the solution were sampled into 2 ml polypropylene tubes to determine in quadruplicate in the pH and the concentration of formed colistin after 0, 3 and 6 hours and after 1, 3, 8 and 14 days. Samples were stored at –80°C until analysis. Quantification of colistin A and colistin B was performed with an LC-MS/MS assay using polymyxin B1 and polymyxin B2 as internal standards (IS). Quantification was carried out after diluting all samples to 16 mg/l of CMS with 10% trichloroacetic acid at 4°C. The compounds were separated on a Zorbax EclipsePlus-C18 (2.1×50 mm, 1.8 µm) column, using a linear gradient with a binary mobile phase of 0.1% formic acid in highly purified water (A) and 0.1% formic acid in acetonitrile (B). A triple quadrupole mass spectrometer (Agilent Technologies 6460) was operating in the ESI positive mode, and the double charged molecular ion was used as the precursor ion. The transition ions were m/z 585.4/101.1 for colistin A, m/z 578.4/101.1 for colistin B, m/z 602.4/101.1 for polymyxin B1 (IS for colistin A) and m/z 595.4/101.1 for polymyxin B2 (IS for colistin B). Good linearity was achieved ($r^2 \geq 0.99$) for colistin and intraday variation was 3.6%. Colistin A plus colistin B were expressed as colistin, and the amount hydrolysed was expressed as percentage of CMS. Conversion into molar units was done by intermediate calculations using 1749.82, 1735.79, 1169.46 and 1155.43g/mol as the molecular masses of CMS A, CMS B, colistin A and colistin B (free bases), respectively.[12]

RESULTS

In the initial solution of 2 MU CMS in 100 ml saline sampled immediately after reconstitution and dilution, 1.5% of CMS was hydrolysed to colistin. When stored at room temperature and exposed to natural light, colistin concentration in elastomeric infusion pumps increased to 2.6% in 8 days and to 2.1% when stored at 4°C as depicted in *figure 1*. In infusion bags (2 MU CMS in 100 ml saline) at day 8, 1.7% of colistin was formed when stored at 4°C and 2.1% when stored at room temperature and exposed to natural light, 3.7% of CMS was hydrolysed in 8 days in a 1 MU CMS in 100 ml solution, in a 2 MU in 100 ml solution this was 2.6% and in a 3 MU in 100 ml solution 2.3% of CMS was hydrolysed. The ratio of colistin A to colistin B was determined to be 3.7:1 and remained stable over time (*figure 2*). When stored at room temperature and exposed to natural light, the pH in elastomeric infusion pumps (2 MU in 100 ml) increased from 7.77 to 8.35 at day 1 and decreased to 7.87 at day 14.

DISCUSSION

This *in vitro* study showed that CMS was converted to 1.5% free colistin immediately after reconstitution and dilution. Eight days of storage in elastomeric infusion bags containing 2 MU CMS in 100 ml infusion solution (0.9% saline) at room temperature lead to 2.6% hydrolisation of CMS to colistin. Similar CMS stability was observed for CMS solutions kept in natural light and protected from light. CMS was more stable at lower temperatures and at higher concentrations.[13]

In addition, there was slightly more colistin formation in elastomeric infusion pumps than in infusion bags as shown in *figure 3*. Minor changes in pH were observed, with pH values ranging between 7.77 and 8.35. We cannot exclude some colistin formation during the analytical process, but this appears to be limited to a maximum conversion to colistin of 1.5%. Therefore, the current data are representative for the CMS solution administered in the clinical setting.[14]

Wallace *et al*[9] found <4% of colistin after 48 hours at 25°C and 0.3% at 4°C in a 4000 mg/l solution of CMS in glucose or normal saline. These results show a higher level of colistin formation at room temperature and a larger difference in colistin formation between room temperature and 4°C than our results. In comparison with our data, we must keep in mind the differences in concentration (800–2400 mg/l in our study) and in analytical technique. The HPLC assay applied by Wallace was able to distinguish colistin from (partially) sulfated colistin, but in-between formed derivates could not be excluded. Our LCMS assay only included colistin A and colistin B in unsulfonated form; other derivates were excluded due to separation on differences in molecular mass. Furthermore, the source of CMS differed (Coly-Mycin M vs Tadim).

Abdulla *et al*[13] found no CMS degradation (<0.5%) after 3 days at 4°C and <5% after 7 days and concluded a CMS solution of 800 mg/l can be stored up to 3 days at 4°C.

Our data and the data of Wallace and Abdulla cannot explain colistin formation as possible cause of the unfortunate death of a patient after CMS inhalation, which lead to an FDA warning and a restraint against in advance preparation of CMS solutions. In current practice, however, there is a need for safe, in-advance preparation of an intravenous medicine for home therapy, which can only be fulfilled when sufficient stability data are available.

While the SPC of Tadim states a shelf life of 24 hours after reconstitution and dilution, and several dosing guidelines also indicate that CMS infusion solutions should be used within 24 hours, based on the current data, chemical stability of CMS infusion solution was sufficient for a shelf life of 7 days refrigerated plus 1 day at room temperature.[4-6,15]

These data support the administration of colistin using elastomeric home infusion pumps. The difference in colistin concentration immediately after preparation (1.5%) and after 8 days storage at room temperature (2.6%) was small, and the remaining CMS concentration was well within the specification of 90%–110%.[16] The formation of colistin was limited when compared with the reported *in vivo* conversion of 30%. Potential tolerability issues cannot be entirely excluded based on this *in vitro* study and should be monitored by pharmacovigilance in clinical practice.[6]

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FIGURE 1 Time course of colistin formation in elastomeric infusion pumps containing 2 MU colistin methanesulfonate in 100 ml 0.9% saline when stored under different conditions.

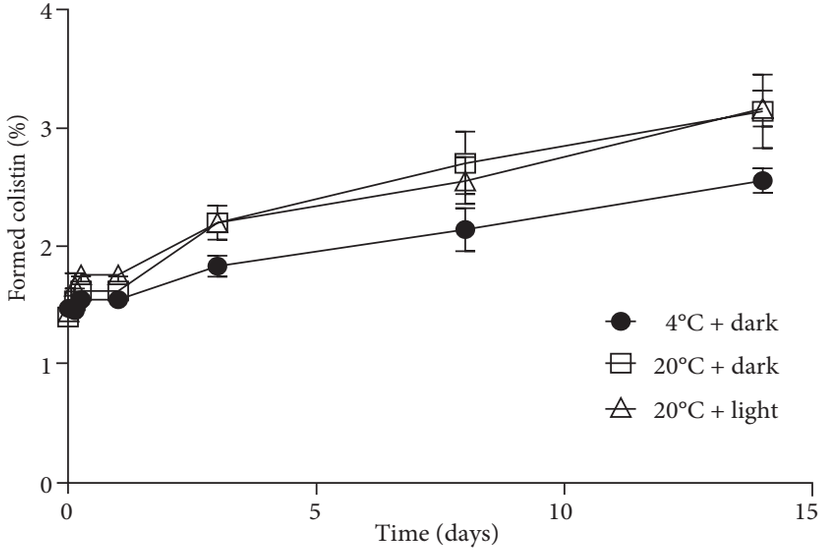


FIGURE 2 Time course of ratio colistin A to colistin B in elastomeric infusion pumps containing colistin methanesulfonate in 100 ml 0.9% saline when stored under different conditions.

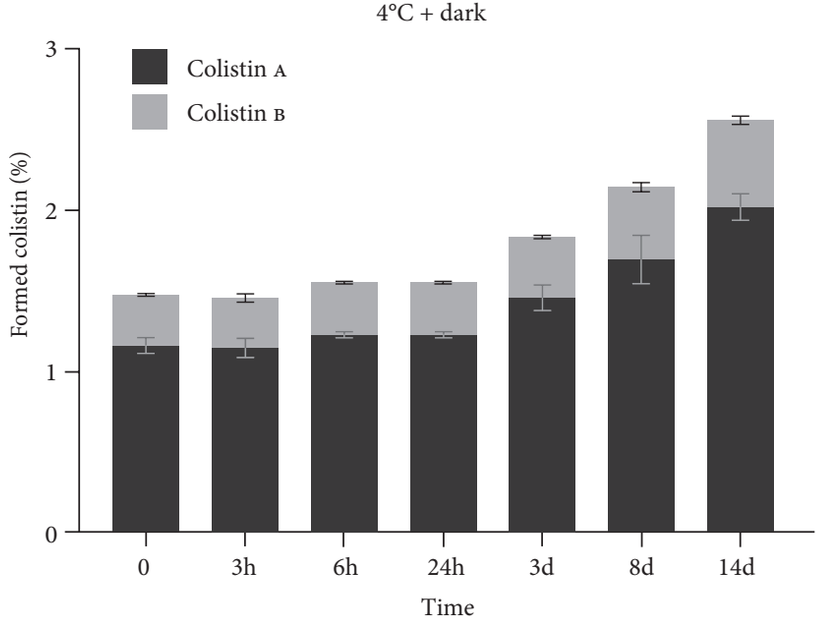
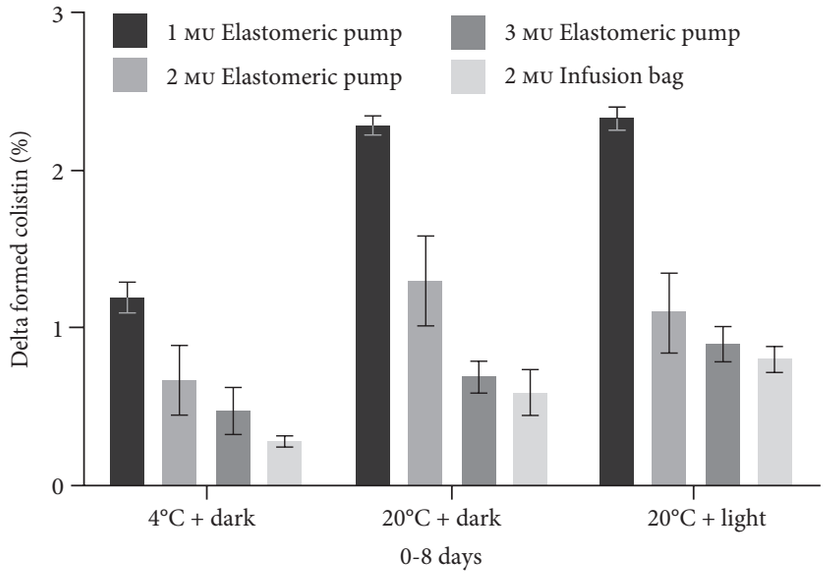


FIGURE 3 Colistin concentration 8 days after preparation (baseline delta) in elastomeric infusion pumps containing 1, 2 and 3 MU CMS in 100 ml 0.9% saline and in infusion bags containing 2 MU CMS in 100 ml 0.9% when stored under different conditions. CMS, colistin methanesulfonate.



CHAPTER 8

FOSFOMYCIN: PHARMACOLOGICAL, CLINICAL AND FUTURE PERSPECTIVES

Antibiotics (Basel) 2017; 6(4)

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ABSTRACT

Fosfomycin is a bactericidal, low-molecular weight, broad-spectrum antibiotic, with putative activity against several bacteria, including multidrug-resistant Gram-negative bacteria, by irreversibly inhibiting an early stage in cell wall synthesis. Evidence suggests that fosfomycin has a synergistic effect when used in combination with other antimicrobial agents that act via a different mechanism of action, thereby allowing for reduced dosages and lower toxicity. Fosfomycin does not bind to plasma proteins and is cleared via the kidneys. Due to its extensive tissue penetration, fosfomycin may be indicated for infections of the CNS, soft tissues, bone, lungs, and abscesses. The oral bioavailability of fosfomycin tromethamine is <50%; therefore, oral administration of fosfomycin tromethamine is approved only as a 3-gram one-time dose for treating urinary tract infections. However, based on published PK parameters, PK/PD simulations have been performed for several multiple-dose regimens, which might lead to the future use of fosfomycin for treating complicated infections with multidrug-resistant bacteria. Because essential pharmacological information and knowledge regarding mechanisms of resistance are currently limited and/or controversial, further studies are urgently needed, and fosfomycin monotherapy should be avoided.

INTRODUCTION

The discovery of antibiotics in the 1920s was one of the greatest breakthroughs in the history of healthcare, leading to a marked decrease in both morbidity and mortality associated with bacterial infections.[1] However, the intensive and extensive use and misuse of antibiotics over the past 50 years has contributed to the emergence and spread of antibiotic-resistant bacterial strains.[2-4] This increase and global spread of multidrug-resistant (MDR) bacteria is particularly alarming,[3,5] and the World Health Organization has identified antibacterial drug resistance as a major threat to global public health.

The decrease in the number of effective antibiotics – together with a relative paucity of new antimicrobial drugs – is particularly relevant for treating infections with Gram-negative MDR bacteria.[6-8] To overcome this problem, the reassessment and reintroduction of 'old' antibiotics has emerged as a viable strategy.[9,10] However, these antibiotics were never subjected to the rigorous drug development program that is currently mandatory for receiving marketing authorization. Thus, the pharmacological information needed in order to develop optimal dosing regimens with maximal activity and minimal toxicity is limited.[9,11] One such 'old' antibiotic is fosfomycin, a broad-spectrum antibiotic that was originally developed more than 45 years ago. Because it has both *in vitro* and *in vivo* activity against a wide range of MDR bacteria, as well as XDR (extensively drug-resistant) and PDR (pan-drug-resistant) bacteria, fosfomycin is potentially a good candidate for treating infections with these bacteria.[12-18]

In this review, we discuss the potential for using fosfomycin to treat MDR bacterial infections. Specifically, we review the currently available pharmacological data, with a focus on the chemistry, pharmacokinetics, pharmacodynamics, and clinical use of fosfomycin.

METHODS

Systematic search strategy

The PUBMED/MEDLINE and OVID/EMBASE databases were searched systematically in February 2016 to identify all published relevant articles regarding fosfomycin. To be as comprehensive as possible, the search terms included synonyms of fosfomycin in the article titles.

The search strategies were designed and performed by a specialist librarian and were restricted to journals published in English or Dutch. No other publication or date restrictions were applied. A comprehensive database of the retrieved articles was created, and duplicate publications were removed. The abstract of each identified publication was then independently reviewed by the first author (A.C. Dijkmans) and last author (I.M.C. Kamerling). We then obtained and reviewed the full-text version of all articles that focused on multidrug-resistant Gram-negative

bacteria (e.g., Enterobacteriaceae, *A. baumannii*, and *P. aeruginosa*), pharmacokinetics, pharmacodynamics, critically ill patients, treatment outcome, and/or mode of action. To search for any additional relevant articles, we screened the reference lists of the full-text articles, as well as relevant guidelines and references from the cited product information.

A final check was performed prior to submission of the manuscript in order to update the systematic search and include any new publications.

PUBMED/MEDLINE

PUBMED/MEDLINE was searched using the following terms: ('Fosfomycin'[Majr] OR phosphomycin[ti] OR fosfomycin[ti] OR phosphonomycin[ti] OR fosfonomycin[ti] OR monuril[ti] OR tromethamine[ti] OR trometamine[ti] OR trometamol[ti] OR tromethamol[ti]) AND (eng[la] OR dut[la]).

OVID/EMBASE

OVID/EMBASE was searched using the following terms: (exp *fosfomycin/ OR phosphomycin.ti. OR fosfomycin.ti. OR phosphonomycin.ti. OR fosfonomycin.ti. OR monuril.ti. OR tromethamine.ti. OR trometamine.ti. OR trometamol.ti. OR tromethamol.ti.) AND (english.lg. OR dutch.lg.).

RESULTS

In total, our combined search of the databases PUBMED/MEDLINE and OVID/EMBASE retrieved 3422 records; after 2135 duplicates were removed, 1287 unique publications were screened (*figure 1*). Of the remaining 1287 records that were screened by title and abstract, 975 were excluded as they were judged not relevant to the topic. The remaining 312 records were examined as full-text articles, and an additional 251 were excluded, leaving 61 articles. An additional 31 articles were identified by manually checking the included publications and product information. Thus, a total of 92 articles were included in our analysis.

Pharmacology of fosfomycin for treating MDR bacteria

CHEMISTRY

Fosfomycin is a bactericidal broad-spectrum antibiotic first isolated in 1969 from cultures of *Streptomyces* spp.[19] Fosfomycin, which is currently produced using a synthetic process, is a low-molecular weight (138g/mol), highly polar phosphonic acid derivative (cis–1,2-epoxypropyl phosphonic acid) that represents its own class of antibiotics.[20] Fosfomycin was initially marketed as both a calcium salt formulation (fosfomycin calcium) for oral administration and a more hydrophilic disodium salt (fosfomycin disodium) for parenteral administration. Later, because of its improved bioavailability, fosfomycin tromethamine became the standard formulation for oral administration.[20,21] The chemical structures of the various formulations of fosfomycin are shown in *figure 2*.

PHARMACOKINETICS OF FOSFOMYCIN

ABSORPTION Orally administered fosfomycin is absorbed partially in the small intestine via two proposed mechanisms: (*i*) a saturable carrier-mediated system associated with a phosphate transport system, and (*ii*) a non-saturable process with first-order kinetics.[22] Studies with fosfomycin calcium have shown that before reaching the small intestine, fosfomycin undergoes acid-catalyzed hydrolysis in the stomach, where intragastric acidity and gastric emptying rate can affect the extent of fosfomycin's hydrolytic degradation and – consequently – its bioavailability.[23] Variations between individuals with respect to intragastric acidity and gastric emptying rate may also explain the high variability in serum levels achieved after oral administration of fosfomycin.[23,24]

Tromethamine is a pH-elevating (i.e., alkaline) organic compound believed to slow acid-catalyzed hydrolysis. As mentioned above, fosfomycin tromethamine is now the preferred oral formulation due to its improved properties compared to fosfomycin calcium, including higher bioavailability (F) which ranges from 33% to 44%[21,25,26] (compared to 12–37% for the calcium salt[21,27,28]). When bioavailability was calculated from urinary excretion data following oral and IV administration of fosfomycin tromethamine, values as high as 58% have been calculated.[25] Although the bioavailability of both salts is reduced when taken orally following food,[24,29] when taken under fasting conditions, serum concentrations of the tromethamine salt are approximately 2–4-fold higher than the calcium formulation.[21,30] However, because no cross-over study has been performed, a systematic study of bioavailability is recommended.

