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

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ABSTRACT

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

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

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

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

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

INTRODUCTION

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

PATIENTS AND METHODS

Study design and participants

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

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

Enrollment, randomization and intervention

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

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

Nitrofurantoin assay

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

Pharmacokinetic analysis

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

Statistical methods

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

RESULTS

Study population

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

Plasma and urinary concentrations of nitrofurantoin

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

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

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

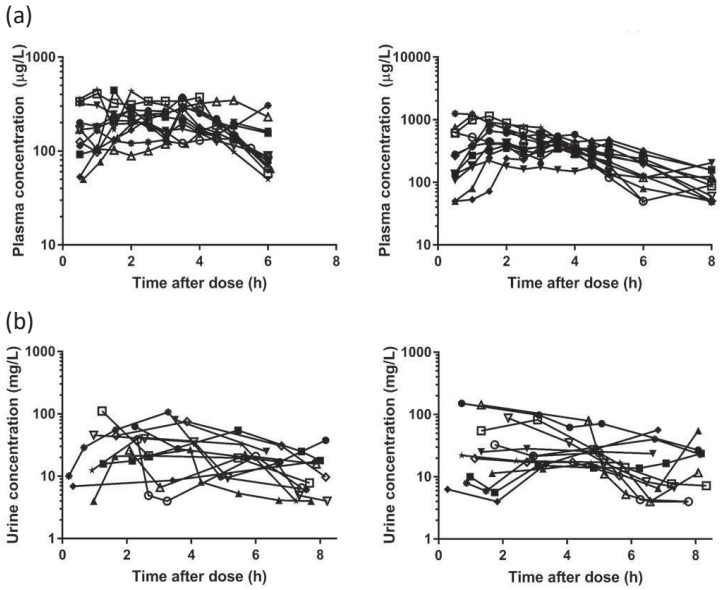

Figure 1. Nitrofurantoin concentration–time curves for plasma (a) and urine (b) samples.

Side effects

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

Table 1. Pharmacokinetic parameters in plasma and urine.

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

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

DISCUSSION

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

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

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

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

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

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

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

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

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

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