Pharmacokinetic profiling of fosfomycin and nitrofurantoin to optimize the treatment of uncomplicated urinary tract infections

Rixt Wijma

Pharmacokinetic Profiling of Fosfomycin and Nitrofurantoin to Optimize the Treatment of Uncomplicated Urinary Tract Infections

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Pharmacokinetic Profiling of Fosfomycin and Nitrofurantoin to Optimize the Treatment of Uncomplicated Urinary Tract Infections

De farmacokinetiek van fosfomycine en nitrofurantoïne voor de optimalisatie van de behandeling van ongecompliceerde urineweginfecties

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1

Introduction and outline

This introduction provides a concise overview of the global epidemiology of uncomplicated urinary tract infections (uUTIs) and describes how uUTIs are treated in clinical practice. The necessity to treat uUTIs with old antibiotics and its consequences for patient safety, effectiveness of the treatment, and the emergence of drug resistance among uropathogens is described. Furthermore, the current knowledge of the pharmacokinetics (PK) of the two antibiotics that are the focus of this thesis, e.g. fosfomycin and nitrofurantoin, is reviewed. This chapter will be concluded with an overview of the research questions formulated at the start of this project.

Urinary tract infections

uUTIs are the most common infections among women worldwide and typically occur in the community and in first line health care (1–3). The incidence of uncomplicated UTIs is high. Up to 70% of women will experience uUTI symptoms during her life and 30% of women up to the age of 24 years have had at least one uUTI in their lifetime for which antibiotic treatment was required (1, 2). Men are less likely to develop an uUTI because of the different anatomy of the male urinary tract (2). In the Netherlands, uUTI symptoms are the most common reason for women to contact the General Practitioner (GP) (4). The incidence of uUTIs in women visiting Dutch GPs was 7/1000 per year in 2015. The majority of these patients were older than 60 years.

Various definitions of uUTIs are used in the literature. In this thesis, the definition described by Hooton and Gupta is used (5). They define uUTIs by infections in non-pregnant and non-immunocompromised female patients in which only the lower urinary tract regions (figure 1) are involved, and both fever and tissue invasion are absent. The updated guidelines for Dutch GPs for UTIs ('*NHG-Standaard Urineweginfecties*') abandoned the terms 'complicated' and 'uncomplicated' because 'complicated' can refer to the course of the UTI, but also to the increased risk of a complicated course of the UTI (6). Alternatively, the terms 'cystitis' and 'pyelonephritis' are being used. The term 'uncomplicated' by Hooton and Gupta is similar to the term 'cystitis' in the Dutch GP guidelines so these terms can be used interchangeably.

The term 'uncomplicated' implies that this type of infection may not be serious and/or (life)threatening, but this is incorrect. uUTIs may progress to complicated UTIs and eventually to bloodstream infections if they are not treated properly in the first phase of infection. They account for almost 40% of the hospital-acquired cases of sepsis. This percentage emphasizes the importance of optimally treating uUTIs (7, 8).

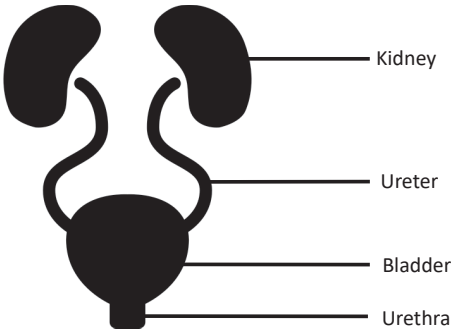

Figure 1. Anatomy of the female urinary tract. Urine is produced in the two kidneys, is transported via the ureter to the bladder and leaves the female body through the urethra. Only the bladder is infected in case of an uncomplicated UTI.

Urinary tract infection in clinical practice

The most common symptoms of uUTI are increased urinary frequency, increased urgency and dysuria (3, 5). It is important to distinguish between uUTIs (cystitis) and complicated UTIs (pyelonephritis or prostatitis in men) before starting the antibiotic therapy because complicated UTIs should be treated longer than uUTIs, but it is difficult to differentiate as they can present with similar symptoms (3). In GPs, culturing (with or without susceptibility testing) of a pretreatment urine sample is often not of added value since the type of uropathogen can be predicted based on epidemiology data or on a previously experienced uUTI, and culture results only become available after the antibiotic treatment has already started. Because UTI symptoms are urgent which requires immediate relieve, culturing only plays a role in confirmation of the UTI, susceptibility testing for epidemiology purposes or mapping the development of drug resistance. The latter may be of particular importance if the patient has been treated before and/or failed on earlier treatment. The Dutch GP guidelines also recommends a urine culture after failure of two empirical courses of antibiotics. In general, further testing is not necessary in female patients with standard uUTI symptoms with no other symptoms that indicate a possible alternative diagnosis (e.g. sexually transmitted diseases and early pyelonephritis) or underlying complicating conditions. A patients description of symptoms via telephone, followed by prescribing an antibiotic might be appropriate in these patients, therefore this is common in clinical daily practice at GPs today (3, 6). If the patients is already in the GP office, a dipstick (e.g. nitrite test with- or without leukocyte and erythrocyte testing) might be helpful to confirm the uUTI (9–11).

Treatment of uUTIs

The treatment strategy of suspected or proven uUTIs is dependent on geographical location. The Dutch GP guidelines recommends the use of nitrofurantoin (50 mg 4dd

of Macrodantin/Furadantin® or 100 mg 2dd Macrobid/Furabid®) for 5 days as the first choice treatment option, followed by fosfomycin (single dose of 3 grams) as the second option, and trimethoprim (300 mg 1dd) for 3 days as the third (6). An extended course of nitrofurantoin or trimethoprim for 7 days is recommended for patients with comorbidities and in pregnant women, while fosfomycin is not recommended in these patients.

German guidelines are comparable to the Dutch guidelines, but they recommend to use nitrofurantoin (Macrodantin®/Furadantin®) 50 mg q6 hours for 7 days instead of 5 days (12). They also recommend the use of pivmecillinam and nitroxoline as other oral treatment options (**Chapter 3.1**), and they specifically mention not to use trimethoprim if local resistance to *E. coli* exceeds 20%. The guidelines of the Infectious Diseases Society of America (IDSA) in collaboration with the European Society for Microbiology and Infectious Diseases (ESCMID) recommend to use either nitrofurantoin (Macrobid®/Furabid®), trimethoprim as a combination Tablet with sulfamethoxazole, or fosfomycin (13, 14). They also suggest pivmecillinam as treatment option in countries where it is available. The Australian clinical guidelines were recently changed and now recommend to use trimethoprim or nitrofurantoin (product is not specified), and cephalexin as an alternative treatment option. The treatment strategy of nitrofurantoin will be discussed in **Chapter 5.2** (15). The Dutch guidelines are the only guidelines that specifically distinguish between first, second, and third treatment option. The other guidelines leave the choice of order to the prescriber. The details of all treatment recommendations are given in table 1 below.

WHY IS DOSE OPTIMIZATION OF OLD ANTIBIOTICS NECESSARY?

Antimicrobial resistance is the development of changing susceptibility of microorganisms (e.g. bacteria) when they are exposed to antimicrobial drugs (e.g. antibiotics) (16). In an era of multidrug resistance, pathogens continue to show increasing resistance rates to many of the commonly used antibiotics (17). This is a worrying situation that increases the risk of being unable to treat infections, such as UTIs effectively with antibiotics. This means that we would go back to an era before antibiotics existed, and infections that we currently consider as easy to treat, may be soon be fatal. The most straightforward solution for this problem would be to develop new antibiotics, but this has proven to be difficult. In general, drug development is a complex, time consuming and costly process with a high degree of uncertainty around drug approval. Pharmaceutical companies are reluctant to invest in the development of new antibiotics because this class of drugs is known for its high investment costs compared to other classes (18,

19). For the time being, no new antibiotics are on the horizon therefore clinicians have to move to other treatment options, including the use of old and 'forgotten' antibiotics registered decades ago (20). In general, old antibiotics are still active but have not been used extensively in recent years due to the development of new antibiotics (21). As such, microorganisms have rarely been exposed to these antibiotics over the last few decades and therefore the process of developing resistance mechanisms has almost not taken place. However, history teaches us that this resistance process can develop fast and that extensive use and misuse of antibiotics in daily practice are the most important drivers for the emergence of resistance (22). It is therefore important to reintroduce old antibiotics in a well-considered way (20).

Table 1. recommended treatment for uUTIs in non-pregnant and non-immunocompromised female patients without fever and/or tissue invasion.

Country	Antibiotic	Daily dose	Duration
The Netherlands	Nitrofurantoin	50 mg q6 h (Furadantin®)	5 days [a]
		100 mg q12 h (Furabid®)	
	Fosfomycin	3 gram daily	1 day
	Trimethoprim	300 mg q24 h	3 days [a]
Germany	Fosfomycin	3 gram daily	1 day
	Nitrofurantoin	50 mg q6 h (Furadantin®)	7 days
		100 mg q12 h (Furabid®)	5 days
	Nitroxoline	250 mg q8 h	5 days
	Pivmecillinam	400 mg q12 h or q8 h	3 days
	Trimethoprim [b]	200 mg q12 h	3 days
IDSA-ESCMID	Nitrofurantoin	100 mg q12 h (Furabid®)	5 days
	Trimethoprim-sulfamethoxazole [b]	160/800 mg daily	3 days
	Fosfomycin	3 gram daily	1 day
	Pivmecillinam	400 mg q12 h	5 days
Australia	Trimethoprim	300 mg q24 h	3 days
	Nitrofurantoin	100 mg q6 h	5 days
	Cefalexin	500 mg q12 h	5 days

[a] 7 days for patients with comorbidities and pregnant women
[b] do not use as first choice if local resistance (for *E. coli*) exceeds 20%

WHAT IS THE CONSEQUENCE OF USING OLD ANTIBIOTICS?

The process of drug development and registration has drastically improved and changed over time. The Food and Drug Administration (FDA) and the European Medi-

cines Agency (EMA) are the institutions responsible for regulating the safe marketing of drugs in the United States and Europe, respectively (23, 24). They ensure that pharmaceutical companies submit a complete registration dossier according to established guidelines. Over the years, novel Chapters have been added to this dossier to include information about (pre-) clinical studies conducted to find the optimal dose, the concentration of the drug to be expected in patients/volunteers, and the response (e.g. desired and toxic effects) (25). These types of studies are also known as dose-finding studies and are key in the process of drug development (23–25). Pharmacokinetic (PK) knowledge of antimicrobial drugs forms the basis of dose-finding studies, and serves as input for pharmacodynamic (PD) experiments in which the effect of the antimicrobial drug on the target microorganism is investigated (figure 2) (25, 26). Together, they are needed to investigate the relevant PK/PD index with the corresponding PD target. The PK/PD index describes the relationship between the effect of the drug on the microorganism, taking into account the changing drug concentration over time. For antibiotics, this relationship can be either time-dependent (time above the minimal inhibitory concentration; T>MIC), or concentration-dependent (maximum concentration over MIC; C_{max}/MIC or area under the concentration-time curve over MIC; AUC/MIC). The PD target indicates either the percentage of time of the dosing interval in which the antibiotic concentration exceeds the MIC, the C_{max}/MIC ratio, or the AUC/MIC ratio for which the effect of the antibiotic is maximized. This will then form the target for treating patients in clinical practice. For the majority of the antibiotic-microorganism combinations, these targets can be found in the clinical breakpoint Tables provided by European Committee on Antimicrobial Susceptibility Testing (EUCAST) (27).

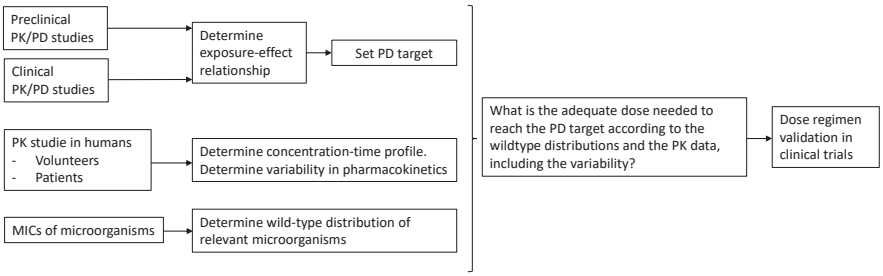

Figure 2. The role of pharmacokinetic data in the process finding the optimal dosing regimen (figure adapted from Muller et. al (25)).

Old antibiotics were registered before this structured process of drug development was mandatory and therefore have not undergone this process (figure 2). This means that neither PK/PD studies, nor dose-finding studies in which PK/PD data served as input were performed at the time of registration (20). Therefore, old antibiotics are

being prescribed in clinical practice to varying patient populations based on limited data, obtained by old-fashioned bioanalytical methods (20, 25). The lack of data on old antibiotics impacts patient safety with regards to the significant risk for inadequate dosing, resulting in an increased occurrence of unwanted side effects and the emergence of resistance among (uro)pathogens (20, 25, 28).

FOSFOMYCIN AND NITROFURANTOIN

Two examples of these old antibiotics are fosfomycin-trometamol (fosfomycin) and nitrofurantoin. They both are narrow spectrum, oral antibiotics indicated for the treatment of uncomplicated urinary tract infections (UTIs) (29, 30). Nitrofurantoin was registered in 1953 and fosfomycin in 1969 for the treatment of uUTIs. Both antibiotics have been used to treat these infections for many years after registration, but have been slowly pushed to the background since the registration of beta-lactam and fluoroquinolone antibiotic classes in the 1970s. These new antibiotics were registered based on complete registration dossiers in which the safety and effectiveness studies were extensively described according to FDA and EMA guidelines. Using these antibiotics was therefore considered to be safer and more evidence based compared to the use of fosfomycin and nitrofurantoin. The marketing that accompanied the registration of these new antibiotics also played an important role in their increasing popularity.

Although the popularity of fosfomycin and nitrofurantoin for uUTIs is increasing today, resistance rates remain low (21, 31, 32). This makes them important candidates for the treatment of (multidrug resistant, MDR) uUTIs, but the risk for emergence of resistance due to extensive, non-PK based and therefore sub-optimal use, also applies for these two antibiotics.

PHARMACOKINETIC STUDIES

Studying the PK of a drug includes investigation of the disposition of the drug throughout the body (33). After oral administration, a drug must first dissolve in the gastrointestinal (GI) tract to be absorbed into the blood circulation. The drug will then be distributed throughout the body, possibly metabolized by enzymes in the liver and/or the GI tract, and leave the body via excretion in urine or feces. The process of absorption, distribution, metabolism and excretion is known as ADME and must be studied during the drug development phase (figure 3). Today, ADME studies are also part of the registration dossier discussed earlier. Each drug has its own PK properties which are closely related to its chemical characteristics. The following paragraphs provide a

concise overview of what has been studied in the field of ADME for oral fosfomycin and nitrofurantoin to date.

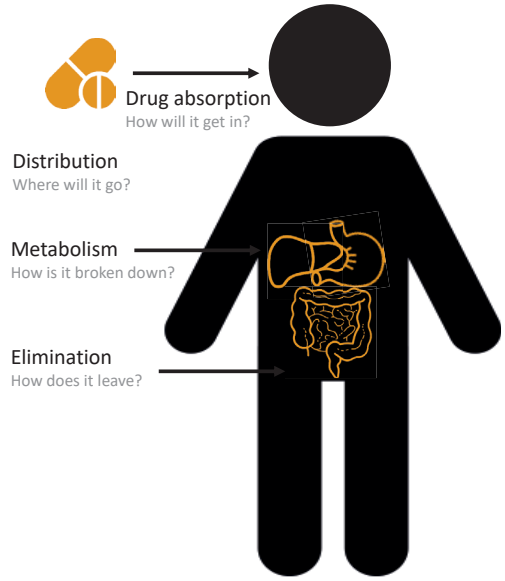

Figure 3. The principles of Absorption, Distribution, Metabolism and Excretion.

FOSFOMYCIN

As discussed, the PK of fosfomycin was investigated in only a few small studies using old-fashioned analytical methods, resulting in poor and outdated PK parameters. The urinary PK was hardly investigated since the majority of these studies focused on the plasma PK. This is quite remarkable, given the fact that fosfomycin is supposed to treat infections in urine.

Chemistry and mechanism of action

Fosfomycin is a small molecule (138.06 g/mol) with a chemical structure of $C_3H_7PO_4$ (figure 4) (34). Its unique chemical structure and mechanism of action explains why cross-resistance with other agents in uncommon (35).

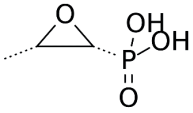

Figure 4. The chemical structure of fosfomycin-trometamol.

Currently, oral fosfomycin is used in its fosfomycin-tromethamol (Monuril ®) form because of a higher bioavailability compared to the fosfomycin calcium salt and the fosfomcyin disodium salt formulations, which were marketed initially (29, 36–38). The antibiotic is also available in intravenous formulations (Fomicyt®) were it is used to treat patients with complicated infections (39). Its spectrum of activity includes both Gram-negative and Gram-positive pathogens including the most important uropathogens, *Escherichia coli (E.coli)* and *Klebsiella pneumoniae (K. pneumoniae),* and extended spectrum beta-lactamase–producing (ESBL) and MDR pathogens (32, 40). Its mechanism of action is based on interfering with bacterial cell wall synthesis by inhibiting several enzymes which are crucial for the synthesis of peptidoglycan, the most important component of the bacterial cell wall, which is crucial for its survival (41).

Absorption, distribution and metabolism

After oral administration, fosfomycin is absorbed from the GI tract and distributed to the kidneys, and in a smaller amount to the bladder wall and the prostate (29, 42). Fosfomycin is not metabolized and leaves the body unchanged via urine and feces. No active tubular secretion is reported so creatinine clearance can be used to guide dose-adjustment decisions in patients with renal impairment (29). The following PK values were reported in the product information of fosfomycin (29): the volume of distribution is 136.1 ± 44.1 L and it hardly binds to plasma proteins. Its bioavailability (F) is ~37%, but this dependents on the feeding status of the patient. Simultaneous food intake decreases bioavailability which eventually leads to decreased urine and plasma concentrations (38). The concentration half-life in plasma is 1.5-2 hours, and maximum concentrations are found after approximately 2 hours and can increase to 4 hours by simultaneous food intake (29, 43, 44).

Table 2 provides an overview of the PK parameters in urine and plasma after administration of fosfomycin-trometamol in the clinically relevant dose of either 3 grams or 50 mg/kg (≈ 3 gram). Nine studies are included in this table, the majority of which dates from the 1980s and 1990s. The study of Segre et al. could be considered as the dose finding study because they also examined doses of 2, 4 and 5 grams of fosfomycin in addition to the 3 gram and 50 mg/kg dose as described in table 2 (45). They concluded that the PK of fosfomycin is dose-dependent, and that the 3 gram dose results in antibacterial activity for at least 2 days based on the time with which urinary concentrations exceed the MIC of the most common uropathogens. Of course, this study has not been conducted according to the current guidelines for dose-finding studies as described above, emphasizing that the use of fosfomycin in current clinical practice is based on outdated studies.

Table 2. PK of fosfomycin-trometamol in urine and plasma after administration of 3 grams or 50 mg/kg.

General information of the study				PK parameters							
				Plasma PK			Urine PK				Analytical method
Reference	Subjects	Dose	Fasting status	C_{max}	T_{max}	F	C_{max}	T_{max}	Recovery	time	
				(mg/L)	(h)	(%)	(mg/L)	(h)	(%)	(h)	
Segre (45)	5 HV (m)	50 mg/kg	-	32.1 ± 0.3	2.2 ± 0.4	58.0 ± 4.0	3178 ± 958	2-4	50.4 ± 5.9	48	MB
	4 HV (m)	3 g		15.6 ± 0.9	3	-	2895 ± 842		31.8 ± 5.3		
Bergogne-Berezin (46)	10 HV (?)	50 mg/kg	-	21.0 ± 6.9	2	-	2000-2750[b]	0-2	25.0	8	MB
Bergan (47)	7 HV (m)	50 mg/kg	-		-		4415 ± 1055	2-4	36.0 ± 6.0	48	MB
Bergan (38)	8 HV (m)	50 mg/kg	f	26.2 ± 2.5	2.5 ± 0.8	40.6 ± 7.9	-	-	-	48	MB
Bergan (48)	12 HV (m+f)	3g	-	21.8 ± 4.8	2.0 ± 0.6	32.9 ± 7.9	1750 (range 1053-3749)	0-2	39.1 ± 6.7	72	MB
Janknegt (49)	7 pt [a]	3g	nf		-		1383 ± 1354 (range 314-4200)	0-12	37.0 ± 15.0 (range 15-60)	84	MB
Zambon® (29)	? HV	3g	f		-		706 ± 466	2-4	38.0	-	MB
Wenzler (50)*	28 HV (m+f)	3g	f	26.8 ± 6.4	2.25 ± 0.4	52.8	1049 ± 867.8	0-4	37.0	48	LC-MS/MS
Wijma (51)*	40 HV (f)	3g	nf		-		1982 ± 1257.4	4.2	47.0	48	LC-MS/MS

HV = healthy volunteers, pt = patients, m = male, f = female, MB = microbiologically, f = fasting, nf = non fasting

[a] elderly patients (>65 years) with impaired renal function. [b] only the range was reported.

Fosfomycin concentrates in urine as typically, urine concentrations are 200-fold higher than those in plasma. Maximum plasma concentrations in healthy volunteers range from 15.6 ± 0.9 mg/L to 32.1 ± 0.3 mg/L, measured 2 to 3 hours after dosing (38, 45–50).

Studies marked with an asterix in table 2 were performed using the novel analytical methods standard for PK/PD research and therapeutic drug monitoring today (50, 51). The remainder of the studies presented in table 2 used data collected via old-fashioned microbiological assays. These methods include mass spectrometry (MS) as detection method combined with (ultra) high performance liquid chromatography ((U) HPLC). In **Chapter 2.2**, the development and validation of an UPLC-MS/MS method is described for quantification of fosfomycin concentrations in urine and plasma. The method described in this chapter is more sensitive compared to the methods reported by others. This offers the possibility to also quantify concentrations in the lower concentration range so that the whole range of minimal inhibitory concentrations (MICs) of the most important uropathogens is covered. This makes the method suitable for PK/PD research purposes aiming for the previously mentioned dose-finding studies.

Excretion

Urine is the clinically relevant matrix in which the PK of fosfomycin should be investigated if it is used for the treatment of uUTIs. Urine concentrations directly represent the concentrations to which the uropathogen is exposed, therefore the efficacy of the treatment can be evaluated based on these concentrations. The number of studies in which urinary PK parameters following a clinically relevant dose were reported, is limited. Table 2 provides an overview of these parameters. Maximum concentrations range from 706 ± 466 mg/L to almost 4400 mg/L, but are highly variable between subjects and are directly influenced by the voiding rhythm of the subject (29, 38, 45–51). In most studies, volunteers were instructed to follow a strict voiding schedule so voiding times were standardized. All C_{max} values were therefore found during the first or the second 2-hour time interval after dosing in all studies. The urinary recovery was found to be approximately 40% and reached a plateau after approximately 48 hours, but Segre et al. found relatively high recovery levels of more than 50% in some of the subjects. This again demonstrates the highly variable PK pattern of fosfomycin between subjects (45).

Effect of renal function on the PK

Renal function, measured by creatinine clearance, directly influences the PK of fosfomycin. This is because fosfomycin does not undergo active tubular secretion (29, 52). Patients with impaired renal function are at risk for sub-therapeutic urinary concentra-

tions because the excretion of fosfomycin in the urine is reduced, resulting in lower urinary concentrations and therefore lower uropathogen exposure. This may lead to less effective treatment, however sufficient research data to validate this hypothesis is lacking. Many randomized controlled trials, investigating the effectiveness of fosfomycin for the treatment of uUTIs, exclude patients with impaired renal function. However, the impact of renal function on fosfomycin urinary PK was investigated by Janknegt et. al in a small study of seven elderly patients with a renal function of 21-65 mL/min (49). Maximum urinary concentrations were found to be relatively low compared to those in healthy volunteers, but these differences were only observed in the first 12 hours after dosing (table 2). Concentrations were even higher in the patients with impaired renal function after 24 hours. There are mixed views on the whether renal function should be accounted for in the dosing of fosfomycin, with some suggesting it as unnecessary, and others suggesting it may be indicated in patients with impaired renal function. However, sufficient evidence to support these suggestions is lacking (53, 54).

PK/PD relation

Most studies report that urine concentrations remain sufficiently high for 24-48 hours after administration of a 3 gram dose. However, what should be considered as 'high' is unclear. 'High' should mean 'high enough to treat the most important uropathogens (PD related) in the majority of the patients (PK related).' Therefore, it is required to know which PK/PD index is relevant for fosfomycin and what the clinical PD target should be. This is still unclear for fosfomycin (26). This also applies to clinical cases in which the uropathogen and its susceptibility to fosfomycin are known as result of additional diagnostic tests.

Much like to the PK data, limited data is available about the relevant PK/PD index with the corresponding PD target for fosfomycin. The studies where this was investigated report conflicting results. Some suggest that fosfomycin has a time-dependent killing pattern, for which the time above the MIC of the uropathogen should be optimized. Others report that fosfomycin exhibits concentration-dependent killing (53). It was even suggested that fosfomycins killing behavior not only differs between species, but also within species. This is because time-dependent killing and concentration-dependent killing was found in different *E. coli* strains (55). It is clear that more research is needed to find the relevant PK/PD index. The first step in this process is to investigate the urinary PK of fosfomycin. This will be discussed in **Chapter 2.1**, in which we present the results of a PK study into the urinary concentrations of fosfomycin, followed during 7 days in 40 female volunteers (51). The fact that concentrations were followed during this long time period and that volunteers were allowed to void freely instead of in a predefined schedule, makes this study unique in a way that the PK results give a good reflection of what one can expect in real patients. The PK data obtained in this study

could serve as the base for PD research presented in **Chapter 4.1**, in which the urinary antibacterial activity of fosfomycin and nitrofurantoin were studied.

Resistance

There are three known mechanisms for the development of resistance (34, 56). The first mechanism includes the inactivation of fosfomycin by cleavage of the molecule by bacterial enzymes. The second mechanism includes modification of the bacterial enzyme murA to which fosfomycin must bind in order to exhibit its antibacterial effect. The third is the mutation of the gene responsible for the expression of the fosfomycin transporter, resulting in an reduced uptake of fosfomycin by the pathogen. Resistance can either be intrinsic or acquired due to exposure to fosfomycin. The first two mechanisms are primary associated with intrinsic resistance whereas the third is usually acquired by pathogens (56). Although the emergence of resistance occurs fast *in vitro*, resistance rates in clinical isolates are still relatively low (e.g 1.4% in the Netherlands in *E.coli* isolates from GP patients in 2017). However, there is an increasing trend of resistance observed in countries where the it is extensively used (31, 34, 57, 58).

Clinical use

In the Netherlands, GP guidelines recommend fosfomycin as a second treatment option. It is only registered for the treatment of uncomplicated urinary tract infections as a single, oral dose of 3 grams. However, it is also used outside the registered label in clinical daily practice (59–61). Clinicians prescribe the fosfomycyin for UTI prophylactically to pregnant women and to male patients with UTIs, as well as to more complex patients at risk for complicated UTIs (such as patients with diabetes mellitus, immunocompromised patients, and patients with renal tract abnormalities). It is also used in children (<12 years) (34). Prescribers usually adhere to the dosage of 3 grams, which likely relates to the fact that the only available formulation is the 3 gram sachet (62). However, prescribers may be inclined to deviate from the single dose.

NITROFURANTOIN

A more detailed overview of the PK(/PD) of nitrofurantoin can be found in the reviews in **Chapter 3.1** and **3.2** (63, 64). This section includes only the highlights of these two reviews. It should be noted that most PK research was performed in the phase of drug development in which nitrofurantoin was used in different, unstandardized, crystal sizes, and it became clear that nitrofurantoin PK was highly influenced by simultaneous food administration. Therefore, the PK data are hard to interpret since no uniform dose, crystal size, formulation, or fasting status was used. Nitrofurantoin is also unstable in

daylight, complicating the PK sampling and quantification of concentrations. Analytical errors are particularly relevant in studies conducted during the era in which analytical methods, such as microbiological assays, were used. Collectively, these factors reduce the reliability and accuracy of the currently published PK data. In this thesis, **Chapter 3.5** is attributed to the development and validation of a UHPLC-UV method to quantify nitrofurantoin levels in plasma and urine.

Chemistry, mechanism of action and dosing

Nitrofurantoin is one of the nitrofuran antibiotics of which several members were used in clinical studies in order to investigate their antibacterial effect in patients, but only nitrofurantoin found its way to the market (65). Its chemical structure is displayed in figure 5 below were the typical furan ring is circled. Nitrofurantoin is a weak acid and is poorly soluble in water (66).

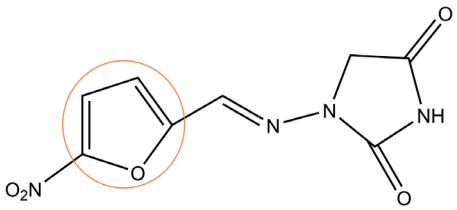

Figure 5. Chemical structure of nitrofurantoin.

Its spectrum of activity is narrow and only includes gram positive aerobe organisms such as *Staphylococcus aureus* and (vancomycin-resistant) *Entercocci*, as well as gram negative aerobes like ESBL-producing Enterobacteriaceae and the most common uropathogen, *E. coli* (30). Its activity is enhanced under acidic conditions (67).

The mechanism of action of nitrofurantoin is not fully understood, but it has been suggested that it has several mechanisms of action all related to the formation of reactive compounds that are toxic for the bacterial cell (68). The multiple mechanisms of action may relate to its low resistance rates and the absence of cases of cross-resistance with other antibiotic classes.

Today, nitrofurantoin is only used in its macrocrystalline form (Macrodantin®/Furadantin®), whereas it was also used in its microcrystalline form until several years ago (30). This crystalline form was abandoned from clinical practice since it was related to more GI side effects. It is also available in several countries in a slow-release formulation (Macrobid®/Furabid®) and oral suspension (69, 70). The Macrodantin®/Furadantin® 50 mg capsule is used as a four time daily dose, and 2 to 4 times daily as a 100 mg capsule, however the preferred dosing regimen of the 100 mg capsule can differ

between countries (71). Macrobid®/Furabid® is registered as a 2 times daily dose. 50-100 mg daily is the registered dose for prophylactic use.

Absorption, distribution and metabolism

There is little research into the PK of nitrofurantoin in clinically relevant dosages and the formulations commonly used today (64). The crystal size of nitrofurantoin highly influences the absorption and excretion pattern (72). After absorption from the GI tract, maximum plasma concentrations in healthy volunteers vary and can range from 0.21-0.45 mg/L after a dose of 50 mg 4dd and from 0.221.26 mg/L after a dose of 100 mg 3dd (73). These concentrations have been shown to be in the same order of magnitude as those found in patients who administered a dose of 50 mg or 100 mg 1dd for UTI prophylaxis (74–78). Nitrofurantoin is distributed to most body fluids and concentrates in the bladder, the only compartment where antibacterial concentrations are reached (30, 79).

Nitrofurantoin is metabolized into several metabolites, some of which might also have antibacterial activity. The full metabolic pathway is not yet known, but it is known that nitrofurantoin is also metabolized by bacterial enzymes (68, 80). This knowledge gap further complicates the interpretation of the published PK data, particularly in quantifying the clinical effect of nitrofurantoin as unidentified metabolites may be partly responsible for the effects observed.

Excretion

After oral absorption and distribution to the body fluids, nitrofurantoin is rapidly excreted in bile and urine (30, 79). The highly variable plasma concentrations are also observed in urine, but are not linearly related to the administered dose, suggesting that the PK pattern of nitrofurantoin is complicated and difficult to predict based solely on the dose.

This conclusion was supported by the results of the only available study in which the PK of nitrofurantoin was investigated after administration of a clinically relevant dose in the formulation we also use today (73). In this study, urinary concentrations were 100 times higher than plasma concentrations and these concentrations did not significantly differ between the two dosing regimens (e.g. 50 mg q6 hours and 100 mg q8 hours). Taken into account all urinary PK studies found in literature, maximum urinary concentrations were found to range from 15 mg/L to 230 mg/L after 3 to 10 hours after administration of macrocrystalline nitrofurantoin (63). Most studies that report urinary PK data only report recovery values, expressed as the amount excreted in urine reported as percentage of the administered (daily) dose. The cumulative recovery in urine of microcrystalline nitrofurantoin over 7 days was found to be 43.6% compared to 35.0% when macrocrystalline nitrofurantoin was administered (63). Recovery is also

influenced by the formulation because the recovery was found to be 33.7% to 47.7% over 24 hours for the slow-release capsule (81). A complete overview of nitrofurantoin urinary PK data can be found in **Chapter 3.1**.

Effect of renal function on the PK

The effect of renal function on the excretion and effectiveness of treatment with nitrofurantoin has been poorly studied and the studies that have been published report conflicting results (82–85). International guidelines discourage its use in patients with estimated Glomerular Filtration Rate (eGFR) <30 mL/min because of the increased risk of toxic effects due to high plasma concentrations and the risk of inappropriate treatment of the UTI due to reduced urine concentrations (13). Reduced eGFR was associated with low urine concentrations and specific for nitrofurantoin, this resulted in reduced time above MIC (82, 83). No consensus has been reached regarding the influence of renal function on the excretion of nitrofurantoin and whether this significantly effects the effectiveness of treatment. The influence of reduced renal function on the clinical effectiveness of treatment with nitrofurantoin is discussed in **Chapter 5.1**.

PK/PD relation

EUCAST and CLSI do report clinical breakpoints for nitrofurantoin, but these breakpoints are not based on PK/PD data obtained with modern analytical methods (64). These breakpoints are therefore not reliable, and further research is needed in order to establish these breakpoints using modern techniques and PK/PD data. It was suggested that the PK/PD index of nitrofurantoin may differ between species: a concentration-dependent pattern was observed for *E. cloacae*, and a time-dependent pattern was found in *E. coli* and *K. pneumoniae* (86, 87). The first step in this process is to investigate the urinary PK of nitrofurantoin will be discussed in **Chapters 3.3** and **3.4**, in which we present the results of a PK study in healthy volunteers and in UTI patients by quantifying urinary concentrations of nitrofurantoin. The PK data of the study described in **Chapter 3.3** served as the base for the previously mentioned PD study, discussed in **Chapter 4.1**.

Resistance

Comparable with fosfomycin, resistance rates for nitrofurantoin among *E. coli* and most other ESBL-producing Enterobacteriaceae are still <2.0%, despite the fact that it is being used extensively as first line treatment for uUTIs (**Chapter 3.2**) (31). Nitrofurantoins popularity may be due to the several unique mechanisms of action, of which at least one is accompanied by formation of reactive compounds which damage the uropathogen at different sites, rendering it unable to repair the several damaged sites at the same time (88–91).

Clinical use

Nitrofurantoins clinical use is extensively discussed in **Chapter 3.1** and **3.2**, and in two-meta analyses based on controlled trials (21, 92). Nitrofurantoin was found to be clinically effective with clinical cure rates of at least 79% (21). A 5-day treatment was found to be as good as a 7-day treatment, but a 3-day treatment resulted in diminished clinical efficacy. Nitrofurantoin was also found to be effective when used for UTI prophylaxis (92). The most feared toxic effects are pulmonary fibrosis and hepatotoxicity which was associated with increased duration of prophylactic therapy. However, a meta-analysis demonstrated that these effects were never reported when using nitrofurantoin for uUTI treatment (21), with only mild and reversible GI side effects reported when nitrofurantoin was used for this indication. When using nitrofurantoin for prophylactic use, severe toxic effects have also been reported to be rare, but the risk of these kind of effects increase when using nitrofurantoin prophylaxis for a longer period of time (92).

AIM AND RESEARCH QUESTIONS

In summary, renewed research using modern analytical methods is needed to obtain the lacking PK data for fosfomycin and nitrofurantoin in order to optimize patient treatment and to minimize the emergence of drug resistance. The aim of this thesis is to provide these missing PK data which can then serve as the base of further PD research to establish the relevant PK/PD index and corresponding PD target. For this purpose, bioanalytical methods for accurate quantification in the relevant biological fluids are needed. This thesis addressed the call of the World Health Organization and European Union for PK knowledge of old antibiotics as part of the European AIDA study (93).

This thesis answers the following research questions:

1. What are the pharmacokinetic properties of fosfomycin and nitrofurantoin?
2. Which (patient specific) covariates influence the pharmacokinetics?
3. How can pharmacokinetic data serve as input for pharmacodynamic studies?
4. To what extend is renal function of influence with regards to treatment effectivity?

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2

Fosfomycin

2.1

High interindividual variability in urinary fosfomycin concentrations in healthy female volunteers

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Category: *Fosfomycin, Fosfomycin trometamol, Pharmacokinetics, Urinary tract infections, Healthy volunteers*

ABSTRACT

Objectives

Fosfomycin is increasingly being prescribed for the treatment of uncomplicated urinary tract infections in an era of emerging drug resistance. Surprisingly, little is known of the urinary concentrations of fosfomycin and its interindividual variation after the standard single 3 gram oral dose. We aimed to gain more insight into urinary fosfomycin pharmacokinetics to evaluate its effectiveness.

Methods

Three grams of fosfomycin trometamol was administered to 40 healthy female volunteers with an estimated mean glomerular filtration rate of > 90 mL/min/1.73m^2. Urine samples were collected from every urination during 48 hours, and then twice daily for up to 7 days. Time, volume and pH were recorded. Concentrations were quantified with UPLC-MS/MS. Effectiveness was evaluated based on urinary concentrations and the target MIC of *E. coli*, the most common uropathogen.

Results

A high interindividual variability was found. Peak concentration was 1982.0 ± 1257.4 mg/L, urinary half-life 12.4 ± 5.7 hours and excretion rate over 48 hours 29.9 ± 7.1 mg/h. Recovery was 44.5 ± 12.6% after 48 h and 47.0 ± 10.4% after 7 days. Concentrations remained above the EUCAST breakpoint of 32 mg/L in 100% of the volunteers over the first 24 h, 67.5% for 48 h and 30% for 72 h. A high urinary output was associated with low urinary concentrations and consequently reduced time > MIC, $AUC_{0\text{-}7days}$/MIC and C_{max}/MIC values.

Conclusions

Considerable interindividual variability observed in the pharmacokinetics of fosfomycin signifies a risk for inadequate drug exposure in a significant proportion of the population. The current dosing regimen should therefore be reevaluated.

INTRODUCTION

Uncomplicated urinary tract infections (UTIs) are the most common bacterial infections among otherwise healthy, premenopausal, non-pregnant women (1). In most cases, these infections are caused by *Escherichia coli* (*E.coli*), but an increased prevalence of infections caused by extended-spectrum beta-lactamase (ESBL) producing *Enterobacteriaceae* and multi drug resistant (MDR) pathogens has been observed, which is a concerning development (2–4).

Oral fosfomycin is gaining more attention as an alternative or even a first line treatment due to the increased incidence of UTIs caused by ESBL-producing or MDR pathogens (2, 5, 6). Clinical studies have demonstrated the efficacy of fosfomycin in the treatment of lower UTI caused by resistant (ESBL-producing) *E. coli* (7, 8). However, only 70 – 85% of the treatments with fosfomycin result in a clinical success (9). One of reasons of treatment failure might be inadequate urinary concentrations and/or a large interindividual variation.

Despite fosfomycin having been used clinically for decades, little is known about its pharmacokinetic (PK) and pharmacodynamic (PD) characteristics. A few small pharmacokinetics studies have been conducted, however none inferred a relationship between urinary fosfomycin concentrations and the effectiveness of the treatment (10–13). Furthermore, no concentrations were measured beyond 72 hours (h) in order to fully describe the elimination process. This is an important limitation since the time-course of urinary drug concentrations directly influence the uropathogen kill-rate and thereby the efficacy of the antibiotic treatment (14). Knowledge of these concentrations (PK) serves as the base of therapy optimization and the prevention of the emergence of resistance (15, 16).

This study aimed to gain more insight into the population distribution of urinary concentrations of fosfomycin to evaluate the effectiveness of the standard treatment based on the expected uropathogen fosfomycin minimal inhibitory concentrations (MIC) .

METHODS

Study design and drug administration

The study was designed as a single center, open label, single dose study in the home setting. Volunteers received a single oral dose of 3 grams of fosfomycin trometamol (*Monuril®, Zambon Nederland B.V., Amersfoort, the Netherlands*). Fosfomycin was administered under supervision during the first visit with a standardized volume of 250 mL water to rule out drug non-adherence. Urine was collected during one week after

fosfomycin administration. No restrictions were placed on food or fluid intake prior to fosfomycin administration or during the study week.

The study was approved by the ethical committee of the Erasmus Medical Center (MEC-2016-121) and registered with EudraCT (2015-005700-28).

Study population

Written informed consent was obtained from all volunteers prior to participation. Inclusion criteria were (1) female, (2) age ≥ 18 years and (3) healthy. Health status was assessed by taking the medical history and an interview, and was confirmed during the first visit before fosfomycin administration by a general blood test. To that purpose, two capillary blood samples of ~0.5 mL each were taken from a finger. Besides creatinine (50-90 μmol/L) also electrolytes and blood counts were checked.

Exclusion criteria included menstruation during the sampling week; known severe renal impairment (defined as eGFR < 30 mL/min1.73m^2); co-medication with any antimicrobial agent within 1 month prior or with metoclopramide; history of intolerance/allergy to fosfomycin; pregnancy or lactation.

Sample collection

Urine samples were self-collected at home from every urination during the initial 48 h, and then twice daily up until 7 days after fosfomycin administration. Urine was collected in a 1000 mL measuring cup, subsequently 1 mL was transferred to a tube (1.5 mL safe-lock, Eppendorf) and immediately stored in a freezer (≈-20°C). A portable cooling box was provided to keep the samples cool when the volunteer was not at home. The volume and time of the urination were recorded in a schedule. Volunteers measured pH of each sample with a dipstick (pH-range 0-14, Boom BV, Meppel, the Netherlands). After one week, all collected samples were delivered to the researchers and stored at -80°C until analysis. Stability of the samples at 18°C, -20°C, and -80°C was confirmed during the method validation (17).

Quantification of fosfomycin in urine

Fosfomycin concentrations in urine were assayed using a validated ultra performance liquid chromatography tandem mass spectrometric (UPLC-MS/MS) method as described in detail elsewhere (17). Samples expected to fall outside the validated concentration range (0.75 to 375 mg/L) were diluted with drug free urine as described before (17).

Pharmacokinetic analysis

Urinary concentrations from each volunteer were plotted against time after administration in a semi-logarithmic graph, from which the maximum concentration ($C_{max,}$ mg/L) and corresponding time (T_{max}, h) were established. The mean population urinary con-

centrations with standard deviation (SD) were plotted against time. The concentration elimination half-life ($T_{1/2}$) was estimated from the individual concentration-time graphs. The fosfomycin excretion (in mg) was calculated by multiplying the urinary fosfomycin concentrations by the volume of urine collected for each urination and calculated for specific time intervals. The cumulative recovery (%) was expressed as percentage of the fosfomycin dose. The urinary output (mL) was defined as the total volume of the produced urine per time interval. Fosfomycin excretion rate (mg/h) was calculated over time periods of 12 h, 24 h and 48 h and calculated from the total amount fosfomycin excreted (mg) divided by the time interval (h).

The influence of the following volunteer characteristics: urinary output, number of urinations, estimated glomerular filtration rate (eGFR), BMI, urinary pH, time/type of the last meal prior to fosfomycin administration and fluid intake on the PK parameters (C_{max}, T_{max}, $T_{1/2}$, excretion and recovery) was explored to explain the interindividual pharmacokinetic variability (IIV). The CKD-EPI equation was applied to estimate eGFR using the mean value of the two capillary creatinine measurements (18).

Pharmacodynamic analysis

PK/PD indices were calculated using GraphPad Prism 7.01 based on individual concentration-time graphs and MICs of 0.5 – 128 mg/L. This range was chosen based on MICs of possible uropathogens (European Committee on Antimicrobial Susceptibility Testing; EUCAST) (19). Effectiveness was defined as the ability to reach adequate concentrations in urine using different measures of PK/PD targets as presented for the EUCAST fosfomycin trometamol clinical breakpoints in the EUCAST rationale document (20).

Safety assessment

Safety evaluations included the collection of volunteer-reported adverse events (AEs) and serious AEs.

RESULTS

Study population

Forty volunteers participated in the study meeting all inclusion criteria. All completed the full sampling week. Their characteristics are presented in table 1. Three cases of diarrhea, two cases of abdominal pain, one case of headache, and one case of dizziness were reported. No serious AEs were reported.

Table 1: Volunteer characteristics and pharmacokinetic parameters presented as population mean and standard deviation (SD).

Characteristics		Mean	SD
Age	(years)	24.3	7.9
Height	(cm)	170.0	6.4
Weight	(kg)	64.1	8.4
BMI		22.1	2.4
eGFR	(mL/min/1.73m^2)*	112.9	72-133**
Pharmacokinetic parameter			
C_{max}	(mg/L)	1982.0	1257.4
T_{max}	(h)	7.5	4.2
$T_{1/2}$	(h)	12.4	5.7

*reported as > 90 mL/min/1.
**range

Sample collection

A total of 891 urine samples with a mean of 22.3 (± 2.9) samples per volunteer were collected. The number of collected samples was dependent of the individual urination rhythm. The pH of the samples was comparable between the volunteers (pH of 5.5 (± 0.5)).

Pharmacokinetic analysis

The mean concentrations for the time intervals are demonstrated in figure 1. As demonstrated in table 1, a high IIV was observed for all PK parameters. A log-linear relationship (R^2=0.95) was found between mean urinary concentration and time after

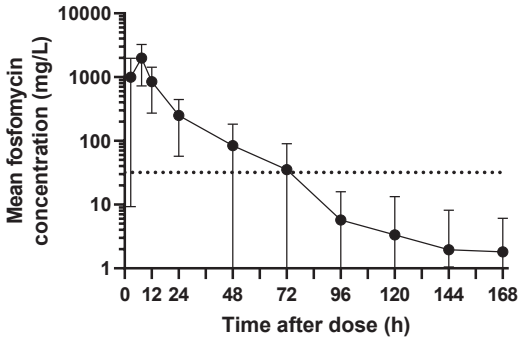

Figure 1: Mean urinary fosfomycin concentration – time curve
Mean concentration-time curve with on the vertical axis the urinary fosfomycin concentration on a log scale and the time after the fosfomycin dose on the horizontal axis. The variability is presented as the SD (vertical bars). The dotted line represents the clinical breakpoint of 32 mg/L for susceptible Enterobacteriacea according to the EUCAST.

dose. The C_{max} obtained in the 2-4 h; 4-6 h; 6-8 h; 8-10 h; 10-12 h and > 12 h time intervals in respectively 23.1%; 23.1%; 20.5%; 7.7%; 7.7% and 17.9% of the volunteers. Urinary concentrations remained above the EUCAST breakpoint of 32 mg/L (dotted line in figure 1) in 100% of the volunteers for 24 h, in 67.5% for 48 h and in 30% for 72 h. Fosfomycin was still detectable (C_{7days}=1.8mg/L) after 7 days in 18% of the volunteers.

The cumulative recovery over 7 days is demonstrated in figure 2. An average of 47.0% (± 10.4%) was excreted over 7 days of which 44.5% (± 12.6%) was excreted over the initial 48 h. In fact, 90% of the excretion occurred within the initial 32 h, 95% within 42 h and 99% within 60 h. Most fosfomycin (36.1%) was excreted during the first 6 h. Table 2 presents an overview of the excretion results. The range of urinary output was similar for day 1 and day 2 (Supplementary data 1).

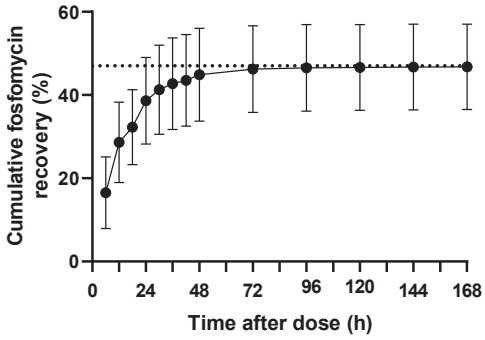

Figure 2: Mean cumulative fosfomycin recovery – time curve
Mean and SD (vertical bars) of the cumulative fosfomycin recovery (%) for the specific time intervals. The recovery reaches a plateau of 47.0% which represents the dotted line.

Table 2: Summary of the excretion parameters presented as population means and SD during the first 48 h after fosfomycin administration

Time interval	Excretion		Number of samples		Excretion rate		Recovery	
(h)	(mL)				(mg/h)		(%)	
0-12	1035	(501)	3.7	(1.3)	71.6	(24.1)	28.6	(9.7)
12-24	802	(451)	2.5	(1.1)	25.0	(12.5)	9.6	(5.0)
24-48	1866	(814)	6.0	(1.8)	7.8	(4.0)	6.3	(3.2)

Volunteers with a high urinary output over 48 h (6386 mL) out of a mean of 13 samples, had a higher recovery (56.9%) and a lower C_{max} (1051.0 mg/L) compared to the mean population (3702 mL; 12 urine samples; 44.5% recovery; C_{max} of 1982.0 mg/L). The recovery was found to be lower (43.4%) and C_{max} was high (3206.8 mg/L) in volunteers with a lower urinary output (1850 mL) out of 10 samples. No relationship

was found between neither the eGFR and the excretion rate nor between one of the other volunteer characteristics and the PK parameters.

