48th ICAAC / 46th IDSA,
Washington DC 2008

F1- 2036

New Fluoroquinolone Finafloxacin HCl (FIN): Route of Synthesis, Physicochemical Characteristics and Activity under Neutral and Acid Conditions

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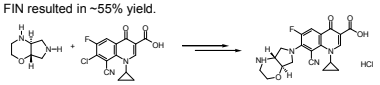
Revised Abstract

Background: FIN, a novel fluoroquinolone (FQ), is a representative of a new 8-cyano subclass. FIN exhibits optimal efficacy at slightly acidic pH under which other FQs lose activity. FIN is therefore intended for therapeutic use against bacterial infections in acidic environments, e.g. *H. pylori* eradication, UTI.

Methods: I and II were synthesized and combined to III (FIN). Physicochemical characterization was performed by; NMR, X-ray, HPLC (solubility), titration (ionisation constants). MICs were determined using CLSI methodology for broth microdilution at different pH.

Results: I and II were synthesized in 7 steps at ~25% and ~30% yield, respectively. Coupling of I and II and subsequent crystallization into FIN resulted in ~55% yield.



Characterization of FIN included elucidation of the chemical and crystal structure, determination of solubility (mg/mL; 5.5 (pH 7), 1.9 (pH 4.5)) and ionisation constants (pK_{a1}=5.6, pK_{a2}=7.8). FIN MICs (mg/L) against *E. coli* ATCC 25922 and *S. aureus* ATCC 29213 were 0.06 and 0.25 (pH 7.2) and 0.008 and 0.06 (pH 5.8), respectively.

Conclusions: FIN displays exceptional antibacterial activity at low pH, unlike other FQs, making it a prime candidate to treat infections in acidic environments, such as the gastrointestinal or urogenital tract.

Introduction

Finafloxacin (FIN, Figure 1) is a novel, broad spectrum fluoroquinolone (FQ) that belongs to a new 8-cyano subclass. 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 [1].

FIN exhibited superior activity to comparator FQs against adherent bacteria *in vitro* [2] and in a wide range of rodent infection models [3,4]. Additionally, FIN displayed an excellent safety profile in a wide range of predictive, *in vitro,* toxicity assays [5] and was well tolerated in healthy human volunteers [6]. These attributes suggest that FIN warrants clinical investigation for bacterial infections that are associated with low pH such as urinary tract infection and *Helicobacter pylori* eradication.

The present study was performed to determine the physiochemical characteristics of FIN and the effect of pH on its basic antibacterial activity.

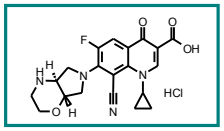


Figure 1. Finafloxacin hydrochloride (FIN).

Methods

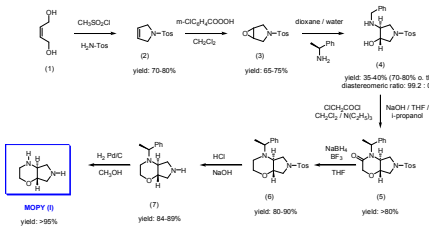
Chemistry: Finafloxacin HCl (FIN, III) was synthesized by combining MOPY,-(1*S*,6*S*)-Morpholinopyrrolidine (I), with Cyano-FQA, 7-chloro-8-cyano-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic acid (II), followed by crystallization to the hydrochloride in two steps with a ~55% overall yield. The two components, MOPY (I) and Cyano-FQA (II) were prepared in 7-step syntheses each, with ~25% yield and 30% yield, respectively. The synthesis of MOPY (I) started from 2-butendiol (1) and p-toluenesulfonamide to form 1-tosylpyrroline (2), which was converted into epoxide (3) by 3-chloro-perbenzoic acid. Chirality was introduced by opening the epoxide ring with (*S*)-1-phenylethylamine and retrieval of the desired diastereomere (4) by crystallization. Oxo-morpholine (5) was synthesized by acylation of (4) with chloro-acetylchloride and subsequent cyclization. (5) was reduced to (6) with a sodium borhydride bortrifluoride-THF-complex prior to de-tosylation to (7) and final hydrogenation to MOPY (I). The Cyano-FQA (II) synthesis started with fluoro-*m*-xylene (8) reacting to (9), which was chlorinated to form hepta-chloro-xylene (10) under UV-irradiation. Starting from (10) formylbenzoic acid (11) and the corresponding cyanobenzoic acid (12) were subsequently formed before cyano-benzoyl-chloride (13) resulted from reacting with thionylchloride. Esterification of (13) with β-ethyl-3-dimethylaminoacrylate (β-DAASE), reaction with cyclopropyl-amine followed by cyclization lead to (14), which after acidic ester-hydrolysis yielded Cyano-FQA (II).

Physicochemical characterisation: Chemical and physical properties of FIN (III) were determined by 1- and 2D-NMR as well as X-ray crystal analysis, using standard methods. Polymorphism studies comprised spectroscopical (IR, Raman, X-ray powder diffraction), thermal (differential scanning cloriometry, thermomicroscopy, thermogravimetry) and hygroscopical (dynamic vapor sorption) analysis of various crystallization experiments (phase equilibration, evaporation, vapor diffusion, precipitation, drying, desolvation).

***In vitro* activity:** All minimum inhibitory concentration (MICs) were performed using CLSI methodology for broth microdilution with the pH being adjusted to 7.2 or 5.8 with 1M NaOH or 1M HCl.

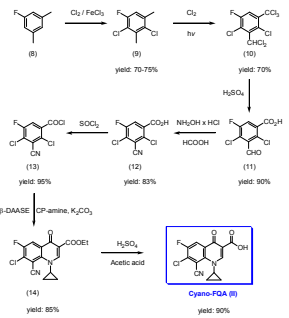
Results and Discussion

Scheme 1. Synthesis of the MOPY (I) building block.



Results and Discussion

Scheme 2. Synthesis of the Cyano-FQA (II) building block.



Physicochemical characteristics of FIN (III).

Description
white to yellowish substance

Average molecular weight
434.8545 g/mol - $C_{20}H_{19}FN_4O_4$ x HCl

Optical purity
α^{20}_D: -129° (based on dried substance)

Water content (25°C, 60% rh; 40°C, 75% rh; in PE bags)
~ 7.7% or ~2x H_2O per molecule FIN (III)

Table 1. *Solubility* (at 25±5 °C)

Solvent	mg/mL
Water	5.5
Water, pH 4.5	1.9

Table 2. *Partition coefficients (P)*

System	log P
Octanol/Water	-1.5
Octanol/pH1	-1.7
Octanol/pH7	-0.6

Table 3. *Ionisation constants* (by potentiometric titration)

	pK_a values
pK_{a1} (carboxylate function)	5.6
pK_{a2} (nitrogen at C7 substitute)	7.8

Polymorphism

FIN (III) crystallized in at least 9 modifications (2 non solvated and 7 solvated forms) with the dihydrate and an anhydrate form as the most stable and preferred variants under ambient conditions.

Scheme 3. Synthesis of FIN (III).

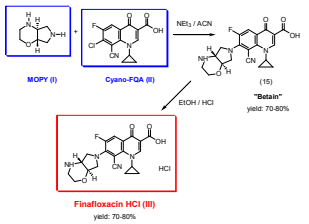
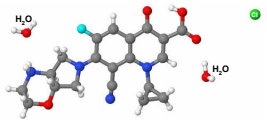
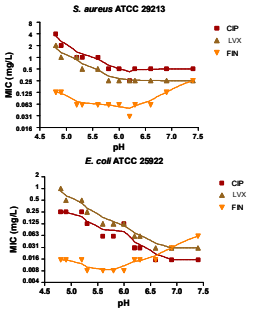


Figure 2. X-ray crystal structure of FIN (III).



Antibacterial properties of FIN (III).

Figures 3 and 4. pH dependant MIC.



pH dependent MIC: MICs of FIN (III), ciprofloxacin (CIP) and levofloxacin (LVX) were determined over a range of pH. The antibacterial activity of FIN was optimal between pH 5 – pH 6, under which the activities of CIP and LVX were significantly lower.

Table 5. Antibacterial activity of FIN (III) compared to ciprofloxacin (CIP) and levofloxacin (LVX) against a panel of pathogenic bacteria.

Organism	Strain	MIC (mg/L)					
		FIN		CIP		LVX	
		pH 7.2	pH 5.8	pH 7.2	pH 5.8	pH 7.2	pH 5.8
E. coli[1]	ATCC 25922	0.06	0.0078	0.015	0.06	0.03	0.125
E. coli[1]	ATCC 700928	0.125	0.015	0.015	0.125	0.03	0.25
E. coli[1]	ATCC 10536	0.125	0.0078	0.0078	0.03	0.03	0.125
K. pneumonia[2]	52145	0.125	0.03	0.03	0.25	0.06	0.5
P. aeruginosa[3]	ATCC 27853	8	1	0.25	1	1	2
P. aeruginosa[3]	PA01	4	0.5	0.125	0.25	0.5	1
P. mirabilis[4]	ATCC 14153	1	0.125	0.03	0.125	0.125	0.25
S. aureus[5]	ATCC 29213	0.25	0.06	0.5	0.5	0.25	0.25
S. aureus[5]	ATCC 33591	0.25	0.06	0.5	0.5	0.25	0.5
S. saprophyticus[5]	ATCC 15305	0.5	0.125	1	0.5	1	0.5
E. faecalis[6]	ATCC 29212	1	0.25	1	2	1	2

[1]*Escherichia coli*, [2]*Klebsiella pneumoniae*, [3]*Pseudomonas aeruginosa*, [4]*Proteus mirabilis*, [5]*Staphylococcus aureus*, [5]*Staphylococcus saprophyticus*, [6]*Enterococcus faecalis*.

MIC: FIN (III) showed good activity compared to CIP and LVX at pH 7.2. Overall, under acidic pH conditions, FIN (III) was superior to both competitors against a range of pathogens.

Conclusions

- A scalable synthesis of the two building blocks and the novel fluoroquinolone finafloxacin hydrochloride (FIN, III) was established.
- The chemical structure of FIN (III) and important physiochemical characteristics were determined.
- The basic antibacterial activity of FIN (III) against Gram-negative and Gram-positive pathogens was determined.
- FIN (III) exhibited excellent and overall superior antibacterial activity at low pH, demonstrating an exceptional potential to treat infections in acidic environments, such as in the gastrointestinal or urogenital tract, in abscesses, intra-abdominal infections, TB, CF and others.
- In acidic environment FIN (III) outperformed relevant reference quinolones, most likely due to its lower intrinsic basic capacity enabling a more efficient uptake into the cell at lower pH.

Literature

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[3] Endermann *et al.*, 48th ICAAC, Washington DC 2008, Poster No.F1-2044.
[4] Endermann *et al.*, 48th ICAAC, Washington DC 2008, Poster No. F1-2045.
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48th ICAAC / 46th IDSA,
Washington DC 2008

F1-2037

Effect of pH on the *In Vitro* Activity of Finafloxacin against Gram-negative and Gram-positive Bacteria

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Revised Abstract

Background: Finafloxacin (FIN) is a novel 8-cyano-fluoroquinolone, that exhibits an *in vitro* spectrum of activity similar to that of ciprofloxacin (CIP). The present study was performed to study the effect of pH on the *in vitro* activity of FIN in comparison to CIP against selected strains of various aerobic Gram- and Gram+ bacterial species known to cause genito-urinary tract infections.

Methods: The susceptibilities of 100 clinical isolates to FIN and CIP were tested at pH 5, 6, 7.3, and 8. There were 22 *Escherichia coli* (ECO), 13 *Klebsiella pneumoniae* (KPN), 11 *Morganella morganii* (MOM), 10 *Proteus mirabilis* (PRM), 10 *Pseudomonas aeruginosa* (PSA), 12 *Staphylococcus aureus* (SAU), 11 *Staphylococcus saprophyticus* (SSA), and 11 *Streptococcus agalactiae* (SAG). Of these, 66 were sensitive and 34 exhibited reduced susceptibilities to CIP. MICs were determined using the CLSI broth microdilution method.

Results: Results are presented in Table 1.

Conclusions: Overall, FIN demonstrated superior activity to CIP under acidic conditions against isolates of all species including resistant strains. Furthermore, FIN showed comparable activity to CIP against Gram+ cocci at pH 7.3. Hence, FIN appears to be a promising new antimicrobial agent for the treatment of infections at acidic sites.

Methods

Bacterial strains: A total of 100 clinical isolates predominantly collected from 15 microbiology laboratories during a multi-centre study conducted between April and August 2005 in Germany were tested: *Escherichia coli* (n=22), *Klebsiella pneumoniae* (n=13), *Proteus mirabilis* (n=10), *Morganella morganii* (n=11), *Pseudomonas aeruginosa* (n=10), methicillin-susceptible *Staphylococcus aureus* (MSSA, n=6), methicillin-resistant *S. aureus* (MRSA, n=6), *Staphylococcus saprophyticus* (n=11), and *Streptococcus agalactiae* (n=11). Of these, 66 were susceptible to CIP (CIP-S) and 34 were either intermediate or resistant to CIP (non-susceptible, CIP-NS) according to the interpretive criteria defined by EUCAST [5].

Antibacterial agents: FIN (batch no. CBC000288; potency 84.5%) and CIP (batch no. CBC000290; potency 84.8%) were provided by MerLion Pharmaceuticals.

Susceptibility testing: The CLSI broth microdilution procedure with geometric twofold serial dilutions in cation-adjusted Mueller-Hinton broth (CAMHB) purchased from Becton Dickinson (Heidelberg, Germany; BBL™ Cation Adjusted Mueller Hinton II Broth, lot no. 6317238) was used to determine MICs [6]. Each strain was tested at the following pH values: 5.0, 6.0, 7.3 (standard), and 8.0. The pH was adjusted by adding drop by drop 1N HCl or 1N NaOH to the test medium. The pH was checked before autoclaving, after autoclaving and after addition of each antimicrobial agent.

Introduction

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

In addition, FIN exhibited superior activity to comparator FQs against adherent bacteria, *in vitro*, that was especially notable at low pH [2]. FIN also exhibited superior activity in rodent infection models [3,4] which involved inflammation, abscess formation or other acidic foci of infection.

The present study was performed to study the effect of the pH on the *in vitro* activity of FIN and CIP against 100 clinical isolates of various aerobic Gram-positive and Gram-negative bacterial species known to cause genito-urinary tract infections.

Results and Discussion

Results are presented in Table 1. Overall, FIN exhibited the highest *in vitro* activity at acidic conditions, while CIP was most active at pH 7.3 or 8.0. MICs of FIN and CIP against CIP-S *E. coli* are also illustrated in Figure 2 to demonstrate the opposing effect of pH on the inhibitory activity of these FQs.

***Enterobacteriaceae*:** Median MICs of FIN for CIP-S isolates of *E. coli*, *K. pneumoniae*, *P. mirabilis* and *M. morganii* were ≤0.25 mg/L each at pH 5.0 and pH 6.0, 0.125-1 mg/L at pH 7.3 and 0.25-2 mg/L at pH 8.0. FIN was more active than CIP against CIP-S isolates of all species at pH 5.0, while it was less active than CIP at pH 7.3 and 8.0. At pH 6.0, FIN showed superior activity to CIP against *E. coli* and *K. pneumoniae* and comparable activity to CIP against *P. mirabilis*, but was 4-fold less active than CIP against *M. morganii*. Similar differences were found for CIP-NS isolates.

***P. aeruginosa*:** Based on median MICs, FIN showed comparable activity to CIP at acidic pH, but was less active than CIP at pH 7.3 and 8.0, respectively.

***S. aureus*:** At pH 5.0 and 6.0, median MICs of FIN for CIP-S isolates (0.125 mg/L and 0.031 mg/L) were four dilution steps lower than those of CIP. Moreover, FIN was 2-fold more active than CIP at pH 7.3 and 8.0. This trend was also observed for CIP-NS isolates.

***S. saprophyticus*:** Based on median MICs, FIN showed three and two dilution steps higher activity than CIP at pH values of 5.0 and 6.0, respectively, while it was one dilution step less active than CIP at pH 7.3 and 8.0.

***S. agalactiae*:** FIN demonstrated superior activity to CIP, at pH 5.0 and 6.0 and showed equal activity at pH 7.3, while it was one dilution step less active at pH 8.0.

