A1-679

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Pharmacokinetics / Pharmacodynamics of Finafloxacin in the Murine Thigh Infection Model with *S. aureus* and *E. coli*

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Abstract

Background: Finafloxacin (FIN), a novel fluoroquinolone (FQ) in clinical development, has the unique property of being activated under acidic conditions, unlike other marketed FQs. Since local acidic environments are a hallmark of bacterial infection, FIN may have an advantage over existing agents in treating these infections. This study was performed to determine the PK/PD parameter that best correlates to FIN efficacy. **Methods:** MICs for FIN and other FQs were determined at pH 5, 6 and 7.2. Female CD-1 mice were rendered neutropenic by IP injection of Cytoxan (150/100 mg/kg at days -4/-1 pre-infection). Infection was established by injection of 10^5 CFU of and MSSA or *E. coli* (Ec) strain in the right thigh. Dose fractionation studies (q24h, q12h and q6h) were performed from 0.25 - 150 mg/kg SC. All thighs were removed 26 hrs post-infection and processed for CFU counts. FIN was administered SC from 1 to 100 mg/kg to determine PK parameters (C_{max}, AUC, T>MIC) in neutropenic, thigh-infected animals. The dose vs. change in log CFU/thigh relationship vs. untreated controls was determined and related to the PK parameters at each dose. **Results:** FIN was more active than the other FQs tested at pH5. The static dose for both the MSSA and Ec was 10.7 mg/kg. The correlation coefficients of the PD parameters to efficacy in the thigh model for the 24 hr AUC/MIC, C_{max}/MIC and %T>MIC were 90, 79 and 57% for MSSA and 89, 77 and 67% for Ec, respectively. The 24 hr total AUC/MIC (pH 7.2) ratio necessary to achieve a static effect was 132.5 for the MSSA and 88.1 for the Ec. The corresponding C_{max}/MIC (pH 7.2) ratio for the static effect was 30.9 for the MSSA and 22.4 for the Ec. **Conclusion:** The efficacy of FIN in the neutropenic thigh model, for both MSSA and *E. coli* correlated best to the AUC/MIC and further investigations are warranted to determine the effect of pH at the site of infection on the magnitude of this parameter.

Introduction

Finafloxacin is a novel member of the fluoroquinolone class of antibiotics with a new pH activated profile offering therapeutic potential for severe and difficult to treat bacterial infections. Some of the characteristics of finafloxacin which set it aside from other members of the fluoroquinolone (FQ) class can be summarized as follows: pH activation and activity under infection relevant conditions; more active than other marketed FQs against the growth / physiological forms of bacteria which cause the most serious and recurrent infections; an all inclusive spectrum of activity that covers Gram positive, Gram negative, anaerobic and atypical pathogens; more effective than the classical FQs over a range of sepsis, cSSSI, RTI, UTI and IAI infection models and safety, finafloxacin has an outstanding safety profile compared to other fluoroquinolones. The current study was performed to determine the PK/PD parameter that is most predictive for the efficacy of finafloxacin.

Methods and Materials

Mice: Female 5 - 6 wk old CD-1 mice (18-22 gm) rendered neutropenic by IP injection of Cytoxan (cyclophosphamide) 150 mg/kg (-4 days) and 100 mg/kg (-1 day) pre-infection.

Thigh Infection: A fresh overnight culture of a *S. aureus* and *E. coli* strains diluted to approx. 2 x 10^6 CFU/mL and 0.1 mL injected (5x10^5 final CFU) IM into the thighs of the pre-treated mice.

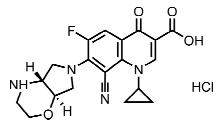
MICs: MICs for FIN at different pH were determined by microbroth dilution in accordance with CLSI guidelines.

PK: FIN was administered SC at 1 – 100 mg/kg in order to determine PK parameters (Cmax, AUC, T>MIC) and their relationship to administered dose. PK was performed in neutropenic, *S. aureus* thigh-infected animals to best predict compound levels in the efficacy studies.