Pharmacodynamic analysis

The mean values and ranges of the PK/PD indices are demonstrated in figure 3. An exponential relationship was found between the T>MIC and the MIC values (R^2=0.97; figure 3a). Concentrations exceeded 32 mg/L for 60 h with a wide range from 33.6 h to 111.0 h. Strains with MIC < 16 mg/L would be exposed to concentrations exceeding this concentration for at least 36 h (dotted line). Figure 3b demonstrates that $AUC_{0\text{-}7days}$/MIC values exceed the EUCAST breakpoint value of 3994 for bacteriostasis (dotted

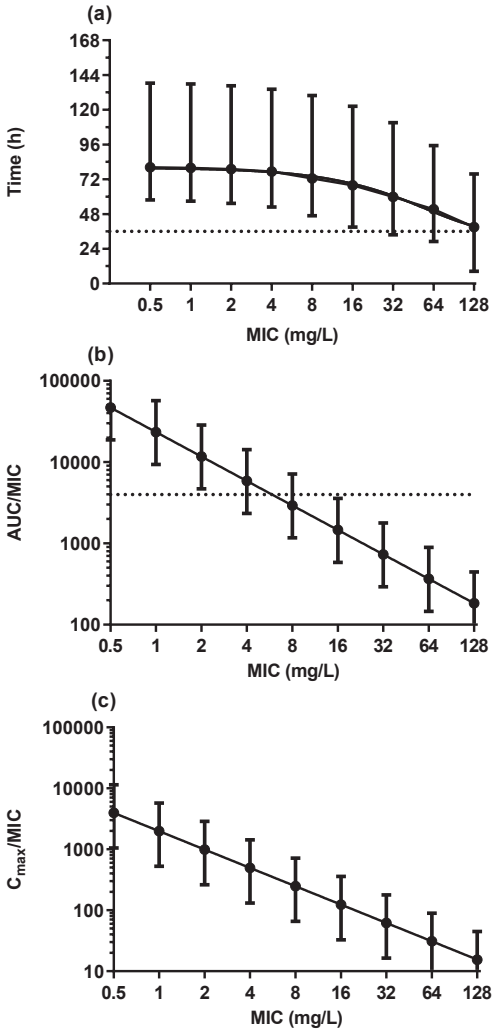

Figure 3: Mean PK/PD index values for *E. coli* MICs

Mean and range (vertical bars) of the T>MIC **(3a)**, $AUC_{0\text{-}7days}$/MIC **(3b)** and C_{max}/MIC **(3c)** for all MICs with the MICs on the horizontal axis and the three different PK/PD indices on the vertical axis. Both axes have logarithmic scale. The dotted line in figure 3a represents the 36 h time point and the dotted line in figure 3b represents the EUCAST AUC/MIC value for bacteriostasis.

line) only for strains with MIC < 2 mg/L (20). Given the observed high IIV in urinary concentrations, effective killing of strains with MICs of 4-8 mg/L is questionable. Strains with MICs of > 16 mg/L are never exposed to concentrations exceeding the MIC so effective killing would be unlikely. When considering the peak concentration, strains with MICs of < 32 mg/L would achieve a minimal average C_{max}/MIC value of 61.9 (16.4-179.1). This is demonstrated in figure 3c.

DISCUSSION

This study provides a full perspective of the urinary PK profile of fosfomycin and its interindividual variability during both the absorption and elimination phases after a single oral dose of 3 grams. PK parameters were calculated based on urinary concentrations of 40 volunteers from 7 days.

The urinary output and C_{max} appeared to be correlated. High urinary output and number of urinations were associated with a lower C_{max} and a higher recovery compared to volunteers with a low urinary output and number of urinations over 48 h. This supports the importance of informing patients on the need to administer fosfomycin after emptying the bladder as stated in the product information in order to minimize the urge to urinate so that C_{max} values could be tripled (21).

C_{max} values in reported studies are comparable with our findings, but T_{max} values were found to be somewhat lower (≈ 4 h) (10, 11, 22–25). Urinary concentrations were followed between 8 h and 72 h in 4-13 volunteers in these studies. This resulted in recovery values ranging from 25% (over 8 h) to 51% (over 48 h).

The presence of the high IIV is our most important finding. This variability might be a reason for the treatment failures in 15 – 30% of the patients (9). It also causes uncertainties in the prediction of the PK/PD indices and therefore in the ability to evaluate the effectiveness of the treatment. Effectiveness was evaluated based on the clinical breakpoint for *Enterobacteriacea* (S) according to the EUCAST (≤ 32 mg/L (20)). The 36 h time point was chosen based on the therapeutic concentration as stated in the product information of fosfomycin (21). Based on these two numbers and the T>MIC as PK/PD index, it was concluded that fosfomycin treatment should be effective for strains with MIC values up to 16 mg/L. The EUCAST clinical breakpoint for *Enterobacteriaceae* bacteriostasis in urine of 3994 was chosen to evaluate the therapy based on the $AUC_{0\text{-}7days}$/MIC value as PK/PD index (20). Recent observations confirm this value (26). When taking the IIV into account, only strains with MICs ≤2 mg/L are treatable with the current therapy. C_{max}/MIC, with breakpoint value 32 mg/L, was also calculated to give a complete view of the PD. Of note, the clinical breakpoints from EUCAST are based

on limited evidence since urinary data for fosfomycin are sparse which is important to keep in mind when interpreting the PD related results (20).

Urinary concentrations were followed during 7 days. This offered the opportunity to study the complete course of both absorption and elimination phases. We have shown that this was of added value since fosfomycin excretion was still not fully completed after 7 days in 18% of the volunteers. This is a strength of this study since previous studies reported PK parameters based on samples obtained after maximum 72 h (10, 11, 22–25). The high number of 40 volunteers strengthens the results of our study. Since the volunteers all collected the samples based on their own urinary rhythm, variation exists in the time points on the horizontal axis in figure 1. This can be seen as a limitation, but we consider this as a strength since it reflects the real world situation.

Although volunteers were instructed to make notes of a possible missed urine sample, we cannot exclude the possibility that one or more volunteers did not report such an event. This could have led to results that are somewhat biased regarding recovery and urinary output results.

Another limitation of our study was that the group of volunteers was relatively homogeneous, impeding exploration of the influence of covariates such as GFR or food intake on the PK parameters. This will be different in the patient population were more variability in these characteristics can be expected. The influence of the urination frequency is important if our results are translated to the clinical situation. A frequent and strong urge to urinate resulting in a high amount of small urine portions is one of the effects of an UTI. Using standard PK equations, the effect of increased urinary frequency can be simulated in a mathematical model. This demonstrates that increased urinary frequency will only slightly increase the C_{max} and shorten the T_{max}, while only decreasing the AUC (which represents total drug exposure in the bladder) by less than 5% (27). These negligible changes in PK parameters will therefore not alter the conclusions regarding the effectiveness of fosfomycin treatment in this clinical scenario. When fosfomycin is administered to an elderly patient with renal impairment the elimination half-life could be longer. However, the reported half-life values are comparable with what is found in the elderly, indicating that the influence of renal impairment falls within the high variation of which we reported (13).

E.coli, the most common uropathogen, is rarely associated with micro-organism induced changes in urinary pH so we consider the influence of pH differences due to infection negligible small (28). On the contrary, pH can influence the PD effect of the treatment, but this was not within the scope of this research.

Our data can serve as a base for in vitro models to investigate the influence of the distribution of urinary concentrations on the killing-rate of pathogens. Hereby, the relevant PK/PD index and the corresponding breakpoints can be found in order to optimize patient outcomes and minimize the emergence of resistance for fosfomycin.

In conclusion, this is the first study in a large cohort monitoring urinary fosfomycin concentrations during one week. This provides more insight into the full PK profile and the effectiveness of the current treatment of UTIs in the population of healthy females. The high IIV and/or inadequate drug exposure can be an explanation for the observed treatment failures in part of the patient population.

Funding

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Transparency declarations

All authors have no conflicts of interest to disclose.

Supplementary data

Figure S1 demonstrates the distribution of the volume of urine during the first and second day of the sample collection week of the volunteers. This figure is available as Supplementary data.

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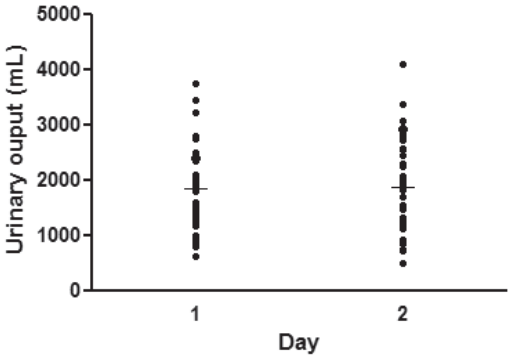

Figure S1: Distribution of the urinary output during day 1 and day 2

Scatterplot of the urinary output during day 1 and day 2 from all 40 volunteers. The mean values of each day (–) are 1836 mL on day 1 and 1866 mL on day 2. These values are not significantly different ($p>0.05$ in Wilcoxon matched pairs test).

2.2

A fast and sensitive LC-MS/MS method for the quantification of fosfomycin in human urine and plasma using one sample preparation method and HILIC chromatography

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ABSTRACT

Fosfomycin is an old antibiotic that is increasingly prescribed because of emergence of the antibiotic resistance and the growing incidence of multi-drug resistant infections. Surprisingly, little is known about its pharmacokinetics (PK) and the pharmacodynamics (PD). Quantification of fosfomycin in both urine and plasma provides insight into the PK/PD characteristics of fosfomycin, which is crucial for the optimization of the therapy and the prevention of the emergence of resistance. An analytical method is therefore needed for the quantification of fosfomycin in both urine and plasma. A fast and sensitive tandem mass spectrometry method in combination with HILIC chromatography for the quantification of fosfomycin with a universal sample preparation method for urine and plasma was developed and validated according to FDA guidelines. The universal sample preparation method only requires 100 µL of a sample, the addition of the internal standard fosfomycin-$13C_3$ benzylamine and an ultrafiltration step. The method is applicable for the concentration range of 0.75 to 375 mg/L (R^2 of 0.9998 in both matrices) encompassing the clinically relevant concentration range based on the susceptibility of possible (uro)pathogens in the clinical setting. The validation results for urine and plasma for all QC levels, were <2.1% and <3.2% for accuracy, <1.5% and <1.7% for within day precision and <5.0% and<3.8% for between day precision, respectively. No matrix effects were encountered and the total recovery in urine and plasma was high (102.5% and 99.4%). Prepared samples were stable at 4°C and 15°C for at least 72 hours and stored samples at -80°C were stable for at least 6 months. Selectivity and sensitivity were confirmed and no carry-over was observed. The method was successfully applied in two pharmacokinetic studies in healthy volunteers and patients respectively.

Keywords

Fosfomycin; Pharmacokinetics/Pharmacodynamics; UPLC-MS/MS; HILIC; Antibiotics

INTRODUCTION

In an era of emerging drug resistance and lack of new antibiotics, old off-patent antibiotics are increasingly being prescribed. Oral fosfomycin has gained more attention as an alternative, or even as first line treatment, for uncomplicated urinary tract infections (UTIs) caused by extended spectrum beta lactamase (ESBL)-producing bacteria (1–3). Oral fosfomycin is also used for the treatment of complicated urinary tract infection in some countries and has been used as prophylactic therapy in prostate resection procedures. Fosfomycin remains active against many multidrug-resistant (MDR) pathogens (4–6). For this reason, intravenous (IV) fosfomycin is now given a more prominent role in the treatment of (critically ill) patients due to the higher prevalence of MDR pathogens (7). The IV formulation of fosfomycin was recently approved in several countries worldwide, thereby it is expected that the use of this administration form will increase over the next few years. Fosfomycin, discovered in the late 1960s, is an old antibiotic agent, specifically suited to the treatment of UTIs (4). The chemical structure of fosfomycin (figure 1a) is unique and not related to any other antibiotic drug: it is small (138 Dalton) and highly hydrophilic.

Urinary or plasma concentrations (PK) directly influence the kill-rate of the (uro) pathogen in vitro and hereby the effectivity of the antibiotic treatment (8). Since resistance rates have dramatically increased over the last few years (9), it is important to investigate the pharmacokinetics of fosfomycin in order to optimize the treatment response (PD), minimize the duration of treatment and minimize the risk of the development of resistant pathogens (10).

Several methods for the quantification of fosfomycin in urine and/or plasma have been developed during the last years. These vary from an older microbiological assay (11), gas chromatography methods (12, 13), a flow injection spectrophotometric method (14) and ion exchange chromatography (15) to the more sophisticated method as high performance liquid chromatography tandem mass spectrometry (HPLC-MS) (16), LC-MS combined with atmospheric pressure chemical ionization (17) and Hydrophilic Interaction Liquid Chromatography (HILIC) (18). They were all successfully validated, but lack the ability to quantitate fosfomycin in the lower range of the clinically relevant concentrations, relating to the wild-type distribution of (uro)pathogens, with a minimal inhibitory concentration (MIC) less than (or equal to) 8 mg/L (19). The MIC is a measure for the susceptibility of the pathogen to fosfomycin. Therefore, concentrations from 1 to 256 mg/L in both urine and plasma should be able to be quantified (19). Earlier published methods using LC-MS/MS are lacking this sensitivity (16, 18). Also, retention times of these methods are long which indicates a longer runtime (8-10 minutes). Only two of these methods are applicable for both urine and plasma samples (12, 18), but they use different sample preparation methods for both matrices. The aim of this study

was to develop a sensitive and rapid ultra performance LC-MS/MS method with HILIC chromatography for the quantification of fosfomycin in urine and plasma.

EXPERIMENTAL

Chemicals and reagents

Fosfomycin was purchased from Santa Cruz Biotechnology Inc. (Huissen, the Netherlands, purity >98%) and racemic fosfomycin-13C_3 benzylamine salt, which was used as the internal standard, was purchased from Toronto Research Chemicals (North York, Canada, purity 96%). Acetonitrile and methanol were both purchased from Biosolve BV (Valkenswaard, the Netherlands) and were of LC-MS quality. Ammonium formate was purchased from Sigma-Aldrich (Zwijndrecht, the Netherlands). The water was purified using a Milli-Q Ultrapure Water System (Merck Millipore, Darmstadt, Germany). Antibiotic free urine was donated just prior to analysis by five healthy volunteers without history of antibiotic use including fosfomycin over the past four weeks. Blank plasma was obtained from drug-free volunteers who donated blood in the blood donation center (Sanquin, Rotterdam, the Netherlands). After donation the blood was centrifuged and plasma was pooled and stored at -18°C prior to analysis.

Solutions

A stock solution of 10000 mg/L fosfomycin disodium salt in Milli-Q Ultrapure water was used to prepare calibration standards in blank urine or plasma at eight concentration levels between 3.75 and 375 mg/L. The stock solution was stored at -20°C. Quality control samples were prepared in the same manner as the calibration standards at concentrations of 7.5 mg/L (QC low (L)), 115 mg/L (QC medium (M)) and 335 mg/L (QC high (H)). Also, a lower limit of quantification (LLOQ) standard was prepared in a concentration of 0.75 mg/L. 100 µL of each standard or quality control sample was transferred to a 1.5 mL safe-lock Eppendorf tube and stored at -80°C until to analysis.

A stock solution of 100 mg/L racemic fosfomycin-13C_3 benzylamine salt was prepared in an ammonium formate/ultrapure water solution (pH 7; 4 mM).This ammonium formate solution was also used in the preparation of the mobile phase (see section 2.4). The stock solution of the internal standard was stored in a refrigerator at 2-8°C and was brought to room temperature before use.

Instruments

The equipment used was a Dionex Ultimate UPLC system which was connected to a triple Quadrupole mass spectrometer with a Heated Electrospray Ionization-probe operating in the negative mode (Thermo Scientific, Waltham, MA). A spray voltage of

4000 kV with a capillary temperature of 250°C and a vaporizer temperature of 400°C were used to produce the parent ions with a mass/charge (m/z) ratio of 137.040 for fosfomycin and 137.021 for the internal standard. With nitrogen used as sheet gas and auxiliary gas, and collision gas pressure of 1.5 mTorr, the product ions with m/z=79.170 for fosfomycin and m/z=79.171 for the internal standard were produced. The fragmentation energies were respectively 26 eV (S-lens of 10 V) and 41 eV (S-lens of 76 V). The UPLC system consisted of a UPLC-pump, an auto sampler with flow through needle injection and a column compartment (all RS 3000 Ultimate). The software programs Chromeleon 6.80 (Dionex, Thermo Scientific), LCquan 2.6.1.32 (Thermo Scientific) and Xcalibur 2.1 (Thermo Scientific) were used for data processing.

LC-MS/MS conditions

A HILIC column (2.1 x 100 mm Acquity UPLC BEH Amide 1.7 µm, Waters, Etten-Leur, the Netherlands), operating at 40°C was used to perform the chromatographic separation. An isocratic mobile phase containing a mixture of the previous described ammonium formate solution in ultrapure water (pH 7; 4 mM) and acetonitrile (20:80, v/v) at a flow rate of 0.4 mL/min. The retention time for both components was 1.8 minutes. The column was intensively preconditioned with the mobile phase for stabilization prior to the analysis.

Sample preparation

The following sample preparation method was applicable to both urine and plasma samples: 100 µL of a sample and the same volume of the internal standard solution were added together and mixed on a shaker for 10 seconds. The mixture was transferred to an ultrafilter tube (Amicon Ultra 0.5 ml Ultracel 10k, Millipore) and then centrifuged at 16.100 g for 5 minutes. Ultrafiltration is a method to determine the free, protein unbound fraction of a drug. 50 µL of the filtrate was mixed with 200 µL of acetonitrile in an auto sampler insert vial (snap ring vial, 32 x 11.6 mm with integrated 0.2 ml glass micro-insert, VWR). 2 µL for urine or 4 µL for plasma was injected into the LC-MS/MS system.

Analytical validation

The following validation parameters were investigated, according to the US Food and Drug Administration guidelines for bio analytical method validations (20):

Linearity

To investigate the linearity of the method, eight calibration standards (table 1) were prepared together with two blank samples (n=2 per concentration). The responses, defined as the ratio between the response of fosfomycin and the internal standard,

were plotted against the theoretical sample concentrations. The determination coefficient (R^2) was calculated and had to be at least 0.995.

Limits of quantification and detection

To assess the ability of the method to quantitate the LLOQ precise and accurate, six replicates of the LLOQ standard (table 1) were prepared where after the mean and the standard deviation of the response ratios were calculated as well as the accuracy and precision. The LLOQ concentration should be below the lowest calibration standard (standard 1), the accuracy between 80%-120% and the precision ≤20%. The lower limit of detection (LOD) was determined by the measurement of five blank samples . The LOD was defined as the mean response plus three times the standard deviation (SD)..

Table 1: Concentrations of eight calibration standards (S1-S8), quality control samples (QC-L, QC-M and QC-H) and the LLOQ standard. The concentrations are the same in both matrices. Standard 3 and 6 are used during routine analysis to prepare the calibration line.

Calibrations standards (mg/L)								Internal quality control samples (mg/L)			
S1	S2	S3	S4	S5	S6	S7	S8	QC-L	QC-M	QC-H	LLOQ
5	15	35	75	150	225	300	375	7.50	110	335	0.75

Accuracy and Precision

Six QC samples of each level (table 1) were prepared in order to investigate the accuracy of the method. The deviation of the measured concentrations compared to the theoretical concentration was calculated. Two types of precision were investigated: the within-day precision by analyzing the 18 QC samples which were also used for the accuracy and the between-day precision by analyzing two QC samples of each level on six different days. For accuracy as well as the precision, the measured concentrations should be within the acceptance criteria of ±15% of the nominal concentration.

Matrix variability and Recovery

The method of Matuszewski et al. was used to investigate the presence of matrix effects (21). Human urine and plasma were collected from five different sources. QC samples of the highest and the lowest concentration (table 1) were prepared in the six different matrices together with blank samples. In total, three sets (A, B and C) of samples were prepared (QC-L,QC- H and blanks). In set B, fosfomycin was added after the ultrafiltration step during sample preparation. These samples were prepared in the five different sources of urine or plasma (2 samples per source; 30 samples in total). The samples for set C, also consisting of 30 samples, were prepared with the standard sample preparation method and the last set (A) was prepared without using urine of plasma. These samples were prepared in ultrapure water (6 samples in total).

All samples were measured with the standard analysis and the responses were normalized for response of the internal standard. Matrix effects were defined as the ratio of the response from the samples in set B and the samples from set A. Recovery was defined as the ratio of the response from the samples in set C and the samples from set A. Process efficiency is the product of the matrix effects and the recovery. Matrix effects, recovery and process efficiency are expressed as a percentage which should be between 80% and 120%. Deviation of the measured concentrations and the theoretical concentrations of the QC samples was required to be ≤15% in all cases.

Stability

To investigate the auto sampler stability and the in-process stability of prepared samples, QC samples of three levels were prepared, analyzed and then stored during 24, 48, 72 and 168 hours in the auto sampler at15°C or at 4°C. The recovery was calculated after each storage time and was compared to the recovery of the same sample after initial testing. Stability of stored samples over 168 hours was tested for three different conditions: room temperature (18°C), refrigerator (4°C) and freezer (-20°C). Six QC samples (n=2 for per level) were freshly prepared at T=0 hours and fosfomycin concentrations were quantified. After 168 hours of storage at the three different conditions, a second set of six QC samples was prepared and analyzed. The recovery was calculated at T=168 hours and was compared to the recovery at T=0 hours. Long term stability of stored samples in the freezer (-80°C) was investigated by the same process after a period of six-months storage had transpired. The recoveries should not deviate more than 10% from each other.

Clinical Validation

As part of two research projects into the PK of fosfomycin in urine, four healthy, female volunteers collected urine samples during 48 hours after they received a single, oral dose of 3 grams fosfomycin. Only females were included since this is the population who are most likely to have a UTI in daily practice.. In a second research project, three plasma samples of six male patients were collected after receiving an oral dose of 3 grams fosfomycin two hours before surgery as part of a prophylactic treatment. Plasma samples were immediately centrifuged and all samples were stored at -80°C prior to analysis. From all samples (urine and plasma), 100 µL was prepared as described in section 2.5.

Both studies were approved by the local ethical committee (MEC-2012-121 and MEC-15-047) and registered with EudraCT (2015-005700-28 and 2015-000626-11). Participation in both studies was voluntary and enrollment occurred after informed written consent had been obtained.

RESULTS AND DISCUSSION

Optimization of the method

Infusion experiments were conducted to determine the m/z values of the parent- and product ions together with the optimal MS settings. Therefore, two separate 1 mg/L solutions of fosfomycin and the internal standard in methanol were directly injected in the MS without chromatographic separation. The optimal MS setting of fosfomycin could be replicated for the internal standard.

This method is unique in the application of the $13C_3$-labeled internal standard. The use of a stable isotope, (C13) labeled internal standard is superior compared to an unlabeled standard, since the $13C_3$-labeled fosfomycin salt is a structure analogue of fosfomycin (figure 1a, 1b). This internal standard is perfectly able to adjust for variations other than those related to those associated with different fosfomycin concentrations, and thereby to improve quantitative detection (22).

This HILIC chromatographic method makes use of a hydrophilic stationary phase which binds hydrophilic compounds so that they are separated from the less hydrophilic matrix. The fact that fosfomycin has a highly hydrophilic character makes this chromatographic method very suitable for the quantification of this compound (23). The sensitivity of a method can be enhanced by increasing the organic content of the mobile phase whereby also the flow rate can be increased due to the reduced viscosity of the mobile phase (24). As the retention time of the analytes can be directly influenced by the pH of the mobile phase, it is of great importance to investigate the optimal contents of the mobile phase regarding the ratio between the organic solvent (acetonitrile), water and the buffer (ammonium formate in ultrapure water, pH 7; 4 mM). The buffer is added to regulate the pH of the mobile phase during the analysis to ensure a reproducible retention time (25). Different buffer concentrations, as well as different pH levels of the buffer were tested in order to achieve an optimal separation of fosfomycin and the internal standard from the matrix components as recommended by Kahsay et al. (23).

Based on recommendations from a previous study with HILIC, buffer concentrations ranging from 2, 4, 5, 10 and 20 mM were tested with a pH of 4, 6 and 9 (26). The optimal appearance of the peak was observed with a buffer concentration of 4 mM and a pH of 6. Unfortunately, the retention time of fosfomycin under these circumstances was greatly reduced compared to a buffer concentration of 20 mM: 1.40 min to 0.98 min, which was slightly more than the column dead time. To increase the retention time, increased concentrations of acetonitrile were tested (26). 75%, 78% and 80% acetonitrile have been tested where after it could be concluded that the optimal peak appearance and retention time were obtained at a percentage of 80% acetonitrile. The retention time was eventually increased to 1.8 minutes so the combination of UPLC

in combination with HILIC chromatography resulted in a shorter run time (4 minutes) compared to other methods (16, 17). This is an advantage regarding the applicability of the method in daily lab routine.

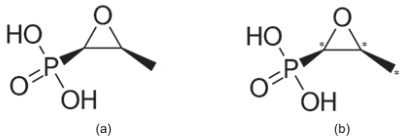

Figure 1: (a) Chemical structure of fosfomycin and **(b)** the internal standard: fosfomycin-$13C_3$. Only one of the enantiomers from the racemic mixture is depicted. The three 13C atoms are marked as C*.

The sample preparation method was initially developed for urine samples and consisted of the addition of the internal standard, an ultrafiltration step and a dilution step with acetonitrile to meet the starting conditions of the UPLC mobile phase. This sample preparation method was found to be applicable to the plasma samples. This is seen as a major advantage over previously developed methods because of its applicability to determine concentrations in both urine and plasma, thereby describing the PK process of drug distribution and elimination, contributing to the optimization of therapy and minimize the risk of emergence of resistance.

Analytical validation

Linearity

The method was successfully validated over a range of 3.75 to 375 mg/L in urine (R^2=0.9998, with a maximum deviation of 6%) and plasma (R^2=0.9998, with a maximum deviation of 5%) with a weighting factor of 1/x. Since the highest reported plasma concentration following oral administration of fosfomycin is 32 mg/L based on an earlier publication, it was expected that this calibration range was sufficient for the clinical plasma samples and no dilution step would be needed in the preparation of these samples (27). Standards 3 and 6 were used as calibration standards when the method was used in routine practice.

Urinary concentrations measuring up to 4000 mg/L were expected from clinical samples based on reports from earlier publications (28–30). Accuracy problems with such a wide range of concentrations were observed during the method development. Therefore, it was determined to validate the method for a smaller range, such that some urine samples would require dilution before analysis. This dilution step is not needed for plasma samples. With this method, all samples could be quantified with one calibration range. Another possible solution for the problem was used by Martens-Lobenhoffer et al. These authors used two calibration lines to cover the whole thera-

peutic range (16). As a consequence, one should estimate the expected concentration of a sample prior to analysis in order to decide which calibration range should be used. As this is usually difficult to predict, unknown samples always have to be prepared in both calibration ranges and thus have to be analyzed twice. The method presented here was developed to quantify fosfomycin concentrations in a specific, and clinically relevant concentration range. Therefore, the decision to validate the method over a smaller concentration range is not a limitation for the applicability of the method because it still covers the relevant concentration range (see also section 3.2.2). Preference was given here to develop the method over a smaller concentration range.

Limits of quantification and detection

The LLOQ, based on five LLOQ standards, was determined as 0.75 mg/L for both urine and plasma. The LOD for plasma was 0.65 mg/L and 0.70 mg/L for urine. The concentration range, together with the low LLOQ and LOD, appears adequate for studying fosfomycin in clinically relevant concentrations, based on the range of MICs of possible (uro)pathogens. This is based on the fact that oral fosfomycin is primary used for the treatment of uncomplicated urinary tract infections caused by gram-negative organisms of which *E. coli* is the most important (31). The concentrations in urine required to exceed the fosfomycin MICs of *E.coli* (uro)pathogens fall within the quantification range of the method, based on data from the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (19). Plasma levels of fosfomycin that are expected in order to treat the infectionare also within the calibration range of this method (32–34).

There were no interfering peaks found in both matrices at the retention times of fosfomycin and the internal standard. The areas in the blank samples were negligible compared to the fosfomycin area in the LLOQ standard. Therefore, the method was found to be selective and sensitive. Importantly, no carry over was observed when analyzing a sample with the highest concentration (calibration sample 8) after a blank sample. A typical chromatogram of fosfomycin (35 mg/L) and the internal standard in urine and in plasma is presented in figure 2.

Accuracy and Precision

The accuracy and precision data are shown in table 2. All calculated values were within the acceptance criteria of ±15% of the mean concentrations. Therefore, the within-day and the between-day variability of the method is low.

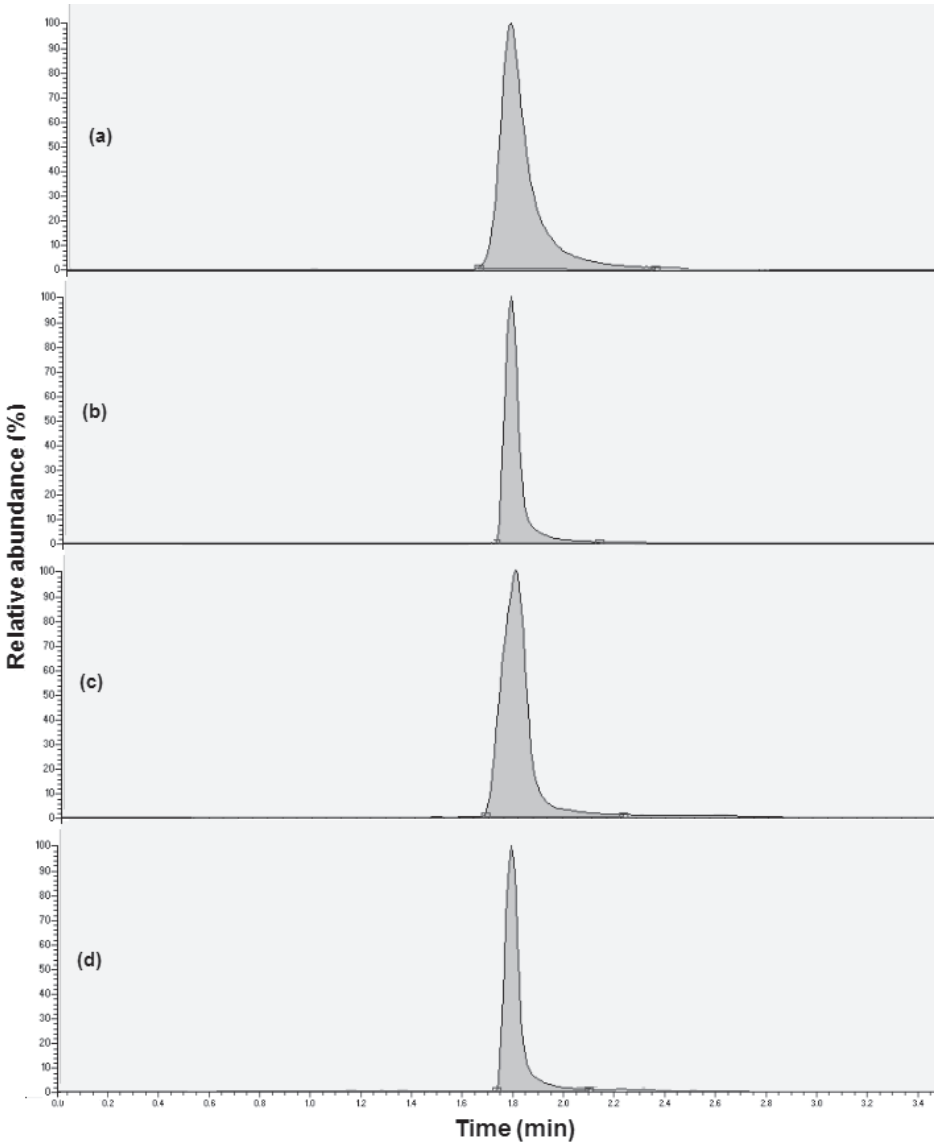

Figure 2: Chromatograms of fosfomycin in urine (a) and plasma (b) and the internal standard in urine (c) and in plasma (d) after injection of calibration standard 3.

Table 2: Accuracy and within-day and between-day precision was tested. The values represent the deviation of the measured concentrations compared to the theoretical concentrations, expressed as a percentage of the theoretical concentrations. This value should be within the acceptance criteria of ±15%.

Matrix	Sample [a]	Accuracy (%) [b]	Within-day precision (%) [b]	Between-day precision (%) [c]
Urine	QC-L	-0.5	1.5	4.2
	QC-M	-2.1	0.5	5.0
	QC-H	0.0	1.1	1.0
Plasma	QC-L	3.2	1.7	3.8
	QC-M	1.9	0.8	1.2
	QC-H	0.7	1.2	1.1

[a] The sample concentrations are: QC-L = 7.50 mg/L, QC-M = 115 mg/L and QC-H=335 mg/L.
[b] Values are means and based on six QC samples for each level measured on one day.
[c] Values are means and based on two QC samples for each level measured on six different days.

Matrix Variability and Recovery

Matrix effect, recovery and process efficiency of fosfomycin in urine and in plasma are presented in table 3. No ion suppression and/or enhancement effects due to compound of the matrices was observed. Recoveries were high in both urine and plasma for both QC levels. This is remarkable for plasma samples since low recoveries were reported before (68% compared to 99.4% here) (18). This also resulted in higher LLOQ limits in urine (100 mg/L) and in plasma (1 mg/L) compared to this method (0.75 mg/L) (18). Ultrafiltration appears to result in a higher recovery compared to protein precipitation during the sample preparation. This can be explained by the fact that protein binding of fosfomycin is negligible (35) so the concentrations were not significantly influenced by the ultrafiltration step.

Table 3: Matrix effect, recovery and process efficiency of fosfomycin in urine and in plasma. The presented values, all expressed as a percentage, are corrected for the internal standard and obtained when using five different sources of urine and plasma. 'Diff' represents the deviation of the measured concentration compared to the theoretical concentration of the QC samples and should not exceed 15%.

	Matrix effect (%)				Recovery (%)				Process Efficiency (%)			
	QC-L	QC-H	mean	Diff	QC-L	QC-H	mean	Diff	QC-L	QC-H	mean	Diff
Urine	85.0	104.0	94.6	14.0	103.0	102.0	102.5	0.5	88.0	106.0	96.9	13.5
Plasma	93.0	102.0	97.4	6.1	100.0	99.0	99.4	0.2	93.0	101.0	96.8	5.9

Stability

Stability data of prepared samples and stored samples are presented in table 4. All QC levels for the prepared urine and plasma samples were stable for at least 72 hours with the exception of the QC-L sample in urine at 4°C. This sample was stable for at least 48 hours. No further time points were tested for the samples, but it is expected that the samples at both temperatures will have a longer expiration time than 72 hours. This is based on additional tests at 4°C were the QC-M and QC-H samples showed stability up to 168 hours in both matrices (data not shown). However, stability of 72 hours was found to be sufficient in the lab and did not provide practical problems. All stored urine samples are stable at each condition for one week and even for six months at -80°C. Plasma samples showed to be a little less stable since recoveries slightly decreased

Table 4: Stability data of the assay where auto sampler (15°C) stability and in-process (4°C) stability was tested for prepared samples. Stability of stored samples was tested at 18°C, 4°C and -20°C during one week and at -80°C during six months. The values represent the mean recovery (%) of two QC samples of each level.

Matrix	Condition	Time	QC-L	QC-M	QC-H
Urine		24h	98.4	98.3	99.5
	15°C	48h	101.6	98.4	99.7
		72h	100.9	98.1	100.7
		24h	103.9	100.3	99.1
	4°C	48h	107.5	98.8	100.6
		72h	236.6	95.8	98.2
	18°C	1 week	102.3	99.3	98.2
	4°C	1 week	103.0	100.4	100.8
	-20°C	1 week	99.1	98.1	98.3
	-80°C	6 months	101.1	92.4	93.8
Plasma		24h	105.1	101.1	100.2
	15°C	48h	100.5	99.1	100.6
		72h	100.2	100.0	109.7
		24h	102.7	100.0	101.3
	4°C	48h	97.9	89.5	99.3
		72h	101.8	98.3	100.2
	18°C	1 week	89.1	87.4	87.8
	4°C	1 week	102.3	88.3	89.0
	-20°C	1 week	101.4	90.7	101.8
	-80°C	6 months	94.0	99.6	98.8

to <90 % at -18°C for all QC levels and at 4°C for QC-M and QC-H. Stability for six months at -80°C was considered to be the most important finding since QC samples and calibration standards, which are used during every analysis, are stored at -80°C.

Clinical validation

Concentrations in urine of four volunteers and in plasma of six patients are presented in figure 3a and 3b. No interfering peaks were found from the matrices. Also, distortion of the chromatograms by co-medication was not seen. The maximum urinary concentrations ranged from 1050.3 mg/L to 4378.9 mg/L and maximum plasma concentrations ranged from 17.5 mg/L to 47.7 mg/L. All concentrations were comparable with those found in previous studies (28–30, 36–38).

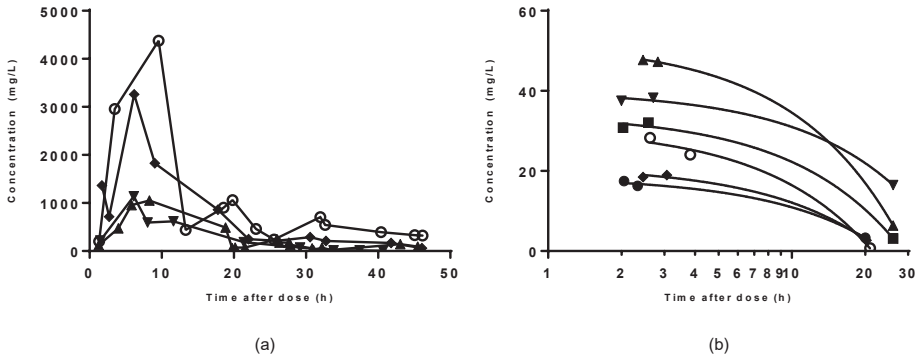

Figure 3: (a) Urinary concentration – time curves of four healthy, female volunteers after receiving 3 grams of oral fosfomycin at T=0 h. A sample was collected from every urination in the following. **(b)** Plasma concentration – time curves of six male patients after receiving 3 grams of oral fosfomycin. The horizontal axis is presented on a logarithmic scale. Each symbol represents one subject.

The presented method was developed to quantify fosfomycin concentrations in the range achieved clinically and could also serve as a method for therapy optimization (19). Since this method is more sensitive compared to methods described earlier, it appears to be more suitable for monitoring the extent to which the (time above) MIC is achieved. Therefore, distinctions can be made in particular for strains with relatively low MIC values, such as *E. coli*. This is an important advantage since regrowth of bacteria is suspected to be correlated to low urinary concentrations. This highlights the importance of monitoring of these concentrations in order to optimize patient outcomes and to minimize the risk of emergence of resistance. The method may therefore serve as the basis of more individualized therapy with fosfomycin rather than the 'one size fits all' strategy which is currently used (39).

CONCLUSIONS

To the best of our knowledge, this is the first method suitable for the quantification of fosfomycin in urine and in plasma using one sample preparation method that includes an isotope labeled internal standard. The method is highly sensitive allowing quantification of fosfomycin concentrations in the clinically relevant concentration range. This makes the method applicable for optimization of both oral and IV therapy with fosfomycin. The use of ultra performance chromatography instead of high-pressure chromatography, offers the possibility to use a short retention time and therefore a shorter total runtime. The method has proven its applicability in daily clinical practice, and has been used in two clinical studies in which fosfomycin concentrations were quantified in urine and plasma samples.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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3

Nitrofurantoin

3.1

Review of the pharmacokinetic properties of nitrofurantoin and nitroxoline

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ABSTRACT

Nitrofurantoin and nitroxoline are oral antibiotics for the treatment or prophylaxis of acute urinary tract infections. New interest in both drugs is increasing because of the emergence of resistance to other antibiotics, but knowledge of their pharmacokinetics (PK) is lacking since they were developed before the advent of standardized research for drug approval. The aims of this review were to (1) summarize the PK data reported in literature and (2) to identify PK knowledge gaps. The current body of PK knowledge of both drugs appears to be poor and mainly based on old studies. Nitrofurantoin PK values were obtained from studies using many variables, *e.g.*, formulations, crystal sizes and analytical methods, resulting in high inter-individual variability in PK parameters and no uniform PK profile. Clinical experience and PK data for nitroxoline are even more limited since the drug is registered in only Germany and a few (eastern European) countries. Clinical studies in relevant patient populations are needed with commercially available nitrofurantoin and nitroxoline formulations at approved dosing regimens to more fully characterize their PK profiles and to investigate the influence of patient characteristics on these profiles in order to optimize efficacy and avoid toxicity and emergence of resistance. Only with this updated knowledge and efficacy data from well-structured trials can both drugs maintain their antimicrobial activity against uropathogens.

INTRODUCTION

In an era of increasing multi-drug resistance, old antibiotics such as nitrofurantoin and nitroxoline are gaining renewed interest as oral therapeutic or prophylactic agents for acute urinary tract infections (UTI) (1–4). Nitrofurantoin was approved by the FDA in 1954 and has been in clinical use ever since in many countries.(5) It is currently recommended as first-line UTI therapy because of the emergence of resistance to other antibiotics such as cotrimoxazole, trimethoprim, the fluoroquinolones, and amoxicillin. (2, 6) Nitrofurantoin is mainly bacteriostatic, but can also have a bactericidal effect when present in high concentrations (≥2x MIC) (7, 8). Nitroxoline has been used since 1962 for the treatment and prophylaxis of acute and recurrent UTIs caused by *Escherichia coli* in children and adults (9, 10). It is recommended by some for catheter-related infections because of its anti-biofilm properties (11–13), but is available only in Eastern European countries as well as Germany, where resistance among *E. coli* still seems to be rare (12). This fact makes nitroxoline a promising candidate as an oral treatment option for UTIs in more countries worldwide. Where nitrofurantoin proved to be effective in the treatment and prophylaxis of UTIs, the clinical effectiveness of nitroxoline has not been convincingly demonstrated (14–16). Though several *in vitro* studies have demonstrated its activity against uropathogens and a meta-analysis of four clinical studies demonstrated comparable efficacy when nitroxoline was compared to cotrimoxazole or norfloxacine, well-structured randomized clinical trials to prove its early effectiveness in UTI are lacking (9, 11, 17).

In general, for old antibiotics such as nitrofurantoin and nitroxoline, crucial data on the pharmacokinetics (PK) in relevant matrices such as plasma and urine are sparse or even lacking (18). This is an important limitation since the consumption of nitrofurantoin and nitroxoline has increased and because optimizing PK improves patient outcomes and minimizes the risk of emergence of drug resistance (19, 20). Knowledge about the PK properties is also needed as input for *in vitro* pharmacodynamics (PD) models in order to investigate the effect of the antibiotic concentrations obtained in human subjects on a pathogen and thus to establish clinical breakpoints.

We aimed to map the size of this knowledge gap by giving an overview of the papers wherein PK data on both commercially available and unavailable formulations of nitrofurantoin and nitroxoline were mentioned. We selected digital as well as publications on paper in which PK parameters describing the absorption, distribution, metabolism and elimination of nitrofurantoin or nitroxoline were reported after oral administration in volunteers or in patients. The chemical and pharmacological properties of both nitrofurantoin and nitroxoline will be described, followed by the different analytical methods, which are used in the reviewed papers. The major part of this review will be devoted to nitrofurantoin; nitroxoline will be discussed in the last section.

CHEMISTRY, MECHANISM OF ACTION AND CURRENTLY USED DOSING REGIMENS

Nitrofurantoin (figure 1a) is a member of the nitrofuran family. The defining structural component is a furan ring (five-membered aromatic ring with four carbon (C) atoms and one oxygen (O)) directly connected to a nitro group (-NO_2). Nitrofurantoin is commercially available as capsules containing 50 mg or 100 mg of the macrocrystalline form of nitrofurantoin, which is also available as slow-release formulation and as a suspension (21–23). Microcrystalline nitrofurantoin is still available, but is not a first-line product because of its higher rate of gastrointestinal (GI) side effects. The standard dose of nitrofurantoin depends on the indication and on the geographical location: regimens of either 50-100 mg q6h (regular-release formulation) or 100 mg q12h or q8h (slow-release formulation) are prescribed for the treatment of acute UTI, while 50-100 mg q24h is prescribed for prophylactic use. Both nitrofurantoin and its metabolites have antibacterial activity, which is enhanced under acidic conditions (24–26). Metabolites are formed by reduction by bacterial enzymes, but the exact structure and antibacterial activity of each metabolite is still unclear (24, 27). The spectrum of activity includes (vancomycin-resistant) enterococci and ESBL-producing Enterobacteriaceae with the exception of *Pseudomonas aeruginosa* and several *Proteae* strains because they carry intrinsic resistance for nitrofurantoin (8, 28, 29). Resistance among *E. coli* and most other ESBL-producing Enterobacteriaceae to nitrofurantoin is still low, likely because nitrofurantoin has a different mode of action compared to other antibiotics and has multiple mechanisms of action (30)(14).

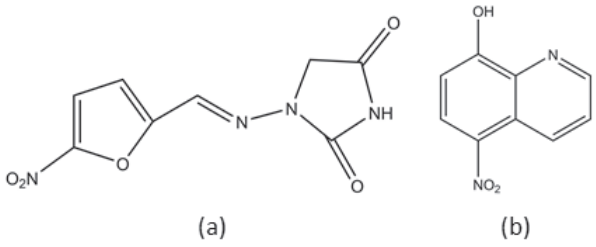

Figure 1: the chemical structures of nitrofurantoin **(a)** and nitroxoline **(b)**.

While its name may suggest the opposite, nitroxoline (8-hydroxy-5-nitroquinolin) is not a member of the nitrofurans. This hydroxychinolin derivate is considered structurally unrelated to any other drug class (figure 1b), and is available as soft capsules containing 250 mg nitroxoline. The standard dose is 250 mg q8h (12). Nitroxoline's mechanism of action is based on the chelation of cations resulting in the inhibition of bacterial adhesion to the bladder epithelial cells, which in turn results in a bacteriostatic effect

that is enhanced under acidic conditions (11, 31, 32). Its spectrum of activity includes *E. coli*, (multidrug resistant) *Staphylococcus aureus* and *Acinetobacter baumannii* strains (13). Resistance rates of *E. coli* to nitroxoline are still reported to be low, probably because of the drug's low prescription rates (12).

ANALYTICAL METHODS

Nitrofurantoin

Since nitrofurantoin is sensitive to photochemical degradation, it is important to take the analytical method together with the corresponding sample preparation method into account when interpreting the reported concentrations of nitrofurantoin (33).

The first method for nitrofurantoin detection in urine was reported by Bender, Nohle and Paul in 1956 (34). The paper describes the chromatographic separation followed by colorimetric or spectrophotometric detection. The majority of the papers included in this review, however, used the spectrophotometric method of Conklin and Hollifield, described in 1965 (35). This method is applicable for quantification of urinary concentrations, but no distinction was made between detecting different metabolites. The method uses a liquid-liquid extraction for preparation of the samples followed by spectrophotometric detection. An updated version of this method was developed by Mason *et al* (36). Nitrofurantoin was separated from the urine matrix with HPLC. From that moment on, more HPLC methods with UV detection were developed for nitrofurantoin alone or for the quantification of the amino and the cyano metabolite in both plasma and urine (37–39). All these methods took the photochemical degradation of nitrofurantoin into account. The only method for which the photochemical degradation was not mentioned specifically was the method of Arancibia *et al*. for the quantification of nitrofurantoin and its toxic metabolite in urine (40). The most recently developed method consists of HPLC with MS detection for nitrofurantoin in plasma; amber-colored vials are used in order to protect the nitrofurantoin content from light (41). It appears that concentrations can be well quantified using UV detection since most PK paper report concentrations using this detection method (tables 1 and 2) and concentrations in urine are relatively high. MS detection therefore does not seem to be specifically necessary to determine nitrofurantoin concentrations. However, MS detection may be of added value in the elucidation of nitrofurantoins metabolism pattern since these concentrations may fall out of the UV detection range.

Nitroxoline

When considering the nitroxoline concentrations, one should keep in mind that about 99% of the excreted nitroxoline is eliminated in the urine as (conjugated) metabolites

Table 1: The PK parameters of nitrofurantoin in **urine**.