Results and Discussion

Table 1: MICs of 66 Gram-negative and 34 Gram-positive organisms

Species	Pheno-type	No. of strains	Para-meter	pH 5.0		pH 6.0		pH 7.3		pH 8.0	
				FIN	CIP	FIN	CIP	FIN	CIP	FIN	CIP
Escherichia coli	CIP-S	12	Median	0.031	0.5	0.016	0.125	0.125	0.016	0.25	≤0.008
			Range	0.016-0.25	0.25-8	≤0.008-0.125	0.063-2	0.031-1	≤0.008-0.125	0.031-2	≤0.008-0.125
	CIP-NS	10	Median	8	≥16	8	≥16	≥32	≥16	≥32	≥16
			Range	4-≥16	≥16	4-≥16	≥16	≥32	≥16	≥32	8-≥16
Klebsiella pneumoniae	CIP-S	5	Median	0.063	1	0.031	0.25	0.125	0.031	0.5	≤0.016
			Range	0.062-0.125	0.5-2	0.016-0.063	0.125-0.5	0.063-0.25	0.031	0.25-0.5	≤0.016
	CIP-NS	8	Median	2	≥16	1.5	≥16	6	3	12	1
			Range	0.5-≥32	≥16	0.25-≥32	8-≥16	0.5-≥32	1-≥16	1-≥32	0.25-≥16
Proteus mirabilis	CIP-S	7	Median	0.188	1	0.25	0.25	1	0.031	1.5	≤0.031
			Range	0.063-0.25	0.25-1	0.063-0.25	0.063-0.25	0.25-1	0.016-0.031	0.5-2	≤0.008-0.031
	CIP-NS	3	Median	4	≥16	4	8	16	2	16	1
			Range	4	8-≥16	4-8	8-≥16	16-≥32	1-2	16-≥32	0.5-1
Morganella morganii	CIP-S	9	Median	0.25	1	0.25	0.063	0.5	≤0.008	2	≤0.008
			Range	0.125-0.5	0.25-1	0.031-0.25	0.016-0.125	0.125-1	≤0.008-0.063	0.5-2	≤0.008
	CIP-NS	2	Median	-	-	-	-	-	-	-	-
			Range	4-≥32	≥16	4-16	≥16	16	4-16	16-≥32	2-16
Pseudomonas aeruginosa	CIP-S	5	Median	1	1	0.5	0.5	4	0.125	8	0.063
			Range	0.25-2	0.125-2	0.25-2	0.125-1	2-16	0.063-0.5	2-16	0.031-0.5
	CIP-NS	5	Median	8	≥16	8	≥16	≥32	≥16	≥32	8
			Range	4-≥32	4-≥16	4-≥32	2-≥16	≥32	1-≥16	≥32	0.5-≥16
Staphylococcus aureus	CIP-S	7	Median	0.125	2	0.031	0.5	0.25	0.5	0.25	0.5
			Range	0.031-0.125	0.5-8	0.031-0.125	0.25-2	0.125-0.25	0.25-1	0.25-16	0.25-4
	CIP-NS	5	Median	8	≥16	2	≥16	8	≥16	≥32	16
			Range	0.5-8	≥16	0.125-4	≥16	1-16	≥16	2-≥32	8-≥16
Staphylococcus saprophyticus	CIP-S	11	Median	0.063	0.5	0.125	0.5	0.5	0.25	1	0.5
			Range	≤0.008-0.125	≤0.008-1	0.125	0.25-1	0.25-0.5	0.25-0.5	1	0.25-0.5
Streptococcus agalactiae	CIP-S*	10	Median	0.25	2	0.25	1	1	1	2	1
			Range	0.125-0.5	2-4	0.125-0.5	0.5-2	0.5-2	0.5-2	1-4	0.5-2
	CIP-NS*	1	Median	-	-	-	-	-	-	-	-
			Range	2	16	4	4	4	4	8	4

Abbreviations: CIP-S, ciprofloxacin-susceptible; CIP-NS, non-susceptible to ciprofloxacin; *Epidemiological cut off value defined by EUCAST

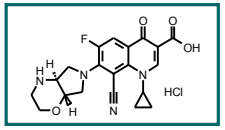

Figure 1. Finafloxacin hydrochloride.

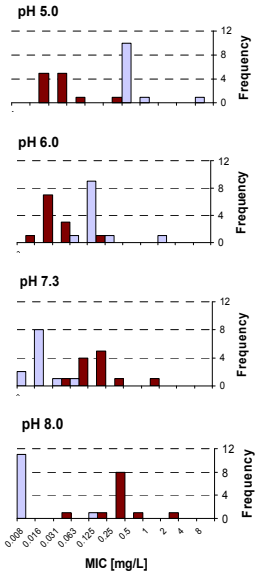

Figure 2. MIC distribution of FIN ( )and CIP ( ) at pH 5.0, 6.0, 7.3 and 8.0 against CIP-S *E. coli* (n = 12).

Conclusions

- FIN demonstrated superior activity to CIP under acidic conditions against isolates of all species, except *P. aeruginosa* for which both drugs showed similar potency under these conditions.
- FIN appears to be a promising new antimicrobial agent for the treatment of infections in acidic environments.

Literature

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48th ICAAC / 46th IDSA,
Washington DC 2008

F1-2038

Antimicrobial Activity of Finafloxacin (FIN) against *Helicobacter pylori In Vitro* and *In Vivo*

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Revised abstract

Introduction: 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. FIN is intended for therapeutic use against bacterial infections associated with an acidic environment such as *H. pylori* eradication. The antibacterial activity of FIN, was determined against FQ^{RES} and susceptible strains at acidic pH, and against *H. felis in vivo*.

Methods: *H. pylori* strains were obtained from patients gastroscoped in France. MICs for FIN and levofloxacin (LVX) were performed by agar dilution at 3 different pHs: 7.3, 6.3 and 5.3. The propensity for emergence of resistance *in vivo* was determined in a murine model in which *H. felis* was passaged until persistent infection was established that required triple therapy to eradicate.

Results: MIC_{50} and MIC_{90} values of FIN and LVX for 31: (18 FQ^{RES} and 13 susceptible) strains are shown in Table 1. Additionally, MICs were determined for a panel of 24 FQ susceptible isolates (Fig. 2). Emergence of resistance was determined by pre-treating infected animals with sub therapeutic levels of FIN 1mg/kg or ciprofloxacin (CIP) 2.5mg/kg, (o.d., 7d) before treatment with FIN or CIP (10mg/kg, o.d., 7d). FIN cleared infection (negative urease test, 24h post-therapy) in 100% of pre-exposed animals whereas subsequent CIP treatment failed.

Conclusions: FIN exhibited increased efficacy at acidic pH compared to LVX. This was especially true against the FQ resistant strains. Additionally, FIN pre-exposure did not select for resistance *in vivo*. This unusual acid dependent activity seems particularly well suited for Helicobacter eradication and warrants a clinical evaluation.

Introduction

Finafloxacin (FIN, Figure 1) is a novel, 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 also 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 associated with low pH such as urinary tract infection and *Helicobacter pylori* eradication.

FQs such a levofloxacin (LVX) have shown good antibacterial activity against *H. pylori* and a successful eradication rate when used in triple combination therapy. The antibacterial activity of FIN was investigated against FQ susceptible and resistant strains at acidic pH and against *H. felis* in a novel murine infection that was developed to be a stringent evaluator of anti-helicobacter therapy.

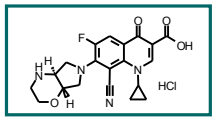

Figure 1. Finafloxacin hydrochloride.

Methods

Minimum inhibitory concentration (MIC) determination

MICs were determined for FIN and LVX against *H. pylori* strains (n= 55) that were obtained from patients gastroscoped in the Southwest of France. MICs were performed by agar dilution at 3 different pHs: 7.3, 6.3 and 5.3. An inoculum (equivalent to a McFarland 3 opacity standard) from a 48 h culture was plated on Mueller Hinton agar enriched with 10% sheep blood prepared extemporaneously and containing progressive concentrations of the FQs (0.015 - 128 mg/L). Reading was performed after 2 - 3 days of incubation at 37°C in a microaerobic atmosphere.

Murine model of *Helicobacter felis* infection

H. felis was passaged in female Swiss-Webster mice by repeated feeding of colonised gastric homogenate, achieving a persistent infection which could not be eradicated with conventional antibacterial monotherapy but could be eradicated following FIN monotherapy. Eradication was defined as a negative urease test on gastric tissue, 4 weeks post-therapy.

The propensity for resistance emergence to FIN and ciprofloxacin (CIP) was investigated by pre-treating infected animals (n = 5) with sub therapeutic doses of FIN (1mg/kg) or CIP (2.5mg/kg), (once daily, 7d) before treatment with therapeutic doses of FIN or CIP (10mg/kg, once daily, 7d). The therapeutic endpoint was defined by a negative urease test, 24h post treatment which could be attained, only if resistance to the test drug did not emerge in the colonising bacteria during pre-treatment.

Results and Discussion

Susceptibility of *H. pylori* isolates to FIN and LVX under standard conditions

In total, 55 *H. pylori* isolates were investigated for their susceptibility to FIN and LVX. Initially, a panel of 31 strains were investigated (Table 1). These were pre-defined as FQ susceptible (n = 18) or resistant (n = 13), based on their susceptibility to LVX.

Under standard susceptibility testing conditions (pH 7.3), FIN and LVX exhibited similar activities against the tested strains (Table 1).

Effect of pH on the activity of FIN against *H. pylori*

Agar dilution MICs were determined at pH 7.3, 6.3 and 5.3 against a second panel of 24 LVX susceptible strains. The antibacterial activity of FIN, as seen by its MIC distribution (Figure 2), increased in a step-wise manner as the pH became more acidic. Shifting from neutral to acidic pH had a minimal effect on the activity of LVX.

FIN exhibits improved antibacterial activity, under conditions of acidic pH, against a wide range of species [1,2]. Under the same conditions, other marketed FQs exhibit significantly reduced activity. This unusual property has been attributed, at least in part, to the relatively low intrinsic basic capacity (pK_b) of FIN compared to that of other FQs [1]. This most probably results in an increased cellular accumulation of FIN under acidic conditions. This is illustrated in Figure 3, in which a correlation is drawn between low intrinsic basic capacity of experimental and commercially available FQs and their improved therapeutic efficacy in an *in vivo* model of Helicobacter colonisation [8].

Results and Discussion

pH	Finafloxacin MIC_{50} [mg/L]	Finafloxacin MIC_{90} [mg/L]	Levofloxacin MIC_{50} [mg/L]	Levofloxacin MIC_{90} [mg/L]
FQ susceptible (n = 18)				
7.3	0.125	0.5	0.25	0.5
6.3	0.125	0.25	0.25	0.5
5.3	0.125	0.25	0.25	0.5
FQ resistant (n = 13)				
7.3	8	16	4	8
6.3	8	8	4	16
5.3	2	4	4	16

Table 1. MIC_{50} and MIC_{90} of finafloxacin (FIN) and levofloxacin (LVX) against a panel of fluoroquinolone susceptible (n = 18) and FQ resistant (n = 13) clinical isolates of *H. pylori*.

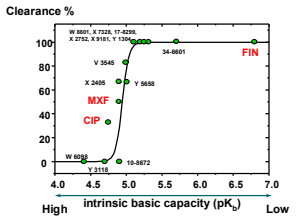

Figure 3. Capacity of various commercially available (CIP; ciprofloxacin, MXF, moxifloxacin) and experimental FQs (FIN; finafloxacin) to clear a *Helicobacter* infection in mice *vs.* the intrinsic basic capacity of the test compounds [8].

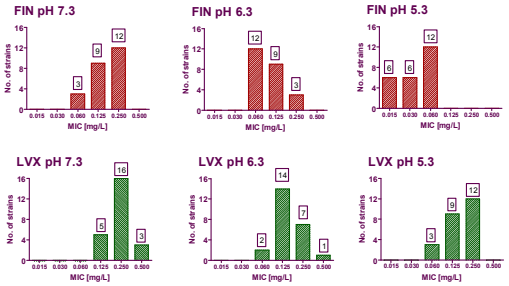

Figure 2. MIC distribution of FIN (top row) and LVX (bottom row) against 24 FQ susceptible *H. pylori* strains at pH 7.3 (left) pH 6.3 (middle) and pH 5.3 (right).

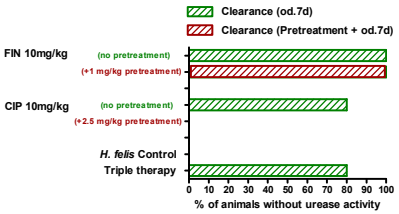

Figure 4. Clearance (as determined by negative urease test, 24h post-therapy) of persistent *H. felis* infection following seven day pre-treatment of mice with sub therapeutic doses of CIP (2.5 mg/kg, 7d) or FIN (1 mg/kg, 7d) before seven day treatment with therapeutic doses (10 mg/kg).

Murine model of *H. felis* infection

H. felis was passaged in mice to achieve a persistent infection that exhibited a similar response to therapy as *H. pylori* in humans. Triple therapy (bismuth citrate, amoxicillin (AMX) and metronidazole (14 d) could sucessfully eradicate infection (endpoint: negative urease test on gastric tissue, 4 weeks post-therapy) whereas monotherapies of clarithromycin, AMX or CIP all failed. FIN was the only drug able to successfully eradicate infection when administered as a monotherapy.

The present study was performed to investigate whether pre-exposure to FIN and CIP could select for resistance *in vivo* and lead to subsequent treatment failure. Both drugs could clear (negative urease test, 24 h post-therapy) infection from animals with no prior antibiotic exposure (Figure 4).

Sub therapeutic (FIN 1mg/kg or CIP 2.5mg/kg) doses were administered to infected mice, once daily for 7d. The mice were then administered therapeutic doses (10 mg/kg). The data for clearance in pre-exposed mice are summarised in Figure 4.

These findings show that pre-exposure to CIP leads to an total failure of the subsequent treatment (failure to clear infection in 100% of animals) where as pretreatment with FIN did not alter the success subsequent therapy (0% failure). Selection of resistance during pre-treatment was the most probable reason for the subsequent treatment failure seen with CIP.

Conclusions

- FIN exhibited improved antibacterial activity, *in vitro,* against a panel of both FQ resistant and susceptible recent clinical isolates of *H. pylori* at low pH.
- In addition to exhibiting clearly superior efficacy in an murine model of persistent Helicobacter infection, FIN (sub therapeutic dose) did not select for resistance in this model.
- The pH activation observed with FIN against *H. pylori in vitro* and its efficacy in a difficult to treat model of *H. felis* colonisation, suggest that FIN may be a promising treatment that could improve *H. pylori* eradication therapy in humans.

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] Bishop *et al.*, European Helicobacter Study Group Meeting, Istanbul, 2007. Poster No. P-054.

Comparison of Methods for Finafloxacin MIC Testing at Acidic and Neutral pH

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F1-2039

OBJECTIVES

MIC testing of aerobic bacteria with finafloxacin (FIN) at acidic and neutral pH was studied by comparing Etest®, CLSI agar dilution (AD) and broth microdilution (BMD) methods.

INTRODUCTION

Finafloxacin is a novel broad spectrum fluoroquinolone that exhibits optimal activity at slightly acidic conditions (pH 5-6) where other fluoroquinolones lose some of their activity. Hence, finafloxacin is intended for therapy of bacterial infections associated with an acidic environment such as *H. pylori* eradication and complicated urinary tract infections.

Studies, thus far, have also shown that finafloxacin retains additional various positive features of other marketed fluoroquinolones, including a good safety profile.

MATERIALS AND METHODS

Bacterial strains

Test strains: *A. anitratus* (1), *E. coli* (8), *E. cloacae* (2), *K. pneumoniae* (2), *P. vulgaris* (1), *P. rettgeri* (1), *P. stuartii* (1), *P. aeruginosa* (3), *S. aureus* (13), *S. haemolyticus* (3), *S. marsescens* (2), *S. saprophyticus* (1) and *S. warneri* (1).

Quality control strains: *E. coli* ATCC® 25922, *P. aeruginosa* ATCC 27853 and *S. aureus* ATCC 29213.

Reagents

Finafloxacin powder (MerLion Pharmaceuticals Pte. Ltd, Singapore); Etest Finafloxacin (FIN) MIC range 0.002 – 32 µg/mL (AB bioMérieux, Solna, Sweden); Mueller Hinton agar and broth (BBL, Maryland, USA) at pH 5.8 and 7.2.

