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.

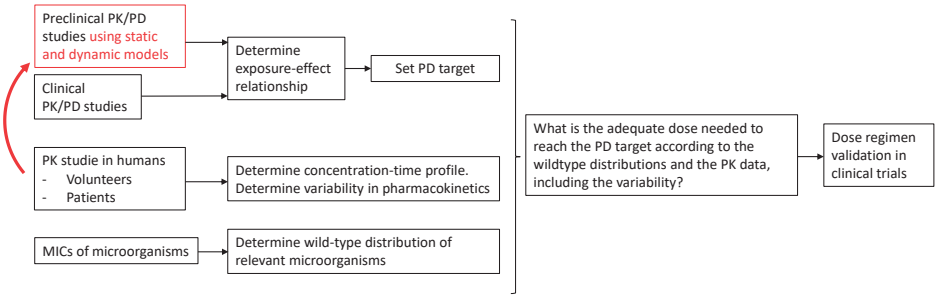

Figure. The role of pharmacokinetic data in the process of finding the optimal dosing regimen for UTI antibiotics, as presented in figure 2 of **Chapter 1**, adjusted (in red) based on the content of this thesis (34).

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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