Despite the improved bioavailability achieved with orally administered fosfomycin tromethamine, maximum concentrations (C_{max}) of fosfomycin are still well below the C_{max} values achieved following IV administration.[21,31] For example, 2–2.5 h after a single fasting oral dose of fosfomycin tromethamine at 3g (approximately 50 mg/kg body weight), C_{max} is 21.8–32.1 mg/l, with a total area under the serum concentration-time curve (AUC) of 145–193 mg·h/l[21,25,26]. In contrast, after IV administration of the same dose of fosfomycin disodium, C_{max} was 276–370 mg/l, with an AUC of 405–448 mg·h/l.[21,25,26]

DISTRIBUTION AND TISSUE PENETRATION Fosfomycin binds to plasma proteins at only negligible levels[31] and is distributed widely into a variety of tissues; in addition to serum, biologically relevant concentrations of fosfomycin have been measured in the kidneys, bladder, prostate, lungs, bone, and cerebrospinal fluid, as well as in inflamed tissues and abscess fluid.[32-40]

The apparent volume of distribution (V_d /F) following oral administration of fosfomycin tromethamine is approximately 100–170 l for a 70-kg individual[29,30]. In contrast, because of its higher bioavailability, IV-administered fosfomycin disodium

has a reported V_d of 9–30 l at steady state, and values of 3–12 l have been reported for both the central (Vc) and peripheral (Vp) compartments.[25,27,28,32,36,41,42]

metabolism and excretion Approximately 90% of an IV dose of 3g fosfomycin disodium is recovered unchanged in the urine 36–48 h after dosing.[21,25,26] In contrast, only 40–50% of a 3g oral dose of fosfomycin disodium is recovered; this difference compared to an IV dose is due primarily to incomplete absorption of oral fosfomycin disodium.[21,25,26,29] Following an oral dose of fosfomycin tromethamine, approximately 10% of the original dose is recovered unchanged in the feces.[29]

Segre et al. reported that the fraction of the original dose excreted in the urine decreases as the oral dose increases,[25] suggesting decreased absorption at higher doses. However, their study used a relatively limited range of doses (2, 3, and 4g) in a small number of individuals (n=12). On the other hand, urinary concentrations >128 mg/l are maintained 24–48 h after an oral dose of 2, 3, or 4g and 12–24 h after an IV dose of 3g.[26]

In general, the total clearance rate ranges from 5 to 10 l/h, whereas renal clearance ranges from 6 to 8 l/h.[25,27,31,32,35,36,41,43] Fosfomycin has also been detected in the bile, with biliary concentrations of approximately 20% of the serum concentration.[31,44,45] Given this finding, Segre et al. suggested that fosfomycin undergoes biliary recirculation, based on the presence of secondary peaks in serum drug concentration following oral administration and based on the concentrations of fosfomycin measured in the bile.[25,31,38,44,45]

In healthy individuals, IV fosfomycin is distributed in and eliminated from the serum in a bi-exponential manner; the serum disposition half-life ($t_{½\alpha}$) of fosfomycin is 0.18–0.38 h,[28,43] and the terminal (or elimination) half-life ($t_{½\beta}$) of fosfomycin is 1.9–3.9 h.[21,25-28,32,35,36,43] In contrast, the $t_{½\beta}$ is longer following an oral dose of fosfomycin tromethamine (3.6–8.28 h), [21,26,30] which can be explained by a longer absorption phase. In patients who have renal failure and/or are receiving hemodialysis, the $t_{½\beta}$ of fosfomycin can be as long as 50 h, depending on the level of renal function; therefore, the dosing schedule should be adjusted accordingly, particularly if creatinine clearance (CLcr) drops below 40 ml/min.[43,44,46] An overview of the farmacokinetics is given in *table 1*.

PHARMACODYNAMICS OF FOSFOMYCIN

mechanism ofaction In general, antibiotics exert their bactericidal or bacteriostatic activity by targeting the microorganism's essential physiological and/or metabolic functions, including protein, DNA, RNA, or cell wall synthesis and cell membrane organization. Fosfomycin has a unique mechanism of action in which it irreversibly inhibits an early stage of bacterial cell wall biosynthesis.

In order to exert its bactericidal activity, fosfomycin must reach the bacterial cytoplasm. To enter the cell, fosfomycin uses the active transport proteins GlpT and UhpT by mimicking both glucose-6-P (G6P) and glycerol-3-P (G3P). Thus, fosfomycin can be imported into the bacterial cell via the hexose monophosphate transport system (which is induced by G6P) and via the L-a-glycerophosphate transport system (which is induced by G3P).[20,47] Once in the cytoplasm, fosfomycin acts as an analog of phosphoenolpyruvate (PEP) and binds MurA (UDP-GlcNAc enopyruvyl transferase), thereby inactivating the enzyme enolpyruvyl transferase, an essential enzyme in peptidoglycan biosynthesis.[48] Thus, fosfomycin prevents the formation of UDP-GlcNac-3-O-enolpyruvate from UDP-GlcNAc and PEP during the first step in peptidoglycan biosynthesis, thereby leading to bacterial cell lysis and death (*figure 3*).[47] In addition, fosfomycin also decreases penicillin-binding proteins.[49]

antibacterial activity Because both Gram-negative and Gram-positive bacteria require *N*-acetylmuramic acid for cell wall synthesis, fosfomycin is as a broad-spectrum antibiotic with activity against a wide range of bacteria, including *Escherichia coli*, *Proteus mirabilis*, *Klebsiella pneumoniae*, *Enterobacter* spp., *Citrobacter* spp., and *Salmonella typhi.*[12,20,50-52] However, due to a paucity of preclinical and clinical data, no universally accepted minimum inhibitory concentration (MIC) values have been defined for the susceptibility and resistance to fosfomycin; overall, the MIC for susceptibility ranges from ≤32 to ≤64 mg/l, and the MIC for resistance ranges from >32 to >256 mg/l, according to the Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST).[14,53]

Several studies have investigated the microbiological activity and efficacy of fosfomycin against several MDR, XDR, and PDR strains of Gram-negative bacteria. In this respect, fosfomycin has been reported to have both *in vitro* and *in vivo* activity against several MDR and XDR species of Enterobacteriaceae, including species that express extended-spectrum β-lactamases (ESBL) and metallo-β-lactamases (MBL).[14-18] Due to the broad range of MIC values and differences in methods used to test susceptibility (e.g., agar dilution, microdilution, E-test), it is difficult to compare the results of different studies. However, given that some studies found that more than 90% of MDR and XDR isolates are susceptible to fosfomycin, fosfomycin is a promising candidate for treating infections with these pathogens,[15,16] provided that *in vivo* results support the *in vitro* data.

MDR P. aeruginosa and *A. baumannii* are Gram-negative pathogens primarily responsible for nosocomial (i.e., hospital-acquired) infections, particularly in intensive care units.[54] A systematic review of microbiological, animal, and clinical studies using non-fermenting Gram-negative bacilli concluded that using fosfomycin in combined therapy may provide a safe and effective therapeutic option for treating infections due to *MDR P. aeruginosa.*[13] The clinical efficacy of fosfomycin against MDR-bacteria, including *P. aeruginosa*, has been suggested in patients with severe infections and critical conditions,[18] and in cystic fibrosis patients with infective pulmonary exacerbations.[55,56] However, when used as monotherapy, *P. aeruginosa* should generally be regarded resistant to fosfomycin[57] and its use in *P. aeruginosa* infections should ideally be reversed for additional evaluation in clinical studies

because the increased bacterial killing of combination therapy does not prevent the emergence of fosfomycin resistance.[58] In contrast, nearly all isolates of *A. baumannii* are resistant to fosfomycin, with a MIC_{90} value higher than 512 mg/l and there are no data on its use in combination therapy.[14]

Fosfomycin resistance

Three separate mechanisms of fosfomycin resistance have been reported.[59] The first mechanism is based on decreased uptake by the bacterium due to mutations in the genes that encode the glycerol-3-phosphate transporter or the glucose-6-phosphate transporter.[47,60,61] The second mechanism is based on point mutations in the binding site of the targeted enzyme (MurA),[62] and several isolates of *E. coli* have clinical resistance levels (32 mg/l) due to increased expression of the *murA* gene.[63] The third mechanism of resistance is based on the inactivation of fosfomycin either by enzymatic cleavage of the epoxide ring or by phosphorylation of the phosphonate group. In the presence of the metalloenzymes FosA, FosB, and FosX, the epoxide structure is cleaved, with glutathione (FosA), bacillithiol and other thiols (FosB), or water (FosX) serving as the nucleophile.[64] With respect to the phosphorylation of the phosphonate group, FomA and FomB are kinases that catalyze the phosphorylation of fosfomycin to the diphosphate and triphosphate states, respectively.[65,66] Fosfomycin dosing regimens that include a total daily dose of up to 24g per day resulted in the emergence of a resistant subpopulation within 30–40 h of drug exposure, suggesting that resistance can occur rapidly.

In vitro synergy between fosfomycin and other antibiotics

The use of combined antimicrobial therapy is recommended in specific patient populations and indications, including critically ill patients who are at high risk for developing an MDR bacterial infection and patients with a *P. aeruginosa* infection.[11,67,68] In this regard, fosfomycin has an *in vitro* synergistic effect of up to 100% when combined with other antimicrobial agents.[69]

The synergistic effect between fosfomycin and β-lactam antibiotics is proposed to arise from the inhibition of cell wall synthesis at separate steps; fosfomycin inhibits the first enzymatic step, whereas β-lactam antibiotics inhibit the final stage in the cell wall synthesis process.[70] In addition, fosfomycin may modify the activity of penicillin-binding proteins, which may account for the synergistic effect between fosfomycin and β-lactam antibiotics.[49,71,72] Another study found that the synergistic effect between fosfomycin and ciprofloxacin is due to ciprofloxacin-mediated damage to the outer membrane, which increases the penetration and activity of fosfomycin.[73] With respect to *P. aeruginosa*, several *in vitro* studies found synergy between fosfomycin and a variety of other antibiotics, including aztreonam, cefepime, meropenem, imipenem, ceftazidime, gentamycin, amikacin, ciprofloxacin, and others.[70,74,75] In addition, a few studies measured the synergistic effect of combining fosfomycin with amikacin or sulbactam against *A. baumannii* strains, providing evidence that these drugs might provide an effective combination therapy for infections with this pathogen.[76,77] Fosfomycin also has synergistic effects when combined with other antibiotics for treating methicillin-resistant *S. aureus*, *Streptococcus*, *Enterococcus*, and Enterobacteriaceae species.[69,70] In addition to increasing antibacterial efficacy, fosfomycin can also reduce toxicity associated with other antibiotics such as aminoglycosides, glycopeptides, and polymyxin B, as lower doses of these drugs can be prescribed.[78-80]

Properties of fosfomycin

The reintroduction of 'old' antimicrobial agents to treat MDR bacteria requires optimization of the dosing regimen. This optimization includes obtaining a thorough understanding of the drug's pharmacokinetic (PK) and pharmacodynamic (PD) properties, thereby providing maximal antibacterial activity while minimizing toxicity and the development of resistance.[11]However, some 'old' antibiotics, including fosfomycin, are currently used clinically despite uncertainty regarding the required and/or optimal exposure.[11] Therefore, it is essential to determine a rational dosing regimen based on the drug's PK/PD properties when introduced as a therapy against MDR bacteria.

PK/PD properties

Because the exposure-response relationship can differ between antibiotics, it is important to define the correct PK/PD index for each antibiotic in order to establish the PK/PD target value that will maximize clinical efficacy.[11,81,82] With respect to antimicrobials, three PK/PD indices are commonly used: $T_{>MIC}$, which is the duration of time in which the drug concentration remains above the MIC during a dose interval; C_{max} /MIC, which is the drug's C_{max} divided by the MIC; and AUC/MIC, which is the AUC measured over a 24-h period divided by the MIC.

Relatively few *in vitro* studies have been performed to characterize fosfomycin's PK/PD properties. Some such studies suggest that fosfomycin has a time-dependent bactericidal activity, specifically against the Gram-positive *S. aureus* and *S. pyogenes* strains[32,35]; therefore, based on these results $T_{>MIC}$ should be optimized. However, *in vitro* studies by Mazzei et al.[83] and VanScoy et al.[84] suggest that fosfomycin shows a tendency towards a concentration-dependent bactericidal activity against *E.coli* and *P. mirabilis* strain, achieving complete sterilization at concentrations ≥4×MIC and ≥8×MIC, respectively. Moreover, an *in vitro* concentration-dependent post-antibiotic effect (PAE) was observed for both *E.coli* and *P. mirabilis* 3.2–3.4 h at 0.25× MIC and 3.5–4.7 h at 8×MIC.[83] However, with respect to these studies, it is not clear whether the bactericidal activity is concentration-dependent and/or time-dependent.[85] These studies however, do not provide conclusive data on the concentrationor time depending nature of bactericidal activity. Therefore, the target PK/PD to achieve

during therapy remains unknown, which is a major hurdle that must be overcome in order to optimize therapy.

Current clinical indications for fosfomycin and potential future applications

INTRAVENOUS ADMINISTRATION

Fosfomycin disodium is currently available in only a few European countries – namely, Spain, France, Germany, the United Kingdom, the Netherlands, Austria, and Greece – where it is approved for the treatment of soft-tissue infection and sepsis. A fosfomycin disodium adult dose of 12–24g daily is commonly administered in 2–4 separate infusions.[51]

Due to is extensive tissue penetration, fosfomycin has emerged as a potential therapy for treating infections in the central nervous system (CNS),[32] soft tissues,[33,39,40] bone,[39] lungs,[34] and abscesses.[36] Fosfomycin has high penetration into the interstitial fluid of soft tissues,[40] reaching 50–70% of the levels measured in plasma, reaching sufficiently high levels to eliminate relevant pathogens.[33,40] Moreover, Schintler et al. reported that fosfomycin might also be effective in treating 'deep' infections involving the osseous matrix.[39]

With respect to CNS infections, Pfausler et al. reported that three daily IV doses of 8g provided a steady-state concentration of 16 mg/l in the cerebrospinal fluid (CSF) for more than 90% of the interval between doses.[32] Moreover, the concentration of fosfomycin in the CSF can increase by nearly threefold with meningeal inflammation.[86] With respect to suppurative lesions, Sauermann et al. reported that repeated doses of IV fosfomycin can yield a concentration of 32 mg/l fosfomycin in the abscess, albeit with high inter-individual variability in the PK of fosfomycin in the abscess fluid.[36,41]

MDR bacteria such as ESBL-producing bacteria and carbapenem-resistant bacteria are still susceptible to fosfomycin,[17,18] and fosfomycin is used in combination therapy for treating these infections.

The repurposing of fosfomycin based on its activity against MDR Enterobacteriaceae is an important strategy for addressing the ever-present threat of antimicrobial resistance. The AUC/MIC seems to be the dynamically linked index for determining resistance suppression. In this respect, it is essential to develop optimal dosing strategies for each MDR Enterobacteriaceae species based on PK/PD data; moreover, additional dosing regimens may need to be developed for targeting different tissue sites of infection in order to prevent the development of resistance. Another promising approach is the use of combination therapy; for example, combining fosfomycin and meropenem yielded a significant synergistic effect, but also yielded a significantly additive effect in the fosfomycin-resistant subpopulation.[87]

Currently, the FOREST study group is comparing the efficacy of combining fosfomycin with meropenem in treating urinary tract infections (UTIs) with ESBL-producing *E. coli*.[88]

ORAL ADMINISTRATION

Fosfomycin tromethamine is currently approved for use in several European countries and is only approved as a single 3-g dose for treating uncomplicated UTIs in women, specifically UTIs due to *E. coli* infection.[29] Fosfomycin tromethamine has also been investigated as a potential therapy for surgical prophylaxis in order to prevent prostate infection and even as a treatment for prostatitis due to MDR Gram-negative bacteria.[37] The use of a multiple-dose regimen with fosfomycin tromethamine has emerged as a potential strategy for treating of complicated and/or recurrent UTI, as well as infections due to MDR bacteria.[89–91] In this respect, simulations of the urinary concentrations of fosfomycin have been developed in order to determine the optimum dosing regimen that can provide a urinary concentration above the MIC (i.e., >16 mg/l) for seven days;[89] these simulations suggest that a single dose of 3g administered every 72 h is sufficient to achieve the appropriate concentration. In addition, an uncontrolled, open-label, multicenter study conducted in China found that a regimen of single 3-g doses of fosfomycin tromethamine administered at two-day intervals might provide a safe, effective, and well-tolerated option for treating recurrent and/or complicated lower UTIs.[90] Thus, although the currently approved 3-g single dose of fosfomycin tromethamine is sufficient to reach efficacious concentrations in the urine, it might not be sufficient to achieve serum and/or tissue concentrations that are relevant for a clinical cure. A multiple-dose regimen of fosfomycin tromethamine might therefore be warranted for the oral treatment of more severe infections.

Ortiz et al. conducted simulations of several multiple-dose regimens using a wide range of daily doses of fosfomycin tromethamine and fosfomycin disodium.[92] The authors calculated PK/PD indices, including C_{max}/MIC, AUC/MIC, and $\%T_{>MIC}$, for each dosing regimen using a MIC of 8 mg/l. They concluded that a total daily dose of 6–12g for microorganisms with a MIC of 8 mg/l well exceeds the currently approved single dose of 3g. However, the safety and tolerability of fosfomycin tromethamine at such high doses has not been investigated. Nevertheless, further studies are urgently needed in order to assess the PK, safety, tolerability, and efficacy of fosfomycin in both multiple-dose regimens and synergistic combinations.

CONCLUSIONS

The World Health Organization currently recognizes that antibacterial drug resistance is one of the major threats facing global public health, particularly given the reduction in the number of effective antibiotics. In this respect, reassessing and reevaluating 'old' antibiotics such as fosfomycin has been proposed as a possible strategy in treating drug-resistant bacterial infections. Fosfomycin is a broad-spectrum antibiotic with both *in vivo* and *in vitro* activity against a wide range of bacteria, including MDR, XDR, and PDR bacteria. Thanks to its high tissue penetration, fosfomycin may be used in a broad range of tissues and targets, including the CNS, soft

tissue, bone, lungs, and abscess fluid. Oral fosfomycin in a multiple-dose regimen has emerged as a potential strategy for treating complicated UTIs and prostatitis; however, given the relative lack of essential information regarding the pharmacological properties and mechanisms of resistance, additional studies are urgently needed. In the meantime, using fosfomycin as a monotherapy should be avoided due to the rapid development of resistance *in vitro*.