Reference	Subjects	Drug information		Fasting status	PK parameters						Analytical method
		Crystal size	Formulation		C_{max}	T_{max}	Recovery		Max. excretion rate		
					(mg/l)	(h)	(%)	(h)		(h)	
Multiple dose: 4x50 mg (therapeutic use)											
Lippman (68)	9[a]	micro	tablet	nf	0-230	5-10	-		-		Chrom. + UV
Multiple dose: 4x100 mg (therapeutic use)											
Lippman (68)	6[a]	micro	tablet	nf	60-230	10-15	-		-		Chrom. + UV
Jawetz (61)	3[a]	micro	tablet	-	15-60[b]		-		-		Micro. [e]
Conklin (47)	10	micro	*capsule*	nf	-	4-8	42.7	24	14.1 mg/h	4-8	LLE + UV
						4-8	43.6	24	15.4 mg/h	4-8	
		macro	capsule	nf	-	12-24	37.9	24	10.5 mg/h	4-8	
						12-24	35,0	24	9.9 mg/h	4-8	
Single dose: 1x50 mg (prophylactic use)											
Meyer (54)	14	micro	tablet	f	-	-	15-39	12	1.3-3.3%/h	-	LLE + UV
Hoener (66)	6	micro	tablet	f	132	1,8	-		-		HPLC + UV
				nf[c]	50	2,8					
Gröning (58)	4	micro	tablet	f	-	-	34.5	8	-		Polarogr.
Meyer (62)	14	micro	4 tablets	f	-	-	34.3-42.1	23	13.4-17.9 %/h	1.4-2.3	HPLC + UV
Single dose: 1x100 mg (prophylactic use)											
Meyer (54)	14	micro	tablet	f	-	-	25-38	12	2.1-3.2%/h	-	LLE + UV
Meyer (62)	14	micro	3 tablets	f	-	-	31.6-33.7	23	11.1-11.7 %/h	2.0-2.6	HPLC + UV

Table 1: The PK parameters of nitrofurantoin in **urine**. (*continued*)

Reference	Subjects	Drug information		Fasting status	PK parameters						Analytical method
		Crystal size	Formulation		C_{max}	T_{max}	Recovery		Max. excretion rate		
					(mg/l)	(h)	(%)	(h)		(h)	
Paul (46)	15	micro	tablet	nf	151	3	36.1	24	10 %/h	3.6	Chrom. + UV
		macro	4 capsules		83-159	3.4-5.5	19.6-35.4	24	4.2-8.9 %/h	3.6-4.9	
Stoll (53)	4	micro	suspension	nf	-	-	14.8	24	-	3,6	Chrom. + UV
Albert (48)	10	micro	tablet	f	-	-	56.7	24	-		LLE + UV
		macro	capsule				59.2	24			
Bates (64)	4	micro	tablet	f	-	-	36.1	24	10.9 mg/h	2.6	Chrom. + UV
				nf			44.4	24	14.4 mg/h	4.5	
		macro	capsule	f	-	-	22.4	24	7.2 mg/h	2.3	
				nf			40.4	24	10.4 mg/h	3.5	
Rosenberg (65)	4	micro	3 tablets	f	-	-	4.3-36.1	24	-		LLE + UV
		micro	suspension				37.2	24			
		macro	capsule				22.4	24			
		micro	3 tablets	nf			21.45-44.4	24	-		
		micro	suspension				43.7	24			
		macro	capsule				40.4	24			
Mendes (55)	5	micro	tablet	nf	-	-	41.5	24	13.4 mg/h	2.4	LLE + UV
Naggar (57)	6	micro	tablet	f	-	-	22	12			LLE + UV
Ogunbona (67)	8	micro	tablet	f	-	-	33	24	10.2 mg/h	3.3	LLE + UV
				nf[c]			46.2	24	14.7 mg/h	2.5	

Table 1: The PK parameters of nitrofurantoin in **urine**. (*continued*)

Reference	Subjects	Drug information		Fasting status	PK parameters						Analytical method
		Crystal size	Formulation		C_{max}	T_{max}	Recovery		Max. excretion rate		
					(mg/l)	(h)	(%)	(h)		(h)	
Panayotis (59)	4	macro	capsule	fd	-	-	26.3	24	6.1 mg/h	-	LLE + UV
							27.6	24	5.3 mg/h		
							21.2	24	5.3 mg/h		
Mason (36)	24	macro	capsule	f	95	5	24.5	24	5.6 mg/h	4.7	LLE + UV
			3 slow-release		120-150	3-5	30.4-34.1	24	7.5-8.3 mg/h	3.7-4.2	
Slow release formulation: 2x100 mg (therapeutic use)											
Maier-Lenz (56)	6	-	slow-release	-	-	-	47.5	24	-		LLE + UV [e]
							33.7	24			
Mason (36)	24	macro	capsule	f	95	5	24.5	24	5.6 mg/h	4.7	LLE + UV
			3 slow-release		120-150	3-5	30.4-34.1	24	7.5-8.3 mg/h	3.7-4.2	

Abbreviations used: [nf = non-fasting] [f = fasting] [Photodeg. = photodegradation] [Chrom. = chromatography] [LLE = liquid-liquid extraction] [micro. = microbiologically] [Polarogr. = polarographical].
The '-' sign is used when data are missing.
[a] Patients with recurrent UTI
[b] After 14 days of treatment
[c] After high fat meal
[d] Administered with milk
[e] It was not specifically mentioned if the photochemical degradation of nitrofurantoin was taken into account for this method. All the other methods did took this into account.

Table 2: The PK parameters of nitrofurantoin in **plasma**.

Reference	Subjects	Drug information		Fasting status	PK parameters					Analytical method[c]
		Crystal size	Formulation		C_{max}	T_{max}	AUC		$T_{1/2}$	
					(mg/l)	(h)	(mg/l.h)	(h)	(h)	
Multiple dose: 4x100 mg (therapeutic use)										
Carroll (60)	2[a]	macro	capsule	f	1.11	> 48	-	-	-	Chrom . + UV
	10[a]	macro	capsule[b]	f	0.8-1.81					
Single dose: 1x50 mg (prophylactic use)										
Liedtke (51)	10	macro	tablet	f	0.26	2.1	1.5	-	1.7	HPLC+ polarogr.
Hoener (66)	6	micro	tablet	f	0.428	2,3	1.05	0-∞	-	HPLC + UV
				nf	0.427	2,6	1.13			
Single dose: 1x100 mg (prophylactic use)										
Felts (69)	11		-	nf	0.9-4.6	2-4	-	-	-	Chrom.+ color.
	6				0.75-3.7					
Albert (48)	10	micro	tablet	f	0.986	2	2,10	0-4	-	LLE + UV
		macro	capsule		1.47		3,28			
Adkison (70)	36	macro	capsule	f	0.88	2-2.3	2.21	0-∞	0.78	LC-UV or LC-MS/MS
					0.96		2.42		0.76	
					0.96		2.32		0.72	
Patel (41)	36	macro	capsule	nf	0.51	4.5	2.62	0-∞	1.66	LC-MS/MS
						4.7	2.56		1.55	

Abbreviations used: [nf = non-fasting] [f = fasting] [Chrom. = chromatography] [LLE = liquid-liquid extraction] [polarogr. = polarographical] [color. = colorimetric].

[a] UTI patients with loading dose of 100 mg.

[b] UTI patients with loading dose of 200 mg.

[c] All methods took the photochemical degradation of nitrofurantoin into account.

and only 1% as the unconjugated form (3, 10, 42). This factor seems to be less important when plasma concentrations are considered (43).

Three of the reported papers used an UV spectrophotometric method in order to quantify urinary levels or urinary and plasma levels of both unconjugated and conjugated nitroxoline (42–44). An electrochemical method for the quantification of unconjugated nitroxoline in plasma was used by Ghoneim *et al* (45). The first HPLC method for quantification on unconjugated and conjugated nitroxoline in plasma and urine was used by Bergogne-Berezin *et al* (10). Wagenlehner *et al*. and Forstner *et al*. reported urinary concentrations of unconjugated and conjugated nitroxoline measured by HPLC with MS detection (3, 16). Since nitroxoline concentrations of both unconjugated and conjugated nitroxoline vary (table 3), it is difficult to make a statement about the applicability of UV or MS detection. The high nitroxoline concentrations in urine and plasma seem to be enough to quantify with the less sensitive UV detection method, but the lower concentrations of the unconjugated nitroxoline form may need the more precise MS detection (3, 10, 16, 43–45). Especially if PK research is conducted in patient populations in whom lower nitroxoline concentrations can be expected, for example elderly patients with renal impairment (44). More clinical research is needed to establish in which order of magnitude the concentrations of unconjugated nitroxoline in urine can be expected to determine whether UV or MS detection is preferred.

PHARMACOKINETIC PROPERTIES OF NITROFURANTOIN

The current knowledge of nitrofurantoins PK profile is poor and mainly based on old studies from the fifties. The majority of the publications report data obtained in a phase of product development during which pharmaceutical companies attempted to maximize bioavailability and minimize toxicity. Therefore, nitrofurantoin was administrated in different crystal sizes (46–48), formulations (36, 49–59), dosages (60–62), fasting states (63–67) and/or populations (68–70). The influence of these different parameters on nitrofurantoins PK is discussed in the following sections. A summary of the literature search results is found in the supplementary tables S1 (urine PK) and S2 (plasma PK).

Effect of crystal size on the pharmacokinetics

It is important to keep in mind the crystal sizes of nitrofurantoin in the commercially available products when interpreting PK results. Nowadays, the macrocrystalline formulations have almost completely replaced microcrystalline formulations because microcrystals were more associated with unwanted side effects due to their rapid absorption (46, 64, 71).

Table 3: The PK parameters of nitroxoline in urine and plasma.

Reference	Subjects	Dose	Fasting status	Metabolite	PK parameters						Analytical method
					C_{max}	T_{max}	Recovery		Max. excretion rate	Time to max. excretion rate	
		(mg)			(mg/l)	(h)	(%)	(h)	(%/h)	(h)	
URINE											
Mrhar (42)	8	400	f	Uncj.	17	4	1.8	24	-		UV
				Conj.			60.0	24			
	8	200	f	Uncj.	9	2	1.5	24	-		
				Conj.			50.0	24			
Makhailova (43)[a]	8	200[b]	nf	Uncj.	380	2-4	53.0	24	10.8	4	UV
				Uncj.	400	0-2	63.0	24	9	2	
Mikhailova (44)[c]	7	200	nf	Uncj.	160	6	50	24	6.6	3.5	UV
	6	200			120	6	39	24	4.5	5	
	7	200			30	6	21	24	2.1	6.5	
Wagenlehner (3)	6	250	nf	Uncj.	0.5	0-4	0.2	24	-		HPLC-MS
				Conj.	27.8	0-4	11.7	24			
Forstner (16)[c]	30	3x250	nf	Uncj.	5.4		-		-		HPLC-MS
				Conj.	210.6						

Table 3: The PK parameters of nitroxoline in urine and plasma. (*continued*)

Reference	Subjects	Dose	Fasting status	Metabolite	PK parameters						Analytical method
					C_{max}	T_{max}	Recovery		Max. excretion rate	Time to max. excretion rate	
		(mg)			(mg/l)	(h)	(%)	(h)	(%/h)	(h)	
PLASMA											
Makhailova (43)	8	200[b]	nf	Uncj.	9.5	1.5	-		-		UV
				Uncj.	8.5	1.5					
Mikhailova (44)	7	200	nf	Uncj.	7	2.5	-		-		UV
	6	200			5.5	4.5					
	7	200			5	7					
Bergogne (10)[d]	8	200	-	Uncj.	5.50	1.75	-		-		HPLC
		3x200									
Ghoneim (45)	2	100	f	-	5.4	1.75	-		-		Voltammetric

The abbreviations for non-fasting (nf) and fasting (f) are used in the fasting column. The '-' sign is used when data are missing.

[a] two different nitroxoline products were administered

[b] dose based on bodyweight (3 mg/kg). one subject received 250 mg

[c] PK parameters obtained in kidney failure patients (Mikhailova) or elderly patients at steady-state (Forstner)

[d] mean of 1x200 mg and 3x200 mg dose

The effect of different nitrofurantoin crystal sizes on the absorption and excretion profile of nitrofurantoin was clearly demonstrated by Conklin *et al* (47). Figure 2 demonstrates their findings. More inter-individual variability in recovery values is observed when macrocrystalline nitrofurantoin is administered compared to microcrystalline nitrofurantoin. Urinary excretion was monitored for 24 hours on the first day and on the last day after administration of 100 mg q6h microcrystals or macrocrystals for 7 days. On days 1 and 7, 42.7% and 43.6% was excreted in the microcrystal group, respectively, and 37.9% and 35.0% in the macrocrystal group. An overall higher percentage was found for the microcrystals because more microcrystalline nitrofurantoin was excreted in the first 12 hours compared to the percentage of macrocrystalline nitrofurantoin. Yet from 12 to 24 hours, the opposite was observed, reflecting a slower rate of absorption of the macrocrystal form (not shown in figure 2). In daily clinical practice, this slow absorption property of the macrocrystal form is overcome by administering the drug with food which prolongs the residence time in the GI tract and therefore the time in which the drug can be absorbed (21–23, 64, 65).

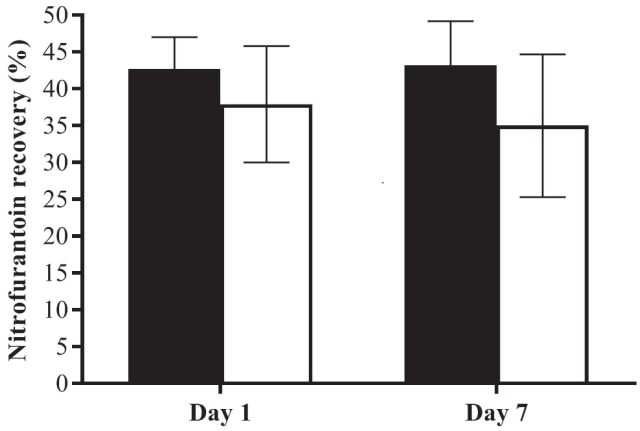

Figure 2: Recovery of nitrofurantoin (%) on the first day and on the seventh day of the treatment with microcrystals (black) or macrocrystals (white). Brackets represent the standard deviation of the mean recovery.

Effect of product formulation on pharmacokinetics

The slow-release formulation was investigated only by Maier-Lenz *et al.*; recovery values ranged from 33.7% to 47.7% over 24 hours (56). Total excretion (%) as well as excretion rates over 24 hours for slow-release products were found to be higher compared to a normal macrocrystal capsule (36). When the absorption of the regular-release formulation was defined as 100%, the absorption of the slow-release products were 129.8%, 130.2% and 145.3%. This indicates that prolongation of the residence

time in the GI tract results in a higher amount of nitrofurantoin absorption and therefore higher amounts excreted in urine.

Effect of food on pharmacokinetics

The presence of food in the GI tract increases the gastric emptying time and therefore increases residence time so that more nitrofurantoin can be dissolved in the gastric fluids before passing to the duodenum, where absorption of nitrofurantoin is maximal (72). The influence of food is stronger when macrocrystalline nitrofurantoin is administered compared to microcrystalline nitrofurantoin, suggesting that the critical, time-dependent step in the absorption of nitrofurantoin is its dissolution in GI fluids rather than its residence time in the GI tract (73). This results in a smaller absorption rate compared to the excretion rate, which is also known as 'flip-flop kinetics'(74). The hypothesis of dissolution time is supported by the results of Naggar *et al.*, who showed that absorption was increased when nitrofurantoins solubility was increased by the addition of $Mg_2O_8Si_3$ (57). The occurrence of side effects is increased with smaller nitrofurantoin crystal size (75). To counter these side effects, manufacturers suggested administering nitrofurantoin with food. It was then demonstrated that food increases the bioavailability of nitrofurantoin, reflected by increased urinary recovery (64, 65). Thus the administration of the macrocrystalline nitrofurantoin formulation with food both increases the drug's exposure to uropathogens and minimizes its side effects.

General discussion: urinary pharmacokinetics

An overview of the urinary PK data after administration of a clinically relevant dose in a commercially available formulation is given in table 1. The following PK data were considered clinically relevant: all data after a multiple dose of 50 mg q6h or 100 mg q6h; data after a single dose of 50 mg or 100 mg; data after administration of the slow-release formulation in a dose of 100 mg q12h or 100 mg q24h (up to 12 hours). The following PK parameters were investigated in urine: maximum concentration (C_{max}), time to maximum concentration (T_{max}), recovery, maximum excretion rate and maximum absorption rate. The recovery represents the cumulative excretion and is expressed as a percentage of the total administered dose. The maximum excretion rate was reported as percentage per time or as amount per time, based on what was reported in the reference paper. C_{max} or excretion rates were reported as a range instead of one value if the reference reported values of different subjects separately instead of one mean value. If the crystal size of the product under investigation was not specifically mentioned, tablets were considered to contain microcrystals and capsules contained macrocrystals based on the product information (21–23).

Only one study investigated PK after the standard dose of 50 mg q6h: this dose was administered to nine patients with recurrent UTI when nitrofurantoin was given

as a prolonged administration from 1 month to 3 years (68). A high inter-individual variability in maximum urinary concentrations was found in this population with concentrations of 0-140 mg/l; 20-230 mg/l; 5-160 mg/l; and 40-210 mg/l after one to four administrations, respectively. The researchers concluded that in the group with normal renal function, concentrations usually exceeded 50 mg/l throughout the day. The maximum concentration after administration of 100 mg q6h was lower in the first time interval compared to the 50 mg q6h dose, but remained higher for a longer time (120 mg/l after the fourth administration compared to 90 mg/l after 50 mg q6h) (68). This is remarkable and points to the hypothesis that the PK pattern of nitrofurantoin is not linear, but may be dose-dependent. Preliminary data from a PK study in healthy volunteers in which nitrofurantoin concentrations in plasma and urine after a dose of 50 mg q6h or 100 mg q8h were compared confirms the pattern in urine found by Lippman *et al.*, but also reveals that plasma concentrations change in proportion to the dose received (76). More research is needed to fully understand the absorption and excretion pattern of nitrofurantoin.

The mean recovery over 24 hours after administration of 100 mg q6h of the microcrystalline form could be calculated based on the values reported in several papers (36, 46, 48, 53, 55, 59, 64, 65, 67). The recovery was not found to be different between the microcrystalline and macrocrystalline forms (32.9 ± 13.9% versus 32.1 ± 11.6%). Recovery after a single dose of 100 mg was also found to be comparable with that after 200 mg, so the dose seems not to influence the total recovery over 24 hours.(56) The maximum excretion rate of the microcrystalline formulation was 50% higher (≈15 mg/h) compared to the macrocrystalline formulation (≈10 mg/h) so absorption of nitrofurantoin can be increased by using macrocrystalline nitrofurantoin and administering the drug with food.

General discussion: plasma pharmacokinetics

The AUC, the elimination half-life ($T_{1/2}$) and the elimination constant (K_{el}) were investigated as specific plasma parameters together with the PK parameters which were also investigated for describing the urine PK. A complete overview of these parameters is presented in table S2. An overview of the plasma PK data after administration of a clinically relevant dose in a commercially available formulation is given in table 2.

No plasma PK values after administration of the clinically relevant dosing regimen were reported. In general, less research has been conducted on the PK properties of nitrofurantoin in plasma versus urine. This is not surprising since nitrofurantoin needs to be active in the bladder and is known to have minimal and transient plasma concentrations. Nonetheless, an idea of the PK pattern in plasma can be derived from the PK pattern in urine since urinary excretion reflects the time course of drug in the blood (71). Concentrations in plasma never exceed 2 mg/l under fasting conditions regardless of

the dose, the formulation and the time after dose. Concentrations of macrocrystalline nitrofurantoin are higher (up to 1.8 mg/l) compared to those of the microcrystalline form (maximum of 0.99 mg/l). When administered with food, nitrofurantoin concentrations were even higher: maximum 4.6 mg/l compared to non-fasting conditions (69).

Four papers report the plasma concentration-time profiles of individual subjects after administration of macrocrystalline nitrofurantoin under fasting conditions (48, 51, 60, 70). These values are presented in figure 3. A high inter-individual variability in nitrofurantoin PK, which was already observed in the urine PK, is also present in plasma. The wide distribution of plasma concentrations is probably a result of administration of different nitrofurantoin dosages, but even when only the 100 mg dose is considered, the high variability remains (figure 3b) (48, 60, 70). Differences in drug levels may be explained by different nitrofurantoin formulations since no details of the administered nitrofurantoin capsule were given (60). Also, differences in analytical methods may be responsible for the high variability in concentrations. For example, it was not clear whether the analytical method used by Carroll *et al*. takes into account the degradation of nitrofurantoin in daylight.

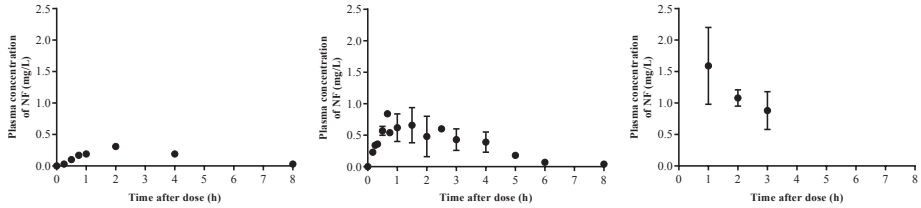

Figure 3: Plasma concentrations after administration of clinically relevant dosages of a single dose of 50 mg (a), 100 mg (b) or 200 mg (c) of macrocrystalline nitrofurantoin (NF) under fasting conditions. The concentrations in figure a are reported by Liedtke (mean of 10 subjects per value). Since several concentrations were reported for the 100 mg and the 200 mg dose for the majority of the time points, the concentrations in figure b and c are demonstrated as means and standard deviations, based on data from Adkison (n=12), Albert (n=10) and Carroll (n=2) for figure b and based on data from Carroll (n=10 for T1, n=6 for T2 and n=4 for T3) for figure c.

Pharmacokinetics in patients with renal dysfunction

Both treatment failures and high plasma levels have been observed in UTI patients with impaired renal function (69, 77, 78). Plasma levels can increase up to 5-6 mg/l and are associated with potential toxicity (78). Dose adjustments in order to avoid toxic plasma levels in these patients might only lead to subtherapeutic urine levels, so exploring the relationship between renal function and plasma and urine levels of nitrofurantoin is necessary. Several papers included subjects with impaired renal function; a linear relationship between creatinine clearance and urinary nitrofurantoin concentrations or urinary nitrofurantoin recovery was found (69, 77, 78).

Lippmann *et al*. administered the standard nitrofurantoin dose of 50 mg q12h to eight UTI patients with low creatinine clearance in the microcrystalline form as prolonged administrations over several months (68). The urinary C_{max} in nine UTI patients with normal renal clearance ranged from 70 mg/l to 110 mg/l during 24 hours after administration when nitrofurantoin concentrations were measured in five-hour interval urine portions. Concentrations were 10-20 mg/l in the patients with poor renal function. The excretion of nitrofurantoin was minimal in patients with creatinine clearances <20 mL/min, so its use should be avoided in these patients (79). However, some official guidelines recommend avoiding nitrofurantoin in patients with moderate renal insufficiency (creatinine clearance <60 mL/min) because of probable reduced efficacy (78). New guidelines have increased this limit to <30 mL/min, but the quality of evidence for either recommendation remains low (80). A recent study demonstrated that nitrofurantoin can be used safely in patients with mildly to moderately reduced renal function (81).

Pharmacokinetics in children

Nitrofurantoin is approved for the treatment and prophylaxis of UTI in children ≥12 years old (21–23). The only PK data are from a case report of Jawetz *et al*. describing a 9-year-old girl with recurrent UTI (61). A daily dose of 100 mg was administered for 14 days; a maximum urinary nitrofurantoin level of 240 mg/l was found. Karpman *et al*. reviewed the literature regarding the safety and toxicity of nitrofurantoin and two other drugs (82). They concluded that nitrofurantoin is safe in children for long-term prophylactic use, but no PK parameters were reported.

Effect of genotype

The only known gene associated with the PK of nitrofurantoin is the breast cancer resistance protein (BCRP) gene. Several *in vitro* studies have demonstrated that nitrofurantoin is a substrate of this transporter (83–85). Nitrofurantoin may thus be suitable as clinical probe substrate for BCRP activity, polymorphisms of which can reduce the absorption and/or increase the elimination of drugs. Urinary and plasma concentrations of nitrofurantoin were quantified in 36 Chinese male subjects with different BCRP polymorphisms, but there was no significant effect on plasma and urine concentrations in these subjects (70). Thus there is currently no evidence that different BCRP genotypes influence nitrofurantoins PK.

PHARMACOKINETIC-PHARMACODYNAMIC RELATIONSHIPS OF NITROFURANTOIN

In past years, a considerable amount of research has been conducted on nitrofurantoins PD properties. Yet the PK/PD index that best correlates with the drug's antibacterial effect is still unknown. The EUCAST does not mention a PK/PD index for nitrofurantoin, but does mention 64 mg/l as cut-off value (ECOFF) for susceptibility for *E. coli*, *S. saprophyticus*, *Enterococcus* spp and *Streptococcus agalactiae* (86). There are not enough PK/PD data available to set breakpoints in urine, the clinically relevant matrix for UTI.

It is therefore difficult to associate the nitrofurantoin concentrations with a therapeutic effect. Fransen *et al*. demonstrated bactericidal effect at ≥2x MIC against several *E. cloacae* after 4-8 hours, against *K. pneumoniae* after 8-10 hours and against *E. coli* (ESBL+ and ESBL-) species after 12-16 hours in an *in vitro* time-kill assay.(8) This effect was not uniform among the Enterobacteriaceae family: a concentration-dependent pattern was observed for *E. cloacae* and a time-dependent pattern was demonstrated for *E. coli* (as well as for *K. pneumoniae*). These findings are in line with the study of Lindgren *et al.*, where no behavioral differences between ESBL-producing and non-ESBL-producing species were found (7).

CLINICAL USE OF NITROFURANTOIN

The clinical efficacy and toxicity of nitrofurantoin when used as treatment of lower UTIs or as prophylaxis have been recently described in two meta-analyses based on controlled trials (14, 15). Twenty-seven controlled trials including 4807 patients were assessed to investigate nitrofurantoin as treatment for UTIs; nitrofurantoin was found to be clinical and microbiologically effective with clinical cure rates ranging from 79%-92% and microbiological eradication rates of 80%-92%. Treatment for 7 days was not found to be more effective compared to the standard treatment of 5 days, but its clinical efficacy was diminished (61%-70%) when given for only 3 days. Toxicity was found in 5% to 16% of the cases and was mild, reversible and mainly limited to GI related side effects. Twenty-six controlled trials including 3052 patients were assessed to determine nitrofurantoins efficacy and safety as prophylaxis against UTI; the drug is effective in the prevention of UTIs, and side effects were noted again to be mild, with the risk of (severe) toxicity increasing with the duration of the prophylactic use (14, 15, 87). Not only crystal size, but also (high) plasma levels are associated with toxicity (21). It would therefore be interesting to compare plasma concentrations of patients who do experience side effects to those who experience no side effects.

NITROXOLINE

The nitroxoline papers in which PK data are mentioned are older and more limited than those reporting nitrofurantoin data (3, 10, 16, 42–45). The results of the literature search are demonstrated in table 3.

Urine and plasma pharmacokinetics

Absorption, distribution and metabolism

The C_{max} of both unconjugated and conjugated forms obtained in the first time interval in all studies indicate rapid absorption from the GI tract and rapid conjugation in the liver. Mrhar *et al*. demonstrated complete and rapid absorption even when a high dose of 400 mg was administered (42). When comparing urine concentrations after a dose of 400 versus 200 mg, higher concentrations with a longer time period >6 mg/l were observed, indicating a linear relationship between dose and C_{max} in urine (42).

Almost all absorbed nitroxoline is metabolized in the liver and subsequently excreted in the urine (10, 42). There seems to be inter-individual variability in the metabolism pattern since varying concentrations of the conjugated metabolite were found, but this could also be a result of differences in excretion patterns between subjects, since no data are available on plasma concentrations of the conjugated form (10). Metabolism occurs soon after absorption, based on early levels of conjugated nitroxoline (10). The consensus seems to be that while the unconjugated form of nitroxoline exhibits antibacterial activity, its metabolites may also significantly contribute to its antibacterial effect (3, 9, 12). This would appear plausible, as it seems unlikely that the almost negligible amount of unconjugated nitroxoline would be fully responsible for the drug's total antibacterial effect. However, the relative contribution of these metabolites remains unclear; more research is clearly needed.

Excretion

The majority of the papers reported that approximately 60% of the total administered dose is eliminated in the urine, from which only ~1% in the unconjugated form (3, 10, 11, 42). The only study investigating nitroxoline PK after administration of the clinically relevant dose of 250 mg found a recovery of the unconjugated form of approximately 1.7% versus 98.3% for the conjugated sulfate form over 24 hours (3). Recovery levels after a registered dose of 250 mg q8h were not reported (16). The ratio between recovery of the conjugated and the unconjugated form was consistent with data from other studies.

Urinary elimination of both unconjugated and conjugated nitroxoline occurs rapidly: high urine concentrations of both forms are found already one hour after

administration (10). Two Bulgarian studies indicate that the majority is excreted within 10 hours after administration; the process is delayed in patients with renal insufficiency (43, 44). Impaired renal function not only influences recovery but also C_{max} levels of the unconjugated nitroxoline form in urine.(44) Urinary C_{max} levels of the unconjugated nitroxoline form decreased from 160 to 30 mg/l when creatinine clearance decreased from 40-90 mL/min to less than 20 mL/min. This decrease in urinary concentrations points to the hypotheses that either more nitroxoline is conjugated in the liver (due to decreased excretion of unconjugated nitroxoline in the urine, in turn resulting in a longer residence time in the central circulation, during which metabolism will occur) and/or less nitroxoline is excreted in the urine. An increase in nitroxoline metabolism would be reflected in high plasma levels of the conjugated nitroxoline form and/or in low unconjugated nitroxoline plasma levels, but these low plasma levels were not observed in patients with renal impairment (7 versus 5 mg/l) (44). This hypothesis may therefore not be valid. What is striking, however, is that the C_{max} in plasma is found much later when renal function decreases (2.5 versus 7 hours), which is was not observed in urine (C_{max} after 6 hours) (44). This again points to the hypothesis that a higher fraction of nitroxoline is metabolized when renal function decreases due to a longer residence time in the central circulation. The mechanism behind this observation is unclear; more research on conjugated nitroxoline concentrations is needed in order to clarify the influence of the renal function on the metabolism and excretion of nitroxoline. This is important information because the extent to which the antibacterial active form of nitroxoline is metabolized to (in)active metabolites would presumably influence the overall antibacterial activity of the administered therapy. It should be noted that the validity of the two Bulgarian studies is questionable since their reported concentrations are high and not in line with those reported by others (table 3). They also did not distinguish between the conjugated and the unconjugated nitroxoline forms. Nonetheless, though the values for absolute concentrations may be unreliable, the relationship between creatinine clearance and C_{max} seems valid and consistent with expectations, since renal excretion is the primary route of nitroxoline removal from the body (42). Forstner *et al*. confirm this relationship between urinary concentrations and creatinine clearance (16).

Pharmacokinetic-pharmacodynamic relationships

EUCAST and the German National Antibiotic Susceptibility Committee describe 16 mg/l as the clinical breakpoint for *E. coli* susceptibility for nitroxoline when treating uncomplicated UTI (17). This breakpoint was used in an *in vitro* study in which the activity of nitroxoline against clinical *E. coli* isolates was tested (12). Nitroxoline was efficacious in all 499 tested isolates, regardless of their baseline susceptibility for nitroxoline. Based on this breakpoint and in theory, effective urinary concentrations of

the conjugated sulfate form of nitroxoline can be reached in volunteers after a single dose of 250 mg measured over 24 hours (C_{max} 0.3-27.8 mg/l) and in patients after the registered dose of 250 mg q8h at steady-state (C_{max} 0.8-210.6 mg/l) (3, 16). No effective concentrations of the unconjugated form were reached in either of the groups. This is also true when the breakpoint of 6 mg/l for the unconjugated form, which has an *in vitro* antimicrobial effect in urine at a concentration of 6 mg/l, is considered (42).

No PK/PD index has been reported; there are yet few clinical data available for its determination. More research is needed in order to investigate the PK/PD index and the corresponding breakpoint. Only with this knowledge can treatment with nitroxoline be carried out in an effective and safe manner (18, 20).

Nitroxoline's activity is mainly bacteriostatic and seems to be dependent on urinary pH (3). The highest reciprocal inhibitory titers were found for *E. coli* ATCC 25922 with a relatively low MIC of 2 mg/l for nitroxoline. No *E. coli* strains with higher MICs were tested, but a *Proteus* and a *S. saprophyticus* strain with a MIC of 8 mg/l were tested, which both showed lower inhibitory titers (mostly 0) compared to the *E. coli* ATCC strain. These results support the hypothesis that nitroxolines activity is also dependent on the species and less dependent on the MIC.

Clinical use

In vitro data suggest that nitroxoline would be effective in the treatment of uncomplicated UTI's (11, 17). A meta-analysis of four unpublished, controlled trials examined the outcomes of 234 female UTI patients treated with 250 mg q8h nitroxoline versus 232 women treated with cotrimoxazole or norfloxacin (9), results suggested comparable toxicity and microbiologic efficacy, defined as a reduction of bacteriuria from $\geq 10^5$ to $<10^4$ cfu/mL, 7-13 days after end of therapy; this reduction was observed in over 90% of the patients after five (acute UTI) or 10 (recurrent UTI) days of treatment. Clinical efficacy was defined as symptom improvement and was found to be similar between the treatment groups (9). Additionally, Wagenlehner *et al*. concluded that urinary steady-state concentrations after a dose of 250 mg q8h in healthy volunteers had an *in vitro* bacteriostatic activity during 24 hours (3).

The opposite conclusion, however, was drawn in a prospective study in which nitroxoline was administered at the registered dose for 7 days to 30 hospitalized geriatric patients with lower UTIs (16). Only 17 patients were treated as planned, so conclusions were limited. Microbiological success, defined as a urine culture taken on day 12 showing reduction of bacteriuria from $\geq 10^5$ to $<10^4$ cfu/mL, was limited especially in elderly patients with either comorbidities (*e.g.*, diabetes) or those with catheter-associated or otherwise complicated UTIs. Clinical symptoms were still observed in 23.5% of the patients at day 7. The study was terminated early because of disappointing microbiological and response results. Since urinary concentrations of the

unconjugated form and the conjugated form were comparable with those of healthy volunteers, it is unlikely that the difference in microbiological success was due to a difference in exposure. The static effect of nitroxoline may therefore not be sufficient to successfully treat UTIs in this specific patient population, about half of which had complicated (catheter-associated) UTI (3). Antibiotics with a bactericidal effect may be needed in order to treat this group of patients. On the contrary, this static effect of nitroxoline was enough to successfully treat 90% of the patients with uncomplicated UTIs as shown in a meta-analysis (9).

Overall, there is currently only modest evidence for early microbiological and clinical success with nitroxoline as therapy for UTI. However, a distinction must be made between different patient groups if treatment with nitroxoline is considered. In primary care, where in general young and relatively healthy women are treated for UTI, nitroxoline could be a possible oral treatment option. When older, more comorbid patients are considered, nitroxoline appears to be microbiologically inferior. Randomized clinical trials with longer follow-up and clearly defined clinical outcomes are needed to assess the drug's true efficacy. Additional research is needed in order to investigate the antibacterial activity of its metabolites; in the meantime, its use should be considered carefully.

CONCLUSIONS

Pharmacokinetic data on both nitrofurantoin and nitroxoline are sparse and have been obtained largely via outdated analytical methods. High inter-individual variability was found for the PK profile of nitrofurantoin, which can (partly) be explained by inconsistencies in crystal size, product formulation and/or dose. New PK studies using commercially available nitrofurantoin products at approved dosages and current analytical methods are needed. More knowledge of these two drugs' metabolism, including the activity and toxicity of their metabolites, will help to optimize their use. Nitroxoline's potential as a therapeutic agent for uncomplicated UTI was demonstrated in a non-inferiority study in relative healthy patients, but its short-term effect and its effect in patients with comorbidities remain unknown. In an age of reduced oral options for UTI, pharmacokinetic results from much needed randomized clinical trials would help to optimize therapy and minimize emergence of resistance.

Transparency declarations

The authors declare that there are no conflicts of interest.

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Table S1: Complete overview of the PK parameters of nitrofurantoin in urine.

Reference	Subjects	Drug information			Fasting status	PK parameters						Analytical method
		Dose (mg)	Crystal size	Formulation		C_{max} (mg/l)		T_{max} (h)	Recovery (%)	(h)	Max. excretion rate (time. h)	
Carroll 1955	9	200 + 4x100	macro	Furadantin (capsule)	f	-		-	10,6 29,0 34,2	3 12 24	-	Chrom. + UV
Richards 1955	1	4x200	-	-	f	-	500	0-1	-		-	Micro.
		2x200			nf		450	2-3				
Jawetz 1957	1 child	4x50	micro	tablet	-	Day 14:	240	-	-		-	Micro.[d]
	1	4x75				Day 14:	15					
	3	4x100				Day 14-15:	15-60					
	1	4x125				Day 15:	60-120					
	5	4x150				Day 14-19:	60-240					
Lippman 1958 [a]	4	4x25	micro	tablet	nf + normal weight		80-70	5-10	-		-	Chrom. + UV
	3	4x25			nf + low weight		70-200	10-15				
	9	4x50			nf + normal GFR		40-230	5-10				
	8	4x50			nf + renal imp.		0-50	0-5				
	6	4x100			nf		60-230	10-15				
Henry Paul 1967	15	100	micro	tablet	nf		151	3	36,1	24	10 %/h	3,6	Chrom. + UV
			macro	4 capsules			83-159	3,4-5,5	19,6-35,4		4,2-8,9 %/h	3,6-4,9	
Stoffels 1968	10	200	micro	tablet	nf	-	-	-	62,5	12	7,3 %/h	0-4	Colorimetric
				coated dragee					64,9		11,4 %/h		
	16[b]			tablet					33,6		3,6 %/h		
				coated dragee					43,8		5,4 %/h		

Table S1: Complete overview of the PK parameters of nitrofurantoin in urine. (*continued*)

Reference	Subjects	Drug information			Fasting status	PK parameters					Analytical method
		Dose (mg)	Crystal size	Formulation		C_{max} (mg/l)	T_{max} (h)	Recovery (%)	Max. excretion rate (h)	(time. h)	
Conklin 1969	10	4x100[c]	micro	capsule	nf	Day 1: 56,5 mg	4-8	42,7	24	14,1 mg/h	4-8h	LLE + UV
						Day 7: 61,7 mg		43,6		15,4 mg/h		
			macro			Day 1: 60,4 mg	12-24	37,9		10,5 mg/h		
						Day 7: 49,4 mg		35,0		9,9 mg/h		
Felts 1971	11	100	-	-	nf + normal GFR	1,1-104	2-4	-		-		Chrom. + colorimetric
	6				nf + renal imp.	1,7-27,6						
Schwartländer 1972	5	150	macro	capsule	-	195,7	2-4	38,3	8	-		Colorimetric
		150	macro	capsule		273,5	0-2	35,1				
		150	micro	tablet		225	0-2	22,5				
		150	micro	tablet		295,1	2-4	34,8				
		3x50	macro	capsule		124,9	0-2	39,4	12	-		
		3x50	micro	tablet		94,3	0-2	30	12			
		75	-	Slow release tablet		166,4	2-4	26,5	10			
Stoll 1973	4	100	micro	suspension	nf	-	-	14,8	24	-	3,6h	Chrom. + UV
Albert 1974	10	100	micro	tablet	f + 4h	-	-	56,7	24	-		LLE + UV
			macro	capsule				59,2				

Table S1: Complete overview of the PK parameters of nitrofurantoin in urine. (*continued*)

Reference	Subjects	Drug information			Fasting status	PK parameters					Analytical method
		Dose (mg)	Crystal size	Formulation		C_{max} (mg/l)	T_{max} (h)	Recovery (%)	(h)	Max. excretion rate	(time. h)	
Bates 1974	4	100	micro	tablet	f	-	-	36,1	24	10,9 mg/h	2,6h	Chrom. + UV
					nf			44,4		14,4 mg/h	4,5h	
			macro	capsule	f			22,4		7,2 mg/h	2,3h	
					nf			40,4		10,4 mg/h	3,5h	
Meyer 1974	14	50	micro	tablet	f +4h	-	-	36	12	3,0%/h	-	LLE + UV
		50	micro	tablet				33		2,8%/h		
		50	micro	tablet				37		3,1%/h		
		50	micro	tablet				39		3,3%/h		
		50	macro	capsule				24		2,0%/h		
		50	micro	tablet				18		1,5%/h		
		50	micro	tablet				15		1,3%h		
		100	micro	tablet				30		2,5%/h		
		100	micro	tablet				32,5		2,7%/h		
		100	micro	tablet				38		3,2%/h		
		100	micro	tablet				33,5		2,8%/h		
		100	macro	capsule				25		2,1%/h		
		100	micro	tablet				30		2,5%/h		
		250	micro	tablet				31,5		2,6%/h		

Table S1: Complete overview of the PK parameters of nitrofurantoin in urine. (*continued*)

Reference	Subjects	Drug information			Fasting status	PK parameters					Analytical method
		Dose (mg)	Crystal size	Formulation		C_{max} (mg/l)	T_{max} (h)	Recovery (%)	(h)	Max. excretion rate (time. h)	
Rosenberg 1976	4	100	micro	3 tablets	f	-	-	4,3-36,1	24	-	LLE + UV
			micro	suspension				37,2			
			macro	capsule				22,4			
			micro	3 tablets	nf			21,45-44,4			
			micro	suspension				43,7			
			macro	capsule				40,4			
Mendes 1978	5	100	micro	2 chewable tablets	nf	-	-	40,7	24	13,6 mg/h 1,6h	LLE + UV
				2 swallow tablets				32,0		10,0 mg/h 2,6h	
				2 hard gelatin capsules				39,5		15,9 mg/h 2h	
				tablet				41,5		13,4 mg/h 2,4h	
Maier-Lenz 1979	6	200	micro	tablet	-	-	-	35,3	24	-	LLE + UV[d]
			-	Slow release tablet				47,5			
								33,7			
Naggar 1979	6	100	micro	tablet	f	-	-	22	12	-	LLE + UV
Hoener 1981	6	50	micro	tablet	f	132	1,8	-		-	HPLC + UV
					nf [e]	50	2,8				
Gröning 1981	4	50	micro	tablet	f +4h	-	-	34,5	8	-	Polarogr.
Ogunbona 1986	8	100	micro	tablet	f	-	-	33	24	10,2 mg/h 3,3h	LLE + UV
					nf [e]			46,2		14,7 mg/h 2,5h	
Panayotis 1986	4	100	macro	capsule	f + 100 mL milk	-	-	26,3	24	6,1 mg/h -	LLE + UV
					f + 200 mL milk			27,6		5,3 mg/h	
					f + 400 mL milk			21,2		5,3 mg/h	

Table S1: Complete overview of the PK parameters of nitrofurantoin in urine. (*continued*)

Reference	Subjects	Drug information			Fasting status	PK parameters					Analytical method
		Dose (mg)	Crystal size	Formulation		C_{max} (mg/l)	T_{max} (h)	Recovery (%)	(h)	Max. excretion rate	(time. h)	
Mason 1987	24	100	macro	Macrodantin	f +1h	95	5	24,5	24	5,6 mg/h	4,7h	LLE + UV
				3 slow release forms		120-150	3-5	30,4-34,1		7,5-8,3 mg/h	3,7-4,2h	
Meyer 1989	14	50	micro	4 tablets	f +4h	-	-	34,3-42,1	23	13,4-17,9 %/h	1,4-2,3h	HPLC + UV
		100		3 tablets				31,6-33,7		11,1-11,7 %/h	2,0-2,6h	
Ertan 1994	6	50	micro	pure NF in hard capsule	f +1h	14,3 mg	0-2	55,0	12	7,2 mg/h	0-2	TLC
				hard gelatin capsule		10,4 mg	2-4	47,9		5,2 mg/h	2-4	
				tableted capsule		12,5 mg	2-4	49,2		6,3 mg/h	2-4	
Adkison 2008	36	100	macro	capsule	f + CC genotype	-	-	43,1	30	-		LC-UV or LC-MS/MS
					f + CA genotype			44,3				
					f + AA genotype			38,8				

Abbreviations used: [nf = non-fasting] [f = fasting] [Photodeg. = photodegradation] [Chrom. = chromatography] [LLE = liquid-liquid extraction] [micro. = microbiologically] [Polarogr. = polarographical] [TLC] Thin layer chromatography.

[TLC] Thin layer chromatography

The '-' sign is used when data are missing.

[a] Patients with recurrent UTI

[b] UTI patient with a gastric resection

[c] Treated for 7 days

[d] It was not specifically mentioned if the photochemical degradation of NF was taken into account for this method. All the other methods did took this into account.

[e] After high fat meal

Table S2: Complete overview of the PK parameters of nitrofurantoin in plasma.

Reference	Subjects	Drug information				Cmax	T_{max}	AUC		T1/2	Analytical method[b]
		Dose (mg)	Crystal size	Formulation	Fasting status	(mg/l)	(h)	(mg/l .h)	(h)	(h)	
Carroll 1955	2[a]	100 + 4x100	macro	Furadantin (capsule)	f	1.11	> 48	-		-	Chrom. + UV
	10[a]	200 + 4x100				0.8-1.81					
Felts 1971	11	100	-	-	nf + normal GFR	0.9-4.6	2-4	-		-	Chrom. + color.
	6				nf + renal imp.	0.75-3.7					
Albert 1974	10	100	micro	Furadantin (tablet)	f +4h	0.986	2	2,10	0-4	-	LLE + UV
			macro	capsule		1.47		3,28			
Maier-Lenz 1979	6	200	micro	tablet	-	0.75	1.5	3885,00			
			-	Slow release tablet		1,00	4	2896,00	0-∞	-	LLE + UV[c]
						0.8	3	2419,00			
Liedtke 1980	10	50 mg	macro	sugar coated tablet	f	0.26	2.1	1.5		1.7	HPLC + polargr.
	7	150 mg				0.59	1.9	3.1	-	1.2	
	10			capsule[d]		0.71	2.6	3.6		1.2	
Hoener 1981	6	50	micro	tablet	f	0.428	2,3	1.05	0-∞	-	HPLC + UV
					nf	0.427	2,6	1.13			
Adkison 2008	36	100	macro	capsule	f + CC genotype	0.875	2-2.3	2.21	0-∞	0.78	LC-UV or LC-MS/MS
					f + CA genotype	0.961		2.42		0.76	
					f + AA genotype	0.963		2.32		0.72	
Patel 2013	36	100	macro	capsule	nf	0.513	4.5	2.62	0-∞	1.66	LC-MS/MS
						0.51	4.7	2.56		1.55	

Abbreviations used: [nf = non-fasting] [f = fasting] [Chrom. = chromatography] [LLE = liquid-liquid extraction] [polarogr. = polarographical] [color. = colorimetric].

[a] UTI patients with loading dose of 100 mg.

[b] All methods took the photochemical degradation of NF into account.

[c] It was not specifically mentioned if the photochemical degradation of NF was taken into account for this method. All the other methods did took this into account.

[d] Capsule with three different granulations.

3.2

Optimizing dosing of nitrofurantoin from a PK/PD point of view: what do we need to know?

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ABSTRACT

Nitrofurantoin is an old antibiotic and an important first-line oral antibiotic for the treatment of uncomplicated urinary tract infections. However despite its long term use for over 60 years, little information is available with respect to its dose justification and this may be the reason of highly variable recommended doses and dosing schedules. Furthermore, nitrofurantoin is not a uniform product -crystal sizes of nitrofurantoin, and therefore pharmacokinetic properties, differ significantly by product. Moreover, pharmacokinetic profiling of some products is even lacking, or difficult to interpret because of its unstable chemical properties. Pharmacokinetic and pharmacodynamic data is now slowly becoming available. This review provides an overview of nitrofurantoins antibacterial, pharmacokinetic and pharmacodynamic properties. This shows that a clear rationale of current dosing regimens is scanty.

Keywords: pharmacokinetics; pharmacodynamics; urinary tract infections; antibiotic resistance

INTRODUCTION

Nitrofurantoin is an old antibiotic used for the treatment of uncomplicated urinary tract infections (UTI) for decades (1–3). Registered in 1953, its popularity has been increasing recently mainly because of the emergence of multi-drug resistance (including β-lactam and quinolone resistance) amongst gram-negative micro-organisms (2, 4). Resistance rates for nitrofurantoin are still low despite its extensive use (5, 6). Its spectrum of activity includes (vancomycin-resistant) enterococci and Enterobacterales -including extended beta-lactamase (ESBL) producers, but with the exception of some *Klebsiella* strains and *Proteae (e.g. Proteus, Morganella, and Providencia spp) which are intrinsically resistant* (7–10).

Positive clinical outcomes of percentage up to 90% for uncomplicated UTIs are reported for nitrofurantoin (6, 11, 12). The most recent international guidelines therefore lists nitrofurantoin as a first line treatment option for uncomplicated UTIs in many countries worldwide (2).

Nitrofurantoin is the only member of the nitrofuran family currently in use in human medicine and is available as an oral formulation only. There are various nitrofurantoin products on the market of which the 50 mg and the 100 mg capsules are the most commonly prescribed products in clinical practice. Other formulations available are the slow-release capsule and the oral suspension.

Despite its long time availability pharmacokinetic and pharmacodynamic (PK/PD) data are scarce, and the relationship between exposure and response is not clear, although it is well know that these data are crucial in treatment optimization and prevention of emergence of resistance (13, 14). The aim of this paper is therefore to provide an overview of existing clinical and *in vitro* PK/PD data. This may serve as a basis to provide guidance to assess missing PK/PD related information.

NITROFURANTOIN FORMULATIONS

Being a member of the nitrofuran family, nitrofurantoin's chemical structure shows the typical five-membered furan ring containing four carbon atoms and one oxygen directly connected to a nitro group (-NO_2) (figure 1). The drug is a weak acid (pKa of 7.2) and is poorly soluble in water. Its solubility is enhanced under acidic conditions allowing good absorption of the gastrointestinal tract (15).

Nitrofurantoin is a synthetic product and has the appearance of a yellow crystalline powder (16, 17). There are different formulations of nitrofurantoin, those containing microcrystals (~10 micrometer in diameter) and those containing macrocrystals (75-180 micrometer in diameter). Macrocrystal formulations are available as such (Mac-

rodantin® or Furadantin®), as the slow-release formulation containing a mixture of macrocrystalline nitrofurantoin and its monohydrate form (Macrobid® or Furabid®) and as an oral suspension (16–19). However, an important defect of the marketed nitrofurantoin products is that there is no fixed cut-off value in crystal diameter for defining microcrystals and macrocrystals. Thus macrocrystalline nitrofurantoin crystal sizes can vary between products from different manufacturers. Crystal size impacts PK properties since macrocrystals are more slowly absorbed from the gastrointestinal tract and are excreted less in urine, both cumulatively (%) and in speed (%/h) compared to microcrystals (19–22). This issue of crystal size heterogeneity also applies to the slow-release and the oral suspension products. An additional problem for the slow-release product is that there is no fixed ratio between macrocrystalline nitrofurantoin and monohydrate nitrofurantoin so this ratio can vary between products. It is therefore almost impossible to describe one uniform PK profile since all published studies may have used different products with different crystal sizes (however, with the same product name).

The rapid absorption of the microcrystalline products are associated with more (gastrointestinal related) side effects so these tablets are completely replaced as first-line agents by the Macrodantin®/Furadantin® capsules and the Macrobid®/Furabid® capsules nowadays (19, 21). The drug is mainly used in a dose of 50 mg q6 hours, 100 mg q8 hours (both macrocrystalline nitrofurantoin) or 100 mg q12 hours (slow-release product) when used for the treatment of an UTI, but this can be different between countries (AFSSAPS, 2008; Gupta et al., 2011; TherapeuticGuidelines, 2018). 50-100 mg q24 hours is the registered dose for prophylactic use (16).

