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Urinary Pharmacokinetics and Bactericidal Activity of Finafloxacin (200 and 800 mg) in Healthy Volunteers Receiving a Single Oral Dose

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Key Words
Finafloxacin · Urine pharmacokinetics · Pharmacodynamics · Urinary bactericidal titers

Abstract
Background: Finafloxacin is a novel 8-cyano-fluoroquinolone under investigation for treatment of urinary tract infection. ***Methods:*** Urinary concentrations and urinary bactericidal titers (UBT) of finafloxacin 200- and 800-mg single doses in 6 healthy volunteers were measured up to 48 h. UBT were determined for a reference strain and 9 selected clinical uropathogens at the pH of native, acidified (pH 5.5) and alkalinized (pH 8.0) urine. ***Results:*** The mean maximum urine concentrations for 200 and 800 mg finafloxacin were 69.3 mg/l (0–2 h) and 150 mg/l (4–8 h). Median UBT were between 0 and 1:>2,048 and were in general agreement with minimal inhibitory concentrations of strains and urinary pH values. UBT in alkaline urine were significantly lower than those in native or acidic urine, except for *Enterococcus faecalis*. ***Conclusions:*** Finafloxacin exhibited significant bactericidal activity against susceptible uropathogens. The urinary bactericidal activity of finafloxacin was enhanced in acidic urine and significantly lower in alkaline urine.

Introduction

Urinary tract infection (UTI) is one of the most common reasons for medical consultation. Uncomplicated UTI are predominantly caused by *Escherichia coli*, but also by *Proteus mirabilis* and occasionally *Klebsiella* spp., other Enterobacteriaceae and *Staphylococcus saprophyticus* (<5% each) [1–3]. Infections with *P. mirabilis* are significantly more common in patients over 50 years, whereas infections with *S. saprophyticus* are more common in younger patients. Nosocomial UTI, almost always complicated, are caused by a wide range of pathogens, most frequently *E. coli* (33.5%) and other Enterobacteriaceae such as *Proteus, Klebsiella, Enterobacter* and *Citrobacter* spp. (20.7%), but also enterococci (22.7%), coagulase-negative staphylococci (8.9%) and nonfermenters such as *Pseudomonas aeruginosa* (11.9%) are isolated from hospitalized patients [4, 5]. The characteristics of an antimicrobial agent to be used for the treatment of UTI should include: (1) activity against those most anticipated uropathogens; (2) activity at the site of infection, predominantly the urine, i.e. minimal loss of antimicrobial activity in urine; (3) relatively high and prolonged urinary concentrations, and (4) pronounced bactericidal activity [6]. Recent studies suggest that fluoroquinolones are still

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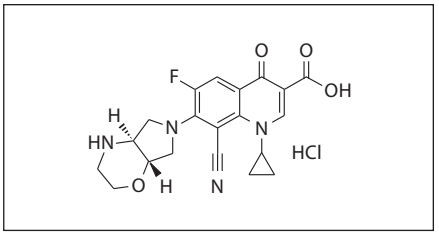

Fig. 1. Structure of finafloxacin HCl.

considered the drugs of choice. An international survey of the antimicrobial susceptibility of pathogens from uncomplicated UTI (ECO.SENS Project) revealed that almost all of the pathogens isolated from patients who presented with symptoms of acute UTI at 252 community health care centers in 17 European countries were still quinolone susceptible. Overall, 2.3% of *E. coli,* 2.1% of *P. mirabilis,* ≤1% of other Enterobacteriaceae, and 0% of *S. saprophyticus* were resistant to ciprofloxacin [2]. However, more recent studies showed a general increase in fluoroquinolone resistance to depend very much on the country [3].

The antibacterial activity of commercially available fluoroquinolones that are labeled for UTI, such as ciprofloxacin, norfloxacin and ofloxacin/levofloxacin, is significantly reduced in urine, depending on urine pH and composition [7, 8]. Higher urinary concentrations of magnesium, which are commonly in the range of 8–10 mM, account in large part for this reduction in activity. Supplementation of standard media with magnesium to levels present in human urine results in 2- to 16-fold increases in minimal inhibitory concentrations (MIC) of quinolones for various bacterial species. Reduced potencies of the quinolones in urine can also be ascribed in part to the diminished activities of many quinolones at low pH levels prevailing in these media. Both the high magnesium concentrations and the low pH of normal and, in particular, of infected urine contribute to the reduced activities of quinolones in such media.

Finafloxacin is a novel fluoroquinolone which is under development for infections in the hospital and critical care setting and is being investigated for treatment of UTI [9, 10]. Finafloxacin is a zwitterion with an isoelectric pH of 6.7 and two dissociation constants at a pK_{a1} of 5.6 (carboxylate function) and a pK_{a2} of 7.8 (nitrogen at C7 substitute) (fig. 1), in contrast to ciprofloxacin with an isoelectric pH of 7.4 and two dissociation constants at a pK_{a1} of 6.1 and pK_{a2} of 8.7 [9–14].

In this ex vivo study, the urinary antibacterial activity of finafloxacin was evaluated at different pH values which may be encountered in UTI. In order to integrate pharmacokinetic and pharmacodynamic parameters, the concentrations in urine and the urinary bactericidal titers (UBT) for common uropathogens were determined. The UBT is the highest dilution of urine after ingestion of an antibiotic with antibiotic-free urine still bactericidal. This approach mirrors more closely the antibacterial activities of antibacterials at the site of infection [15–17].