Dose Ranging Study: An initial dose-ranging study (single dose at +1.5 hrs post-infection) was performed over a wide range (0.25 – 150 mg/kg) in *S. aureus* thigh-infected animals in order to determine the defined range that will be used in the dose fractionation studies.

Dose Fractionation: FIN was administered by the same route used for the PK and dose-ranging study at up to 8 different total daily doses (selected from the dose ranging studies and covering a range from maximal to the no-antibacterial effect level). Each total dose was given at 3 different regimens; q24hr, q12hr and q6hr. Efficacy in the thigh infection model was compared to calculated PK parameters at each of the dose fractionations in order to determine the PK/PD parameter that is most predictive of efficacy.

Panel 1: Chemical Structure of Finafloxacin



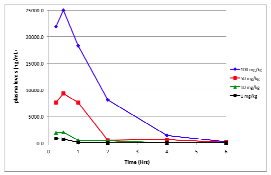
(-)-8-cyano-1-cyclopropyl-6-fluoro-7-[(4aS,7aS)-hexahydropyrrolo[3,4-b]-1,4-oxazin-6(2*H*-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylc acid hydrochloride

Panel 2: Minimum Inhibitory Concentration (MICs) of Finafloxacin and Other Fluoroquinolones

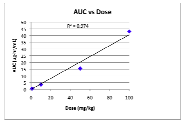
	S. aureus ATCC 29213			*E. coli* ATCC 25922		
Compound	pH 5	pH 6	pH 7.2	pH 5	pH 6	pH 7.2
Finafloxacin	**0.06**	**0.03**	**0.03**	**0.06**	**0.03**	**0.03**
Ciprofloxacin	2	0.5	0.25	0.5	0.12	0.015
Levofloxacin	1	0.25	0.12	1	0.25	0.03
Gatifloxacin	1	0.25	0.06	1	0.25	0.03
Norfloxacin	8	2	2	2	0.5	0.06

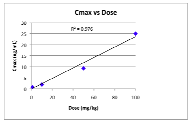
- FIN exhibited excellent activity at pH 7.2 against both organisms. Unlike all the other FQs, FIN maintained this activity at lower pH values with MICs of 0.03 µg/mL at pH 6 and 0.06 µg/mL at pH 5.

Panel 3: Pharmacokinetics of Finafloxacin following Subcutaneous Administration in Thigh Infected CD-1 Mice

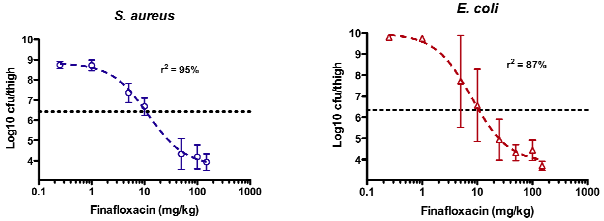


	Finafloxacin Dose - Subcutaneous			
Parameter	1 mg/kg	10 mg/kg	50 mg/kg	100 mg/kg
C_{max} (ng/mL)	0.8	2	9.3	25
AUC $_{0-inf}$ (ug-hr/mL)	0.5	3.6	15.5	43.9
MRT (hr)	0.5	3.6	1.2	1.3
T_{max} (hr)	0.3	0.5	0.5	0.5



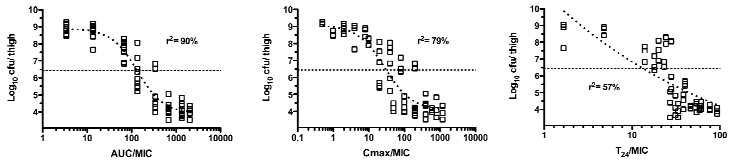


Panel 4: Efficacy of Finafloxacin in the Neutropenic Mouse Thigh Infection Following a Single Dose Administration



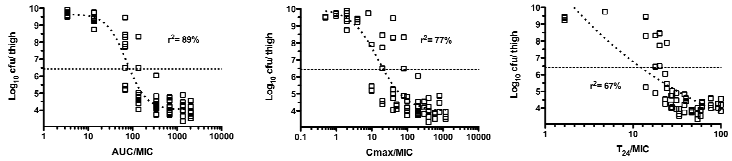
- The static dose, no change in CFU counts in treated groups compared to the bacterial burden at the start of treatment, was calculated at 10.7 mg/kg for both the *S. aureus* and *E. coli* infections. Doses corresponding to 1 and 2 log reductions in thigh CFU were 20.4 and 52.5 for *S. aureus* ATCC 29213 and 19.1 and 52.5 mg/kg for *E. coli* ATCC 25922.