The metabolic pathway of nitrofurantoin is unclear, but it was suggested that metabolites are formed by reduction through bacterial enzymes (8, 25). It is not clear to what extent this reduction is required for its antibacterial activity (8). Both nitrofurantoin and its metabolites contain antibacterial activity which is enhanced under acidic conditions, but there is still a knowledge gap bout the exact identity and activity of the metabolites (8, 26–28).

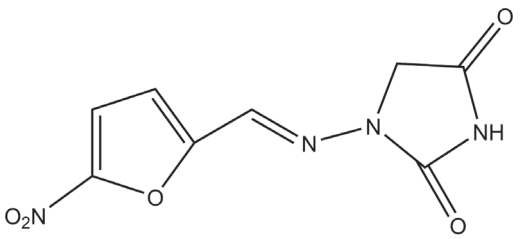

Figure 1. Chemical structure of nitrofurantoin. The chemical formula of nitrofurantoin is $C_8H_6N_4O_5$ and the average molecular weight is 238.2 g/mol.

BIOANALYSIS

Analytical methods

Two issues are important when interpreting papers describing analytical methods for nitrofurantoin. First, most methods describe the analysis of nitrofurantoin only, but do not include the metabolites. This may be relevant since metabolites may also be responsible for nitrofurantoin's clinical and/or microbiological effect (section 2). It is therefore difficult to fully link the measured concentration of nitrofurantoin to the observed effect. Second, since nitrofurantoin degrades under the influence of light, samples should be protected against (day)light and papers should specifically mention this (29).

Several analytical methods for the quantification of nitrofurantoin in human blood and/or urine were published over the last decades of which the first one was published in 1956 by Bender, Nohle and Paul (30). The method of Conklin and Hollifield from 1965 served as the base of most of the following published methods (31). Their paper described liquid-liquid extraction for urine samples followed by spectrophotometric detection. Aufrere, Hoener and Vore were the first in 1977 to describe a method applicable for both urine and plasma using HPLC with UV detection and subsequently, more methods followed for nitrofurantoin with or without metabolites (32–34). The first method using MS detection, the most used detection method nowadays for both research and therapeutic drug monitoring purposes, was published in 2013 (35).

3.2 Issues in the preparation of nitrofurantoin stock solutions

Besides its instability in light (29), nitrofurantoin also decomposes upon contact with metals other than stainless steel and aluminium (36). It has been demonstrated that the degradation of nitrofurantoin is enhanced in alkaline media (pH 10) compared to acidic media (pH 1.2) (37, 38).

Nitrofurantoin is practically insoluble in water and can, in contrast to most other antibiotics, not be dissolved in sterilized water to prepare stock solutions. Nitrofurantoin is therefore dissolved in a minimal volume of either dimethylformamide (DMF) or dimethyl sulfoxide (DMSO). DMF is recommended by the International Organization for Standardization (ISO) and the European Committee on Antimicrobial Susceptibility Testing EUCAST, whereas DMSO is recommended by Clinical & Laboratory Standards Institute (CLSI). Subsequently, the solution is diluted using a phosphate buffer (PBS) 0.1 M pH 8.0, followed by further dilution in PBS.

Since the amount of DMF to use is not expressly stated by ISO nor EUCAST, we performed several experiments to test the solubility of nitrofurantoin. These showed that the maximum stock concentration was 1024 mg/L using 5% DMF and only if dilution was performed using prewarmed diluents; otherwise precipitation and/or crystalliza-

tion would occur. We performed similar experiments with DMSO and concluded that it was easier to keep nitrofurantoin dissolved in DMSO compared to DMF, even though the published solubility for DMSO is considered equal to DMF (39–44).

Even after stock solutions have been prepared, precipitation of nitrofurantoin crystals may (re)appear when nitrofurantoin stock solutions are stored overnight at +/- 20 °C (room temperature), 4 °C, or kept at -80 °C. As a consequence, the nitrofurantoin concentration may deviate from the expected concentration. It is therefore recommended to only use freshly prepared nitrofurantoin (stock) solutions.

MECHANISM OF ACTION

It has been suggested that nitrofurantoin has multiple mechanisms of action, but none of these are fully understood. Nitrofurantoin is considered a prodrug that requires a reduction of the nitro group by bacterial nitroreductase enzymes in order to exert their antimicrobial activity. Nitrofurantoin is reduced to reactive electrophilic intermediates by bacterial flavoproteins that either alter or inactivate bacterial micro and macro molecules (45, 46). Besides, nitrofurantoin inhibits certain enzymes that have a role in the bacterial carbohydrate metabolism at 3 different locations in the citric acid cycle which prevents the generation of essential ATP (15). In addition the reactive intermediates attack/inhibit the initiation of ribosomal protein translation causing complete inhibition of protein synthesis and bind to DNA, and as a consequence strand breakage and/or DNA-damage may occur (46–50). The fact that the reactive compounds interfere in multiple biochemical processes, contributes to its low resistance rates since the bacteria are damaged in different ways and are not able to repair the damaged processes at the same time. This also contributes to the absence of cases of cross-resistance with other antibiotic classes.

SUSCEPTIBILITY TESTING

The ISO standard for broth microdilution method is considered as the reference method used for minimum inhibitory concentration (MIC) determination according to CLSI, EUCAST and ISO (51–53). However, there are other methods available that are also being used, e.g. the agar dilution method and the gradient diffusion method (for example the Etest® (Biomerieux, Marcy-l'Etoile, France), Liofilchem® MIC test strip (Liofilchem, Teramo, Italy) Overall, gradient test MICs correlated well with MICs observed by broth or agar dilution methods (54, 55). Disk diffusion has been used since the 1960 (56). The results of the disk diffusion test are qualitative and will indicate the

category of susceptibility (i.e., susceptible, (intermediate), or resistant). It should be noted here that the EUCAST has renamed its intermediate category to susceptible, dose dependent. There is a difference in recommended disk loads between EUCAST/ISO (100 µg) and CLSI (300 µg) and interpretation of zone-diameters is therefore different.

ANTIBACTERIAL ACTIVITY

Nitrofurantoin antibacterial activity differs by species. Table 1 provides the Wild-Type distributions and the ECOFF (the epidemiological cut-off; the MIC delineating the Wild-Type distribution) of species commonly encountered in uncomplicated UTI.

The ECOFF of *E. coli* is 64 mg/L which is also the susceptibility breakpoint for nitrofurantoin published by EUCAST. Susceptibility breakpoints for other species - Staphylococcus spp; *S. saprophyticus,* Enterococcus spp, *E. faecalis and Streptococcus agalactiae* are 64 mg/L as well at present; that for *Aerococcus sanguinicola* and *A. urinae* is 16 mg/L. EUCAST breakpoints are in principle based on PK data, microbiological data and clinical experience (57), but nitrofurantoin breakpoints are largely based on historic values used before the advent of PK/PD. Likewise, the susceptibility breakpoints published by CLSI are primarily based on historical set values. A difference with EUCAST is the recognition of an intermediate susceptibility category for microorganisms with an MIC of 64 mg/L, (53).

Table 1. MIC distribution and epidemiological cut-off value (ECOFF) (88).

Concentration mg/L	0.002-0.5	1	2	4	8	16	32	64	128	256	512	ECOFF
Species												
E. faecalis	0	1	1	31	535	163	7	7	1	0	0	32
E. faecium	0	0	0	1	15	40	331	754	781	263	0	256
E. coli	0	1	15	155	1304	2022	323	96	17	5	0	64
S. aureus	0	2	9	35	742	794	34	0	0	0	0	32
S. saprophyticus	0	0	0	3	40	28	0	0	0	0	0	32
S. agalactiae	0	0	3	31	10	2	0	0	0	0	0	16

CLINICAL PHARMACOKINETICS

Because of the different crystal sizes, formulations and recommendations for simultaneous food intake, PK profiling shows significant variation between products. In

addition, differences in the assay used in order to quantify the drug levels may have had a significant impact on the results (22, 31–35). It is therefore almost impossible to combine these data to provide a general PK profile of nitrofurantoin. In addition, in a recent review of the published PK data in urine and plasma of nitrofurantoin, the most important conclusion was that urine as well as plasma concentrations are highly variable between subjects (58). Thus, the PK profile of nitrofurantoin is complicated, can be influenced by several factors such as the crystal size and formulation of the product and characteristics of the patient such as fasting status and urination frequency, and is difficult to predict. This makes it complicated to review the effectivity of the current dosing regimens, to investigate the appropriate PK/PD index, and to set the corresponding PK/PD susceptibility breakpoint (section 8).

Urinary pharmacokinetics

Table 2 summarizes a selection of the PK parameters of nitrofurantoin in urine and plasma. The table displays PK parameters after the administration of macrocrystalline nitrofurantoin as microcrystalline formulations are not in current use. In general, maximum urine concentrations of nitrofurantoin vary from 15 mg/L to 230 mg/L and were found between ~3 and 10 hours after dosing, depending on the crystal size, formulation of the nitrofurantoin product and the fasting status of the subject (58). One study investigated urine excretion after a therapeutic dose of 100 mg macrocrystalline nitrofurantoin q6 hours (20). Although urinary concentrations were not reported, the recovery was found to be ~36% over 24 hours. Two other studies reported urine concentrations and recovery values after a single, prophylactic dose of 100 mg macrocrystalline nitrofurantoin. Concentrations varied from 83 to 159 mg/L after ~5 hours and recovery values were comparable with those after a multiple 100 mg dose (19, 20, 22). It should be noted that in general, urinary concentrations and recovery values are comparable between the studies wherein different dosages and dosing schedules were investigated. It therefore appears that the urinary PK of nitrofurantoin is not linearly related to the administered dose. PK data from a study in 12 healthy volunteers who received a dose of 50 mg q6 hours or 100 mg q8 hours of nitrofurantoin in its macrocrystalline form support this observation (59). In this study, urinary concentrations were comparable between the two dosing regimens.

The bioavailability of nitrofurantoin is ~20-30% and can increase to ~40% when administering the drug with food (21, 60). Urine concentrations were higher (>120 mg/L versus 95 mg/L), but were not found to occur later when comparing the slow-release formulation to the normal capsule (22). The total recovery over 24 hours however was higher (>30% versus 24.5%) for the slow-release formulation (22, 61). Of note, this slow-release formulation was not the slow-release formulation of macrocrystalline nitrofurantoin/monohydrate (Macrobid®/Furabid®) as being used nowadays in some

Table 2. The pharmacokinetic parameters of nitrofurantoin in urine and plasma after administering of macorcrystalline nitrofurantoin, modified from Wijma et al. (58).

	Reference	Subjects	Drug information		Fasting status	PK parameters						Analytical method
			Crystal size	Formulation		C_{max}	T_{max}	Recovery		Max. excretion rate		
						(mg/L)	(h)	(%)	(h)	(mg/h)	(h)	
Urine	**Multiple dose: 4x50 mg (therapeutic use)**											
	Huttner 2019	12	macro	capsule	f	26.8-176.3	3.3-5.5	-		-		LC-UV
	Multiple dose: 3x100 mg (therapeutic use)											
	Huttner 2019	12	macro	capsule	f	40.1-209.4	1.3-8.1	-		-		LC-UV
	Multiple dose: 4x100 mg (therapeutic use)											
	Conklin 1969	10	macro	capsule	nf	-	12-24	37.9	24	10.5	4-8	LLE + UV
							12-24	35.0		9.9	4-8	
	Single dose: 50 mg (prophylactic use)											
	Meyer 1974	14	macro	capsule	f	-		24.0	12	2[e]	-	LLE + UV
	Single dose: 100 mg (prophylactic use)											
	Paul 1967	15	macro	4 capsules	nf	83-159	3.4-5.5	19.6-35.4	24	4.2-8.9[e]	3.6-4.9	Chrom. + UV
	Albert 1974	10	macro	capsule	f	-		59.2	24	-		LLE + UV
	Bates 1974	4	macro	capsule	f	-		22.4	24	7.2	2.3	Chrom. + UV
					nf			40.4		10.4	3.5	
	Meyer 1974	14	macro	capsule	f	-		25.0	12	2.1[e]	-	LLE + UV
	Rosenberg 1976	4	macro	capsule	f	-		22.4	24	-		LLE + UV
					nf			40.4				

Table 2. The pharmacokinetic parameters of nitrofurantoin in urine and plasma after administering of macorcrystalline nitrofurantoin, modified from Wijma et al. (58). (*continued*)

	Reference	Subjects	Drug information		Fasting status	PK parameters						Analytical method
			Crystal size	Formulation		C_{max}	T_{max}	Recovery		Max. excretion rate		
						(mg/L)	(h)	(%)	(h)	(mg/h)	(h)	
Urine	Panayotis 1986	4	macro	capsule	f[a]	-		26.3 27.6 21.2	24	6.1 5.3 5.3	-	LLE + UV
	Macheras 1986	4	macro	capsule	f[f]	-		26.3 27.6 21.2	24	6.1 5.3 5.3	-	LLE + UV
	Mason 1987	24	macro	capsule	f	95	5	24.5	24	5.6	4.7	LLE + UV
	Adkison 2008	36	macro	capsule	f[g]	-		43.1 44.3 38.8	30	-		LC-UV or LC-MS/MS
	Slow release formulation: 2x100 mg (therapeutic use)											
	Maier-Lenz 1979	6	-	slow-release	-	-	-	47.5 33.7	24 24	-		LLE + UV[b]
	Mason 1987	24	macro	3 slow-release forms	f	120-150	3-5	30.4-34.1	24	7.5-8.3	3.7-4.2	LLE + UV
	Other dosages											
	Carroll 1955	9	macro dose: 200 + 4x100 mg	capsule	f	-		10.6 29.0 34.2	3 12 24	-		Chrom. + UV

Table 2. The pharmacokinetic parameters of nitrofurantoin in urine and plasma after administering of macorcrystalline nitrofurantoin, modified from Wijma et al. (58). (*continued*)

	Reference	Subjects	Drug information		Fasting status	PK parameters						Analytical method
			Crystal size	Formulation		C_{max}	T_{max}	Recovery		Max. excretion rate		
						(mg/L)	(h)	(%)	(h)	(mg/h)	(h)	
Urine	Schwartländer 1972	5	macro	capsule 150 mg	-	195.7	2-4	38.3	8	-		Color.
						273.5	0-2	35.1				
				capsule 3x50 mg		124.9	0-2	39.4	12			
				Slow-release tablet 75 mg		166.4	2-4	26.5	10			

	Reference	Subjects	Drug information		Fasting status	PK parameters					Analytical method
			Crystal size	Formulation		C_{max}	T_{max}	AUC		$T_{1/2}$	
						(mg/L)	(h)	(mg/L.h)	(h)	(h)	
Plasma	**Multiple dose: 4x50 mg (therapeutic use)**										
	Huttner 2019	12	macro	capsule	f	0.21-0.45	0.5-5.0	3.01-6.40	24	0.9-6.3	LC-UV
	Multiple dose: 3x100 mg (therapeutic use)										
	Huttner 2019	12	macro	capsule	f	0.22-1.26	0.5-5.0	0.94-10.97	24	0.8-2.7	LC-UV
	Single dose: 1x50 mg (prophylactic use)										
	Liedtke 1980	10	macro	tablet	f	0.26	2.1	1.5	-	1.7	HPLC + polarogr.
	Single dose: 1x100 mg (prophylactic use)										
	Felts 1971	11		-	nf	0.9-4.6	2-4	-		-	Chrom. + color.
		6				0.75-3.7					

Table 2. The pharmacokinetic parameters of nitrofurantoin in urine and plasma after administering of macorcrystalline nitrofurantoin, modified from Wijma et al. (58). (*continued*)

	Reference	Subjects	Drug information		Fasting status	PK parameters						Analytical method
			Crystal size	Formulation		C_{max}	T_{max}	Recovery		Max. excretion rate		
						(mg/L)	(h)	(%)	(h)	(mg/h)	(h)	
Plasma	Albert 1974	10	macro	capsule	f	1.47	2	3.28	0-4	-		LLE + UV
	Adkison 2008	36	macro	capsule	f	0.88	2-2.3	2.21	0-∞	0.78		LC-UV or LC-MS/MS
						0.96		2.42		0.76		
						0.96		2.32		0.72		
	Patel 2013	36	macro	capsule	nf	0.51	4.5	2622.3	0-∞	1.66		0
							4.7	2563.9		1.55		

Abbreviations used: [nf = non-fasting] [f = fasting] [Chrom. = chromatography] [Color. = colorimetric] [LLE = liquid-liquid extraction] [micro. = microbiologically] [Polarogr. = polarographical] [AUC = area under the concentration-time curve).
The '-' sign is used when data are missing.
[a] Administered with milk
[b] It was not specifically mentioned if the photochemical degradation of NF was taken into account for this method.
[c] UTI patients with loading dose of 100 mg.
[d] UTI patients with loading dose of 200 mg.
[e] unit: %/h.
[f] fasting + 100 mL, 200 mL or 400 mL milk, respectively.
[g] fasting + three different genotypes for the breast cancer resistance protein gene.

countries. PK data of this slow-release formulation given 100 mg q12 hours is lacking, as well as PK data in patients with impaired renal function to support or refute the recommended restrictive use of nitrofurantoin in this patient group (62, 63).

Plasma pharmacokinetics

Plasma concentrations of nitrofurantoin are less important than urine concentrations since urine is the clinically relevant compartment. Only one study investigated the plasma PK of nitrofurantoin (59). As demonstrated in table 2, plasma concentrations were significantly higher for the 100 mg q8 hour dose (up to 1.26 mg/L after ~2 hours) compared to those after the 50 mg q6 hour dose (up to only 0.45 mg/L after ~2 hours) (p-value <0.05), resulting in an overall higher exposure of the plasma compartment (expressed as AUC over 24 hours). Similar to urine, plasma concentrations are highly variable and are dependent of the crystal size of nitrofurantoin and fasting status of the subject (table 2). In general, maximum plasma concentrations are a 100 fold lower than urine concentrations (~1 mg/L) and were observed already 2 hours after dosing, suggesting a rapid absorption from the gastrointestinal tract (58). No plasma data are available after administration of the slow-release formulation.

PHARMACODYNAMICS

8.1 *In vitro* models

8.1.1 Static models: time-kill curves

To the best of our knowledge, only three published papers evaluated the effect of nitrofurantoin against several *Enterobacterales* species by time-kill assays (Fransen et al., 2016, 2017; Komp Lindgren et al., 2014). The studies used time-kill experiments to study several PD parameters, e.g. the kill rate (log10 cfu/mL×h^{-1}). This parameter represents the rate at which different concentrations of the antibiotic have a bactericidal effect and the degree of concentration-dependence. The relationship between concentration and kill rates was analysed by non-linear regression analysis using a sigmoidal E_{max} model with variable slope.

Early-phase PD analysis showed a higher maximal killing rate for *E. cloacae* compared *E. coli* (7) (figure 2). A concentration dependent kill pattern was observed for *E. cloacae* with significant increased killing over a wide concentration range, which resembles the PD efficacy of aminoglycosides (65, 66). Remarkably, this effect was not uniform among the Enterobacterales family. For the various *E. coli* strains (as well as for *K. pneumoniae*), the killing behaviour appeared to be much less concentration dependent as represented by a steeper Hill slope in the concentration-kill rate diagram

of *E. coli* (figure 2a) compared to the Hill slope of *E. cloacae* (figure 2b). The range for maximal killing of *E. coli* was considered narrow and resembles a β-lactam antimicrobial type of killing behaviour comparable to meropenem (65). A similar relationship for *E.coli* was found by Komp Lindgren et al. (64).

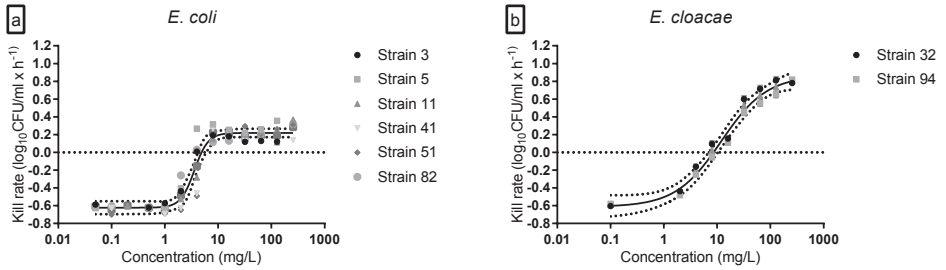

Figure 2. Early-time pharmacodynamics of nitrofurantoin for *E. coli* and *E. cloacae* strains after exposure to nitrofurantoin for 6 hours demonstrating a difference in pharmacodynamic effects. Kill rate data are plotted against concentration and best fitted sigmoid curves obtained from sigmoid maximum effect (E_{max}) model. The 95% confidence bands (dashed lines) are also plotted. The horizontal dotted line represents stasis i.e. no cfu reduction compared to the initial inoculum. Adapted from Fransen et al. (7).

8.1.2 Effect of matrix on pharmacodynamics

Since the matrix (urine) and the compartment (bladder) where nitrofurantoin has to exerts its effect is different from other body sites, it is important to determine the activity of nitrofurantoin under those circumstances. Both the composition of urine as well as its pH differ from the body in general.

Since the pH of urine may vary considerably, the effect of pH on the efficacy of nitrofurantoin was determined (67). Time-kill assays were performed at four pH levels (5.5, 6.5, 7.5 and 8.5) exposing the bacteria to 2-fold increasing concentrations of nitrofurantoin. Figure 3 shows the relationship between efficacy and pH for three species represented as the concentration required for static effect and 1 10log kill normalized to MIC. At lower pH values, the efficacy of nitrofurantoin is increased towards *E. coli* and *E. cloacae* relative to the MIC, whereas at higher pH values nitrofurantoin becomes less efficacious. This indicates that acidifying urine may be beneficial for the activity of nitrofurantoin.

MICs and time-kill curves are usually determined in Mueller-Hinton broth. This may provide only an estimate of nitrofurantoin activity because circumstances in the bladder differ, primarily because the composition of urine is different from that of Mueller Hinton. MICs determined in Mueller Hinton may therefore overestimate or underestimate local effects, as has been shown for several other antibiotics, such as ampicillin, ciprofloxacin and Trimethoprim-Sulfamethoxazole (68) and fosfomycin (69). To determine the activity of nitrofurantoin in urine, time-kill experiments were car-

ried out in urine and broth (Fransen et al 2017a). Figure 4 shows a typical example of the relationship between concentration and effect in both matrices. It is apparent that the maximum growth is higher in Mueller Hinton, but more importantly, that the EC50 and the concentration for a static effect in Mueller Hinton is much higher than in urine – indicating a significantly higher activity of nitrofurantoin in urine as opposed to laboratory conditions. Thus, nitrofurantoin may be more effective in patients than sometimes thought.

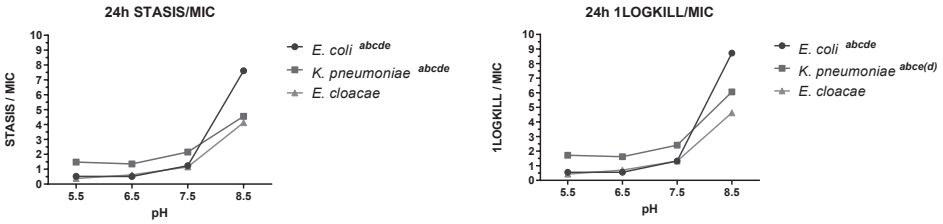

Figure 3. Geometric mean MIC-normalized stasis and 1 log kill at 24 h for *E. coli*, *K. pneumoniae* and *E. cloacae* at four different pH levels. Redrawn/adapted from Figure 3 Fransen et al, 2017 (26).

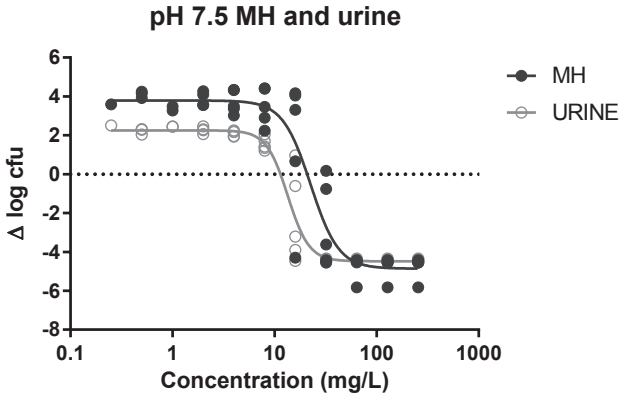

Figure 4. Typical example showing the relationship between concentration and effect at 24h incubation for nitrofurantoin in Mueller Hinton broth and urine for five E. coli strains. The lines are the fitted sigmoid curves obtained from the E_{max} model. The difference in static effect is significant ($p<0.01$)

8.1.3 Dynamic models

Currently, only one *in vitro* study addressed some dynamic nitrofurantoin concentrations (64). In this *in vitro* model, based on the dilution model described by Löwdin et al. (70), an *E. coli* isolate (MIC 2 mg/L) was initially exposed to a static nitrofurantoin concentration (16 mg/L), either for 24 hour or followed by a dilution phase with a half-life of 1 hour. In addition, experiments with starting concentrations of 12, 24, 32 and 100 mg/L nitrofurantoin and varying T>MIC were performed. When exposing *E.*

coli to dynamic nitrofurantoin concentrations, the PK/PD index that best correlated to the antibacterial activity of nitrofurantoin against *E. coli* was T>MIC. Thus, both the data from this study as well as the data from Fransen et al. (2016) indicate that T>MIC is the pharmacodynamic driver of nitrofurantoin for *E. coli* (section 8.1.1) (7). However, this study did not take into account the pathophysiology related factors of patients with UTI e.g. an increased urination frequency and experiments were carried out in Mueller-Hinton as opposed to the natural environment of urine. That this difference in medium may have consequences for interpretation of drug activity was recently shown for fosfomycin in a comparative study performed by Abbott et al (2018b) in a newly developed in vitro dynamic bladder model (I Abbott et al., 2018a). This in vitro model was constructed to reflect normal human urodynamics. Following simulation of a single dose 3g fosfomycin dose, the pharmacodynamic activity appeared to be reduced in urine as compared to Mueller-Hinton. Further studies preferably in urinary bladder models incorporating these items are required.

8.1.4 Urinary antibacterial activity

As already touched upon in the previous section, *in vitro* PD models often lack the ability to include patient related factors, which limits the translation of the results to the clinical situation. The urinary antibacterial activity of a drug is a measure for the antibacterial activity in the biological, clinically relevant matrix and is an alternative method using *ex vivo* PK data in order to obtain PD knowledge (71–73). Briefly, the method includes a2-fold serial dilution of a urine sample with drug-free urine in a microtiter plate, inoculation to a final concentration of 2.5×10^5 CFU/mL, and incubated overnight. The urinary antibacterial activity is then described by the urinary inhibitory titer (UIT), which is the largest dilution of the urine sample that inhibits visible bacterial growth or the urinary bactericidal titer (UBT) which is largest dilution of the urine sample that is bactericidal. A high titer thus indicates a relative high activity. The UIT and UBT values provide a reflection of the total activity of the drug in urine against the pathogen as it includes the microbiological activity of metabolites and other constituents (8, 28).

We recently investigated the urinary antibacterial activity of nitrofurantoin in healthy volunteers (59). Urine samples were collected during 6 or 8 hours in steady state to determine the UIT and the UBT. The major conclusion was that UITs are comparable to UBTs for nitrofurantoin, suggesting a bactericidal activity of the drug. Maximum titers were obtained in the first 2 hours after dosing, but no bactericidal or inhibitory effect was found during the complete 8 hour period in the majority of the samples (titers of <2). Higher titer values were observed after the 50 mg q6 hour dose compared to the 100 mg q8 hour dose in *E. coli* supporting more frequent dosing and a time dependence of nitrofurantoin. Of note, UITs and UBTs were comparable for *E. coli* and

K. pneumoniae strains although it is known that *E. coli* is in general highly susceptible to nitrofurantoin whereas *K. pneumoniae* carries often intrinsic resistant genes (10).

8.2 Animal models

Studies in animal models have not been published. However, for the last 15 years several murine models for urinary tract infections with *E. coli* have been developed (74–76). The mouse represents a desirable model system for mammalian UTI, as the bladder structure and cellular composition mimic those found in the human bladder. These mouse models use different permutations of intra-urethral or transurethral inoculation, with, e.g., variations in the compositions of urinary catheters and inoculum sizes, to introduce bacteria into the mouse bladders. Similar to other infection models, therapy can be administered and the pharmacodynamic effects determined by colony counts. The difference with other models such as the standard thigh model however, is that concentrations of the drug should (also) be measured in urine as this is the relevant matrix. It would be relevant to study nitrofurantoin in such a setting.

RESISTANCE

Nitrofurantoin resistance in E. coli results primarily by stepwise mutations in two chromosomal genes encoding for oxygen insensitive nitroreductases: nitrofuran sensitivity (nfs) genes A and B (77). The majority consists of the insertion of insertion sequence elements, but also deletions and missense mutations have also been observed (78). The mutations hinder the reduction of nitrofurantoin, thereby preventing the formation of toxic intermediate compounds (79). Resistance has also been generated in vitro as a results of deletion(s) in the ribE gene, encoding for lumazine synthase, an essential enzyme involved in the riboflavin biosynthesis pathway. The deletion in ribE leads to nitrofurantoin resistance by inhibiting the synthesis of riboflavin/Flavin mononucleotide, which is considered an important cofactor of nfsA and nfsB (80). Recently, the plasmid-mediated efflux genes oqxAB have also been associated with nitrofurantoin resistance, however there is a great need to study the dissemination of this plasmid (81).

The probability of resistance development to nitrofurantoin in *E. coli* is high *in vitro.* (79). However, resistant mutants appear to have a significant decrease in fitness as characterized by a lower growth rate compared to the susceptible wild-type population. Thus, resistant mutants will be outcompeted by the wild-type population in the absence of antibiotic pressure. Due to the physiology of the dynamic bladder and repeated voiding, a period of antibiotic absence is not rare even during treatment. This may explain the relative low resistance rates clinically (79). Despite its extensive use during the last decades, resistance rates for nitrofurantoin are still low. *E. coli* is sensi-

tive to nitrofurantoin in more than 95% when considering western European countries and the US (5, 82, 83). An additional reason for the *in vitro* and *ex vivo* discrepancy in resistance rates might be that oxygen levels *in vitro* are different from those in the human body. Since reduction of nitrofurantoin, which is important for its activity (section 2), is influenced by the presence of oxygen, this might result in a different antibacterial effect of nitrofurantoin *in vitro* and *ex vivo* (79).

Relationships between exposure and emergence of resistance have so far not been studied, but to test the hypotheses stated would be worthwhile to pursue.

ADVERSE EVENTS

Nitrofurantoins toxicity has recently been extensively investigated in two meta-analyses, one for UTI treatment (≤14 days) and one for short-term prophylaxis (3-14 days),for long term prophylaxis (9-28 days), or post-surgery prophylaxis (29-34 days) (6, 84). The results demonstrated that mild adverse events were found in 5%-16% of the cases when nitrofurantoin was used for UTI treatment. Patients receiving nitrofurantoin as UTI prophylaxis had an increased risk of 2.24 (95% CI 1.77-2.83) for non-severe side effects. If occurred, toxicity was primarily mild, reversible and limited to GI-related side effects. Pulmonary and hepatotoxicity are considered as serious adverse events of nitrofurantoin (16–18). However, only one out of 3052 patients in studies published experienced a severe pulmonary side effect when nitrofurantoin was used as UTI prophylaxis (84). It was concluded that severe side effects are rare and only related to UTI prophylaxis.

In neither of the two meta-analyses a relationship with exposure was apparent except long term use. However, It seems reasonable to assume that the severity of side effects can be different between nitrofurantoin products since the crystal size of macrocrystalline nitrofurantoin differs between products and the crystal size is associated with the severity of side effects due to the rapid absorption of microcrystalline nitrofurantoin (section 2) (19, 21, 85). This would warrant further investigation.

CONCLUDING REMARKS

In summary, there are few dose justification data for nitrofurantoin following the current standards which may be the reason that different dosing regimens are recommended. In addition, there are different formulations of nitrofurantoin in use that each have their own characteristics of disposition, but are not available for all formulations. There would be a clear benefit if the formulations of nitrofurantoin were standardized. Exposure response data of nitrofurantoin are not readily available. Yet in a recent ran-

domized controlled study comparing nitrofurantoin 100 mg q8 hours (macrocrystalline, normal-release) for five days with a single 3 gram dose of fosfomycin, nitrofurantoin was clinically and microbiologically more effective. However, treatment failures are not rare (e.g. 70% of the patients clinically improved and microbiologically resolution occurred in 74%) (86) and optimizing exposure could benefit patients and reduce failures. It is therefore imperative that more PK/PD data become available. One such approach could be the use of a dynamic bladder infection model as recently by Abbott et al. (87). This *in vitro* model was constructed to reflect normal human urodynamics on a 1:15 scale over a period of several days. Alternatively, studies in UTI animal models could verify (or refute) that T>MIC is the driving PD index as was suggested by the time-kill experiments.

Another important knowledge gap is the relationship between exposure and the occurrence of adverse events, although there are some indications that, with a decline of renal function as a result of aging, more side effects occur. However, PK related evidence for this is lacking.

Finally, although it is known that metabolites are formed, the exact structure and antibacterial activity and/or toxicity of each metabolite is still unclear and needs to be resolved.

Although nitrofurantoin has been available for over 60 years and is the first choice to treat lower UTI in many countries, the lack of a scientific basis for optimal dosing is alarming.

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Transparency declaration

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3.3

The pharmacokinetics of nitrofurantoin in healthy female volunteers: a randomized cross-over study

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ABSTRACT

Background. Nitrofurantoin's use has increased significantly since its recent repositioning as a first-line agent for uncomplicated cystitis by multiple guidelines. However, current dosing schemes were developed in an era predating robust pharmacokinetic testing and may not be optimal. Furthermore, formulations have been modified over the years.

Objectives. To reassess the plasma and urinary pharmacokinetic profile of macrocrystalline nitrofurantoin at two commonly used dosing regimens.

Methods. In this open-label, randomized cross-over pharmacokinetic trial, 12 healthy adult female volunteers were randomized to receive oral nitrofurantoin 100 mg q8h on days 1 and 2 and, after a washout period, 50 mg q6h on days 30 and 31, or the same dosing schemes in reversed order. Urine and blood were collected in steady state and analyzed by HPLC. Pharmacokinetic analysis was performed by WinNonlin.

Results. Plasma peak concentrations were low (mean 0.33 mg/L, SD 0.08 and 0.69 mg/L, SD 0.35 after 50 mg and 100 mg, respectively) and dose-dependent. The $AUC_{0\text{-}24h}$ for the 100 mg q8 hours dosing regimen was higher (6.49 h*mg/L versus 4.43 h*mg/L, p=0.021), but the dose-normalized AUC similar. In contrast, urinary concentrations were dose-independent: increasing the nitrofurantoin dose delayed the time to peak urinary concentration, while $AUC_{0\text{-}24,ss}$ values remained unchanged (943.49 h*mg/L and 855.95 h*mg/L at 50 mg q6h and 100 mg q8h, respectively).

Conclusions. Plasma concentrations were relatively low and dose-dependent. The dose-independent urinary concentrations suggest that excretion of nitrofurantoin into the urine is saturable. Pharmacodynamic studies are urgently required to determine the impact of these findings.

INTRODUCTION

Nitrofurantoin has been in clinical use since 1953 (1). Its consumption has increased exponentially (2) since international guidelines for the management of uncomplicated urinary tract infections (UTI) were updated in 2011 to position nitrofurantoin as a first-line agent for the treatment of cystitis (3). Two key advantages of nitrofurantoin are the current low prevalence of nitrofurantoin resistance amongst Enterobacteriaceae and a lower propensity for "collateral damage" amongst commensal flora in comparison to that of the quinolones and beta-lactam antibiotics (4, 5). Yet while a meta-analysis of randomized trials conducted between 1946 and 2014 comparing nitrofurantoin to other UTI agents showed equivalent clinical efficacy, it also showed that comparator drugs had a slight but statistically significant advantage in terms of microbiologic efficacy (6). A more recent randomized trial comparing macrocrystalline nitrofurantoin to single-dose fosfomycin demonstrated superiority of nitrofurantoin in both clinical and microbiologic outcomes (7), but nitrofurantoin's success rates in this trial were still lower than those reported in earlier studies. It is unclear whether current dosing schemes, established in an era predating standardized, methodologically robust approaches to drug testing, are optimal. The current body of pharmacokinetic knowledge regarding nitrofurantoin in both healthy subjects and patients with UTI is poor and based mainly on decades-old studies using comparatively archaic laboratory and analytic techniques (8). In addition, the formulations of nitrofurantoin have changed over the years. And nowadays, the most commonly used dosing regimen varies per country. The dose of 50mg q6h and the 100mg q8h are both regularly used. Given the resurgence of nitrofurantoin's clinical use, a re-examination of its pharmacokinetic profile at frequently used dosing schemes is warranted. The purpose of this study was to document the pharmacokinetics of nitrofurantoin in healthy female volunteers receiving the drug at two commonly used dosing regimens (9–11), comparing the regimens in terms of exposure.

PATIENTS AND METHODS

Study design and participants

For this phase I randomized, open-label crossover trial conducted in March and April of 2015 at the Geneva University Hospitals (HUG) in Geneva, Switzerland, 12 non-pregnant female volunteers aged 18 to 75 years and in good health, without clinically significant medical history, physical examination findings, or clinical laboratory abnormalities as per clinical judgment of the investigator, were recruited by means of flyers posted locally. Exclusion criteria were (1) receipt of concomitant medications besides

estrogen-based oral contraceptives, (2) receipt of any antibiotic within four weeks of inclusion, and (3) creatinine clearance < 60 ml/min. Participants were screened one week before enrollment and randomization. The study and all protocol amendments were reviewed and approved by the Cantonal Ethics Commission of Geneva (13-036) and by the Swiss Agency for Therapeutic Products (2014DR1008). All participants provided written, informed consent before their inclusion.

Enrollment, randomization and intervention

Participants were enrolled by study investigators and randomized to receive one of two nitrofurantoin regimens over a two-day period; after a 28-day washout period, they then received the alternate regimen for a final two days. The randomization sequence was computer-generated and used randomly permuted blocks of varying sizes. Assignments were concealed from investigators by means of opaque, sealed envelopes until volunteer enrollment, and allocated treatment to either dosing scheme in a 1:1 ratio.

Participants randomized to Group 1 received oral nitrofurantoin at a dose of 100 mg q8 hours (Furadantine® macrocrystalline [MC] 100 mg capsules) for two days (days 1 and 2) and, after a washout period of 28 days, a dose of 50 mg q6 hours (Furadantine® MC 50 mg capsules) for two days (days 30 and 31); those randomized to Group 2 followed the same scheme but with the dosing regimens switched. Study visits occurred only on days 2 and 31, when steady state (after 24 hours of nitrofurantoin intake) was presumably reached. On days 1 and 30, just before self-administering nitrofurantoin, participants voided and collected a baseline 5 ml urine sample from the total void, which they refrigerated (5°C) before bringing it to the clinical trials unit (CTU) on the following day. They then took the assigned nitrofurantoin regimen for the following two days, reporting to the clinical trials unit (CTU) on day 2 (and 31) for sampling. All volunteers arrived fasting on day 2 (and 31) and then ate breakfast at the CTU after the first blood draw. The first blood sample was taken just before the subject received the first daily dose of NF at the research centre (t=0h). Afterwards, blood samples of 2 ml each were collected at the following fixed time points: 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6 and 8h. For the urine samples there were no fixed sampling times. When the participant voided 5 ml of the urine was stored and times recorded. To simulate the real life situation they were permitted to urinate at liberty. For both serum and urine, the exact sampling times were recorded. The washout period began at the end of the study visit on day 2; in this 28-day span, participants took no nitrofurantoin or other medications (except estrogen-based oral contraceptives). All potentially related side effects were recorded. The nature of the event, the date and time it occurred were recorded.

Nitrofurantoin assay

During study visits, urine samples were stored at -30°C for a maximum of two hours in the dark and then transferred to plastic storage tubes for freezing at -80°C until analysis. Blood samples drawn on days 2 and 31 were placed immediately on ice in the dark, allowed to clot for a minimum of 15 min, and centrifuged at 1,200 × g for approximately 10 min at room temperature. Supernatants were transferred to plastic storage tubes and frozen at −80°C until analysis. All samples were shipped at -80°C to Radboudumc (Nijmegen, the Netherlands) for analysis. Urinary and plasma nitrofurantoin concentrations were analyzed by means of ultra-high performance liquid chromatography with diode array detection (UPLC-DAD) (12). The sample preparation method consisted of protein precipitation for plasma and liquid-liquid extraction for urine. 100 µL was needed for the sample preparation. Linearity was confirmed over a concentration range from 0.05 to 1.25 mg/L in plasma and from 4 to 200 mg/L in urine ($r^2 > 0.95$). Within-day accuracy was <± 13% in both matrices, between-day accuracy <±7% and <±9%, within-day precision <10% and <4% and between-day precision <10% and <5%. Plasma samples are stable for seven days at 4°C, and for six months at -20°C and -80°C. Urine samples are stable for at least seven days at 4°C or room temperature and during three months at -20°C or -80°C, except from the lower concentrated samples, which are only stable at -80°C. All samples were kept from daylight using amber-coloured glassware. This method was validated according to the Food and Drug Administration's guideline for bioanalytical method validation, 2018 (13).

Pharmacokinetic analysis

The maximum concentration (C_{max}, mg/L), time to maximum concentration (T_{max}, h), volume of distribution (V_d/F, L), clearance (CL/F, L/h), concentration half-life ($T_{1/2}$, h) and the area under the concentration-time curve (AUC, h*mg/L) for one dosing interval (AUC, were calculated as pharmacokinetic parameters using non-compartmental analysis (Phoenix® WinNonlin™ version 6.4; Pharsight Corporation). The $AUC_{0\text{-}24,ss}$, (at steady-state) h*mg/L was calculated by multiplying the $AUC_{0\text{-}6h}$ by 4 or the $AUC_{0\text{-}8h}$ by 3 since the samples were collected in steady state. In addition, a dose normalized $AUC_{0\text{-}24h,ss}$ was determined.

Statistical methods

Descriptive statistics were used to report volunteers' clinical characteristics; when normally distributed, continuous data are presented as the mean (± standard deviation [SD]), and medians with IQR are reported for unevenly distributed data. To compare the values in the analysis a paired, 2-tailed t-test was used. Non-parametric or parametric tests were performed where appropriate using Stata v15.0, Statacorp, College Station, Tx. Associations with p values of ≤0.05 (two-sided) were considered statistically significant .

RESULTS

Study population

Fifteen women were screened for eligibility; two were excluded due to undiagnosed anemia and peripheral eosinophilia, respectively, while the third was not included because the required number of participants had been reached. All participants were of European (Caucasian) ancestry; median age was 25 years (IQR 24-33, full range 18-46); median weight was 61.5 kg (IQR 60.0-68.4, full range 50.0-85.0), median BMI 22.3 kg/m^2 (IQR 21.0-24.5, full range 18.8-34.7), mean serum creatinine at screening 64.0 mmol/L (SD 10.2), and mean estimated glomerular filtration rate (estimated by Cockcroft-Gault equation) of 118 mL/min (SD 23.6).

Plasma and urinary concentrations of nitrofurantoin

The plasma and urinary concentrations of study volunteers are depicted in Figure 1. The total number of plasma samples and urine samples included in the analysis is 312 and 140, respectively. The percentage of samples with values below-the limit of quantification in serum was 5.1% and in urine 7.1%. Since both values are below 10% these values were deleted from the analysis. Peak plasma concentrations were achieved at a mean of 2.4 h (SD 1.4) after administration of the 50 mg dose and at a mean of 2.1 h (SD 1.4) after the 100 mg dose. Considerable inter-individual variability was observed, with a full range of 0.5-5.0 h for both the 50 mg and 100 mg doses (figure 1). Maximal plasma concentrations were low, ranging from 0.21 to 0.45 mg/L for the 50 mg dose and 0.22 to 1.26 mg/L for the 100 mg dose. The $AUC_{0\text{-}6h}$ in plasma for the 50 mg q6 hours regimen ranged from 0.76 to 1.60 h*mg/L, with a mean value of 1.11 h*mg/L. For the 100 mg q8 hours regimen, $AUC_{0\text{-}8h}$ varied from 0.31 to 3.66 h*mg/L, with a mean of 2.16 h*mg/L. The mean plasma V/F and CL/F were comparable for both dosing regimens (100.0 L (SD 49.6) versus 103.8 L [SD 65.9] (p=0.8167) and 36.4 L/h (SD 11.4) versus 46.2 L/h (SD 18.6) (p=0.116) for the 50 mg q6h and 100 mg q8h dose respectively). This difference in concentrations results in a significantly higher AUC over 24 hours for the 100 mg q8 hours dosing regimen (6.49 h*mg/L versus 4.43 h*mg/L, p=0.021), meaning a higher exposure of the plasma compartment to nitrofurantoin for the 100 mg q8 hours regimen. The dose normalized AUCs were not significantly different (table 1). There was no significant relation between creatinine clearance and nitrofurantoin clearance. A summary of the pharmacokinetic parameters in plasma is presented in table 1.

The mean pharmacokinetic parameters in the urine are presented in table 1. Urinary concentrations were considerably higher compared to the plasma concentrations. Despite the difference in dose and interval, the PK parameters for the two dosing regimens did not significantly different from each other in general. Maximum concentrations for

the 50 mg q6 hours dose ranged from 26.8-176.3 mg/L and from 94.1-49.9 mg/L for the 100 mg q8 hours dose, but mean concentrations were comparable (94.4 mg/L versus 94.1 mg/L, p=0.9871). The concentration-time profile for the two regimens in the urine is shown in figure 1. The $AUC_{0\text{-}6h}$ in urine for the 50 mg q6 hours regimen ranged from 57.04 to 553.01 h*mg/L, with a mean value of 235.87 h*mg/L. For the 100 mg q8 hours regimen, $AUC_{0\text{-}8h}$ varied from 126.19 to 699.62 h*mg/L, with a mean of 285.32 h*mg/L. Thus, the urine exposure over 24 hours for the 50 mg q6 hour regimen (mean $AUC_{0\text{-}24h}$ of 943.49 h*mg/L) as compared to the 100 mg q8 hour regimen (mean $AUC_{0\text{-}24h}$ of 855.95 h*mg/L) was similar (p=0.507). However, the dose normalized AUCs differed significantly (mean 4.717 h*mg/L per mg dose for the 50mg q6h regimen vs 2.85 h*mg/L per mg dose for the 100mg q8h regimen, p=0.039).

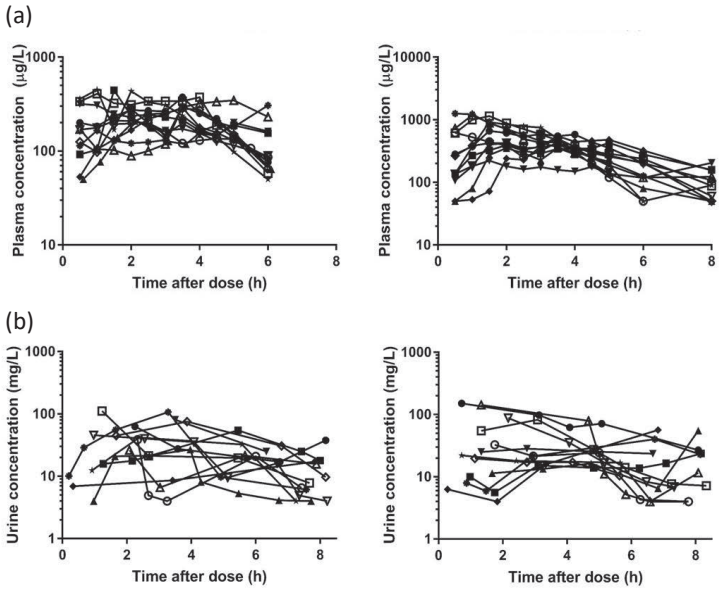

Figure 1. Nitrofurantoin concentration–time curves for plasma (a) and urine (b) samples.

Side effects

In total 10 events were recorded of which 8 occurred during the first phase of the study during both dosing regimens, 1 during the second and of 1 the date was unknown. Five individuals reported mild gastro-intestinal side effects (all on the first day during the first phase of the study). Other reported events were tiredness (N=2), mild neurologic symptoms (N=1), mild chest discomfort (N=1), and short term heat sensation (N=1).

Table 1. Pharmacokinetic parameters in plasma and urine.

Plasma							
	50 mg q6h			**100 mg q8h**			**P value**
Pharmacokinetic parameter	Mean ± SD	Minimum-maximum value	CV (%)	Mean ± SD	Minimum-maximum value	CV (%)	
C_{max} (mg/L)	0.326 ± 0.081	0.21 – 0.45	24.77	0.69 ± 0.35	0.22 – 1.26	51.00	.0029
T_{max} (h)	2.4 ± 1.4	0.5 – 5.0	58.3	2.1 ± 1.4	0.5 – 5.0	66.6	.6825
V_d/F (L)	100.0 ± 49.6	37.4 – 183.3	49.5	103.8 ± 65.9	44.1 – 287.4	63.5	.8167
CL/F (L/h)	36.4 ± 11.4	20.0 – 58.9	31.4	46.2 ± 18.6	25.5 – 78.7	40.3	.1116
$T_{1/2}$ (h)	2.3 ± 1.8	0.9 – 6.3	80.7	1.7 ± 0.6	0.8 – 2.7	33.3	.1901
$AUC_{0\text{-}24\ ,ss}$ (h*mg/L)	4.43 ± 0.96	3.04 – 6.40	21.65	6.5 ± 2.9	0.94 – 10.97	44.32	.0211
Dose-normalised $AUC_{0\text{-}24}$ (per mg dose) (h*mg/L)	0.0221 ± 0.0096	0.00479-0.032	21.65	0.0216 ± 0.0096	0.0031-0.037	44.32	.8480
Urine							
	50 mg q6h			**100 mg q8h**			**P value**
Pharmacokinetic parameter	Mean ± SD	Minimum-maximum value	CV (%)	Mean ± SD	Minimum-maximum value	CV (%)	
C_{max} (mg/L)	94.4 ± 47.8	26.8 – 176.3	50.6	94.1 ± 49.9	40.1 – 209.4	53.0	.9871
T_{max} (h)	5.1 ± 0.7	3.3 – 5.5	13.6	6.8 ± 1.8	1.3 – 8.1	25.8	.0112
$AUC_{0\text{-}24,ss}$ (h*mg/L)	943.49 ± 539.60	228.2 – 2212.0	57.19	855.95 ± 581.0	378.6– 2098.9	67.88	.507
Dose-normalised $AUC_{0\text{-}24}$ (per mg dose) (h*mg/L)	4.717 ± 2.698	1.14- 11.06	57.19	2.85 ± 1.94	1.3 – 7.0	67.88	.0390

C_{max}: maximal concentration; T_{max}: time after administration to C_{max}; V_d/F: volume of distribution after oral administration; CL/F: apparent total clearance from plasma after oral administration; $T_{1/2}$: elimination half-life; $AUC_{0\text{-}24,ss}$: area under the concentration time curve within 0 and 24h measured at steady state. AUC calculated by trapezoideal rule.