Methods

Study Design and Subjects

The study followed the guidelines for a first human dose study in accordance with good clinical practice (CPMP/ICH/135/95). This trial is part of a double-blind, placebo-controlled, randomized dose escalation study [13] in healthy adult volunteers receiving single or multiple oral doses of finafloxacin performed in the phase I unit of Swiss Pharma Contract Ltd. (now Covance Basel Clinical Research Unit), Allschwil, Switzerland. The study reported here is an ex vivo annex to this phase I clinical study, using the urine from 12 healthy volunteers, 6 receiving 200 mg and 6 receiving 800 mg of finafloxacin as a single oral dose each. The phase I study was approved by the independent Ethics Committee (Ethikkommission beider Basel – EKBB; EK: 104/07) Canton Basel, Switzerland. Regulatory approval was received from the Swiss Agency for Therapeutic Products (Swissmedic; 2007DR1190). The study was registered at ClinicalTrials.gov, registry No. NCT00483158 (http://www.clinicaltrial.gov/ct2/show/NCT00483158). The volunteers were healthy as shown by medical history, physical examination, hematology and serum chemistry as well as urinalysis. Absence of antibacterial activity in urine was shown by the lack of inhibition of *Bacillus subtilis* in the conventional cup-plate agar diffusion test. Only postmenopausal or surgically sterilized female volunteers were included.

Drug Administration

After having given written informed consent to participate in this study, each volunteer received 1 oral dose of 200 or 800 mg finafloxacin (MerLion Pharmaceuticals GmbH, Berlin; batch No. BX02H5N and BX02H5L). The study drug was administered after an overnight fast. After drug administration, the subjects fasted for another 2 h. Alcoholic beverages were not allowed within 48 h of drug administration.

Acidic drinks like grapefruit or orange juice were not allowed 48 h before and 72 h after drug administration. Xanthine-containing beverages and food were avoided during the entire study period. The volunteers were asked to drink sufficient amounts of water throughout the collection period to ensure adequate urine production. A physical examination, electrocardiography and laboratory tests were performed before and after the study. Adverse events were recorded throughout the study period.

Sample Collection

All urine voided was collected prior to drug administration (to ascertain that the urine was antibiotic free) and at the following time intervals thereafter: 0–2, 2–4, 4–8, 8–12, 12–24 and 24–48 h; samples from 0–4 h were combined for UBT determination. The volumes were recorded and all samples were stored at –20°C.

Drug Concentrations in Urine

The concentrations of finafloxacin in urine samples were measured by HPLC with mass spectrometry (HPLC-MS/MS) using a series of finafloxacin concentrations for calibration and ciprofloxacin as an internal standard. To 50 μl urine, 25 μl methanol was added; thereafter, 2.0 ml formic acid in water was added, the mixture vortexed for 5 s, and 2 μl injected onto the LC-MS/MS. The lower limit of quantification in urine was 0.1 mg/l.

Spiked quality controls were used for the determination of interassay variation. No interference was observed for finafloxacin or the internal standard in urine. The overall coefficient of variance for the assay of finafloxacin in urine was between 2.9 and 11.7% across the calibration range, which was within the predefined acceptance criterion of 15% (MerLion Pharmaceuticals; data on file).

Pharmacokinetic and Statistical Analysis of Urine Samples

Urine pharmacokinetic parameters of finafloxacin were calculated by noncompartmental analysis. Urinary recovery (percent of the dose administered) and renal clearance (CLR) were calculated by using WinNonlin Professional version 4.1. For the variables including mean values ± standard deviations (SD), medians and minimum and maximum values, descriptive statistics were applied (SAS version 9.1).

Determination of MIC

MIC of finafloxacin, levofloxacin and ciprofloxacin for the strains tested were determined by broth microdilution using cation-adjusted Mueller-Hinton broth (CAMHB; Oxoid, Wesel, Germany) at pH 5.8, 7.2 and 8.0 according to the CLSI (Clinical and Laboratory Standards Institute) guidelines [18]. MIC were also determined in artificial urine medium ($CaCl_2 \cdot 2\ H_2O$: 0.65 g/l; $MgCl_2 \cdot 6\ H_2O$: 0.65 g/l; NaCl: 4.6 g/l; Na_2SO_4: 2.3 g/l; Na_2 citrate$\cdot 2\ H_2O$: 6.25 g/l; Na_2 oxalate: 0.02 g/l; KH_2PO_4: 2.8 g/l; KCl: 1.6 g/l; NH_4Cl: 1 g/l; urea: 25 g/l; creatine: 1 g/l) adjusted to pH 5.8, and trypticase soy broth was added to 5% [19]. MIC was defined as the lowest concentration inhibiting visible growth after incubation at 37°C for 18 h in ambient air [18].

Determination of UBT

For the determination of UBT, 2-fold serial dilutions (dilution range 1:0, 1:1 to 1:2,048) of the urine samples were prepared. Antibiotic-free urine from healthy subjects was used as diluent, with a mean (SD) pH of 5.7 (0.4) for the 200-mg (table 1), and 5.6 (0.5) for the 800-mg phase (table 2) [15, 20, 21]. A microdilution test was used to determine the UBT. Each well of the microplates contained 100 μl of the prepared urinary dilution. Bacterial strains were then added to the wells of the microplates using a multipoint inoculator (10 μl). The final inoculum, which was confirmed by actual counting, ranged from $2.4 \cdot 10^5$ to $6.1 \cdot 10^5$ CFU/ml. The plates were then incubated at 37°C for 18 h in ambient air.

UBT were determined in a second step after having transferred 10 μl of the subcultured urine onto Isosensitest agar supplemented with 5% sheep blood (Oxoid). The plates were incubated for 24 h at 37°C before the number of colonies was counted. Urinary bactericidal activity was defined as a >99.9% (>3 $\log_{10}$) reduction of the initial counts. The experiments were each performed on native urine and on urine with pH values adjusted to 5.5 and 8.0 by hydrochloric acid and sodium hydroxide, respectively.