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FIGURE 1 **Flow-chart depicting the systematic search process and articles included.**

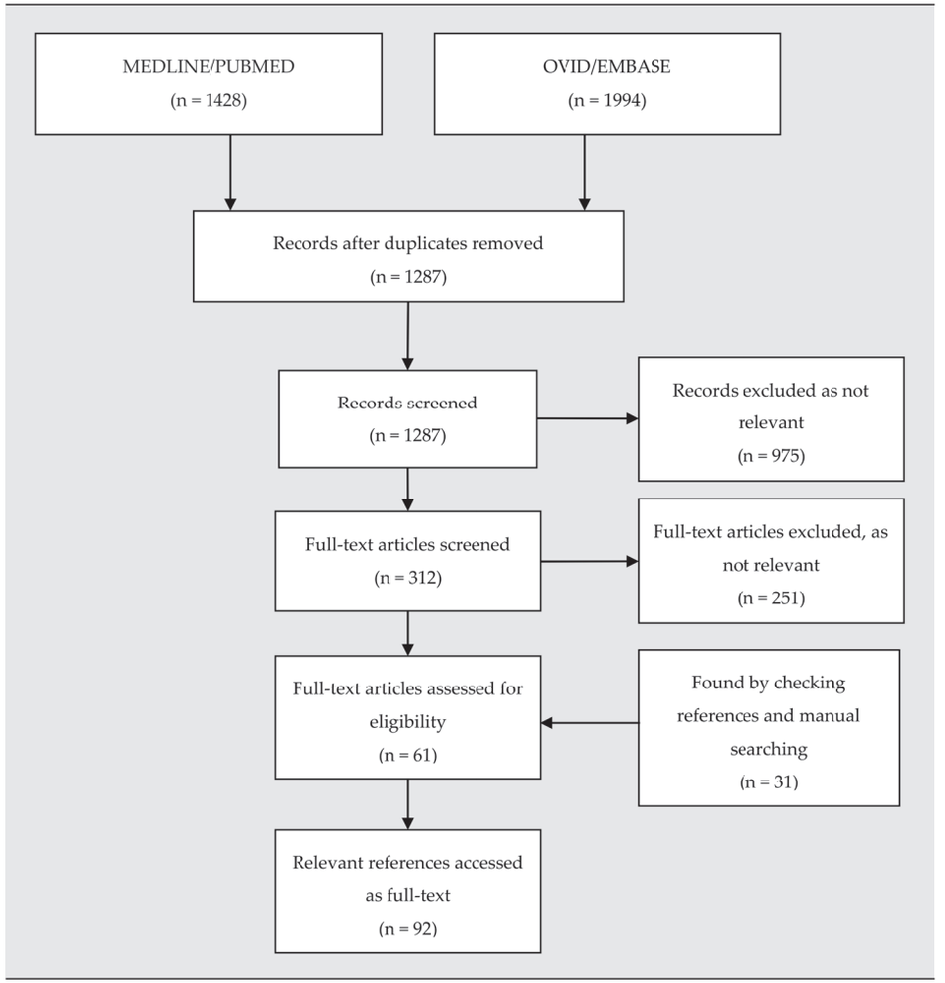

FIGURE 2 **Chemical structures of fosfomycin calcium (A), fosfomycin disodium (B) and fosfomycin tromethamine (C).**

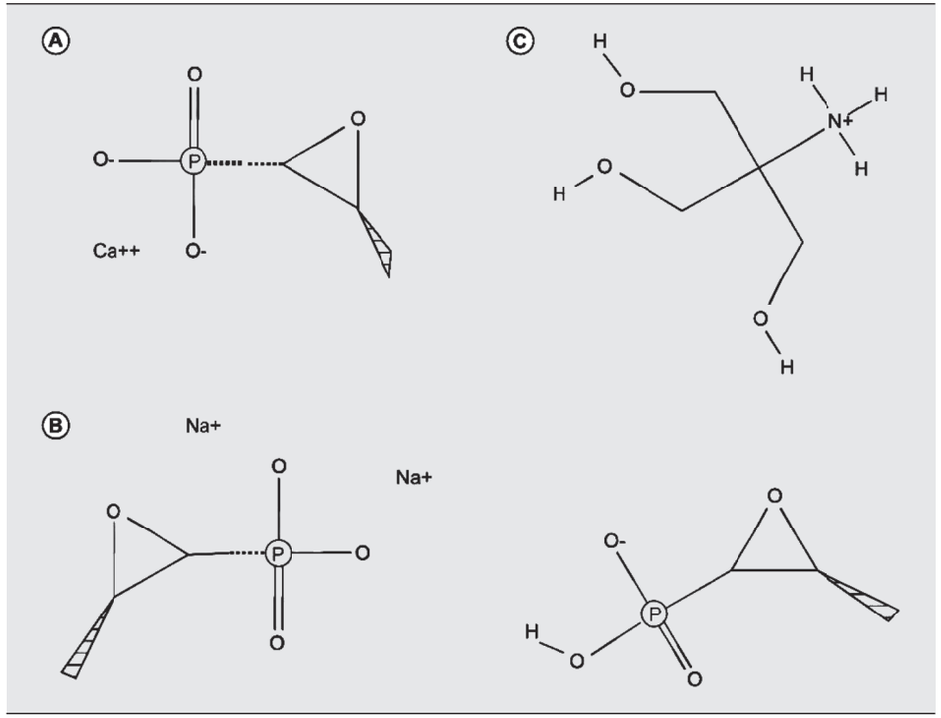

FIGURE 3 **Mechanism of action of fosfomycin ('F').**

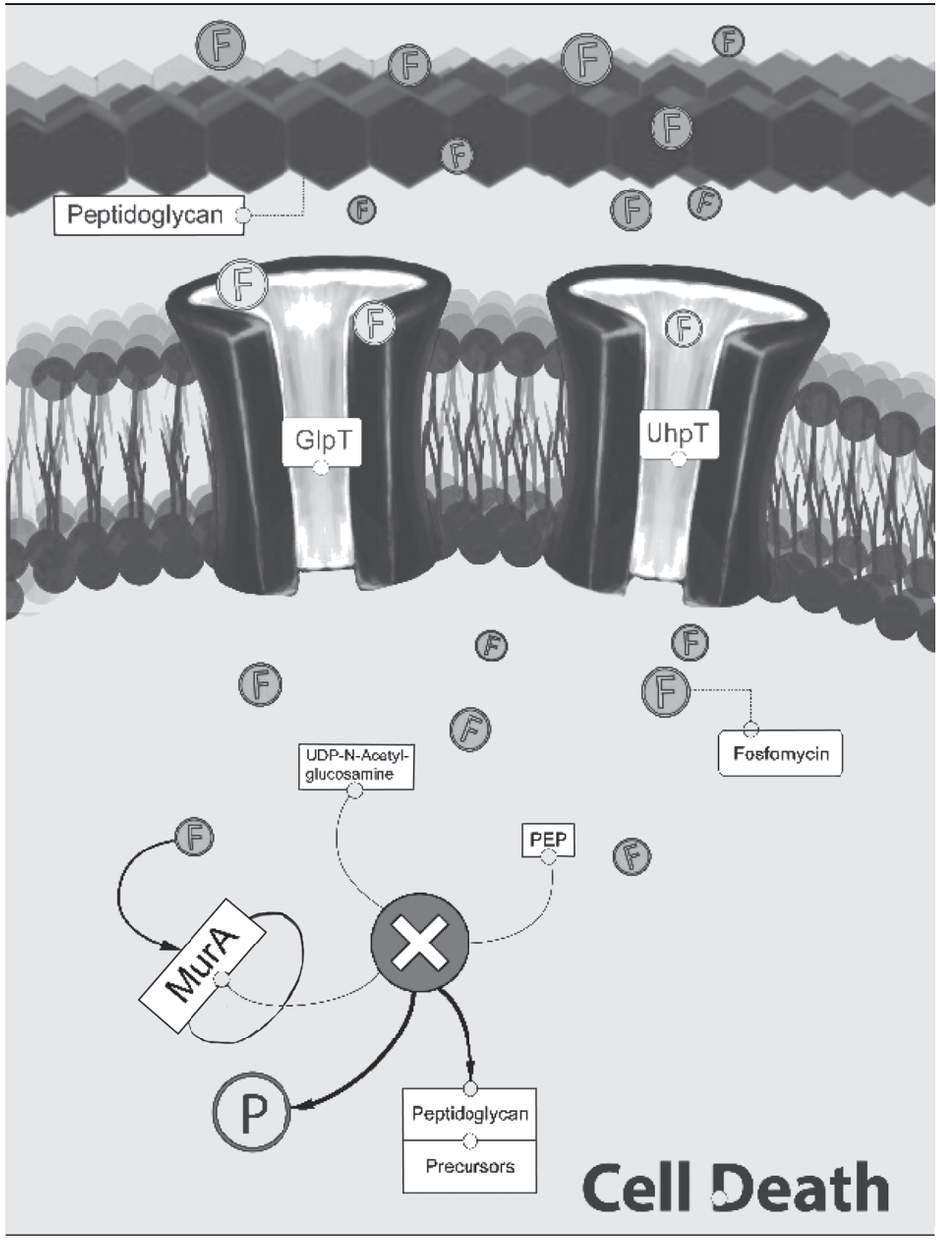

TABLE 1 Overview of the reported pharmacokinetic properties of fosfomycin calcium, fosfomycin tromethamine, and fosfomycin disodium.

Ref	Dose	Study Group (N)	T_{max} (h)	$t_{1/2\beta}$ (h)	V_d (l)	CL (l/h)	CL_R	F (%)	k_a	k_{el}	Q
FOSFOMYCIN CALCIUM											
Cadorniga et al., 1977[28]	500 mg	HV (6)	2–2.5	2.04	20.7	ND	ND	37	ND	0.12	NA
Goto et al., 1981[27]	20 mg/kg	HV (7)	2.3 (0.3)	3.01 (0.67)	30.1 (4.6)	7.1 (1.5)	ND	28 (7.0)	1.03 (0.38)	0.24 (0.05)	NA
	40 mg/kg	HV (7)	2.7 (0.2)	5.05 (0.81)[g]	60.2 (17.4)	9.0 (1.7)	ND	28 (8.0)	0.92 (0.40)	0.14 (0.02)	NA
Borsa et al., 1988[30]	40 mg/kg SD	Young HV (5)	1.41 (0.67)	4.81 (1.90)	435.0 (144.0)	59.3 (23.3)[a]	5.0 (1.1)[a]	ND	ND	0.170 (0.084)	NA
		Elderly HV (8)	2.58 (0.54)	11.80 (6.86)[g]	409.4 (100.4)	33.4 (23.1)[a]	3.3 (1.1)[a]	ND	ND	0.082 (0.047)	NA
Bergan et al., 1990[21]	50 mg/kg	HV (8)	2.9 (0.6)	5.6 (1.8)	ND	ND	ND	12.0 (7.5)	ND	0.135 (0.053)	NA
FOSFOMYCIN TROMETHAMINE											
Segre et al., 1987[25]	50 mg/kg	HV (5)	2.2 (0.44)	2.43 (0.31)	10.4 (1.5)	8.3 (1.6)	7.0 (0.9)	0.44 (0.09)	0.58 (0.04)[e]	Transit model k_{10}: 1.24 (0.55) k_{12}: 1.69 (0.62) k_{23}: 0.34 (0.10) k_{35}: 0.69 (0.07)[f]	NA
Borsa et al., 1988[30]	25 mg/kg SD	Young HV (5)	1.61 (0.23)	5.37 (2.56)	186.3 (129.4)	19.4 (8.4)[a]	10.8 (1.5)[a]	ND	ND	0.156 (0.073)	NA
		Elderly HV (8)	2.16 (0.72)	8.28 (5.51)[g]	101.1 (61.2)	9.7 (4.2)[a]	2.9 (1.0)[a]	ND	ND	0.124 (0.078)	NA
Bergan et al., 1990[21]	25 mg/kg	HV (8)	2.6 (0.5)	3.9 (0.65)	ND	ND	ND	ND	ND	0.183 (0.031)	NA
	50 mg/kg	HV (8)	2.5 (0.8)	3.6 (0.44)[g]	ND	ND	ND	40.6 (17.9)	ND	0.197 (0.024)	NA
Bergan et al., 1993[26]	2g	HV (12)	2.2 (0.9)	4.1 (0.8)	ND	ND	ND	ND	ND	0.17[b]	NA
	3g	HV (12)	2.0 (0.6)	4.5 (2.1)[g]	ND	ND	ND	32.9 (7.9)	ND	0.15[b]	NA
	4g	HV (12)	2.0 (0.0)	3.9 (2.7)[g]	ND	ND	ND	ND	ND	0.18[b]	NA
FOSFOMYCIN DISODIUM											
Kwan et al., 1971[42]	250 or 500 mg, 10-min infusion, Single dose 500 mg every 6 h, 8 times	HV (17)	NA	1.1 c	V_c: 12.9	7.5	7.1	NA	NA	K_{13}: 0.62	12.4[b] k_{12}: 0.96 k_{21}: 1.19
Cadorniga et al., 1977[28]	500 mg, 5-min infusion	HV (6)	NA	$t_{1/2\alpha}$: 0.38 $t_{1/2\beta}$: 2.04	V_c: 12.9 V_p: 7.8 V_{dss}: 20.7	ND	ND	NA	NA	K_{13}: 0.67	6.9[b] k_{12}: 0.54 k_{21}: 0.88
Goto et al., 1981[27]	20 mg/kg, 5-min infusion	HV (7)	NA	2.25 (0.74)	V_c: 8.7 (2.9) V_p: 9.8 (1.7) V_{dss}: 18.5 (4.6)	7.2 (1.6)	6.0 (2.2)	NA	NA	β: 0.34 (0.12) k_{10}: 0.92 (0.31)	14.2[b] k_{12}: 1.62 (0.76) k_{21}: 1.45 (0.75)
	40 mg/kg, 5-min infusion	HV (7)	NA	2.22 (0.46)	V_c: 8.7 (2.9) V_p: 12.7 (2.9) V_{dss}: 20.8 (3.5)	8.0 (0.8)	6.6 (0.9)	NA	NA	β: 0.32 (0.06) k_{10}: 0.99 (0.22)	16.2[b] k_{12}: 1.84 (0.85) k_{21}: 1.30 (0.49)
Lastra et al., 1983[43]	30 mg/kg	Patients with normal renal function (9)	NA	$t_{1/2\alpha}$: 0.18 (0.09) $t_{1/2\beta}$: 1.91 (0.50)	21.2 (10.4)	7.9 (3.2)	ND	NA	NA	k_{13}: 1.91 (1.29)	k_{12}: 2.22 (1.49) k_{21}: 1.18 (0.68)
		Patients with impaired renal function (8)	NA	$t_{1/2\alpha}$: 0.61 (0.18) $t_{1/2\beta}$: 16.3 (11.9)	17.8 (6.8)	1.1 (0.8)	ND	NA	NA	k_{13}: 0.21 (0.17)	k_{12}: 0.66 (0.38) k_{21}: 0.43 (0.13)
Segre et al., 1987[25]	50 mg/kg, Single injection	HV (5)	NA	2.43 (0.31)	10.4 (1.5)	8.3 (1.6)	7.0 (0.9)	NA	NA	k_{35}: 0.69 (0.07)[f]	10.6[b] k_{34}: 1.00 (0.92) k_{43}: 1.40 (0.91)
Bergan et al. 1990[21]	50 mg/kg, 5-min infusion	HV (8)	NA	3.4 (1.1)	ND	ND	ND	NA	NA	0.206 (0.048)	ND
Bergan et al., 1993[26]	3g	HV (12)	0.02 (0.0)	2.1 (0.1)	ND	ND	ND	NA	NA	0.33[b]	ND
Joukhadar et al., 2003[35]	8g, 20-min infusion	Critically ill patients (9)	0.4 (0.1)	3.9 (0.9)	31.5 (4.5)	7.2 (1.3)	ND	NA	NA	0.18	ND
Pfausler et al., 2004[32]	8g, 30-min infusion, Single dose	Patients requiring extraventricular drainage (6)	1.2 (0.4)	3.0 (1.0)	30.8 (10.2)	7.4 (2.3)	ND	NA	NA	ND	ND
	8g, 30-min infusion, every 8 h for 5 days	Patients requiring EVD	1.5 (1.2)	4.0 (0.5)	26.3 (9.7)	5.0 (2.0)	ND	NA	NA	ND	ND
Sauermann et al., 2005[36]	8g, 30-min infusion, Single dose	Patients (12)	0.47 (0.12)	3.7 (2.2)	V_c: 15.5 (4.5) V_{dss}: 28.6 (9.9)	7.6 (4.1)	ND	NA	NA	0.19[b]	ND
Kjellsson et al., 2009[41]	8g, 30-min infusion, Single dose	Patients (12)	NA	1.2[c]	V_c: 10.1(5.4-14.8) V_p: 9.80 (5.7-13.9)	5.8 (3.8–7.8)	ND	NA	NA	0.58[d]	15.4 (9.1–21.6)

*HV, healthy volunteers; N, number of subjects; Vd, apparent volume of distribution (unless specified as another reported volume); CLR, renal clearance; F, bioavailability; ka, apparent first-order absorption rate constant; kel, apparent first-order elimination rate constant; Q, intercompartmental clearance. A calculated in l/h per 1.73 m^2. B Calculated from kel and k12, k21. Q=k12 *V1 and Q=k21 *V2, C Calculated using the equation t1/2=0.693/kel, D Calculated from CL and central Vd the equations Kel=CL/VC and Kel=0.693/t1/2, E Bioavailability calculated using the PK model (F=k12 /(k12 +k10)) and the ratio of the amount excreted in the urine after oral and IV administration, F Rate of elimination in the urine, G Apparent terminal half-life.*

CHAPTER 9

FOSFOMYCIN AS A POTENTIAL THERAPY FOR THE TREATMENT OF SYSTEMIC INFECTIONS: A POPULATION PHARMACOKINETIC MODEL TO SIMULATE MULTIPLE DOSING REGIMENS

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ABSTRACT

Fosfomycin has emerged as a potential therapy for multidrug-resistant bacterial infections. In most European countries, the oral formulation is only approved as a 3g single dose for treatment of uncomplicated cystitis. However, for the treatment of complicated systemic infections, this dose regimen is unlikely to reach efficacious serum and tissue concentrations. This study aims to investigate different fosfomycin-dosing regimens to evaluate its rationale for treatment of systemic infections. Serum concentration-time profiles of fosfomycin were simulated using a population pharmacokinetic model based on published pharmacokinetic parameter values, their uncertainty, inter-individual variability and covariates. The model was validated on published data and used to simulate a wide range of dosing regimens for oral and intravenous administration of fosfomycin. Finally, based on the minimum inhibitory concentration for *E. coli*, surrogate pharmacodynamic indices were calculated for each dosing regimen. This is the first population pharmacokinetic model to describe the oral pharmacokinetics of fosfomycin using data from different literature sources. The model and surrogate pharmacodynamic indices provide quantitative evidence that a dosing regimen of 6–12g per day divided in 3 doses is required to obtain efficacious exposure and may serve as a first step in the treatment of systemic multi-drug-resistant bacterial infections.

INTRODUCTION

Antibacterial resistance remains one of the major threats to human health, despite its identification as one of the worldwide priority conditions by the WHO over a decade ago.[1-3] Particularly alarming is the rise in number and spread of multi-drug resistant (MDR) bacterial strains and a poor pipeline of new Gram-negative antibiotics.[4-7]

To battle MDR bacteria strains, the reassessment and reintroduction of 'old' antibiotics have emerged as alternative solution to circumvent the long and costly process of developing new antibiotics.[8,9] One of such 'old' antibiotics is fosfomycin, developed more than 40 years ago.[10] Fosfomycin is a broad spectrum antibiotic which exerts its bactericidal activity by irreversibly inhibiting the early stages of the bacterial cell wall synthesis.[11]

MDR Gram-negative bacteria are responsible for around twothirds of the deaths by MDR-bacterial infections in Europe.[6] Fosfomycin exhibits *in vitro* and *in vivo* antibacterial activity against a wide range of both Gram-positive and Gram-negative bacteria, including several MDR-strains.[12-17] Even most of the extensively drug-resistant (XDR) Enterobacteriaceae strains still remain susceptible to fosfomycin, including those expressing extended-spectrum beta-lactamases (ESBL) or metallo-β-lactamases (MBL).[14-16,18] In addition, fosfomycin has been suggested as add-on therapy for infections caused by *MDR-P.aeruginosa*, one of the main pathogens associated with nosocomial-acquired infections.[16,17,19]

Fosfomycin has been marketed in different formulations including fosfomycin tromethamine for oral administration and fosfomycin disodium for intravenous administration.[20] In most European countries, only the oral formulation is available and approved as a single 3g dose for the treatment of uncomplicated urinary tract infections (UTIS) in women. This single-dose regimen is not efficacious for the treatment of systemic MDR bacterial infections, making the prospective evaluation of new oral dosing regimens a necessity. A multiple-dose regimen of oral fosfomycin tromethamine has been proposed for the treatment of complicated UTIS, including those due to MDR-bacteria.[21,22] However, more studies are urgently needed to determine the optimal oral dose regimen to achieve efficacious systemic exposure.

Few pharmacokinetic (PK) models for fosfomycin have been described in literature, which were developed on different study designs, limited numbers of subjects and different model structures.[23-26] PK modeling techniques allow integration of different study designs, on the basis that despite study differences the underlying population pharmacokinetics are similar, as commonly applied in dose-regimen selection.[27]

To assess the feasibility of a multiple oral-dose regimen with fosfomycin tromethamine for systemic infections, a combined PK model for intravenous and oral administration was built on PK parameters reported in literature in order to simulate various serum-concentration time profiles. In addition, surrogate pharmacodynamic indices were calculated, based on the minimum inhibitory concentration (MIC) representing the epidemiological cut-off value for *E. coli*,[28] to estimate its clinical efficacy.

METHODS

PK model

The structural model for intravenous administration was based on a previously reported two-compartment population PK model of fosfomycin, developed on 12 patients scheduled for abscess drainage.[25]

The model was parameterized in terms of elimination rate constant (k_e), volumes of distribution for the central (Vc) and peripheral compartments (Vp) and intercompartmental clearance (Q). The rate and duration of infusion were parameterized by Q_{inf} and t_{inf}, respectively.

To include oral administration of fosfomycin tromethamine, the model was extended with a gastrointestinal (GI) and a transit component (TRANS), based on a PK model published by Segre et al., that was developed after oral and intravenous administration in 5 healthy volunteers.[24] This model was parameterized in terms of rate constants k_{ij}, representing the different rates of drug transfer from the *i^{th}* compartment to the *j^{th}* compartment, including a k_{10}, representing the first order loss of dose, hence correcting for oral bioavailability. Additionally, a transfer constant representing biliary clearance of the drug (k_b) was included in the oral PK model. As literature is inconclusive on reabsorption of fosfomycin,[24,29,30] models with and without enterohepatic recirculation were compared to published data in order to evaluate its descriptive impact on the simulations. The PK model structures used for the simulations of different multiple-dose regimens after intravenous and oral administration of fosfomycin are presented in *figure 1*.

