48th ICAAC / 46th IDSA, Washington DC 2008

F1-2049

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

F. M. E. WAGENLEHNER[1], C. WAGENLEHNER[2], B. BLENK[2], H. BLENK[2], W. STUBBINGS[3], H. PATEL[3], S. SCHUBERT[4], A. DALHOFF[4], **K. G. NABER**[2];

[1]Dept. Urology, Justus Liebig-Univ., Giessen, Germany, [2]Eukarion Inst., Nuremberg, Germany, [3]MerLion Pharmaceuticals GmbH, Berlin, Germany, [4]Univ. Hosp. Schleswig-Holstein, Kiel, Germany.

Contact information:

KURT G. NABER
Karl Bickleder Str. 44C
94315 Straubing
Germany

kurt.naber@nabers.de

Revised abstract

Background: FIN is a novel fluoroquinolone (FQ) belonging to a new 8-cyano subclass. FIN exhibits optimal efficacy at slightly acidic pH (5.0 - 6.0), under which other FQs lose activity. Therefore, FIN is intended for bacterial infections associated with a usually acidic environment (such as UTI). This study assessed the urinary pharmacokinetics (PK) and bactericidal activity of FIN in 6 healthy volunteers receiving a single, 800 mg oral dose.

Methods: Urinary concentrations were determined over a 24h period. Urinary bactericidal titers (UBTs) were defined as the highest dilution of subject urine (following dilution in antibiotic free urine) that exhibited bactericidal activity. UBTs were determined at intervals over 24h to produce an area under the 24h UBT time-curve (AUBT) for FIN in native and acidified urine against 1 test strain (*E.coli* ATCC 25922) and 6 ciprofloxacin (CIP) susceptible uropathogens.

Results: The mean (median) maximum concentration of FIN in urine was 150 (137) mg/L at 4 to 8 hours.

Median UBT (0-4h) and AUBT 24h [h] of FIN against 1 test strain and 6 uropathogens

Strain	CIP MIC (in CAMH broth) pH7.2 (µg/mL)	CIP MIC (in artificial urine pH 5.8) (µg/mL)	FIN MIC (in artificial urine pH 5.8) (µg/mL)	UBT* (0-4h)	AUBT* [h] 24h native urine	AUBT* [h] 24h urine pH 5.5
E. coli ATCC25922	≤0.004	1	0.03	>2048	41,984	35,840
E. coli #523 (NR)	0.060.03	16	2	384	2,944	3,264
E. coli MI-4	V0.5	128	4	48	504	488
K. pneumoniae #95	≤0.125	2	0.25	512	9,472	8,448
P. mirabilis #414	≤0.004	2\1	4	32	448	552
P. aeruginosa #568	0.25	8	2	48	568	776
E. faecalis #60	2	16	2	64	624	360

*Reciprocal values

Conclusions: FIN (800mg) exhibits bactericidal activity in ex vivo urine against a range of UTI pathogens and warrants further investigation for this indication.

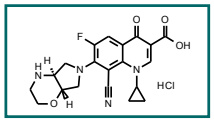
Introduction

Finafloxacin (FIN, Figure 1) is a broad spectrum fluoroquinolone (FQ) that belongs to a new 8-cyano subclass [1]. FIN contains a novel chiral base component which confers improved antibacterial activity at slightly acidic pH (pH 5.0 – 6.0) under which other marketed FQs exhibit significantly reduced activity [2].

FIN exhibited superior activity to comparator FQs against adherent bacteria *in vitro* [3] and in a wide range of rodent infection models [4,5]. Additionally, FIN displayed an excellent safety profile in a wide range of predictive, *in vitro*, toxicity assays [6] and was well tolerated in healthy human volunteers [7]. These attributes suggest that FIN warrants clinical investigation for bacterial infections that are usually associated with low pH environments such as urinary tract infection (UTI) and *Helicobacter pylori* eradication.

The urinary pharmacokinetics (PK) of FIN were determined in six healthy volunteers following an oral dose of 800mg. Bactericidal activity of FIN in *ex vivo* urine from these subjects was then quantified against a panel of UTI pathogens.

Figure 1.
Finafloxacin hydrochloride.



Methods

Study design and subjects: Urine from six healthy volunteers [7] was collected before and following an oral dose of 800mg FIN according to the following time intervals: 0 to 2, 2 to 4, 4 to 8, 8 to 12, 12 to 24, and 24 to 48 hours. Urine from 0 to 4 hours was then pooled for bactericidal titer determinations. Absence of antibacterial activity in pre-dose urine was shown by the lack of inhibition of *Bacillus subtilis*.