DISCUSSION

In this cohort of healthy volunteers, we observed a higher total exposure in plasma with the 100 mg q8h dose, but the total exposure in the urine was similar for the two regimens. When the $AUC_{0\text{-}24,ss}$ was normalized per 1 mg administered nitrofurantoin, the dose-normalized $AUC_{0\text{-}24}$ in plasma was similar, but in urine it was significantly higher after the 50 mg q6h dose. In general, there was high inter-individual variability in both plasma as well as urine concentrations. We confirm earlier observations of low (≤1 mg/L) plasma concentrations peaking within two hours after oral intake and higher, more durable urinary concentrations appearing within minutes after oral intake (14–17), findings that support the continued use of nitrofurantoin for lower UTI only.

The absorption of macrocrystalline nitrofurantoin from the gastrointestinal (GI) tract into the central compartment depends more on the drug's ability to dissolve in GI fluids than on its residence time in the GI tract (18). Based on the dose-dependent increase of plasma concentrations, it seems that the dose of 100 mg dissolves as well as the 50 mg dose. However, the speed of absorption is not higher for the 100 mg dose based on the comparable T_{max} values in plasma. Considering these two dosages, we conclude that absorption of nitrofurantoin from the GI tract is insaturable within the dose range of the study. It would be interesting to investigate to which extent the absorption is insaturable by investigating the PK in plasma and urine after administration of dosages higher than 100 mg. If also insaturable at higher dose levels, then this might result in higher plasma levels and prolonged urine concentrations, which will contribute to the time-dependent killing of nitrofurantoin for *E. coli* and *K. pneumonia* (19). More research is needed to investigate both dose dependency and formulation dependency of the PK of nitrofurantoin in plasma.

To our knowledge, the only recent study reporting nitrofurantoin PK was conducted by Adkison *et al*. in 2008 (17). In this single dose study, plasma and urine concentrations were measured in 36 healthy Chinese men with different ABCG2 polymorphisms after administration of a single dose of 100 mg (MC). Reported PK parameters were comparable with those reported here.

Older literature would appear to support the findings of the non-compartmental analysis. In 1975, Sullivan *et al*. showed in patients with unequally functioning kidneys that macrocrystalline nitrofurantoin concentrations in urine produced by the compromised kidney were significantly elevated throughout the dosing interval as compared to concentrations in urine produced by the better-functioning kidney after oral administration of the same dose (20). In 1958, Lippman and others investigated urinary PK after administration of a clinically relevant dose of 50 mg (microcrystalline)q6 hours (21). Similar to our results, they found lower concentrations after administering 100 mg q6 hours compared to 50 mg q6 hours in nine patients with recurrent UTI. However,

underlying mechanisms remain unclear and require further elucidation, as only a better understanding of the pharmacokinetic profile of nitrofurantoin will allow for optimization of its use.

The inter-individual variability in urine concentrations was high. Part of the variability might be explained by differences in renal function between subjects. Although all the eGFR values were >90 mL/min, there was of course a difference in renal function values (118 mL/min, SD 23.6). The fact that the fluid intake was not standardised, might also partially explain this, since it effects the extent of dilutions of the urine samples. Fluid intake 'ad lib' is a known limitation of urinary PK research. However, we consider this not as a limitation of the study, but more as a strength since is gives a better reflection of the real-world situation. Our results therefore show clearly the variation that is expected in patients.

The pharmacodynamic driver for nitrofurantoin has not been clearly established. In a recent study, it was suggested that the pharmacodynamic characteristics might be species-dependent. Using time-kill curve methodology, Franssen *et al*. showed that the effect of nitrofurantoin against *Enterobacter cloacae* appeared to be concentration-dependent, whereas for *Escherichia coli* strains as well as for *Klebsiella pneumoniae* strains the effect was relatively time-dependent (19). These findings would support the use of lower frequent doses instead of higher, less frequent doses. Alternatively a slow release formulation may also lead to extended exposure. However, up to now, no specific minimum value of the exposure needed for clinical efficacy is available to correlate with the PK results found in this study. Based on the total exposure no difference in efficacy between the two dosing regimen is to be expected.

In summary, we have described the pharmacokinetic profiling of two commonly used dosing regimens of nitrofurantoin. Whereas plasma profiling was dose dependent in a linear fashion, urine concentrations at the 50 mg dose were relatively high compared to the 100 mg dose. More pharmacodynamic studies are required to determine the impact of these findings.

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Transparency declarations

AH, RAW, AJS, FO, EvD, RJMB, and AEM have no conflict of interest to declare. SH has consulted for Sandoz and DNA Electronics. JWM has received research funding from Adenium, Astra-Zeneca, Basilea, Cubist, Helperby, Nordic-Pharma, Polyphor, Roche, Eumedica, Basilea, VenatorX, AiCuris, Gilead and Wockhardt.

Contribution of the authors: the authors were involved in the following:

AH: design of the study, data collection, analysis of the data and writing the manuscript. RAW: analysis of the data and writing the manuscript. AJS: design of the study, writing the manuscript , FO: recruitment, data collection, designing database, writing the manuscript , EvD: recruitment, data collection, writing the manuscript, RJMB: analysis of the samples and data and writing the manuscript. SH: design of the study, data collection, writing the manuscript JWM: design of the study, data collection, analysis of the data and writing the manuscript. AEM: design of the study, data collection, analysis of the data and writing the manuscript.

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3.4

The urinary pharmacokinetics of nitrofurantoin in patients with uncomplicated urinary tract infections: interim analysis

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[Inclusion of patients in this study is still ongoing so interim data are reported in this paper.]

ABSTRACT

Background: Nitrofurantoin was registered as first-line treatment option of uncomplicated urinary tract infections in 1954, a time before a structured process of drug development was mandatory. As a consequence, nitrofurantoin is being prescribed in daily clinical practice based on limited data supporting the currently used dosing regimen. A better knowledge of the pharmacokinetic (PK) properties of nitrofurantoin would help to achieve optimal dosing regimens.

Objectives: To investigate and compare the urinary PK profile of nitrofurantoin in two commonly used dosing regimens in patients with uncomplicated urinary tract infections.

Methods: Urine samples were collected during 24 hours by 19 female patients who were prescribed nitrofurantoin in a dose of 50 mg q6h (Macrodantin®/Furadantin®) or 100 mg q12h (Macrobid®/Furabid®) by their treating general practitioner. Nitrofurantoin concentrations were quantified by UHPLC-UV. PK analysis was performed by PKSolver® using non-compartmental analysis.

Results: Mean peak concentrations of nitrofurantoin in urine after 100 mg were higher compared to those after 50 mg (104.2 mg/L ± 74.7 versus 84.7 mg/L ± 64.6), but the range in maximum concentrations was comparable (<4 mg/L to 264.8 mg/L or to 267.7 mg/L). The $AUC_{0\text{-}24h,ss}$ was higher (931.5 mg.h/L ± 672.3 versus 881.9 mg.h/L ± 347.1) after the 100 mg dosing regimen. The slow-release effect of the 100 mg capsule, demonstrated as a delayed peak concentration, but none of the PK parameters differed significantly between the dosing regimens.

Conclusion: The urinary PK of nitrofurantoin was comparable between the two studied dosing regimens and therefore independent of the administered dose and formulation. Additional PK studies, including more patients administering Macrobid®/Furabid®, are needed.

INTRODUCTION

In an era of emergence of drug resistant pathogens, there is more interest in the use of old antibiotics in the treatment of infections in daily clinical practice (1,2). Nitrofurantoin is one of those "old" drugs. It was registered in 1954 as oral antibiotic for the treatment of uncomplicated urinary tract infections (UTIs) (3). It is a synthetic agent with a broad spectrum of activity, including (vancomycin-resistant) enterococci and Extended Spectrum Beta-Lactamase (ESBL) producing Enterobacteriaceae (4–6). Its popularity is increasing as resistance rates for nitrofurantoin are still low (7), while increasing for former treatment options for uncomplicated UTIs like beta-lactam antibiotics and quinolones (2). Despite the fact that the drug has been used since its registration, little is known about its pharmacokinetic (PK) properties. The majority of published studies in which PK parameters are reported, were performed at the time of drug development and therefore different crystal sizes, formulations and patient populations were investigated. This variability complicates translation of these older PK data to the use of nitrofurantoin today. Therefore, renewed studies on the PK properties of nitrofurantoin, using clinically relevant formulations, dosages and patients with uncomplicated UTIs is needed. In this study, we investigated and compared the PK profile of nitrofurantoin in its macrocrystalline form after two commonly used dosing regimens e.g. Furadantin®/Macrodantin® (50 mg q6 hours) or Furabid®/Macrobid® (100 mg q12 hours). Furthermore, we explored the influence of different patient specific covariates on the PK of nitrofurantoin. This interim analysis presents the first findings.

METHODS

Study design and drug administration

The study was designed as a multicenter, open label study. Patients were approached to participate in the study after the treating physician prescribed nitrofurantoin for a suspected or proven uncomplicated UTI in the registered dose of 50 mg (Furadantin®/Macrodantin®) q6 hours or 100 mg (Furabid®/Macrobid®) q12 hours according to the Dutch Guidelines for general practitioners (GPs) (8). Patients were enrolled in one of the two clinical departments of the Erasmus Medical Center (EMC), e.g. neurosurgery and neurology, or at one of the four GPs after written informed consent was provided. The study was approved by the ethics committee of the EMC (MEC-2017-526).

Study population

Female patients of at least 18 years of age, who were prescribed nitrofurantoin, and with a recent (≤ one year) measurement of their creatinine clearance, were allowed to

participate in the study. The creatinine clearance was additionally measured as part of the study after consent of the patient if the latter requirement was not met. Patients were excluded from participation in this study if they were being treated with any other antibiotic within one week of the potential urine collection period, if the patient was known for porphyria or an allergy related to nitrofurantoin use or with a creatinine clearance of ≤30 mL/min, calculated using the CKD-EPI equation.

Sample collection

EMC patients collected urine samples during hospitalization with help from a nurse and GP patients self-collected urine samples in the home setting. Urine was collected during 24 hours and started on day 2, 3 or 4 of the 5-day treatment to ensure the steady state situation was reached. Patients were instructed to record date, time and total volume of each void. Volume could be accurately measured by voiding in a measuring cup. After recording the volume, ± 5 mL of urine was transferred to a small 50 mL-urine container. Urine containers were covered in aluminum foil to protect the content from daylight. The samples were stored in the freezer at approximately -20°C at the patients' home during the urine collection period of 24 hours. After the researchers collected the urine samples from the patients, samples were stored at -80°C in the EMC until the day of analysis. Stability of the samples at these two conditions was confirmed during validation of the method (9).

Quantification of nitrofurantoin in urine

Nitrofurantoin concentrations were determined using a ultra-high performance liquid chromatography (UHPLC) method with ultra violet (UV) detection at a wavelength of 369 nm (9). The method was validated according to the guidelines for bio analytical method validation of the Food and Drug Agency (FDA) (10). One-hundred microliter urine was needed for the sample preparation which consisted of liquid-liquid extraction. Linearity was confirmed over a concentration range from 4 to 200 mg/L. Samples were diluted with drug free urine if the concentration exceeded the upper end of this concentration range during the initial analysis. Concentrations below the lower limit of this concentration range were reported as '<4 mg/L'. Urine samples were found to be stable for at least seven days at 4°C and at room temperature and for 2 years at -20°C and at -80°C. Since short-term stability at 4°C for 7 days of lower concentrated urine samples could not be confirmed, all urine samples were stored in the patients' freezer (at approximately -20°C) for a maximum of 48 hours to guarantee stability.

Pharmacokinetic analysis

The following PK parameters were calculated with PKSolver® using non-compartmental analysis (11) based on the nitrofurantoin concentrations in the urine samples: maximum

concentration (C_{max}, mg/L) time to maximum concentrations (T_{max}, hours) cumulative amount excreted over 24 hours (mg), recovery expressed as percentage of the total daily dose (%), area under the concentration-time curve in steady-state calculated over 24 hours ($AUC_{0\text{-}24h,ss}$). The linear trapezoidal approach was used to calculate the AUC.

Data analysis

The mean, standard deviation (SD), range and coefficient of variation (CV, %) of the patient characteristics and the PK parameters were calculated for the total population and per dosing regimen. PK parameters were compared between the two dosing regimens using an unpaired, two-tailed t-test. P values ≤ 0.05 (two-sided) were considered statistically significant.

The influence of the following covariates on the PK parameters was investigated: renal function, demonstrated as the estimated glomerular filtration rate (eGFR) calculated with the CKD-EPI equation; urinary output, demonstrated as the total volume of urine excreted in 24 hours (mL), the number of voids, and the nitrofurantoin excretion rate (mg/h).

Effectiveness of the treatment was evaluated by asking the GP if the patient returned with UTI symptoms (e.g. dysuria with or without frequency, urgency, suprapubic pain, or hematuria) within two weeks and/or four weeks after finishing of the initial antibiotic course with nitrofurantoin (12).

Safety assessment

Safety evaluation included the collection of adverse events (AEs) and serious AEs reported by the patients to the GP.

RESULTS

Study population

A total of 20 patients were included in this interim analysis. One patient was not able to complete the 24-hour urine collection so the reported data are based on the samples of 19 patients. All patients were Caucasian with a median age of 62.5 years (range 19-84). Patient characteristics are shown in Table 1. Twelve patients were prescribed the dosing regimen of 50 mg q6 hours was and seven patients were prescribed the 100 mg q12 hours dosing regimen. Baseline patient characteristics were comparable for the two dosing groups (Table 1).

Sample collection

A total number of 201 samples were collected by the 19 patients with a mean of eleven samples per patient (± 3) (Table 1). There was a wide range in the number of samples per patient (7-16 samples), as a result of varying voiding rhythms.

Table 1. Patient characteristics demonstrated as the population mean, SD, range and CV (%) for the total population and per dosing regimen.

	mean	SD	range	CV (%)
Total population (n=19)				
Age (years)	62.5	14.9	19-84	24.0
Height (cm)	164	5.0	151-178	3.3
Weight (kg)	72.6	13.1	52.0-102.0	18.0
BMI	27.0	5.6	19.1-42.5	20.8
eGFR (mL/min/1.73m2)	78.2	17.0	50.0-114.0	20.8
Urine samples (n=201)	11	3	7-16	27
50 mg q6 hours (n=12)				
Age (years)	64.0	12.9	45-84	20.1
Height (cm)	165	5.0	159-178	3.1
Weight (kg)	71.5	13.5	52.0-102.0	18.9
BMI	26.2	4.8	19.1-36.6	18.4
eGFR (mL/min/1.73m2)	78.3	18.3	50.0-114.0	23.3
Urine samples (n=121)	10	3	7-16	29
100 mg q12 hours (n=7)				
Age (years)	59.9	18.9	19-74	31.6
Height (cm)	163	6.0	151-168	3.5
Weight (kg)	74.6	13.0	56.0-97.0	17.5
BMI	28.5	6.9	20.8-42.5	24.3
eGFR (mL/min/1.73m2)	81.3	14.1	58.0-97.0	17.3
Urine samples (n=80)	11	3	8-16	24

Pharmacokinetic analysis

The concentration-time curves of the patients receiving the 50 mg regimen and the 100 mg regimen are presented in Figures 1a and 1b respectively. Concentrations in the 50 mg dosing group ranged from <4 mg/L (LLOQ) to 267.7 mg/L, and from <4 mg/L to 264.8 mg/L in the 100 mg group. There was one patient in each dosing group with an extraordinary high urinary nitrofurantoin concentration (267.7 mg/L in the 50 mg group and 264.8 mg/L in the 100 mg group (Figure 1)).

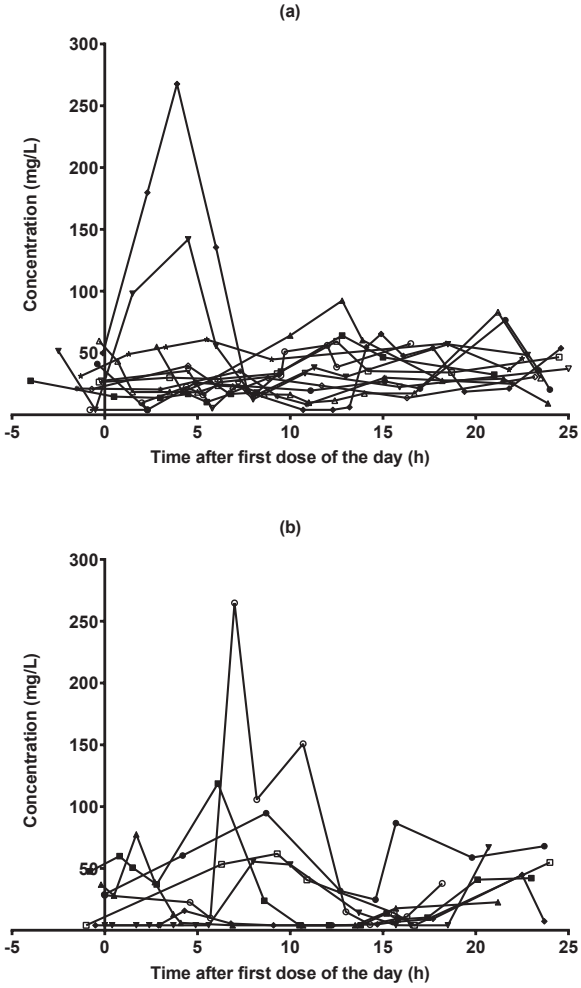

Figure 1. The individual concentration-time curves of the patients receiving a dosing regimen of 50 mg q6 hours **(a)** or 100 mg q12 hours **(b)**. Each line represents one patient.

In Table 2, the corresponding PK parameters are shown, calculated based on the individual concentration-time curves for the samples collected over 24 hours. Urinary concentrations of nitrofurantoin were highly variable between the patients, demonstrated as high SD and CV values in Table 2. Mean maximum concentrations in the 100 mg group were higher compared to those in the 50 mg group (104.2 mg/L ± 74.7 versus 84.7 mg/L ± 64.6), but the range in maximum concentrations was comparable (Table 2). The slow-release effect of the 100 mg capsule is reflected in the prolonged T_{max} (TALD) of 6.7 hours ± 6.0 compared to 4.3 hours ± 3.0 for the 50 mg dose. However, none of the PK parameters differed significantly between the two dosing regimens, probably because the range in concentrations was wide.

The higher C_{max} values found after a longer time after dose, did not result in differences in excretion and recovery values, but did result in an overall higher bladder exposure as the $AUC_{0\text{-}24h,ss}$ in patient receiving the 100 mg dose was higher compared to patients receiving the 50 mg dose (e.g. 931.5 mg.h/L ± 672.3 versus 881.9 mg.h/L ± 347.1) (Table 2).

Table 2. Pharmacokinetic parameters in urine for the two dosing regimens when the full sample collection period of 24 hours was considered.

	50 mg q6 hours (n=12)				100 mg q12 hours (n=7)				P value
	mean	SD	range	CV (%)	mean	SD	range	CV (%)	
Cmax (mg/L)	84.7	64.6	35.3-267.7	76.4	104.2	74.7	44.8-264.8	71.7	0.575
Tmax (TALD) (h)	4.3	3.0	0.02-11.3	69.7	6.7	6.0	0.3-16.6	90.1	0.354
Urinary output (mL)	2905	1065	1252-4600	36.6	2696	864	1550-3630	32.0	0.648
Cumulative recovery (%)	44.8	16.5	16.3-78.6	36.7	41.3	15.9	21.2-58.4	38.6	0.672
AUC0-24h,ss (mg.h/L)	881.9	347.1	533.4-1647.8	39.4	931.5	672.3	323.14-2217.58	72.2	0.860

TALD, time after last dose

The mean urinary output and number of voids during 24 hours was comparable between the two treatment groups (Table 1 and 2). There were three patients with a relatively high urinary output (>3700 mL in a relatively low number of voids; 7-8 voids). Despite a high recovery in these three patients (57.9 % ± 18.0), C_{max} values were low (44.8 mg/L ± 12.9) compared to those in the overall population, probably due to the high urinary output resulting in highly diluted urine and low urinary concentrations of nitrofurantoin. This is in line with the lower total bladder exposure ($AUC_{0\text{-}24h,ss}$ 679.8 mg.h/L ± 192.9). There were four patients with a relatively low urinary output (<1700 mL in a relatively high number of voids; 8-16 voids). C_{max} values and the total bladder exposure were high in these four patients compared to the overall population being 121.8 mg/L ± 98.6 and $AUC_{0\text{-}24h,ss}$ 1208.6 mg.h/L ± 868.8 respectively, but the recovery was low (28.3 % ± 12.2).

No relationship was found between the renal function of the patient and any of the following parameters: C_{max}, cumulative recovery, $AUC_{0\text{-}24h,ss}$ or the nitrofurantoin excretion rate as the graphs demonstrated a random distribution of the data points. In Figure 2, two examples of these graphs are shown. Figure 2a demonstrates the eGFR versus the nitrofurantoin clearance and Figure 2b demonstrates the eGFR versus $AUC_{0\text{-}24h,ss}$.

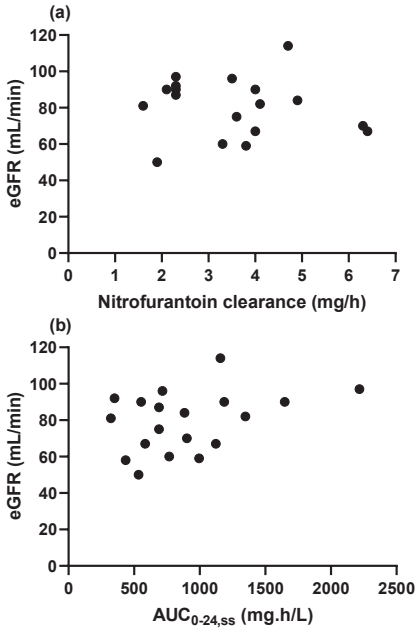

Figure 2. The renal function of the patients, demonstrated as eGFR, versus (a) nitrofurantoin clearance and (b) versus AUC0-24h,ss. The graphs demonstrated that the eGFR is not related to the nitrofurantoin clearance or the AUC0-24h,ss.

Three patients (15%) returned to their GP within two weeks with recurrent UTI symptoms (one patient had used the 50 mg dose). After four weeks, one additional patient returned to her GP with uUTI symptoms. This patient had been treated with the 50 mg dose.

DISCUSSION

The PK of macrocrystalline nitrofurantoin was studied in a group of 19 female patient with suspected or proven uUTIs, treated with 50 mg q6 hours or 100 mg q12 hours in line with the Dutch GP guidelines (8). Urinary concentrations of nitrofurantoin were studied over 24 hours and were found to be highly variable between patients, which is typical for UTI drugs, especially in studies without predefined times for urine sampling (13,14). The urinary concentrations after the 100 mg dose were slightly higher and were found after a longer time after dose compared to the 50 mg dose. This resulted in an overall bladder exposure which was slightly higher in patients being treated with the 100 mg dose. However, differences in PK parameters between the dosing regimens were small and not significant, questioning the clinical relevance of the PK differences.

The results of this interim analysis therefore point to the hypothesis that the urinary PK of nitrofurantoin is independent of the administered dose and formulation within the studied dosing regimens. The PK parameter that differed most between the dosing regimens was the T_{max} (not significant), which could be related to the slow-release formulation of the 100 mg capsule. In view of the time-dependent killing of *Escherichia coli* and *Klebsiella pneumoniae* (15), some investigators have suggested that the slow-release capsule of nitrofurantoin may lead to higher cure rates than the immediate release formulation (13). This could not be validated in our data set. More patients using Macrobid®/Furabid® should be included in the study to demonstrate this possible difference in PK.

A relationship was found between a high urinary output in a relatively low number of voids and lower C_{max} and $AUC_{0\text{-}24h,ss}$ values, but a higher urinary recovery. The opposite for patients with a low urinary output was also observed. This relationship was also found for fosfomycin (11). These observations indicate that minimizing the urinary output contributes to a higher bladder exposure and therefore a better treatment outcome. Whether patients should be advised to minimize voiding episodes is controversial since on the other hand, it is important to regularly void and thereby flush out the uropathogen together with the urine. Voiding regularly may hereby reduce the urinary antibiotic exposure, but still promote clinical cure. More research is needed to reveal how to balance between these factors (either maximizing the bladder exposure by minimizing the number of voids or clearing the uropathogen by maximizing the number of voids) in order to improve treatment outcome.

In the scarce number of publications in which the PK of nitrofurantoin after a clinically relevant dose was reported, PK results were in line with those reported in this interim analysis (16–19). The study of Huttner et al. reported the urinary PK in 12 healthy volunteers after administration of macrocrystalline nitrofurantoin (Macrodantin®/Furadantin®) in a dose of 50 mg q6 hours or 100 mg q8 hours (13). Urine samples were collected in steady state during one dose interval of 6 hours or 8 hours. Mean values of the PK parameters of this study were comparable to those found in our patients, but the variability in the PK parameters in our study was higher (Table 3). This is likely to be related to differences in patient specific factors, which can influence the urinary concentrations such as renal function, fluid intake, voiding time, urine frequency and drug absorption.

The finding that the urinary PK of nitrofurantoin based on these two studies was comparable, is interesting in the context of the administered dose and formulation. Regarding the dose, the results of the study in healthy volunteers revealed that, while

plasma concentrations are doubled when comparing the dose of 50 mg with the 100 mg, urinary concentrations are in the same order of magnitude (Table 3) (13). This points to the hypothesis that plasma concentrations are dose-dependent, but that urinary concentrations are independent of the administered dose. Regarding the formulation, the finding that the PK of 100 mg Macrodantin®/Furadantin® in volunteers is comparable with the PK of 100 mg Macrobid®/Furabid® in patients, indicates that the slow-release mechanism of nitrofurantoin from the Macrobid®/Furabid® capsule, was not reflected in higher and/or delayed urinary concentrations. Moreover, a slow-release (delayed release) effect was only observed when comparing the PK after the 50 mg dose (Macrodantin®/Furadantin®) with the PK after 100 mg Macrodantin®/Furadantin® or Macrobid®/Furabid®. Based on all the above, it became clear that the PK of nitrofurantoin is complicated and not predictable based on the administered dose and the theoretical mechanism of the Macrobid®/Furabid® nitrofurantoin formulation. Future research should focus on revealing the complete process of absorption and elimination of nitrofurantoin so this knowledge can serve as the base for treatment optimization (20). For this purpose, both plasma and urine samples must be investigated in subjects where voiding and fluid intake are standardized so that the influence of one's own voiding rhythm on the PK is minimized.

Table 3. Pharmacokinetic parameters in urine for the 50 mg dosing regimen (upper part) and for the 100 mg dosing regimen (lower part) in patients (left) and in healthy volunteers (right). The PK parameters in healthy volunteers are based on the study of Huttner et al. (13).

		Patients			Healthy volunteers		
50 mg regimen		50 mg q6 hours (n=12)			50 mg q6 hours (n=12)		
		Mean	SD	range	Mean	SD	range
	C_{max} (mg/L)	84.7	64.6	35.3-267.7	94.4	47.8	26.8-176.3
	T_{max} (TALD) (h)	4.3	3.0	0.02-11.3	5.1	0.7	3.3-5.5
	$AUC_{0\text{-}24,ss}$ (mg.h/L)	881.9	347.1	533.4-1647.8	943.5	539.6	228.2-2212.0
100 mg regimen		100 mg q12 hours (n=7)			100 mg q8 hours (n=12)		
		Mean	SD	range	Mean	SD	range
	C_{max} (mg/L)	104.2	74.7	44.8-264.8	94.1	49.9	40.1-209.4
	T_{max} (TALD) (h)	6.7	6.0	0.3-16.6	6.8	1.8	1.3-8.1
	$AUC_{0\text{-}24,ss}$ (mg.h/L)	931.5	672.3	323.1-2217.6	856.0	581.0	378.6-2098.9

TALD, time after last dose

The initial course with nitrofurantoin was not sufficient to treat the infection in four patients. The success rate of the treatment (e.g. 80% [16/20]) is comparable to what was found in a randomized clinical trial in which a clinical resolution of 70% was reported in patients through day 28 after completion of antibiotic therapy (21). Microbiological

resolution occurred in 74% of the patients (21). Because of the large interindividual variability in urinary concentrations, it is likely that urinary concentrations in some patients will be sub-therapeutic. This may explain the cases of clinical and microbiological failure, but this interim analysis was underpowered to demonstrate a difference in treatment effect for the two dosing regimens. Future research will focus on the enrollment of more patients in this ongoing study.

The small sample size is the most important limitation of the study at the time of this interim analysis. The study was underpowered to demonstrate a difference in treatment effect between the two dosing regimens and to perform a covariate analysis. The results of this interim analysis should therefore only be considered as explorative. However, the fact that the results are in line with what was found in literature about the urinary PK (13,16) and the clinical effectiveness (21), support the validity of the explorative results.

In conclusion, the urinary PK of nitrofurantoin was comparable between the two studied dosing regimens and therefore independent of the administered dose and formulation. Additional PK studies, including more patients administering Macrobid®/ Furabid®, are warranted to further investigate and compare the PK pattern of both nitrofurantoin formulations. Because of the small sample size of this interim analysis, the presented results are only explorative. The hypotheses raised in this study must be validated in additional studies with more patients.

Transparency declaration

The authors declare no conflicts of interest.

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3.5

Development and validation of a fast and sensitive UHPLC-DAD assay for the quantification of nitrofurantoin in plasma and urine

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ABSTRACT

Nitrofurantoin is an antimicrobial drug that has been used in the treatment of lower urinary tract infections for more than 50 years. Despite its long use, surprisingly little is known of the pharmacokinetics of nitrofurantoin, whereas this is essential to optimize patient treatment. We developed a novel analytical method for the quantification of nitrofurantoin in plasma and urine using ultra-high performance liquid chromatography and diode array detection to allow pharmacokinetic studies in these two matrices. The sample preparation method consisted of protein precipitation for plasma and liquid-liquid extraction for urine. 100 µL was needed for the sample preparation. Furazolidone was used as internal standard. Gradient chromatographic separation was performed on a HSS-T3 column. UV detection was performed at a wavelength of 369 nm. The analysis time was 5 minutes. The method was successfully validated according to the FDA-guidelines (2018). Linearity was confirmed over a concentration range from 50 to 1250 µg/L in plasma and from 4 to 200 mg/L in urine ($r^2 > 0.95$). Validation results of five QC concentrations for plasma and urine, respectively, are for within-day accuracy <± 13% in both matrices, for between-day accuracy <± 7% and <± 9%, for within-day precision <10% and <4% and for between-day precision <10% and <5%. Plasma samples are stable for seven days at 4°C, and for 2 years at -20°C and -80°C. Urine samples are stable for at least seven days at 4°C and at room temperature and for 2 years at -20°C and at -80°C, except from the lower concentrated samples, which are only stable at -80°C. All samples were kept from daylight using amber colored glassware. The presented method meets all validation requirements and was successfully used in a clinical study where the pharmacokinetics of nitrofurantoin were investigated in healthy volunteers. The easy sample preparation method and the short analysis time make this method suitable for use during routine clinical practice to study the pharmacokinetics of nitrofurantoin.

Keywords: Nitrofurantoin; Pharmacokinetics/Pharmacodynamics; UHPLC-DAD; Antimicrobial drug; Urinary tract infections

INTRODUCTION

Urinary tract infections (UTIs) are one of the most common infections worldwide (1). These infections became more difficult to treat due to the increasing prevalence of multi-drug resistance (including β-lactam resistance and quinolone-resistance) among Gram-negative uropathogens (2–4). In the search for alternative treatment options, there is a renewed interest in old antimicrobial drugs. However, an important disadvantage of these old drugs is that important pharmacokinetic (PK) information is lacking. It is well known that this information is important for dosing optimization and therefore maximizing treatment effectivity and minimizing the risk of emergence of resistance among pathogens (5).

One of these old antimicrobial drugs is nitrofurantoin. The drug is a member of the nitrofuran group and was marketed in the early 1950s for the treatment of uncomplicated UTIs. The chemical structure of nitrofurantoin is demonstrated in figure 1a. Its spectrum of activity includes (extended spectrum beta-lactamase-producing (ESBL)) *Enterobacteriaceae* and (vancomycin-resistant) *Enterococci*. Nitrofurantoin is registered for the treatment of UTIs in the standard dose of 50 mg q6h (regular release capsule) or 100 mg q8h or q12h (slow release formulation), depending on the geographical location. A single daily dose of 50-100 mg is registered for UTI prophylaxis. Maximum plasma concentrations after a dose of 50 mg q6h were never reported, but are expected to be low (around 700 μg/L) and therefore sub therapeutic, based on plasma concentrations after other dosages of nitrofurantoin (6–8). Around 40% of the dose is excreted unchanged in the urine after oral administration (9–11). Microbiological effective urine concentrations are expected to be around 200 mg/L (9, 12, 13).

The current body of PK knowledge of nitrofurantoin in UTI patients is poor and mainly based on decades-old studies using comparative, archaic laboratory and analytical techniques (14). Development of a sensitive analytical method for the quantification of nitrofurantoin in human plasma and urine is an important first step to fill in this PK knowledge gap.

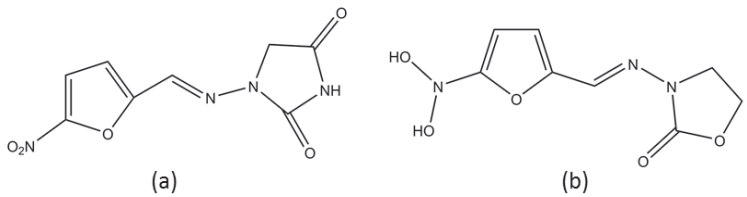

Figure 1. (a) The chemical structure of nitrofurantoin and (b) the internal standard furazolidone.

The first analytical method for nitrofurantoin in urine was the chromatographic method with spectrophotometric or colorimetric detection described by Bender, Nohle and Paul in 1956 (15). This method served as the base for the first methods using high performance liquid chromatography (HPLC) with UV-detection for the quantification of nitrofurantoin in plasma and/or urine (9, 16–20). LC methods with mass-spectrometry (LC-MS/MS) detection followed thereafter (8, 21). These methods are in general more specific compared to the HPLC-UV methods. Limits of quantification in plasma range from 5 to 20 µg/L with LC-MS and from 10 to 500 µg/L with LC-UV. No LC-MS methods were described for the quantification of nitrofurantoin in urine. The only methods describing the quantification of nitrofurantoin in both matrices are based on UV-detection where total analysis times up to 15 minutes are reported (20). One method was designed for the quantification of (some of) its metabolites because it is known that also metabolites are responsible for its antibacterial activity (18, 22). We aimed to develop an analytical method for the quantification of nitrofurantoin in plasma and urine using ultra-high performance liquid chromatography and diode array detection (UHPLC-DAD) to allow pharmacokinetic studies in these two matrices which can be used in daily clinical practice as well as for research purposes.

MATERIAL AND METHODS

Chemicals and reagents

Nitrofurantoin and the internal standard (IS) furazolidone (figure 1b) were purchased from *Sigma Aldrich* (Zwijndrecht, the Netherlands, purity 99.90% and 99.60%, respectively). Methanol was purchased from *Biosolve B.V.* (Valkenswaard, the Netherlands). Acetonitrile, dimethylsulfoxide (DMSO), acetic acid, hydrochloric acid (HCl), phosphoric acid (25%), zinc sulfate heptahydrate ($ZnSO_4 \cdot 7H_2O$), ammonium acetate (CH_3COONH_4), potassium chloride (KCl) and sodium phosphate dibasic dihydrate ($Na_2HPO_4 \cdot 2H_2O$) were purchased from *Merck Millipore* (Darmstadt, Germany). The water was purified using a Millipore Advantage A10 System also from *Merck Millipore*. All chemicals were of LC-MS quality.

Drug free plasma was obtained from volunteers who donated blood in the national blood donation center. The blood was centrifuged after donation and plasma was pooled and stored at -20°C prior to analysis. Drug free urine was obtained from drug-free subjects who donated urine voluntary. The urine was pooled and stored for maximum 3 days at 4°C after collection.

Table 1. The used gradient for the mobile phases A and B.

Time (min)	Mobile phase A (%)	Mobile phase B (%)
0-2.5	96.0	4.0
2.5-4	33.3	66.7
4-5	96.0	4.0

Preparation of calibration curve, quality controls samples and internal standard.

Stock solutions of nitrofurantoin and furazolidone of 1000 mg/L were prepared in Methanol-DMSO (1:1, v:v). The working solutions of 10 mg/L were prepared by diluting these stock solutions 100 times with purified water. Calibration standards and the quality control (QC) samples were prepared by diluting this working solution with drug free urine or plasma. The final concentrations of these samples are demonstrated in table 2. The stock solutions and working solutions were stored at 4°C and were brought to room temperature prior to use. Amber color glassware was used to protect the content from daylight.

Table 2. Concentrations of the six calibration standards and the quality control samples (QC-L, QC-M, QC-H, LLOQ and ULOQ).

	Calibrations standards						**Internal quality control samples (μg/L)**				
	1	2	3	4	5	6	QC-L	QC-M	QC-H	LLOQ	ULOQ
Plasma (μg/L)	50	100	200	500	750	1250	100	600	1000	50	1250
Urine (mg/L)	4	24	40	60	100	200	10	80	150	4	200

Sample preparation

Mobile phase A was prepared by dissolving 3.85 mg CH_3COONH_4 in 1000 mL purified water so that the molarity of the final solution was 0.05M. pH was adapted to 5.8 with acetic acid and the solution was mixed with acetonitrile (90:10, v:v). Mobile phase B consisted of acetonitrile. The buffer of pH 2 was a 6.57g/L solution of KCl in HCl and water and was prepared by dissolving 0.657 g KCl in 11.9 mL of 0.1 M HCl (conform The Dutch Pharmacopoeia, 8th edition, part I). Purified water was then added to the final volume of 100 mL. The 0.05 M phosphate buffer (pH 4.8) was prepared by dissolving 0.89 g Na_2HPO_4 . $2H_2O$ in 100 mL of purified water. The pH was adjusted to 4.8 with 25% phosphoric acid. The protein precipitation solution was prepared by mixing methanol with a $ZnSO_4$ solution and the 10 mg/L internal standard solution (20:20:1, v:v). The $ZnSO_4$ solution was prepared by dissolving 178 mg $ZnSO_4$. $7H_2O$ in 1000 mL purified water (stored in portions of 35 mL at -20°C, freshly prepared every week). 100

µL of a plasma sample was needed to perform the sample preparation using protein precipitation. The glass tubes were vortexed for one minute and thereafter centrifuged for five minutes at 16,1 **g**. 50 µL of the 0.05 M phosphate buffer (pH 4.8) and 100 µL of the protein precipitation solution were added. 100 µL of the supernatant was then transferred to an 1.5 mL tube (Greiner Bio-One™ Reaction tube) and mixed with 100 µL purified water. 40 µL of the final mixture was injected in the LC-apparatus.

Urine samples were prepared using liquid-liquid extraction. 100 µL of an urine sample was mixed with 900 µL purified water, 200 µL of the pH 2 buffer, 50 µL of the 100 mg/L internal standard solution, and 3 mL dichloromethane. The 100 mg/L internal standard solution was freshly prepared each time by ten-fold dilution of the stock solution with purified water. The mixture was vortexed for five minutes and centrifuged for five minutes at 6,0 **g**. The lower organic layer was separated from the upper aqueous layer by removing the aqueous layer using vacuum and then transferring the organic layer into a clean tube. After centrifugation during five minutes at 6,0 **g**, the organic layer was again transferred to a clean tube. The solvent was evaporated under nitrogen flow at room temperature and the residue was reconstituted in 200 µL of the mobile phase A. The mixture was vortexed for five minutes and then transferred to an ultrasonic bath for two minutes operating at room temperature. The mixture was then vortexed for five minutes and 5 µL of the final mixture was injected in the LC apparatus.

All samples were brought to room temperature one hour before use and were protected from light during the preparation using amber colored glass ware and plastic disposables.

Chromatography and detection

The UHPLC system consisted of a UHPLC-pump from *Waters Acquity Quaternary Solvent Manager,* an auto sampler operating at 15°C with flow through needle injection mode, a column compartment and a DA detector (all *Waters Chromatography B.V.*, Etten-Leur, the Netherlands). The software program *Empower version 3.0* (all *Waters Chromatography B.V*) was used for data processing.

Chromatographic separation was achieved using an Acquity UHPLC HSS-T3, 100 x 2.1 mm, 1.8 µm column (*Waters Chromatography B.V.*). Gradient elution was performed using mobile phases A and B. The used gradient is described in table 1 where the composition changed linear over time. The total analysis time was five minutes and the flow rate was stable at 0.6 mL/min. The retention times of nitrofurantoin and the internal standard were 1.7 and 2.3 minutes, respectively. Detection was performed at 369 nm and the column temperature was 45°C.

Analytical validation

UHPLC-UV settings and the sample preparation procedure were adapted from in-house methods for other compounds. Validation was performed according to the Food and Drug Administration (FDA) guideline for bioanalytical method validation, 2018 (23).

Selectivity

The interference from endogenous compounds was investigated by analyzing blank plasma and urine of six different individuals who did not use nitrofurantoin. Furthermore, we tested the following frequently used co-administered drugs: acenocoumarol, acetylsalicylic acid, enalapril, ethinyl estradiol, furosemide, ibuprofen, metoprolol, paracetamol, simvastatin, and metformin. No interfering peaks were allowed.

Accuracy, precision and limits of quantification

Five quality control (QC) samples of each level (25 samples in total) were prepared and the deviations of the measured concentrations were compared to the theoretical concentrations of the samples (table 2). To define the within-day accuracy, the mean of five replicates was calculated. For the between-day accuracy, all QC samples were prepared in triplicate (15 samples in total) and were measured on three different days. The claim for the accuracy parameters is that the measured concentrations should be within the acceptance criteria of ± 20% for the lower limit of quantification (LLOQ) concentration and ± 15% of the nominal concentration for the other QC concentrations.

The same 25 samples were used to investigate the within-day precision and the same 15 samples were used to investigate the between-day precision. For these purposes, the coefficient of variation was calculated for each concentration level. The claim for the precision parameters is that the coefficient of variation should be under 20% (LLOQ) or 15% (other QCs).

The LLOQ of the method was investigated by calculating the coefficient of variation for the analysis of six LLOQ samples and should be less than 20%. Additionally to the aforementioned FDA guidelines, the lower limit of detection (LOD) was investigated by analyzing five drug free samples of each matrix. The LOD was defined as the difference between the minimal and the maximum background signal at the retention time of nitrofurantoin and could be calculated with the following formula: LOD = (3 x background signal/LLOQ signal) x concentration of LLOQ sample.

Linearity

Linearity across the therapeutic range was evaluated in order to confirm the presence of the linear relationship between the concentration of the calibration standards and the response (correlation coefficient (r^2) > 0.95). Therefore, six calibration standards were prepared in duplicate (table 2) together with two blank samples. Concentrations

of these standards were based on the expected concentrations of nitrofurantoin in the two matrices, reported in previous publications (6–9, 12, 13). The specification of the correlation coefficient is part of the in-house aims used for method validation. The calibration line was not forced to the origin.

Recovery

Recovery was tested by analyzing two sets of QC samples in duplicate for each level. The first set was prepared using the standard sample preparation method as described in section 2.3. The second set was prepared by spiking drug free urine just before injection into the LC-apparatus at the same concentration level as the first set. The recovery was then calculated as the ratio of the response from the samples in set one and two. The response was normalized for the response of the internal standard.

Stability

Stability of stock solutions and working solutions was investigated by quantifying nitrofurantoin and furazolidone concentrations in the solutions just after preparation and after storages in the fridge (4°C) for successive periods of three months, up to 2 years. The time periods were chosen based on recommendations by the World Health Organization and are part of in-house procedures (24). The concentrations after three months should not deviate more than 10% from the original concentrations. Additionally, the following four stability forms of spiked samples during storage were investigated as part of the validation. Stability of plasma samples was tested at two different QC levels (low and high) and stability of urine samples was tested at all three different QC levels:

Short-term stability: QC samples were prepared in duplicate. Short-term stability was investigated by placing the samples on the workbench at room temperature (± 18°C) and in the fridge at 4°C during seven days without protecting them from light.

Long-term stability: Several sets of the QC samples were prepared in duplicate and were stored at -20°C or at -80°C. Stability was then confirmed every three months afterwards for up to 2 years.

Freeze-thaw stability: Stability during three freeze-thaw cycles was tested for the QC samples in duplicate.

Auto sampler stability: Stability in the auto sampler was tested by preparing all five QC samples in duplicate and by storing them in the auto sampler (15°C) during one week.

The measured concentration of the QC samples should not deviate more than 15% from the theoretical concentration for all stability forms.

Clinical Validation

The method was developed in order to quantify nitrofurantoin concentrations in plasma and urine samples from a clinical study. The study was approved by the local ethical committee (CER 13-036) and registered with FDA number (2014DR1008). Participation was voluntary and enrollment occurred after informed written consent had been obtained. The study aimed to investigate the PK of nitrofurantoin in twelve healthy, female volunteers after administering a standard dose of 50 mg q6h (normal release capsule) or 100 mg q8h (slow release capsule) with food at steady state. Plasma samples were collected during six or eight hours after administration, depending on the dose and were immediately centrifuged after taking the sample. Urine samples were also collected during the same time. All samples were immediately kept from light using amber colored glassware and disposables. Samples were stored at -80°C and were analyzed within three months after storage, taking into account the stability testing results. 100 µL of each sample was used for analysis as described in section 2.3.

RESULTS

Analytical validation

Selectivity

Selectivity of the method was confirmed because no interfering peaks from endogenous compounds or co-medication were found around the retention times of nitrofurantoin or the internal standard [data not shown]. Blank samples were used to determine matrix effect. No matrix effects were found since the response of the blank samples was negligible compared to the nitrofurantoin response in the LLOQ standard. The chromatograms of five drug free samples did not show any peaks after injection of a ULOQ sample so no carry-over was observed.

Accuracy, precision and limits of quantification

Accuracy and precision data are demonstrated in table 3. All values were within the accepted range of 15%.

The LOD was calculated with the equation described in section 2.5.2 and was found to be 27 µg/L in plasma and 0.046 mg/L in urine. Calibration standard 1 and 6 were considered to be the LLOQ and ULOQ in both matrices.

Table 3. Within-day accuracy and precision and between-day accuracy and precision results of the assay.

Matrix	[a] Sample	Within-day [b] accuracy (%)	Between-day [b] accuracy (%)	Within-day [c] precision (%)	Between-day [c] precision (%)
Plasma	LLOQ	110.3	99.9	6.7	9.3
	QC-L	86.6	96.3	9.6	8.8
	QC-M	105.7	102.5	5.4	3.8
	QC-H	105.1	103.2	5.4	1.9
	ULOQ	113.6	106.6	5.6	5.5
Urine	LLOQ	113.0	109.0	1.0	4.1
	QC-L	108.7	103.0	2.4	4.8
	QC-M	104.3	101.6	3.2	2.2
	QC-H	107.6	103.2	1.4	3.7
	ULOQ	108.6	104.9	2.2	2.9

[a] The sample concentrations are presented in table 2.
[b] The accuracy values represent the deviation of the measured concentrations compared to the theoretical concentrations, expressed as a percentage of the theoretical concentrations.
[c] The precision values are expressed as coefficients of variation. All values should be within the acceptance criteria of ± 20% for the LLOQ samples and ± 15% for the other QC samples.

Linearity

The method was successfully validated over a range from 50 to 1250 µg/L in plasma ($r^2 > 0.95$) and the clinically relevant range from 4 to 200 mg/L in urine ($r^2 > 0.95$) with a weighting factor of 1/x so the target for linearity was reached in both matrices. All calibration standards (singular) and QCs of five levels (duplicate) were prepared when the method was used for routine analysis. Patient samples were prepared in singular.

Recovery

The following percentages were measured when testing the recovery of the method in urine: 60% (RSD = 5.1%) for nitrofurantoin and 105% (RSD = 3.3%) for the internal standard. Recovery of nitrofurantoin was low, but consistent. No recovery was tested for plasma samples since this is not customary when using protein precipitation.

Stability

The two stock solutions and the two working solutions were found to be stable at 4°C for 2 years since the concentrations were still within the 10% range of the initial concentrations (+6.4% and +6.0% for the stock solutions and -1.5% and +3.1% for the working solutions).

Stability data of the assay is presented in table 4. QCs in plasma are stable for seven days at 4°C, for 2 years at -20°C and at -80°C (only QC-H), but for less than

three hours at room temperature (± 18°C). Plasma samples of all levels are stable for seven days in the auto sampler (15°C). There is however a stability problem for low concentrated plasma samples when stored for more than three months and for low concentrated urine samples in general, based on the stability testing results for QC-L samples in both matrices. Long-term stability of low concentrated urine samples was only confirmed for samples stored at -80°C (stable for at least 2 years). On the contrary, QC-M and QC-H samples in urine are stable at 4°C and at room temperature for at least 7 days, and at -20°C and -80°C during at least 2 years. Only higher concentrated samples in both matrices are stable during three freeze-thaw cycles.