Individual PK parameters were simulated according to Equation 1.

$$\theta_i = \theta_{TV} * \exp(\eta_i), \qquad (1)$$

where θ_i is the PK parameter for the i^{th} individual, θ_{TV} the typical population PK parameter, and ηi the interindividual variability (IIV) for the i^{th} individual. Here, IIV was reported to be log-normally distributed for CL, Vc, and Vp,[25] and incorporated as such in the model; η is assumed to be normally distributed around 0 with its reported variance ω^2.

The θ_{TV} is simulated based on literature values of mean population PK parameters (θ_p) and their uncertainty in terms of variance [based on reported standard deviation (SD) and/or 90% confidence intervals (CI)], thus resulting in an uncertainty distribution of the population PK parameter. Both θ_p and its variance were log-transformed to avoid negative values, according to Equation 2 and Equation 3.[31]

$$\theta_{p,LN} = ln\theta_{p,N} - \frac{1}{2}\omega^2_{LN} \qquad (2)$$

$$\omega^2_{LN} = ln\left(\frac{\sigma^2_N}{\theta^2_{p,N}} + 1\right), \qquad (3)$$

where subscript LN refers to the log domain, and $_N$ refers to the normal domain. Subsequently, θ_{TV} was calculated according to Equation 4.

$$\theta_{TV} = exp(\theta_{p,LN} + \omega^2_{LN}) \qquad (4)$$

Covariates

A mean-centered linear relationship between creatinine clearance (CL_{CR}) and clearance (CL) was reported,[25] and incorporated as such in the simulated clearance for the i^{th} individual (CL_i, Equation 5).

$$CL_i = (CL_{TV} + (0.0141 * (CL_{CR,i} - 103))) * \exp(\eta_i), \qquad (5)$$

where CL_{TV} is the literature derived mean population parameter with its uncertainty (Equation 4), $CL_{CR,i}$ is the creatinine clearance and η_i the IIV for the ith individual. The $CL_{CR,i}$ and normalization factor (103) were obtained from Sauermann et al.[32] To simulate $CL_{CR,i}$, samples were drawn from a distribution with a mean of 103 and standard deviation 41, which was limited between the minimal and maximal reported values.[32]

Simulations

One thousand (1000) individual PK parameter sets (θ_i) were randomly sampled using the distributions for parameter uncertainty and IIV, with resampling. The resulting individual PK parameter sets were then used to simulate individual plasma fosfomycin concentrations over time. The mean PK parameters, uncertainty and IIV used for the simulations are listed in *table 1*. All simulations were performed in R (version 2.13.1)[33] using the lsoda (deSolve Package 1.10-3) and; mvrnorm functions (MASS Package v7.3-8), within the RStudio[34] interface (version 0.98.501).

Model validation

The validation of the PK models was performed by simulating previously published study designs and visually comparing the 90% prediction interval (PI) of the simulations to the observed data reported in literature. In short, the previously published study designs in healthy volunteers were, for intravenous administration, 8 doses of 500 mg every 6 hours[35]; 500 mg in 5 min infusion[23]; and 50 mg/kg bolus.[24] For single-dose oral administration, dosing regimens were 50 mg/kg, 2g and 5g.[24]

Alternative dosing regimens and calculation of PK/PD indices

Once validated, the different oral dosing regimens were simulated to assess the feasibility of a multiple dosing regimen. These scenarios included the simulation of total daily doses ranging from 3g to 45g once or divided into two or three times per day for oral fosfomycin tromethamine.

PK parameters were obtained in R and included: maximum serum concentration (C_{max}), time to reach C_{max} (T_{max}), area under the serum concentration–time curve (linear trapezoidal rule with 0.1 h time-steps) over the dosing interval ($AUC_{o\text{-}tau}$), and AUC from time 0 to time of the last simulated concentration ($AUC_{o\text{-}last}$).

Surrogate pharmacodynamic indices were based on the minimum inhibitory concentration (MIC) of 8 mg/l, as this represents the epidemiological cut-off value for *E. coli* according to EUCAST[28] and include: C_{max}/MIC, AUC/MIC, time above MIC ($T_{>MIC}$) and percentage of $T_{>MIC}$ during the dose interval ($\%T_{>MIC}$). Primarily, the mean estimated values of C_{max} and AUC during 24 hour at steady state were used. The C_{max}/MIC and $\%T_{>MIC}$ were calculated over the length of a dose interval at steady state, while AUC/MIC was calculated over a period of 24 hours at steady state as defined by Mouton et al.[36] Secondly, the lower 90% prediction interval (PI) of the simulated plasma concentration-time profiles was used, e.g., 95% of all subjects will have higher exposure compared to this PI.

RESULTS

PK Models

The contribution of enterohepatic recirculation on improvement of descriptive properties of the model proved to be marginal; the median concentrations and 90% PI did not differ substantially. The slight changes were considered to be of no clinical relevance. Secondly, as there is also no consistent proof for enterohepatic recirculation in literature, it was decided to exclude this PK property from the model. The parameter k_b was kept in the model as this rate constant for apparent biliary elimination is required to attest for the total elimination of fosfomycin.

All observations following intravenous (*figure 2*) and oral dosing (*figure 3*) lie within the 90% PI of the PK model. For the intravenous simulations, C_{max} is well described and the median slope of the terminal elimination phase follows the slope of the data. However, the terminal elimination phase and trough concentrations seem overpredicted by the model. Following the multiple 500 mg dose in 8 hours dosing intervals, no accumulation occurs and the simulated median concentration remains above the MIC until approximately 5 hours after dosing. For the oral simulations, the median C_{max} seems well predicted although the shape of the concentration-time curve in the terminal phase seems steeper compared to the data. Following the single 50 mg/kg dose, the simulated median serum concentration remains above the MIC until approximately 10 hours after dosing. As all data points lie within the 90% PI of the simulations, the PI is wider than expected based on the data, indicating that the variability of the model is overestimated.

Simulation of different multiple-dose regimens and calculation of PK/PD indices

Different multiple-dose regimens after oral administration of fosfomycin were simulated using the validated PK model. *Figure 4* shows the medians of the predicted PK profiles of 1000 subjects after intravenous administration of 3, 4, 6, or 8g of fosfomycin every 8 hours by 30 min infusion, as well as the MIC. In addition, simulation of different dosing schedules such as 4g and 6g every 6 hours were also conducted (data not shown). All simulated intravenous regimens reached serum concentrations above the MIC. The surrogate pharmacodynamic indices and mean PK measures for each dosing regimen are shown in *table 2*. All intravenous dosing regimens simulated produced C_{max} levels of at least 18-fold over the MIC, AUC/MIC values from 180 to 500, and a $100\%T_{>MIC}$.

Several oral dose regimens were simulated for doses of 3g and 6g of fosfomycin tromethamine, including a single dose per day (qd), two times daily (bid) and three times daily (tid). The predicted medians of these different dose regimens as presented in *figure 5* show that the medians of all first doses reached serum concentrations above the MIC. For both dose groups, concentrations only maintain above the MIC for the entire duration of the day following tid dosing. As shown in *table 3*, a 2g tid dose would also not suffice to reach a $\%T_{>MIC}$ of 100%. Interestingly, the currently clinically approved 3g single oral dose for UTIs may achieve efficacious concentrations in urine, however, it only achieves a $\%T_{>MIC}$ of around 30% in serum. Although most of the regimens reached a high $\%T_{>MIC}$, comparable to the intravenous regimens, the C_{max}/MIC and AUC/MIC values are lower than those in intravenous regimens: the C_{max}/MIC is 17.78 after 15 mg bid and the AUC/MIC values range from 37 to 300. *Table 3* also represents the pharmacodynamic indices based on the lower 90% PI of the plasma concentration-time simulations. These results show that for some individuals, a minimum dose of 4g tid will be required in order to reach a C_{max} that exceeds the MIC, and remains above the MIC for more than 50% of the dose interval.

DISCUSSION

This is the first population PK model to describe the oral pharmacokinetics of fosfomycin, using data from different literature sources. The study provides quantitative evidence that an oral dosing regimen of 6–12g per day divided in 3 doses is required to obtain serum concentrations above the MIC for at least 50% of the dose interval. This may serve as a first step in the treatment of systemic infections by MDR bacteria with a similar MIC compared to *E.coli*. Model validation showed a slight bias in the description of literature data and overprediction of variability. The slight bias can be explained by the use of few subjects in the development of the literature models causing relatively high parameter uncertainty and IIV, which accumulates in large prediction intervals. Following intravenous simulation, late PK time points seem

overestimated while for oral simulations time points after 15 hours seem underestimated, which may lead to bias in accumulation following multiple dosing regimens. In general, the reported population PK parameters used in our simulations were within the CI reported in a recent publication on intravenous fosfomycin infusion in critically ill patients. Compared to the volume of distribution in our simulations, the publication reports a relatively high volume of distribution, which the authors attest to pathophysiological changes in their critically ill patient population.[26] We acknowledge the quantitative and qualitative lack of data in literature, which is the case for many drugs that have been developed in the past. For this reason, we stress the importance of additional clinical data to ascertain whether oral fosfomycin may be used for the treatment of systemic.

The suggested daily oral doses of fosfomycin tromethamine to achieve an effective serum concentration exceed the currently approved single dose of 3g. To our knowledge, safety and tolerability has not been investigated *in vivo*, using higher oral doses. Alternative approaches to avoid such higher doses when dealing with systemic MDR infections may lie in synergistic combinations with other antibiotics, such as imipenem for treatment of methicillin-resistent *Staphylococcus aureus*,[37] or approval of intravenous fosfomycin formulations. Yet, more studies are urgently needed to assess the PK, safety, tolerability, and efficacy of fosfomycin in multiple-dose regimens and synergistic combinations.

The broad range of daily doses suggested with these simulations (from 6 up to 12g per day) can be explained, in part, by the relatively large parameter uncertainty and IIV reported in literature. To our knowledge, serum creatinine clearance is the only reported covariate in literature that explains part of the IIV. In addition, disease state may explain IIV of volume of distribution.[26] These aspects contribute to wide prediction intervals around the means of the simulations. An effect of bodyweight on volume of distribution has been used in a study but was not statistically supported.[26] Inclusion of more data and demographics would reduce the parameter uncertainty and improve quantitation of the IIV and is anticipated to provide a more precise prediction interval. With the current available literature data, the current dosing results based on the lower 95% prediction interval may prove to be a relatively conservative approach.

In this study, different surrogate pharmacodynamic indices were used to evaluate the effect of different dose regimens on the epidemiological cut-off value for *E. coli*. However, an important limitation in the evaluation of different dose regimens and optimization of therapy is the lack of information regarding the PD properties of fosfomycin. Few studies have attempted to characterize the PD properties of fosfomycin, but results are conflicting. Some studies pointed to a time-dependent bactericidal activity,[38,39] while others have suggested a concentration-dependent bactericidal activity.[40] This again stresses the need for more data.

The lack of PD data has also affected the clinical and PD breakpoints for MDR-bacterial infections from a regulatory perspective. In the case of fosfomycin tromethamine, the EUCAST has established clinical breakpoints for Enterobacteria-ceae (Susceptible ≤32 mg/l and Resistant >32 mg/l) which are only applicable to uncomplicated UTIs caused by Enterobacteriaceae, using a single dose of 3g.[28] As clinical breakpoints depend on the clinical features of the disease and the dose regimen, we chose the epidemiological cut-off value of fosfomycin for *E. coli* to calculate the PD indices. This value is independent of the dose regimens and exclusively determined by the MIC values distribution and therefore not used to advise on clinical therapy.[41] In this regard, further studies are urgently needed to establish the PK/PD relationships of fosfomycin. Microbiological susceptibility information could also be included in Monte Carlo simulations in order to define oral dosing regimens based on potential PK/PD targets with high probability of microbiological cure. This has been recently reported following intravenous infusion of fosfomycin in the treatment of *Klebsiella pneumoniae*,[42] and *Pseudomonas aeruginosa*.[43]

Literature review on fosfomycin PK and simulations clearly indicate the need for further clinical research to characterize the PK and PD properties of fosfomycin tromethamine. Previous studies reported potential decreased absorption at higher doses [24,44] and fosfomycin recirculation.[24] In the model building, these concepts were considered but did not improve the descriptive properties of the model with regards to the available data. Also, when administering doses that are higher than the current recommended dose in the clinic, this may result in nonlinear PK.[24,44] Hence, in the design of a future clinical trial, dose regimens as well as sampling times should be chosen to optimally address these potential PK characteristics. Characterization of these processes is the key to the design of optimal multiple-dose strategies, as saturable absorption or elimination can limit the use of higher doses and recirculation can lead to clinically relevant accumulation.

Simulations and PD indices show that a total daily oral dose of at least 6–12g of fosfomycin tromethamine are required to achieve a therapeutic concentration to treat systemic infections, based on the epidemiological cut-off value for *E. coli*. In light of the reported simulations, the population PK model can be used to optimize a new clinical trial to assess the PK, safety, and tolerability of fosfomycin tromethamine in multiple-dose regimens.

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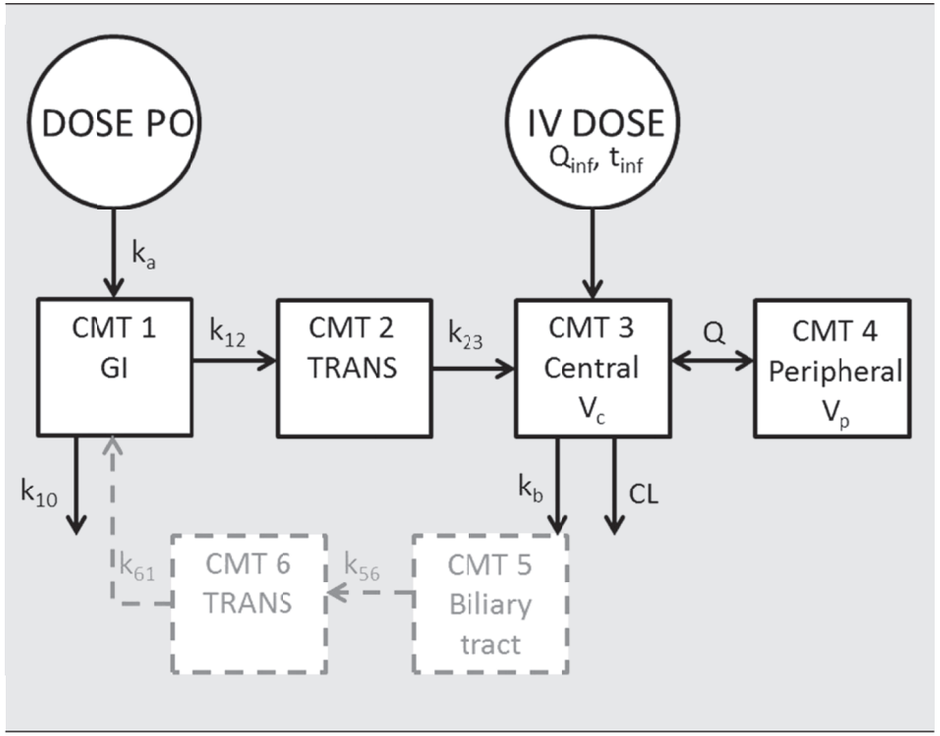
FIGURE 1 **The two compartment PK model structure used for the simulations of fosfomycin multiple-dose regimens (black), together with the excluded enterohepatic recirculation (gray).**



CL, clearance; CMT, compartment with associated number; k10, the first-order loss prior to reaching CMT 2; k12, k23, k56, k61, rate constants between compartments; kb, biliary elimination; GI; gastrointestinal; Q, intercompartmental clearance; Q_{inf} infusion rate constant; t_{inf}, infusion time; TRANS, transit; Vc, central volume of distribution; Vp, peripheral volume of distribution. Individual PK parameters were simulated according to Equation 1.

FIGURE 2 **Mean plasma fosfomycin concentration-time profiles (black line) and 90% prediction interval (gray area) of 1000 simulated subjects with observations (circles): (A) simulations and data after 1 minute IV bolus injection of 50 mg/kg fosfomycin disodium[24]; (B) simulations after 500 mg of fosfomycin disodium in a 5-10 minute short IV infusion with data (grey; data obtained by Kwan et al.,[23] black; data obtained by Cadorniga[35]). The dashed line represents the minimum inhibitory concentration**

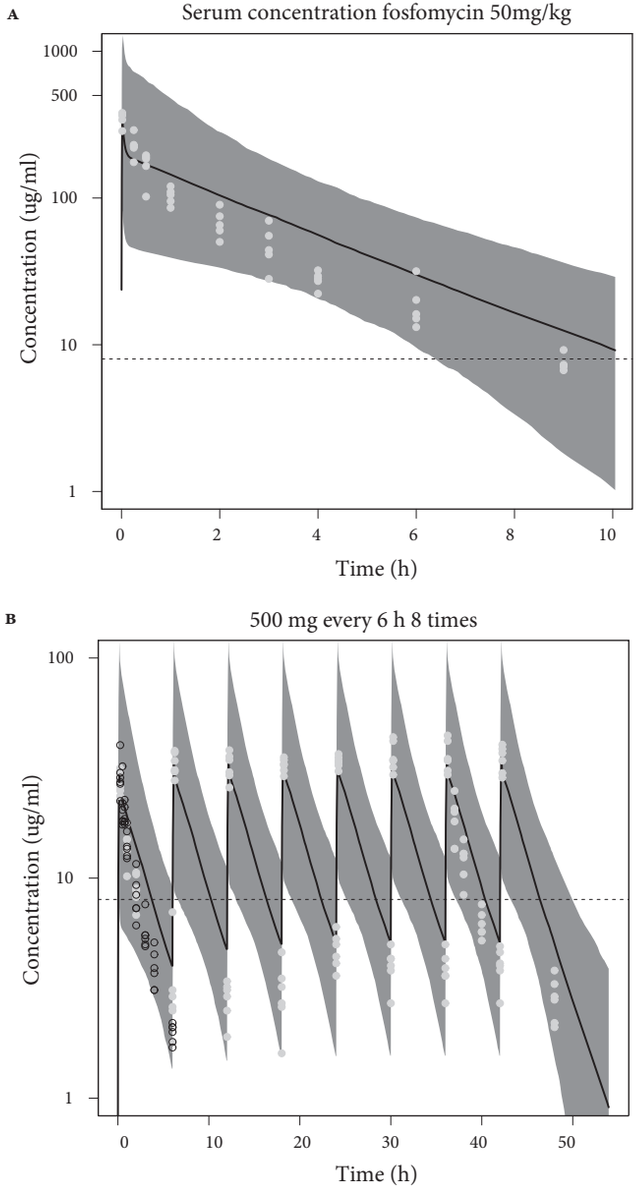


FIGURE 3 **Mean serum fosfomycin concentration-time profiles (black line) and 90% prediction interval (gray area) of 1000 simulated subjects with reported observations [24] after oral administration of fosfomycin tromethamine: (A) 50 mg/kg with data (black circles,[24] (B) 2g with reported mean values ± SD and (C) 5g with reported mean values ± SD. The dashed line represents the minimum inhibitory concentration**