Drug concentrations in urine: The concentration of FIN in urine samples was measured by HPLC and tandem (MS/MS) mass spectrometry using a concentration range of FIN for calibration.

Determination of MIC: Minimal inhibitory concentrations (MICs) of FIN, levofloxacin (LVX) and ciprofloxacin (CIP) were determined by broth microdilution procedures according to the CLSI at pH 5.8, 7.2 and 8.0. MICs were also determined in synthetic urine medium, pH 5.8 [8].

Determination of UBTs and $AUBT_{24h}$: Two-fold serial dilutions of urine samples were prepared in antibacterial-free urine and used to prepare a microdilution test with an inoculum of 2.4×10^5 to 6.1×10^5 CFU/mL. Bactericidal activity in urine was determined by plating 10 µL of each well onto fresh agar. Urinary bactericidal titers (UBTs) were defined as the reciprocal value of the highest dilution to produce a >99.9% (>3 10_{log}) reduction of the initial count. The area under the UBT-versus-time (24h) curve ($AUBT_{24h}$) was calculated as the sum of the products of the UBTs and the respective time intervals for 24h post-dose for each test organism and drug. Data analysis has been described previously [9].

Results and Discussion

Susceptibility

MIC data for FIN, CIP and LVX against the 10 test organisms in CAMHB at various pH and in synthetic urine, pH 5.8 are shown in Table 1. In standard growth media, FIN was, on average, 4 – 8-fold more active at pH 5.8 compared to at pH 7.2 whereas CIP and LVX exhibited an 8 – 16-fold reduction in activity under conditions of lower pH.

The net difference in activity between FIN and CIP (or LVX) at pH 5.8 was, on average, 8 – 64-fold in favor of FIN against all strains tested except *P. mirabilis* and *P. aeruginosa* against which the activities were equipotent.

FIN MICs were, on average, at least 32-fold lower than CIP or LVX in synthetic urine. This effect may be partly due to the lower pH of this medium (which is optimal for FIN activity) however, this does not account for all of the difference. For example, against *E. coli* ATCC 25922, FIN is 8-fold more active than CIP in CAMHB pH 5.8, but 32-fold more active in synthetic urine (also pH 5.8). It is possible that the activity of FIN is less affected by components of urine than CIP and LVX.

Urinary pharmacokinetics

Urinary parameters and pharmacokinetics following 800mg dose are summarized in Table 3. FIN reached mean peak levels of 150 mg/L in urine collected between 4 – 8 h and remained above the MIC (as determined in synthetic urine) for the tested strains (with the exception of *E. coli* with synthetic urine FIN MICs of ≥32 mg/L), for 48h.

Results and Discussion

Bacteria	Minimum Inhibitory Concentration [mg/L]											
	CAMHB pH 5.8			CAMHB pH 7.2			CAMHB pH 8.0			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	0.0075	0.004	0.015	0.03	0.004	0.0075	0.03	1	2	0.03
***E. coli* #523 (CIP^{RS})**	2	2	0.03	0.06	0.125	1	0.03	0.03	2	16	16	2
***E. coli* #MI-4 (CIP^{RS})**	8	8	0.5	1	0.5	4	0.25	0.5	16	128	64	4
***E. coli* #MI-3-1-M4 (CIP^{R})**	64	32	4	4	4	32	1	2	128	128	128	64
***E. coli* #1135121 (CIP^{R})**	32	16	2	2	2	8	0.5	2	32	128	128	32
***E. coli* #1949820 (CIP^{R})**	128	64	8	16	8	64	8	8	128	128	128	128
***K. pneumoniae* #595**	0.06	0.125	0.015	0.125	0.0075	0.015	0.004	0.0075	0.06	2	2	0.25
***P. mirabilis* #414**	0.125	0.25	0.25	0.004	0.015	1	0.0075	0.03	1	1	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

Table 1. Minimum inhibitory concentration (MIC) of FIN, CIP and LVX against *E. coli* exhibiting a range of susceptibilities to fluoroquinolones and wild type *K. pneumoniae*, *P. mirabilis*, *P. aeruginosa* and *E. faecalis*. MICs were determined in CAMHB at different pH (5.8, 7.2 and 8.0) and in synthetic urine, pH 5.8. CIP^{RS}: ciprofloxacin - borderline susceptible, CIP^{R}: ciprofloxacin resistant, as determined under standard MIC test conditions.

