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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.

1. Introduction

Nitrofurantoin is an old antibiotic used for the treatment of uncomplicated urinary tract infections (UTI) for decades ("Antimicrobial Consumption Trends, European Center for Disease Prevention and Control. Accessed 6 September 2018.,"n.d.; Gupta et al., 2011; Shah and Wade, 1989). 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 (Garau, 2008; Gupta et al., 2011). Resistance rates for nitrofurantoin are still low despite its extensive use (de Greef and Mouton, 2018; Huttner et al., 2015). 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 (Fransen et al., 2016; McOsker and Fitzpatrick, 1994; Mezzatesta et al., 2017; Zhanel et al., 2003).

Positive clinical outcomes of percentage up to 90% for uncomplicated UTIs are reported for nitrofurantoin (Huttner et al., 2015; Iravani et al., 1999; Stein, 1999). The most recent international guidelines therefore lists nitrofurantoin as a first line treatment option for uncomplicated UTIs in many countries worldwide (Gupta et al., 2011).

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 (Mouton et al., 2011; Roberts et al., 2012). 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.

2. 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

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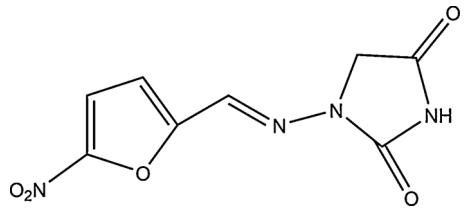

Fig. 1. Chemical structure of nitrofurantoin. The chemical formula of nitrofurantoin is $C_8H_6N_4O_5$ and the average molecular weight is 238.2 g/mol.

(-NO_2) (Fig. 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 (Cunha, 1989).

Nitrofurantoin is a synthetic product and has the appearance of a yellow crystalline powder (FDA, 2009a, 2009b). There are different formulations of nitrofurantoin, those containing microcrystals (˜10 μm in diameter) and those containing macrocrystals (75–180 micrometer in diameter). Macrocrystal formulations are available as such (Macrodantin® 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 (FDA, 2009a, 2009b, 2008; Paul et al., 1967). 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 (Bates et al., 1974; Conklin and Hailey, 1969; Mason et al., 1987; Paul et al., 1967). 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 (Bates et al., 1974; Paul et al., 1967). The drug is mainly used in a dose of 50 mg q6 h, 100 mg q8 h (both macrocrystalline nitrofurantoin) or 100 mg q12 h (slow-release product) when used for the treatment of an UTI, but this can be different between countries (AFSSAPS, 2008; Gupta et al., 2011; TherpeuticGuidelines, 2018). 50–100 mg q24 h is the registered dose for prophylactic use (FDA, 2009a).

The metabolic pathway of nitrofurantoin is unclear, but it was suggested that metabolites are formed by reduction through bacterial enzymes (Beckett and Robinson, 1959; McOsker and Fitzpatrick, 1994). It is not clear to what extent this reduction is required for its antibacterial activity (McOsker and Fitzpatrick, 1994). 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 (Cunha et al., 2017; Fransen et al., 2017b; Hahn, 1979; McOsker and Fitzpatrick, 1994).

3. Bioanalysis

3.1. 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 (Vree et al., 1979).

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 (Bender et al., 1956). The method of Conklin and Hollifield from 1965 served as the base of most of the following published methods (Conklin and Hollifield, 1965). 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 (Aufrere et al., 1977; Hoener and Wolff, 1980; Roseboom and Koster, 1978). The first method using MS detection, the most used detection method nowadays for both research and therapeutic drug monitoring purposes, was published in 2013 (Patel et al., 2013).

3.2. Issues in the preparation of nitrofurantoin stock solutions

Besides its instability in light (Vree et al., 1979), nitrofurantoin also decomposes upon contact with metals other than stainless steel and aluminium (Cadwallader and Jun, 1976). It has been demonstrated that the degradation of nitrofurantoin is enhanced in alkaline media (pH 10) compared to acidic media (pH 1.2) (Ertan et al., 1993; Vishnupad, 1980).

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 crystallization 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 (Barry et al., 1976; Cayman Chemical, 2018; CliniSciences, 2018; Keith and Walters, 1992; Selleckchem, 2018; Sigma-Aldrich, 2007).

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.

4. 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 (Gleckman et al., 1979; Mc Osker and

Fitzpatrick, 1994). 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 (Cunha, 1989). 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 (Chamberlain, 1976; Edwards, 1993; Mc Osker and Fitzpatrick, 1994; McCalla, 1977; McCalla et al., 1971). 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.

5. 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 (CLSI, 2019; EUCAST, 2003; ISO, 2006). 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 (Baker et al., 1991; Huang et al., 1992). Disk diffusion has been used since the 1960 (Bauer et al., 1966). 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, increased exposure. 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.