Test Organisms

A reference strain (*E. coli* ATCC 25922), 5 selected *E. coli* strains with genetically defined mutations in the quinolone resistance-determining region representing ciprofloxacin borderline-susceptible and -resistant strains, and 4 wild-type, fluoroquinolone-susceptible clinical uropathogens isolated from patients with hospital-acquired UTI were tested:

E. coli Nal R 523 (gyrA S83L)
E. coli 1135121 (gyrA D87G)
E. coli 1949820 (gyrA S83N, gyrA D87N, parC S80I)
E. coli MI-4 (gyrA S83L, parC S80I)
E. coli WT-3-1-M4 (gyrA S83L + D87G, parC S80R)
Klebsiella pneumoniae 595 (wild type)
P. mirabilis 414 (wild type)
P. aeruginosa 586 (wild type)
Enterococcus faecalis 60 (wild type)

Statistical Analyses

UBT data are represented by the reciprocals of the factor of the highest dilution showing bactericidal action. When bactericidal activity was observed at the highest dilution (1:>2,048), a reciprocal value of 4,096 was used in subsequent calculations. The area under the 24-hour UBT versus time curve ($AUBT_{24}$) was calculated as the sum of the products of each UBT in the sampling period times the respective time interval (in hours) for each test organism and for each drug. UBT and AUBT data were compared between the different pH values by the paired t test. $\alpha = 0.05$ was chosen to determine statistical significance. Statistical calculations were performed by using the Microsoft Excel 97 program (1985–1998; Microsoft Co., Redmond, Wash., USA).

Results

Volunteers

The volunteers were 5 men and 1 woman in both the 200-mg and the 800-mg dose groups. The mean age was 36.7 years (median: 33.5 years; range: 25–53 years), the mean body weight was 74.1 kg (median: 76.5 kg; range: 65–80.4 kg), the mean height was 174.5 cm (median: 176.5 cm; range: 161–181 cm), and the mean body mass index was 24.4 (median: 24.8; range: 20.5–26.9) for the 200-mg dose group. For the 800-mg dose group, the mean age was 37 years (median: 35.5 years; range: 22–55 years), the mean body weight was 73.3 kg (median: 74.1 kg; range: 66.9–79.4 kg), the mean height was 175.8 cm (median: 175.5 cm; range: 170–184 cm), and the mean body mass index was 23.7 (median: 24.3; range: 20.1–25.6).

Table 1. Urinary pH, volume and parent drug concentration in urine of volunteers[1] after single oral administration of 200 mg finafloxacin

Collection period	Urinary pH	Volume, ml	Concentration, mg/l
Predose	5.7 ± 0.4 (5.6; 5.4–6.4)	n.a.	n.a.
0–2 h	6.4 ± 0.8 (6.7; 5.4–7.2)	483 ± 196 (538; 150–650)	69.3 ± 48.3 (52.1; 30.7–169)
2–4 h	6.5 ± 0.8 (6.7; 5.2–7.3)	358 ± 224 (363; 70–650)	68.2 ± 48.4 (48.4; 34.9–164)
4–8 h	7.3 ± 0.6 (7.1; 6.7–8.4)	406 ± 192 (365; 205–660)	33.9 ± 29.1 (22.4; 11.9–91)
8–12 h	7.0 ± 0.6 (7.1; 6.2–7.8)	597 ± 468 (440; 200–1,300)	12.5 ± 11.8 (10.5; 2.1–31.7)
12–24 h	6.5 ± 0.6 (6.3; 6.0–7.7)	1,158 ± 716 (1,025; 300–2,400)	4.3 ± 4.6 (2.3; 0.9–13)
24–48 h	6.6 ± 0.4 (6.6; 6.0–7.0)	2,317 ± 496 (2,350; 1,600–2,900)	0.8 ± 0.4 (0.7; 0.5–1.6)

Values denote means ± SD with medians and ranges in parentheses. [1] A total of 6 volunteers were tested.

Table 2. Urinary pH, volume and parent drug concentration in urine of volunteers[1] after single oral administration of 800 mg finafloxacin

Collection period	Urinary pH	Volume, ml	Concentration, mg/l
Predose	5.6 ± 0.5 (5.4; 5.2–6.3)	n.a.	n.a.
0–2 h	6.8 ± 0.8 (6.8; 5.6–7.8)	792 ± 351 (850; 350–1,200)	114 ± 71.1 (90; 38–242)
2–4 h	6.9 ± 1.0 (6.8; 5.4–8.1)	1,055 ± 344 (1,165; 500–1,400)	112 ± 45.7 (112; 63–193)
4–8 h	7.1 ± 0.3 (7.1; 6.5–7.4)	442 ± 228 (395; 220–820)	150 ± 90.6 (137; 44–256)
8–12 h	6.8 ± 0.4 (7.0; 6.3–7.4)	592 ± 287 (575; 300–1,000)	33.0 ± 29.1 (22.2; 12–87)
12–24 h	5.9 ± 0.4 (6.1; 5.2–6.4)	755 ± 310 (745; 320–1,280)	17.9 ± 18.0 (11.5; 7.6–54.1)
24–48 h	7.0 ± 0.6 (6.9; 6.3–8.0)	2,333 ± 1,087 (2,450; 750–3,550)	13.6 ± 28.9 (1.9; 0.7–72.3)

Values denote means ± SD with medians and ranges in parentheses. [1] A total of 6 volunteers were tested.

Table 3. Summary of urine pharmacokinetic parameters of finafloxacin by dose

	Number	%R	CLR, liters/h	AUC (urine) 24 h
200 mg	6	32.1 ± 12.2 [29.9 (20.5–54.0)]	15.8 ± 2.95 [15.3 (12.6–19.7)]	511.7 ± 338.4 [350.6 (218.6–962.5)]
800 mg	6	33.4 ± 7.0 [31.6 (24.6–43.2)]	11.0 ± 0.78 [11.0 (10.2–12.39)]	1,397.6 ± 824.7 [1,143.4 (675.1–2,892.2)]

Values denote means ± SD [medians (min.–max. values)].
%R = Urinary recovery in percent of dose administered; AUC = area under the curve.

Safety and Laboratory Test Results

Finafloxacin was well tolerated by all volunteers. No serious adverse events occurred during the study. There were no clinically relevant changes in the laboratory test results of blood and urine.