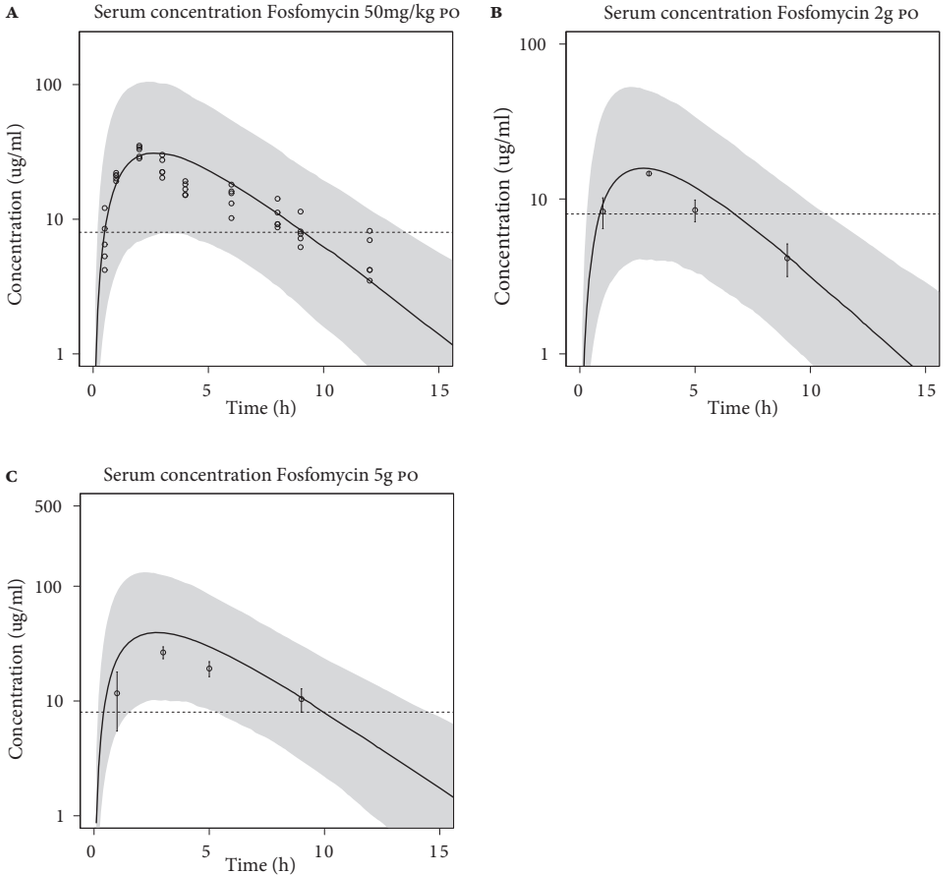


FIGURE 4 **Median serum fosfomycin concentration-time profiles of 1000 simulated subjects after three times daily (tid) IV bolus dosing of 3, 4, 6 and 8 mg fosfomycindisodium. Horizontal dashed line represents the minimum inhibitory concentration**

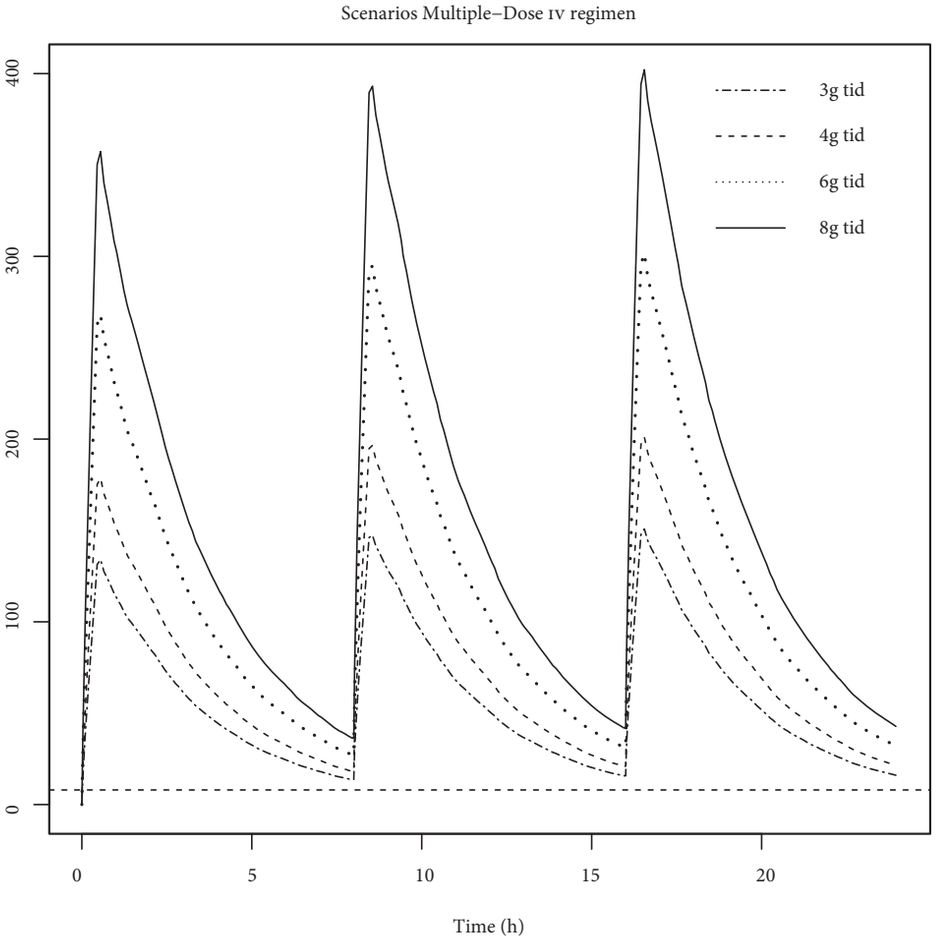


FIGURE 5 Scenarios Multiple–Dose Oral regimen. Median serum concentration-time profiles of fosfomycin simulated in 1000 subjects following oral administration of 3 or 6g of fosfomycintromethamine with various dose regimens: single dose (sd), two times daily (bid) or three times daily (tid). Dashed blue line represents the minimum inhibitory concentration of 8 mg/l

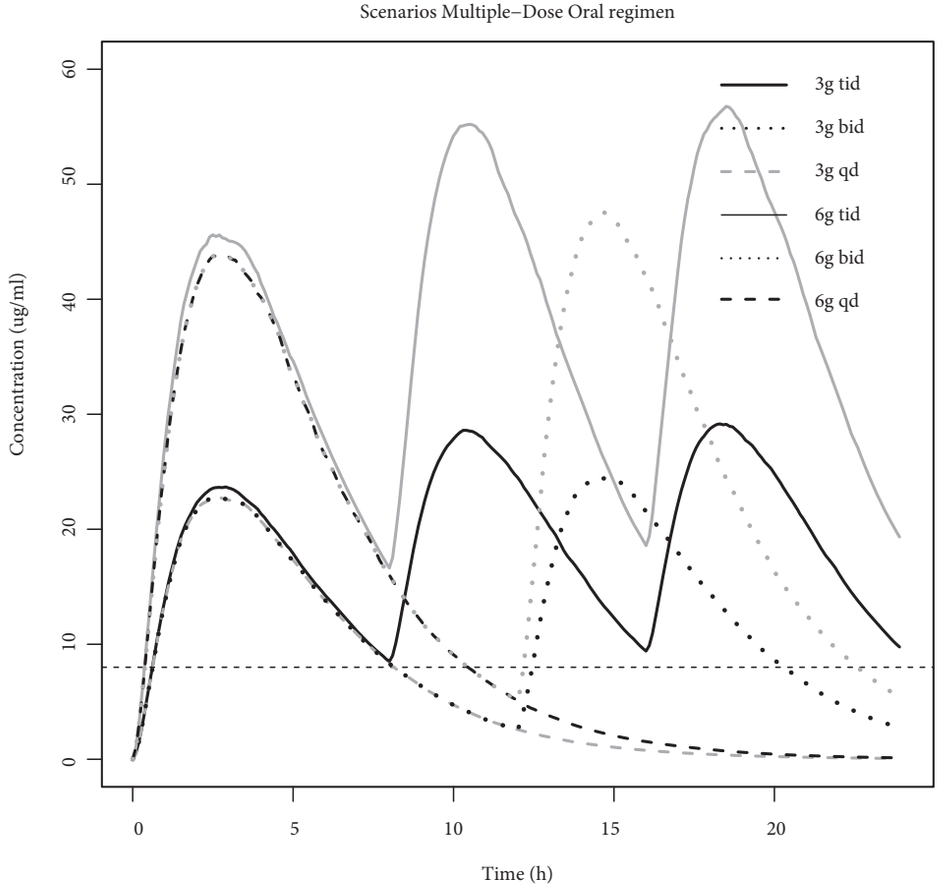


TABLE 1 Pharmacokinetic parameter values used in the simulations

Parameter	Mean estimate (90% CI or ± SD)	IIV	Uncertainty (variance)[a]	Reference
CL (l/h)[b]	5.808 (3.792–7.80)	0.238	1.4841	Kjellsson et al.[25]
Vc (L)	10.1 (5.36–14.8)	0.238*1.64	8.2329	Kjellsson et al.[25]
Vp (L)	9.80 (5.70–13.9)	0.197	6.2120	Kjellsson et al.[25]
Q (l/h)[b]	15.36 (9.12–21.6)	NI	14.3892	Kjellsson et al.[25]
COV_{CLCR-C}	0.0141	–	–	
k_{10} (h-1)	1.24 ± 0.55	ND	0.3025	Segre et al.[24]
k_{12} (h^{-1})	1.69 ± 0.62	ND	0.3844	Segre et al.[24]
k_{23} (h^{-1})	0.34 ± 0.10	ND	0.0100	Segre et al.[24]
k_b (h^{-1})	0.50 ± 0.18	ND	0.0324	Segre et al.[24]

CL, clearance; Vc, volume of distribution of central compartment; Vp, volume of distribution of peripheral compartment; Q, intercompartmental clearance; $COV_{CLCR-CL}$ linear relationship between creatinine clearance and CL; $k_{x,y}$, rate constants from compartment x to y; NI, not identified; k_b, rate constant biliary elimination; ND, no data available / a=Calculated from the 90% CI or SD / b=Value converted to match units.

TABLE 2 Mean surrogate pharmacodynamic indices for different intravenous dosing regimens of fosfomycin disodium, using a MIC of 8 mg/l.

Dose(g)	Interval (h)	C_{max} (mg/l)	C_{max}/MIC	AUC (mg/l*h)	AUC/MIC8	$\%T_{>MIC}$
3	8	151.41	18.93	1490.82	186.35	100
4	8	201.88	25.24	1987.76	248.47	100
4	6	224.04	28.00	2684.44	335.55	100
6	8	302.83	37.85	2981.64	372.70	100
6	6	336.05	42.01	4026.66	503.33	100
8	8	403.77	50.47	3975.52	496.94	100

C_{max}, maximum concentration; MIC, minimum inhibitory concentration; AUC, area under the concentration-time curve; %T>MIC, time above the MIC during a dose interval, expressed as percentage.

TABLE 3 **Mean surrogate pharmacodynamic indices based on the median (med) and lower limit of the 90% prediction interval(90PI) PK simulations for different oral dosing regimens of fosfomycin trometh-amine, using a MIC of 8 mg/l**

Dose (g)	Interval (h)	C_{max} (mg/l) med/90PI	C_{max}/MIC med/90PI	AUC (mg/l*h) med/90PI	AUC/MIC med/90PI	$\%T_{>MIC}$ med/90PI
2	8	18.96/5.16	2.37/0.65	316.95/92.18	39.62/11.52	84/0
3	8	28.44/7.75	3.56/0.97	475.42/138.26	59.43/17.28	100/0
3	12	24.52/6.60	3.07/0.82	313.48/88.52	39.19/11.06	66/0
3	24	22.87/6.05	2.86/0.76	154.26/41.58	19.28/5.20	31/0
4	8	37.93/10.33	4.74/1.29	633.89/184.35	79.24/23.04	100/51.57
5	8	47.41/12.91	5.93/1.61	792.36/230.44	99.05/28.80	100/67.63
6	8	56.89/15.50	7.11/1.94	950.84/276.53	118.85/34.57	100/78.75
6	12	47.70/13.34	5.96/1.67	602.87/178.67	75.36/22.33	87/45.76
6	24	44.12/12.12	5.51/1.52	296.83/83.11	37.10/10.39	42/20.44
7	8	66.37	8.30	1109.31	138.66	100
8	8	75.85	9.48	1267.78	158.47	100
9	8	85.33	10.67	1426.26	178.28	100
10	8	94.81	11.85	1584.73	198.09	100
11	8	104.30	13.04	1743.20	217.90	100
12	8	113.78	14.22	1901.67	237.71	100
15	8	142.22	17.78	2377.09	297.14	100

C_{max}, maximum concentration; MIC, minimum inhibitory concentration; AUC, area under the concentration–time curve; $\%T_{>MIC}$, time above the MIC during a dose interval, expressed as percentage.

CHAPTER 10

PHARMACOKINETICS OF FOSFOMYCIN IN PATIENTS WITH PROPHYLACTIC TREATMENT FOR RECURRENT *E. COLI* URINARY TRACT INFECTION

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ABSTRACT

OBJECTIVES To evaluate pharmacokinetics and clinical effectiveness of intravenous and oral fosfomycin treatment in patients with recurrent urinary tract infection (RUTI) with *Escherichia coli*.

PATIENTS AND METHODS Patients with RUTI treated with oral fosfomycin 3 gram every 72 hours for at least 14 days were included in a prospective open label single-center study. Serum samples were taken after oral and intravenous administration of fosfomycin. Urine was collected for 24 hours at 3 consecutive days. Fosfomycin concentrations in serum and urine were analysed using a validated ultra performance chromatography tandem mass spectrometry. Pharmacokinetics were evaluated using a population model.

RESULTS Twelve patients were included, of whom nine also administered intravenous fosfomycin. Data were best described by a two-compartment model with linear elimination and a transit-absorption compartment. Median values for absolute bioavailability and serum half-life were 18% and 2.13h, respectively. Geometrical mean urine concentrations on day 1, 2 and 3 were above an MIC of 8 mg/L after both oral and intravenous administration. Quality of life reported on a scale of 1-10 increased from 5.1 to 7.4 (p=0.001). The average score of urinary tract infection symptoms decreased after fosfomycin dosing (3.1 points, 95% CI: -0.7 – 7.0, p=0.10).

CONCLUSIONS Oral fosfomycin provides urine levels of fosfomycin above MIC for *E. coli* and seems to improve symptoms. The pharmacokinetic model can be used to develop dosing regimes of fosfomycin in patients with *E. Coli* RUTI.

INTRODUCTION

Urinary tract infections are common and associated with a considerable burden of hospital admissions and associated healthcare costs.[1] Management of patients with recurrent urinary tract infections (RUTI) is challenging, particularly given the increasing prevalence of antimicrobial resistance.[2] Continuous antimicrobial prophylaxis is one of the strategies for the prevention of RUTI. The choice of antimicrobial should be based on patterns of resistance, tolerability, side effects, availability and costs. Commonly used agents for this purpose are fluoroquinolones, nitrofurantoin, trimethoprim-sulfamethoxazole and oral cephalosporins.[3]

Fosfomycin is considered the first choice of treatment for RUTI because of its favorable side effect pattern compared to other antibiotics.[4] Fosfomycin was discovered in 1969 and has sustained activity against several multidrug-resistant uropathogenic Enterobacteriaceae.[5-8] Fosfomycin has been considered to be less useful for the treatment of systemic infections, because of its rapid clearance after oral administration. However, increased and sustained urinary drug concentrations are observed after systemic administration.[9] Given the trend of increasing antimicrobial resistance, fosfomycin may be an appealing alternative for the treatment and prophylaxis of RUTI caused by multidrug-resistant uropathogens.[10]

What remains unclear is the optimal dosing regimen of fosfomycin treatment in patients with RUTI, despite the numerous studies that have reported the pharmacokinetic and pharmacodynamic characteristics of fosfomycin, especially when administered intravenously for the treatment of various infections.[3,11-19] Most of these studies lack accurate measurements of fosfomycin levels, especially in the lower range of clinically relevant concentrations. The recent development of liquid chromatography – mass spectrometry to measure fosfomycin levels in serum and urine now allow for an accurate analysis of fosfomycin in serum and urine of patients.[20,21]

The aim of the present study was to evaluate pharmacokinetics and clinical effectiveness of intravenous and oral fosfomycin treatment in patients with RUTI with *E. coli*.

PATIENTS AND METHODS

Ethics

The study was conducted at the Haga Teaching Hospital, The Hague, The Netherlands. The study protocol was approved by the Medical Ethics Committee of South-West Holland (protocol 18-050) and the Institutional Scientific Review Board of the Haga Teaching Hospital. This study was registered under EudraCT number 2018-000616-25. Written informed consent of all participants was obtained.

Study design and patients

This study was a prospective open label single-center study including patients with rUTI, defined as at least three UTIS per year or two during the last six months.[3] Inclusion criteria were: age ³ 18 years, treatment of rUTI with oral fosfomycin 3 gram every 72 hours for at least 14 days as instignated by the treating physician, ability to communicate in Dutch and written informed consent. Exclusion criteria were: renal insufficiency (estimated glomerulair filtration rate (eGFR) <30ml/min/1.73 m^2), known allergy for fosfomycin, pregnancy or breast feeding, active malignancy, loss or donation of ³ 500 ml of blood within 90 days prior to screening, participation in an investigational drug study within 90 days prior to day 1, use of metoclopramide, and any condition that might interfere with treatment compliance or study conduct (e.g., use of illicit drug, alcohol dependence).

Study procedures

Data on patient demographics (age and gender), medical history, medication use, height and weight and renal function (calculated using the CKD-EPI method) were collected at baseline. [22]

Fosfomycin tromethamine (5,63 g, Monuril®, Zambon S.p.A.) was used for the oral administration and fosfomycin disodium (3,96 g, Fomicyt®, Nordic Pharma BV) was administered in a 30 minutes intravenous infusion. Sampling of blood and urine was performed around a planned dose of 3 gram oral fosfomycin and, optionally, when an oral dose was replaced by the equivalent intravenous dose.

Blood samples were collected pre-dose and after oral (at t = 30, 60, 90, 120, 180, 240, 300, 360 minutes) and intravenous fosfomycin administration (at t = 10, 20, 30, 60, 90, 120, 180, 240, 300 and 360 minutes) in plain serum tubes. After collection, samples were centrifuged at 3500 rounds per minute at room temperature and serum was transferred to a storage tube and frozen at -80° C unil analysis. Urine was collected for 24 hours on 3 consecutive days, starting at the time of administration of fosfomycin. Total 24-hour urine volume was measured and an aliquot was frozen at -80° C until analysis.

Fosfomycin analysis

Fosfomycin concentrations in serum and urine were analysed using a validated ultra performance chromatography tandem mass spectrometry (LC-MS/MS) method.[21] Analysis of the samples was performed at the Department of Pharmacy, Erasmus University Medical Centre, Rotterdam, The Netherlands. The upper and lower limits of quantification (ULOQ and LLOQ) were 375 mg/L and 0.75mg/L for both matrices. Results above the ULOQ were diluted and re-analysed.

Pharmacokinetic analysis

Population pharmacokinetics modelling using nonlinear mixed-effects modelling methods was carried out based on serum fosfomycin concentration data using NONMEM 7.3.[23] Visual exploratory inspection of the data revealed multi-exponential decay in the individual serum fosfomycin concentration versus time profiles. Therefore, two- and three-compartmental models with linear and nonlinear elimination were developed using physiological parameterization, e.g., absolute clearances (CL), absolute volumes of distribution (V) and absolute bioavailability (F). Various absorption models with and without delay in absorption were explored. Mixed-effects models were evaluated using first-order conditional estimation with interaction (FOCEI) maximum likelihood estimation. Interindividual variability was assumed to be log-linear distributed and covariance between the estimated parameters was explored. Proportional, additive and combined residual error structures were tested. Potential covariate relationships between Bayesian post-hoc parameter estimates and individual covariate values were formally tested in the model if the Pearson correlation coefficient was >0.5. Potential covariates were age, sex, race, height, weight, serum creatinine concentrations and body mass index. Criteria for model selection and evaluation were based on numerical and graphical evaluation as described previously, using the minimum objective function value (MOFV, 3.84 points resembling p=0.05), standard goodness-of-fit plots (including Visual Predictive Check of 1000 simulations), residual standard error (RSE) of the population parameter estimates and the coefficient of variation (%CV).[24]

Urine fosfomycin concentrations were graphically represented by geometric boxplots. Renal excretion in 72 hours was calculated by multiplying the volume of urine and the urinary fosfomycin concentration. Serum fosfomycin levels were presented as individual plots.