6. 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 (EUCAST, 2019a), 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 micro-organisms with an MIC of 64 mg/L, (CLSI, 2019).

7. 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 (Aufrere et al., 1977; Conklin and Hollifield, 1965; Hoener and Wolff, 1980; Mason et al., 1987; Patel et al., 2013; Roseboom and Koster, 1978). 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 (Wijma et al., 2018). 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).

7.1. 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 h after dosing, depending on the crystal size, formulation of the nitrofurantoin product and the fasting status of the subject (Wijma et al., 2018). One study investigated urine excretion after a therapeutic dose of 100 mg macrocrystalline nitrofurantoin q6 h (Conklin and Hailey, 1969). Although urinary concentrations were not reported, the recovery was found to be ~36% over 24 h. 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 h and recovery values were comparable with those after a multiple 100 mg dose (Conklin and Hailey, 1969; Mason et al., 1987; Paul et al., 1967). 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 h or 100 mg q8 h of nitrofurantoin in its macrocrystalline form support this observation (Huttner et al., 2019). 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 (Bates et al., 1974; Rosenberg, 1976). 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 (Mason et al., 1987). The total recovery over 24 h however was higher (> 30% versus 24.5%) for the slow-release formulation (Maier-Lenz, 1979; Mason

Table 1
MIC distribution and epidemiological cut-off value (ECOFF) (EUCAST, 2019b).

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

Table 2
The pharmacokinetic parameters of nitrofurantoin in urine and plasma after administering of macorcrystalline nitrofurantoin, modified from Wijma et al. (Wijma et al., 2018).

	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 et al., 2019	12	macro	capsule	f	26.8-176.3	3.3-5.5	–		–		LC-UV
	Multiple dose: 3x100 mg (therapeutic use)											
	Huttner et al., 2019	12	macro	capsule	f	40.1-209.4	1.3-8.1	–		–		LC-UV
	Multiple dose: 4x100 mg (therapeutic use)											
	Conklin and Hailey, 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 et al., 1974	14	macro	capsule	f	–		24.0	12	2[c]	–	LLE + UV
	Single dose: 100 mg (prophylactic use)											
	Paul et al., 1967	15	macro	4 capsules	nf	83-159	3.4-5.5	19.6-35.4	24	4.2-8.9[c]	3.6-4.9	Chrom. + UV
	Albert et al., 1974	10	macro	capsule	f	–		59.2	24	–		LLE + UV
	Bates et al., 1974	4	macro	capsule	f	–		22.4	24	7.2	2.3	Chrom. + UV
					nf			40.4		10.4	3.5	
	Meyer et al., 1974	14	macro	capsule	f	–		25.0	12	2.1[c]	–	LLE + UV
	Rosenberg, 1976	4	macro	capsule	f	–		22.4	24	–		LLE + UV
					nf			40.4				
	Panayotis, 1986	4	macro	capsule	f[a]	–		26.3	24	6.1	–	LLE + UV
								27.6		5.3		
								21.2		5.3		
	Macheras and Reppas, 1986	4	macro	capsule	f[d]	–		26.3	24	6.1	–	LLE + UV
								27.6		5.3		
								21.2		5.3		
	Mason et al., 1987	24	macro	capsule	f	95	5	24.5	24	5.6	4.7	LLE + UV
	Adkison et al., 2008	36	macro	capsule	f[e]	–		43.1	30	–		LC-UV or LC-MS/MS
								44.3				
								38.8				
	Slow release formulation: 2x100 mg (therapeutic use)											
	Maier-Lenz, 1979	6	–	slow-release	–	–	–	47.5	24	–		LLE + UV[b]
								33.7	24			
	Mason et al., 1987	24	macro	3 slow-release	f	120-150	3-5	30.4-34.1	24	7.5-8.3	3.7-4.2	LLE + UV
	Other dosages											
	Carroll et al., 1955	9	macro	capsule	f	–		10.6	3	–		Chrom. + UV
			dose: 200 + 4x100 mg					29.0	12			
								34.2	24			
	Schwartländer, 1972	5	macro	capsule	–	195.7	2-4	38.3	8	–		Color
						273.5	0-2	35.1				
				capsule		124.9	0-2	39.4	12			
				Slow-release		166.4	2-4	26.5	10			