Table 4. Stability data of the assay.

Matrix	Condition	Time	QC-L	QC-M	QC-H
Plasma	[b] Short-term 4°C	7 days	97%	-	91%
	[b] Short-term room temp. (± 18°C)	3h	**80%**	-	**84%**
	[c] Long-term -20°C	3 months	115%	-	99%
		6 months	**119%**		113%
		2 years	-		115%
	[c] Long-term -80°C	3 months	114%	-	100%
		6 months	**116%**		108%
		2 years	-		110%
	Freeze-thaw cycles	3 cycles	**133%**	-	114%
	[a] Auto sampler (15°C)	7 days	All QC samples meet requirement		
Urine	[b] Short-term 4°C	7 days	**71.6%**	87.2%	90.1%
	[b] Short-term room temp. (± 18°C)	3 h	**67.3%**	85.3%	91.7%
		7 days	**64.8%**	89.4%	88.8%
	[c] Long-term -20°C	3 months	**73.9%**	88.7%	91.6%
		6 months	**-**	88.0%	87.0%
		2 years	**-**	87.1%	86.1%
	[c] Long-term -80°C	3 months	94.1%	113.8%	103.2%
		6 months	92.0%	114.1%	104.0%
		2 years	90.1%	114.6%	106.5%
	Freeze-thaw cycles	3 cycles	**84.9%**	90.9%	91.5%
	[a] Auto sampler (15°C)	24 hours	All QC samples meet requirement		

[a] tested for QC-L, QC-M, QC-H, LLOQ and ULOQ
[b] Short-term stability was tested for stored samples.
[c] Long-term stability was tested for at least three months.
The values represent the mean recovery (%) of two (plasma) or three (urine) QC samples of each level.

Clinical validation

A chromatogram obtained after injection of a plasma sample and a urine sample of a volunteer is demonstrated in figure 2. The concentration of the plasma sample was 199 µg/L and 56.4 mg/L for the urine sample. No interfering peaks were observed in both matrices when analyzing the samples. Maximum plasma concentrations ranged from 209 to 450 µg/L after a dose of 50 mg q6h and from 222 to 1255 µg/L after 100 mg q8h. Urine concentrations after these dosages ranged from 26.8 to 176.3 mg/L and from 40.1 to 209.4 mg/L, respectively. Concentrations were comparable with those found in literature (6, 8, 9, 12, 13). The samples of which the initial concentrations exceeded the validated concentrations ranges were re-analyzed after diluting the samples with drug free plasma or urine. This dilution step did not affect the quality of the method, as confirmed during additional analysis as part of the method validation.

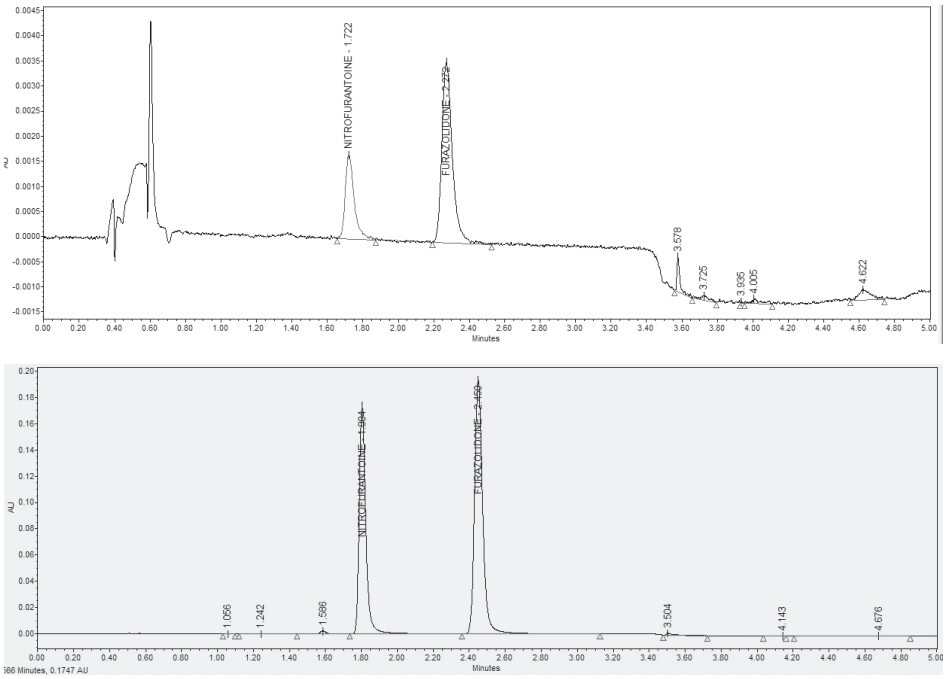

Figure 2. Chromatograms of a volunteers' plasma sample (upper) and urine sample (bottom). The retention time is presented on the horizontal axis and the absorption at 369 nm is demonstrated on the vertical axis. The number above the individual peaks represents the retention time of nitrofurantoin (first peak) and furazolidone (second peak).

DISCUSSION

Recent guidelines for drug registration include PK/PD research to provide the rationale for dose and dose frequency selection (5, 25, 26). This information is missing for old antimicrobial drugs as this was not required at the time of registration. This is an important and worrisome knowledge gap since it is known that this information is necessary for therapy optimization and the prevention of emergence of drug resistance (5).

We developed this method to quantify nitrofurantoin concentrations in plasma and urine. Based on these concentrations, we will be able to study the PK after administration of nitrofurantoin in different dosages and in different dosing regimens. This is of specific interest because the registered dose, frequency, duration and formulation of nitrofurantoin is different per indication and/or per country (27). A scientific base for dose justification for nitrofurantoin is lacking which is alarming, especially for an old drug which has been in clinical use for decades. We believe that it is highly important to investigate the effectivity and safety of the dosing regimens which are currently used in clinical daily practice in order to keep nitrofurantoin as a possible oral treatment option for uncomplicated UTIs in an era of emergence of resistance among common uropathogens (2–4).

An important advantage of the method we present is the short analysis time of only five minutes in comparison with other published methods (19, 20). This advantage ensures that the method can be integrated easily in daily lab routine for research and therapeutic drug monitoring purposes. The method is sensitive enough in order to quantify nitrofurantoin concentrations in the clinically relevant concentration areas, but cannot match the sensitivity of a LC-MS/MS method that can achieve a quantification limit of 5 µg/L in plasma (21). Although the majority of the nitrofurantoin dose is excreted unchanged in urine, it is important to mention that it cannot be ruled out whether other drugs and/or metabolites with coincidently the same retention time and/or UV absorption spectrum as nitrofurantoin are detected too (28). To overcome this limitation as much as possible, peak height and shape of the internal standard in the sample needs to be judged for every analysis and compared to those from the QC samples, which are measured simultaneously. This is a common strategy to rule out the presence of interfering peaks. Any deviation from the peak pattern of the internal standard must be mentioned when reporting the measured concentrations when using the method during clinical daily practice. This is a general limitation of the published analytical methods using UV based detection methods for nitrofurantoin and is of course closely related to the fact that the metabolic pattern of nitrofurantoin has still not been fully elucidated (18, 22). In order to make the measured concentrations be more translatable to treatment effectivity, selecting which metabolites to include in the method would have relied on guesswork and was therefore considered not war-

ranted for inclusion. This factor should to be taken into account when interpreting the concentrations and translating them to antibacterial activity, clinical effectivity and/or toxicity, since the measured concentrations may not be fully 'responsible' for these effects.

Several steps had to be taken to optimize the analytical assay before final validation. These steps will be discussed. We started using an unbuffered protein precipitation solution for the plasma sample preparation. Measured calibration standard concentrations and QC concentrations were different for different batches of drug free plasma. Based on this finding, we concluded that the pH differences between plasma batches caused this deviation and we started to use a buffered protein precipitation solution. pH 4.8 was found to be the optimal. We aimed to apply the plasma sample preparation method to the urine samples, but this resulted in varying concentrations of the calibration standards and disruptions in the chromatogram due to pollution of the urine matrix. We therefore decided to use liquid-liquid extraction with dichloromethane instead of nitromethane, based on a previous method (29). The final sample preparation method for urine samples consists of extraction with a buffer with KCl and HCl of pH 2 since HCl has no buffering effect by itself.

CONCLUSIONS

This is the first UHPLC-DAD method suitable for the quantification of nitrofurantoin concentrations in plasma and urine with a small sample volume and a short analysis time. The method was found to be selective ad sensitive with low LLOQ concentrations. These properties ensure that the method is highly suitable for use during the daily routine for analyzing patients' samples in the context of clinical care and research where it can serve as a base for therapy evaluation and optimization. The applicability of the method was demonstrated during its use in a clinical study.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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4

Urinary antibacterial activity (PD) of fosfomycin and nitrofurantoin

4.1

Urinary antibacterial activity of fosfomycin and nitrofurantoin at registered dosages in healthy volunteers

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ABSTRACT

Given emerging uropathogen resistance to more recent antibiotics, old antibiotics used for uncomplicated urinary tract infection (UTI) warrant reexamination. We investigated the urinary antibacterial activities of fosfomycin and nitrofurantoin by determining the urinary inhibitory titer (UIT) and the bactericidal titer (UBT) against uropathogens in urine samples from female volunteers after the administration of single-dose fosfomycin (3g) or nitrofurantoin (50 mg q6h or 100 mg q8h). Urine samples were collected over 48h (fosfomycin) or 8h (nitrofurantoin) with drug levels quantified with every void. Fosfomycin concentrations ranged from <0.75 (below the limit of quantification [LOQ]) to 5729.9 mg/l, and nitrofurantoin concentrations from <4 (below LOQ) to 176.3 mg/l (50 mg q6h) or 209.4 mg/l (100 mg q8h). There is discrepancy in the response to fosfomycin between *Escherichia coli* and *Klebsiella pneumoniae* species because fosfomycin displayed strong bactericidal activity for 48h for *Escherichia coli*, and moderate bactericidal activity for 18h against *Klebsiella pneumoniae* isolates. This effect was not related to the strain's baseline minimal inhibitory concentration (MIC), but rather to the presence of a resistant subpopulation. Maximum titers of nitrofurantoin were obtained during the first 2h, but no antibacterial effect was found in most samples, regardless of the dose. In the rare samples in which antibacterial activity was detectable, titers were comparable for both species tested. These findings confirm the doubts on fosfomycin administration in UTIs caused by *Klebsiella pneumoniae* and reveal discrepancy between nitrofurantoin's measurable *ex vivo* activity and its clinical effect over multiple dosing intervals.

Keywords: fosfomycin; nitrofurantoin; urinary tract infections; pharmacokinetics; pharmacodynamics; urine; healthy volunteers

INTRODUCTION

Fosfomycin and nitrofurantoin are recommended first-line for urinary tract infection (UTI), the most common bacterial infection among otherwise healthy women (1). Though resistance among uropathogens is increasing, it remains relatively low for fosfomycin and nitrofurantoin (2–4). Despite their use over several decades, these antibiotics' pharmacokinetic (PK) and pharmacodynamic (PD) properties remain poorly defined, though such information is essential for therapy optimization and the prevention of the emergence of resistance (5, 6). While new data are beginning to emerge on the PK (7–11) and PD properties (12, 13) of both drugs, most *in vitro* PD studies have been conducted in a non-biological matrix and/or did not take into account drug-concentration changes over time *ex vivo*, thus limiting the clinical translation of these results.

A method to address these limitations is the determination of the urinary antibacterial activity of antimicrobial agents in which *ex vivo* PK data are used within a static *in vitro* model (14–16). The urinary antibacterial activity of an antimicrobial agent is described by the urinary inhibitory titer (UIT) and the urinary bactericidal titer (UBT). These are measures of antibacterial activity over time in urine, the relevant biological matrix. Thus providing *in vitro* data that more closely reflects the clinical scenario by describing antibiotic activity against the pathogen within the host's environment.

We determined the urinary antibacterial activity of fosfomycin and nitrofurantoin against common uropathogens, after administration of registered doses for the treatment of UTI, to evaluate the effectiveness of these drugs.

MATERIAL AND METHODS

Study design, subjects, drug administration and sample collection

Urine samples to determine the UIT and UBT were obtained in two previous studies evaluating the PK properties of the both fosfomycin and nitrofurantoin (7, 8). Briefly, the fosfomycin urinary PK study was a single-center study to examine the urinary PK following a single, oral 3 gram dose of fosfomycin trometamol (Monuril, Zambon Nederland B.V., Amersfoort, the Netherlands) in 40 healthy, female volunteers (7). Fosfomycin was administered under supervision of one of the researchers. Urine samples were collected in a home setting over 48 hours with every void and two times daily from 48 hours until 7 days after administration. For the present study, we used only the samples collected in the first 48 hours. There were no dietary restrictions prior to or after drug administration. Samples were kept in home freezers until handed in to investigators. The nitrofurantoin PK study was a single-center study in which macrocrystalline nitro-

furantoin was administered either 50 mg q6 hours (Furadantine MC, Mercury Pharma Ltd, Croydon UK) or 100 mg q8 hours (Furadantine® retard, Mercury Pharma Ltd, Croydon UK) in a crossover design to 12 healthy, female volunteers (8). The drug was administered with food and administration began in a home setting 24 hours prior to sample collection to achieve steady state. The last dose was administered in the hospital at the start of the 8-hour visit, during which urine samples were collected for 6 hours or 8 hours, depending on the assigned dosing interval. Volunteers were instructed to protect the nitrofurantoin samples from daylight using aluminum foil to avoid photodegradation of the drug.

Total volume, pH and time of each sample were recorded for both fosfomycin and nitrofurantoin samples prior to storage at -80°C. Stability of the samples under these conditions was confirmed during the validation of the analytical methods (17, 18). Drug levels were quantified using ultra-high performance liquid chromatography (UHPLC) with tandem mass spectrometry (MS/MS) detection for fosfomycin and ultraviolet (UV) detection for nitrofurantoin. Both methods were validated according to FDA guidelines, as described elsewhere (17, 18).

Test organisms and MICs

Isolates were obtained from clinical sources with the exception of the ATCC reference strain and were selected with a range of fosfomycin and nitrofurantoin MIC values (table 1). Fosfomycin susceptibility was determined by agar dilution using 10^4 CFU/spot of each isolate inoculated on Mueller–Hinton II agar (MHA; BD Diagnostics, Franklin Lakes, NJ, USA) containing 25 mg/L glucose-6-phosphate (G6P; Sigma, Taufkirchen, Germany) and fosfomycin (InfectoPharm, Heppenheim, Germany) following CLSI recommendations in a concentration range of 0.25 – 1024 mg/L. Isolates were tested in triplicate. Nitrofurantoin susceptibility was determined by broth microdilution according to ISO guidelines (19).

Fosfomycin containing urine samples from the volunteers were divided in two sets to allow for the limited volumes of material. Set 1 consisted of samples of the initial 20 volunteers and the second set consisted of those from the remaining 20 volunteers. Both sets were tested against two *Escherichia coli* strains, two *Klebsiella pneumoniae* strains and the ATCC strain (table 1). All strains were used for testing the nitrofurantoin samples.

Determination of UITs and UBTs

All urine samples were filtered before analysis by centrifugation (10 minutes at 13.000 rpm) using Amicon Ultra-0.5 centrifugal filter units with a 10 kDa cutoff Ultracel-10 membrane (UFC5010BK; Merck, Amsterdam, the Netherlands). The large volumes of antibiotic-free urine were filtered over 0.2 μm bottle-top vacuum filters (CLS430756;

Table 1. MICs of fosfomycin and nitrofurantoin. The MIC represents the modal value based on the results of agar dilution (fosfomycin) or micro dilution (nitrofurantoin) performed in triplicate.

Test strain	Source	MIC Fosfomycin (mg/liter)[a]	MIC Nitrofurantoin (mg/liter)
		E. coli	
ATCC 25922	Laboratory strain	1	16
51[b]	Blood	2	32
03 [b]	Urine	0.25	16
1231	Urine	16	512
4807	Rectal swab	32	16
		K. pneumoniae	
58 [b]	Urine	8	64
20 [b]	Rectal swab	32	256
31865	Blood	2	128
55	Sputum	4	256

[a] The MIC represents the modal value based on the results of agar dilution (fosfomycin) or microdilution (nitrofurantoin) performed in triplicate.
[b] Strains used for set 1 of the urine samples of volunteer 1 to 40 in the fosfomycin study.

Corning, Taufkirchen, Germany). UITs and UBTs were determined by microdilution. Urine samples underwent a serial two-fold dilution series in antibiotic-free urine from healthy volunteers such that the first well of the microtiter plate contained a 2-times diluted sample. The final bacterial inoculum within the microtiter tray was approximately 2.5×10^5 CFU/ml. Inoculated plates were incubated for 18 ± 2 hours at 35 ± 2°C. After which every well was checked visually for growth. The UIT represents the bacteriostatic activity and was defined as the highest dilution that inhibited visible growth. The UBT represents the bactericidal activity and was defined by the absence of bacterial growth following a subculture from the microtiter tray onto an antibiotic-free Tryptic Soy agar plate supplemented with 5% sheep blood (TSB, 254087, Becton Dickinson, Franklin Lakes, NJ, USA). The limit of detection was 50 cfu/ml. TSB plates were incubated for 18 ± 2 hours at 35 ± 2°C. The UBT was defined as the highest dilution of the sample that still exhibited bactericidal activity. Comparable UIT and UBT reflect antibiotic bactericidal activity, while a UIT exceeding the UBT reflects bacteriostatic activity. UITs and UBTs are presented as reciprocal values of the titers and could therefore range from <2 (no antibacterial activity observed) to 1024, with higher titers indicating greater antibacterial activity.

Determination of fosfomycin-resistant subpopulation

To determine the presence of fosfomycin low-level resistant (LLR) or high-level resistant (HLR) subpopulations, isolates were cultured overnight in both Muller-Hinton

broth (MHB) and antibiotic-free urine using a starting inoculum of 2.5 x 10^5 CFU/ml. Quantitative cultures were then performed in parallel on antibiotic-free Muller-Hinton agar (MHA) and MHA supplemented with 25 mg/liter glucose-6-phosphate together with 64 or 512 mg/liter of fosfomycin. Total bacterial density, and the comparative density of any growth on the fosfomycin-containing media, was determined by plating 20 µl from a serial 10-fold dilution of the incubated liquid media. Growth capacity and resistant-subpopulation proportions were compared between MHB and urine. MHA plates were incubated overnight at 35 ± 2°C. The limit of detection was considered to be 1.4 $\log_{10}$ CFU/ml. This additional analysis was performed for fosfomycin based on previous studies in which a resistant subpopulation was identified in susceptible *Enterobacteriales* (9, 20).

Statistical analysis

Microsoft Excel 2013, IBM SPSS Statistics 24 and Graphpad Prism (San Diego, CA, version 7.0) were used for processing the data. Fosfomycin samples were grouped in 6-hour time intervals and nitrofurantoin samples were grouped in 2-hour time intervals. The median and range of UITs and UBTs were calculated for each interval. The area under the inhibitory titer–time curve (AUIT) and the area under the bactericidal titer–time curve (AUBT) where calculated to give an indication of the inhibitory and bactericidal activity for each strain using the trapezoidal rule (14). A period of 48 hours was considered for fosfomycin and 6 and 8 hours for nitrofurantoin for the 50 mg q6 and 100 mg q8 hour dosing regimens, respectively. Titer values were compared using a two-sided Wilcoxon matched-pairs rank test ($p<0.0001$) to compare the titers of the two species and a one-sided Wilcoxon matched-pairs rank test ($p<0.0001$) was used to compare the UIT values and the UBT values per time interval. The d'Agostino-Pearson test was used to check normal distribution of data. Untransformed data were used for the statistical analysis. Titer values of <2 were transformed into 1 for statistical analysis. The UBT in the most concentrated sample was used to calculate the percentage of volunteers in whom bactericidal activity (UBT ≥2) could be measured.

RESULTS

Subjects and urine samples

Volunteers in both studies were Caucasian females with a mean age (SD) of 24.3 (± 7.9) years and 28.5 (± 7.9) years in the fosfomycin and nitrofurantoin groups, respectively. A more detailed overview of volunteer characteristics can be found in the original studies (7, 8). The number of samples collected by the volunteers varied from 6 to 19 for fosfomycin and from 3 to 9 for nitrofurantoin because they were not instructed to follow a

voiding schedule. Fosfomycin urinary concentrations ranged from <0.75 (lower limit of quantification [LOQ]) to 5729.9 mg/l and did not differ significantly between the two sets ($p<0.05$; table S1). Nitrofurantoin concentrations ranged from <4 mg/l (LOQ) to 176.3 mg/l (nitrofurantoin 50 mg q6 hours), and from <4 to 209.4 mg/l (nitrofurantoin 100 mg q8 hours; table S1). Nitrofurantoin concentrations were slightly higher for the 100 mg dose, but peak concentrations were almost equal (mean C_{max} 94.4 mg/l [± 47.8] for 50 mg q6 hours versus 94.1 mg/l [± 49.9] for 100 mg q8 hours).

(A)UITs and (A)UBTs

Fosfomycin

The high interindividual variability in urinary drug concentrations was reflected by the wide range in UITs and UBTs (7). For *E. coli*, fosfomycin UITs ranged from <2 to 256 and maximum titers were obtained during the first 12 hours after dosing (figure 1A and table S2). Likewise, UBTs ranged from <2 to 512 and were comparable to the UITs for *E. coli*. Thus, fosfomycin was bactericidal against *E. coli* (figure 1B and table 2). There was still reasonable bactericidal activity after 48 hours for *E. coli* because UBTs were ≥2 in the majority (95%) of the samples. The only exception was the *E. coli* 1231 strain (MIC 16 mg/liter) where UITs and UBTs did not exceed 2 for the full 48 hours. The $AUIT_{0\text{-}48h}$ values between the five *E. coli* strains were comparable, again with the exception of *E. coli* 1231 (table 2 and table S2). The same is true for the $AUBT_{0\text{-}48h}$ values (table 2). The difference in $AUIT_{0\text{-}48h}$ and $AUBT_{0\text{-}48h}$ values between the *E. coli* strains did not reflect their varying baseline MICs for fosfomycin (table 1).

UITs for *K. pneumoniae* ranged from <2 to 128 and maximum titers were found during the first 6-hour time period (figure 1A and table S2). UITs and UBTs were comparable, reflecting bactericidal activity of fosfomycin in *K. pneumoniae* (figure 1B and table 2). In contrast to *E. coli*, no antibacterial activity of fosfomycin in *K. pneumoniae* was observed in the majority (86%) of samples throughout the complete 48 hours. Where an antibacterial effect was detected, it was bactericidal in the majority (90%) of the samples, but it was present only during the first 18 hours after administration. UITs and UBTs declined dramatically after that 18-hour time point. UITs and UBTs for *K. pneumoniae* were significantly lower than those for *E. coli* ($p<0.0001$ for all time intervals) (figure 1A and 1B). $AUIT_{0\text{-}48h}$ and $AUBT_{0\text{-}48h}$ values ranged from 47 to 110 and were independent of strains' baseline MICs for fosfomycin (table 2 and table S2).

Nitrofurantoin

For *E. coli*, nitrofurantoin UITs ranged from <2 to 16 for the 50 mg q6 hour regimen and from <2 to 32 for the 100 mg q8 hour regimen and were generally within the same range for both dosing regimens (figure 1C and table S3). Maximum titers were

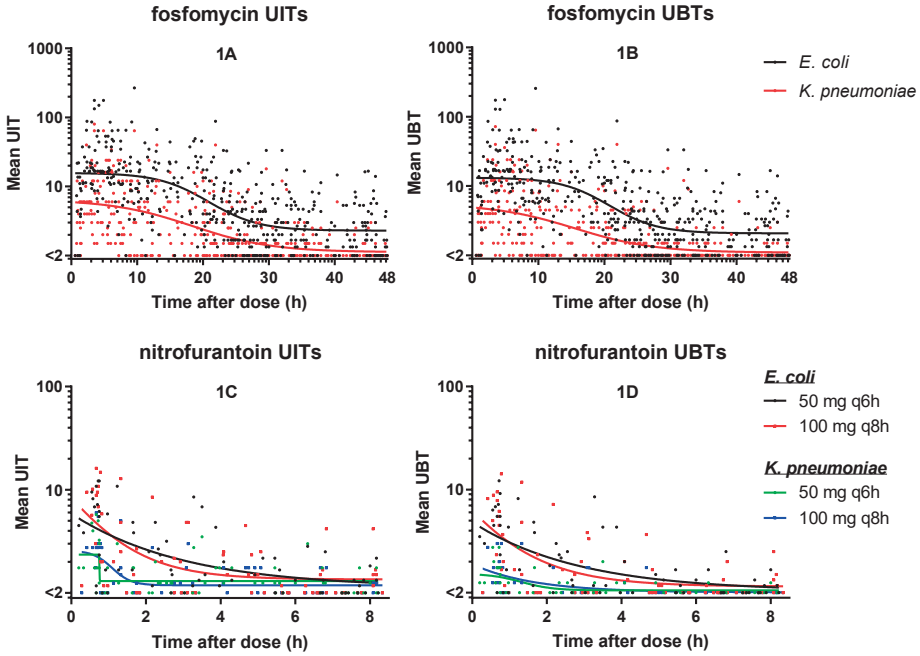

Figure 1. Fosfomycin UIT values (**1A**) and UBT values (**1B**) for *E.coli* (black) and for *K. pneumoniae* (red) for all samples. Below, nitrofurantoin UITs (**1C**) and UBTs (**1D**) for both dosing regimens for *E.coli* (black and red) and for *K. pneumoniae* (green and blue). Every dot represents the mean UIT or UBT for each sample for the *E. coli* and *K. pneumoniae* strains, respectively.

obtained within the first 2 hours after administration. UBTs for *E. coli* were comparable with the UIT values, demonstrating bactericidal activity of nitrofurantoin against *E. coli* (figure 1D and table 3). After 2 hours, no detectable antibacterial activity was found in the majority of the samples (titers of <2).

For *K. pneumoniae,* nitrofurantoin UITs ranged from <2 to 16 for both dosing regimens; maximum titers were found in the first 2 hours after administration (figure 1C and table S3). UBTs ranged from <2 to 8 and did not differ between dosing regimens (figure 1D and table 3). UITs and UBTs were comparable in these first two 2 hours, again reflecting bactericidal activity of nitrofurantoin in the few samples in which antibacterial activity was detectable.

The UITs and UBTs were higher for *E. coli* compared to those for *K. pneumoniae* for both dosing regimens (figure 1C and 1D). Similar to fosfomycin activity, the AUIT and AUBT values were found to be independent of the baseline nitrofurantoin MICs of the isolates (tables 3 and S3). This is true for both dosing regimens.

Table 2. Median (range) of UBTs and $AUBT_{0\text{-}48h}$ values for fosfomycin over time for each strain.

Strain (MIC, mg/liter)	UBT (median (range)) for the indicated time periods (h)								$AUBT_{0\text{-}48h}$
	0-6	6-12	12-18	18-24	24-30	30-36	36-42	42-48	
E. coli									
ATCC25922 (1)	16 (<2-256)	16 (<2-512)	16 (<2-128)	4 (<2-128)	4 (<2-64)	2 (<2-64)	3 (<2-32)	2 (<2-32)	152 (97-303)
51 (2)	8 (<2-64)	4 (<2-32)	4 (<2-16)	4 (<2-16)	2 (<2-16)	2 (<2-8)	2 (<2-8)	2 (<2-4)	115 (53-163)
03 (0.25)	16 (<2-64)	16 (4-64)	16 (2-32)	8 (<2-32)	3 (<2-16)	2 (<2-16)	2 (<2-8)	2 (<2-8)	143 (101-192)
1231 (16)	2 (<2-16)	2 (<2-8)	<2 (<2-8)	<2 (<2-8)	<2 (<2-4)	<2 (<2-4)	<2 (<2-4)	<2 (<2-2)	63 (46-112)
4807 (32)	16 (<2-256)	16 (<2-256)	16 (<2-64)	6 (<2-128)	3 (<2-32)	2 (<2-32)	3 (<2-16)	2 (<2-16)	162 (88-275)
K. pneumoniae									
58 (8)	2 (<2-8)	<2 (<2-16)	<2 (<2-2)	<2 (<2-4)	<2 (<2-2)	<2 (<2-2)	<2 (<2-4)	<2 (<2-2)	47 (39-102)
20 (32)	4 (<2-32)	2 (<2-32)	<2 (<2-8)	2 (<2-16)	<2 (<2-2)	<2 (<2-2)	<2 (<2-2)	<2 (<2-2)	69 (44-114)
31865 (2)	8 (<2-128)	8 (<2-64)	4 (<2-64)	2 (<2-64)	<2 (<2-32)	<2 (<2-16)	<2 (<2-8)	<2 (<2-4)	106 (74-218)
55 (4)	4 (<2-16)	2 (<2-64)	<2 (<2-16)	<2 (<2-16)	<2 (<2-4)	<2 (<2-8)	<2 (<2-4)	<2 (<2-2)	68 (44-153)

Table 3. Median (range) of UBTs and $AUBT_{0\text{-}6h}$ or $AUBT_{0\text{-}8h}$ values for nitrofurantoin over time for each strain (MIC).

Dose, strain (MIC, mg/liter), and isolate	UBT (median (range)) for the indicated time periods (h)				
	0-2	2-4	4-6	6-8	$AUBT_{0\text{-}6h}$ or $AUBT_{0\text{-}8h}$
Nitrofurantoin 50 mg q6 hours					
E. coli					
ATCC25922 (16)	8 (<2-16)	2 (<2-16)	<2 (<2-4)	<2 (<2-4)	18 (10-31)
51 (32)	4 (<2-16)	<2 (<2-8)	<2 (<2-2)	<2 (<2-2)	14 (9-25)
03 (16)	4 (<2-16)	<2 (<2-8)	<2 (<2-2)	<2 (<2-2)	15 (9-25)
1231 (512)	<2 (<2-<2)	<2 (<2-<2)	<2 (<2-<2)	<2 (<2-<2)	9 (9-22)
4807 (16)	2 (<2-8)	<2 (<2-4)	<2 (<2-<2)	<2 (<2-2)	12 (8-11)
K. pneumoniae					
58 (64)	2 (<2-8)	<2 (<2-4)	<2 (<2-8)	<2 (<2-2)	11 (7-9)
20 (256)	<2 (<2-2)	<2 (<2-2)	<2 (<2-<2)	<2 (<2-<2)	9 (9-17)
31865 (128)	<2 (<2-<2)	<2 (<2-<2)	<2 (<2-<2)	<2 (<2-<2)	9 (7-9)
55 (256)	<2 (<2-2)	<2 (<2-<2)	<2 (<2-<2)	<2 (<2-<2)	9 (7-11)
Nitrofurantoin 100 mg q8 hours					
E. coli					
ATCC25922 (16)	4 (<2-32)	2 (<2-16)	<2 (<2-8)	<2 (<2-4)	15 (10-25)
51 (32)	2 (<2-16)	<2 (<2-8)	<2 (<2-2)	<2 (<2-2)	12 (8-21)
03 (16)	2 (<2-16)	<2 (<2-8)	<2 (<2-2)	<2 (<2-4)	13 (11-24)
1231 (512)	<2 (<2-<2)	<2 (<2-<2)	<2 (<2-<2)	<2 (<2-<2)	9 (8-21)
4807 (16)	<2 (<2-8)	<2 (<2-4)	<2 (<2-2)	<2 (<2-2)	11 (7-11)
K. pneumoniae					
58 (64)	2 (<2-8)	<2 (<2-4)	<2 (<2-4)	<2 (<2-<2)	11 (7-9)
20 (256)	<2 (<2-2)	<2 (<2-<2)	<2 (<2-<2)	<2 (<2-<2)	9 (7-20)
31865 (128)	<2 (<2-2)	<2 (<2-<2)	<2 (<2-<2)	<2 (<2-<2)	9 (7-10)
55 (256)	<2 (<2-2)	<2 (<2-<2)	<2 (<2-<2)	<2 (<2-<2)	9 (7-10)

Bactericidal effect in the samples and in volunteers

To correlate with clinical antibiotic effectiveness for UTI treatment, the percentages of volunteers in which bactericidal activity was found was calculated for sequential time intervals for the five *E. coli* and four *K. pneumoniae* strains. Figure 2 demonstrates these percentages over time. A higher percentage reflects a more effective treatment.

Considering fosfomycin, the percentages for *E. coli* were higher than for *K. pneumoniae*. Bactericidal activity against *E. coli* was found in a mean percentage of 90% of the volunteers during 24 hours, but this declined to <60% thereafter. This applies to all *E. coli* strains with the exception of the *E. coli* 1231 strain, against which fosfomycin was not bactericidal in the least diluted sample in 50% of volunteers (figure 2A). This finding was supported by the detection of a resistant subpopulation in this isolate (section 3.3). Against *K. pneumoniae*, bactericidal activity of fosfomycin was found in only an average percentage of 60% of the volunteers during the first 18 hours after administration (figure 2A). Percentages declined quickly thereafter to <20% beyond 24 hours after dosing. Thus, fosfomycin remained bactericidal against *K. pneumoniae* isolates after 24 hours in a very small number of volunteers.

Nitrofurantoin was bactericidal in *E. coli* ATCC 25922, *E. coli* 51 and *E. coli* 03, regardless of the administered dose (figure 2B and 2C). However, this bactericidal activity was only found in 0% - 50% of volunteers. Percentages of >60% were found only in the first 2 hours after administration. In *K. pneumoniae*, percentages never exceeded

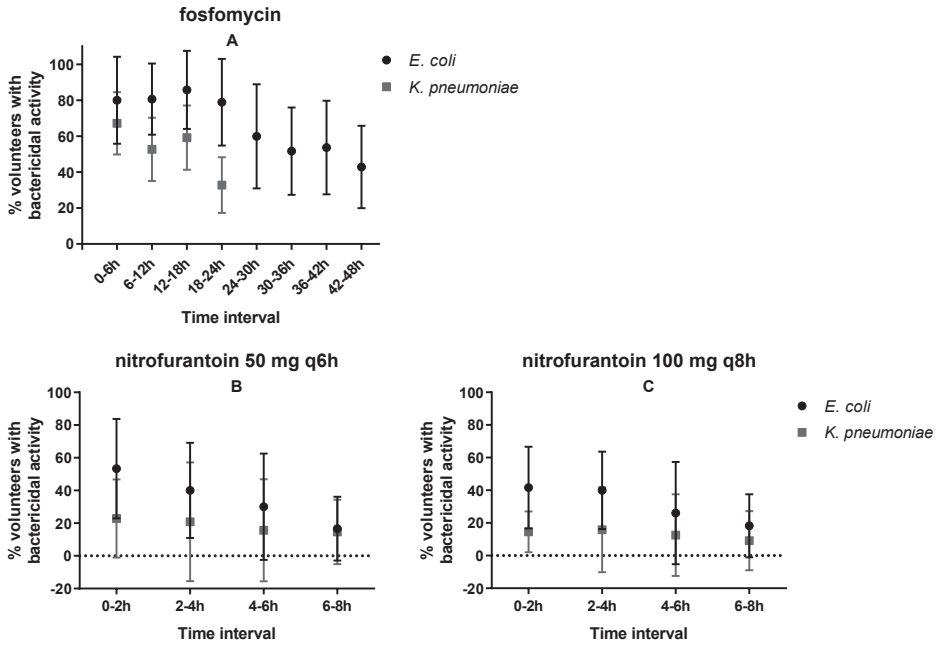

Figure 2. Percentage of volunteers where a bactericidal effect was found of fosfomycin during 48 hours (**2A**), and nitrofurantoin 50 mg q6 hours during 8 hours (**2B**) 100 mg q8 hours or (**2C**) in *E. coli* (black) and in *K. pneumoniae* (red). The numbers are expressed as the mean (± SD) percentages for both species (y axis) of the total number of volunteers that had produced urine samples in the considered time interval (x axis). Because bactericidal activity in *K. pneumoniae* was found in only a small number of volunteers (ranging from 1 to 3 volunteers) in the time intervals after 24 hours, these percentages are negligibly small and are therefore not presented in figure 2A.

17% in strains 58, 20, and 31865. This was independent of the administered dose. Only in *K. pneumoniae* 55, bactericidal activity was found in approximately 40% (100 mg q8 hours) and 60% (50 mg q6 hours) of volunteers. These percentages remained consistent over the 8-hour urine collection time period for this strain.

Fosfomycin-resistant subpopulation

Only one out of five *E. coli* isolates had a detectable fosfomycin-resistant subpopulation when grown in both standard laboratory media and human urine. Whereas, all of the *K. pneumoniae* isolates had a detected resistant subpopulation (table 4). The resistant subpopulation detected in *E. coli* 1231 and in the *K. pneumoniae* isolates had a fosfomycin MIC of >1024 mg/liter, after subculturing off the fosfomycin containing MHA onto TSA. This result is consistent with the low antibacterial activity of fosfomycin in these strains.

Table 4. Presence of a fosfomycin LLR and/or a HLR subpopulation of the strains in urine or MHB.

Strain (MIC, mg/liter)	LLR/HLR subpopulation present	
	Urine	MHB
E. coli		
ATCC25922 (1)	No	No
51 (2)	No	No
03 (0.25)	LLR	LLR
1231 (16)	HLR	HLR
4807 (32)	LLR	LLR
K. pneumoniae		
58 (8)	HLR	HLR
20 (32)	LLR	HLR
31865 (2)	HLR	HLR
55 (4)	HLR	HLR

MHB, Muller-Hinton Broth; LLR, low level resistant; HLR, high level resistant

DISCUSSION

While fosfomycin exhibited bactericidal activity for at least 48 hours against *E. coli*, no antibacterial activity was detected in the majority of *K. pneumoniae* samples. In contrast to fosfomycin, nitrofurantoin showed low antibacterial activity in both species regardless of the administered dose, though only one dose interval was examined among the many intervals intended with a course of nitrofurantoin.

In general, fosfomycin exhibited bactericidal activity as demonstrated by comparable UIT and UBT values. The duration of activity was strongly species-dependent, with ≥48 hours for *E. coli* and only 18 hours for *K. pneumoniae*. Indeed, 48-hour antibacterial activity against *K. pneumoniae* could be demonstrated in only a small subset. These findings are supported by earlier *in vitro* research demonstrating that fosfomycin was not able to reliably kill *K. pneumoniae* isolates (9, 20).

It was suggested that fosfomycin is able to kill (or at least inhibit growth of) *E. coli*, but regrowth occurs thereafter. The extent of regrowth depends on the presence of a resistant subpopulation and this is not predicted based on the baseline fosfomycin MIC for the strain (21). This is corroborated by our finding that bactericidal activity against *E. coli* over 24 hours was found in approximately 90% of volunteers, but quickly fell below 60% thereafter. This was true for all *E. coli* strains with the exception of *E. coli* 1231, the strain harboring a HLR subpopulation. For *K. pneumoniae*, we found moderate (or almost totally absent) antibacterial activity of fosfomycin in the majority of the samples, confirming other reports (9, 10, 22). All *K. pneumoniae* strains had a HLR subpopulation. This may be more a matter of intrinsic rather than acquired resistance after antibiotic exposure (9, 23). These findings suggest that a single, 3g dose may be sufficient for UTIs caused by *E. coli* without HLR subpopulations, and that fosfomycin is inappropriate for UTIs caused by *K. pneumoniae*, regardless of the MIC for the strain and the fosfomycin dose.

Maximum UITs and UBTs of nitrofurantoin were obtained in the first two-hour time interval. Titers were low but comparable for both species tested, demonstrating reasonable bactericidal activity of nitrofurantoin only in the first two hours, confirming a previous report describing early activity against extended spectrum beta lactamase (ESBL)-producing pathogens like *E. coli* and *K. pneumoniae* (12). We did not find any significant differences in antibacterial activity between the two dosing regimens; the slightly higher urinary concentrations of nitrofurantoin after 100 mg versus 50 mg did not result in more antibacterial activity in our experiment (8).

The major advantage of the method used here is that it is a*n ex-vivo* model combining patient-related pharmacokinetic properties of a drug with its PD effect. The *ex-vivo* results obtained with this method therefore may reflect the antimicrobial clinical effectiveness against uropathogens better than most other *ex-vivo/in-vitro* methods. This is important, as bacterial growth *ex-vivo/in-vitro* can be different from that in humans (24). Yet we found bactericidal activity of nitrofurantoin in less than 50% of volunteer samples, and this only for a short period of time. This contrasts with fosfomycin's bactericidal activity, which was detected in 90% of volunteer samples. These results are in conflict with what was found in a recent randomized clinical trial comparing five days of nitrofurantoin (100 mg q8 hours) to single-dose fosfomycin (3g) for acute lower UTI (25). Seventy percent of those receiving nitrofurantoin had clinical success versus

only 58% of those receiving fosfomycin. Microbiologic resolution was achieved in 74% versus 63%, respectively. There is thus discrepancy between the *ex-vivo* activity of nitrofurantoin in a single dosing interval (and also, but to a smaller extent, of fosfomycin) and its clinical efficacy.

There are several possible factors that could explain this discrepancy. Fosfomycin requires glucose-6-phosphate to enter the bacterial cell to exert its antibacterial activity so it is standard practice to add 25 mg/l glucose-6-phosphate to the laboratory media when performing in vitro experiments with fosfomycin (26). Because human urine normally does not contain glucose-6-phosphate in significant amounts, the ex vivo antibacterial activity was measured without adding glucose-6-phospate. It should be noted, however, that the baseline MICs for fosfomycin were measured in the presence of glucose-6-phosphate, as per the reference standard for fosfomycin susceptibility testing (27). This could party explain the discrepancy between the fosfomycin MICs at baseline and the urinary antibacterial activity. For nitrofurantoin, we investigated its activity during only one dosing interval of a drug intended to be administered over at least five days. It would therefore seem likely that the short period of antibacterial activity we found, would be sufficient to achieve clinical success in the majority of the patients when administered as a course of multiple oral doses. The cumulative effect of the full nitrofurantoin course after repetitive dosing has not been investigated, such that our results would underestimate the effect of the antimicrobial agent. Secondly, we considered the bactericidal activity only when calculating the percentages of bactericidal success, but whether pathogen killing is needed to achieve clinical success is questionable. Bacteriostatic activity, or a bactericidal effect during a short period of time (*e.g.*, less than 2 hours), might be sufficient to promote clinical success. In particular because of the natural urodynamics of regularly voiding episodes, during which uropathogens are flushed out together with the urine. Finally, the percentages of bactericidal success gives an underestimation of daily clinical practice since we were not able to measure the antibacterial activity in the undiluted sample due to limited sample volumes.

CONCLUSION

We found strong bactericidal activity of fosfomycin against *E. coli* over at least 48 hours after administration and moderate bactericidal activity against *K. pneumoniae* over 18 hours. High-level resistant subpopulations were found in all *K. pneumoniae* strains and in one of the *E. coli* strains, findings that further support the likelihood of intrinsic resistance of *K. pneumoniae* against fosfomycin, and highlight that MIC measurements might not be the best measure for predicting *ex-vivo* activity of fosfomycin. Titers

of nitrofurantoin were comparable for both *E. coli* and *K. pneumoniae*, demonstrating moderate bactericidal activity in the first 2 hours after dosing. In the majority of subsequent samples, however, no antibacterial activity was detected, regardless of the administered dose. This finding is in contrast to nitrofurantoin's well-observed clinical effects over multiple dosing intervals. Our findings reveal a discrepancy between nitrofurantoin's measurable *ex vivo* activity in a single dosing interval time period and its clinical effectiveness. For fosfomcyin, our findings suggest that the current single-dose approach to fosfomycin administration in UTIs caused by *E. coli* without HLR may be sufficient, but confirm the doubts of the use of fosfomycin in general in UTIs caused by *K. pneumoniae*.

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Table S1. Characteristics of the fosfomycin and nitrofurantoin samples. Median (range) of the drug concentration, number of samples and pH of the urine samples for both drugs during each time interval. Samples were grouped based on their time after dose.

Collection period	Concentration [a]		Number of samples	pH	
(h)	(mg/liter)				
Fosfomycin					
0-6	808.1	(19.9-5729.9)	77	5	(5-7)
6-12	744.4	(90-4375.9)	68	5.5	(5-7)
12-18	512.7	(145.1-1866.6)	33	6	(5-7)
18-24	348.3	(51.5-2189.1)	67	5	(5-7)
24-30	124	(<0.75-947.9)	82	5	(5-7)
30-36	78.5	(9.1-707.9)	64	5	(5-7)
36-42	80.8	(<0.75-454.8)	32	5	(5-7)
42-48	62.3	(<0.75-495.7)	61	5	(5-7)
Nitrofurantoin (50 mg q6 hours)					
0-2	53.6	(4-176.3)	23	5.6	(5-6)
2-4	26.8	(<4-106.7)	17	5.8	(5-6)
4-6	19.7	(5.3-54.4)	9	6	(6-6)
6-8	15.7	(<4-37.6)	19	5.8	(5-6)
Nitrofurantoin (100 mg q8 hours)					
0-2	32.6	(4-209.4)	25	5.7	(5-7)
2-4	21.3	(13.4-97.9)	11	5.6	(5-6)
4-6	17	(5.2-79.3)	16	5.8	(5-6)
6-8	10	(<4-56.4)	20	5.8	(5-6)

[a]The lower limit of quantification of the analytical method for fosfomycin was 0.75 mg/liter and 4 mg/liter for nitrofurantoin.

Table S2. UITs and $AUIT_{0\text{-}48h}$ values of fosfomycin. Median (range) of UITs and $AUIT_{0\text{-}48h}$ values of fosfomycin over time for each strain.

Strain (MIC, mg/liter)	UIT (median (range)) for the indicated time periods (h)								
	0-6	6-12	12-18	18-24	24-30	30-36	36-42	42-48	$AUIT_{0\text{-}48h}$
E. coli									
ATCC25922 (1)	16 (<2-256)	16 (<2-256)	16 (<2-128)	8 (<2-128)	4 (<2-64)	2 (<2-64)	4 (<2-32)	2 (<2-32)	164 (98-309)
51 (2)	16 (<2-128)	8 (2-32)	4 (<2-16)	4 (<2-16)	2 (<2-16)	2 (<2-8)	2 (<2-8)	<2 (<2-8)	117 (74-164)
03 (0.25)	16 (<2-64)	16 (4-64)	16 (2-32)	4 (<2-64)	2 (<2-16)	2 (<2-16)	2 (<2-8)	<2 (<2-8)	150 (96-199)
1231 (16)	2 (<2-16)	2 (<2-32)	<2 (<2-8)	<2 (<2-8)	<2 (<2-4)	<2 (<2-4)	<2 (<2-4)	<2 (<2-2)	69 (46-144)
4807 (32)	32 (<2-256)	16 (<2-256)	16 (<2-64)	8 (<2-128)	3 (<2-32)	4 (<2-32)	4 (<2-16)	2 (<2-16)	171 (91-279)
K. pneumoniae									
58 (8)	4 (<2-16)	2 (<2-16)	<2 (<2-8)	<2 (<2-8)	<2 (<2-2)	<2 (<2-2)	<2 (<2-<2)	<2 (<2-2)	57 (45-100)
20 (32)	4 (<2-32)	4 (<2-64)	2 (<2-8)	2 (<2-16)	<2 (<2-4)	<2 (<2-4)	<2 (<2-2)	<2 (<2-<2)	79 (41-129)
31865 (2)	8 (<2-128)	8 (<2-64)	8 (<2-64)	3 (<2-64)	<2 (<2-32)	<2 (<2-16)	<2 (<2-8)	<2 (<2-4)	110 (74-224)
55 (4)	4 (<2-64)	4 (<2-64)	4 (<2-16)	2 (<2-16)	<2 (<2-8)	<2 (<2-8)	<2 (<2-4)	<2 (<2-4)	90 (58-174)

Table S3. UITs and $AUIT_{0-6h}$ or $AUIT_{0-8h}$ values of nitrofurantoin. Median (range) of UITs and $AUIT_{0-6h}$ or $AUIT_{0-8h}$ values for nitrofurantoin over time for each strain.

Dose, strain (MIC, mg/liter), and isolate	UIT (median (range)) for the indicated time periods (h)				
	0-2	2-4	4-6	6-8	$AUIT_{0-6h}$ or $AUIT_{0-8h}$
Nitrofurantoin 50 mg q6 hours					
E. coli					
ATCC25922 (16)	8 (<2-16)	2 (<2-16)	1 (<2-4)	<2 (<2-4)	20 (12-31)
51 (32)	4 (<2-16)	2 (<2-8)	<2 (<2-4)	<2 (<2-4)	17 (10-26)
03 (16)	4 (<2-16)	2 (<2-8)	2 (<2-2)	<2 (<2-2)	18 (12-26)
1231 (512)	<2 (<2-<2)	<2 (<2-<2)	<2 (<2-<2)	<2 (<2-<2)	9 (11-32)
4807 (16)	2 (<2-8)	<2 (<2-8)	<2 (<2-<2)	<2 (<2-2)	13 (8-15)
K. pneumoniae					
58 (64)	4 (<2-16)	2 (<2-8)	2 (<2-4)	<2 (<2-8)	18 (7-9)
20 (256)	<2 (<2-4)	<2 (<2-4)	<2 (<2-<2)	<2 (<2-8)	10 (9-24)
31865 (128)	<2 (<2-2)	<2 (<2-<2)	<2 (<2-<2)	<2 (<2-<2)	9 (7-11)
55 (256)	<2 (<2-2)	<2 (<2-<2)	<2 (<2-<2)	<2 (<2-<2)	9 (7-11)
Nitrofurantoin 100 mg q8 hours					
E. coli					
ATCC25922 (16)	4 (<2-32)	2 (<2-16)	1 (<2-8)	<2 (<2-8)	18 (13-30)
51 (32)	4 (<2-16)	2 (<2-8)	2 (<2-2)	<2 (<2-4)	15 (10-23)
03 (16)	4 (<2-16)	2 (<2-8)	1 (<2-4)	<2 (<2-4)	16 (11-26)
1231 (512)	<2 (<2-<2)	<2 (<2-<2)	<2 (<2-<2)	<2 (<2-<2)	9 (11-30)
4807 (16)	<2 (<2-16)	<2 (<2-4)	<2 (<2-4)	<2 (<2-4)	12 (7-14)
K. pneumoniae					
58 (64)	4 (<2-16)	2 (<2-8)	<2 (<2-4)	<2 (<2-4)	14 (7-9)
20 (256)	<2 (<2-4)	<2 (<2-2)	<2 (<2-<2)	<2 (<2-2)	9 (7-24)
31865 (128)	<2 (<2-2)	<2 (<2-<2)	<2 (<2-<2)	<2 (<2-<2)	9 (7-11)
55 (256)	<2 (<2-2)	<2 (<2-<2)	<2 (<2-<2)	<2 (<2-<2)	9 (7-11)

5

Clinical use of fosfomycin and nitrofurantoin

5.1

The effectiveness of nitrofurantoin, fosfomycin and trimethoprim for cystitis in relation to renal function

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ABSTRACT

Objective

We evaluated the effect of renal function on the effectiveness of nitrofurantoin, fosfomycin and trimethoprim for cystitis in primary care patients.