Strain	$AUBT_{24h}$ [h]		
	Native urine	Acidified (pH 5.5) urine	Alkaline (pH 8.0) urine
***E. coli* ATCC 25922**	**41,984** (3,840 - 55,296)	**35,840** (3,840 - 98,304)	**28,160** (2,688 - 37, 888)
***E. coli* #523 (CIP^{RS})**	**2,944** (1,184 - 5,376)	**3,264** (1,184 - 11,264)	**552** (256 - 1,184)
***E. coli* MI–4 (CIP^{RS})**	**504** (144 - 926)	**488** (136 - 1,664)	**82** (20 - 256)
***E. coli* MI-3-1-M4 (CIP^{R})**	**30** (0 - 160)	**70** (8 - 232)	**0** (0 - 12)
***E. coli* 1135121 (CIP^{R})**	**146** (16 - 336)	**154** (40 - 544)	**30** (0 - 96)
***E. coli* 1949820 (CIP^{R})**	**0** (0 - 8)	**0** (0 - 12)	**0** (0 - 0)
***K. pneumoniae* #595**	**9,472** (3,072 - 13,312)	**8,448** (4,096 - 37,888)	**1, 312** (384 - 5,888)
***P. mirabilis* #414**	**448** (272 - 1,536)	**552** (192 - 1,920)	**236** (96 - 1,088)
***P. aeruginosa* #568**	**568** (192 - 928)	**776** (160 - 4,352)	**56** (24 - 336)
***E. faecalis* #60**	**624** (40 - 1,344)	**360** (112 - 768)	**328** (136 - 1,344)

Table 2. Bactericidal activity ($AUBT_{24h}$) of FIN in *ex vivo* urine sampled over a 24h period from six healthy volunteers following 800mg oral dose. $AUBT_{24h}$ were calculated as the sum of the products of the UBTs and the respective time intervals for each test organism and for each drug.

Collection period (hours)	Urinary parameters - Mean ± SD (median; range)		
	Urinary pH	Volume [mL]	Concentration [mg/L]
0-2	6.8 ± 0.8 (6.8; 5.6-7.8)	792 ± 351 (850; 350-1,200)	114 ± 71.1 (90; 38-242)
2 - 4	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	7.1 ± 0.3 (7.1; 6.5-7.4)	442 ± 228 (395; 220-820)	150 ± 90.6 (137; 44-256)
8 - 12	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	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	7.0 ± 0.6 (6.9; 6.3-8.0)	2,333 ± 1087 (2,450; 750-3,550)	13.6 ± 28.9 (1.9; 0.7-72.3)

Table 3. Mean (median; range) urinary pH, volume and finafloxacin concentrations from six volunteers, at timed collection periods following 800mg oral dose of FIN.

Table 4. Urinary bactericidal activity of FIN in *ex vivo* urine from six healthy volunteers following 800mg dose. Organisms tested included *E. coli* exhibiting a range of susceptibilities to fluoroquinolones (see Table 1) and wild type *K. pneumoniae*, *P. mirabilis*, *P. aeruginosa* and *E. faecalis*. CIP^{RS}: ciprofloxacin - borderline susceptible, CIP^{R}: ciprofloxacin resistant, as determined under standard MIC test conditions.