Procedure

Etest was used according to the manufacturer's instructions and tested at both pH 5.8 and pH 7.2. AD and BMD were performed using the CLSI procedures and tested at both pH 5.8 and pH 7.2. The MIC was read at complete inhibition of growth.

CONCLUSIONS

- MIC testing of finafloxacin with Etest, agar and broth dilution reference methods provides substantially equivalent results at both neutral and acidic pH (EA ±1 dilution 88-100 %), and demonstrates higher activity of FIN at slightly acidic pH.
- Etest agreement with CLSI methods was lower at pH 5.8 primarily due to Etest being more efficient in detecting the resistant subpopulations.
- Etest MIC values at pH 5.8 were approximately 2-4 dilutions lower than those at pH 7.2.
- Etest with a wide concentration range (15 dilutions) comprise a useful MIC tool for drug development studies with FIN and for future studies with *H. pylori*.

RESULTS

Table 1. Etest MIC for different species at acidic and neutral pH

Species	N	MIC range (µg/mL)	
		pH 5.8	pH 7.2
A. anitratus	1	0.25	1
E. coli	8	0.016 – 32	0.094 – 32
E. cloacae	2	0.012 – 32	0.047 – 32
K. pneumoniae	2	0.023 – 0.5	0.19 – 1.5
P. vulgaris	1	0.19	0.5
P. rettgeri	1	0.5	3
P. stuartii	1	0.094	0.38
P. aeruginosa	3	0.38 – 4	3 – 32
S. marcescens	2	0.5 – 32	2 – 32
S. aureus	13	0.023 – 3	0.094 – 32
S. haemolyticus	3	0.023 – 0.032	0.094 – 0.125
S. saprophyticus	1	0.19	0.38
S. warneri	1	0.064	0.25

Finafloxacin activity at acidic pH was 2-4 dilutions greater than at neutral pH

Illustrations of Etest Finafloxacin results at neutral and acidic pH

Figure 1. *P. stuartii* CDC 2083

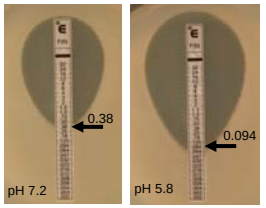


Figure 2. *S. aureus* ATCC 29213

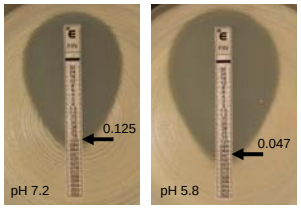
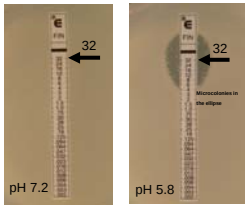


Figure 3. *E. coli* ECI 2119 FQR



MIC endpoints were generally clear-cut for most organisms. Macro- and microcolonies were occasionally seen in Etest FIN inhibition ellipses for a few *E. coli* and *E. cloacae* strains, such colonies are not specific to finafloxacin. The MIC was read at complete inhibition of growth.

Table 2. Comparison of MIC methods at acidic and neutral pH

Comparator	Regression analysis		% EA ± 1 dil.
	Equation	r	
AD vs. BMD, pH 5.8	y = 0.94 x + 0.27	0.99	100
AD vs. BMD, pH 7.2	y = 0.97 x + 0.36	0.98	100
Etest vs. AD, pH 5.8	y = 1.11 x - 0.45	0.97	87.5
Etest vs. AD, pH 7.2	y = 0.99 x + 0.28	0.97	100
Etest vs. BMD, pH 5.8	y = 1.05 x - 0.27	0.97	87.5
Etest vs. BMD, pH 7.2	y = 0.97 x + 0.55	0.98	97.9

EA = Essential Agreement

Figure 4. Comparison of Etest MICs at acidic and neutral pH

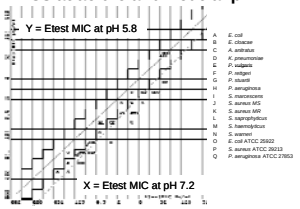


Table 3. Quality control results and tentative QC ranges for Etest FIN (µg/mL)

Strain	Etest (N 20)		Agar dilution (N 8)		Broth dilution (N 8)		Etest Tentative QC	
	pH 5.8	pH 7.2	pH 5.8	pH 7.2	pH 5.8	pH 7.2	pH 5.8	pH 7.2
E. coli ATCC 25922	0.008-0.012 **Mode: 0.012**	0.047-0.094 **Mode: 0.047**	0.008-0.016 **Mode: 0.008**	0.032-0.064 **Mode: 0.064**	0.008-0.016 **Mode: 0.008**	0.032-0.064 **Mode: 0.064**	**0.008-0.032**	**0.032-0.125**
S. aureus ATCC 29213	0.032-0.047 **Mode: 0.032**	0.094-0.125 **Mode: 0.125**	0.032 **Mode: 0.032**	0.125 **Mode: 0.125**	0.032-0.064 **Mode: 0.064**	0.125 **Mode: 0.125**	**0.032-0.125**	**0.064-0.25**
P. aeruginosa ATCC 27853	0.38-0.75 **Mode: 0.5**	2-4 **Mode: 3**	0.25 **Mode: 0.25**	2-4 **Mode: 4**	0.25-0.5 **Mode: 0.5**	4 **Mode: 4**	**0.25-1**	**2-8**

MIC Testing of *Helicobacter pylori* using Etest® Finafloxacin and the Reference Agar Dilution Method

F1-2040

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OBJECTIVES

The aim of the study was to compare MIC testing with Etest Finafloxacin and the CLSI agar dilution reference method using clinical *Helicobacter pylori* isolates.

INTRODUCTION

Helicobacter pylori (HP) is a Gram negative bacteria commonly found in the gastric mucosa, where it can reside without any clinical symptoms. However, this pathogen has been associated with e.g. peptic ulcers and gastric cancer.

Since the treatment of HP infections used today is complex and can lead to side effects and cross-resistance, it is of great importance to find the most suitable drug.

Finafloxacin hydrochloride (FIN) is a novel 8-cyano fluoroquinolone that exhibits optimal activity at slightly acidic conditions, where other fluoroquinolones lose activity. The intended use of FIN is for the therapy of bacterial infections such as HP infections, since the bacteria harbours in an acidic environment.

The Etest FIN gradient is preformed, predefined and stable which makes the system suitable for testing of fastidious organisms like HP with varying growth rates.

MATERIAL AND METHODS

Strains

Test isolates: A total of 36 *H. pylori* clinical isolates, including fluoroquinolone resistant strains, were tested in quadruplicate.

Quality control strain: *H. pylori* ATCC 43504 was tested in quintuplicate.

Reagents

Finafloxacin powder (MerLion Pharmaceuticals Pte. Ltd, Singapore); Mueller Hinton agar (BBL, Maryland, USA); Etest Finafloxacin (FIN) MIC range 0.002 – 32 µg/mL (AB bioMérieux, Solna, Sweden).

Procedure

Etest was used according to manufacturers instruction and agar dilution was performed according to CLSI guidelines. Both methods were read after 3 and 5 days of incubation.

RESULTS

Figure 1. Etest FIN vs. AD; 3 days

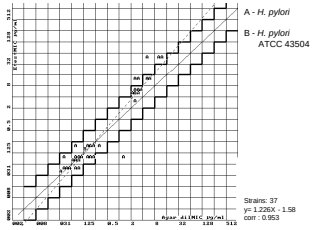
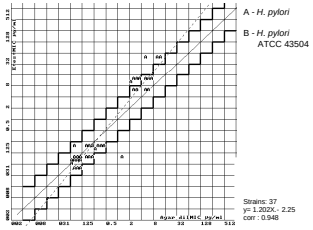
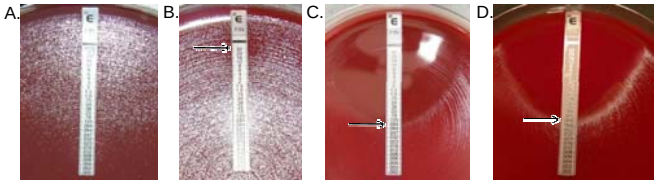


Figure 2. Etest FIN vs. AD; 5 days



Etest FIN and CLSI agar dilution method were shown to be comparable, although the correlation between Etest and AD were slightly better after 3 days (table 1), i.e. the recommended incubation time. Resistant micro/macro subcolonies were seen in the inhibition ellipse for a few isolates when tested by Etest, especially after 5 days of incubation (figure 3A and 3B). These subcolonies are not specific to finafloxacin and the clinical relevance needs to be further investigated.

Figure 3. Illustrations of Etest Finafloxacin results



A. Etest MIC >32 AD MIC 8
B. Etest MIC 32 AD MIC 4
C. Etest MIC 0.094 AD MIC 0.25
D. Etest MIC 0.094 AD MIC 0.125

CONCLUSIONS

- Etest FIN vs. CLSI agar dilution MIC results showed good agreement after 3 and 5 days of incubation.
- Recommended incubation time of *Helicobacter pylori* is 3 days.
- Etest FIN was more efficient than AD in detecting resistant subpopulations.
- Etest is a useful tool for testing new agents against *Helicobacter pylori*.

Table 1. Essential agreements Etest vs. agar dilution

Incubation time	% EA, modal MIC	
	± 1 dil	± 2 dil
3 days	83.3	94.4
5 days	77.8	94.4

Table 2. Intralaboratory reproducibility of Etest and agar dilution

Incubation time	% Reproducibility, modal MIC ± 1 dil	
	Etest (36 strains x4)	Agar dilution (36 strains x3)
3 days	93.1	97.2
5 days	90.3	97.2

Table 3. Tentative quality control ranges for Etest Finafloxacin (µg/mL)

Organism	3 and 5 days incubation		Tentative Etest QC range
	AD (n=30)	Etest (n=20)	
H. pylori ATCC 43504	0.125 - 0.25 Mode: 0.125	0.064 - 0.19 Mode: 0.125	0.064 - 0.25

48th ICAAC / 46th IDSA,
Washington DC 2008

F1-2041

Comparative Inhibitory and Bactericidal Activities of Finafloxacin and Ciprofloxacin against Gram-Negative and Gram-Positive UTI-pathogens under Physiological Conditions and at Varying pH-values

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Revised Abstract

Background: FIN is a novel fluoroquinolone (FQ) belonging to a new 8-cyano subclass which exhibits improved *in vitro* activity at slightly acidic pH and is therefore intended for treatment of UTI. The antibacterial and bactericidal activities of FIN and CIP were compared in artificial urine medium which reflects the physiological conditions of pH, ionic strength and chemical composition, encountered *in vivo*.

Methods: The MICs of FIN and CIP were determined against 34 strains (*S. aureus, S. saprophyticus*, Enterobacteriaceae, *P. aeruginosa*, incl. CIP-res and ESBL producers) using CLSI methodology in cation adjusted Mueller-Hinton Broth (CAMHB) at pH 7.2 and 5.8 and in artificial urine (pH 5.8). Bactericidal activity was determined against 10 strains exposed to 1 x, 4 x and 16 x MIC. During the initial log-linear phase of CFU-decline, single point kill rates (k= -ln(N/No))/t) were calculated.

Results: FIN MICs were 1 - 3 dilutions lower at pH 5.8 compared to those at pH 7.2, whereas CIP MICs increased by 1 - 3 dilutions at the lower pH. In artificial urine (pH 5.8), FIN exhibited MICs similar to those obtained in CAMHB pH 7.2, whereas CIP MICs increased by 10 - >100-fold. On average, FIN MICs were 4 - 5 dilutions lower than CIP in artificial urine, regardless of Gram type or susceptibility profile. Bactericidal activities of both FIN and CIP (kill-rates normalised to concentration) demonstrate that FIN is about 2- to >20-fold more active than CIP in both media.

Conclusions: The bacteriostatic (MICs) and bactericidal activities (time kill curves) of FIN differ favourably from those of CIP under conditions mimicking UTIs. The activity of FIN in artificial urine is quantitatively and qualitatively different from that of CIP. These findings indicate that FIN may be effective in the treatment of UTIs.

Introduction

Finafloxacin (FIN, Figure 1) is a novel, broad spectrum fluoroquinolone (FQ) belonging 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). Other marketed FQs have significantly reduced activity over this pH range [2].

FIN exhibited superior activity compared with 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 associated with low pH such as urinary tract infection and *Helicobacter pylori* eradication.

The antibacterial activity of FIN and ciprofloxacin (CIP) were compared in a medium that mimics, in part, the environment encountered during UTI.

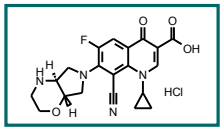


Figure 1. Finafloxacin hydrochloride.

Methods

MIC determinations

MIC testing was performed using a microdilution method according to CLSI (formerly NCCLS) guidelines [8]. MICs were determined in cation adjusted Mueller Hinton broth (CAMHB) at pH 7.2 and pH 5.8 and in artificial urine pH 5.8 [9]. The final inoculum was 5 x 10^4 CFU/mL.
35 strains of Gram-positive and Gram-negative bacteria were tested; these included a number with resistance determinants.

Time-kill experiments

These were performed with the following panel of 10 strains:

- *Enterobacter cloacae* ATCC 13047
- *Enterococcus faecalis* ATCC 29212
- *Escherichia coli* ATCC 25922
- *Escherichia coli* WT-2 (CIP^{BS})
- *Escherichia coli* M1-4 (CIP^{R})
- *Escherichia coli* WT-4-M2-1 (CIP^{R})
- *Proteus mirabilis* ATCC 9240
- *Pseudomonas aeruginosa* ATCC 10145
- *Staphylococcus aureus* ATCC 29213
- *Staphylococcus saprophyticus* ATCC 15305

CIP^{BS}; ciprofloxacin - borderline susceptible, CIP^{R}; ciprofloxacin resistant, as determined under standard MIC test conditions.

The strains were stored frozen at – 80°C in a volume of 100 µL.

Time-Kill curve kinetics

Kill curve kinetics were carried out using a modified CLSI method [10]. FIN and CIP were tested at multiples (x 1, x 4, and x 16) of the MIC value in mg/L against each strain.
Samples were taken at 0h, 1h, 2h, 4h, 6h, 8h and 24 h after incubation. Ten-fold serial dilutions were inoculated onto Mueller-Hinton agar and colonies enumerated following 24 h incubation at 37°C.

Results and Discussion

Effect of pH and medium on activity of FIN and CIP

The MIC values in mg/L of FIN and CIP against the 35 strains tested in CAMHB at pH 7.2 and 5.8 and in artificial urine at pH 5.8 are shown in Figure 2.

FIN MICs were lower at an acidic pH value in CAMHB (pH 5.8) and were also low in artificial urine (pH 5.8), despite the high levels of divalent cations which inactivate most of the commercially available FQs like CIP. In contrast CIP MICs increased strain dependently from 2- to greater than 10-fold in acidic CAMHB and increased >10- to >100-fold in artificial urine (pH 5.8).

Bactericidal effects of FIN and CIP

The bactericidal activity of FIN against two of the strains, at multiples of the MIC, in CAMBH pH 7.2 and artificial urine pH 5.8 are shown in Figure 3 (*E. coli*) and Figure 4 (*P. mirabilis*).

When compared on the basis of MIC (under the prevailing conditions) the bactericidal activities of both FIN and CIP were comparable. However, the concentration normalised kill-rates (basis 1mg/L) clearly demonstrate that FIN is approximately 2-fold to >20-fold more active than CIP in CAMHB or synthetic urine. Normalised kill rates for selected organisms are illustrated in Fig. 5 where it can be seen that FIN is more active than CIP.