Methods

Data was retrospectively obtained from 75 Dutch primary care practices between 2013 and 2019. Episodes were classified as uncomplicated or complicated cystitis based on the medical records and prescription according to the guidelines. Renal function was categorized as normal to mildly decreased (eGFR ≥60 mL/min), or moderately decreased to kidney failure (eGFR <60 mL/min). Clinical failure was defined as a second antibiotic prescription for cystitis or pyelonephritis within 28 days post-prescription. We used mixed effects regression analysis, with patient and GP practice as random effects.

Results

In total, 40,916 episodes were included, of which 29,873 for uncomplicated cystitis (NF5: 23,793, FT1: 5,048, TMP3: 1,032) and 11,043 for complicated cystitis (NF7: 10,236, TMP7: 807). An eGFR below 60 mL/min was observed in 8.8% (3612/40,916) of episodes and clinical failure occurred in 7.0% (2867/40,916). After adjustment, renal function was not significantly associated with clinical failure in any regime, with odds ratios of 0.96 for NF5 (95%CI: 0.90-1.01), 0.93 for FT1 (95%CI: 0.76-1.08), 0.93 for TMP3 (95%CI: 0.80-1.07), 0.95 for NF7 (95%CI: 0.65-1.26), 1.03 for TMP7 (95%CI: 0.90-1.16). In the treatment of uncomplicated and complicated cystitis no differences were observed between regimens in normal or decreased renal function after adjustment for confounders.

Conclusion

Renal function was not independently associated with increased clinical failure in patients with cystitis.

Key words Renal impairment, Cystitis, Nitrofurantoin, Trimethoprim, Fosfomycin

INTRODUCTION

Cystitis is a common bacterial infection with an annual incidence of approximately 70 per 1000 in adult women and 10 per 1000 in adult men (1). The most common causative pathogen for community-acquired cystitis is *Escherichia coli* (75-90%) (2). The aim of antimicrobial treatment is to eradicate the pathogen from the urogenital tract, to reduce symptom duration and to prevent aggravation and re-infections (3).

In primary care in the Netherlands, nitrofurantoin for five days (NF5) is recommended as first-choice oral treatment for acute uncomplicated cystitis, with a single dose of fosfomycin-trometamol (fosfomycin, FT1) as second choice and trimethoprim for three days (TMP3) as third choice (1). An extended seven-day regimen of nitrofurantoin (NF7) is the first choice and seven days of trimethoprim (TMP7) the second choice in patients with complicated cystitis, defined as having risk factors for a complicated course such as male gender, diabetes mellitus (DM), urologic abnormalities and immunosuppression (1). The efficacy of antimicrobial treatment for cystitis largely depends on its antimicrobial activity against the pathogen and the achieved concentration in urine (4). Nitrofurantoin, fosfomycin and trimethoprim are active against most uropathogens and are eliminated by renal excretion resulting in high concentrations in urine (4–6).

Lower urinary concentrations have been reported for all three antibiotics in patients with impaired renal function (4–7). The concern is that efficacy declines if insufficient drug concentrations are achieved in urine, although strong, pharmacokinetic-based, evidence for this is lacking (6, 7). Retrospective cohort studies do not show a clear answer about the effect of impaired renal function on the clinical effectiveness of nitrofurantoin for the treatment of cystitis (8, 9). To the best of our knowledge, no such studies have been conducted for fosfomycin and trimethoprim. Consequently, little evidence exists to guide the choice of antibiotic treatment of cystitis for patients with decreased renal function in primary care.

The aim of this study was to evaluate the effect of renal function on the occurrence of clinical failure when using nitrofurantoin, fosfomycin or trimethoprim for the treatment of cystitis. Furthermore, the effectiveness of nitrofurantoin, fosfomycin and trimethoprim for cystitis was compared for normal and decreased renal function.

METHODS

Design and data collection

Data were retrospectively obtained from the Julius General Practitioners' Network (JGPN) consisting data from 75 general practices (GPs) in the province of Utrecht, the Netherlands, between January 2013 and June 2019 (10). The database consists

of all antibiotic prescriptions to treat cystitis and includes information on patient characteristics, comorbidities and co-medication. Diagnoses were coded according to the International Classification of Primary Care (ICPC). Medication prescriptions were coded according to the Anatomical Therapeutic Chemical (ATC) classification system.

Study population

Episodes were eligible for analysis if antibiotic therapy was prescribed by the GP for the treatment of cystitis according to the Dutch guideline in patients of at least 12 years of age. Diagnoses were classified as uncomplicated or complicated according to the duration of treatment. For uncomplicated cystitis, the guideline advices a regimen consisting of five days nitrofurantoin 100 mg extended release (Furabid®) every 12 hours or 50 mg normal release (Furadantin®) every 6 hours (NF5), a single gift of fosfomycin 3000 mg (FT1) or a three-day treatment with trimethoprim 300 mg every 24 hours (TMP3). Cystitis episodes that were treated as being uncomplicated in which one of the following risk factors were present for a complicated course were excluded: male gender, pregnancy, DM, urologic abnormalities and immunosuppression. For complicated cystitis, the guideline recommends nitrofurantoin and trimethoprim in an extended treatment duration for seven days (NF7 and TMP7). Patients without documented risk factors and receiving the extended course were included in the complicated cystitis group. Episodes that are assumed to represent treatment failures of a prior cystitis episode were excluded, that are episodes within 28 days of a prior antibiotic prescription for cystitis, i.e. short-course nitrofurantoin, trimethoprim or fosfomycin.

Renal function was based on the most recent estimated Glomerular Filtration Rate (eGFR) value measured within six months before or after the prescription date. Episodes were excluded from analysis if no eGFR was measured in this period. The eGFR was calculated with the Chronic Kidney Disease Epidemiology (CKDepi) formula using plasma creatinine values, age and gender (11).

Outcome

Clinical failure was defined as the prescription of one of the following antibacterial agents within 28 days of the initial prescription: nitrofurantoin, fosfomycin and trimethoprim, with exclusion of prophylactic use of trimethoprim or nitrofurantoin (>7-day use), or one of the following antimicrobials in combination with an ICPC code for cystitis or pyelonephritis: ciprofloxacin, co-trimoxazole or amoxicillin-clavulanic acid.

Statistical analysis

Effect of renal function on clinical failure per antibiotic class

Odds ratios were calculated to determine the association between the patients' renal function and the risk of clinical failure (crude analysis) within each of the antibiotic classes. Renal function was analysed in the model as a continuous variable. Odds ratios for clinical failure were calculated per 10 mL/min increase of eGFR. eGFR values ≥90 mL/min were truncated, as no effect is expected across the range of normal glomerular filtration rates on the effectiveness of these antibiotics (12).

For the multivariable analysis, a logistic model with mixed effects was used, that incorporated the correlation among repeated episodes within one patient and within one GP practice using a random intercept. The adjusted model was corrected for the following fixed variables: in the population of uncomplicated cystitis: age, gender, socio-economic status, number of cystitis prescriptions in the previous year, year of prescription, a history of dementia, cognitive impairment other than dementia, depression, a (presumed) sexually transmitted disease, and oral contraceptives use. In the population of complicated cystitis, the analysis was additionally corrected for the following variables: solid organ transplantation, diabetes mellitus, anatomic/functional deficits in the urinary tract or kidney, and immunosuppressive medicine use. For nitrofurantoin, the dosing regimen (50 mg normal release every 6 hours vs. 100 mg slow release every 12 hours) was included as confounding variable. For the crude and the multivariable model, the assumption of linearity was tested by visually inspecting the residuals. Missing data of socio-economic status (n=172) and number of cystitis prescriptions (n=1) were imputed using multiple imputation. As a sensitivity analysis, a logistic model was performed in which we included patients with unknown serum creatinine as having an eGFR of 90 mL/min. In this analysis the same fixed and random effects were used as above for all regimen including a binomial determinant that indicates whether eGFR had been measured or not.

Effect of antibiotic class on clinical failure rate within strata of renal function

To compare the effect of antibiotic classes on clinical failure within strata of renal function, a crude and multivariable mixed effects logistic regression model was used with only first cystitis episodes per patient included for analysis. The same fixed effects as described above were used with additionally eGFR as a continuous variable and GP practice as random effect. We compared the short regimens for uncomplicated cystitis (NF5, FT1, TMP3) and extended regimens for complicated cystitis (NF7, TMP7). These comparisons were performed for episodes in patients with normal to mild decreased renal function (eGFR ≥60 mL/min; Kdigo stage G1 or G2) and in moderately decreased renal function to kidney failure (eGFR <60 mL/min; Kdigo stage G3-G5) (13). In all

cases, P-values less than 0.05 were considered statistically significant. The same sensitivity analysis as above was performed for episodes in patients with normal to mild decreased renal function (eGFR ≥60 mL/min) including patients with unknown serum creatinine as having an eGFR of 90 mL/min.

The models were fit to maximum likelihood using the Laplace approximation. All analyses were performed using R software (version 3.4.1), using the lme4 package (version 1.1-21).

Ethics

Approval for the study was obtained with a waiver for informed consent from the ethical board of the University Medical Centre Utrecht, the Netherlands, with reference WAG/mb/18/022909.

RESULTS

Study population

The complete dataset consisted of 160,399 episodes of nitrofurantoin, fosfomycin and trimethoprim prescriptions. After exclusion of children younger than 12 years (n=4,416), episodes that were not in accordance with the guideline (n= 35,821), episodes with a cystitis prescription in the prior 28 days (31,034), and episodes in which the eGFR value was not measured (n= 48,212) 40,916 episodes remained for analysis. Of these, 29,873 consisted of a short regimen for uncomplicated cystitis and 11,043 consisted of an extended regimen for complicated cystitis (Figure 1).

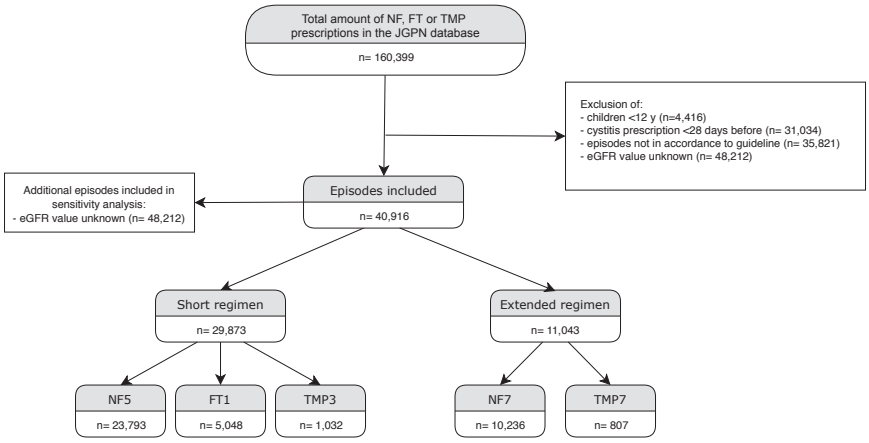

Figure 1: Flowchart for inclusion of episodes from the Julius General Practitioners' Network (JGPN) consisting of data from 75 general practitioner practices (GP practices') in the province of Utrecht, the Netherlands, between January 2013 and July 2019.

Table 1 shows the patient characteristics at baseline in the five treatment arms. In the past six years the amount of FT1 prescriptions for cystitis increased yearly whereas the amount of NF5, NF7, TMP3 and TMP7 prescriptions each year were stable. Of all episodes, 8.8% (3,612/40,916) had an eGFR below 60 mL/min. The mean eGFR was higher (85.5 mL/min) in the NF5 arm in comparison to FT1 (80.0 mL/min) and TMP3 (79.1 mL/min). In patients that were prescribed NF5, the median age was lower (61 years vs. resp. 72 and 73 years), and the amount of cystitis episodes in the previous year was lower (median 1 vs. resp. 0 and 0) compared to patients that were prescribed FT1 or TMP3. Among those treated with extended regimens, the median age was lower (71 vs. 77 years), the eGFR was higher (78.9 vs. 73.3 mL/min), and the number of cystitis episodes in the previous year was lower (median 0 vs. median 1) when being treated with NF7 compared to TMP7.

The population with cystitis that is included for the sensitivity analysis in which no renal function was measured had a median age of 33 years in the NF5 population up to 55 in the TMP7 population, with 31% (1,891/6,057) of patients being male and 9.0% (547/6,057) having DM in the NF7 population.

Effect of renal function on clinical failure per antibiotic class

Clinical failure occurred in 7.0% (2,867/40,916) of all episodes: 7.1% after using NF5, 6.0% after using FT1, 7.7% after using TMP3, 7.3% after using NF7, and 6.8% after using TMP7.

No significant associations were found in any of the treatment arms after adjusting for confounders (table 2). In the sensitivity analysis a higher eGFR was associated with a lower risk of clinical failure for NF5 and TMP3, but not for FT1, NF7 or TMP7.

Effect of antibiotic class on clinical failure rate within strata of renal function

Table 3 shows the odds ratios of the antibiotic regimens for clinical failure within categories of renal function. The probability of treatment failure among patients with moderate to severely decreased renal function (eGFR <60 mL/min) was not significantly different between the treatment regimens for uncomplicated cystitis, nor between the treatment regimens used for complicated cystitis. Similar non-significant results were observed in the sensitivity analysis.

Table 1: The baseline characteristics of cystitis episodes classified on the prescribed antimicrobial therapy.

Patient characteristics	Treatment (n=49,115)				
	Short regimen			Extended regimen	
	NF5 (n=23,793)	**FT1 (n=5,048)**	**TMP3 (n=1,032)**	**NF7 (n=10,236)**	**TMP7 (n=807)**
Age (years)					
Median	61	72	73	71	77
Interquartile range	43 – 75	57 - 83	56 - 84	59 – 81	66 - 85
Gender					
Male (%)	NA	NA	NA	3,238 (31.6%)	210 (26.0%)
eGFR (mL/min)					
Mean ± SD	85.5± 10.1	80.0 ± 17.1	79.1 ± 18.2	78.9 ± 16.0	73.3 ± 19.4
eGFR levels					
≥90	17,125 (72.0%)	2,850 (56.4%)	606 (58.7%)	5,256 (51.3%)	327 (40.5%)
60-90	5,618 (23.6%)	1,527 (30.2%)	262 (25.3%)	3,458 (33.8%)	275 (34.1%)
30-60	1,014 (4.3%)	541 (10.7%)	129 (12.5%)	1,415 (13.8%)	176 (21.8%)
0-30	36 (0.15%)	130 (2.6%)	35 (3.4%)	107 (1.0%)	29 (3.6%)
Prescription year					
2013	3,189 (13.4%)	364 (7.2%)	193 (18.7%)	1,183 (11.6%)	81 (10.0%)
2014	3,995 (16.8%)	622 (12.3%)	157 (15.2%)	1,555 (15.2%)	102 (12.6%)
2015	4,190 (17.6%)	832 (16.5%)	200 (19.4%)	1,717 (16.8%)	131 (16.2%)
2016	4,178 (17.6%)	954 (18.9%)	167 (16.2%)	1,824 (17.8%)	149 (18.5%)
2017	4,061 (17.1%)	1,163 (23.0%)	161 (15.6%)	1,874 (18.3%)	166 (20.6%)
2018	3,523 (14.8%)	919 (18.2%)	130 (12.6%)	1,729 (16.9%)	144 (17.8%)
2019-June	657 (2.8%)	194 (3.8%)	24 (2.3%)	354 (3.5%)	34 (4.2%)
Pregnancy	NA	NA	NA	396 (3.9%)	13 (1.6%)
STD	1,144 (4.8%)	337 (6.7%)	55 (5.3%)	586 (5.7%)	62 (7.7%)
Cognitive impairment*	29 (0.12%)	3 (0.06%)	5 (0.48%)	17 (0.17%)	0 (0.0%)
Dementia	428 (1.8%)	176 (3.5%)	20 (1.9%)	317 (3.1%)	45 (5.6%)
Use of OAC	2,556 (10.7%)	394 (7.8%)	90 (8.7%)	363 (3.5%)	16 (2.0%)
Depression	1,815 (7.6%)	395 (7.8%)	91 (8.8%)	675 (6.6%)	53 (6.6%)
Diabetes Mellitus	NA	NA	NA	4,894 (47.8%)	433 (53.7%)
Urologic abnormalities	NA	NA	NA	394 (3.8%)	19 (2.4%)
Use of immunosuppressant's	NA	NA	NA	360 (3.5%)	37 (4.6%)

Table 1: The baseline characteristics of cystitis episodes classified on the prescribed antimicrobial therapy. (*continued*)

Patient characteristics	Treatment (n=49,115)				
	Short regimen			Extended regimen	
	NF5 (n=23,793)	**FT1 (n=5,048)**	**TMP3 (n=1,032)**	**NF7 (n=10,236)**	**TMP7 (n=807)**
Socio-economic status score					
Median	0.19	0.19	0.32	0.19	0.19
Interquartile range	-0.19 – 1.24	-0.12 – 1.81	-0.48 – 1.31	-0.94 – 0.97	-1.16 – 0.97
N episodes of cystitis previous year					
Median	0	1	1	0	1
Interquartile range	0 - 1	0 - 3	0 - 2	0 - 1	0 - 2

eGFR = estimated glomerular filtration rate
STD = sexually transmitted diseases
OAC = oral anticonception
NF5 = NF five-day treatment
NF7 = NF seven-day treatment
FT1 = FT one day treatment
TMP3 = TMP three-day treatment
TMP7 = TMP seven-day treatment
*other than dementia

Table 2: The effect of every 10 mL/min increase in eGFR on the odds ratio of clinical failure within 28 days post-prescription.

	Crude analysis	Multivariable analysis	Multivariable sensitivity analysis
Therapy	Odds ratio (95% CI)	Odds ratio (95% CI)	Odds ratio (95% CI)
NF5	0.87 (0.78-0.97) **	0.96 (0.90-1.01)	0.65 (0.54-0.80) ***
FT1	1.02 (0.96-1.08)	0.93 (0.76-1.08)	0.97 (0.89-1.06)
TMP3	0.96 (0.87-1.05)	0.93 (0.80-1.07)	0.81 (0.70-0.94) **
NF7	0.94 (0.91-0.98) *	0.95 (0.65-1.26)	0.84 (0.68-1.04)
TMP7	1.04 (0.93-1.14)	1.03 (0.90-1.16)	0.56 (0.20-1.56)

Adjusted for the following confounding variables: gender, age, year of prescription, pregnancy, sexual transmitted diseases, cognitive impairment other than dementia, oral contraceptive use, depression, dementia, use of immunosuppressant's, socio-economic status, number of episodes of cystitis in the previous year and in the previous 28 days, the use of normal or slow release nitrofurantoin formulation, with as random effects the patient and the general practitioners practice. For complicated cystitis additionally for fixed variables diabetes mellitus, urologic abnormalities, solid organ transplantation
Significance levels: * $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$
TMP3 = trimethoprim three-day treatment
NF5 = nitrofurantoin five-day treatment
NF7 = nitrofurantoin seven-day treatment
FT1 = fosfomycin single dose treatment
TMP7 = trimethoprim seven-day treatment

Table 3: The incidence of clinical failure at 28 days compared between the treatment arms within renal function groups.

			Crude analysis	Multivariable analysis	Multivariable sensitivity analysis
eGFR (mL/min)	**UTI group**	**Therapy**	Odds ratio (95% CI)	Odds ratio (95% CI)	Odds ratio (95% CI)
<60	Uncomplicated	FT1 vs. NF5†	0.53 (0.28-1.00)	0.54 (0.27-1.09)	NA
		TMP3 vs. NF5†	1.04 (0.45-2.40)	1.21 (0.49-2.98)	NA
		TMP3 vs. FT1	1.95 (0.75-5.10)	2.24 (0.79-6.33)	NA
	Complicated	TMP7 vs. NF7†	0.55 (0.29-1.07)	0.62 (0.31-1.24)	NA
≥60	Uncomplicated	FT1 vs. NF5	0.94 (0.75-1.18)	0.91 (0.73-1.15)	1.01 (0.85-1.19)
		TMP3 vs. NF5	1.03 (0.68-1.56)	1.05 (0.69-1.60)	1.19 (0.88-1.61)
		TMP3 vs. FT1	1.10 (0.69-1.74)	1.15 (0.72-1.84)	1.18 (0.84-1.65)
	Complicated	TMP7 vs. NF7	1.05 (0.77-1.44)	2.39 (0.86-6.63)	0.79 (0.50-1.26)

Adjusted for the following confounding variables: gender, age, year of prescription, pregnancy, sexual transmitted diseases, cognitive impairment other than dementia, oral contraceptive use, depression, dementia, use of immunosuppressant's, socio-economic status, number of episodes of cystitis in the previous year and in the previous 28 days, the use of normal or slow release nitrofurantoin formulation, with as random effects the general practitioners practice. For complicated cystitis additionally for fixed variables diabetes mellitus, urologic abnormalities, solid organ transplantation.

NF5 = NF five-day treatment
NF7 = NF seven-day treatment
FT1 = FT single dose treatment
TMP7 = TMP seven-day treatment
TMP3 = TMP three-day treatment
† Nitrofurantoin is contraindicated in patients with eGFR <30 mL/min (1)

DISCUSSION

The results of this study suggest that no large effect exists of (decreased) renal function on the effectiveness of nitrofurantoin, fosfomycin or trimethoprim for cystitis. This is surprising given the fact that all three antibiotics are being eliminated by glomerular excretion (14–17). Especially for nitrofurantoin, it was expected that a decreased renal function could have led to low, sub-therapeutic urinary concentrations of nitrofurantoin as urinary concentrations of nitrofurantoin are relatively low compared to those for fosfomycin and trimethoprim (18–20). The crude results suggest that in patients with moderately decreased renal function to kidney failure, treatment with FT1 for uncomplicated and TMP7 for complicated are associated with lower clinical failure rates than respectively treatment with NF5 and NF7. However, this difference was not observed after adjustment for confounders.

The population of analysis is relatively old and has much comorbidities. As these are both reasons to measure serum creatinine, a sensitivity analysis was performed to evaluate the effect of renal function on clinical failure when also including the population with unknown serum creatinine. This population consisted of younger patients with less comorbidities, in which we assumed the renal function to be normal (eGFR =90ml/min). This sensitivity analysis indicate that a higher clinical failure rate is observed in patients with decreasing renal function after using NF5 or TMP3 for cystitis. This was not observed for FT1. These results point to the hypothesis that the effectiveness of fosfomycin is less affected by renal function compared to nitrofurantoin or trimethoprim in the primary care population. This is likely to be related to the relatively high urinary concentrations of fosfomycin.

Little is known about the effect of decreased renal function on clinical efficacy of cystitis treatment as previous randomized controlled trials excluded patients with decreased renal function (21–26). A retrospective cohort study investigated the effect of renal function on the effectiveness of nitrofurantoin and used trimethoprim as a control arm. A decreased renal function (eGFR <50 mL/min/1.73m^2) was not associated with a decreased effectiveness of nitrofurantoin or trimethoprim, although confidence intervals were wide (9). In the same study, a significant association between a decreased renal function and the occurrence of pulmonary reactions leading to hospitalization was found for nitrofurantoin. Another retrospective cohort study evaluated the effectiveness of nitrofurantoin in the treatment of cystitis in outpatient males with varying renal functions. It was found that for every 10 mL/min decrease in eGFR, the odds of clinical failure increased by 13% (8). No clinical studies have been performed that evaluated the effect of decreased renal function on the efficacy or effectiveness of oral fosfomycin for the treatment of cystitis.

Little pharmacokinetic data is available to support our findings. In a small study with 28 kidney failure patients, it was found that urinary concentrations of nitrofurantoin were lower in patients with severely reduced renal function (4-11 mL/min) compared to patients with normal renal function (27). Taken into account the ECOFF of *E.coli* for nitrofurantoin of 64 mg/L as reported by the EUCAST, urinary drug concentrations in these patients did not exceed the ECOFF so urinary concentrations in these patients were sub therapeutic (28). We therefore expected to find a relationship between renal function and clinical treatment failure for nitrofurantoin, but this was not found. However, an effect of kidney failure on the effectiveness of nitrofurantoin could not be excluded with this study because our database contains only few patients with kidney failure that were treated with nitrofurantoin for cystitis. This is likely to be a consequence of the Dutch guideline that advices against using nitrofurantoin in eGFR below 30 mL/min. In contrast to nitrofurantoin, high urinary concentrations of trimethoprim and fosfomycin are reached after administration of the registered dose, making it less likely that a decrease in renal function will result in sub therapeutic concentrations in urine (19, 20). This hypothesis was supported for fosfomycin by the findings of a small study in seven patients with different levels of renal function (eGFR ranged from 21-72 mL/min). Urinary concentrations of fosfomycin were found to be lower and excretion was delayed with progressive renal failure compared to those in healthy subjects (29). Despite these concentrations were lower, they remained higher than the breakpoint of 128 mg/L for susceptible *E. coli* for as long as 48 hours in all seven patients. No data is available on the urinary concentrations of trimethoprim in patients with decreased renal function (30). More research is needed into the effect of the renal function on the treatment outcome of fosfomycin and trimethoprim.

Our study has some limitations. The most important limitation was the retrospective nature of the data. It is known that the JGPN database provides reliable quantitative estimates of demographic data, drug prescriptions (ATC codes), symptoms (ICPC codes) and laboratory values, but detailed information is lacking. For example, details about dipstick results, drug concentrations, microbiological cultures, and decision to treat are missing (10). However, it is known that Dutch GPs usually confirm the presence of cystitis using dipstick before prescribing antibiotics, making it more likely that a true cystitis was treated (31). Second, clinical failure rates may have been underestimated as prescription data from hospitals and out of office GP services were lacking so the follow-up was limited. However, only 6% of total antibiotic prescriptions in primary care occurs out of office hours in the Netherlands and the occurrence of pyelonephritis after treatment for uncomplicated cystitis was found to be low (around 1%) in patients with uncomplicated cystitis, diminishing the effect of underestimation (32, 33).

Second, confounding by indication could not be excluded when comparing the treatment with nitrofurantoin, fosfomycin and trimethoprim because nitrofurantoin is

the first choice option for cystitis according to the treatment guideline and nitrofurantoin is contraindicated in patients with a severely decreased renal function (GFR <30 mL/min) (1). Although we only included first episodes of cystitis and we adjusted for the number of cystitis prescriptions in the previous year, residual confounding is possible. If so, we expect bias in favour of NF5 and NF7.

In conclusion, we found no important effect of (decreased) renal function on the effectiveness of nitrofurantoin, fosfomycin or trimethoprim. New studies, including patients with kidney failure as well as young healthy patients are needed to confirm our findings. Next, more studies are warranted investigating the PK/PD profile of nitrofurantoin, fosfomycin and trimethoprim for cystitis in patients with decreased renal function. Our findings suggest that the impact of renal function on the treatment outcome with one of the three drugs for cystitis might be overestimated during clinical practice nowadays.

Conflict of interest

The authors declare that there are no conflicts of interest.

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5.2

An audit of nitrofurantoin use in three Australian hospitals

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ABSTRACT

Background: International guidelines have recommended the long-acting formulation of nitrofurantoin as first-line treatment for uncomplicated urinary tract infections (UTIs) since 2010. Australian guidelines have only recently listed nitrofurantoin as a first-line agent, but the long-acting formulation is not available. In the setting of increasing multidrug-resistance, the unavailability of the long-acting formulation of nitrofurantoin in Australia, and anecdotal perception of confusion regarding dosing, we audited nitrofurantoin use.

Methods: We performed a retrospective audit of nitrofurantoin use at Alfred Health. All patients dispensed nitrofurantoin from January 2016 to June 2018, as identified from pharmacy dispensing records, were eligible. We used a standardised case report form to extract data from medical records, including dosing regimen and indication.

Results: We included 150 patients with 151 nitrofurantoin prescriptions in the analysis, of whom 74% [111/150] were female. Nitrofurantoin was most commonly dispensed for the treatment of UTIs (68% [103/151] versus 32% [48/151] for UTI prophylaxis). For the treatment of uncomplicated UTIs, the most frequently used dose was 100 mg twice daily for five days. In male patients, the 100 mg twice daily for seven days was the most popular regimen. The prophylactic dose of 50 mg once daily was used in women but rarely in men. We did not find evidence of dose adjustment for renal impairment.

Conclusion: While treatment duration was consistent with guidelines, the dosage and frequency used was often incorrect for the formulation and was not adjusted for renal function. Nitrofurantoin use is likely to increase, so clarification regarding optimal nitrofurantoin dosing regimens may be appropriate.

Keywords: nitrofurantoin; urinary tract infections; antibiotic use; oral drugs; infectious diseases

INTRODUCTION

Nitrofurantoin is an oral antibiotic that has been recommended as first-line therapy for uncomplicated urinary tract infections (UTIs) in Europe and the United States (US) since 2010 (1, 2). First registered in 1952, nitrofurantoin has undergone a recent resurgence in popularity in parallel with the emergence of multidrug-resistance among bacteria that cause UTIs. This is because of the low prevalence of nitrofurantoin resistance and the minimal impact of nitrofurantoin on commensal microbiota in comparison with other oral treatment options, such as beta-lactams and fluoroquinolones (1, 3). Its spectrum of activity includes most uropathogens, including extended spectrum beta-lactamase (ESBL)-producing *Enterobacteriaceae* and vancomycin resistant enterococci (VRE) (4, 5).

In Australia, nitrofurantoin is only available as 50 mg and 100 mg oral capsules (Macrodantin®). The product information recommends a dose of 50-100 mg four times daily for five days (6). Internationally, nitrofurantoin is also produced as an oral suspension (Macrodantin®) and as slow-release 100 mg capsules (Macrobid®) for twice daily dosing, but these formulations are not registered in Australia (4, 5, 7). These products all contain nitrofurantoin in its macrocrystalline form, the microcrystalline containing products being less popular due to gastrointestinal side effects (8). There is substantial heterogeneity in nitrofurantoin dosing recommendations internationally, with total daily dose ranging from 150 mg to 400 mg divided in two, three or four doses per day (9). This is of concern because suboptimal nitrofurantoin dosing regimens may lead to selection of resistant isolates (1, 10).

Despite its popularity in Europe and the US, at the time of this study, the 'Therapeutic Guidelines: Antibiotic' (Version 15) recommended trimethoprim (300 mg once daily for three days), cephalexin (500 mg twice daily for five days) and amoxicillin-clavulanic acid (500/125 mg twice daily for five days) as preferable to nitrofurantoin for the treatment of uncomplicated UTIs in non-pregnant women (11). A seven-day course was recommended for male patients. Nitrofurantoin was listed as a fourth-line therapy, but with twice daily dosing, which is usually reserved for the slow-release formulation, Macrobid® (not registered in Australia). Given the differing recommendations regarding indication and dosing of this antibiotic, and the local increase in Enterobacteriaceae resistant to first-line agents (12), we performed an audit of nitrofurantoin use across three hospitals within Alfred Health, with particular focus on the indications and dosing regimens being used.

METHODS

Design, setting and population

We performed an audit of nitrofurantoin use at the three hospital campuses within Alfred Health in Melbourne, Australia; the Alfred Hospital, Caulfield Hospital and Sandringham Hospital. Any patient prescribed nitrofurantoin from 1 January 2016 to 30 June 2018 was eligible. We obtained a list of prescriptions from the pharmacy dispensing records. We reviewed how the antibiotic was used, with a specific focus on (1) the patient groups in whom this drug was used, (2) indication, and (3) variability in dosing (schedule, formulation and duration). Study data were collected and managed using REDCap electronic data capture tool hosted at Alfred Health (13).

Definitions

Uncomplicated UTIs were defined as UTIs in non-pregnant women who are not immunocompromised, have no urinary tract abnormalities, and no symptoms of tissue invasion and/or systemic infections (1). The following patients were considered to be at risk for developing a complicated UTI: male patients, pregnant women, patients with diabetes mellitus, patients with renal tract abnormalities, and patients with an impaired immune system (for example patients with transplants, Human Immunodeficiency Virus [HIV] infection, or cancer who receive immunosuppressive drugs).

Data collection and analysis

The extract from the pharmacy dispensing records included medical record number, nitrofurantoin brand name, date dispensed, and quantity dispensed. We then manually extracted data from paper-based patient medical records (including medication charts) using a standardized case report form. We recorded the laboratory accession number for relevant microbiology samples (blood/urine cultures) then extracted antimicrobial susceptibility testing results from the Alfred Health Department of Infectious Diseases microbiology database.

We used standard descriptive statistics. For most analyses, we stratified patients by nitrofurantoin indication into a therapeutic treatment group (suspected or confirmed UTI or other infection) and a prophylactic treatment group (use in asymptomatic patients to prevent UTIs). We also examined how nitrofurantoin was used in the following specific sub-groups; patients with renal impairment and patients at risk of complicated UTIs.

RESULTS

Patient characteristics

We collected information regarding 150 patients with 151 nitrofurantoin prescriptions. The majority of these prescriptions were for therapeutic use (68% [103/151]). The median age was 75 years (interquartile range [IQR] 61-84) and 74% [111/150] were female. Three patients in this cohort were pregnant. Table 1 demonstrates the patient characteristics, stratified by indication.

Therapeutic use of nitrofurantoin

Among patients prescribed nitrofurantoin for the treatment of an infection, the most common indication was uncomplicated UTI (56% [58/103]), followed by complicated UTI (22% [23/103]). The indication was not specified in 6 of the 151 prescriptions (4%). Thirty-five (34%) of the 103 patients prescribed nitrofurantoin for the treatment of an infection had documentation of an intolerance or allergy to amoxicillin-clavulanic acid.

Dosage

The most commonly prescribed dosage in this population are displayed in Table 2.

The 100 mg capsule was more commonly prescribed in the study population compared to the 50 mg capsule (72% [109/151] versus 28% [42/151] for all prescriptions and 81% [83/103] versus 17% [17/103] when used for UTI treatment). The most commonly prescribed therapeutic dose was 100 mg twice daily for five days (22% [23/103]) or seven days (18% [19/103]). The 50 mg dose was most frequently prescribed four times daily for five days (8% [8/103]). In total, 38 patients were treated with four doses per day. Only one patient was treated with three doses per day (100 mg for seven days).

There were 112 prescriptions of nitrofurantoin for female patients. The 100 mg capsule was more commonly prescribed than the 50 mg capsule (71% [79/112] versus 29% [33/112]). When stratified by indication, 100 mg twice daily for five days was the most frequently prescribed dose in female patients to treat uncomplicated UTIs (30% [21/71]) (Table 2).

There were 39 nitrofurantoin prescriptions for male patients (Table 2). The 100 mg capsule was more commonly prescribed than the 50 mg capsule (79% [31/39] versus 21% [8/39]). The most frequently prescribed dosing regimen was 100 mg twice daily for seven days (41% [13/32]) for the treatment of complicated UTI.

Pathogens

Ninety-eight microorganisms were isolated in urine samples obtained from 60 patients. *Escherichia coli* was the most frequently isolated bacteria (42% [41/98]). Six of these isolates were ESBL-producers, of which five were reported as resistant to trimethoprim,

cephalexin and amoxicillin-clavulanic acid, and one as resistant to trimethoprim and cephalexin. Additional susceptibility testing was performed for all six isolates, which were reported as susceptible to nitrofurantoin. The second most commonly isolated bacteria were *Enterococcus faecalis* (30% [29/98], of which 3 were VRE), followed by *Staphylococcus aureus* (10% [10/98], of which six were methicillin resistant). Two of the VRE isolates were also resistant to amoxicillin and were susceptible to nitrofurantoin.

Prophylactic use of nitrofurantoin

The most frequently prescribed dose of nitrofurantoin for UTI prophylaxis was 100 mg once daily (50% [24/48]), followed by the 50 mg once daily (46% [22/48]) (Table 2). No additional information was available about the duration of use. Among 41 female patients, the 50 mg once daily dose and 100 mg once daily dose were used with similar frequency (49% [20/41] and 46% [19/41]). Among the seven males prescribed nitrofurantoin for prophylaxis, 100 mg once daily was more frequently prescribed than 50 mg once daily (71% [5/7] and 29% [2/7]).

Nitrofurantoin use in patients with renal impairment

The distribution of estimated glomerular filtration rate (eGFR) in this patient population is presented in Table 1. Among patients treated for a UTI, 100 mg twice daily was the most common daily dosage regardless of renal function, when categorised into the following groups: eGFR <60 mL/min, 60-89 mL/min, and ≥90 mL/min. The most common dosages of prophylactic nitrofurantoin varied with renal function; 50 mg daily and 100 mg daily, respectively, for patients with an eGFR <89 mL/min, and ≥90 mL/min.

Nitrofurantoin dose adjustment for impaired renal function was mentioned in medical records for two female patients. The first patient was commenced on 100 mg four times daily and was changed to 100 mg twice daily in the context of an eGFR of 45 mL/min. The second patient was started on 100 mg twice daily but changed to 50 mg twice daily in the setting of an eGFR of 30 mL/min.

We identified five patients with an eGFR of less than 30 mL/min, all of whom were prescribed nitrofurantoin for UTI treatment (Table 1). Of these five patients, three were prescribed 100 mg twice daily for five days, one with 100 mg four times daily for five days, and one with 50 mg four times daily for five days. Three of these five patients had bacteria isolated from a urine sample, but none of these were multidrug-resistant.

None of the patients receiving nitrofurantoin for UTI prophylaxis had an eGFR of less than 30 mL/min.

Nitrofurantoin use in patients at risk of complicated urinary tract infections

The patients at risk of developing a complicated UTI are described in Table 1. The three pregnant women in our study were all treated for seven days. The three patients with

Table 1. Baseline patient characteristics stratified by indication for nitrofurantoin treatment.

Baseline patient characteristics	Nitrofurantoin prescriptions *(n=151)*	
	Therapeutic *(n=103)*	**Prophylactic *(n=48)***
Sex		
Female	71 (69%)	41 (85%)
Male	32 (31%)	7 (15%)
Age (years)		
Median (IQR)	72 (59-84)	77 (65-85)
Range	25 - 95	35 - 94
eGFR groups* (mL/min/1.73m^2)		
< 30	5 (5%)	0 (0%)
30-59	21 (20%)	9 (19%)
60-89	41 (40%)	25 (52%)
≥ 90	24 (23%)	8 (17%)
Unknown	12 (12%)	6 (13%)
Comorbidities/medical diagnosis		
Pregnancy	3 (3%)	0 (0%)
Immunosuppression	3 (3%)	0 (0%)
Diabetes Mellitus	13 (13%)	14 (29%)
Renal tract abnormalities		
Functional	10 (10%)	6 (13%)
Structural	16 16%)	16 (33%)
Antibiotic allergy		
Amoxicillin-clavulanic acid	35 (34%)	2 (4%)
Trimethoprim	4 (4%)	0 (0%)
Cephalexin	4 (4%)	0 (0%)
Indications for nitrofurantoin use		
UTI prophylaxis	NA	48 (100%)
Uncomplicated UTI	58 (56%)	NA
Complicated UTI	23 (22%)	
Asymptomatic bacteriuria	14 (14%)	
Prostatitis	2 (2%)	
Other infection/unknown*	6 (6%)	

eGFR = estimated glomerular filtration rate; mL/min = millilitre/minute; IQR = inter quartile range; urinary tract infection (UTI)

* therapeutic treatment course used, but indication not documented.

an impaired immune system were treated for 5 days. Among the thirteen patients with diabetes mellitus treated therapeutically with nitrofurantoin, five (56%) were treated for seven days. Six (60%) patients with functional renal tract abnormalities and nine (56%) patients with structural renal tract abnormalities were treated for seven days. Male patients were discussed above.

Table 2. The most frequently prescribed nitrofurantoin regimens, stratified by indication and sex.

	Total	**Females**	**Males**
Therapeutic use	***(n=103)***	***(n=71)***	***(n=32)***
100 mg BD 5d	23 (22%)	21 (30%)	2 (6%)
100 mg BD 7d	20 (19%)	7 (10%)	13 (41%)
100 mg QID 5d	11 (11%)	9 (13%)	2 (6%)
50 mg QID 5d	8 (8%)	8 (11%)	0
100 mg QID 7d	4 (4%)	4 (6%)	0
100 mg BD 10d	4 (4%)	4 (6%)	0
Other	33 (32%)	18 (25%)	15 (47%)
Prophylactic use	***(n=48)***	***(n=41)***	***(n=7)***
100 mg daily	24 (50%)	19 (17%)	5 (71%)
50 mg daily	22 (46%)	20 (49%)	2 (29%)
50 mg BD	2 (4%)	2 (5%)	0

Percentages in parentheses correspond to the proportion of times each regimen was used for the corresponding group (i.e. total, female, or males). d=days, BD=twice daily, QID=four times daily.

DISCUSSION

We described the use of nitrofurantoin in three hospitals within our health service. The most common therapeutic dose prescribed was 100 mg twice daily for five days. A regimen of 100 mg twice daily for seven days was also commonly used, possibly because our study population contained a relatively high number of males and patients with comorbidities, which may place the patients at risk for a complicated UTI. The duration of treatment was predominantly consistent with (international) guidelines (1, 11, 14). Nitrofurantoin was also used for the prophylaxis of UTIs despite not being recommended for this indication in 'Therapeutic Guidelines: Antibiotic' (version 15) during the study period (11).

The most common frequency used for treatment of UTIs was twice daily, a substantial departure from the Macrodantin® recommendation of four times daily (6). This difference may be explained by the fact that the 'Therapeutic Guidelines: Antibiotic' (version 15) recommended 100 mg twice daily for nitrofurantoin without specifying which for-

mulation. In contrast, the 2010 US-European guidelines also recommend 100 mg twice daily, but specified the Macrobid® (long-acting) formulation. There are pharmacokinetic differences between Macrodantin® and Macrobid®, so substituting these nitrofurantoin products may result in different antibiotic concentration profiles in the lower urinary tract (15–17). The impact of twice daily dosing of Macrodantin® on therapeutic effectiveness is unknown, however we did not observe major problems with relapse or recurrence in this study (albeit that we had limited capacity to evaluate this).

Among the patients in our cohort, the median eGFR was 74 mL/min/1.73m^2 in the treatment group and 76 mL/min/1.73m^2 in the prophylactic group, with 35 patients having an eGFR of <60 mL/min. While the Australian Macrodantin® product information leaflet lists a creatinine clearance of less than 60 mL/min as a contraindication, the United Kingdom Medicines and Products Regulatory Agency revised this to a threshold eGFR of 45 mL/min/1.73m^2, adding that short courses (5-7 days) may be used with caution in patients with an eGFR of 30 to 45 mL/min/1.73m^2 in the setting of multidrug-resistant bacteria (6). The Australian Medicines Handbook provides the same recommendation (18). Patients with impaired renal function excrete less nitrofurantoin in the urine so urinary concentrations may be sub-therapeutic, which could lead to treatment failure, the selection of nitrofurantoin-resistance, or toxicity due to higher plasma concentrations (19–21). In this study, eight patients with an eGFR between 30 and 45 mL/min were treated with nitrofurantoin, one of which had a UTI caused by multidrug-resistant bacteria. Of five patients with an eGFR <30 mL/min treated with nitrofurantoin, none had a UTI caused by a multidrug-resistant bacteria. We did not find any evidence that the prophylactic dose was changed (or discontinued) based on impaired renal function. The prophylactic use of nitrofurantoin is not mentioned in the 'Therapeutic Guidelines: Antibiotic' (Version 15) or the Australian Medicines Handbook (2019). However, it may be reasonable for a prescriber to adjust the duration of the prophylactic use because long term use of nitrofurantoin is associated with (severe) side effects (22) as side effects are more likely to occur in patients where higher plasma concentrations of nitrofurantoin can be expected due to slower excretion from the plasma to the urine compartment (16). We identified no patients with an eGFR < 30 mL/min being treated with prophylactic nitrofurantoin in this cohort.

A new version (Version 16) of the Australian 'Therapeutic Guidelines: Antibiotic' was published after the completion of this study (23). The two first-line options for treatment of uncomplicated lower UTIs are now nitrofurantoin, 100 mg four times daily for five days, and trimethoprim 300 mg once daily for three days (23). The nitrofurantoin formulation is not specified, but given Macrobid® is not available in Australia, prescribers will most likely prescribe Macrodantin®. This total daily dosage of nitrofurantoin (400 mg) is higher than recommended in all but one European national guideline

(9). A recent review of national treatments guidelines for UTIs in European countries highlighted the lack of clarity around optimal nitrofurantoin dosing, with recommendations ranging from 150 mg daily (50 mg 8-hourly in Norway and Sweden, and 75 mg 12-hourly in Finland) to the one country that recommends up to 400 mg daily (Russia). This diversity in recommendations demonstrates the need for further research on the pharmacokinetics and pharmacodynamics of nitrofurantoin, and evidence regarding efficacy of different regimens.

We acknowledge the limitations of this study. First, our cohort comes from three hospitals in one health service, and may not be generalisable to other settings in Australia. Second, we don't have information about the total number of UTIs at Alfred Health during the study period, and therefore can't calculate the relative frequency of nitrofurantoin usage for the treatment of UTIs. Finally, we were not able to systematically collect information regarding clinical or microbiological cure from this retrospective audit.

CONCLUSIONS

Nitrofurantoin is considered an important antibiotic for the treatment of UTIs in the context of increasing multi-drug resistance. In our study, while the most common indication for nitrofurantoin use (uncomplicated lower UTIs) and treatment duration were consistent with national and international guidelines, the dosage frequency used was not correct for the formulation that is available in Australia. This study and the wide range of different recommendations for nitrofurantoin dosing internationally, highlight the need for further research into optimal nitrofurantoin dosing regimens to maximise efficacy while avoiding dose-dependent toxicities and the emergence of resistance.

Ethics

The study was approved by the Human Research and Ethics Committee at Alfred Hospital [Project Number: 577/18].

Author statement

RAW, AYP and AJS designed the study. RAW, SJC and KAC obtained the data. RAW analysed the data and drafted the report. All authors critically reviewed the report and approved the final version.

Conflicts of interest

All authors have no conflicts of interest relevant to this study.

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6

Summary, discussion and perspectives

This thesis describes essential steps in optimizing the treatment of uncomplicated urinary tract infections (UTIs) using fosfomycin and nitrofurantoin. The pharmacokinetics (PK) of both antibiotics are studied in order to optimize the effect of the treatment and to minimize the emergence of drug resistance among uropathogens as part of the redeveloping process of old antibiotics. Analytical methods were developed and validated to quantify concentrations of both antibiotics in human urine and plasma to support the PK studies.

The results demonstrate that the urinary PK of both antibiotics is highly variable between subjects. This may help explain the reason for treatment failures that occur in clinical practice and identifies opportunities to optimize treatments given for this common clinical condition. The urinary output and number of voids were found to be the variables with greatest influence on the urinary PK of the studies antibiotics. The varying number of voids complicates PK research based on urinary concentrations since voiding is a conscious act, which is mainly influenced by the subject itself. For both antibiotics, discrepancy was found between the *in vitro* activity and the clinical response. This highlights the importance of using (pre)clinical PK and PD data as the basis for designing more robust and effective dosing regimens for subsequent validation in clinical trials as described in **Chapter 1**.

FOSFOMYCIN

In **Chapter 2.1**, the PK of fosfomycin was studied in a group of 40 healthy, female volunteers during a period of seven days. Drug concentrations were measured using the UPLC-MS/MS method which was developed and validated (**Chapter 2.2**) (1). High urinary concentrations were found in the majority of the volunteers in the first two hours after dosing, although urinary concentrations were highly variable between subjects (2). Urinary concentrations of fosfomycin exceeded the EUCAST clinical breakpoint of 32 mg/L for susceptibility in all volunteers for 24 hours. However, there was uncertainty in predicting treatment outcome by calculating the probability of PK/PD target attainment because of highly variable urinary drug concentrations.

The PK samples obtained in this study were used as input for a static *in vitro* model in which the urinary antibacterial activity of fosfomycin and nitrofurantoin were measured (**Chapter 4.1**). A strong bactericidal effect of fosfomycin was found in the samples to which *E. coli* was added. This bactericidal effect was present for at least 48 hours in *E. coli*. For *K. pneumoniae*, only a moderate bactericidal effect was found which was only present for approximately 18 hours (3).

Overall, the bactericidal effect in *E. coli* could be measured in the urine samples of 90% of the volunteers during 24 hours after dosing. In the remaining 10% of the vol-

unteers' samples , no bactericidal effect of fosfomycin was observed. This is surprising because the urinary concentrations in 100% of the volunteers exceeded the breakpoint of 32 mg/L (2), and the baseline MIC of all strains was ≤ 32 mg/L (3). Therefore, it seems that the *ex vivo* activity of fosfomycin is not predicted based on the level of the urinary concentrations and/or antibacterial activity is not well predicted based on the baseline MIC of the strain for fosfomycin (3, 4). These discrepant results are also clinically relevant since in a recently conducted randomized clinical trial, fosfomycin was found to be clinically effective in only 58% of the uUTI patients and microbiological resolution occurred in only 63%, while in our study a percentage of *in vitro* antibacterial success of 90% was reached (3, 5). An explanation for these discrepant results is more likely to be related to the intrinsic and/or acquired fosfomycin resistance among uropathogens rather than PK related.