Panel 5: PK/PD Parameter Determinations from Dose Fractionation of Finafloxacin Against *Staphylococcus aureus*



- For *S. aureus*, the correlations achieved were 90% for AUC/MIC, 79% for C_{max}/MIC and 57% for %T24>MIC
- The AUC/MIC ratios at stasis, 1 log and 2 log CFU reductions were 132.5, 235.4 and 581.3, respectively.

Panel 6: PK/PD Parameter Determinations from Dose Fractionation of Finafloxacin Against *Escherichia coli*



- For *E. coli*, the correlations achieved were 89% for AUC/MIC, 77% for C_{max}/MIC and 67% for %T24>MIC
- The AUC/MIC ratios at stasis, 1 log and 2 log CFU reductions were 88.1, 134.5 and 312.2, respectively.

Summary and Conclusions

- Finafloxacin was 4- to 16-fold more active than the other fluoroquinolones by MIC testing at pH 5 – pH 6.
- Finafloxacin exhibited a good correlation for the pharmacokinetic parameters of AUC_{0-inf} and C_{max} to dose.
- Finafloxacin exhibited a good correlation between total administered dose and antibacterial effect against both *E. coli* and *S. aureus* in the murine thigh infection model.
- The PK/PD parameter which best predicts finafloxacin activity in this model was AUC/MIC, closely followed by C_{max}/MIC. These parameters are also used to describe the clinical efficacy of marketed fluoroquinolones and could also be utilized to set target exposures in the clinical evaluation of finafloxacin.
- The preliminary PK/PD target of an AUC/MIC of 88.1 for *E. coli* is in the region of those described for other fluoroquinolones to Gram negative organisms (~125).
- Further testing is warranted with a larger strain set to more accurately define the magnitude of the PK/PD parameters which describe the *in vivo* efficacy of finafloxacin.

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Acknowledgments

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Determination of the Effect of Age and Gender on the Pharmacokinetics (PK) and Tolerability of a Single Dose of Finafloxacin HCl (FIN) in Healthy Volunteers

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Abstract

Introduction: FIN is a novel fluoroquinolone that exhibits improved antibacterial and pharmacodynamic properties under acidic conditions which often characterize infection sites. Previous clinical studies have indicated that FIN is well tolerated with few treatment related adverse events (AEs). This study aimed to determine primarily the effect of age and gender on the PK of FIN and assess its safety profile.

Methods: The study was conducted under approval of the Celerion IRB, the FDA IND # 106,696, and in accordance with GCP. This was a single center, open label study in 40 healthy subjects (10 young adult males, 10 young adult females, 10 elderly adult males, and 10 elderly adult females). All subjects received a single oral dose of 400 mg FIN (2 x 200 mg tablets).

Results: Following a single dose of 400 mg, the mean peak exposure (C_{max}) of FIN was similar in elderly (4630 - 6190 ng/mL) and young (5160 - 6440) subjects. The average exposure (AUC_{0-inf}) was approximately 18% greater in elderly (20250 - 23477 ng.h/mL) versus young (17240 - 19942 ng.h/mL) subjects (not statistically significant). Mean exposure was similar in males and females (<16% difference) and Cmax was statistically higher in females (6190 - 6440 ng/mL) than males (4630 - 5160 ng/mL). The mean $t_{1/2}$ of FIN was comparable in both genders within each age group. Renal clearance was reduced in the elderly group. In total, 23 AEs (22 mild and 1 moderate) and no serious AEs were observed. There were no significant findings in clinical laboratory values, vital signs, ECGs, and physical examinations.

Conclusions: There were no statistically significant age or gender effects on FIN PK except on urinary excretion (age) and peak exposure (gender). The administration of a single oral dose of 400 mg FIN was safe and well tolerated in the young and elderly male and female subjects in this study.