Results and Discussion

Bacterial Strain	CAMHB pH 7.2 FIN	CAMHB pH 7.2 CIP	CAMHB pH 5.8 FIN	CAMHB pH 5.8 CIP	Art. urine pH 5.8 FIN	Art. urine pH 5.8 CIP
S. aureus ATCC 29213	0,25	0,5	0,125	2	0,25	8
E. coli ATCC 25922	0,03	0,03	0,015	0,06	0,06	0,5
E. faecalis ATCC 29212	1	1	0,25	2	1	8
K. pneumoniae ATCC 13883	0,06	0,03	0,015	1	0,25	2
E. coli ATCC 11775	0,25	1	0,015	0,06	0,125	2
P. mirabilis ATCC 9240	0,5	0,015	0,25	0,06	0,5	2
E. cloacae ATCC 13047	0,25	0,015	0,03	0,015	0,25	2
S. marcescens ATCC 13880	2	0,03	0,5	0,5	0,125	2
P. aeruginosa ATCC 10145	4	0,06	1	0,5	1	4
S. aureus ATCC 12600	0,25	0,5	0,015	1	0,5	8
S. saprophyticus ATCC 15305	0,25	0,5	0,015	0,5	0,5	8
S. agalactiae ATCC 13813	0,25	0,5	0,125	0,5	1	8
E faecalis ATCC 19433	1	1	0,5	4	2	16
C. freundi ATCC 8090	0,0075	≤0,0075	≤0,0075	≤0,0075	0,06	≤0,0075
K. pneumoniae ESBL ATCC 700603	4	0,25	1	4	4	32
K. pneumoniae ESBL 20 SHV 27 TEM116	32	4	4	64	16	>128
K. oxytoca ESBL 23 SHV 12 TEM 1	128	>128	16	>128	128	>128
E. coli ESBL CIA TEM 7	0,5	0,06	0,03	4	64	>128
E. coli ESBL 85 (U 4731/06 from Kiel)	64	64	8	64	64	>128
S. aureus 133	0,25	0,06	0,015	0,5	0,25	8
S. aureus clone 16 (CIP^{R})	0,25	1	0,03	2	2	64
S. aureus 105-11 (CIP^{R})	1	4	0,5	16	4	128
S. aureus 104-13 (CIP^{R})	2	16	1	32	8	>128
S. aureus 103-17 (CIP^{R})	8	64	4	128	32	>128
E. coli WT	0,015	0,015	0,015	0,015	0,06	2
E. coli WT-2 (CIP^{BS})	1	0,125	0,125	2	1	16
E. coli WT-3-M4 (CIP^{R})	128	32	16	>128	128	>128
E. coli WT-3-1-M4 (CIP^{R})	32	2	4	32	32	>128
E. coli WT-3-M21 (CIP^{R})	64	8	8	64	64	>128
E. coli WT-4-M2-1 (CIP^{BS})	0,25	0,03	0,015	0,03	0,25	2
E. coli M (CIP^{BS})	4	0,5	0,5	4	4	64
E. coli M1-4 (CIP^{BS})	4	0,5	1	8	4	128
E. coli M8 (CIP^{R})	16	2	2	32	16	>128
E. coli M8 (CIP^{R})	>128	128	64	>128	>128	>128

Figure 2. Activity of FIN and CIP in CAMBH at pH 7.2 and 5.8 and in artificial urine pH 5.8. CIP^{BS}; ciprofloxacin - borderline susceptible, CIP^{R}; ciprofloxacin resistant, as determined under standard MIC test conditions.

Figure 5. Comparative bactericidal effects of FIN and CIP in synthetic urine, pH 5.8 at 1 x MIC (■), 4 x MIC (■) and 16 x MIC (■). Relative kill rates normalised to 1 mg/L.

Figure 3. The bactericidal effect of FIN at 1 x MIC (■), 4 x MIC (▲) and 16 x MIC (■) on *E. coli* ATCC 25922.

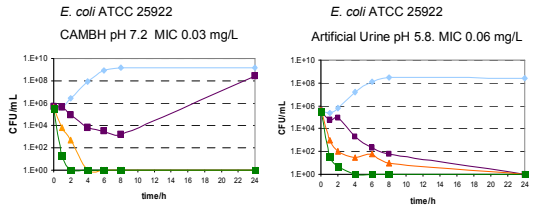
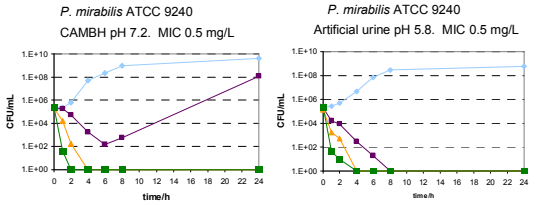
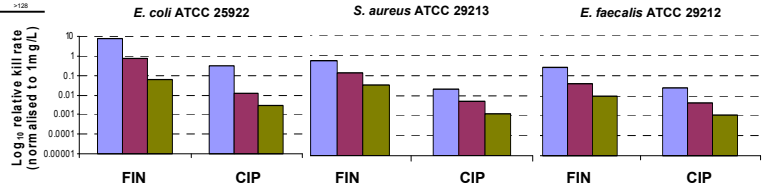


Figure 4. The bactericidal effect of FIN at 1 x MIC (■), 4 x MIC (▲) and 16 x MIC (■) on *P. mirabilis* ATCC 9240.





Conclusions

- FIN was more active in CAMHB at an acidic pH (5.8) than at pH 7.2, unlike CIP, which had reduced activity at an acidic pH.
- These bacteriostatic (MICs) and bactericidal activities (time kill curves) of FIN also differ favourably from those of CIP under conditions mimicking UTIs. The activity of FIN in artificial urine was both quantitatively and qualitatively different from that of CIP.
- These properties, plus the excellent tolerance seen by the oral route in Phase I studies in man [7] and the lack of toxicity seen in predictive *ex vivo* toxicity tests [6], indicate that finafloxacin is an excellent candidate for progression to the clinic.

Literature

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48th ICAAC / 46th IDSA,
Washington DC 2008

F1-2042

Bactericidal Activity Of Finafloxacin Against Difficult To Kill Growth Forms of *Escherichia coli*

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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 FQ lose activity. Therefore, FIN is intended for bacterial infections associated with an acidic environment. During infection, bacteria may exist as adherent populations and form persistent subpopulations. This study assessed the ability of FIN to kill these difficult to treat growth forms.

Methods: Adherent populations of *E. coli* C600 were grown on 0.45µm membrane filters perfused with BHI (pH 6.2) to a steady state of 10^7 - 10^8 CFU/mL of perfusate. FIN, ciprofloxacin (CIP), levofloxacin (LVX) or moxifloxacin (MXF) (all 5mg/L) were then perfused for 3d, followed by 1d of drug free media. Persistent subpopulations (persister frequencies) were defined as the fraction of viable cells that were recovered following exposure of high cell densities of *E. coli* ATCC 25922 (1 - 5 x10^8 CFU/mL) to FIN, CIP or LVX (10mg/L, 24h) in Mueller-Hinton, pH 7.2. Aderent populations of *E. coli* 25922 and *S. aureus* 29213 were also grown on segments of Foley catheters suspended in artificial urine before exposure to FQs.

Results: FIN resulted in a 5-log reduction of adherent *E. coli*, to below the limit of detection (<10_2 CFU/mL) within 5h, the nearest comparator to this was LVX (2-log reduction). All drugs had significantly reduced viability by day 3, however rapid regrowth was then observed following perfusion with drug-free media in the comparator-treated populations but no regrowth was observed after FIN treatment. The frequency of persisters that remained following high cell density killing in MH were FIN (5.2 x 10^{-7}) , CIP (1.6 x 10^{-3}) and LEV (1.6 x 10^{-4}). Overall, FIN eradicated catheter adherent populations by Δ 1 log greater than CIP.

Conclusions: Adherent populations of *E. coli* were killed more rapidly by FIN and did not regrow following cessation of treatment, which was observed with the comparators. Such superior degree of killing may be related to the lower numbers of persistent bacteria isolated following exposure to FIN.

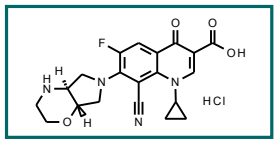
Introduction

Finafloxacin (FIN, Figure 1) is a novel, 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 also exhibited superior activity to comparator FQs in a wide range of rodent infection models, including several models in which adherent bacterial populations are formed [3,4].

Additionally, FIN displayed an excellent safety profile in a wide range of predictive, *in vitro*, toxicity assays [5] and was well tolerated in healthy human volunteers [6]. These attributes suggest that FIN warrants clinical investigation for bacterial infections that are associated with low pH such as urinary tract infection and *Helicobacter pylori* eradication.

Bacterial growth, *in vivo*, is thought to be often associated with slow generation time and formation of adherent populations or biofilms that exhibit phenotypic resistance to antibiotics and disinfectants. Despite this, most *in vitro* assessment of antibacterials involves testing only planktonic cultures. FIN and other FQs were evaluated in a series of *in vitro* models that involved formation of stationary or adherent populations to determine how effectively they eradicated these difficult to treat growth forms.

Figure 1. Finafloxacin hydrochloride.



Methods

Membrane filter model

E. coli C600 were inoculated onto 0.45 µm filter cartridges under a continuous flow of brain heart infusion broth (BHI), pH 6.2. Once steady state had been established (10^7 – 10^8 CFU/mL of perfusate), FIN, ciprofloxacin (CIP), moxifloxacin (MXF) or levofloxacin (LVX) were perfused at 5mg/L for 3 days followed by drug-free media for a further 24h. Samples of perfusate were taken for CFU determination at T_0, T_{5h}, T_{3d} and T_{3d+1}.

Stationary-phase killing

E. coli 25922 were grown in cation-adjusted Mueller-Hinton broth (CAMHB) for 24h. FIN, CIP or LVX (all 10 mg/L) were then added to these stationary-phase broths for a further 24h. Viable counts were performed on washed culture samples before and after drug exposure. Counts plates were incubated for 72h before reading and the recovered (persistent) bacteria expressed as a fraction of the starting cell number.

Catheter adherent population

E. coli 25922 or *S. aureus* 29213 were grown for 24h – 6d on segments of silicone coated Foley urinary catheters suspended in artificial urine medium [7]. Catheter adherent cultures of different ages were washed and exposed to concentration ranges of FIN or CIP for 24h. The surviving catheter-adherent cells were recovered in PBS by sonication and vortexing and plated out for CFUs.

Results and Discussion

Membrane filter model

The comparative bactericidal activity of FIN, CIP, LVX or MXF (5 mg/L) against membrane adherent populations of *E. coli* are summarised in Figure 2.

FIN had a rapid effect on the viability of adherent *E. coli* populations, causing a 5-log reduction to below the limit of detection (<10^2 CFU/mL) within 5 h, the nearest comparator to this was LVX which caused a 2-log reduction. All drugs had significantly reduced viability by day 3, however rapid regrowth of the adherent population was then observed following perfusion with drug-free media in the CIP, LVX or MXF-treated populations but no regrowth was observed after FIN treatment.

Stationary-phase killing

Saturated, stationary-phase cultures of *E. coli* 25922 were exposed to FIN, CIP or LVX for 24h. The extent of killing (Δ $\log_{10}$ CFU), determined from the survival rate are shown in Figure 3. The greater number of surviving CFUs were recovered following exposure to CIP (persister frequency, 1.6 x 10^{-3}), then LVX (1.6 x 10^{-4}) then FIN (5.2 x 10^{-7}). These data indicate, that FIN induces a more thorough eradication of non-dividing, high density *E. coli* populations than CIP or LVX.

Results and Discussion

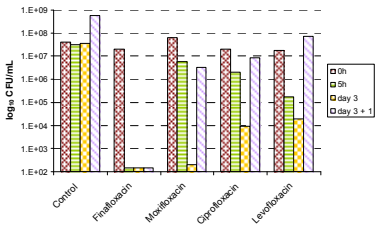


Figure 2. Viability (CFU/mL perfusate) of adherent *E. coli* following 5h ( ) and 3d ( ) exposure to FIN, CIP, MXF or LVX (5 mg/L). Following exposure, the adherent populations were perfused with drug free media for a further 24h and viability measured to determine the extent of recovery ( ).

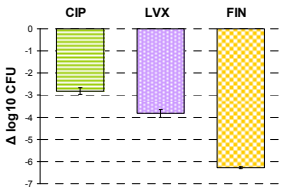


Figure 3. Killing (Δ $\log_{10}$ CFU) of stationary phase *E. coli*. Saturated 24h broth cultures were exposed to CIP ( ), LVX ( ) or FIN ( ) (10 mg/L) for 24h, at which point surviving (persistent) cells were recovered.

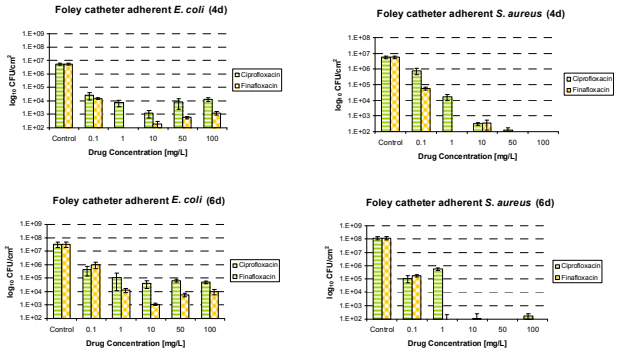


Figure 4. Catheter-adherent populations of *E. coli* and *S. aureus* following exposure to CIP ( ) and FIN ( ). Populations were grown for 4 or 6 days on silicon-coated Foley catheters in artificial urine medium, before drug exposure to concentration ranges of FIN or CIP for 24h. Following treatment, viable cells were recovered and enumerated. FIN exhibited a greater degree of killing than CIP, in terms of eradication, on both populations.

Catheter-adherent populations

Catheter-adherent populations of *E. coli* and *S. aureus* exhibited age-dependent susceptibilities to antibiotics. For example at 3 days, adherent populations of both species were completely eradicated from the catheter following exposure to 0.1 mg/L of FIN or CIP. Older populations began to exhibit phenotypic resistance to these drugs and hence were more difficult to treat. FIN exhibited superior bactericidal activity to CIP at concentrations of 1mg/L and above against 4- and 6- day old catheter adherent populations of *E. coli* and *S. aureus* (Figure 4). On average, FIN reduced such populations to 1 – 2 $\log_{10}$ CFU lower than equivalent CIP treated populations.

Conclusions

- FIN exhibited superior killing to CIP, LVX and MXF against filter membrane-adherent *E. coli*. Killing was faster and FIN was the only drug to prevent the treated population from re-growing in drug-free media.
- FIN also exhibited superior bactericidal activity to CIP against catheter adherent *E. coli* and *S. aureus*. Killing was superior in terms of the lower numbers of surviving cells.
- Exposure of stationary-phase *E. coli* to FIN also resulted in a more extensive killing than CIP and LVX.
- These findings show that FIN is superior to other FQs in terms of the speed and extent of its bactericidal activity against non-growing and adherent *E. coli*.

Literature

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48th ICAAC / 46th IDSA
Washington DC 2008

F1-2043

Selection and Characterisation of Finafloxacin, Ciprofloxacin and Levofloxacin Resistant Mutants of *Escherichia coli*

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Revised Abstract

Background: Finafloxacin (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 show decreased activity. Therefore, FIN is intended for therapeutic use against bacterial infections associated with an acidic environment. The *in vitro* emergence and genotypic mechanism of resistance to FIN, ciprofloxacin (CIP) and levofloxacin (LVX) was investigated in *E. coli* at pH 7.2 and pH 5.8.

Methods: Single-step mutants of *E. coli* ATCC 25922 were selected against FQ concentration gradients in Mueller-Hinton (MH) agar by plating 10^{10} CFU. MICs of stable mutants were determined by CLSI broth microdilution procedures at pH 7.2 and 5.8. Target mutation in gene segments of *gyrA* and *parC* of CIP, LVX and FIN resistant mutants were sequenced from PCR products. DNA sequences of mutants were aligned with parent.

Results: Resistance frequencies (8 x MIC) of first step mutants to FIN, CIP and LVX were 4.1 x 10^{-10}, 2.2 x10^{-9} and 1.3 x 10^{-9} respectively. First step mutants exhibited an 8 - 32-fold decrease in susceptibility over the parent and a relative decrease in susceptibility to the comparator FQs. All first step mutants (FIN, CIP & LVX) developed mutations within the quinolone resistance determination region (QRDR) of *gyrA*, the following substitutions were identified; G81D, S83L and D87N. No mutations in the QRDR of *parC* were detected.

Conclusion: FIN mutants arose at similar frequencies to the CIP and LVX mutants and exhibited similar decreases in susceptibility suggesting that FIN has the same low potential for resistance development. Mutations within the QRDR of *gyrA* were identified in FIN, CIP and LVX first step mutants of *E. coli* indicating this as a primary target.