Clinical effectiveness

After inclusion, each patient filled out a questionnaire with questions about symptoms of cystitis, quality of life and adverse events six weeks before and after having started fosfomycin treatment for rUTI. A questionnaire based on the Acute Cystitis Symptom Score was used, consisting of a 4-point scale indicating the severity of each symptom ranging from 0 (no symptom) to 3 (severe symptoms), with a maximum total score of 30 (most severe symptoms).[25] Questions on adverse events included gastro-intestinal compliants, paresthesias, rash or itching, headache and tiredness. Quality of life was assessed on scale of 1 (worst) till 10 (best). Paired t-tests were performed to compare symptoms of cystitis, quality of life and adverse events before and after fosfomycin treatment.

Information about known urinary cultures (routinely performed before and after start of fosfomycin treatment) and the total duration of fosfomycine treatment in months were retrieved from the patient's medical records.

RESULTS

Patients characteristics

In total, 3 men and 9 women with rUTI on stable oral fosfomycin treatment were included. Nine participants (3 men and 6 women) also received an intravenous fosfomycin dose. The median (range) demographics were: age 66 (44-76) years, BMI 26.8 (20.4-28.7) kg/m^2, weight 79.9 (57-97) kg, height 169.5 (153-186) cm and eGFR 83 ml/min/1.73m^2 (63-103). All participants had *E. Coli* as cause of rUTI. Detailed patient characteristics are listed in table 1.

Pharmacokinetic analysis

SERUM PHARMACOKINETICS

Initial data fitting started using a two-compartmental model structure with proportional residual error. The individual data after oral administration were best described by a transit compartment, as a standard lag time absorption model resulted in a higher MOFV (79 points).[26] Expanding the model to a three-compartment model reduced the bias in the conditional weighted residuals with interaction *vs.* time but caused structural bias and overparameterisation (condition number > 100000), so model development was continued with a two-compartmental model structure. A combined residual error structure proved most fit for purpose as the use of an additive residual error structure resulted in problems in the minimisation and a proportional error structure resulting in a significant higher MOFV (137 points). Interindividual variability was identified on the central volume of distribution, clearance and bioavailability. Additional sources for interindividual variability resulted in unacceptable levels of overparameterization (condition number > 1000). No covariates were identified that could explain variability.

In general, the pharmacokinetics of fosfomycin were adequately captured by the model. The central and individual trend of the data were well described as the population predictions (Figure 1A) and individual predictions (Figure 1B) closely followed the line of unity for both oral and iv fosfomycin data. The conditional weighted residuals with interaction showed no bias over the range of population predictions (Figure 1C) but a slight underprediction for the late time points (Figure 1D). The parameter estimates of the population pharmacokinetic model are displayed in Table 2. All parameters were estimated with reasonable precision as all relative standard errors (RSEs) are below 30%. Between-subject variability was relatively low for V, CL and F (with CVs of 25.5%, 22.7% and 40.2%). The condition number was 50.9, which is well below the threshold of overparameterisation. The shrinkages of the empirical Bayes estimates that characterize the inter-individual variability and the residual error were well below 20%. The visual predictive check (VPC) is displayed in Figure 2, which demonstrates that both the variability and the structural trend of the data are adequately captured by the model. The 10th, 50th and 90th percentiles of the observed serum-concentrations are within the 95% confidence intervals (CI) of the 10th, 50th and 90th percentiles of the model predicted serum-concentrations.

URINE PHARMACOKINETICS

Urine data are represented in Figure 3. For 1, 2 and 3 days after oral fosfomycin dosing, the geometric mean (SD) urine concentrations were 622.3 (± 335.1), mg/L 41.41 (± 17.1) mg/L and 20.5 (± 45.60) mg/L. After intravenous administration these concentrations were 1512.17 (± 788.27) mg/L, 43.55 (± 43.62) mg/L and 25.37 (± 45.65) mg/L. Mean total amount renally excreted fosfomycin (SD) was 1.21 (± 0.37) gram after oral intake, and 2.96 (± 0,52) gram after intravenous administration.

Clinical effectiveness

Eleven participants completed the questionnairre (92%). The average score of urinary tract infection symptoms decreased after fosfomycin dosing (3.1 points, 95% CI: -0.7 – 7.0, p=0.10). Quality of life improved by 2.2 points (95% CI: 3.4 – 1.2, p=0.001). Most reported side effects were gastro-intestinal complaints (n=8), tiredness (n=8) and headache (n=7). The details of the questionnaire are provided in supplementary 1.

DISCUSSION

In this study, we evaluated pharmacokinetics and clinical effectiveness of intravenous and oral fosfomycin treatment in patients with rUTI with *E. coli*. The two-compartmental pharmacokinetic model accurately described the individual serum fosfomcyin concentration-time profiles after oral and intravenous administration. The total volume of distribution at steady state (central and peripheral volumes of distribution) was approximately 9.5 L, which is comparable to previous reported literature (range: 9.8-30.2L).[27-32] All model parameters were estimated with high accuracy and resulted in a half-life of 2.13 h which is also in line with previously reported values (range: 1.2-4.0 hours).[16,17,28-31,33-35] This indicates that our pharmacokinetic model resulted in physiological plausible parameter estimates. The estimated bioavailability was 18% (95% confidence interval: 11.5 -23.7%) which is markedly lower than earlier reported bioavailability estimations (range: 33-58%).[16,17,27,33] All previous reported bioavailabity estimations were measured in a healthy population whereas our populations is older and has more co-morbidities, like diabetes mellitus (n=2). Diabetes mellitus may reduce resorption as has been shown for rifampin and fluxcloxacillin.[36,37] Furthermore, the use of other medication may be another explanation for the difference in bioavailabilty, e.g. bioavailabilty of fosfomycin is lowered by co-administration of metoclopramide. Notable is the total amount renally excreted fosfomycin we found (1.21 gram) after oral intake, which is above the amount

absorbed and the calculated bioavailability. This could be explained by variation in measurement of fosfomycin concentration and urine volume or by underestimation of the bioavailability in our calculations. Further research is needed to explore the factors of decreased bioavailibity of fosfomycin.

In the pharmacokinetic model evaluation, it was shown that there is some bias in the conditional weighted residuals over time (Figure 1D). This could be indicative of a suboptimal structural pharmacokinetic model, e.g., the data was fitted to a two-compartmental model where a three-compartmental model would be more appropriate. As a result, the pharmacokinetic model consequently estimates lower concentrations than observed at the latest sample times. When fitting a three compartmental model, the model was clearly overparameterized, which indicates that the data do not allow identification of a three-compartmental model. A three compartmental model would require the quantification of three distinct exponential declines. However, an already dense sampling strategy was applied. Therefore, it is suggested that the duration of serum sampling should be extended in future study designs. When using this pharmacokinetic model for simulations, the accumulation of drug, and thus also the renal clearance into urine, would be slightly underestimated. Despite the relatively short serum half-life (2.13 hours), urine concentrations remained relatively high, even after 72 hours. This supports the suggestion from the model development process that a three-compartmental model is more appropriate as this would lead to a third exponential decay representing the distribution into deeper tissues that results in a slower release into serum hence a prolonged serum exposure and prolonged accumulation of fosfomycin in urine.

Urine fosfomycin concentrations during 24 hours ranged from 300-1500 mg/L, which is considerably higher than serum exposure ($AUC_{0\text{-}6h}$ oral 22.0 mg·h/L and iv 85.2 mg·h/L). This was an expected finding as the urinary tract has a collective function, and the renal clearance of fosfomycin is high. In our study, oral and intravenous administration of 3 g fosfomycin resulted in average urine fosfomycin concentrations high enough to induce an antibacterial effect based on the the epidemiological cut-off value of *E. coli* (i.e., 8 mg/L).[38] However, individual urine concentrations are average values over a 24 hour period. Fosfomycin concentrations in the urinary tract are highly affected by the amount of urine that is produced and timing of urination, which makes it difficult to relate fosfomycin urine concentrations over a 24 hour period to clinical effectiveness.

Although fosfomycin seemed an effective treatment for rUTI in this study, its added value for the treatment of systemic infections has always been argued, due to its "less-favorable" kinetics, e.g., its relatively short half-life, which would render the time at which concentrations are above the MIC to be relatively short.[39] In this study, serum concentrations remained above the epidemiological cut-off value of *E. coli* (a minimum inhibitory concentration (MIC) of 8 mg/L fosfomycin) for approximately 10 hours after oral administration of 3 g fosfomycin.[38] This would suggest that for multidrug-resistant uropathogenic Enterobacterales with a relative low MIC, 3 g fosfomycin orally or slight increments in dose or dosing regimen could be effective for the treatment of systemic infections. The EUCAST MIC distribution data suggest that many urinary pathogens have an even lower MIC, e.g., half of *E. coli* isolates have a MIC of ≤4 mg/L fosfomycin.

In this study we dosed fosfomycin trometamol 3 gram every 72 hours. Rudenko *et al.* performed a similar study in patients with rUTIs and found a significant decrease of 2.8 UTIs per year after oral dosing of fosfomycin trometamol 3 g every 10 days.[40] Based on the study of Rudenko *et al.*, guidelines recommend to dose fosfomycin 3 g every 10 days for prophylactic purpose.[3] This dosing regime with a prolonged interfall will result in low fosfomycin levels and might induce resistance. Higher concentrations of fosfomycin *in vitro* could decrease resistance development.[41] In this respect a more intensified dosing regime would be justified. The results of a non-inferiority trial of Constanti *et al.* provide support for an intensified dosing regime, as 3 g of fosfomycin every 7 days showed non-inferiority to prulofloxacin in female patients with rUTI.[12] However, it is unknown if any unwanted effects occur with an intensified regime, such as changes in intestinal microbioma, more side effects or development of resistance. It should be noted that high interindividual urinary fosfomycin concentrations were observed in healthy individuals,[42] which makes it difficult to establish a suitable endpoint for effective concentrations, and ultimately to choose the most optimal dosing regime for rUTI.

Our study has several strengths. First of all, the patients in our study reflect real-life practice, which is different to previous studies using healthy and predominantly young individuals. Furthermore, in our this study most participants received both an oral and intravenous dose of fosfomycin (n=9). The dense sampling strategy allowed us to asses the pharmacokinetics accurately. Finally, this is the first study done after multiple doses of fosfomycin trometamol.

It should be noted that our patient cohort was relatively small and heterogenous with rUTIs with varying underlying causes. In addition, all participants had fairly good renal function and normal BMI. No interindividual variability as covariate of the demographic parameters was observed on the pharmacokinetics, though it is a small cohort. Secondly, for the MIC of the *E.coli,* we used the epidemiological cut-off value which may be not applicable to each individual patient. Finally, clinical effectiveness indicated by symptoms of UTI and quality of life was retrospectively assessed through questionnaires, rendering it subject to response bias.

Altogether, our data proof that oral fosfomycin provides adequate urine levels of fosfomycin for *E. coli* and seems to improve symptoms. Given the growing concern of multidrug resistancy in rUTI and the limited amount of treatment alternatives, our study argues that fosfomycin 3g every 72 hours can be an effective oral prophylaxis regimen in patients with *E.coli* rUTIs. Further clinical and dosing studies are now warranted to evaluate dosing regimes in patients with *E. Coli* rUTI.

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FIGURE 1 **Goodness-of fit plots of the fosfomycin pharmacokinetic model with serum data after oral (dark grey) and intravenous (light grey) administration.**

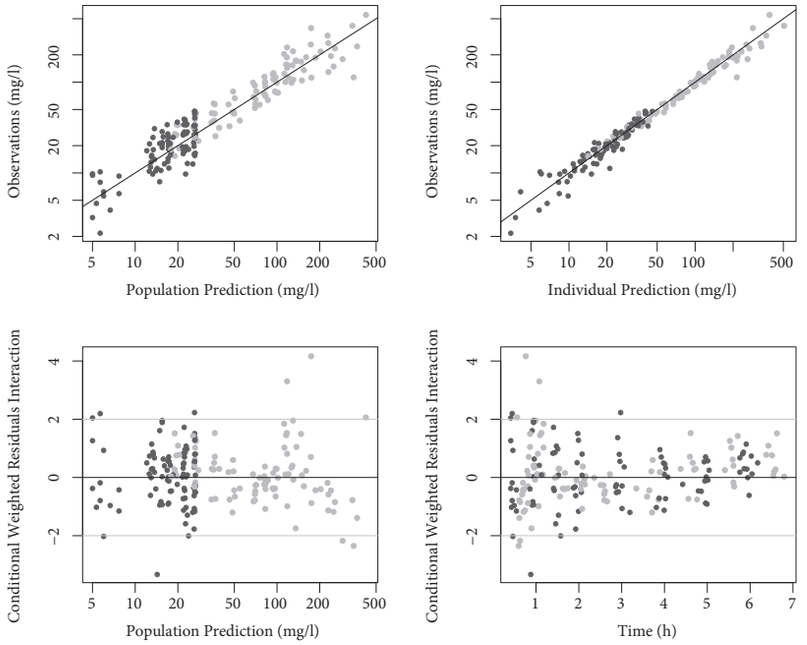


FIGURE 2 **Visual Predictive Check for the fosfomycin pharmacokinetic model after oral and intravenous administration. Solid and dashed lines represent the observed 10th, 50th and 90th percentiles for all observations, shaded area represents the 95% CI for the 10th, 50th and 90th percentiles of the model predictions.**

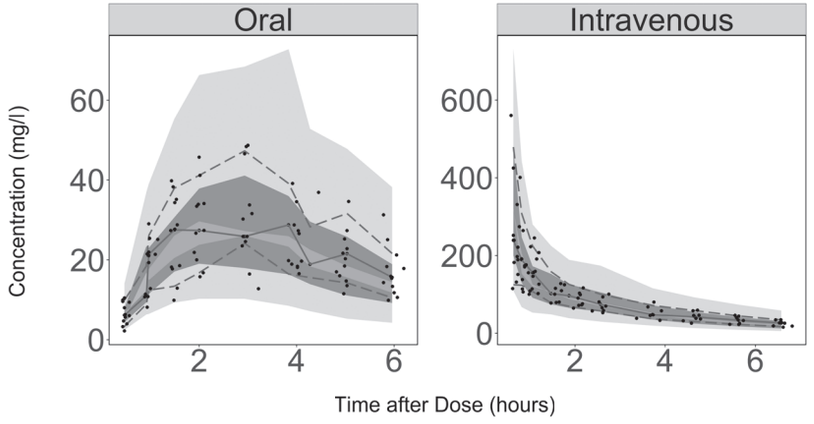


FIGURE 3 **Urine fosfomycin concentrations after intravenous and oral administration of 3 gram fosfomycin. The dotted line represents the MIC of 8 mg/L for *E. coli*. Outliers are depicted as triangles.**

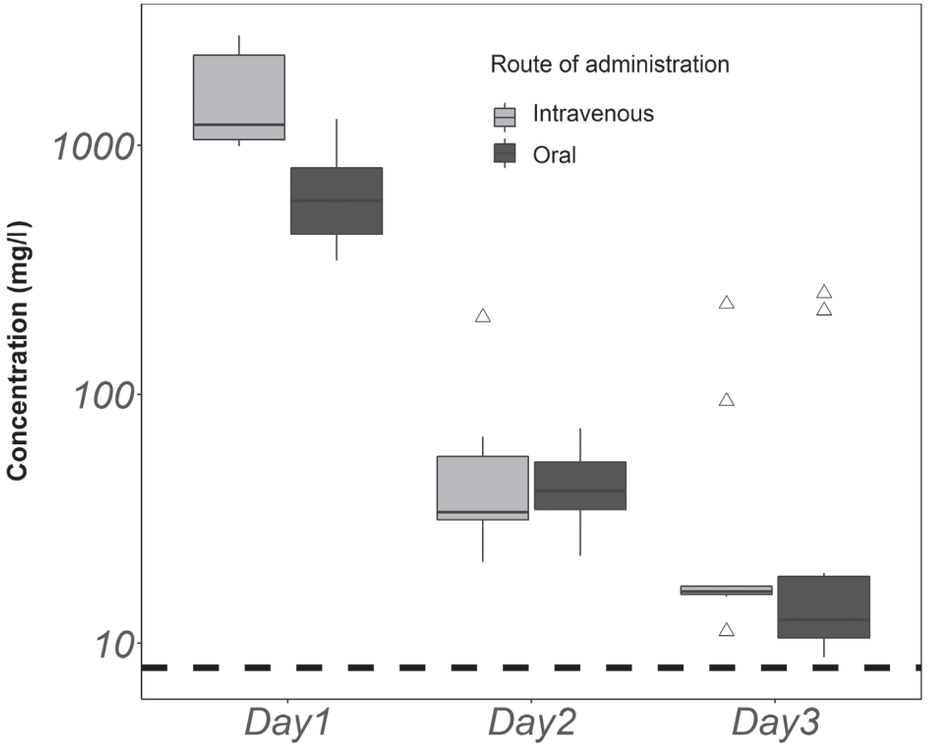


TABLE 1 Patients characteristics.

Patient	Sex	Age (year)	BMI (kg/m²)	eGFR (ml/min/1.73 m²)	Urologic history and co-morbidities	Duration on fosfomycin-treatment (months)	Uro-pathogen	UTIs per year before treatment	UTIs per year on treatment with different micro-organism	Urinary culture during treatment
1	F	63	27.0	103	Pelvic prolapse, Gastro-esophegal reflux disease, Epilepsia	5	*E. Coli*	9	2	negative
2	F	68	27.4	83	Atrial fibrillation, Breast cancer, Nitrofurantoin pneumonitis	13	*E. Coli*	12	2	negative
3	F	69	27.4	95	Acromegaly, Breast cancer, Hypertension	11	*E. Coli*	10	0	negative
4	F	63	27.9	92	Colorectal cancer, T2DM	2	*E. Coli*	12	2	negative
5	M	75	28.7	63	TUR-prostate, Neurogenic bladder, CIC, coronary artery disease, Sleep apnea syndrome	75	*E. Coli*	8	2	negative
6	F	75	28.7	78	Urgency urinary incontinence, T2DM, Hypertension, Aortic aneurysm	6	*E. Coli*	9	0	negative
7	F	74	25.2	66	Breast cancer, Uterus carcinoma, Proctocolitis, Carotic artery disease	2	*E. Coli*	10	0	negative
8	M	57	28.0	83	CBP, Sleep apnea syndrome	7	*E. Coli*	na	0	negative
9	M	76	26.3	85	CBP with prostate stones; TUR-prostate; hypertension	2	*E. Coli*	na	na	positive
10	F	75	19.7	83	Pelvic prolapse, Stress urinary incontinence, ICVA	3	*E. Coli* *Klebsiella pneumoniae*	12	8	positive
11	F	49	26.1	76	Hypospadias repair, Nephrectomy because of chronic pyelonephritis with renal stones	8	*E. Coli*	12	0	negative
12	F	44	28.4	97	None	1	*E. Coli*	12	0	negative

F: female, M: male, BMI: body mass index, eGFR: estimated glomerular filtration rate, CBP: chronic bacterial prostatitis, CIC: clean intermittent catherization, T2DM: type 2 diabetes mellitus, ICVA: ischemic cerebrovascular accident, na; not assessable.

TABLE 2 Population pharmacokinetic parameter and numerical diagnostics.