Background

- Finafloxacin is a novel pH activated, broad spectrum fluoroquinolone in development for infection indications in the hospital and critical care setting [1]
- Finafloxacin exhibits enhanced activity at low pH and under other environmental conditions associated with infection [1, 2]
- Finafloxacin exhibits bactericidal activity against forms of quiescent growth, thought to be relevant *in vivo* e.g. non-growing cells, biofilms and persisters [3]
- Other fluoroquinolones lose activity under such conditions. Consequently, finafloxacin exhibited superior activity in a series of infection models [4, 5]
- The activity of finafloxacin under infection relevant conditions and against infection relevant growth forms in combination with the high dosing potential predicted from its safety profile [6, 7], suggest finafloxacin will offer improved properties over currently marketed fluoroquinolones

Aim

Previous clinical studies have indicated that finafloxacin is well tolerated with few treatment-related AEs. As a part of the clinical development of finafloxacin, other PK studies are required to determine the effect of other variables on the PK profile of finafloxacin.

The primary objective of the study was as follows:

- To assess the PK profile of finafloxacin in healthy young and elderly volunteers.

The secondary objective of the study was as follows:

- To determine the safety and tolerability of finafloxacin in healthy young and elderly volunteers.

Methods

- All pertinent study documents were reviewed by the independently functioning Celerion Institutional Review Board (IRB) prior to study initiation. The IRB operations are in compliance with the U.S. Code of Federal Regulations (21 CFR Part 56) and International Conference on Harmonisation (ICH) guidelines. This study was conducted under the FDA investigational new drug number IND 106,676.
- This was a single-center, open-label, single-dose study in 40 healthy subjects. The subjects were assigned to 1 of 4 groups comprised of the following: 10 healthy young adult males, 10 healthy young adult females, 10 healthy elderly adult males, and 10 healthy elderly adult females.
- The subjects fasted for 10 hours and were administered 400 mg finafloxacin (as 2 x 200 mg tablets) administered with 240 mL of water.
- Blood samples were withdrawn at the following times, predose and at 30 minutes, 45 minutes, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12 and 24 hours postdose.
- Urine samples were collected from subjects over a 24-hour period postdose at the following intervals: baseline (predose) sample, 0 – 4 hours, 4 – 8 hours, 8 – 12 hours, and 12 – 24 hours.
- Finafloxacin PK parameters were summarized using descriptive statistics. The comparisons between the four age-gender groups administered 400 mg finafloxacin were assessed using an analysis of variance (ANOVA).
- The safety assessments included laboratory evaluations, physical examinations, AEs, standard 12-lead ECG parameters, and vital sign assessments

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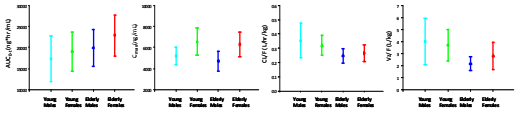
Results

Summary of the Mean Pharmacokinetic Parameters for Plasma Finafloxacin in all Treatment Groups

Pharmacokinetic Parameters	Treatment Group			
	Young Males	Young Females	Elderly Males	Elderly Females
	(N)	(N)	(N)	(N)
Geometric Mean (Geometric CV%)				
C_{max} (ng/mL)	5160 (15.3)	6440 (19.2)	4630 (22.1)	6190 (19.1)
	(10)	(10)	(10)	(10)
AUC_{0-t} (ng·h/mL)	16534 (35.4)	18555 (23.5)	19498 (21.6)	22472 (19.7)
	(10)	(10)	(10)	(10)
AUC_{0-24} (ng·h/mL)	16534 (35.4)	18554 (23.5)	19497 (21.5)	22463 (19.7)
	(10)	(10)	(10)	(10)
AUC_{0-inf} (ng·h/mL)	17240 (40.4)	19942 (25.2)	20250 (22.2)	23477 (18.9)
	(8)	(8)	(10)	(10)
Arithmetic Mean ± SD				
$t_{1/2}$ (h)	7.64 ± 2.01	7.87 ± 1.64	6.06 ± 0.961	7.09 ± 1.39
	(8)	(8)	(10)	(10)
CL/F (L/h/kg)	0.354 ± 0.123	0.319 ± 0.0690	0.247 ± 0.0511	0.264 ± 0.0571
	(8)	(8)	(10)	(10)
Vz /F (L/kg)	4.01 ± 1.92	3.68 ± 1.31	2.16 ± 0.574	2.78 ± 1.11
	(8)	(8)	(10)	(10)
AUC/AUC_{0-inf} (%)	95.6 ± 2.01	96.3 ± 1.47	96.3 ± 1.58	95.7 ± 1.57
	(8)	(8)	(10)	(10)
Median (Minimum – Maximum)				
T_{max} (h)	0.999 (0.500, 1.50)	0.996 (0.749, 1.50)	0.877 (0.499, 2.50)	0.876 (0.509, 1.50)
	(10)	(10)	(10)	(10)