Introduction

Finafloxacin (FIN, Figure 1) is a novel, 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 associated with low pH such as urinary tract infection and *Helicobacter pylori* eradication.

The propensity for the development of resistance to FIN in *E. coli* was investigated in comparison to ciprofloxacin (CIP) and levofloxacin (LVX). Resultant FQ mutants were characterised to a molecular level.

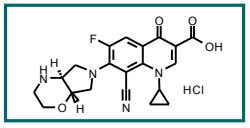

Figure 1. Finafloxacin hydrochloride.

Methods

Minimum inhibitory concentrations (MIC)

MICs were determined according to the CLSI procedure for broth microdilution [8] in cation-adjusted Mueller-Hinton broth (CAMHB) at pH 7.2 and pH 5.8.

Resistance frequencies

Single-step resistance frequencies of *E. coli* ATCC 25922 were determined on sodium phosphate buffered Mueller-Hinton agar (MHA) at pH 7.2 or pH 5.8, containing test drugs up to 32 x MIC. Stability of emergent resistant colonies was confirmed by MIC after three passages on drug free MHA. Mutation prevention concentration (MPC) was defined as the concentration of drug at which no resistant colonies were selected from an inoculum of 10^{10} CFU.

Serial passage

Resistant mutants were also selected following daily serial passage in the presence of subinhibitory FQ concentrations in CAMHB, pH 7.2 or pH 5.8.

Target gene sequencing

Genomic DNA was extracted from mutants and PCR performed according to the method of Lindgreen *et al.*, [9]. DNA sequencing of quinolone resistance determination regions (QRDRs) was performed using standard techniques.

Results and Discussion

The propensity for the development of resistance to FIN, CIP and LVX in *E. coli* 25922 was investigated through comparison of first-step, spontaneous resistance frequencies (Figure 3) and mutation prevention concentrations (Figure 2) at pH 7.2 and pH 5.8.

At pH 7.2, the MPCs for CIP (0.125 mg/L), LVX (0.25 mg/L) and FIN (1 mg/L) were each equivalent to 16 x $MIC_{[pH\ 7.2]}$. Below this threshold, at 8 x $MIC_{[pH\ 7.2]}$, resistance frequencies to each FQ were similar; FIN (4.1 x 10^{-10}), CIP (2.2 x 10^{-9}) and LVX (1.3 x 10^{-9}).

At pH 5.8, the MPCs of FIN (0.25 mg/L), CIP (1 mg/L) and LVX (2 mg/L) were between 16 – 32 x $MIC_{[pH\ 5.8]}$. As at pH 7.2, the FQ resistance frequencies at 8 x $MIC_{[pH\ 5.8]}$ were similar; FIN (1.1 x 10^{-9}), CIP (3.2 x 10^{-9}) and LVX (4.9 x 10^{-10}).

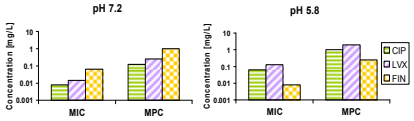

Figure 2. Minimum inhibitory concentration and mutation prevention concentration of FIN, CIP and LVX for *E. coli* 25922 at pH 7.2 (left) and pH 5.8 (right).

Results and Discussion

The potential for resistance development to FIN, CIP and LVX was also compared by serial passage of *E. coli* in the presence of subinhibitory FQ concentrations. The concentration of FQ at which the passaged culture could grow steadily increased over 22 passages, to 8 - 32 times greater than the starting concentration (Figure 4).

These findings, along with the single step resistance frequencies and MPCs (Figure 2 – 3) demonstrate that development of resistance to FIN in *E. coli* occurred at similar frequency to, and to a similar extent as CIP or LVX resistance.

Single-step, agar selected, FIN, CIP and LVX mutants are listed in Table 1. Mutants to each drug exhibited a similar susceptibility profile, comprising of a 8 – 32-fold increase in MIC to each of the FQs tested. Mutants to each drug harbored single point mutations conferring one of the following amino acid substitutions within the QRDR of *gyrA*; G81D, S83L or D87N. No mutations were detected in the QRDR of *parC*. This suggests that FIN shares a common target with CIP and LVX.

Susceptibilities of characterised FQ resistant mutants of common parentage, harboring different combinations of target mutations, to FIN, CIP and LVX were determined at pH 7.2 and 5.8 (Table 2).

The MIC increase exhibited by this panel was dependent on the nature of the target mutation(s). All mutants exhibited relative increases in MIC to FIN, CIP and LVX, when compared under the prevailing conditions of pH. In general, dual target mutants exhibited a 16 – 64 increase in MIC (to FIN, CIP and LVX) and a third mutation resulted in a further 4 – 16-fold increase in MIC.

In summary, the propensity for the development of FIN resistance in *E. coli* was very similar to that of CIP or LVX when compared at concentrations relative to the MIC under the prevailing conditions of pH. FIN exhibits much improved antibacterial activity at low pH. Subsequently, the limits of MPC and mutant selection window were lower at p 5.8 than at pH 7.2. Conversely, the negative effect of low pH on the activity of CIP and LVX is reflected in higher MPCs and concentrations at which mutants arise at pH 5.8.

This would imply that FIN may have an advantage in treating infections in acidic environments, such as in the gastrointestinal or urogenital tract, in abscesses, intra-abdominal infections, TB, CF and others.

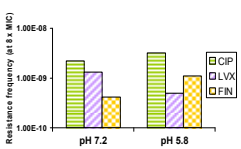

Figure 3. Spontaneous resistance frequencies of *E. coli* 25922 to 8 x MIC of FIN, CIP and LVX at pH 7.2 and pH 5.8.

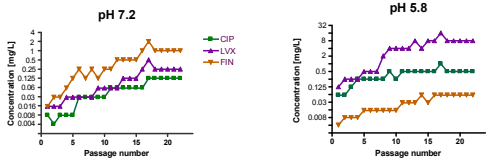

Figure 4. Selection of FQ resistance in *E. coli* 25922 by serial passage at pH 7.2 and pH 5.8.

Strain	*gyrA* mutation			$MIC_{pH\ 7.2}$ [mg/L]			$MIC_{pH\ 5.8}$ [mg/L]		
	Nt	Substitution	Amino acid	CIP	LVX	FIN	CIP	LVX	FIN
ATCC 25922			WT	0.008	0.016	0.063	0.06	0.125	0.008
CIP_14	242	GGT→GAT	G81D	0.125	0.25	0.5	1	2	0.125
CIP_13	248	TCG→TTG	S83L	0.25	0.5	0.5	2	2	0.125
CIP_35	259	GAC→AAC	D87N	0.125	0.25	0.5	2	4	0.06
LVX_17	242	GGT→GAT	G81D	0.125	0.25	0.5	1	2	0.125
LVX_18	248	TCG→TTG	S83L	0.25	0.25	0.5	2	2	0.125
LVX_19	259	GAC→AAC	D87N	0.06	0.125	0.25	1	1	0.06
FIN_21	242	GGT→GAT	G81D	0.125	0.25	0.5	1	2	0.125
FIN_25	248	TCG→TTG	S83L	0.25	0.25	0.5	2	4	0.125
FIN_27	259	GAC→AAC	D87N	0.125	0.25	0.5	1	2	0.125

Table 1. First-step FQ resistant mutants of *E. coli* ATCC 25922, selected against FIN, CIP or LVX. All mutants exhibited an 8 – 32-fold increase in MIC to both the selective and comparator FQs. QRDR sequencing of *gyrA* revealed the following substitutions; G81D, S83L and D87N in mutants selected by each of FIN, CIP and LVX.

gyrA allele	Second *gyrA* allele	*parC* allele	$MIC_{pH\ 7.2}$ [mg/L]			$MIC_{pH\ 5.8}$ [mg/L]		
			CIP	LVX	FIN	CIP	LVX	FIN
ATCC 25922			0.008	0.016	0.063	0.06	0.125	0.008
G81D		wt	0.125	0.25	0.5	1	2	0.125
G81D		S80R	8	16	64	64	128	16
G81D		S80I	1	4	8	8	32	2
G81D		S80I	2	8	32	16	64	4
G81D		G78D	2	16	16	16	128	4
D87N		wt	0.06	0.125	0.25	1	1	0.06
D87N	G81D	wt	2	4	8	16	16	2
S83L	D87N	wt	4	4	16	32	32	4
D87N	A84P	wt	8	8	64	64	64	8
D87N		S80R	2	4	8	16	16	1
D87N	A84P	E84K	128	32	64	>256	256	16
S83L		wt	0.25	0.5	0.5	2	2	0.125
S83L	D87N	S80R	64	32	128	>256	256	16

Table 2. Activity of FIN, CIP and LVX against a panel of double and triple target FQ resistant mutants of *E. coli* ATCC 25922. Mutants exhibited relative increases in MIC to each FQ.

Conclusions

- FIN exhibited improved antibacterial activity at low pH whereas CIP and LVX (along with other marketed FQs) lose activity. The mutant selection window for these FQs changed accordingly (and relative to potency) with pH.
- Resistance frequencies for FIN in *E. coli* were comparable to those for CIP and LVX at 8 x $MIC_{[pH\ 7.2]}$ and at 8 x $MIC_{[pH\ 5.8]}$.
- Single-step mutants to FIN, CIP and LVX exhibited relative decreases in susceptibility and common target allelic profiles, indicating *gyrA* as the primary target.
- These findings imply that FIN would have an advantage, in terms of its mutant selection window, under conditions of low pH, reflecting its unusual pH activation profile.

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] Clinical Laboratory Standards Institute (CLSI). M7-A6. National Committee for Clinical Laboratory Standards, Wayne, Pa., 2003.
[9] Lindgreen *et al.*, (2003). *AAAC* **47(10):** 3222-3232.

48th ICAAC / 46th IDSA,
Washington DC 2008

F1-2044

Pharmacokinetics (PK) and *In Vivo* Efficacy of Oral Finafloxacin (FIN) and Comparators in Rodent Models of Systemic Infection

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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 show decreased activity. FIN was evaluated in comparison with several best in class FQs; ciprofloxacin (CIP), levofloxacin (LVX) and moxifloxacin (MXF), in *in vivo* bacteraemia models with range of pathogens.

Methods: Serum concentrations were quantified from mice (3 / time point) by bioassay. Bacterial inocula were administered intraperitoneally, treatment commenced 0.5h postinfection and survival monitored over 3 - 5 days. Groups of 5 - 6 female CFW-1 mice or 5 Wistar rats were used. Additionally, treatment of *M. catarrhalis* colonisation was assessed by reduction of bacterial counts (Δ $\log_{10}$ CFU/mL) in multiple tissues.

Results: The following PK parameters (normalised to 1mg/kg) were determined for **FIN**, MXF, CIP, LVX following oral administration; AUC [kg*h/L] (**0.57**, 0.15, 0.1, 0.22), Cmax [kg/L] (**0.36**, 0.17, 0.04, 0.18), $t_{1/2}$ [h] (**1.52**, 1.26, 1.84, 0.59). The minimum protective oral (or i.v.) doses (i.e. 100% survival) of **FIN** , MXF , CIP, LVX (all mg/kg) in the following bacteraemia models were; *S. aureus* (**10**, 25, 25, >25), MRSA CIP^{RES} (**50**, 50, >50, >50), *E. faecalis* (**1**, >25, >25, 25), VRE (**10**, 10, 25, 25), *S. pneumoniae* (**25**, 25(MXF)), *S. pneumoniae* PEN^{RES} (**25**, 50, >50, 50), *S. pyogenes* (**50**, 50, >50, >50), *E. coli* (**0.5**, 10, 1, 10), *S. marcescens* (i.v., **5**, 5, 0.2, 1), *K. pneumoniae* (**>2.5**, 1, 2.5, 2.5) and *S. pneumoniae* (rat) (**25**, >25, >25, >25). FIN (10mg/kg p.o.) was considerably more active than the other FQs in reducing the viable load of *M. catarrhalis* from the lungs of colonised mice, exhibiting a Δ $\log_{10}$ CFU/mL of >-3.

Conclusions: FIN, compared with MXF, CIP and LVX exhibited comparative or, in most cases, superior efficacy in rodent bacteraemia models with an extensive range of pathogens.

Introduction

Finafloxacin (FIN, Figure 1) is a novel, 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 also exhibited superior activity against adherent bacteria *in vitro* [3].

Additionally, FIN displayed an excellent safety profile in a wide range of predictive, *in vitro*, toxicity assays [4] and was well tolerated in healthy human volunteers [5]. These attributes suggest that FIN warrants clinical investigation for bacterial infections that are usually associated with low pH such as urinary tract infection and *Helicobacter pylori* eradication.

FIN displayed favorable pharmacokinetic parameters in mice, when compared alongside several best in class FQs, ciprofloxacin (CIP), levofloxacin (LVX) and moxifloxacin (MXF). The therapeutic potential of FIN was then assessed in a series of rodent bacteremia models.

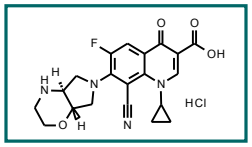


Figure 1. Finafloxacin hydrochloride.

Methods

Pharmacokinetics. Serum concentrations were measured after oral dosing with FIN, MXF, CIP or LVX by bioassay. Blood samples were collected from 3 mice/time point, sera prepared and the concentrations measured by zone diffusion bioassay against *E. coli* or *S. aureus*. AUC, C_{max} and $T_{1/2}$ values were calculated.

Mouse Infections. Overnight cultures of each micro organism were diluted and recultured so that bacteria were in the early logarithmic phase of growth. Female CFW-1 mice, 18 - 20g body weight were infected intraperitoneally (i.p.) with a bacterial suspension in physiological saline or 5% mucin in saline. An inoculum exceeding the LD_{100} was used.

***Enterococcus faecalis* infection** – Groups of 6 mice were infected i.p. with 2.4 x 10^8 CFU/mouse of strain 27159 in 5% mucin. Treatment was by the oral route or by the intravenous (i.v.) route at 30 min post infection with 1, 10, 25 mg/kg of FIN, MXF, CIP, LVX. The survival at 5 days post infection was plotted.

***Moraxella catarrhalis* infection** – Groups of 6 mice were infected i.p. with 1.3 x 10^7 CFU/mouse in 5% mucin. Treatment was by the oral route at 30 min post infection with 1, 10, 25 mg/kg of FIN, MXF, CIP or LVX. On day 1 post infection mice were killed, lungs removed and homogenised (POTTER S Homogeniser) in sterile saline. Viable bacteria were determined by plating serial 10-fold dilutions of the homogenates in duplicate on agar plates. The colony forming units (CFUs) were counted after overnight incubation.

***Escherichia coli* infection** - Groups of 6 mice were infected i.p. with 3.2 x 10^7 CFU /mouse of strain DSM 10650. Treatment was by the oral route at 30 min post infection with 0.1, 0.5, 1.0, or 10 mg/kg of FIN, MXF, CIP or LVX. The survival at 5 days post infection was plotted.

Results and Discussion

Pharmacokinetics (PK)

The PK values are shown in Table 1. FIN had the highest AUC and C_{max} values of the compounds tested. Dose dependency of the C_{max} values after oral administration to mice were almost linear over a dose range of 0 - 225 mg/kg (Figure 2).

Compound	AUC [kg*h/L]	C_{max} [kg/L]	$T_{1/2}$ [Hours]
Finafloxacin	0.571	0.364	1.52
Moxifloxacin	0.153	0.172	1.26
Ciprofloxacin	0.104	0.035	1.84
Levofloxacin	0.223	0.182	0.59

Table 1. Mouse oral PK values (normalised to 1 mg/kg).

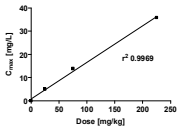


Figure 2. Dose dependency of the C_{max} values after oral administration over a dose range of 0 - 225 mg/kg.

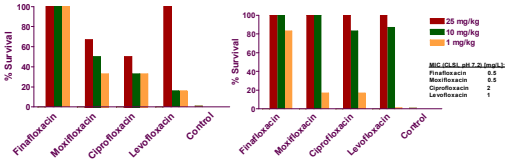
Mouse infection with *Enterococcus faecalis* 27159

FIN was superior to the other FQs when dosed by the oral route with a minimum protective dose of 1 mg/kg in contrast to 25 mg/kg for LVX and >25 mg/kg for MXF and CIP. FIN was also more active when dosed i.v., with 80% protection at 1 mg/kg in contrast to <20% protection with the other three FQs. MXF was as active as FIN *in vitro* (MIC 0.5 mg/L) although being far less active *in vivo* (Figure 3 + 4).