Urinary pH, Volumes and Concentrations

The mean values, medians and ranges of urinary pH, volumes and concentrations following 200- and 800-mg doses of finafloxacin are given in tables 1 and 2. Urinary pH was highest at collection periods 4–8 and 8–12 h after dosing, which was during noon and afternoon of the study. In most collection periods, urinary pH was just slightly acidic. Mean hourly urine volumes were highest in the first collection period of 0–2 h with 242 ml/h for the 200-mg dose, and in the second collection period of 2–4 h with 528 ml/h for the 800-mg dose. Following the 200-mg dose, mean concentration was highest in the pe-

Table 4. MIC[1] of finafloxacin, levofloxacin and ciprofloxacin in CAMHB at various pH and in artificial urine medium (pH 5.8) for test strains used in this study (mg/l)

MIC in CAMHB (pH 5.8)			MIC in CAMHB (pH 7.2)			MIC in CAMHB (pH 8.0)			MIC in synthetic urine (pH 5.8)		
CIP	LVX	FIN	CIP	LVX	FIN	CIP	LVX	FIN	CIP	LVX	FIN
***E. coli* ATCC 25922**											
0.06	0.125	≤0.004/0.0075	≤0.004	0.0075/0.015	0.015/0.03	≤0.004	0.0075	0.03	1	1/2	0.03
***E. coli* 523 (NR)**											
2	2	0.03	0.03/0.06	0.25/0.125	1	0.015/0.03	0.03	2	16	16	2
***E. coli* MI-4 (NR)**[2]											
8	8	0.5	0.5/1	0.5	4	0.25	0.5	16	128	64	4
***E. coli* MI-3-1-M4 (FR)**[2]											
64	32	4	4	4	32	1	2	128	>128	>128	64
***E. coli* 1135121 (FR)**											
32	16	2	2	2	8	0.5	2	16/32	>128	>128	32
***E. coli* 1949820 (FR)**											
128	64	8	16	8	64	8	8	>128	>128	>128	128/64
***K. pneumoniae* 595**											
0.03/0.06	0.06/0.125	0.015	≤0.125	≤0.004/0.0075	0.015	≤0.004	0.0075	0.06	2	2	0.25
***P. mirabilis* 414**											
0.06/0.125	0.25	0.25	≤0.004	0.015	1	≤0.004/0.0075	0.015/0.03	1	2/1	2/4	4
***P. aeruginosa* 568**											
1	2	1	0.25	1	4	0.25	1	16	8	32	2
***E. faecalis* 60**											
4	4	0.5	2	2	2	2	2	4	16	16	2

CIP = Ciprofloxacin; LVX = levofloxacin; FIN = finafloxacin; NR = nalidixic acid resistant; FR = fluoroquinolone resistant.
[1] MIC were determined on 2 separate occasions and listed as 2 values only if a difference was seen.
[2] Strains provided by Axel Dalhoff.

riod of 0–2 h and reached 69.3 mg/l. The concentration decreased to 4.3 mg/l in the period of 12–24 h, and to 0.8 mg/l 24–48 h after dosing. Following the 800-mg dose, the highest mean concentration was in the period of 4–8 h and was 150 mg/l. The concentration decreased to 17.9 mg/l in the period of 12–24 h, and to 13.6 mg/l 24–48 h after dosing. As expected, there was a considerable range of urinary concentrations at each time point within each dose group.

Pharmacokinetic Analysis

The mean/median 24-hour urinary recovery of finafloxacin amounted to 32%/30% for the 200-mg dose, and to 33%/32% for the 800-mg dose (table 3). Median CLR values were 15.3 and 11.0 liters/h in the two dose groups.

Minimal Inhibitory Concentrations

The MIC of finafloxacin, levofloxacin and ciprofloxacin in CAMHB (pH 5.8, 7.2 and 8.0) and artificial urine medium (pH 5.8) are shown in table 4. In general, the MIC of the three fluoroquinolones against *E. coli* varied over a broad range as indicator strains with defined quinolone resistance-determining region mutations had been selected. In standard test conditions (pH 7.2), finafloxacin was less active by a factor of 4–8 than ciprofloxacin or levofloxacin on most Gram-negative strains, and equipotent against *E. faecalis*. However, finafloxacin was more active than ciprofloxacin or levofloxacin by a factor of 4–64 at acidic pH in CAMHB (pH 5.8) against all strains except *P. mirabilis* and *P. aeruginosa*, for which the difference in activity was within a factor of 2. In artificial urine (pH 5.8), finafloxacin was also

Table 5. Reciprocal UBT and $AUBT_{24}$ for finafloxacin in the 6 volunteers tested in native urine, urine at pH 5.5 and urine at pH 8.0 after a single oral dose of 200 mg finafloxacin