Pharmacokinetic parameter	Parameter estimate (RSE%)	IIV in %CV (shrinkage%)
Clearance (L/h)	5.05 (18.6)	25.5 (17.8)
Central volume of distribution (L)	1.32 (16.3)	22.7 (16.9)
Intercompartmental clearance (L/h)	6.31 (10.6)	
Peripheral Volume of distribution (L)	8.19 (7.7)	
Bioavailability (%)	18 (17.8)	40.2 (3.61)
Mean transit time (h)	1.72 (5.16)	
Number of transit compartments	0.60 (29.6)	
	Residual error (shrinkage%)	
Proportional error (ω2)	0.025 (7.34)	
Additive error (ω2)	3.43 (7.44)	

RSE: residual standard error, IIV: interindividual variation.

Supplementary

SUPPLEMENTARY 1 Details questionnaire.

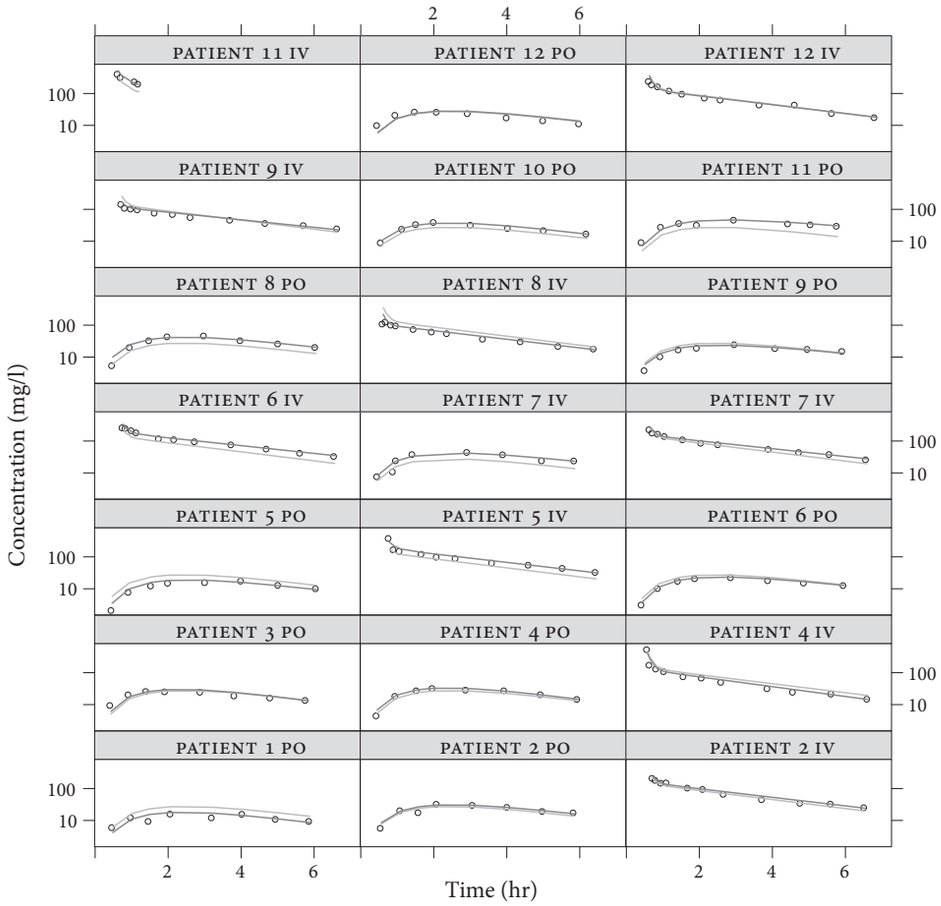
	Frequency		Urgency		Incomplete bladder emptying		Suprapubic pain		Lower back pain		Hematuria	
Patient	B	A	B	A	B	A	B	A	B	A	B	A
1	3	1	2	0	0	0	2	0	1	0	1	0
2	1	1	0	0	2	2	1	1	0	0	3	3
3	2	1	0	0	0	0	1	1	0	0	1	1
5	1	1	1	1	3	3	0	0	0	0	2	2
6	1	1	2	2	0	0	1	1	0	0	0	1
7	2	1	2	2	1	1	0	0	0	0	0	0
8	3	1	3	3	2	2	1	1	0	0	0	0
9	3	3	3	2	3	2	1	0	0	0	0	0
10	0	0	0	0	0	0	2	2	0	0	3	3
11	1	1	1	1	3	3	2	2	0	1	3	3
12	1	0	2	1	1	0	2	0	1	0	1	0

B: before start treatment fosfomycin, A: after start treatment Fosfomycin.

	Dysuria		Fever		General dyscomfort		Impairment daily life		Total score		Quality of life	
Patient	B	A	B	A	B	A	B	A	B	A	B	A
1	2	1	0	0	2	0	2	0	15	2	4	7
2	0	1	0	0	0	1	1	1	8	10	5	9
3	0	0	0	0	0	0	0	0	4	3	4	8
5	2	2	1	1	1	1	0	0	11	11	8	8
6	1	1	0	0	1	0	0	0	6	6	7	7
7	2	0	1	0	2	1	2	1	12	6	4	7
8	0	0	0	0	1	1	1	1	11	9	7	8
9	1	1	1	0	2	1	2	1	16	10	3	7
10	0	0	0	0	0	0	0	0	5	5	4	7
11	1	3	1	1	1	1	2	3	15	19,5	5	5
12	1	0	1	0	2	0	2	0	14	1	5	8

B: before start treatment fosfomycin, A: after start treatment Fosfomycin.

SUPPLEMENTARY 2 **Individual serum concentrations after 3 gram fosfomycin oral and IV. The dark grey line represents patients data, the light grey line represents the predicted concentration based on the pharmacokinetic model.**



CHAPTER 11

RECAPUTILATION AND GENERAL DISCUSSION

The scope of this thesis was to investigate some measures to optimize antibiotic treatment that can be applied to combat the increasing antibiotic resistance. The historical background sketched in the introduction of this thesis showed a timeline, from the time point of the discovery of penicillin in 1928 towards the development of more than 30 antibiotics between 1950 and 1980. However, nowadays, the 'back side', i.e. antibiotic resistance, is considered as one of the biggest threats to global health. At present, the WHO marks antibiotic resistance as a major threat to mankind, ranked along with climate change and terrorism[1]. Several actions have been suggested to tackle the emergence of antibiotic resistance. Cornerstones of an effective strategy to respond to antibiotic resistance include:

1. refining stewardship of existing antimicrobials
2. re-introducing old antibiotics within the framework of antimicrobial stewardship
3. introducing new antimicrobial agents

This thesis focuses on ways to stimulate rational and effective use of antimicrobials, by following the first two action points: (1) refining stewardship of existing antimicrobials and (2) re-introducing old antibiotics within the framework of antimicrobial stewardship.

Refining stewardship of existing antimicrobials

Chapter 2 and **chapter 3** focused on the oral absorption of flucloxacillin. This antibiotic is used specifically to treat infections caused by methicillin susceptible *Staphylococcus aureus* strains, in countries with low prevalence of MRSA, such as in the Netherlands[2]. In case of severe infections flucloxacillin is administered initially intravenously, followed by oral administration. The fraction of flucloxacillin that is absorbed after oral administration is highly variable[3]. Therefore, it is necessary to assure that patients absorb sufficient flucloxacillin, in order to attain efficacy. In case of sufficient absorption, intravenous administration can be switched to oral flucloxacillin. To assess the oral absorption flucloxacillin, therapeutic drug monitoring (TDM) was introduced in LUMC. This test starts after an overnight interruption of intravenous flucloxacillin while the patient fasted. Thereafter, an oral test dose of 1 gram of flucloxacillin is given. Blood samples before and 1 and 2 hours after the oral dose are taken to measure the concentration of flucloxacillin (test A). Though this test worked well, it has the disadvantages of proneness to human error and it is rather laborious. Therefore, we developed an alternative test considering that the therapeutic index of flucloxacillin is wide and hence toxicity at this high concentrations does not occur. This allowed to design a simplified absorption test in which 1 gram of flucloxacillin was administrated orally in the morning with the patient in a common fasted state (eg before breakfast) while the intravenous flucloxacillin was continued, and blood samples were taken before and 1 and 2 hours after the oral dose (test B). To compare the tests a study was performed in 43 patients, data of 19 patients that were previously subjected to test A were compared to data of 24 patients subjected to the new test B. The patient groups were comparable. It was found that the average maximal increase in flucloxacillin levels for both tests were similar, and both tests identified approximately 10% of the patients as poor absorbers. We concluded that the simpler test B yielded similar results compared to the more elaborate test A.

Therefore, the new flucloxacillin absorption test (test B) was introduced in the LUMC and a follow-up study was performed in a larger population (chapter 3). This study was performed in 196 patients receiving intravenous flucloxacillin and for whom oral maintenance therapy was considered. Upon analysis, it was noted that still some old tests A were performed (n=28), but in the majority of the patients (n=168) the new test was performed, Both groups were comparable regarding the baseline characteristics. We found that the average increase in flucloxacillin concentration was 22.0 and 21.5 mg/L for test A and B, respectively. Twenty-six (13%) patients were identified as insufficient absorbers. In this larger study we thus confirmed that both tests yield similar outcomes, and that TDM is indeed needed to assure effective continuation of therapy via the oral route. In conclusion, we advocate the use of the simplified flucloxacillin absorption test. These studies show how rational use of TDM can aid in antibiotic stewardship, and at the same time reduces the chance of infusion errors and prevents an undesirable drop in flucloxacillin concentrations. Thus, it can be used as tool to optimize the use of small spectrum antibiotics, while avoiding rather complicated procedures.

In **chapter 4** we investigated the absorption of oral penicillin, which is known to have large inter-individual and intra-individual variability in bioavailability (4). In the LUMC, an oral penicillin (pheneticillin) absorption test has been integrated in clinical practice. In this thesis, the oral absorption test, executed in 88 hospitalized patients, was evaluated. In brief, patients treated with intravenous penicillin were given an oral dose of 1 gram of pheneticillin in the fasted state and blood samples were taken at 1 and 2 hours. We found that 36% patients absorbed pheneticillin poorly, confirming previous findings and emphasizing the need for TDM in case of switching from intravenous penicillin to pheneticillin. When absorption testing is not available, we advise not to use pheneticillin, but to choose another antibiotic, for instance oral amoxicillin, which is known to be absorbed well.[9-11] With this approach the chance of improper antibiotic use is reduced; it improves patient outcome, reduces the risk on antimicrobial resistance and lastly it reduces medical costs.

In **chapter 5** we evaluated the absorption of the antibiotic rifampicin, a potent antibiotic against a variety of pathogens, including mycobacteria. Rifampicin is mainly used in the first-line treatment of active or latent tuberculosis (TB), due to its high activity against *Mycobacterium tuberculosis*. The worldwide use of rifampicin together with the high risk of developing rifamycin-resistance among all susceptible bacteria is a pervasive concern.[12] In particular, rifamycins are prone to 'endogenous resistance development'[13] resulting from mutations in the target

sites of *Mycobacteria*.[12] This may be aggravated by extrinsic factors resulting in wide-spread resistance of *Mycobacterium tuberculosis* to rifamycins.[14] For the latter, insufficient serum concentrations of rifampin are particularly important in the development of drug resistance.[15,16] Notwithstanding, TDM of rifampin is not part of routine clinical practice. This prompted us to measure rifampicin levels in serum (chapter 5). We conducted a study in 90 patients to measure serum concentrations of rifampin at 0, 3 and 6 hours after drug intake. Furthermore, criteria for interpretation of serum concentrations were established. We found that 63 out of 90 patients (79%) had adequate rifampicin levels in their blood samples drawn at 3 hours after intake. In conclusion, rifampin levels varied but were mostly within the targeted range and a single measurement at 3 hours after intake provided the required information in most cases, indicating that serial measurements could be reserved for specific situations only. We encourage the introduction of a single measurement in clinical practice.

Re-introducing old antibiotics within the framework of antimicrobial stewardship

In addition to improving the use of existing drugs – as described in the first chapters of this thesis – revival of old drugs previously discontinued in routine clinical practice constitutes an opportunity to combat antimicrobial resistance. Polymyxins and fosfomycin are examples of these antibiotics that have barely been used in clinical practice since the seventies of the last century in The Netherlands. After publications about significant side effects – especially nephrotoxicity and neurotoxicity[10] – colistin disappeared from clinical practice in the seventies of the previous century, it was introduced again in first decade of this century due to the need to combat multiple drug resistant organisms. An overview of the potential uses and of the remaining gaps in our knowledge concerning polymyxin E (colistin) is given in **chapter 6**.

At present intravenous colistin is considered as rescue treatment for critically ill patients with infections caused by multidrug resistant Gram-negative bacteria. Further, strict guidelines in terms of antibiotic stewardship have been developed to promote colistin's efficacy and to prevent the emergence of resistance against colistin. No major forms of nephrotoxicity and neurotoxicity, reasons for their earlier withdrawal, have recently been reported.

As before, colistin is currently is mainly used as therapy for the treatment of pulmonary infection by multidrug resistant *Pseudomonas aeruginosa* especially in Cystic Fibrosis patients. Intravenous treatment with colistin is administered in hospitals. To explore whether colistin is suitable for prolonged intravenous administration at patients' homes, we investigated the stability of colistin methanesulfonate (CMS) in **chapter 7**. We found that CMS infusion solution is sufficiently stable for a period of 7 days when refrigerated plus one additional day kept at room temperature. In conclusion, it does appear that CMS is stable for a reasonable time to allow administration of colistin at home, which provides a real benefit for the patient.

Fosfomycin is another antibiotic for which renewed interest is shown. Fosfomycin was discovered in 1969 and is described in detail in **chapter 8**. Fosfomycin has a broad spectrum of activity, including MDR bacteria. In the Netherlands, fosfomycin is currently registered as oral treatment for uncomplicated urinary tract infections. Recently, an intravenous formulation of fosfomycin has been registered for use in the Netherlands.

The oral dose regimen for the treatment of uncomplicated cystitis is unlikely to result effective serum and tissue concentrations for the treatment of complicated systemic infections. To substantiate this assumption, first more detailed information on the pharmacokinetics (PK) of the drug is required. In **Chapter 9** different fosfomycin-dosing regimens were subjected to PK-modelling using surrogate pharmacodynamic (PD) indices to evaluate possible treatment regimens for treatment of systemic infections. Our PK/PD model provided quantitative evidence that a dosing regimen of 6–12 g per day divided in 3 doses is required to obtain effective concentrations in the treatment of systemic multi-drug-resistant bacterial infections.

Chapter 10 describes a prospective clinical trial in patients with recurrent urinary tract infections who were prescribed fosfomycin 3 gram once every three days for at least two weeks. Patients were dosed with 3 gram oral and intravenous administration and blood and urine samples were taken. Based on the finding that fosfomycin urine levels were above an MIC of 8 mg/L for 72 hours, it appears that a dose regimen of 3 gram fosfomycin orally every 72 hours is appropriate to treat patients with recurrent urinary tract infections, who are otherwise unresponsive.

How to combat antibiotics resistance

Now we have explored some cornerstones that could be instrumental in a successful strategy towards the rational use of antibiotics, the following recommendations could be distilled from the results described in this thesis:

- The rules of antibiotic stewardship are essential and must be implemented and maintained in all disciplines of medicine.
- Reliable and easy to use protocols using validated assays for appropriate matrices with a short turn-around time are of great importance to successfully implement therapeutic drug monitoring (TDM) and may overcome practical objections that currently obstruct rational use of antibiotics.
- TDM should involve the assessment of drug concentrations in plasma or serum, and if possible also at the site of the infection
- Assays should measure unbound concentrations of antimicrobials.
- It does seem that personalized antibiotic treatment for patients is best achieved when combining concentration and effect of antibiotics and this should be performed by close collaboration between pharmacies (TDM) and medical

microbiology laboratory (name and MIC of the microorganism), supervised by an antibiotic team that integrates the results and provides therapeutic advice. Our plea is to optimally use the already available infrastructure to implement this.

- More prospective clinical trials, including clinically relevant outcomes, should be performed. The most important reasons that TDM is not commonly used for all antibiotic classes are unclear therapeutic PK/PD targets, the lack of clinical outcome studies and the unavailability of an assay in the hospital.[12-18]
- Both PK studies and PK/PD modeling studies should be performed during drug development for proof-of-concept, for dose and interval selection for clinical trials in humans, to determine susceptibility breakpoints, and evaluation the clinical meaning of antibiotic resistance.[19]

From the results of the studies presented in this thesis, more specific antibiotic-specific recommendations can be generated. We advise the universal introduction in clinical practice of the simple flucloxacillin absorption test that we developed. We advocate to switch from intravenous penicillin to pheneticillin in patients with severe infections after individual proof of sufficient absorption. When absorption testing is not available, we advise not to use pheneticillin, but to choose another antibiotic, for instance oral amoxicillin, which is known to be absorbed well.[4,20,21] With this approach, preferred use of small spectrum antibiotics when available, the chance of improper antibiotic use is reduced; it improves patient outcome, reduces the risk on antimicrobial resistance and, lastly, it reduces medical costs. We also encourage the introduction of a single measurement of rifampin in clinical practice to mitigate the development of resistance to rifampicin during treatment of tuberculosis, a major burden of disease still, particularly in low- and middle income countries.

Old antimicrobial agents have the potential to help combat the result of the emergence of antibiotic resistance, i.e. infections due to multiple drug resistant microorganisms. Colistin can be used parenterally outside the hospital setting, and fosfomycin can be used orally for the treatment of recurrent urinary tract infections, infections which would be difficult to treat without these new regimens applying old antimicrobial agents. It is recommended that re-introduction of these compounds will be based upon population PK/PD models designed for optimal clinical efficacy and for the better prevention of the emergence of antimicrobial resistances due to these therapies.

The third cornerstone of an effective strategy towards antibiotics resistance is the introduction of new agents. Although studies with new antimicrobial drugs have been published[22,23] showing the recent increased activity in the discovery and development of new drugs including plazomicin, a next generation aminoglycoside[23], the pharmaceutical pipeline is quite empty. Although this development possibly marks the beginning of a new era, it can only be part of the solution to overcome resistance to antibiotics.

Last but not least, medical doctors should be more educated in PK/PD and modelling. Extended clinical pharmacology education for medical doctors would give them better insight into dosing recommendations based on modelling and simulation studies, clinical breakpoints and TDM. Therefore, good pharmacology education and education on clinical breakpoints and TDM is highly recommended to improve antimicrobial therapy in clinical practice.

CONCLUSIONS

In conclusion, we conclude that antibiotic stewardship with TDM may assist for the rational use of antibiotics, and may be part of the solution to tackle the problem of increasing antibiotic resistance. Future prospective clinical pharmacological trials are an indispensable part of this strategy. Old antimicrobial agents in combination with PK/PD modeling studies could be helpful to re-use this forgotten antibiotics. Rational use of antibiotics must be on top of the scientific agenda, given the expected disaster of an estimate number of 10 million casualties per year, worldwide in 2050 due to antibiotic resistance.[1]

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NEDERLANDSE SAMENVATTING

INLEIDING

Besmettelijke ziekten zijn van alle tijden. Sinds het ontstaan van de mensheid is zij ontvankelijk voor infectieziekten met ziekte en dood tot gevolg. Pas in de tweede helft van de negentiende eeuw werd duidelijk dat infectieziektes worden veroorzaakt door de aanwezigheid van micro-organismen, zoals bijvoorbeeld bacteriën. Bacteriën kunnen worden bestreden met antibiotica. Sir Alexander Fleming legde in 1928 de basis voor het oudste antibioticum, penicilline. Terwijl hij bacteriën aan het bestuderen was, bleek zijn kweek verontreinigd te zijn met een schimmel (Penicillium fungus). In de buurt van de schimmel groeiden geen bacterien. De schimmel bleek bacterien te kunnen doden. Dit was de basis voor het eerste antibioticum: penicilline. Antibiotica behoren sinds de afgelopen zeventig jaar tot de meest voorgeschreven geneesmiddelen.