The hypothesis that cases of clinical failure can be better explained by arguments related to the susceptibility of the uropathogens to fosfomycin (PD related), are supported by the findings from several studies in which the PD of fosfomycin was studied in a dynamic *in vitro* bladder infection model using the PK samples obtained in the study presented in **Chapter 2.1** (4, 6–9). The most important findings of these studies were that a single 3 gram dose of oral fosfomycin is able to reliably kill (or at least inhibit the growth of) susceptible *E. coli* isolates within a few hours after dosing, but that regrowth occurs thereafter in a significant number of isolates. The extent to which regrowth occurs was dependent on the presence of a pre-existing resistant subpopulation in the bacteria present. Whether the actions of the host immune system can eradicate this resistant subpopulation in time remains uncertain for now. Exposure of *E. coli* isolates to fosfomycin selects out the less susceptible bacteria, resulting in an increased MIC of the strain. This suggests that antibiotic exposure is an important driver for the emergence of resistance in *E. coli* (8). This also highlights the importance of the research presented in this thesis since dose optimization also includes minimizing exposures associated with the development of drug resistance among uropathogens (**Chapter 1**). Another important conclusion was that fosfomycin lacks sustained activity against *K. pneumoniae* isolates that commonly demonstrate fosfomycin resistance and all contain a chromosomal gene that inactivates fosfomycin (4, 6). The non-susceptibility of this species to fosfomycin seems to be more a matter of intrinsic resistance or non-susceptibility rather than acquired resistance due to antibiotic exposure, which was suggested for *E. coli*.

A PK related explanation is less likely because urinary concentrations of fosfomycin are high, for example always exceeding the MIC of the most common uropathogens in the majority of the subjects during at least 24 hours, so underexposure is unlikely (**Chapter 2.1**).

In **Chapter 5.1**, it was investigated to what extend the renal function impacts on treatment failure in patients treated with fosfomycin, nitrofurantoin or trimethoprim

(10)**.** No direct relationship was found between impaired renal function and an increased risk of treatment failure when treated with fosfomycin. This finding also suggests that underexposure, due to a reduced excretion from the plasma compartment into the urine caused by an impaired renal function, is unlikely.

These PK and PD findings ensure that there is now more insight into (1) the possible mechanisms for clinical failure with fosfomycin observed in the treatment of uUTIs, (2) to what extend urinary concentrations may affect antibacterial activity of fosfomycin, and (3) that PD research with dynamic *in vitro* models, in addition to static *in vitro* models, should be part of the drug development process as proposed in **Chapter 1** (figure 2).

NITROFURANTOIN

In **Chapter 3.3**, the PK in 12 healthy, female volunteers after administration of either 50 mg q6 hours or 100 mg q8 hours (both Macrodantin®/Furadantin®) was investigated based on urine and plasma concentrations, using the UPLC-UV method which was developed and validated for both PK studies (**Chapter 3.5**) (11). The results obtained in these PK studies, were in line the PK findings in the literature as described in the two review chapters (**Chapter 3.1** and **3.2**) (12, 13).

Comparable to the conclusions for fosfomycin, highly variable urinary concentrations for nitrofurantoin were observed in the volunteers (**Chapter 3.3**). The PK in plasma was found to be dose dependent, leading to a doubling of the plasma concentrations and thereby to a doubling of the total plasma exposure, presented as $AUC_{0\text{-}24,ss}$ (14). Urinary concentrations, however, were found to be independent of the administered dose as urinary PK was comparable between the two dosing regimens. The underlying mechanism for these observations in plasma and urine is unclear, but it questions the extent to which urinary concentrations can be influenced by making dose adjustments. Revealing this underlying mechanism is an important perspective for future PK studies with nitrofurantoin which will be discussed in the next section.

The slow-release formulation Macrobid®/Furabid® was developed to establish prolonged urinary concentrations (15). Taking into account the finding that nitrofurantoin has a time-dependent killing effect in *E. coli* and *K. pneumoniae*, the expected prolonged urinary concentrations will contribute to the time-dependent effect and therefore improve the effectiveness of the treatment (16). The PK of this formulation was studied in a small group of patients with uncomplicated UTIs in the commonly used dose of 100 mg q12 hours (Macrobid®/Furabid®) together with the PK of macrocrystalline nitrofurantoin in a dose of 50 mg q6 hours (Macrodantin®/Furadantin®) (17). In **Chapter 3.4**, the findings from the interim-analysis including 19 patients are described.

Despite the small number of patients included in this analysis, mean values of the PK parameters were considered as they were comparable with the median values for all PK parameters. Higher urinary C_{max} and $AUC_{0\text{-}24,ss}$ values were found in patients receiving 100 mg q12 hours of Macrobid®/Furabid® (n=7) compared to those receiving 50 mg q6 hours Macrodantin®/Furadantin® (n=12) (17). The effect of the slow-release capsule, demonstrated as a prolonged T_{max} value so a delayed peak concentration in urine, was also observed. However, differences were small and ranges were wide so no significance was found. This limits the clinical relevance of these differences in PK parameters and, together with the findings in **Chapter 3.3**, leads to the hypothesis that the urinary PK of nitrofurantoin is not only independent of the administered dose, but also independent of the formulation.

The study in **Chapter 3.4** will continue until the intended number of 60 patients will be enrolled. It is especially important that patients will be enrolled who receive the Macrobid®/Furabid® capsule to investigate the PK of this formulation and to compare the two dosing regimens in terms of urinary concentrations.

The urine samples obtained in **Chapter 3.3** were, together with the fosfomycin samples from **Chapter 2.1**, used as input for PD research in order to investigate the urinary antibacterial activity of the antibiotics (**Chapter 4.1**) (3). A moderate bactericidal effect of nitrofurantoin was found, but this effect was only present during the first 2 hours after dosing and was only observed in the minority of the volunteers (3). The *ex vivo* antibacterial effect after one dose was small, which is not in line with the well observed clinical effect in, among others, the previously mentioned randomized clinical trial (5). Clinical success in uUTI patients when treated with nitrofurantoin was achieved in the majority of the patients (70%), and microbiological success was found in 74% of the patients.

Comparable with fosfomycin, the *in vitro* percentages of bactericidal success for nitrofurantoin were not representative for the clinical effectiveness observed in patients. However, in contrast to fosfomycin, the discrepancy for nitrofurantoin is more likely to be related to the PK and less likely to be related to non-susceptibility or resistance because resistance rates for nitrofurantoin are still low in *E. coli* (e.g. <3% in 2016, <2% in 2017, and <2% in 2018 (18–20) despite its consumption increased exponentially over the last decade (21). An important reason for the low antibacterial activity of nitrofurantoin *in vitro* is that it was measured after one dose while nitrofurantoin is always prescribed as a course of repeat doses in clinical practice. This is an important observation because it validates the currently used clinical approach of prescribing nitrofurantoin to female uUTI patients in a dosing schedule of 2 – 4 daily doses for 3 to 7 days. This observation is also consistent with the finding of a PD study that demonstrated a time-dependent effect of nitrofurantoin against *E. coli* and *K. pneu-*

moniae. This supports the use of nitrofurantoin in lower, more frequent doses rather than higher, less frequent doses (16).

In general, urinary concentrations of nitrofurantoin are relatively low compared to those for fosfomycin and other UTI antibiotics (14, 17, 22). This implies that nitrofurantoin concentrations are sensitive to small changes in factors that can easily influence these concentrations and can make the difference in effective antibacterial concentrations versus sub-therapeutic concentrations. It was confirmed by others that urinary concentrations of nitrofurantoin were low in patients with severely reduced eGFR of 4-11 mL/min, suggesting that treatment failure in these patients is related to underexposure in the bladder (22). Decreased urinary concentration were neither observed in patients with impaired renal function who were prescribed other UTI antibiotics (although fosfomycin was not tested), nor in patients with moderately impaired renal function (30-60 mL/min) (23). This makes nitrofurantoin an exception with regards to the presence of the relationship between impaired renal function, decreased urinary concentrations, and the probability of treatment failure.

In line with what was found for fosfomycin, no relationship was found between impaired renal function and the probability of treatment failure in patients treated with nitrofurantoin in **Chapter 5.1** (10). The above raised hypothesis about sub-therapeutic urinary levels of nitrofurantoin as a result of impaired renal function could not be supported in this study. It is likely that this can be caused by the fact that patients were grouped in eGFR groups of either <60 mL/min or ≥60 mL/min. As a results, no patients were present with severely reduced renal function so no sub-therapeutic levels would have been reached in these patients (10).

To summarize, uUTI antibiotics like nitrofurantoin with a low bactericidal activity, a good bacteriostatic activity, and relatively low urinary concentrations are in general sufficient to treat uncomplicated UTIs in relatively healthy patients (**Chapter 3.1, 3.3, 3.4** and **4.1**) (24). This is more difficult in the treatment of complicated patients. This shortcoming is of less importance for an UTI antibiotic with relatively high urinary concentrations like fosfomycin.

RECOMMENDATIONS FOR FUTURE (PK) STUDIES

In general, the influence of the urinary output and the number of voids has a greater impact on the PK of fosfomycin and nitrofurantoin than the other parameters. This is a complicating factor in studies aiming to investigate the PK based on urinary concentrations from subjects who were not instructed to follow a predefined voiding schedule. This can be overcome by having subjects follow such a schedule, but this results in urinary PK data that may not be representative of the real clinical situation.

Alternatively, the PK could be studied in patients who are catheterized (for example in nursing home residence) so that urine samples can be drawn continuously and direct from the catheter bag, thereby excluding the impact of voiding, influenced by each subject itself. Future PK research must therefore carefully consider the exact aim of the study when choosing the study set-up. If the aim is to study the 'pure' urinary PK, then the best choice is to perform the study in catheterized patients. However, if the aim is to investigate which urine concentrations can be expected in clinical patients, a study set-up as chosen in the PK studies in this thesis would be the best option.

Additionally, an approach for additional PK studies for both fosfomycin and nitrofurantoin would be to focus on better explaining the cause of the variability in urinary concentrations. Simple behavior changes such as fluid intake and voiding patterns can be altered while collecting urine samples. Subsequent PD studies can then focus on the impact of different PK targets on microbiological outcome. Important, however, is that bacterial clearance of the bladder may be influenced equally by these behavioral changes and thereby influence the effect of the uUTI drug. Such behavioral interventions may in fact reduce the urinary antimicrobial exposure, but still promote clinical cure. How to find the balance between these aspects, and how to optimize therapeutic decisions for individuals and different infecting uropathogens, remains a complex task.

Given that urinary concentrations are a direct measure for antibiotic exposure to the uropathogen and therefore directly influence the probability of treatment success, the most straightforward way to improve the treatment with fosfomycin is to maximize urinary concentrations by adjusting fosfomycin dosing. There appears to be no added value of using fosfomycin in an off-label multiple dose approach rather than the currently used single dose approach with regards to pathogen kill and emergence of resistance (8). Also, the data of the previously mentioned PD studies do not support to increase the fosfomycin dose because re-growth still occurs when the duration of exposure is increased and this increased exposure promotes the selection of resistant subpopulations (4, 6–9). The relationship between exposure and resistance is therefore not linear, but has the shape of an inverted "U" curve. The duration of exposure is a known factor which influences the shape and maximum value of this inverted "U" curve as this was demonstrated by others too (25, 26). This finding discourages the use of fosfomycin in a multiple dose approach and rather confirms the correctness of the single dose approach in the treatment of uncomplicated UTIs. Preliminary data from the same *in vitro* dynamic bladder infection model demonstrated that increasing the dose by administering >3 grams is also not likely to contribute to a better treatment outcome since the variability in urinary exposure seems to have minor impact in the microbiological effect and only promotes the emergence of resistance. Future (PK) research should therefore rather focus on exploring different approaches for treatment optimization. A promising strategy would be to investigate fosfomycin as combina-

tion therapy with other antimicrobial agents. So far, only *in vitro* studies have been conducted using this alternative approach, but the results are promising. For example, synergy was found with various cephalosporins, carbapenems and fluoroquinolones against important uropathogens (27, 28).

Future research requires the development and validation of fast and affordable diagnostic tests for uropathogen identification, fosfomycin susceptibility, and identification of any present resistant subpopulation. These test should be applicable during clinical practice in the first and second line healthcare system which then can direct antibiotic therapy. The added value of these tests will be enormous, not only for treatment optimization, but also for the prevention of emergence of resistance because the numbers of cases in which antibiotics are unnecessary prescribed will decrease.

Regarding the PK of nitrofurantoin, the PK is complicated and not predictable based on the administered dose and formulation. The lack of clarity about (1) the mechanism behind the absorption and excretion pattern of nitrofurantoin, (2) whether or not these mechanisms are dose related, and (3) to what extend the slow-release formulation is influencing this, should be the main focus of future research into the PK of nitrofurantoin, aiming for dose- and treatment optimization.

The first recommendation for future research would be to perform a PK study in patients with uncomplicated UTIs in which nitrofurantoin concentrations are quantified in plasma and urine in subjects who administer linear ascending dosages, starting with a dose lower than the standard minimal dose of 50 mg. This PK study will reveal to what extend the plasma concentrations are linearly related to the administered dose, and whether urinary concentrations are related to the administered dose.

A second study should focus on revealing the metabolic pathway of nitrofurantoin and its role in the process of treatment optimization. It is known that metabolites are formed, but the identity and to what extend these metabolites contribute to nitrofurantoins antimicrobial and toxic effects, are unknown (**Chapter 3.1** and **3.2**). It should be investigated via which route and where in the body these metabolites are formed by investigating plasma, urine and feces. The role of uropathogens in the metabolism of nitrofurantoin should also be investigated by comparing the metabolites in patients with those in healthy subjects (29, 30). Furthermore, the role of pH in the metabolism of nitrofurantoin should be investigated because this might partly explain the difference in the missing relation between the administered dose and the concentrations in plasma and urine. For these future studies, analytical methods are needed to identify and quantify levels of nitrofurantoin and its metabolites.

As mentioned in the previous paragraph, the study in **Chapter 3.4** will continue until the intended number of 60 patients are enrolled. In this study, a specific focus lies on the enrollment of patients who were prescribed the Macrobid®/Furabid® capsule to investigate whether the expected prolonged urinary concentrations are being

achieved and if this contributes to an improved clinical outcome. Ideally, also plasma samples should be collected in these patients in order to investigate the complete absorption and excretion pattern of this nitrofurantoin formulation. The results of this study will be beneficial in guiding nitrofurantoin use and dosing as treatment guidelines for uncomplicated UTIs differ between countries (**Chapter 1** and **5.2**) (31).

Additionally, the plasma and urinary PK should be investigated in a larger group of patients with impaired renal function in order to validate the contraindication of nitrofurantoin use in patients with impaired renal function since there is limited evidence for the eGFR limits of either 60 mL/min (32) or 30 mL/min (33) which are used in current clinical practice (12).his thesis contains the first results following the proposed process for the (re)development of fosfomycin and nitrofurantoin as proposed by Muller et al. (figure 2 in **Chapter 1**) (34). The data presented in this thesis emphasize the importance of studying PK in humans at an early stage of drug development as PK studies reveal changing concentrations over time and therefore wider ranges of concentrations which should be known when designing PD experiments. This is especially important for UTI antibiotics because urine is the clinically relevant matrix in which concentrations are highly variable and hard to predict using well-established PK equations, because next to the influence of the patients renal function, variables such as fluid intake, voiding time, urine frequency and drug absorption influence the urinary concentrations. This is in contrast to other classes of antibiotics where plasma is the clinically relevant matrix in which concentrations in general are more predictable using these PK equations.

The changing concentrations over time can only be considered if dynamic *in vitro* models instead of the static *in vitro* models are used in PD studies that follow the PK studies. The use of these dynamic models offers the possibility to study the antibiotic effect over time, not only with a view to antibacterial activity, but also with a view on the development of resistance due to (the changing) antibiotic exposure (over time).

Based on the knowledge obtained in this thesis, figure 2 as presented in **Chapter 1** can be adjusted for the specific case of UTI antibiotics as demonstrated in figure below. The figure shows that PK data must serve as the input for PD models (red arrow), and that (pre)clinical PD research must be performed using both static and dynamic *in vitro* models (red box). The PK results that will be obtained in the proposed future PK studies should be used according to this new process. In addition to UTI antibiotics, the proposed process in figure below is also applicable to other classes of antibiotics for which the PK may be more constant, but for which it is still important to also consider the varying concentrations over time in PD (pre)clinical studies.

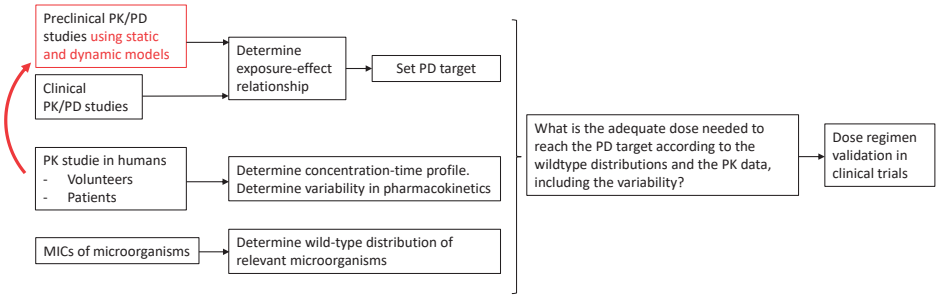

Figure. The role of pharmacokinetic data in the process of finding the optimal dosing regimen for UTI antibiotics, as presented in figure 2 of **Chapter 1**, adjusted (in red) based on the content of this thesis (34).

CONCLUSIONS OF THIS THESIS

The PK of fosfomycin and nitrofurantoin was investigated based on the drug concentrations in urine and/or plasma as part of the redeveloping process of these old antibiotics aiming for treatment optimization. For both drugs, treatment optimization is warranted since cases of clinical failure are common and the lack of alternative oral treatment options due to the alarming increase of (multi)drug resistant among uropathogens. The urinary PK of fosfomycin was found to be highly variable between the healthy volunteers which signifies the risk for underexposure which may partly explain the observed cases of clinical failure when treating patients with uncomplicated UTIs with fosfomycin. Additional PD research based on the PK samples, revealed that the cases of clinical failure can best be explained by the emergence of resistance among uropathogens. Emergence of resistance was found to be related to the duration of exposure and the presence of a pre-existing resistant subpopulation in the bacteria for fosfomycin. Using fosfomycin in a multiple dose approach is therefore not likely to contribute to a better treatment outcome. Future research aiming for treatment optimization should focus on the development of fast and affordable diagnostic tests for fosfomycin susceptibility, and for identification of any present resistant subpopulation. Additionally, the clinical effectiveness of using fosfomycin in combination with other antimicrobial agents should be investigated.

As urinary concentrations of nitrofurantoin are relatively low, the cases of clinical failure are likely to be related to underexposure rather than to resistance of uropathogens for nitrofurantoin. The urinary PK of nitrofurantoin was found to be comparable between healthy volunteers and patients with uncomplicated UTIs even though different dosing regimen and different formulations of nitrofurantoin were used in these groups. The urinary PK was therefore not found to be linear related to the administered dose and formulation whereas the plasma PK was. More research is needed to re-

veal the underlying mechanism of the absorption-, elimination-, and additionally the metabolism pattern of nitrofurantoin in order to find the best strategy for further PK studies aiming for dose- and treatment optimization.

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7

Epilogue

7.1

Nederlandse samenvatting

Ongecompliceerde urineweginfecties (UWIs, ofwel blaasontstekingen) zijn de meest voorkomende infecties bij vrouwen: 70% van de vrouwen in de wereld heeft ten minste één keer in haar leven een UWI waarvoor zij moet worden behandeld met antibiotica. Een UWI is ongecompliceerde infectie als deze zich beperkt tot de blaas van een niet zwangere en koortsvrije vrouw. Klachten bij ongecompliceerde UWIs zijn doorgaans pijn bij het plassen, verhoogde plasdrang en frequent kleine beetjes moeten plassen. In Nederland zijn UWI klachten de meest voorkomende reden om naar de huisarts te gaan.

Fosfomycine en nitrofurantoïne zijn beide oude antibiotica die in de jaren 60-70 zijn ontdekt en die sindsdien gebruikt worden voor de behandeling van ongecompliceerde UWIs. In de afgelopen decennia is hun populariteit echter afgenomen vanwege de komst van nieuwe antibiotica die de laatste jaren vaak zijn gebruikt om patiënten te behandelen. Omdat (overmatige) blootstelling van een bacterie aan een antibioticum een belangrijk stimulans is voor resistentie ontwikkeling (ongevoelig worden van een bacterie voor een antibioticum), zijn bacteriën ongevoelig geworden en kunnen deze nieuwe antibiotica dus niet meer worden gebruikt. Aangezien fosfomycine en nitrofurantoïne minder toegepast zijn in deze periode is er voor deze antibiotica nog weinig bacterieresistentie ontwikkeld. Hierdoor zijn de beide antibiotica weer in populariteit gestegen. Helaas zal dit waarschijnlijk ook betekenen dat de resistentiecijfers voor fosfomycine en nitrofurantoïne ook naar verwachting gaan stijgen. Dit is een zorgwekkende ontwikkeling aangezien er nauwelijks alternatieve behandelopties zijn.

Om te voorkomen dat bacteriën ook resistent worden voor fosfomycine en nitrofurantoïne moet adequate blootstelling worden nagestreefd door een optimale dosering (hoeveelheid van een geneesmiddel die wordt gegeven) vast te stellen. De optimale dosis is die dosis waarbij een zo goed mogelijk bacteriedodend effect wordt bereikt waarbij de patiënt zo min mogelijk last heeft van bijwerkingen. Dit soort studies staan bekend als dosis-effect studies en deze ontbreken over het algemeen voor oude geneesmiddelen. Dit komt doordat oude middelen op de markt zijn gekomen in een tijd waarin het voor farmaceutische bedrijven nog niet verplicht was om een registratiedossier aan te leveren waarin onder andere onderzoeken werden gepresenteerd waarin secuur werd onderzocht wat de optimale dosis is. Het gevolg hiervan is dat er nagenoeg geen bewijs is voor het optimale gebruik van deze geneesmiddelen bij de huidige behandeling van patiënten.

Om het gebruik van fosfomycine en nitrofurantoïne te optimaliseren en hierbij te waarborgen dat ze ook in de toekomst nog effectief zullen zijn bij de behandeling van ongecompliceerde UWIs, is het belangrijk dat deze dosis-effect studies alsnog worden gedaan. De eerste stap hierin is het verkrijgen van kennis van de hoeveelheid fosfomycine of nitrofurantoïne die in de blaas worden bereikt na het innemen van een bepaalde dosis: de urineconcentraties. Studies waarbij de concentraties van genees-

middelen worden onderzocht staan bekend als farmacokinetische (PK) studies welke een cruciale rol spelen in het moderne proces van geneesmiddelontwikkeling. Bij de meeste PK studies wordt er daarnaast ook nog gekeken naar de manier waarop het geneesmiddel vanuit het maag-darm kanaal wordt opgenomen in het bloed (absorptie), hoe het geneesmiddel zich daarna verdeelt over het lichaam (distributie), of het wordt omgezet in andere stoffen (metabolisme) en hoe het vervolgens het lichaam verlaat (excretie). Deze vier processen samen beschrijven het PK profiel van een geneesmiddel. In de studies die in dit proefschrift worden gepresenteerd richten wij ons dus op het excretie proces vanuit het bloed naar de blaas van fosfomycine en nitrofurantoïne omdat ze daar moeten werken. Dit onderdeel van het PK profiel van beide antibiotica kan daardoor worden onderzocht door het meten van urineconcentraties. Met deze kennis kan vervolgens worden onderzocht wat het effect is op de bacterie van deze concentraties. Dit wordt gedaan in zogenaamde farmacodynamiek (PD) studies die volgen op de PK studies. Samen vormen PK/PD studies de basis voor het optimaliseren van de behandeling van ongecompliceerde UWIs met fosfomycine en nitrofurantoïne met als doel om een maximaal bacteriedodend effect te bereiken met daarbij een minimale kans op bijwerkingen en het minimaliseren van de kans op resistentieontwikkeling door bacteriën.

Het doel van dit proefschrift was om het PK profiel van fosfomycine en nitrofurantoïne te onderzoeken om hiermee een eerste stap te zetten in het optimaliseren van de behandeling van UWIs met deze antibiotica. Op basis van deze PK kennis werden vervolgens PD studies gedaan waarbij het effect van de antibiotica concentraties in urine op de bacterie werd onderzocht.

FOSFOMYCINE

Fosfomycine is in Nederland de tweede behandeloptie volgens de behandelstandaard voor ongecompliceerde UWIs van het Nederlandse Huisartsen Genootschap (NHG-standaard). Fosfomycine wordt voorgeschreven in een eenmalige orale (inname via de mond) dosis van 3 gram. De PK van fosfomycine werd onderzocht in **Hoofdstuk 2.1** in een groep van 40 gezonde, vrouwelijke vrijwilligers die gedurende een week urinemonsters verzamelden na het innemen van 3 gram fosfomycine. De belangrijkste bevinding van deze studie was dat er grote verschillen zijn in de urineconcentraties tussen de vrijwilligsters. Dit zorgt ervoor dat het moeilijk is om met zekerheid iets te zeggen over de algemene effectiviteit van de behandeling met 3 gram fosfomycine in UWI patiënten.

De urinemonsters die de vrijwilligsters verzamelden werden daarnaast gebruikt in een *in vitro* model (buiten het menselijk lichaam) waarin de antibacteriële activiteit in

urine van fosfomycine werd onderzocht (**Hoofdstuk 4.1**). Fosfomycine liet gedurende 48 uur een sterke bacteriedodende werking zien in de *Escherichia coli (E. coli)* bacterie, de meest voorkomende veroorzaker van ongecompliceerde UWIs. Het bacteriedodende effect in *Klebsiella pneumoniae (K. pneumoniae)*, tevens een belangrijke veroorzaker van UWIs, was echter zwak en werd slechts gemeten tijdens de eerste 18 uur na inname van fosfomycine. In de urinemonsters van ongeveer 10% van de vrijwilligers werd helemaal geen bacteriedodende werking van fosfomycine gemeten. Dit was onverwacht omdat de minimale inhibitie concentratie (MIC, de concentraties van het antibioticum waarbij er net geen bacteriegroei meer mogelijk is) was voor alle geteste bacteriën ≤32 mg/L terwijl de urineconcentraties in alle vrijwilligers hoger waren dan deze 32 mg/L. Dit betekent dus dat het effect van fosfomycine gemeten in dit *in vitro* model, niet goed te voorspellen is op basis van de urineconcentraties en/of dat het *in vitro* effect niet goed te voorspellen is op basis van de vooraf gemeten MIC. Ook blijkt dat de *in vitro* activiteit die werd gemeten geen goede weerspiegeling is van de effectiviteit van fosfomycine in patiënten (de klinische effectiviteit). Andere onderzoekers toonden aan in een klinische studie dat de behandeling met 3 gram fosfomycine maar in 58% van de patiënten succesvol was terwijl in onze studie een antibacterieel effect in 90% van de vrijwilligers werd gevonden.

De verklaring voor deze verschillende percentages van de *in vitro* antibacteriële activiteit enerzijds en de klinische effectiviteit anderzijds, ligt vermoedelijk aan het feit dat sommige bacteriën resistent zijn voor fosfomycine. Deze hypothese werd bevestigd in een tweede *in vitro* PD onderzoek waarbij wederom de PK kennis uit **Hoofdstuk 2.1** werden gebruikt. Dit onderzoek maakt geen deel uit van dit proefschrift, maar is terug te vinden in de referentielijst in hoofdstuk 7.2. In dit *in vitro* PD model konden ook de veranderde urineconcentraties over de tijd en het vullen- en ledigen van de blaas worden bestudeerd. De belangrijkste conclusie die op basis van dit model kon worden getrokken over het effect van 3 gram fosfomycine was dat een UWI die veroorzaakt wordt door *E. coli* effectief behandeld kon worden, maar dat na een paar uur een deel van de *E. coli* bacteriën weer gaan groeien. De mate van deze groei wordt bepaald door de aanwezigheid van een ongevoelige groep *E. coli* bacteriën. Deze zogenaamde resistente subpopulatie is al aanwezig voordat de behandeling met fosfomycine start. Er werd ook een verband gevonden tussen de duur van de behandeling (dus de duur van de blootstelling van de bacteriën aan fosfomycine) en de hoeveelheid ongevoelige bacteriën na blootstelling aan fosfomycine. Op basis hiervan kon de belangrijke conclusie worden getrokken dat de mate van blootstelling een sterke (misschien wel de sterkste) drijvende kracht is achter resistentieontwikkeling. Dit bevestigt tevens het belang van de onderzoeken die werden gedaan in dit proefschrift aangezien deze allemaal gericht zijn op het optimaliseren van de behandeling en hiermee het minimaliseren van de blootstelling van bacteriën aan antibiotica. Een tweede belangrijkste

conclusie die kon worden getrokken op basis van dit PD onderzoek is dat fosfomycine niet werkt bij infecties die worden veroorzaakt door *K. pneumoniae*. In tegenstelling tot *E. coli* wordt dit voor *K. pneumoniae* vermoedelijk niet veroorzaakt door resistentieontwikkeling door blootstelling aan fosfomycine, maar doordat *K. pneumoniae* per definitie ongevoelig is voor fosfomycine.

Fosfomycine wordt na orale inname opgenomen vanuit het maag-darm kanaal in het bloed en vervolgens uitgescheiden in de urine door de nieren. De nierfunctie heeft daarom theoretisch gezien een grote invloed op de fosfomycine concentraties in urine en daarmee op de effectiviteit van de behandeling. Dit werd onderzocht in **Hoofdstuk 5.1** voor fosfomycine. Er werd geen direct verband gevonden tussen de nierfunctie en de kans op het falen van de behandeling. Dit kan worden verklaard door het feit dat de urineconcentraties van fosfomycine per definitie erg hoog zijn en dat en afname van deze concentratie veroorzaakt door een verminderde nierfunctie, relatief weinig uitmaakt voor het wel of niet bereiken van effectieve urineconcentraties.

Door de hierboven besproken PK en PD onderzoeken is er nu meer kennis over (1) waarom fosfomycine in deel van de patiëntenpopulatie niet effectief blijkt te zijn bij de behandeling van ongecompliceerde UWIs, (2) in hoeverre de urineconcentraties van invloed zijn op de antibacteriële activiteit van fosfomycine en (3) wat de toegevoegde waarde is van dynamische *in vitro* PD modellen bij het doen van PK/PD onderzoeken in het kader van geneesmiddelontwikkeling in het algemeen.

NITROFURANTOÏNE

Nitrofurantoïne is in Nederland de eerste behandeloptie volgens de NHG-standaard voor de behandeling van een ongecompliceerde UWI. Nitrofurantoïne wordt voorgeschreven als dosis van 50 mg die vier keer per dag moeten worden ingenomen (capsule waaruit nitrofurantoïne direct vrijkomt) of in een dosis van 100 mg die slechts twee keer per dag hoeft in te worden genomen (capsule waaruit nitrofurantoïne vertraagt vrijkomt). De manier waarop nitrofurantoïne wordt voorgeschreven verschilt per land. De totale dosis per dag varieert van 150 mg tot 400 mg en wordt doorgaans verdeeld over twee, drie of vier doseringen per dag.

De PK van nitrofurantoïne werd in **Hoofdstuk 3.3** onderzocht in een groep van 12 gezonde, vrouwelijke vrijwilligsters die een dosering van 50 mg vier keer per dag of 100 mg drie keer per dag innamen (beiden keren de capsule met normale afgifte). Bij de vrijwilligsters werden bloedmonsters afgenomen en ze verzamelden zelf urinemonsters gedurende 6 uur (als ze de 50 mg capsule kregen) of gedurende 8 uur (als ze de 100 mg capsule kregen). De concentraties nitrofurantoïne werd vervolgens gemeten in de bloed- en urinemonsters en op basis hiervan werd de PK onderzocht voor beide

doseringen. Net zoals bij fosfomycine werd ook bij nitrofurantoïne vastgesteld dat er grote variaties zijn in de bloed- en urine concentraties tussen de vrijwilligsters. De bloed concentraties verdubbelden als de 50 mg dosis met de 100 mg dosis werd vergeleken. Dit betekent dat de concentraties in bloed dus direct gerelateerd zijn aan de dosis. De urine concentraties waren daarentegen vergelijkbaar tussen de beide doseringen.

Daarnaast werden in een tweede PK studie de urineconcentraties onderzocht in 19 UWI patiënten na het innemen van wederom een dosering van 50 mg vier keer per dag (capsule met normale afgifte) of 100 mg twee keer per dag van de speciale capsule met vertraagde afgifte (**Hoofdstuk 3.4**). De urineconcentraties waren wederom vergelijkbaar na beide doseringen. Dit was opvallend aangezien in tegenstelling tot de eerstgenoemde PK studie in gezonde vrijwilligers, in de studie in patiënten niet alleen de dosering anders was (50 mg versus 100 mg), maar ook de soort capsule (normale capsule versus de capsule met vertraagde afgifte). Theoretisch gezien zouden de urineconcentraties gedurende een langere tijd hoog moeten zijn omdat nitrofurantoïne vertraagd vrijkomt uit de capsule, maar dit verschil werd niet gezien. Op basis van urineconcentraties uit de beide PK studies kon worden geconcludeerd dat de urineconcentraties van nitrofurantoïne niet alleen onafhankelijk van de toegediende dosis lijken te zijn, maar ook van het soort capsule. Het is belangrijk om op te merken dat er slechts 19 patiënten in de tweede PK studie zaten waarvan er maar zeven de capsule met vertraagde afgifte kregen. De studie is nog altijd lopend om het beoogde aantal van 60 patiënten te bereiken. De conclusies op basis van de urineconcentraties in dit kleine aantal patiënten moeten daarom als hypothese genererend worden beschouwd.

Ook de nitrofurantoïne urinemonsters uit **Hoofdstuk 3.3** werden vervolgens gebruikt in het eerdergenoemde *in vitro* PD model uit **Hoofdstuk 4.1** waarin de invloed werd onderzocht van de nitrofurantoïne concentraties op de bacterie groei van *E. coli* en *K. pneumoniae*. De bacteriedodende activiteit van nitrofurantoïne bleek matig te zijn in zowel *E. coli* als in *K. pneumoniae* en dit effect kon alleen worden gemeten in de eerste twee uur na doseren in slechts een klein percentage van de vrijwilligsters. Dit was een onverwacht resultaat want nitrofurantoïne blijkt in de klinische praktijk in 70% van de UWI patiënten effectief te zijn. Wat voor fosfomycine werd geconcludeerd kon daarom ook worden geconcludeerd voor nitrofurantoïne: er is een verschil tussen de *in vitro* antibacteriële activiteit enerzijds en het klinische effect anderzijds. In tegenstelling tot fosfomycine is het voor nitrofurantoïne meer waarschijnlijk dat het PK profiel een verklaring voor deze verschillende percentages kan zijn. In onze PD studie werd namelijk alleen gekeken naar de antibacteriële activiteit na één dosering terwijl nitrofurantoïne in de klinische praktijk altijd wordt gegeven als een meerdaagse kuur waarbij er meerdere doseringen per dag moeten worden ingenomen. Deze resultaten geven daarom een onderschatting van het totale effect van de nitrofurantoïne kuur. Deze

bevinding bevestigt daarom dat het juist is om nitrofurantoïne in een meerdaagse kuur van meerdere doseringen te geven.

Ook voor nitrofurantoïne werd er geen verband gevonden tussen de kans op falen van de behandeling en een verminderde nierfunctie (**Hoofdstuk 5.1**). Dit is een verrassende bevinding aangezien de literatuur laat zien dat er lage urineconcentraties van nitrofurantoïne kunnen worden verwacht in patiënten met een verminderde nierfunctie waardoor het waarschijnlijk is dat het falen van de therapie in deze patiënten wordt veroorzaakt door te lage urineconcentraties en dus een PK gerelateerde oorzaak heeft. Wij hadden verwacht dat we een verband tussen het falen van de behandeling en een verminderde nierfunctie zouden vinden voor nitrofurantoïne, maar niet voor fosfomycine omdat concentraties van nitrofurantoïne die in de urine kunnen worden bereikt bijna 20 keer lager zijn dan die van fosfomycine. De invloed van variabelen zoals de nierfunctie, die van invloed zijn op de urineconcentraties, is dus relatief veel groter voor nitrofurantoïne dan voor fosfomycine. Dit maakt nitrofurantoïne een bijzonder antibioticum waarbij voorkennis van de te verwachten urineconcentraties en de (patiënt gerelateerde) eigenschappen die hier invloed op hebben, waarschijnlijk belangrijker is dan voor andere antibiotica voor UWIs.

AANBEVELINGEN VOOR VERVOLGONDERZOEK

Voor zowel fosfomycine als nitrofurantoïne geldt dat de invloed van hoe vaak de patiënt plast (het plasritme) en hoeveel de patiënt per keer plast (het plasvolume) groot is op de hoogte van de urineconcentraties. Deze twee factoren maken het daarom moeilijk om een goed beeld te krijgen van 'de pure PK' van het antibioticum omdat het nagenoeg onmogelijk is om de storende factor van het individuele plasritme van de patiënt weg te nemen. Dit zou deels kunnen worden voorkomen door de patiënt/vrijwilliger niet vrij te laten in hoe vaak zij plast en hoeveel zij drinkt tijdens het verzamelen van de urinemonsters. Op deze manier kan dan beter onderzoek worden gedaan naar 'het pure PK profiel'. Een nadeel hiervan is echter dat de gemeten urineconcentraties in dit onderzoek dan geen goede afspiegeling zijn van de urineconcentraties die verwacht kunnen worden in patiënten in de klinische praktijk. Het is dus sterk afhankelijk van het doel van de studie welke studieopzet optimaal is voor het verzamelen van de urinemonsters. Een alternatieve methode om het plasritme van de patiënt te standaardiseren is om PK onderzoek te doen in patiënten met een urinekatheter waarbij urine continu kan worden verzameld vanuit de katheterzak die rechtstreeks in verbinding staat met de blaas.

In het belang van het optimaliseren van de behandeling met fosfomycine zouden studies in de toekomst zicht moeten focussen op de ontwikkeling van diagnostische

testen die op een makkelijke in niet-invasieve manier duidelijk kunnen maken of de infectie wordt veroorzaakt door een bacterie die gevoelig is voor fosfomycine. Hierdoor kan het onnodig gebruik van fosfomycine worden geminimaliseerd en dit is belangrijk bij het voorkomen van resistentieontwikkeling door bacteriën. Daarnaast zijn er *in vitro* studies gedaan die laten zien dat de antibacteriële werking van fosfomycine wordt versterkt in combinatie met een ander antibioticum. De resultaten hiervan zijn veelbelovend dus er zouden studies moeten worden gedaan waarin ook de klinische effectiviteit van deze combinatiebehandeling wordt onderzocht in patiënten met ongecompliceerde UWIs. In de klinische praktijk wordt fosfomycine nu ook wel als een meerdaagse kuur voorgeschreven, maar de resultaten vanuit het eerdergenoemde dynamische *in vitro* PD model laten zien dat dit niet bevorderlijk is voor de antibacteriële activiteit en dat dit alleen maar de ontwikkeling van resistente bacteriën in de hand werkt. Het voorschrijven van fosfomycine als een meerdaagse kuur lijkt dus geen goede strategie te zijn voor optimalisatie van de behandeling van ongecompliceerde UWIs.

Aangezien de urineconcentraties van nitrofurantoïne relatief laag zijn en daardoor makkelijk te beïnvloeden door patiënt specifieke eigenschappen zoals de nierfunctie, lijkt het een voordehand liggend idee om de dosis te verhogen met als doel om de urineconcentraties te verhogen en hiermee de behandeling met nitrofurantoïne te optimaliseren. Echter lieten de resultaten uit **Hoofdstuk 3.3** en **Hoofdstuk 3.4** zien dat een dosis verhoging van 50 mg naar 100 mg nauwelijks effect had op de urineconcentraties, maar wel leidde tot hogere concentraties in bloed. Dit is ongewenst omdat juist hogere concentraties in bloed worden geassocieerd met het vergroten van de kans op het krijgen van bijwerkingen. Het mechanisme hierachter is nog onduidelijk en vormt daarmee een goede invalshoek voor vervolgonderzoek. Op basis van deze kennis kunnen dan nieuwe strategieën voor dosis-optimalisatie worden bedacht.

Daarnaast is er nog veel onduidelijkheid over in hoeverre nitrofurantoïne in het lichaam wordt omgezet in andere vormen (metabolieten) van het antibioticum. Het is bekend dat er metabolisme plaatsvindt, maar het is onbekend welke metabolieten er worden gevormd en in hoeverre deze metabolieten bijdragen aan de antibacteriële werking of aan het ontstaan van bijwerkingen door de behandeling met nitrofurantoïne. Meer inzicht in het metabolisme van nitrofurantoïne is belangrijk voor het optimaliseren van de behandeling omdat op basis van deze kennis de nitrofurantoïne formulering kan worden aangepast door een andere vorm van het nitrofurantoïne molecuul in de capsule te stoppen. Hierdoor kan het antibacteriële effect worden gemaximaliseerd waarmee de kans op het krijgen van bijwerkingen wordt geminimaliseerd.

CONCLUSIES VAN DIT PROEFSCHRIFT

De onderzoeken die gepresenteerd zijn in dit proefschrift vormen de eerste stap in het aanvullen van de ontbrekende PK kennis van de oude antibiotica fosfomycine en nitrofurantoïne. Voor beiden antibiotica bleek de gemeten antibacteriële activiteit *in vitro* geen goede weerspiegeling te zijn van het klinische effect. Voor fosfomycine kan dit waarschijnlijk verklaard worden door de (intrinsieke) resistente bacteriën die in de klinische praktijk ongecompliceerde UWIs kunnen veroorzaken. Voor nitrofurantoïne is het meer waarschijnlijk dat de afwijkende *in vitro*- en klinische resultaten worden veroorzaakt doordat er patiënten zijn waarin urineconcentraties te laag zijn om een antibacteriële werking te hebben. Waar voor fosfomycine methodes om de behandeling te verbeteren eerder kunnen worden gezocht in het verbeteren van de diagnostische middelen om ongevoelige bacteriën op te sporen, moet er voor nitrofurantoïne meer onderzoek worden gedaan naar nieuwe doseringen en de soort capsule waarin het wordt toegediend. De PK resultaten in dit proefschrift kunnen als basis dienen voor de ontwikkeling van deze nieuwe doseerstrategieën voor nitrofurantoïne en het doen van aanvullende onderzoeken naar het resistentiemechanisme van bacteriën voor fosfomycine.

De grote variatie in urineconcentraties, als één van de belangrijkste bevindingen in dit proefschrift, tonen het belang aan van het doen van PK onderzoek in een vroege fase van antibiotica ontwikkeling voor UWIs zodat er rekening kan worden gehouden met deze grote variatie in concentraties tijdens het doen van de daaropvolgende PD onderzoeken.

De PK resultaten in de hierboven voorgestelde studies moeten worden vergeleken met de resultaten uit de **Hoofdstukken 2.1, 3.3** en **3.4** om iets te kunnen zeggen over de meerwaarde van de nieuwe dosering ten opzichte van de standaard dosering. Ook moet deze PK kennis worden gebruikt in *in vitro* PD modellen om het effect van deze nieuwe concentraties op de antibacteriële activiteit van fosfomycine en nitrofurantoïne te onderzoeken waarbij ook de veranderende urineconcentraties over de tijd worden meegenomen. Hierbij kan dan niet alleen worden gekeken naar de antibacteriële activiteit, maar kan ook worden onderzocht wat het effect van de urineconcentraties is op de resistentieontwikkeling door de bacteriën. De resultaten in dit proefschrift laten zien dat *in vitro* PD resultaten niet per definitie representatief zijn voor het antibacteriële effect dat in patiënten wordt gezien. Deze bevinding toont aan dat het belangrijk is om nieuwe doseer strategieën die zijn ontwikkeld op basis van PK en PD kennis, te valideren in patiënten studies om de juistheid ervan te controleren. Met dit proefschrift is een belangrijke eerste stap gezet in deze richting en vervolgonderzoek is nodig om deze exploratieve PK en PD kennis toe te passen in het streven naar de optimalisatie van de behandeling van ongecompliceerde UWIs met fosfomycine en nitrofurantoïne.

7.2

List of Publications

LIST OF PUBLICATIONS

Wijma RA, Koch BCP, van Gelder T, Mouton JW. 2018. High interindividual variability in urinary fosfomycin concentrations in healthy female volunteers. *Clinical Microbiology and Infection* 24:528–532.

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ten Doesschate T*, van Haren E*, **Wijma RA**, Koch BCP, Bonten M, van Werkhoven CH. The effectiveness of nitrofurantoin, fosfomycin and trimethoprim for cystitis in relation to renal function. 2019. *Submitted for publication*

* Authors contributed equally.

7.3

Dankwoord

DANKWOORD

Dit laatste deel van mijn proefschrift wil ik wijden aan iets dat nog veel belangrijker is dan urine monstertjes die misschien kunnen leiden tot nieuwe wetenschappelijke inzichten: de mensen die mij in het bijzonder hebben geholpen, bijgestaan, gemotiveerd, gestimuleerd of op welke andere manier dan ook ervoor hebben gezorgd dat ik de eindstreep van deze promotie heb kunnen bereiken met een lach op mijn gezicht.

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7.4

Curriculum vitae

CURRICULUM VITAE

Rixt Anna Wijma was born on the 21st of June 1989 in Sneek, the Netherlands. She followed her pre-university education (VWO) at Bogerman Sneek, which she successfully completed in 2007. Rixt focussed her education in the areas of health and science by completing the profile "Natuur & Gezondheid".

She continued her education at the University of Groningen were she graduated as pharmacist in 2015. As part of her Master programme, she performed her research project at the laboratory of the hospital pharmacy in the Erasmus Medical Centre in Rotterdam for which she left Groningen and moved to Rotterdam. Under the supervision of Dr. Birgit Koch, she developed an analytical method to quantify drug levels of antipsychotic drugs in blood and with a dried blood spots method. As a result of her interest in research of drug concentrations (pharmacokinetics), she started her PhD-training in Rotterdam. This PhD programme was a collaboration between the department of Microbiology & Infectious Diseases and the Hospital Pharmacy of the Erasmus Medical Centre so she was supervised by Prof. Dr. Johan Mouton, Prof. Dr. Teun van Gelder and Dr. Birgit Koch. The results of the research she performed during her PhD-training are presented in this thesis.

As part of her PhD, Rixt went to Australia for six months to work in the research departments of the University of Queensland in Brisbane and Monash University in Melbourne. Under the supervision of Dr. Jason Roberts in Brisbane, she worked as clinical pharmacist in the ICU department were she focused on dose optimisation strategies and therapeutic drug monitoring of antibiotics. Under the supervision of Prof. Dr. Anton Peleg and Dr. Andrew Stewardson, she performed an audit of nitrofurantoin use in the Alfred Hospital in Melbourne.

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PhD portfolio

PhD PORTFOLIO

Name candidate:	Rixt Wijma
Institute:	Erasmus University Medical Center Rotterdam
Department:	Medical Microbiology and Infectious Diseases
PhD period:	2015-2019
Research School:	Molecular Medicine (MolMed) postgraduate school
Promotors:	Prof. dr. J.W. Mouton †
	Prof. dr. T. van Gelder
Copromotors:	Dr. B.C.P. Koch

Courses	Year
Biomedical English Writing and Communication, Erasmus MC	2017
Research Integrity, Erasmus MC	2016
BROK ('Basiscursus Regelgeving Klinisch Onderzoek')	2016
CPO course ('Patient Oriented Research: design, conduct and analysis')	2016
Literatuur zoeken Embase, Erasmus MC	2016
Literatuur zoeken Pubmed, Erasmus MC	2016
NONMEM® (Non-linear mixed effect modelling) course, CHDR Leiden	2015
ECCMID education course, Warszawa	2015
PK/PD course antibiotics ECCMID, Rotterdam	2019
PCDI employability outside academia, Utrecht	2019

(Inter)national conferences and seminars	Year
Research day Clinical Pharmacology, Erasmus MC (oral presentation)	2016
AIDA meeting, Warszawa (oral presentation)	2016
Population Approach Group in Europe (PAGE), Lisbon (poster presentation)	2016
Dutch Society for Clinical Pharmacology & Bio pharmacy spring meeting (poster presentation)	2017
European Committee of Clinical Microbiology & Infectious Diseases conference, Vienna (oral presentation)	2017
AIDA meeting, The Hague (oral presentation)	2017
European Committee of Clinical Microbiology & Infectious Diseases conference, Madrid (oral presentation)	2018
ESCMID PK/PD of Anti-Infective Study Group (EPASG) meeting, Madrid (oral presentation)	2018

Dutch Society for Clinical Pharmacology & Bio pharmacy spring meeting (poster presentation)	2018
International Association of Therapeutic Drug Monitoring and Clinical Toxicology conference, Brisbane (poster presentation)	2018
AIDA meeting, Camogli (oral presentation)	2018
Dutch Federation of Hospital Pharmacists, Bunnik (oral presentation)	2018
Dutch Society for Clinical Pharmacology & Bio pharmacy spring meeting (poster presentation)	2019
European Committee of Clinical Microbiology & Infectious Diseases conference, Amsterdam (poster presentation)	2019

Teaching	Year
Population PK modelling lecture for infectious disease physicians in training, Lunteren	2018
PK/PD lecture for Infection & Immunity students, Erasmus MC	2015-2019
Supervision research internships of pharmacy Master students Utrecht University	2017-2019
Supervision research internships of laboratory sciences students Hogeschool Breda	2017-2019

Grants	Year
FIGON travel grant	2018
Erasmus Trustfonds travel grant	2018