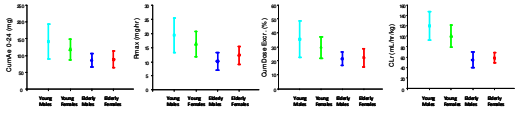
Abbreviations: AUC; area under the plasma concentration-versus-time curve, CL/F; total body clearance calculated as: Dose / AUC_{0-inf}, C_{max}: maximum measured plasma concentration, $t_{1/2}$: apparent terminal elimination half-life, T_{max}: time of the maximum measured plasma concentration, Vz /F; apparent volume of distribution at the terminal phase.



Summary of the Mean Pharmacokinetic Parameters for Urine Finafloxacin in all Treatment Groups

Pharmacokinetic Parameters	Treatment Group			
	Young Males	Young Females	Elderly Males	Elderly Females
	(N)	(N)	(N)	(N)
Geometric Mean (Geometric CV%)				
$CumAe_{0-24}$ (mg)	132 (45.8)	115 (23.4)	84.3 (22.2)	85.5 (28.5)
	(10)	(10)	(10)	(10)
R_{max} (mg/h)	18.2 (39.2)	15.8 (27.7)	9.68 (33.9)	11.9 (26.2)
	(10)	(10)	(10)	(10)
Arithmetic Mean ± SD				
CumDose Excr. (%)	35.5 ± 12.9	29.5 ± 7.5	21.5 ± 4.8	22.2 ± 6.3
	(10)	(10)	(10)	(10)
CLr (mL/h/kg)	120 ± 26.7	100 ± 21.2	54.9 ± 15.0	58.4 ± 9.6
	(10)	(10)	(10)	(10)
Median (Minimum – Maximum)				
T_{max} (h)	2.02 (2.00, 2.03)	2.01 (1.98, 2.02)	2.06 (2.00, 2.08)	2.03 (1.96, 2.05)
	(10)	(10)	(10)	(10)

Abbreviations: CLr; Renal clearance, CumAe0-24; Cumulative amount excreted in urine, CumDose Excr.; finafloxacin urinary excretion, R_{max}: maximum rate of urinary excretion, T_{max}: time of the maximum measured urine concentration,



Summary of Adverse Event (AE) Incidence by Treatment Group

- 22 / 23 AEs were classified by the principal investigator (PI) as mild in intensity and 1 /23 as moderate
- The most common AEs were headache and nausea
- There were no significant findings in clinical laboratory, vital signs, ECGs, and physical examinations.

Treatment Group	Subject Incidence (%)	Number of AEs	Number of Treatment-Related* AEs
Young Males (n=10)	4 (40%)	6	6
Young Females (n=10)	6 (60%)	11	6
Elderly Males (n=10)	2 (20%)	2	2
Elderly Females (n=10)	3 (30%)	4	2
Total (n=40)	15 (38%)	23	16