Blootstelling aan antibiotica kan ertoe leiden dat bacteriën zich aanpassen en ongevoelig worden. Dit wordt (secundaire) resistentie genoemd. Inmiddels is deze resistentie een wereldwijd probleem geworden. Voor sommige infecties zijn geen werkzame antibiotica meer beschikbaar. In landen waarbij antibiotica zonder recept verkrijgbaar zijn, zijn de resistentiecijfers hoger dan in landen met een strenger beleid, zoals Nederland. Om antibiotica succesvol te kunnen blijven inzetten, is het noodzakelijk om resistentievorming te voorkomen en verstandig om te gaan met bestaande antibiotica.

Bestrijden van resistentie tegen micro-organismes

Hoekstenen van een effectieve strategie tegen antibioticaresistentie zijn:

1. Verfijnen van het *antibiotic stewardship* van bestaande antibiotica
2. Opnieuw inzetten van oude antibiotica binnen het raamwerk van *antibiotic stewardship*
3. Ontwikkelen van nieuwe middelen

1 VERFIJNEN VAN ANTIBIOTIC STEWARDSHIP

De belangrijkste maatregel om resistentievorming tegen te gaan is het verantwoord omgaan met antibiotica. Een risico op resistentievorming wordt vooral veroorzaakt door onjuiste indicatiestelling, een (te) lage dosering of een onvoldoende lange behandelduur. Hierbij bestaat de kans dat bacteriën te beperkt blootgesteld worden aan het antibioticum, wat kan leiden tot resistentievorming. Het geheel van maatregelen om beter met antibiotische behandeling om te gaan wordt ook wel *antimicrobial stewardship* genoemd. Hiermee wordt bedoeld dat patiënten met bacteriële infecties zo krachtig en zo kort mogelijk behandeld moeten worden en dat oneigenlijk gebruik van antibiotica moet worden tegen gegaan. Bij dit soort maatregelen moet men denken aan een juiste indicatiestelling, een adequate dosering, en een verantwoorde duur van de behandeling. De regels van *antimicrobial stewardship* gelden voor alle huidige antibacteriële middelen, maar gelden natuurlijk ook voor nieuwe en herontdekte antibiotica.

2 HERONTDEKKING VAN OUDE ANTIBIOTICA

Een andere optie om resistente bacteriën te bestrijden, is het inzetten van 'vergeten' antibiotica. Omdat bacteriën nog niet recent zijn blootgesteld aan deze vergeten antibiotica, is daar weinig resistentie tegen. Voorbeelden van 'vergeten' antibiotica zijn colistine en fosfomycine. Beide middelen zijn ontwikkeld in de jaren zestig van de vorige eeuw, maar zijn vanwege ongewenste bijwerkingen en/of door de ontwikkeling van andere werkzamere middelen in de jaren zeventig 'op de plank' beland (colistine) of hebben zelfs de markt niet goed bereikt (fosfomycine). Door de toename in bacteriële resistentie, zijn deze twee middelen in de afgelopen tien jaar in hernieuwde belangstelling gekomen.

3 ONTWIKKELING VAN NIEUWE ANTIBACTERIËLE MIDDELEN

Een manier om resistente bacteriën te lijf te gaan is het inzetten van nieuwe antibacteriële middelen. Helaas zijn er gedurende de afgelopen tientallen jaren zowel door de farmaceutische industrie als door academische onderzoeksgroepen weinig nieuwe antibacteriële middelen ontdekt, laat staan ontwikkeld. De oorzaken hiervan lijken voornamelijk financieel, het is lucratiever geneesmiddelen te ontwikkelen voor chronisch gebruik, zoals bijvoorbeeld geneesmiddelen tegen reumatische aandoeningen en hart- en vaatziekten. Beleidsmakers zijn de laatste jaren farmaceutische bedrijven tegemoet gekomen door de toegang tot de markt enigszins te versoepelen. Dit heeft ertoe geleid dat er een klein aantal nieuwe antibiotica ontwikkeld is, waaronder plazocin.

DOEL VAN DIT PROEFSCHRIFT

Het doel van dit proefschrift is om rationaal en effectief gebruik van antibiotica te stimuleren middels:

1. Verfijnen van het antibiotic stewardship van bestaande antibiotica
2. Opnieuw inzetten van antibiotica binnen het raamwerk van antibiotic stewardship.

1 VERFIJNEN VAN ANTIBIOTIC STEWARDSHIP

In dit proefschrift wordt beschreven wordt hoe antibiotica beter ingezet kunnen worden. In hoofdstuk 2 en 3 beschrijven we hoe de zogeheten flucloxacilline absorptietest verbeterd is en zinvol ingezet kan worden. Bij behandeling van patienten met ernstige infecties met methicilline gevoelige *Staphylococcus aureus* (*S.aureus*) wordt gestart met het intraveneus toedienen van flucloxacilline, meestal in doseringen van zes tot twaalf gram per 24 uur. Flucloxacilline is een smalspectrum antibioticum, wat betekent dat het slechts een beperkt aantal typen bacteriën doodt. Een smalspectrum antibioticum is gunstiger dan een antibioticum dat meerdere soorten bacteriën aanvalt, omdat dat over het algemeen zorgt

voor minder resistentievorming. De behandeling van een ernstige infectie met flucloxacilline is veelal langdurig (weken tot maanden). Daarbij is initieel intraveneuze toediening noodzakelijk. Intraveneuze toedieningen vinden doorgaans in het ziekenhuis plaats en zijn veelal belastend voor patiënt en ziekenhuispersoneel en brengt ook risico's met zich mee, zoals een verhoogde kans op bijkomende infecties. De duur van de intraveneuze toediening wordt zo kort mogelijk gehouden; er wordt zo snel mogelijk over gestapt op orale toediening. Daarbij is het noodzakelijk te bepalen of oraal flucloxacilline voldoende wordt opgenomen via de darm en in het bloed komt om effectief te kunnen zijn. Om te onderzoeken of flucloxacilline na orale toediening voldoende in het bloed wordt opgenomen, werd in het Leids Universitair Medisch Centrum (LUMC) gebruik gemaakt van een relatief gecompliceerde test. Bij deze test werd het infuus met intraveneus flucloxacilline stopgezet en moest de patiënt gedurende enkele uren vasten. Daarnaast werd vóór én op 1 en 2 uur na inname van de orale dosis flucloxacilline bloed afgenomen om serumconcentraties van flucloxacilline te bepalen. Omdat er nog weleens fouten gemaakt werden bij het stopzetten en opnieuw aanzetten van het infuus, ontwikkelden wij een eenvoudigere absorptietest. Bij deze test hoeft het infuus niet te worden stopgezet. We onderzochten of deze vereenvoudigde test gelijkwaardig is aan de bestaande test. Het onderzoek werd eerst bij 43 patiënten (**hoofdstuk 2)** uitgevoerd. Vervolgens werd de test officieel ingevoerd en werd het onderzoek herhaald bij 196 patiënten (**hoofdstuk 3)**. In beide hoofdstukken werden de 'oude' en de 'nieuwe' (vereenvoudigde) test met elkaar vergeleken.

Het belangrijkste resultaat is dat ongeveer negentig procent van de patiënten voldoende hoge serumconcentraties flucloxacilline na een orale gift had en tien procent onvoldoende hoge concentraties, dus slechte absorbeerders waren. De uitslagen van de vereenvoudigde test waren vergelijkbaar met die van oorspronkelijke test. Dit resultaat betekende dat de gecompliceerde test goed vervangen kon worden door de vereenvoudigde test. Dit betekent een minder grote belasting voor zowel de patiënt als het ziekenhuispersoneel, geen dalende flucloxacillinespiegel na stopzetten infuus en minder kans op fouten omdat er geen infuus stopgezet en weer aangezet hoeft te worden. Het uitvoeren van deze absorptietest kan dus een nuttige bijdrage leveren aan antimicrobial stewardship.

In **hoofdstuk 4** wordt een andere orale absorptietest, nu die van een oraal penicilline (in ons ziekenhuis, het LUMC, is dat pheneticilline) onder de loep genomen. Pheneticilline is één van de orale preparaten, die afgeleid is van penicilline. Penicilline is een bactericide antibioticum en heeft een zeer smal spectrum en wordt in de praktijk ingezet tegen infecties met streptokokken. Van penicillines is bekend dat er tussen personen grote variatie is in serumconcentraties die worden bereikt na orale toediening. Bij de behandeling van bijvoorbeeld ernstige streptokokkeninfecties wordt gestarte met eerst intraveneus penicilline. Indien de patiënt over kan op orale behandeling is een orale resorptietest beschikbaar. We onderzochten deze orale absorptie test voor pheneticilline om patiënten met onvoldoende absorptie van dit antibioticum te identificeren. Dit onderzoek werd verricht bij 88 patiënten; bij 36% van de patiënten bleek de serumconcentraties na orale toediening onvoldoende hoog om werkzaamheid van het dit orale penicilline-preparaat te verwachten. Alleen bij de patiënten waarbij voldoende absorptie is aangetoond, kan worden overgestapt op oraal pheneticilline. In andere gevallen moet worden gegrepen naar een middel met een breder spectrum om de infectie te bestrijden, wat minder wenselijk is in het kader van antimicrobial stewardship.

Hoofdstuk 5 beschrijft het onderzoek waarbij serumconcentraties na inname van rifampicine gemeten worden bij patiënten. Rifampicine heeft een bijzondere positie in de strijd tegen infectieziekten. Het is een krachtig antibioticum met een zeer breed spectrum en kan dus tegen verschillende bacteriële infecties worden ingezet. Wereldwijd heeft het een soevereine positie bij de preventie en de vaak langdurige behandeling van tuberculose, waar miljoenen mensen aan lijden en sterven. Een groot probleem bij het gebruik van dit antibioticum is de beruchte gevoeligheid voor resistentievorming, onder andere als gevolg van te lage spiegels in het serum. Te lage spiegels worden in de hand gewerkt door te lage dosering en/of beperkte therapietrouw bij langdurig gebruik, zoals bij tuberculosebehandeling noodzakelijk is. Te lage spiegels zijn geassocieerd met een falende behandeling van tuberculose. Om bovengenoemde redenen is het gebruik van rifampicine bij uitstek een kandidaat voor antimicrobial stewardship. Retrospectief onderzochten wij rifampicinespiegels bij patiënten om te onderzoeken of adequate concentraties werden bereikt. Vlak vóór en 3 en 6 uur na orale inname werden serumconcentraties bepaald. De belangrijkste conclusie van het onderzoek is dat een enkele meting op 3 uur na inname liet zien dat 66% dan wel 76% van de patiënten, afhankelijk van het gekozen criterium, een adequate serumconcentratie had. Er is kennelijk maar een enkelvoudige bloedafname nodig om te zien is of er sprake is van een adequate absorptie. Zo'n enkelvoudige bloedafname is makkelijk te implementeren in de klinische praktijk en kan als waardevolle toevoeging worden gezien van het antimicrobial stewardship.

2 HERONTDEKKING VAN OUDE ANTIBIOTICA

In de eerste hoofdstukken van dit proefschrift werd beschreven hoe sommige antibiotica beter ingezet kunnen worden. Om antibioticaresistentie te overwinnen, kunnen ook oude antibiotica ingezet worden die niet of nauwelijks meer gebruikt worden in de klinische praktijk. Colistine en fosfomycine zijn hier voorbeelden van.

Hoofdstuk 6 beschrijft een overzicht van colistine. In de jaren zeventig van de vorige eeuw zijn bijwerkingen van dit antibioticum, zoals nefrotoxiciteit en neurologische afwijkingen gerapporteerd. Samen met de opkomst van andere middelen met minder bijwerkingen is de toepassing van colistine in onbruik geraakt. In dit hoofdstuk is, naast de geschiedenis, de huidige positie van het middel beschreven. Colistine is op dit moment gereserveerd als laatste intraveneus redmiddel bij zeer ernstig zieke patiënten als gevolg van een infectie met een multiresistent

micro-organisme. Daarvoor komen in Nederland met name patiënten met taaislijmziekte in aanmerking, die lijden aan een infectie met een multiresistente *Pseudomonas aeruginosa* die behandeld moeten worden met langdurige intraveneuze therapie. Dat gebeurt meestal in het ziekenhuis.

Hoofdstuk 7 beschrijft een onderzoek naar de houdbaarheid van een specifieke colistine-infusieoplossing; het colistine methanesulfaat. Dit onderzoek werd uitgevoerd omdat een voldoende lang houdbare oplossing de thuisbehandeling van patiënten mogelijk zou maken. We vonden dat de colistine-oplossing een houdbaarheid heeft van 7 dagen op koelkasttemperatuur en dat het vervolgens nog een dag houdbaar is op kamertemperatuur. Dit betekent dat colistine thuis in de koelkast bewaard kan worden. Dit is een belangrijke bevinding om thuisbehandeling met colistine van patiënten met taaislijmziekte verder te onderzoeken. Indien succesvol zou dat voor deze patiëntengroep, waarvan velen al eerder langdurig zijn opgenomen in een ziekenhuis, aanzienlijke winst in kwaliteit van leven kunnen opleveren.

Hoofdstuk 8 is een overzichtsartikel van een ander oud antibioticum: fosfomycine. Fosfomycine werd ontdekt in 1969 en is werkzaam tegen een groot aantal grampositieve en -negatieve bacteriën. Het werkingsmechanisme, de farmacokinetiek, -dynamiek, resistentie en mogelijk synergistische combinaties worden beschreven. Oraal fosfomycine wordt toegepast als eenmalige behandeling bij een ongecompliceerde urineweginfectie. Was het geneesmiddel in Nederland alleen de orale vorm verkrijgbaar, sinds een jaar is ook de intraveneus te gebruiken formulering beschikbaar. Er blijkt, zoals in **hoofdstuk 8** beschreven, nog weinig bekend te zijn over de farmacokinetiek van dit middel.

Om meer over de farmacokinetiek te weten te komen is in **hoofdstuk 9** een farmacokinetisch model ontwikkeld. In dit model zijn verschillende doseringsschema's bij patiënten gesimuleerd. Hieruit blijkt dat een doseringsschema van 6-12 gr oraal per dag verdeeld over 3 doses, werkzame spiegels zou kunnen opleveren. Deze bevinding geeft een richting aan het onderzoek dat in de klinische praktijk moet worden uitgevoerd. Om dit model te toetsen werd het onderzoek zoals beschreven in het volgende hoofdstuk opgezet.

In **hoofdstuk 10** is de farmacokinetiek van fosfomycine bij patiënten met fosfomycine als onderhoudstherapie voor recidiverende urineweginfecties in kaart gebracht. Deze patiëntengroep gebruikt een orale dosering van 3 gram per 72 uur vanwege recidiverende urineweginfectie, met een veelvoorkomende veroorzaker van urineweginfecties, namelijk *E.coli*. Het doel van deze studie was om de serum- en urinespiegels van fosfomycine te bepalen. Het blijkt dat bij alle twaalf de patiënten de spiegels in de urine gedurende drie dagen op voldoende peil bleven. Daarnaast ervaren alle patiënten een verbetering van de kwaliteit van hun leven sinds ze begonnen zijn met deze therapie.

CONCLUSIE

Op basis van de resultaten beschreven in dit proefschrift, kunnen de volgende aanbevelingen worden gedaan:

- De regels van *antibiotic stewardship* zijn essentieel en moeten geïmplementeerd worden in alle disciplines van de geneeskunde.
- Voor succesvolle *therapeutic drug monitoring* (TDM) zijn meer snelle en goede toegankelijke testen nodig.
- TDM wordt uitgevoerd in plasma of serum, maar, -indien mogelijk- zou TDM op de plaats van infectie van toegevoegde waarde zijn.
- Assays moeten de ongebonden fractie van het antibioticum meten.
- Een optimale patiëntgerichte behandeling moet worden nagestreefd, gebaseerd op de resultaten van TDM, uitkomsten van het klinisch chemisch laboratorium en de resultaten van het medisch microbiologisch laboratorium (micro-organisme en MIC).
- Het A-team moet deze data standaard verzamelen en integreren tot een behandeladvies op maat. De infrastructuur bestaat hier al voor.
- Prospectieve clinical trials waar antibioticaspiegels en klinische uitkomst gemeten worden, zijn essentieel, net als het goed definiëren van PK/PD targets.

Verder moedigen we het gebruik van de flucloxacilline, pheneticilline en rifampicine absorptietest aan in de klinische praktijk. Voor wat betreft het opnieuw inzetten van oude antibiotica gelden ook de regels van *antibiotic stewardship*. Daarnaast zullen zowel PK studies als PK/PD modellerende studies moeten worden geïnitieerd om het opnieuw inzetten van middelen te verbeteren. Populatie PK modellering wordt aanbevolen om de dosering te optimaliseren, zoals gedaan in de studie naar fosfomycine.

De derde hoeksteen van het reduceren van antibiotica resistentie is het ontwikkelen van nieuwe antibiotica. Momenteel is er behoudens plazomycine weinig in de pijplijn.

Op basis van dit proefschrift kan geconcludeerd worden dat therapeutic drug monitoring een belangrijke bijdrage zou kunnen leveren aan de strategie om antibiotica resistentie tegen te gaan. De combinatie van adequaat doseren, samen met een rationeel *antibiotic stewardship* voor alle antibiotica in alle disciplines van de geneeskunde, moet goed worden geïmplementeerd en moet hoog op de wetenschappelijke agenda staan.

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CURRICULUM VITAE

Anneke Corinne Dijkmans werd geboren op 23 februari 1974 te Rhenen. Na het behalen van haar VWO-diploma in 1993 begon zij aan de studie geneeskunde in Antwerpen, die zij in 1994 voortzette in Leiden. Gedurende de studie was zij werkzaam als student-assistent bij de vakgroep Celbiologie & Histologie en Anatomie & Embryologie. Wetenschappelijk onderzoek werd uitgevoerd op de afdeling Klinische Epidemiologie (hoofd prof. dr. J.P. Vandenbroucke) onder supervisie van prof. dr. T.W.J. Huizinga. Tijdens haar studie heeft zij klinische stages gelopen op de afdeling Interne Geneeskunde in Truro (Verenigd Koninkrijk), op de afdelingen Heelkunde van het Academisch Ziekenhuis en het Diakonessenhuis in Paramaribo, alsmede bij de medische zending in de binnenlanden van Suriname. Na het behalen van het artsexamen in 2001, begon zij haar opleiding tot klinisch farmacoloog bij het Centre for Human Drug Research. Na de start van de opleiding tot arts-microbioloog in 2006, volgden in 2012 de registraties als klinisch farmacoloog (Centre for Human Drug Research; opleider prof. dr. A.F. Cohen) en als arts-microbioloog (Leids Universitair Medisch Centrum; opleider prof. dr. A.C.M. Kroes). Gedurende 2010-2012 was zij voorzitter van de Vereniging voor Arts-assistenten in het LUMC. Na haar registraties werkte zij in verschillende ziekenhuizen als arts-microbioloog. Zij richtte in 2015 het samenwerkingsverband op tussen het CHDR en de afdelingen Medische Microbiologie, Infectieziekten en Farmacologie/Farmacie van verschillende centra, waaronder LUMC, EMC, UMCG en Haga en dit leidde tot de totstandkoming van dit proefschrift (promotor prof. dr. J. Burggraaf en co-promotor dr. I.M.C. de Visser-Kamerling). Sinds begin 2017 is zij, met een onderbreking waarin dit proefschrift is afgerond, werkzaam als arts-microbioloog bij het Antoni van Leeuwenhoekziekenhuis en Ziekenhuis Amstelland (Atalmedial). Zij is sinds 2017 voorzitter van de Commissie Nascholing van de Nederlandse Vereniging voor Medische Microbiologie.

DANKWOORD

Dit proefschrift zou niet tot stand zijn gekomen zonder de bijdrage van alle betrokkenen.

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