Results and Discussion

Figure 3. Survival at 5 days Post Infection with *E. faecalis* Following Oral Dosing.

Figure 4. Survival at 5 days Post Infection with *E. faecalis* Following i.v. Dosing.



Mouse Infection with *Escherichia coli* DSM 10650

All compounds showed good activity but FIN was more effective at low doses, with 0.5 mg/kg producing 100% survival and 0.1 mg/kg protecting 60% of infected mice. Although levofloxacin had a low MIC (0.03 mg/L), it was the least active compound *in vivo* (Figure 5).

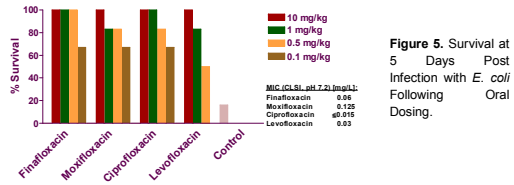


Figure 5. Survival at 5 Days Post Infection with *E. coli* Following Oral Dosing.

Mouse infection with *Moraxella catarrhalis*

FIN showed a greater reduction in the numbers of organisms surviving in the lungs of infected mice at all three dose levels. It was also more active *in vitro* than the three comparator FQs (Figure 6).

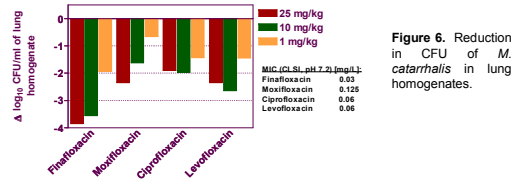


Figure 6. Reduction in CFU of *M. catarrhalis* in lung homogenates.

FIN, MXF, CIP and LVX were evaluated in bacteremia models with an additional and extensive series of pathogens. Efficacy and ranking data are summarised in Table 2.

Table 2. Efficacy of FQs in additional bacteremia models. Minimal protective doses are listed in the order; **FIN**, MXF, CIP, LVX. N; neutropenic.

Infection model	Species	Pathogen	Minimum protective dose (FIN, MXF, CIP, LVX) [mg/kg] Oral	i.v.	Efficacy ranking
Bacteremia	Mouse	*S. aureus*	**10**, 25, 25, >25	**1**, 1, >25, 10	**FIN** ≥ MXF >> LXV > CIP
	Mouse (N)	*S. aureus*		**50**, >50	**FIN** = MXF
	Mouse	*S. aureus* CIP^{RES}	**50**, 50, >50, >50	**50**, 25, >50, >50	MXF ≥ **FIN** >> LVX ≥ CIP
	Mouse (N)	*E. faecalis* (VRE)	**10**, 10, 25, 25		**FIN** = MXF > CIP = LVX
	Mouse	*S. pneumoniae*	**25**, 25	**50**, 50, >50, >50	**FIN** ≥ MXF > LVX = CIP
	Mouse	*S. pneumoniae* PEN^{RES}	**25**, 50, >50, 50		**FIN** ≥ MXF >> LVX ≥ CIP
	Rat	*S. pneumoniae*	**25**, >25, >25, >25		MXF ≥ **FIN** > LVX > CIP
	Mouse	*S. pyogenes*	**50**, 50, >50, >50		**FIN** = MXF >LVX >> CIP
	Mouse (N)	*E. coli*		**1**, 10, 1, 10	**FIN** = CIP > MOX > LEV
	Mouse	*P. aeruginosa*	**>25**, 25	**>25**, 10	CIP >> **FIN**
	Mouse	*S. marcescens*		**5**, 5, 0.2, 1	CIP > LVX ≥ MXF > **FIN**
	Mouse	*K. pneumoniae*	**>2.5**, 1, 2.5, 2.5		MXF ≥ CIP ≥ LVX > **FIN**

Conclusions

- FIN, a new, 8-cyano, broad spectrum FQ has activity *in vivo* against a wide range of pathogenic micro organisms in rodent bacteremia models.
- The results shown here illustrate its superior activity compared with the comparator FQs; MXF, CIP and LVX when administered by the oral route in mouse systemic infections caused by *E. coli* and *E. faecalis*. It was also more active than the other FQs against *M. catarrhalis*, reducing the numbers of organisms in the lungs of infected mice even at low doses.
- These findings, taken together with the good broad spectrum activity *in vitro* against a number of important pathogenic species, including those resistant to other agents, the excellent tolerance seen by the oral route in Phase I studies in man and the lack of toxicity seen in predictive *ex vivo* toxicity tests, indicate that FIN is an excellent candidate for progression to the clinic.

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] Schmuck *et al.*, 48th ICAAC, Washington DC 2008, Poster No. F1-2047.
[5] Patel *et al.*, 48th ICAAC, Washington DC 2008, Poster No. F1-2048.

48th ICAAC / 46th IDSA,
Washington DC 2008

F1 -2045

In Vivo Efficacy of Finafloxacin in Difficult to Treat Animal Models of Infection

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Abstract

Background: Finafloxacin (FIN) is a novel fluoroquinolone (FQ) belonging to a new 8-cyano subclass that exhibits optimal efficacy at slightly acidic pH (5.0 - 6.0), under which other FQs show decreased activity. FIN was evaluated along with ciprofloxacin (CIP), levofloxacin (LVX) and moxifloxacin (MXF), in a wide range of *in vivo* models.

Methods: Female CFW-1 mice (n = 6) were used. Bacterial inocula were administered by the following routes: intraperitoneal, oral application, implantation of colonised catheter material or direct injection into the kidney, bladder, thigh, granuloma pouch or abscess. Treatment was commenced 0.5 - 3h postinfection. End points were determined by % survival (at 3 - 5 days) or by reduction of bacterial counts (Δ $\log_{10}$ CFU/mL) in homogenised tissue.

Results: FIN (10mg/kg s.c.) exhibited greater killing of *S. aureus* in the thigh muscle (Δ $\log_{10}$ CFU/mL: FIN > -4, MXF -3, CIP -1 and LVX -2) than the comparators. FIN (10mg/kg p.o.) exhibited equal killing of *S. aureus* (Δ $\log_{10}$ CFU/mL: FIN -2, MXF -2) and greater killing of *P. aeruginosa* (Δ $\log_{10}$ CFU/mL: FIN -1, CIP, LVX and MXF all <(-1)) than the other FQs in infected abscess models.
FIN (10mg/kg p.o.) exhibited bactericidal activity in severe *E. coli* pyelonephritis (Δ $\log_{10}$ CFU/mL; FIN -4, CIP -3, LVX -4, MXF -3) and also in ascending *P. mirabilis* cystitis (Δ $\log_{10}$ CFU/mL; FIN and CIP (100mg/kg p.o.) -4). Data not shown.
Additionally, FIN exhibited equal, if not superior activity to the comparators in granuloma pouch and implanted catheter models (*S. aureus*), enteritis (*S. typhimurium*), and post surgical polymicrobial peritonitis.

Conclusion: The superior efficacy of FIN over CIP, LVX and MXF in these difficult to treat models was in line with their respective MICs at lower pH values which are anticipated to occur in many infection models, especially those involving inflammation (abscess) or low pH fluids such as urine. These data suggest that the pH activity profile of FIN may be an advantage in combating severe bacterial infection.

Introduction

Finafloxacin (FIN, Figure 1) is a novel, 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 also exhibited excellent activity against adherent bacteria *in vitro* [3].

Additionally, FIN displayed an excellent safety profile in a wide range of predictive, *in vitro*, toxicity assays [4] and was well tolerated in healthy human volunteers [5]. These attributes suggest that FIN warrants clinical investigation for bacterial infections that are associated with low pH such as urinary tract infection and *Helicobacter pylori* eradication.

FIN was a highly efficacious agent in eradicating Helicobacter spp. from a very difficult to treat murine model [6]. To further examine its therapeutic potential, FIN was evaluated in a number of rodent models of infection that were selected to offer a wide range of infection sites and to mimic the type of infections that are difficult to treat in humans, including those involving formation of adherent bacterial populations.

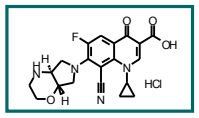


Figure 1. Finafloxacin hydrochloride.

Methods

Thigh Infection Model - *S. aureus*. Mice were rendered neutropenic by the subcutaneous (s.c.) administration of cyclophosphamide 4 days and 1 day prior to infection. Under light anaesthesia (CO_2) 0.1 mL of a log-phase culture of *S. aureus* DSM 11823 (3 x 10^7 CFU/mL) was injected into the right hind leg. Treatment started 30 min post infection (P.I.) with a second dose at 4 h P.I. Mice received doses of 2, 10 and 50 mg/kg s.c. 24 h after infection mice were killed and the number of bacteria remaining in the thigh homogenates was determined by plating out dilutions for CFUs.

Implanted Foreign Body Model - *S. aureus* Catheters were incubated overnight in a *S. aureus* DSM 11823 culture. The catheter was rinsed and then implanted s.c. in mice. Treatment started 3 h later and continued BID until the day prior to removal, with 10 mg/kg/dose. Samples were removed at 4 and 7 days. The explants were homogenised (Ultra Turrax), diluted and plated to determine the CFU remaining.

Infected abscess Model – *S. aureus* and *P. aeruginosa*. Gelfoam™ was cut into pieces 1 x 1 cm and incubated overnight in sterile PBS, pH 7.4. The following day these were implanted s.c. on the back of mice. Within 3 days a capsule formed around the implant and this was infected with 1 x 10^5 *S. aureus* or 4 x 10^4 *P. aeruginosa*. Treatment was with 10 mg/kg 2 h post infection.

Postoperative polymicrobial sepsis. (Caecal ligation and puncture model) Mice were anaesthetised and the peritoneum opened with a small cut. The caecum was moved out of the peritoneum. The caecum was ligated and punctured with a 21G needle. The ligated intestine was replaced and the wound closed. 3 doses of 10 mg/kg were given at 4, 18 and 24 h post operation. Efficacy was determined by survival over 7 days.

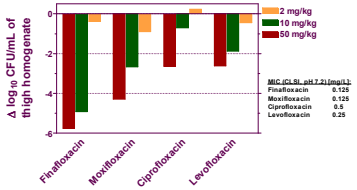
***E. coli* pyelonephritis.** Mice were anaesthetised, the right flank shaved to locate the kidney. Holding the kidney below the skin, 10 µL of a suspension of *E. coli* DSM 10650 (1 x 10^5) was injected directly into the kidney by using a 21G needle. Mice were treated with a single dose of 1 or 10 mg/kg 2 h P.I. Kidneys were removed 2 days later, homogenised and viable counts performed on dilutions of the homogenates.

Results and Discussion

***S. aureus* DSM 11823 infected thigh model**.

The effect of the FQs in reducing the numbers of staphylococci in the thigh tissues is shown in Figure 2. FIN produced a dramatic fall of 5 $\log_{10}$ CFU recovered from the thigh homogenates at 10 mg/kg, far more than was seen with the other compounds. FIN and MXF had the lowest MICs (0.125 mg/L) but MXF, although being more active than CIP and LVX, was less active at all dose levels than FIN.

Figure 2. *S. aureus* infected thigh model – CFU reduction in thigh muscle.

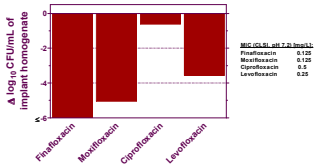


Results and Discussion

Implanted Foreign Body Model *S. aureus* DSM 11823 infected catheter.

The effect of the FQs in reducing the numbers of staphylococci in the catheters at 7 days post -implantation is shown in Figure 3. As in the thigh lesion model, FIN was the most active compound, reducing the numbers by > 6 logs. CIP had little or no effect and had the poorest MIC (0.5 mg/L). MXF was also effective but was still inferior to FIN. LVX had an intermediate effect.

Figure 3. *S. aureus* infected catheter – CFU reduction in catheter.



Infected abscess model (Gelfoam™) – *S. aureus* and *P. aeruginosa.*

Figure 4 shows the reduction in the numbers of *P. aeruginosa* remaining at Day 7 P.I. FIN was the most effective of the four compounds and MXF the least active.

Organs were also sampled in the mice infected with *S. aureus* and Figure 5 shows the reduction in CFU in the abscess and the various organs. Only FIN and MXF were tested and both were effective in reducing CFU counts in the various tissues..

Figure 4. *P. aeruginosa* reduction in abscesses.

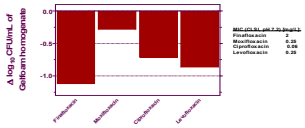
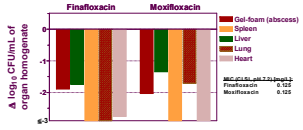


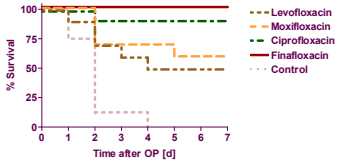
Figure 5. *S. aureus* reduction in various tissues.



Postoperative Polymicrobic Sepsis Model.

The survival of groups of 10 mice treated with three doses of 10 mg/kg of the four FQs is shown in Figure 6. By 7 days post operation all the mice treated with FIN were alive. CIP although being slightly less effective, protected 90% of mice. MXF and LVX were less protective

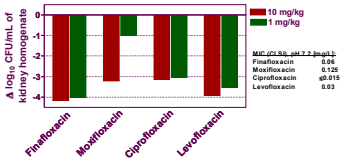
Figure 6. Postoperative Polymicrobic Sepsis Model – survival over 7 days post operation. (N=10).



***E. coli* pyelonephritis**.

FIN was the most active of the four FQs tested, with a 4-log CFU reduction in the kidneys at 2 days P.I. with 10 mg/kg. LVX was equally effective, CIP and MXF were less effective.

Figure 8. *E. coli* pyelonephritis.



Other models.

A variety of other models were used, including granuloma pouches in mice, ascending pyelonephritis using *P. mirabilis*, a peritoneal infection with *L. monocytogenes*, the eradication of *H. felis*, *Salmonella typhimurium* infection and an LPS induced shock model. With the exception of the *P. mirabilis* infection, FIN was the most active compound. The results of all the experiments are summarised in Table 1.

Table 1. Summary of *In vivo* test results

Infection model	Species	Pathogen	Efficacy ranking
UTI (Pyelonephritis)	Mouse	*E. coli*	**FIN** = LEV ≥ CIP > MOX
UTI (ascending)	Mouse	*P. mirabilis*	CIP > **FIN**
SSSI (Infected abscess)	Mouse	*S. aureus*	**FIN** > MOX
	Mouse	*P. aeruginosa*	**FIN** > LEV ≥ CIP > MOX
SSSI (Pouch)	Mouse	*S. aureus*	**FIN** ≥ MOX ≥ LEV
SSSI (Thigh muscle)	Neutropenic mouse	*S. aureus*	**FIN** >> MOX > LEV > CIP
SSSI (Foreign body)	Mouse	*S. aureus*	**FIN** = MOX ≥ LEV >> CIP
	Mouse	*P. aeruginosa*	**FIN** = LEV = CIP >> MOX
Peritonitis	Mouse	*L. monocytogenes*	**FIN** > MOX >> LEV = CIP
Salmonelliosis	Mouse	*S. typhimurium*	**FIN** = MOX = CIP > LEV
Helicobacter eradication	Mouse	*H. felis*	**FIN** >> CIP = MOX
Polymicrobial sepsis	Mouse		**FIN** ≥ CIP > MOX > LEV
LPS - induced shock	Mouse	LPS	**FIN** ≥ MOX = CIP

Conclusions

- FIN had excellent activity in a range of infection models in mice chosen to reflect those that are difficult to treat in the clinic such as peritonitis (with remote organ failure), catheter colonisation and SSTI.
- In general, the efficacy of FIN was superior to that of CIP, LVX and MXF.
- The efficacy of FIN was better than expected from its MIC at pH 7.2, this was especially true in models of serious infection such as peritonitis, pyelonephritis, abscess and thigh muscle infection and may reflect the improved activity of FIN at an acidic pH.
- These findings taken in conjunction with the excellent tolerance seen by the oral route in Phase I studies in man and the lack of toxicity seen in predictive *ex vivo* toxicity tests, indicate that FIN is an excellent candidate for progression to the clinic.