	UBT for the following collection periods					$AUBT_{24}$
	0–4 h	4–8 h	8–12 h	12–24 h	24–48 h	
Native urine						
E. coli ATCC 25922	>2,048 (1,024 to >2,048)	1,024 (512 to >2,048)	512 (128 to >2,048)	128 (64 to >2,048)	32 (16 to >2,048)	24,064 (7,424–98,304)
E. coli 523 (NR)	128 (128–256)	64 (32–128)	12 (8–64)	6 (4–32)	0.5 (0–4)	888 (720–2,176)
E. coli MI-4 (NR)	12 (0–16)	8 (0–16)	2.5 (0–8)	0 (0–2)	0 (0–0)	90 (0–184)
E. coli MI-3-1-M4 (FR)	0 (0–1)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–4)
E. coli 1135121 (FR)	8 (0–16)	2 (0–4)	0 (0–1)	0 (0–0)	0 (0–0)	40 (0–84)
E. coli 1949820 (FR)	0 (0–1)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–4)
K. pneumoniae 595	512 (128–1,024)	160 (64 to >2,048)	64 (16 to >2,048)	24 (8–256)	8 (4–128)	3,232 (928–39,936)
P. mirabilis 414	40 (8–64)	24 (8–64)	6 (2–32)	1.5 (0–8)	0 (0–4)	298 (72–736)
P. aeruginosa 568	0 (0–16)	0 (0–16)	0 (0–4)	0 (0–0)	0 (0–0)	0 (0–144)
E. faecalis 60	16 (8–64)	8 (4–32)	3 (0–8)	1 (1–4)	0 (0–1)	120 (60–464)
Urine pH 5.5						
E. coli ATCC 25922	>2,048 (1,024 to >2,048)	2,304 (512 to >2,048)	192 (128 to >2,048)	96 (64–512)	24 (8–64)	27,520 (7,424–55,296)[1]
E. coli 523 (NR)	128 (32–256)	64 (32–128)	24 (8–128)	6 (2–32)	2 (1–4)	936 (312–2,432)[1]
E. coli MI-4 (NR)	6 (1–32)	4 (0–8)	1.5 (0–8)	0.5 (0–1)	0 (0–0)	52 (4–204)
E. coli MI-3-1-M4 (FR)	1 (0–2)	0 (0–1)	0 (0–1)	0 (0–0)	0 (0–0)	4 (0–16)
E. coli 1135121 (FR)	8 (4–16)	4 (1–8)	1 (0–4)	0 (0–2)	0 (0–0)	52 (20–136)[1]
E. coli 1949820 (FR)	0 (0–2)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–8)
K. pneumoniae 595	384 (64–512)	256 (32–512)	48 (16–256)	16 (8–64)	6 (2–16)	2,944 (544–5,888)[1]
P. mirabilis 414	16 (16–64)	12 (4–64)	3 (0–16)	1.5 (1–16)	0.5 (0–2)	142 (92–768)[1]
P. aeruginosa 568	0 (0–32)	0 (0–32)	0 (0–16)	0 (0–2)	0 (0–0)	0 (0–344)
E. faecalis 60	8 (4–32)	4 (2–8)	3 (1–8)	1 (0–4)	0 (0–1)	72 (28–240)
Urine pH 8.0						
E. coli ATCC 25922	256 (128–1,024)	128 (64–256)	24 (8–128)	8 (2–32)	2 (1–4)	1,728 (824–6,016)[1]
E. coli 523 (NR)	16 (4–64)	8 (4–16)	1.5 (0–4)	0.5 (0–4)	0 (0–0)	108 (32–384)[1]
E. coli MI-4 (NR)	2 (0–4)	0 (0–2)	0 (0–1)	0 (0–0)	0 (0–0)	0 (0–28)
E. coli MI-3-1-M4 (FR)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)
E. coli 1135121 (FR)	0.5 (0–4)	0 (0–1)	0 (0–0)	0 (0–0)	0 (0–0)	2 (0–20)[1]
E. coli 1949820 (FR)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)
K. pneumoniae 595	64 (32–64)	16 (4–32)	5 (1–16)	2 (0–8)	0 (0–0)	364 (148–544)[1]
P. mirabilis 414	6 (1–8)	4 (1–16)	1.5 (0–4)	0 (0–1)	0 (0–0)	46 (8–124)[1]
P. aeruginosa 568	0 (0–2)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–8)
E. faecalis 60	12 (4–4)	4 (2–8)	1.5 (0–8)	0 (0–2)	0 (0–0)	70 (24–344)

Values are medians with ranges in parentheses. NR = Nalidixic acid resistant; FR = fluoroquinolone resistant.
[1] Significantly different ($p < 0.05$, paired t test) for $AUBT_{24}$ in urine at pH 8.0 vs. native urine and urine at pH 5.5.

more active than ciprofloxacin or levofloxacin, exhibiting MIC that were lower by a factor of 4–64 against all strains except *P. mirabilis,* for which the difference in activity was within a factor of 2. This was despite the high levels of divalent cations, which bind most of the commercially available fluoroquinolones like ciprofloxacin and levofloxacin. Therefore, ciprofloxacin and levofloxacin MIC increased strain dependently by a factor of 2 to more than 10 in acidic CAMHB and artificial urine. Both ciprofloxacin and levofloxacin gained activity by a factor of 2–4 against some of the *E. coli* strains tested in CAMHB at an alkaline pH value as compared to their activities at pH 7.2.

UBT and $AUBT_{24}$

The UBT and $AUBT_{24}$ of finafloxacin for the test organisms in native urine and urine adjusted to pH 5.5 and pH 8.0 for the 200- and 800-mg doses are given in tables 5 and 6, respectively. Following the 200-mg dose, the median $AUBT_{24}$ of the susceptible *E. coli* (ATCC 25922), nalidixic acid-resistant *E. coli* 523 and *E. coli* MI-4, fluoroquinolone borderline-resistant *E. coli* 1135121, *K. pneumoniae* and *P. mirabilis* ranged from 52 to 27,520 h in urine adjusted to a pH of 5.5, and from 0 to 1,728 h in urine adjusted to a pH of 8.0. The median $AUBT_{24}$ in native urine were similar to those in urine adjusted to a pH of 5.5 and ranged from 40 to 24,064 h for the listed strains.

Table 6. Reciprocal UBT and $AUBT_{24}$ for finafloxacin in the 6 volunteers tested in native urine, urine at pH 5.5 and at pH 8.0 after a single oral dose of 800 mg finafloxacin