Strain	Urinary bactericidal titer (0 - 4h)			Urinary bactericidal titer (12 - 24h)		
	Native urine	Acidified Urine (pH 5.5)	Alkaline Urine (pH 8.0)	Native urine	Acidified Urine (pH 5.5)	Alkaline Urine (pH 8.0)
***E. coli* ATCC 25922**	**>2,048** (256 - >2,048)	**>2,048** (256 - >2,048)	**>2,048** (256 - >2,048)	**512** (128 - 512)	**512** (64 - >2048)	**64** (32 - 256)
***E. coli* #523 (CIP^{RS})**	**384** (128 - 512)	**256** (128 - 512)	**64** (32 - 128)	**32** (8 - 64)	**48** (8 - 256)	**6** (4 - 8)
***E. coli* MI–4 (CIP^{RS})**	**48** (16 - 64)	**48** (8 - 128)	**12** (4 - 32)	**6** (0 - 8)	**6** (2 - 32)	**1** (0 - 4)
***E. coli* MI-3-1-M4 (CIP^{R})**	**3** (0 - 16)	**8** (2 - 16)	**0** (0 - 2)	**0** (0 - 2)	**0.5** (0 - 2)	**0** (0 - 0)
***E. coli* 1135121 (CIP^{R})**	**16** (1 - 32)	**12** (8 - 32)	**3** (0 - 8)	**2.5** (0 - 4)	**1.5** (0 - 8)	**0** (0 - 2)
***E. coli* 1949820 (CIP^{R})**	**0** (0 - 1)	**0** (0 - 1)	**0** (0 - 0)	**0** (0 - 0)	**0** (0 - 0)	**0** (0 - 0)
***K. pneumoniae* #595**	**512** (256 - 1024)	**512** (512 - >2048)	**128** (32 - 512)	**192** (64 - 256)	**192** (64 - 256)	**16** (8 - 128)
***P. mirabilis* #414**	**32** (16 - 128)	**48** (16 - 64)	**16** (8 - 64)	**8** (4 - 32)	**6** (4 - 32)	**2** (2 - 16)
***P. aeruginosa* #568**	**48** (16 – 64)	**64** (16 – 256)	**8** (4 – 32)	**6** (4 – 8)	**6** (2 – 64)	**0** (0 – 4)
***E. faecalis* #60**	**64** (4 - 128)	**32** (16 - 64)	**32** (16 - 128)	**8** (0 - 16)	**6** (2 - 16)	**4** (2 - 16)

Urinary bactericidal activity

Urinary bactericidal titers (UBTs) of FIN in native, acidified (pH 5.5) and alkaline (pH 8.0) urine, from the initial period after dosing ($UBT_{0 - 4h}$) and in urine sampled 12 – 24h after dosing ($UBT_{12 - 24h}$) are shown in Table 4.

These data demonstrate the bactericidal activity of FIN in *ex vivo* urine, voided between 0 – 4h after dosing, from healthy volunteers. Bactericidal activity was evident against the series of UTI pathogens with the exception of *E. coli* #1949820 (CIP MIC; 16mg/L). Bactericidal activity, as indicated by positives UBTs was also evident in urine collected between 12 – 24h after dosing with the additional exception of *E. coli* MI-3-1-M4 (CIP MIC; 4mg/L). This indicated that urinary FIN concentrations remained at bactericidal levels (for the indicated strains) up to and including the 12-24h period. This is in agreement with the urinary concentrations of FIN (Table 3).

The bactericidal activity of FIN was similar in native and acidified (pH 5.5) urine and slightly lower in alkaline urine (pH 8.0). The degree of bactericidal activity, as determined by UBT and $AUBT_{24h}$ (Table 2), roughly corresponded with the FIN MICs of these strains in synthetic urine

Positive $AUBT_{24h}$ values were determined for all tested strains except *E. coli* #1949820 (CIP MIC; 16mg/L). These data may prove useful in determining target attainment levels that are associated with sucessful treatment of UTI and complicated UTI.

Conclusions

- FIN exhibited superior antibacterial activity to CIP and LVX in synthetic urine medium against a panel of uropathogens.
- FIN was well tolerated in six healthy volunteers receiving a single 800mg dose and reached a mean peak urinary concentration of 150 mg/L.
- FIN remained above the MIC for the tested strains (except those with an MIC in synthetic urine of ≥32 mg/L) in the urine for 48h after dosing.
- This corresponded with the urinary bactericidal activity of FIN, demonstrated by positive UBT and $AUBT_{24h}$ values, against these strains.

Literature

[1] Wohlert *et al.*, 48th ICAAC, Washington DC 2008, Poster No. F1-2036.
[2] Kresken *et al.*, 48th ICAAC, Washington DC 2008, Poster No. F1-2037.
[3] Goh *et al.*, 48th ICAAC, Washington DC 2008, Poster No. F1-2042.
[4] Endermann *et al.* 48th ICAAC, Washington DC 2008, Poster No. F1-2044.
[5] Endermann *et al.*, 48th ICAAC, Washington DC 2008, Poster No. F1-2045.
[6] Schmuck *et al.*, 48th ICAAC, Washington DC 2008, Poster No. F1-2047.
[7] Patel *et al.*, 48th ICAAC, Washington DC 2008, Poster No. F1-2048.
[8] Investigative Urology. Griffith D.P. & Musher D.M. 1976 13 (5): 346-350.
[9] Wagenlehner *et al.*, (2006). *AAAC* **50(11):** p.3947-49.