Plasma	**Reference**	**Subjects**	**Drug information**		**Fasting**	**PK parameters**					**Analytical method**
			Crystal size	**Formulation**		**C_{max} (mg/L)**	**T_{max} (h)**	**AUC (mg/L.h)**	**(h)**	**$T_{1/2}$ (h)**	
	Multiple dose: 4x50 mg (therapeutic use)										
	Huttner et al., 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 et al., 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 et al., 1980	10	macro	tablet	f	0.26	2.1	1.5	–	1.7	HPLC + polarogr.
	Single dose: 1x100 mg (prophylactic use)										
	Felts et al., 1971	11		–	nf	0.9-4.6	2-4	–		–	Chrom. + color
		6				0.75-3.7					
	Albert et al., 1974	10	macro	capsule	f	1.47	2	3.28	0-4	–	LLE + UV
	Adkison et al., 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 et al., 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] unit: %/h.
[d] fasting + 100 mL, 200 mL or 400 mL milk, respectively.
[e] fasting + three different genotypes for the breast cancer resistance protein gene.

et al., 1987). Of note, this slow-release formulation was not the slow-release formulation of macrocrystalline nitrofurantoin/monohydrate (Macrobid®/Furabid®) as being used nowadays in some countries. PK data of this slow-release formulation given 100 mg q12 h 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 (Sachs et al., 1968; Samuel, 2015).

7.2. 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 (Huttner et al., 2019). As demonstrated in Table 2, plasma concentrations were significantly higher for the 100 mg q8 h dose (up to 1.26 mg/L after ~2 h) compared to those after the 50 mg q6 h dose (up to only 0.45 mg/L after ~2 h) (p-value < 0.05), resulting in an overall higher exposure of the plasma compartment (expressed as AUC over 24 h). 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 h after dosing, suggesting a rapid absorption from the gastrointestinal tract (Wijma et al., 2018). No plasma data are available after administration of the slow-release formulation.

8. 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* (Fransen et al., 2016) (Fig. 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 (Mouton and Vinks, 2005; Vogelman and Craig, 1986). 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* (Fig. 2a) compared to the Hill slope of *E. cloacae* (Fig. 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 (Mouton and Vinks, 2005). A similar relationship for *E.coli* was found by Komp Lindgren et al. (Komp Lindgren et al., 2014).

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 (Fransen et al., 2017a). 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. Fig. 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 (Drobot et al., 1996) and fosfomycin (Abbott et al., 2018b). To determine the activity of nitrofurantoin in urine, time-kill experiments were carried out in urine and broth (Fransen et al., 2017a). Fig. 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.

8.1.3. Dynamic models

Currently, only one in vitro study addressed some dynamic nitrofurantoin concentrations (Komp Lindgren et al., 2014). In this in vitro model, based on the dilution model described by Löwdin et al.

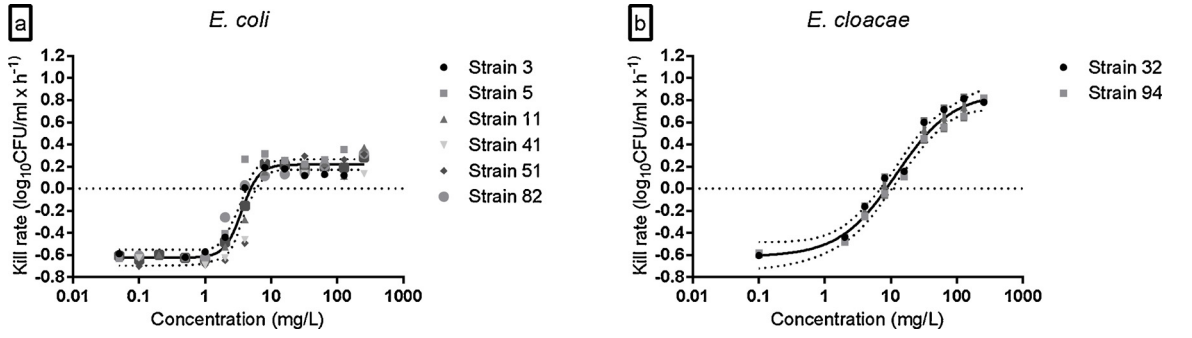

Fig. 2. Early-time pharmacodynamics of nitrofurantoin for *E. coli* and *E. cloacae* strains after exposure to nitrofurantoin for 6 h demonstrating a difference in pharmacodynamic effects. Kill rate data are plotted against concentration and best fitted sigmoid curves obtained from sigmoid maximum effect (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., (Fransen et al., 2016).