	UBT for the following collection periods					$AUBT_{24}$
	0–4 h	4–8 h	8–12 h	12–24 h	24–48 h	
Native urine						
E. coli ATCC 25922	>2,048 (256 to >2,048)	>2,048 (256 to >2,048)	768 (64 to >2,048)	512 (128–512)	32 (16–256)	41,984 (3,840–55,296)
E. coli 523 (NR)	384 (128–512)	192 (128–512)	64 (16–128)	32 (8–64)	5 (0–64)	2,944 (1,184–5,376)
E. coli MI-4 (NR)	48 (16–64)	48 (16–128)	12 (4–16)	6 (0–8)	1.5 (0–16)	504 (144–926)
E. coli MI-3-1-M4 (FR)	3 (0–16)	4 (0–16)	0.5 (0–2)	0 (0–2)	0 (0–0)	30 (0–160)
E. coli 1135121 (FR)	16 (1–32)	10 (2–32)	3 (1–8)	2.5 (0–4)	0 (0–8)	146 (16–336)
E. coli 1949820 (FR)	0 (0–1)	0 (0–1)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–8)
K. pneumoniae 595	512 (256–1,024)	1,024 (256–1,024)	256 (64–512)	192 (64–256)	20 (4–128)	9,472 (3,072–13,312)
P. mirabilis 414	32 (16–128)	32 (32–128)	24 (8–32)	8 (4–32)	2 (0–32)	448 (272–1,536)
P. aeruginosa 568	48 (16–64)	64 (16–128)	12 (4–16)	6 (4–8)	0.5 (0–16)	568 (192–928)
E. faecalis 60	64 (4–128)	48 (4–128)	20 (2–32)	8 (0–16)	1.5 (0–32)	624 (40–1,344)
Urine pH 5.5						
E. coli ATCC 25922	>2,048 (256 to >2,048)	2,560 (256 to >2,048)	768 (256 to >2,048)	512 (64 to >2,048)	64 (32–256)	35,840 (3,840–98,304)[1]
E. coli 523 (NR)	256 (128–512)	320 (128–1,024)	96 (16–512)	48 (8–256)	4 (0–256)	3,264 (1,184–11,264)[1]
E. coli MI-4 (NR)	48 (8–128)	32 (16–128)	24 (4–64)	6 (2–32)	1 (0–32)	488 (136–1,664)[1]
E. coli MI-3-1-M4 (FR)	8 (2–16)	6 (0–32)	2 (0–4)	0.5 (0–2)	0 (0–0)	70 (8–232)[1]
E. coli 1135121 (FR)	12 (8–32)	18 (2–64)	4 (0–16)	1.5 (0–8)	0 (0–8)	154 (40–544)[1]
E. coli 1949820 (FR)	0 (0–1)	0 (0–1)	0 (0–1)	0 (0–0)	0 (0–0)	0 (0–12)
K. pneumoniae 595	512 (512 to >2,048)	768 (256 to >2,048)	256 (64–512)	192 (64–256)	16 (4–64)	8,448 (4,096–37,888)[1]
P. mirabilis 414	48 (16–64)	64 (16–256)	8 (4–64)	6 (4–32)	1.5 (0–32)	552 (192–1,920)[1]
P. aeruginosa 568	64 (16–256)	96 (16–512)	16 (2–128)	6 (2–64)	0.5 (0–64)	776 (160–4,352)[1]
E. faecalis 60	32 (16–64)	32 (4–64)	8 (2–16)	6 (2–16)	1.5 (0–32)	360 (112–768)
Urine pH 8.0						
E. coli ATCC 25922	>2,048 (256 to >2,048)	2,560 (256 to >2,048)	192 (64–512)	64 (32–256)	8 (4–16)	28,160 (2,688–37,888)[1]
E. coli 523 (NR)	64 (32–128)	48 (16–128)	8 (4–16)	6 (4–8)	0 (0–4)	552 (256–1,184)[1]
E. coli MI-4 (NR)	12 (4–32)	4 (1–16)	1.5 (0–4)	1 (0–4)	0 (0–4)	82 (20–256)[1]
E. coli MI-3-1-M4 (FR)	0 (0–2)	0 (0–1)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–12)[1]
E. coli 1135121 (FR)	3 (0–8)	4 (0–8)	0.5 (0–2)	0 (0–2)	0 (0–2)	30 (0–96)[1]
E. coli 1949820 (FR)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)
K. pneumoniae 595	128 (32–512)	128 (32–512)	24 (8–64)	16 (8–128)	2 (1–64)	1,312 (384–5,888)[1]
P. mirabilis 414	16 (8–64)	32 (8–128)	5 (2–32)	2 (2–16)	0 (0–16)	236 (96–1,088)[1]
P. aeruginosa 568	8 (4–32)	6 (2–32)	0 (0–8)	0 (0–4)	0 (0–8)	56 (24–336)[1]
E. faecalis 60	32 (16–128)	32 (8–128)	6 (4–32)	4 (2–16)	0 (0–16)	328 (136–1,344)

Values are medians with ranges in parentheses. NR = Nalidixic acid resistant; FR = fluoroquinolone resistant.
[1] Significantly different ($p < 0.05$, paired t test) for $AUBT_{24}$ in urine at pH 8.0 vs. urine at pH 5.5.

The median $AUBT_{24}$ of the fluoroquinolone-resistant strains *E. coli* 1949820 and *E. coli* MI-3-1-M4 (both of which harbor mutations conferring fluoroquinolone resistance) as well as *P. aeruginosa* approximated 0 in urine at the two pH values and in native urine.

Following the 800-mg dose, the median $AUBT_{24}$ of the susceptible *E. coli* (ATCC 25922), the nalidixic acid-resistant *E. coli* 523 and *E. coli* MI-4, the borderline fluoroquinolone-resistant *E. coli* 1135121, and wild-type clinical isolates *K. pneumoniae, P. mirabilis* and *P. aeruginosa* ranged from 70 to 35,840 h in urine at pH 5.5, and from 30 to 28,160 h in urine at pH 8.0. The fluoroquinolone-resistant *E. coli* MI-3-1-M4 showed an $AUBT_{24}$ of 0 h in urine at pH 8.0. The median $AUBT_{24}$ in native urine were similar to those in urine adjusted to a pH of 5.5 and ranged from 30 to 41,984 h. Only *E. coli* 1949820 (which was resistant to ciprofloxacin with an MIC of 16 mg/l) was not affected ($AUBT_{24}$ = 0 h) following the 800-mg dose.

The median $AUBT_{24}$ for *E. faecalis* were similar in acidic and alkaline urine following both the 200- and the 800-mg doses, with a slightly increased activity in the acidic environment. The median $AUBT_{24}$ was 70–72 h following the 200-mg dose, and amounted to 328–360 h following the 800-mg dose. The activity of both doses against *E. faecalis* in native urine was higher than in ei-

ther acidic or alkaline urine, with median $AUBT_{24}$ of 120 and 624 h for the 200- and 800-mg doses, respectively.