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] Schmuck *et al.*, 48th ICAAC, Washington DC 2008, Poster No. F1-2047.
[5] Patel *et al.*, 48th ICAAC, Washington DC 2008, Poster No. F1-2048.
[6] Buissonière *et al.*, 48th ICAAC, Washington DC 2008, Poster No. F1-2038.

48th ICAAC / 46th IDSA,
Washington DC 2008

F1-2046

Comparative Activity Between Finafloxacin (FIN) and Other Fluoroquinolones Against Bacterial and Eukaryotic Type II Topoisomerases

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Abstract

Background: FIN is a novel fluoroquinolone (FQ) belonging to a new 8-cyano subclass. FIN exhibits optimal efficacy at a slightly acidic pH (5.0 - 6.0) under which other FQ show decreased activity. Because of this property, FIN is intended for therapeutic use against bacterial infections associated with an acidic environment. The selectivity of FIN for eukaryotic and bacterial DNA topoisomerase II enzymes was evaluated using quantitative plasmid DNA cleavage assays *in vitro*.

Methods: The ability of FIN, clinafloxacin (CLX), ciprofloxacin (CIP), moxifloxacin (MXF) and enoxacin (ENX) to induce DNA cleavage from human topo IIα, *E. coli* DNA gyrase and topo IV was quantified and compared based on the cleavage detection limit (CDL), defined as the lowest concentration yielding detectible cleavage product compared with that of the known topo II poison, etoposide (VP16). The CL50 value, defined as the concentration that induces 50% maximum cleavage, was used as an additional endpoint for bacterial enzymes.

Results: The activity of FIN against the human enzyme was 250-fold lower than that of VP16 and places it well amongst the other FQ (in terms of fold lowered activity against the human enzyme) *viz*; CLX (10 - 50), CIP (100 - 250), MXF (500) and ENX (no CDL detectable).
FIN, CLX, CIP and MXF exhibited a CDL of 1ng/mL against bacterial DNA gyrase, ENX exhibited lower activity (10ng/mL). FIN, CLX and MXF displayed comparable activity against topo IV (1ng/mL), while CIP (10ng/mL) and ENX (50ng/mL) were less active. CL50 (ng/mL) against gyrase and topo IV respectively show that FIN (25, 8) was more active against both bacterial targets than CLX (10, 52), CIP (120, 200), MXF (70, 200) and ENX (50, 500).

Conclusions: These data indicate that FIN is highly selective for bacterial type II topoisomerases. FIN exhibited superior activity to the comparator FQs in terms of potency against the individual bacterial enzymes and its relative eqipotency against these dual targets.

Introduction

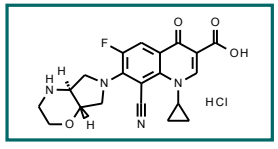
Finafloxacin (FIN, Figure 1) is a broad spectrum fluoroquinolone (FQ) that belongs to a new 8-cyano subclass [1]. FIN contains a novel chiral base component which confers improved antibacterial activity at slightly acidic pH (pH 5.0 – 6.0), under which other marketed FQs exhibit significantly reduced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

In addition, FIN displayed an excellent safety profile in a wide range of predictive, *in vitro*, toxicity assays [3] and was well tolerated in healthy human volunteers [4].

FQs target DNA bound, bacterial type II topoisomerase enzymes, forming a stable complex and halting DNA replication, resulting in the release of double stranded DNA breaks. The selectivity index of FIN and other FQs was investigated by measuring their comparative activities against the human counterpart of these target enzymes, topo IIα.

The relative target activities of FIN and other test FQs against their bacterial targets, were determined *in vitro*, against bacterial DNA gyrase and topoisomerase IV.

Figure 1. Finafloxacin hydrochloride.



Methods

Finafloxacin (FIN), ciprofloxacin (CIP), moxifloxacin (MXF), clinafloxacin (CLX) and enoxacin (ENX) and the topoisomerase poison VP16 were titrated against:

Human Topoisomerase II: Human topo IIα isoform was added to 250 ng plasmid DNA Substrate for 30min at 37ºC. The reactions were terminated with SDS (1%) and digested with proteinase K (50 µg/mL) for 30 min at 56ºC and run on 1% agarose gels. Inhibition of the human enzyme was quantified as a function of released single stranded DNA.

Escherichia coli DNA Gyrase and DNA Topoisomerase IV: Supercoiled pHOT1 was relaxed using human topo I to form open circular DNA. Addition of functional *E. coli* DNA gyrase or topoisomerase IV converted this substrate to supercoiled DNA. Inhibition of gyrase or topoisomerase activity was measured by quantification of released, linear DNA.

Endpoints for topoisomerase inhibition

The cleavage detection limit **(CDL)** was defined as the lowest concentration of drug yielding detectible cleavage product.

The **CL_{50}** value, defined as the concentration that induces 50% maximum cleavage, was used as an additional endpoint for bacterial enzymes.

Results and Discussion

Activity of FQs compared to the topoisomerase poison VP16 against human Topoisomerase II.

The human topo IIα isoform was sensitive to VP16 (CDL = 1 µg/mL, CL_{50} = 25 µg/mL). The activities (CDL and CL_{50}) of the test compounds were determined against the human enzyme for the calculation of their relative selectivity indices [activity of test FQ (CDL or CL_{50}) / activity of VP16 (CDL or CL_{50}].

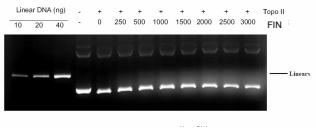
Of these, CLX exhibited the lowest selectivity index (based on CL_{50}; 10, based on CDL; 50) against the eukaryotic enzyme. This was followed by CIP (8, 250) then FIN (100, 250) and MXF (500 - estimated). ENX exhibited the greatest selectivity index of the test compounds (both CDL and CL_{50} were undetectable). These data are summarized in Table 1 and Figure 2.

The CDL values of the FQs against human topo IIα indicate a range of selectivity indices spanning 50- 500-fold. FIN exhibited an index of 250 which placed it well among the other FQs in terms of its ratio of selectivity.

Activity of FQs against *E. coli* DNA gyrase (Table 2, Figure 3).

E. coli DNA gyrase activity in the presence of supercoiled DNA was inhibited by all test FQs in a concentration dependent manner. This inhibition was measured by quantification of released, linear DNA.

Results and Discussion



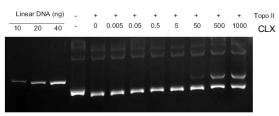


Figure 2. Electrophoretic separation of linear DNA fragments, released as a result of FIN (left) and CLX (right) inhibition of human topo IIα.

Comparator	CDL (µg/mL)	CL_{50} (µg/mL)	Selectivity index (Ratio to VP16)
VP16	1	25	1
CLX	50	250	10 - 50
CIP	100 - 250	200	8 - 250
FIN	250	500	100 - 250
MXF	500 - 1000	Unknown*	500 (estimated)
ENX	Undetectable	-	N/A

Table 1. Comparison of DNA cleavage for FQs with VP16. The ratio value (selectivity index) is a measure of how well a particular FQ targets eukaryotic topo II relative to VP16.
*In experiments with MXF the plateau saturation levels could not be determined and CL_{50} could not be extrapolated.

Comparator	CDL (ng/mL)	CL_{50} (ng/mL)
CLX	1	10
FIN	1	25
MXF	1	70
CIP	1	120
ENX	10	50

Table 2. Comparative cleavage data for *E. coli* DNA gyrase.

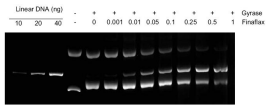


Figure 3. Electrophoretic separation of linear DNA released following FIN inhibition of DNA bound *E. coli* DNA gyrase.

Comparator	CDL (ng/mL)	CL_{50} (ng/mL)
FIN	1	8
CLX	1	52
MXF	1	200
CIP	10	200
ENX	50	500

Table 3. Comparative cleavage data for *E. coli* DNA topoisomerase IV.

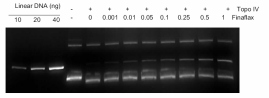


Figure 4. Electrophoretic separation of linear DNA released following FIN inhibition of DNA bound *E. coli* DNA topoisomerase IV.

When compared on the basis of CDL, the test compounds exhibited equipotent activity against *E. coli* DNA gyrase, with the exception of ENX which exhibited a 10-fold lower activity than CLX, FIN, CIP and MXF.

On the basis of CL_{50}, CLX was the most potent inhibitor of *E. coli* DNA gyrase, this was followed by FIN, then MXF, then CIP and ENX.

Activity of FQs against *E. coli* topoisomerase IV (Table 3, Figure 4).

FIN, CLX and MXF exhibited comparatively greater activity than CIP and ENX against topo IV. FIN exhibited equipotent activity to CLX and MXF against topo IV on the basis of CDL (all 1ng/mL) and greater activity than CLX and MXF on the basis of CL_{50} (Table 3). CIP and ENX were less potent than the other test compounds on the basis of both CDL and CL_{50}.

FIN can be classified as a group 4 fluoroquinolone together with MXF and CLX as indicated by its comparably high activity against both bacterial enzymes (dual target activity) [5].

Conclusions

- The test FQs exhibited a range of selectivity against human topo IIα compared with VP16.
- FIN exhibited a high selectivity index against the human enzyme compared with the test FQs.
- Conversely, FIN was one of the most potent inhibitors of *E. coli* gyrase and topo IV, exhibiting a comparatively high level of activity against both bacterial enzymes.
- These data indicate that FIN is at least as potent as a panel of clinically used FQs against bacterial type II topoisomerases and predict a low potential for topoisomerase associated toxicity.

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] Schmuck *et al.*, 48th ICAAC, Washington DC 2008, Poster No. F1-2047.
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48th ICAAC / 46th IDSA,
Washington DC 2008

F1-2047

In Vitro Toxicological Profiling of Finafloxacin

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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 show decreased activity and is therefore intended, e.g., for treatment of *H. pylori* and UTI. Several novel FQs have recently failed during development or shortly after launch due to safety / toxicology concerns. Therefore, prior to start of formal GLP safety / tox studies, FIN was rigorously profiled for the most common class related side effects against a series of predictive *in vitro* tests alongside comparator FQs; Ciprofloxacin (CIP), Trovafloxacin (TRO), and Sparfloxacin (SPA) and standard test controls, where appropriate.

Methods: Cytotoxicity potential was determined against the mouse macrophage line J774.AI and phototoxicity potential against the mouse fibroblast Balb/c 3T3 following exposition to UV irradiation.

Neurotoxic effects like excitatory potentials were tested in extracellular recordings from slices of rat hippocampus and the affinitity to the GABA-A receptor were tested in the guinea pig ileum. Chondrotoxicity potential was studied on primary cartilage cells from dog and man and hepatotoxicity potential on primary rat hepatocytes. Cardiotoxicity (incl. QT effects) was investigated in Langendorff heart preparations and hERG channel experiments.

Results and Conclusions: FIN was examined in a series of *in vitro* cell or tissue based assays that are believed to be predictive of the most common, FQ-associated undesirable side effects. Under the conditions of these assays, FIN displayed a profile indicative of a low potential for the toxicity issues often associated with FQs.

Introduction

Finafloxacin (FIN, Figure 1) is a novel, 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 exhibits superior activity compared with comparator FQs against adherent bacteria *in vitro* [3] and in a wide range of rodent infection models [4,5]. These attributes suggest that FIN warrants clinical investigation for bacterial infections that are usually associated with low pH such as urinary tract infection and *Helicobacter pylori* eradication.

Several novel FQs have failed at late stages of development or shortly after release due to concerns over safety / toxicology. Therefore toxicological profiling of FIN was addressed during early stages of development in an extensive set of predictive, *in vitro* toxicity assays.

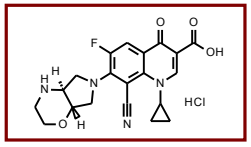

Figure 1. Finafloxacin hydrochloride

Methods

Mouse cytotoxicity – A permanent mouse macrophage cell line (J774.A1) obtained from ATCC was incubated with compounds for 72 h in DNEM in microtitre trays. After washing, cell viability was determined using a neutral red assay.

Excitatory and neurotoxic potential – Brains were removed from young rats and cooled immediately. Slices (450 µm) were made from the hippocampus and incubated in carbon saturated artificial cerebral spinal fluid (ACF) at RT. They were used within 1-2 h. A superfusion chamber was used at 34°C controlled at 2 mL/min ACF. A conventional electrophysiological method was used, with extracellular recordings being made from the pyramidal cell layer. Electric stimulation of 30 min was used and control conditions recorded for 30 min. Slices were then perfused with 2 µM of the test compounds and observed for a further 30 min.

Phototoxicity was determined in a permanent mouse fibroblast cell line (3T3 cells) obtained from ATCC and incubated in DMEM in microtitre trays. Compounds were added for 1 h and the trays exposed to UV irradiation for 20 min or 60 min.

Hepatotoxic potential – Liver perfusion was carried out in Wistar rats and the freshly isolated primary hepatocytes, washed in HBSS and stained with trypan blue to estimate viability (c 80%). A collagen sandwich gel using 6-well plates was used. Cells were incubated for up to 7 d with 7 d recovery. Cytotoxicity was determined by measuring aspartate aminotransferase (AST), alanine aminotransferase (ALT) and lactate dehydrogenase (LDH). Mitochondrial dehydrogenase activity was also measured using the MTT assay.

Chondrotoxic potential – primary cartilage cells from dogs and man were used. Cartilage from the knee joints of a 9 month old beagle dog. Human cartilage tissue was from a 62 year old female undergoing orthopaedic surgery. Direct cytotoxicity estimated by viability (neutral red technique, measured quantitatively with a spectrometer) of cartilage cells. More detailed studies included measurement of mitochondrial dehydrogenase activity (incubated with MTT for 2 h, measured quantitatively with a spectrometer), mitochondrial status (incubation with tetramethylrhodamine for 30 mins, measured with a fluorospectrometer), intracellular ATP (chemiluminescence assay, ATPLite assay), cell proliferation (^{14}C thymidine uptake measured at 2, 24, 48 and 72 h and scintillation counter used to determine uptake) and collagen type II content (cell-ELISA assay over 72 h using monoclonal antibodies and specific epitopes for collagen II).

Affinity to the GABA-A receptors from guinea pig ileum - Portions (4 cm long) from the terminal ileum of freshly killed guinea pigs were used, placed in an organ bath and contractions measured by multichannel recorders. The response to muscimol (EC_{50}) in the presence and absence of FIN at a range of concentrations up to 10^{-4} M was determined.

Cardiotoxic effects - Langendorff heart preparation. Hearts were removed from 3 male guinea pigs (370-410 g, Charles River) and perfused continuously for 15 min with drugs at 10^{-4} and $3x10^{-4}$ M. Left ventricular pressure, heart rate and coronary flow was measured.

hERG channel activity hERG currents were recoded from stably transfected HEK293 cells. FIN was compared with SPAR. Cells were transferred to a recording chamber and continuously perfused with 1-2 mL/min bath solution at RT. A whole cell patch-clamp configuration was established and recordings made. Concentrations used were FIN 300 µM. Sparfloxacin (SPA) 1, 10, 30, 100 and 300 µM. Changes in the magnitude of the current was measured.

Results and Discussion

Mouse cytotoxicity.

FIN had a very low cytotoxic effect, with an EC_{50} of 100 µg/mL and a NOEC of 30 µg/mL.

Phototoxicity – Mouse Fibroblasts.

FIN was classified as non-phototoxic in this system with an EC_{50} of >100 µg/mL after both 20 min and 1 h exposure. CIP was classified as slightly phototoxic (EC_{50} of >50 µg/mL).

hERG channel activity.

hERG channel is responsible for the potassium influx during repolarisation and an inhibition induces effects like QT-prolongation; this was not influenced by FIN up to 300 µM for outward and tail current. SPA had a concentration dependent effect; outward currents were blocked at an IC_{50} of 21.5 µM and tail currents at 25.4 µM.

Primary rat hepatocytes.

FIN was not hepatotoxic at concentrations of up to 100 µg/mL in all 4 test systems. In contrast trovafloxacin (TVO) produced an increase in ALT (Figure 2), LDH, and the MTT test. These results indicate that FIN has a low potential for hepatotoxicity.

