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).

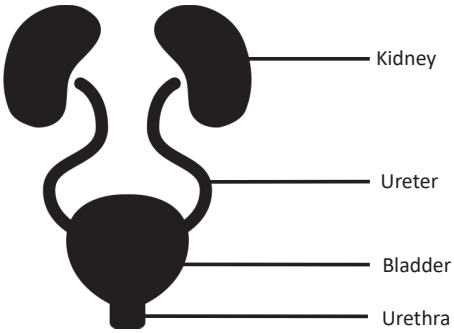

Figure 1. Anatomy of the female urinary tract. Urine is produced in the two kidneys, is transported via the ureter to the bladder and leaves the female body through the urethra. Only the bladder is infected in case of an uncomplicated UTI.

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).

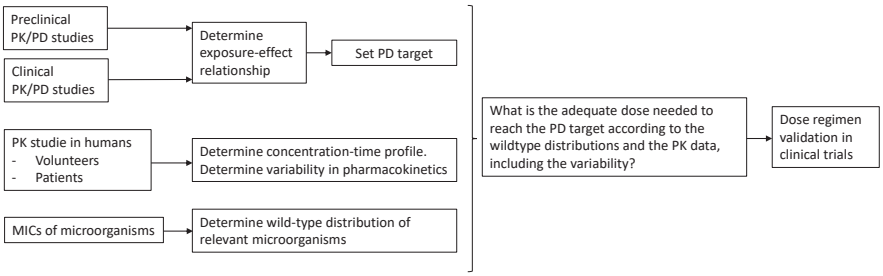

Figure 2. The role of pharmacokinetic data in the process finding the optimal dosing regimen (figure adapted from Muller et. al (25)).

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.

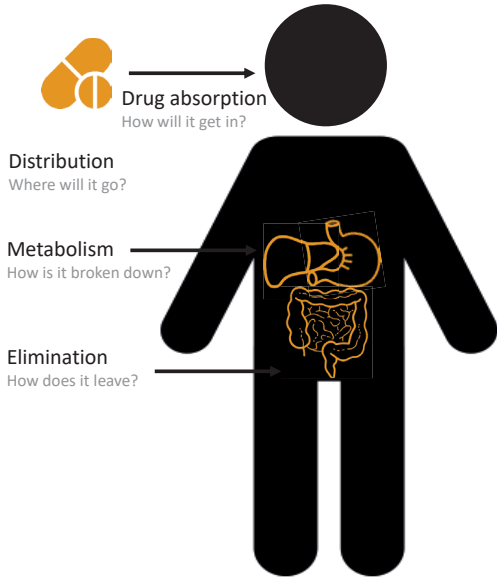

Figure 3. The principles of Absorption, Distribution, Metabolism and Excretion.

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).

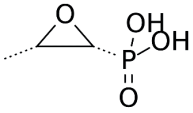

Figure 4. The chemical structure of fosfomycin-trometamol.

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).

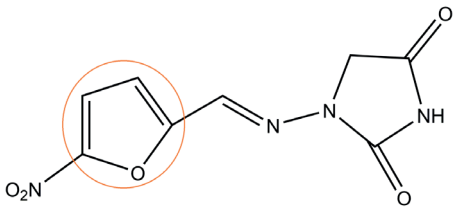

Figure 5. Chemical structure of nitrofurantoin.

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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