The UBT and $AUBT_{24}$ values in urine with pH 5.5 were statistically comparable to those measured in native urine. While this finding correlates well with the almost always slightly acidic mean pH values (tables 1, 2) measured in native urine in both study groups, it was unexpected, given the fact that the pH of the native urine in all groups never reached values as low as 5.5 and in general was much closer to neutrality. In general, mean UBT and $AUBT_{24}$ were significantly lower ($p < 0.05$) in alkalinized urine than in acidified urine, except for *E. faecalis,* where only a slightly lower activity in alkaline conditions was observed.

Discussion

Finafloxacin is a novel developmental fluoroquinolone belonging to a new 8-cyano subclass; the finafloxacin molecule contains a novel base side chain which confers improved antibacterial activity at slightly acidic pH values [11]. Due to its increased antibacterial activity at pH values below neutral, finafloxacin is presently being developed for indications in the hospital and critical care setting such as treatment of UTI and other infections that produce or reside in a low pH environment. The enhanced activity at low pH values contrasts with that of marketed quinolones, which act best at a slightly basic pH and lose antibacterial activity in vitro and in vivo under acidic conditions [8, 22, 23]. In vitro experiments comparing finafloxacin and a range of marketed fluoroquinolones have shown similar activities against uropathogens at a physiologically neutral pH (7.4) but showed markedly increased activity in acidic conditions (pH <7) for finafloxacin [9, 10].

This study was carried out in order to verify or falsify these in vitro findings in an ex vivo pharmacokinetic/pharmacodynamic (PK/PD) setting. First, the MIC at acidic, near neutral and alkaline pH values were determined; second, the urinary concentrations and the urinary bactericidal activity of finafloxacin were evaluated.

The data generated in this study demonstrate that the antibacterial activity of finafloxacin in CAMHB increased as the pH value decreased. In artificial urine (pH 5.8), finafloxacin MIC remained almost unchanged as compared to MIC in CAMHB at pH 7.2 despite the high levels of divalent cations which inactivate most of the commercially available quinolones like ciprofloxacin. This finding may indicate that the high concentrations of Ca^{2+} and Mg^{2+} may counteract the gain in activity mediated by the shift in pH values.

Fluoroquinolones are considered to act concentration dependently. The area under the curve (AUC)/MIC ratios as well as peak/MIC ratios have been used to describe fluoroquinolone pharmacodynamics [24–27]. Fluoroquinolones usually achieve high but strongly variable urinary concentrations [28, 29]. Because of their pharmacodynamics, high urine concentration should translate into high antibacterial activity. However, the activities of commercially available quinolones are substantially reduced in urine-supplemented media or pooled urine, with MIC higher by a factor of up to 64 than those obtained in standard laboratory test conditions. Higher urinary concentrations of magnesium, which are commonly in the range of 8–10 mM, in combination with a low pH value account in large part for this reduction in activity [7, 22, 23]. For the treatment of UTI, the bactericidal activity of an antibacterial agent measured in urine is therefore considered to be a helpful pharmacodynamic parameter [16, 17, 30] that has also been shown to be accurate and reproducible [31]. The urine AUC/minimal bactericidal concentration (MBC) ratio relates directly to the AUBT because the reciprocal UBT indicate the urinary concentrations divided by the MBC as determined in urine. For illustration, AUC_{24}/MIC (CAMHB at pH 7.2 and synthetic urine) values are compared with $AUBT_{24}$ levels in table 7. Due to the inherent turbid nature of urine, for more exact determination, the evaluation of MBC is preferred over MIC in urine. UBT and AUBT are therefore suggested as prognostic PK/PD parameters for antibiotic treatment of UTI, which could be shown already for levofloxacin in part of a clinical study [32]. In this study, UBT values of 100 and higher ($AUBT_{24}$: 2,200 h and higher) were all correlated with clinical and/or microbiologic success, whereas UBT lower than 10 ($AUBT_{24}$: 220 h and lower) were clinical and/or microbiologic failures [32].

The mean concentrations of finafloxacin in urine were between approximately 68 mg/l in the first 4 h and 4 mg/l during the 12- to 24-hour sampling period following the 200-mg dose, and 112 mg/l in the first 4 h (peak of 150 mg/l was reached in the 4- to 8-hour interval) and 18 mg/l during the 12- to 24-hour sampling period following the 800-mg dose. Urinary recovery, however, was approximately 30% after both doses, suggesting also other routes of elimination such as transintestinal elimination, as has been shown for ciprofloxacin as well [33].

Following the 200-mg dose, a bactericidal effect was seen up to 48 h after dosing only against *E. coli* ATCC and

Table 7. Urinary AUC_{24}/MIC values (MIC measured in synthetic urine and CAMHB at pH 7.2)[1] compared with $AUBT_{24}$ levels in native urine