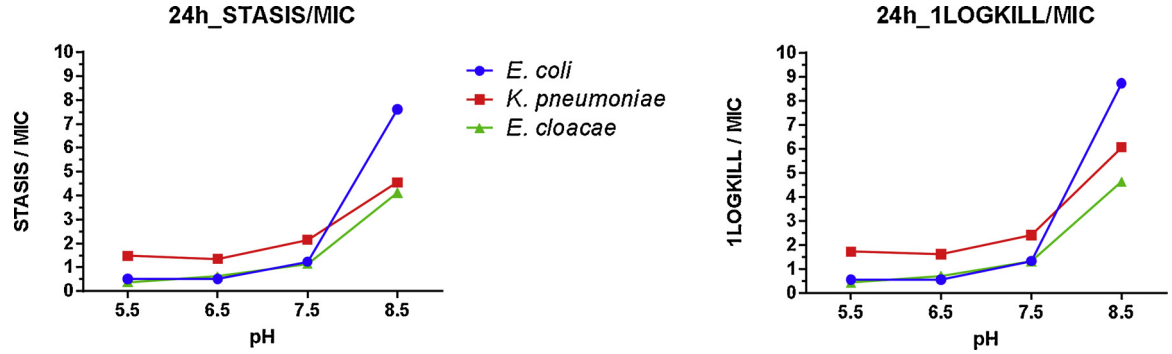

Fig. 3. Geometric mean MIC-normalized stasis and 1 log kill at 24 h for *E. coli, K. pneumoniae* and *E. cloacae* at four different pH levels. Redrawn/adapted from Fig. 3 Fransen et al., 2017 (Fransen et al., 2017b).

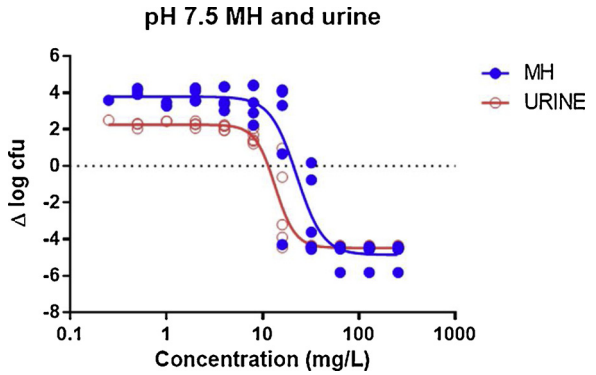

Fig. 4. Typical example showing the relationship between concentration and effect at 24 h incubation for nitrofurantoin in Mueller Hinton broth and urine for five *E. coli* strains. The lines are the fitted sigmoid curves obtained from the E_{max} model. The difference in static effect is significant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

(Löwdin et al., 1996), an *E. coli* isolate (MIC 2 mg/L) was initially exposed to a static nitrofurantoin concentration (16 mg/L), either for 24 h or followed by a dilution phase with a half-life of 1 h. 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) (Fransen et al., 2016). 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 3 g 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 (Boy et al., 2004; Naber et al., 2001; Wagenlehner et al., 2014). Briefly, the method includes a 2-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 (Hahn, 1979; McOsker and Fitzpatrick, 1994).

We recently investigated the urinary antibacterial activity of nitrofurantoin in healthy volunteers (Huttner et al., 2019). Urine samples were collected during 6 or 8 h 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 h after dosing, but no bactericidal or inhibitory effect was found during the complete 8 h period in the majority of the samples (titers of < 2). Higher titer values were observed after the 50 mg q6 h dose compared to the 100 mg q8 h 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 (Mezzatesta et al., 2017).

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 (Hung et al., 2009; Kerrn et al., 2003; Soubirou et al., 2014). 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.

9. 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 (McCalla et al., 1978). The majority consists of the insertion of insertion sequence elements, but also deletions and missense mutations have also been observed (Whiteway et al., 1998). The mutations hinder the reduction of nitrofurantoin, thereby preventing the formation of toxic intermediate compounds (Sandegren et al., 2008). 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 (Sekyere and Asante, 2018). 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 (Ho et al., 2016).

The probability of resistance development to nitrofurantoin in *E. coli* is high in vitro. (Sandegren et al., 2008). 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 (Sandegren et al., 2008). Despite its extensive use during the last decades, resistance rates for nitrofurantoin are still low. *E. coli* is sensitive to nitrofurantoin in more than 95% when considering western European countries and the US (de Greef and Mouton, 2018; Markowitz et al., 2018; Seitz et al., 2017). 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 (Sandegren et al., 2008).

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

10. 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) (Huttner et al., 2015; Muller et al., 2016). 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 (FDA, 2009b, 2009a, 2008). However, only one out of 3052 patients in studies published experienced a severe pulmonary side effect when nitrofurantoin was used as UTI prophylaxis (Muller et al., 2016). 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) (Bates et al., 1974; McGilveray; Mattok; Hossie, 1971; Paul et al., 1967). This would warrant further investigation.

11. 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 randomized controlled study comparing nitrofurantoin 100 mg q8 h (macrocrystalline, normal-release) for five days with a single 3 g 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%) (Huttner et al., 2018) 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. (Abbott et al., 2018a). 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.

Transparency declaration

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