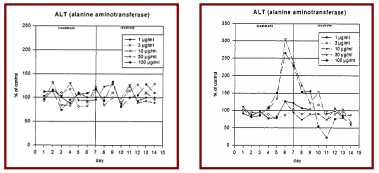

Figure 2. Effect of FIN (left) and TVO (right) on rat hepatocyte ALT levels.

Primary human and dog chondrocytes. FIN showed no toxicity in any of the tests used, resulting in a NOEC level of >100 µg/mL. These are summarised in Figure 3. In contrast, CIP had a NOEC of 10 (dog) and 30 µg/mL (human).

Assay	Dog Chondrocytes	Human Chondrocytes
Neutral red	103 ± 6	94 ± 2
MTT Assay	105 ± 2	95 ± 5
Rhodamine assay	88 ± 5	102 ± 2
ATP	101 ± 12	100 ± 20
Proliferation assay	95 ± 9	97 ± 2
Collagen II determination	120 ± 6	106 ± 0.5

Figure 3. Effect of FIN (100 µg/mL) on Dog and Human Chondrocytes. Expressed at % of control cells.

Langendorff guinea pig heart preparation

No significant change in the left ventricular pressure, its rate of maximal pressure rise, heart rate and coronary flow was noted (p>0.05). No signs of cardiac arrhythmia were observed, NOEC = >300 µM. (Figure 4).

	% change in cardiac parameters			
M (Conc.)	Left Ventricular Pressure	dP/dtmax rate of max. pressure rise	Heart Rate	Coronary Flow
10^{-4}	-4 ± 2	0 ± 0	-3 ± 2	-2 ± 3
3×10^{-4}	+2 ± 3	+5 ± 3	-1± 1	+11 ± 9

Figure 4. Effect of FIN on Langendorff heart preparation.

GABA-A receptors affinity – guinea pig ileum.

These receptors are in the post-ganglionic cholinergic nerve endings in the ileum. When stimulated by the GABA-A receptor agonist, muscimol, contractions occur mediated by acetylcholine released by neurones. FIN had no effect on the EC_{50} of muscimol at concentrations of up to 10^{-4} M (NOEC = >100 µM), and hence had no affinity for GABA-A receptors.

Hippocampus slice test.

FIN showed no excitatory or convulsive potential in this sensitive model (excitatory potential; 98.2% ± 5% of control), confirming its lack of affinity for GABA-A receptors . In contrast, TVO showed an effect (excitatory potential; 276% ± 31% of control) (Figure 5).

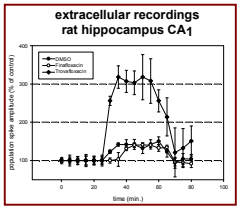

Figure 5. Effect of FIN and TVO on electrically stimulated rat hippocampus slices.

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[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] Patel *et al.*, 48th ICAAC, Washington DC 2008, Poster No. F1-2048.

Conclusions

- FQs suffer from a number of toxic effects, some of which have led to compounds being withdrawn. The test systems used here are all well validated *in vitro* or *ex vivo* systems designed to test possible toxic effects.
- Juvenile dogs have been shown to be more susceptible than rodents to chondrocyte toxicity which is associated with FQ-induced arthropathy. CIP displayed toxicity against the dog and human cell lines whereas the lack of effect with FIN in this model is promising.
- Hepatotoxic, chondrotoxic, phototoxic and neurotoxic potential were determined at concentrations well above those detected in human plasma following dosing [6]. FIN had no effects in these test systems whereas CIP and TRO did display toxicity.
- Compounds inhibiting the hERG channel have been shown to prolong the cardiac action potential and hence the QT interval in man. FIN had no effect in this test (unlike SPA).
- These findings (summarised in Figure 6) taken in conjunction with the excellent tolerability by the oral route in Phase I studies in man [6] and the good activity in animal infection models [4, 5] indicate that finafloxacin is an excellent candidate for progression to the clinic.

Figure 6. Summary of the assessment of FIN in a panel of predictive *in vitro* and *ex vivo* toxicity assays

Test system	Finafloxacin result	Comparator result
Cytotoxicity mouse MΦ J774.A1	EC_{50} = 100 µg/mL	EC_{50} = 80 µg/mL (CIP)
Phototoxicity mouse fibroblasts 20'/1h radiation	EC_{50} > 100 µg mL	EC_{50} > 100 / = 60 µg/mL (CIP)
Hippocampus slice test	98 % of control	276 % of control (TRO)
Primary hepatocytes	NOEC ≥ 100 µg/mL	NOEC 10 µg/mL (TRO)
Primary dog chondrocytes	NOEC ≥ 100 µg/mL	NOEC = 10 µg/mL (CIP)
Primary human chondrocytes	NOEC ≥ 100 µg/mL	NOEC = 30 µg/mL (CIP)
GABA-A receptor	NOEC > 100µM	0.3 µM ((+)-bicuculline)
Langendorff heart preparation	NOEC > 300 µM	-
hERG channel activity	NOEC > 300 µM	ED_{50} ~ 25 µM (SPA)

48th ICAAC / 46th IDSA,
Washington DC 2008

F1-2048

A Phase I Study to Determine Safety, Tolerability and Pharmacokinetics (PK) of Finafloxacin (FIN) in Healthy Subjects

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Revised abstract

Introduction: FIN is a novel fluoroquinolone (FQ) under early clinical development. FIN exhibits optimal activity at slightly acidic pH (pH 5.0 - 6.0). A combined Phase I study protocol was designed to evaluate safety, tolerability and PK of single and multiple ascending oral doses of FIN in healthy adult subjects.

Methods: The study was designed as a single-center, inpatient, double-blind, randomized, placebo-controlled, not weight-adjusted, single and multiple escalating dose study of FIN oral tablets. 75 (64 males, 11 females) subjects were included, 3 of which received a single dose of 25 mg, 40 of which (in groups of 6+2) received single doses of 50 - 800 mg FIN/placebo under fasting conditions (part A). A further 32 were given doses of 150, 300, 600 or 800 mg for 7 consecutive days (part B). The study also included one cohort to evaluate 600 mg multiple dose of 7 days in 20 *H. pylori* carriers (part C). Laboratory safety assessment, vital signs and ECGs were evaluated. Plasma and urine samples for the determination of the PK were collected over a period of 48h post dose.

Results: All enrolled subjects completed the study. No significant changes in laboratory test parameters were observed. Adverse events were recorded for 50 of the 95 subjects including (but not limited to): headache (14 incidents), tiredness (11), feeling of pressure in the head (7), diarrhoea (8) and nausea (3). No serious adverse events were reported. At 400 and 800 mg single doses the plasma $t_{1/2}$ of FIN was 10.1 and 10.5h, C_{max} [µg/mL] was 5.1 and 9.5; and $AUC_{0\text{-}inf}$ [hr*µg/mL] was 14.2 and 24.8 respectively. FIN was readily absorbed with peak plasma concentration achieved at 0.5 - 2h after dosing. The systemic exposure ($AUC_{0\text{-}tlast}$) of FIN increased linearly from 25 to 800 mg. For 400 mg and 800 mg, mean urinary excretion was 28.3% and 33.4%, respectively.

Conclusions: Single and multiple doses were very well tolerated at all evaluated doses. Based on the good safety, tolerability and PK profile, FIN warrants further clinical evaluation.

Introduction

Finafloxacin (FIN, Figure 1) is a novel, broad spectrum fluoroquinolone (FQ) that belongs to a new 8-cyano subclass [1]. FIN contains a novel 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]. Here, the results of pharmacokinetics, safety and tolerability in healthy subjects in a phase I study is reported. These attributes suggest that FIN warrants further clinical investigation for bacterial infections that are associated with low pH such as urinary tract infection and *Helicobacter pylori* eradication.

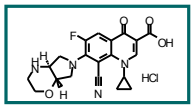


Figure 1. Finafloxacin hydrochloride.

Methods

The study was an inpatient, randomized, double-blind, placebo-controlled, dose-escalating study to evaluate the safety, tolerability and pharmacokinetic profiles of single and multiple doses of finafloxacin hydrochloride administered orally to healthy male and female subjects, aged between 18 and 55 years. Subjects received single doses of 25, 50, 100, 200, 400 or 800 mg. For the multiple dose study, subjects received 7 daily doses of 150, 300, 600 or 800 mg.

ECG rhythm measurements were made from the time of dosing and up to 4 h post-dosing and then at 8h and at 24 h.

Blood and urine samples were collected prior to the study, at entry and at the end of evaluation for clinical chemistry, haematology and urinalysis.

Plasma and urine samples were collected at various intervals from pre-dose till 48 h post dose for pharmacokinetic analysis. Urinary bactericidal activity was determined for 200 and 800 mg single dose. See poster F1-2049 for further details.

FIN concentrations were estimated in plasma and urine samples by a validated LC/MS-MS method. The lower quantification limit was 5 ng/mL in plasma and 100 ng/mL in urine.

Pharmacokinetic parameters were evaluated using non-compartmental analysis. PK parameters, urinary recovery and renal clearance were determined.

All adverse events were reported and assessed as mild, moderate or severe.

Results and Discussion

ECG: No clinically relevant abnormalities were observed.

Clinical laboratory evaluations: Isolated, minor deviations from the normal ranges were observed for various haematology, blood chemistry and / or urinalysis parameters at various time points during the study. None of these deviations were considered clinically significant.

Plasma pharmacokinetics (PK) for single dose:

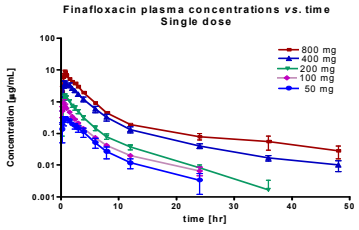
The PK values for subjects receiving single doses are tabulated in Figure 2. Plasma concentration *vs.* time profile of escalating single dose is shown in Figure 3.

Figure 2. Non-compartmental PK parameters determined as mean ± standard deviation in the subjects receiving single doses of FIN orally.

Dose	100 mg	200 mg	400 mg	800 mg
C_{max} [µg/mL]	1.0 ± 0.5	1.9 ± 0.7	5.1 ± 2.1	9.5 ± 2.6
$AUC_{0\text{-}inf}$ [h*µg/mL]	2.2 ± 0.6	4.1 ± 1.0	14.2 ± 4.4	24.8 ± 5.8
$t_{1/2}$ [h]	5.8 ± 2.6	5.0 ± 2.6	10.1 ± 4.5	10.5 ± 2.7

Results and Discussion

Figure 3. Log concentration vs. time curve for single dose of FIN.

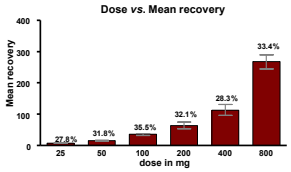


FIN absorption was rapid, with t_{max} values of 0.5 to 2 hour. C_{max} & $AUC_{0\text{-}inf}$ increased almost linearly with dose. The median value of total oral body clearance was 28.0 and 35.8 L/hr for 400 mg and 800 mg dose, respectively.

Urine pharmacokinetics for single dose:

Urinary recovery median values ranged from 26.99% to 34.75%. The absence of clear differences between doses show that urine excretion of unchanged FIN is a relevant elimination route. Figure 4 shows mean urinary recovery after single dose.

Figure 4. Mean urinary recovery for single dose of FIN.



The mean (median) maximum concentration of FIN in urine was 120 (85.2) mg/L at 2 to 4 hours following 400 mg dose and 150 (137) mg/L at 4 to 8 hours following 800 mg dose. The median value of renal clearance was 7.5 and 11 L/hr following 400 mg and 800 mg dose, respectively.

Plasma pharmacokinetics for multiple dose in healthy subjects:

Figure 5 shows plasma concentration *vs.* time curve for 7 day multiple dosing. The PK values for subjects receiving single oral dose of FIN for 7 consecutive days is described in Figure 6.

Figure 5. Log concentration vs. time curve for multiple dose of FIN.

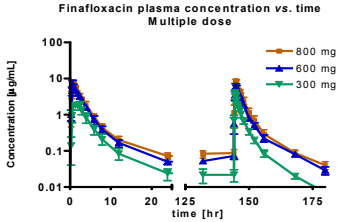


Figure 6. Non-compartmental PK parameters determined as mean ± standard deviation in the subjects on day 7 receiving multiple doses of FIN.

Dose	300 mg	600 mg	800 mg
C_{max} [µg/L]	4.0 ± 2.3	6.8 ± 2.2	9.0 ± 3.1
$AUC_{0\text{-}inf}$ [h*µg/L]	8.9 ± 4.2	20.2 ± 6.7	27.8 ± 9.3
$t_{1/2}$ [h]	6.5 ± 2.7	8.7 ± 3.1	13.8 ± 5.4

Steady state was reached at day 4. The median value of total oral body clearance was 32.4 and 32.2 L/hr for 600 mg and 800 mg dose, respectively.

Safety & Tolerability:

- The subjects enrolled in single and multiple dose group were comparable with regard to age, weight and body mass index.
- The tolerability of FIN / Placebo tablets given as a single dose or in multiple doses over 7 days was considered to be good.
- There was no serious adverse event or death and no discontinuation due to adverse events (AEs).
- No clear dose dependency on the number of subjects reporting AEs per cohort could be detected and none of the reported AEs increased in number or intensity with increasing doses.
- No clear difference of AEs experienced by subjects taking FIN or placebo was observed.
- Figure 7 shows the AEs experienced by single dose, multiple dose and placebo dose group. The most frequent AEs were headache, diarrhoea, nausea, back pain. Flatulence (GI) was more common in *H. pylori* carriers.
- CNS and RTI adverse events were found more frequently in placebo group, where as GI related adverse events were more for subjects taking FIN.

Figure 7. Adverse events reported in subjects receiving single and multiple oral dose of FIN and placebo.

Body system	Part A n = 33	Part B n = 24	Part C n = 20	FIN Total n = 77	Placebo n = 18
	n (%)	n (%)	n (%)	n (%)	n (%)
CNS	13 (39)	9 (37)	4 (20)	26 (34)	8 (44)
Cardiovascular	1 (17)	-	-	1 (1)	-
GI	5 (15)	5 (21)	11 (55)	21 (27)	1 (6)
Musculoskeletal	-	7 (29)	-	7 (9)	1 (6)
RTI	1 (3)	3 (12)	1 (5)	5 (6)	4 (22)
General	2 (6)	1 (4)	1 (5)	4 (5)	1 (6)
Skin	-	-	3 (15)	3 (4)	1 (6)
Eye	-	1 (4)	-	1 (1)	-

Part A = Single dose Part B = Multiple dose in healthy subjects
Part C = Multiple dose in *Helicobacter pylori* carriers

CNS; Central nervous system, RTI; Respiratory tract infection, GI; Gastrointestinal

Conclusions

- This FIN Phase I study in healthy subjects revealed a favorable pharmacokinetics profile with high C_{max} and long half life.
- FIN was well tolerated following single dose and when given for seven days at a range of doses up to 800 mg. Human safety data do not suggest any quantitatively higher or qualitatively different toxicity for FIN as compared with placebo.
- Overall, these findings indicate that the risk of serious adverse reactions to finafloxacin hydrochloride can be expected to be very low. Given the possible therapeutic effects of FIN, further clinical development of the drug appears justified and can be recommended.

Literature

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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

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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) pH 7.2 (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)	0.06/0.03	16	2	384	2,944	3,264
E. coli MI-4	1/0.5	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.

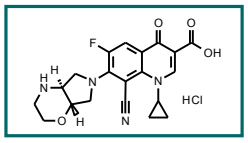
Introduction

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 $\times 10^5$ to 6.1 $\times 10^5$ 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^{BS})**	2	2	0.03	0.06	0.125	1	0.03	0.03	2	16	16	2
***E. coli* MI-4 (CIP^{BS})**	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^{BS}; 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^{BS})**	**2,944** (1,184 - 5,376)	**3,264** (1,184 - 11,264)	**552** (256 - 1,184)
***E. coli* MI–4 (CIP^{BS})**	**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.

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^{BS})**	**384** (128 - 512)	**256** (128 - 512)	**64** (32 - 128)	**32** (8 - 64)	**48** (8 - 256)	**6** (4 - 8)
***E. coli* MI–4 (CIP^{BS})**	**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)

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^{BS}; ciprofloxacin - borderline susceptible, CIP^{R}; ciprofloxacin resistant, as determined under standard MIC test conditions.

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

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