	$AUBT_{24}$	Urine AUC_{24}/MIC synthetic urine	Urine AUC_{24}/ MIC CAMHB pH 7.2
Finafloxacin 200 mg			
E. coli ATCC 25922	24,064 (7,424–98,304)	11,687 (7,288–32,083)	23,374 (14,575–64,167)
E. coli 523 (NR)	888 (720–2,176)	175 (109–481)	351 (219–963)
E. coli MI-4 (NR)	90 (0–184)	88 (55–241)	88 (55–241)
E. coli MI-3-1-M4 (FR)	0 (0–4)	6 (3–15)	11 (7–30)
E. coli 1135121 (FR)	40 (0–84)	11 (7–30)	44 (27–120)
E. coli 1949820 (FR)	0 (0–4)	6 (3–15)	6 (3–15)
K. pneumoniae 595	3,232 (928–39,936)	1,402 (875–3,850)	23,374 (14,575–64,167)
P. mirabilis 414	298 (72–736)	88 (55–241)	351 (219–963)
P. aeruginosa 568	0 (0–144)	175 (109–481)	88 (55–241)
E. faecalis 60	120 (60–464)	175 (109–481)	175 (109–481)
Finafloxacin 800 mg			
E. coli ATCC 25922	41,984 (3,840–55,296)	38,115 (22,503–96,407)	76,230 (45,005–192,813)
E. coli 523 (NR)	2,944 (1,184–5,376)	572 (338–1,446)	1,143 (675–2,892)
E. coli MI-4 (NR)	504 (144–926)	286 (169–723)	286 (169–723)
E. coli MI-3-1-M4 (FR)	30 (0–160)	18 (11–45)	36 (21–90)
E. coli 1135121 (FR)	146 (16–336)	36 (21–90)	143 (84–362)
E. coli 1949820 (FR)	0 (0–8)	18 (11–45)	18 (11–45)
K. pneumoniae 595	9,472 (3,072–13,312)	4,574 (2,700–11,569)	76,230 (45,005–192,813)
P. mirabilis 414	448 (272–1,536)	286 (169–723)	1,143 (675–2,892)
P. aeruginosa 568	568 (192–928)	572 (338–1,446)	286 (169–723)
E. faecalis 60	624 (40–1,344)	572 (338–1,446)	572 (338–1,446)

Values are medians with ranges in parentheses. NR = Nalidixic acid resistant; FR = fluoroquinolone resistant.
[1] See table 4.

K. pneumoniae. For nalidixic acid-resistant *E. coli* and *P. mirabilis,* median UBT indicate bactericidal effects up to 24 h, and for two ciprofloxacin-resistant *E. coli* strains (MI-4 and 1135121), up to 8 and 12 h, respectively. For the two other highly ciprofloxacin-resistant *E. coli* strains (MI-3-1-M4 and 1949820) and the *P. aeruginosa* strain, median UBT were 0 during the study period.

Following the 800-mg dose, bactericidal activity for up to 48 h was additionally observed against nalidixic acid-resistant *E. coli* 523, *P. mirabilis, E. faecalis* and even the borderline ciprofloxacin-susceptible *E. coli* MI-4. Bactericidal effects up to 24 h were additionally observed against the ciprofloxacin-resistant *E. coli* 1135121 and *P. aeruginosa*. The only strain against which no bactericidal activity was observed following the 800-mg dose was the highly ciprofloxacin-resistant *E. coli* 1949820.

The bacterial physiology of bacteria causing acute or chronic infections and of bacteria growing in mucus layers or in biofilms is quite different. Bacteria growing in biofilms are much less susceptible than their planktonic counterparts; on the other hand, *E. coli* growing in mouse bladder mucus was much more susceptible than the same strain growing in broth [34]. Finafloxacin has been shown to exhibit bactericidal activity against adherent bacterial populations and planktonic, slowly growing *E. coli,* as demonstrated by a reduction of the exposed populations by a greater extent than equivalent concentrations of the comparator fluoroquinolones ciprofloxacin and levofloxacin [35]. Although systematic studies on the antibiotic susceptibility of bacterial pathogens in e.g. urine, bladder mucus and biofilms are missing, it is conceivable that PK/PD surrogate parameters will be different for different pathogens and different pathophysiological conditions. It was convincingly demonstrated that a good concordance between in vitro data, studies in infected animals and data from infected patients for a given disease/pathogen association does exist [32]. The PK/PD targets derived from the preclinical and clinical studies are indication, pathogen and drug specific.

In the current study, the experiments were performed on native, acidified (pH 5.5) and alkalinized urine (pH 8.0), urine pH values physiologically seen in healthy persons (tables 1, 2) [16, 36]. In infected patients, the urine pH is usually more in the acidic range (pH 6.0 ± 0.9) [3], with the exception of UTI due to urease-producing bacteria such as *Proteus* spp., *Providencia* spp. or *Morganella* spp. In alkaline urine at a pH of 8.0, however, the observed UBT and $AUBT_{24}$ were significantly lower than those in urine at a pH of 5.5, with the exception of *E. faecalis.* No bactericidal action was measured against the ciprofloxacin-resistant *E. coli* MI-3-1-M4 and 1949820 and *P. aeruginosa* following the 200-mg dose, and against *E. coli* 1949820 following 800 mg.

The results regarding the antibacterial activities of finafloxacin in urine generated in this study are well in agreement with previously published data on commercially available fluoroquinolones. By using the same indicator organisms, i.e. *E. coli* ATCC 25922, *E. coli* 523 NalR, *K. pneumoniae* 595, *P. mirabilis* 414, *P. aeruginosa* 568 and *E. faecalis* 60, the $AUBT_{24}$ calculated for ciprofloxacin at the pH values of freshly voided urine following an oral dose of 500 mg were within the same range as, or tended to be lower than, those calculated for 800 mg finafloxacin [16, 30, 31, 37]. A considerable intra- and interstudy variability is evident from the published data. For example, in three different studies, the mean ciprofloxacin $AUBT_{24}$ for *E. coli* 523 NalR ranged from 528 to 1,632 h, and those for *E. coli* ATCC 25922 ranged from 7,680 to 12,288 h [16, 31, 37]; the intrastudy variability was also comparably high, ranging from 342 to 996 h for *E. coli* ATCC 25922, and from 120 to 570 h for *E. coli* 523 NalR [16]. This high variability of UBT and AUBT values is probably due to the highly variable urinary pH values affecting activity, as well as highly variable urinary concentrations recorded in this and all other UBT studies quoted. Furthermore, this variability mirrors the broad range of urinary recoveries and CLR (15.5–28.7 liters/h per 1.73 m^2) reported for ciprofloxacin [29]. Thus finafloxacin warrants an in-depth analysis of its clinical efficacy. The pharmacodynamic properties of finafloxacin, its physicochemistry and its current safety profile make this new 8-cyano-fluoroquinolone suitable for further clinical assessment.

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