*= At least possibly related to study treatment

Conclusions

- The average systemic availability ($AUC_{0-inf.}$) of finafloxacin was approximately 18% greater in elderly versus young subjects (not statistically significant). Mean systemic exposure was similar in males and females (<16% difference).
- The mean peak exposure (C_{max}) of FIN was similar in elderly (4630 - 6190 ng/mL) and young (5160 - 6440) subjects, but slightly higher in females versus males.
- Urinary excretion of finafloxacin (based on $CumAe_{0-24}$ and R_{max}) was significantly lower (by 42% – 54%) in elderly compared to young subjects and was similar between genders.
- There were no statistically significant age or gender effects on FIN PK except on urinary excretion (age) and peak exposure (gender).
- Overall, there were no major safety concerns found in the vital sign, safety laboratory, ECG, AE, or physical examination assessments associated with the administration of finafloxacin in young and elderly healthy male and female subjects.
- The administration of a single oral dose of 400 mg finafloxacin was safe and well tolerated in the young and elderly male and female subjects in this study.

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In vitro Investigation of Finafloxacin Under Conditions Simulating Lower Respiratory Tract Infection

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Abstract

Introduction Finafloxacin (FIN) is a novel fluoroquinolone (FQ) that exhibits improved antibacterial and pharmacodynamic properties at pH values below neutral which often characterize sites such as in chronic and lower respiratory tract infection (LRTI). The aim of this study was to investigate the activity of FIN under *in vitro* conditions simulating LRTI including pH, low oxygen, presence of sputum components and biofilms.

Methods MICs against LRTI pathogens were determined in an artificial sputum media (ASM) at pH 7.2, 6.2 and 5.2 under both aerobic and anaerobic conditions. Time kill curves were conducted in the presence of sputum from cystic fibrosis (CF) patients. Minimum biofilm eradication concentrations (MBEC) were determined using a modified Calgary device.

Results Finafloxacin exhibited MICs in ASM against MRSA, *P. aeruginosa* (*Pa*), *K. pneumoniae* and *S. maltophilia* which were 2-16-fold lower than those of the other FQs, ciprofloxacin (CIP), levofloxacin or moxifloxacin (MXF), at pH 6.2 and 5.2 under aerobic and at all pHs, under anaerobic conditions. FIN MICs in ASM were 2-64-fold lower than those of tobramycin (TOB), ceftazidime (CAZ) and meropenem (MEM) against all organisms tested in ASM at pH values below neutral or under anaerobic conditions.

FIN, CIP, LVX and MER MICs increased 1-2-fold, TOB 8-fold and CAZ >32-fold when 20mg/mL mucin was included in CAMHB. DNA (up to 10 mg/mL) had minor (1-4-fold MIC increase) effects on all antibiotics. FIN retained bactericidal activity at 4 x MIC against *Pa* 27853 in 20% CF sputum.

MBEC (mg/L) against biofilms of *Pa* 27853 at (pH 7.2, 6.2 and 5.2) were for FIN; 16, 4 and 2, CIP; 1.5, 3 and 1.5, LVX; 6, 12 and 8, TOB; 2, 4 and 4, CAZ; >128, >128 and >128 and MEM; 2, 0.75 and 1.

Conclusions These data highlight some of the factors which could potentially effect antibiotic treatment of LRTI. FIN exhibited robust activity when examined under *in vitro* conditions simulating this environment and warrants further clinical investigation.

Background

- Finafloxacin is a novel pH activated, broad spectrum fluoroquinolone in development for infection indications in the hospital and critical care setting
- Finafloxacin exhibits enhanced activity at low pH and under other environmental conditions associated with infection [1, 2]
- Finafloxacin exhibits bactericidal activity against forms of quiescent growth, thought to be relevant *in vivo* e.g. non-growing cells, biofilms and persisters [3]
- Other fluoroquinolones lose activity under such conditions, therefore finafloxacin exhibited superior activity in a series of infection models [4,5]
- The activity of finafloxacin under infection relevant conditions and against infection relevant growth forms in combination with the high dosing potential predicted from its safety profile [6,7,8], suggest finafloxacin will offer improved properties over currently marketed fluoroquinolones

Aim

Finafloxacin is a candidate for treatment of severe and chronic infections of the lower respiratory tract including: pneumonia, exacerbations of COPD or cystic fibrosis (CF), because of its broad spectrum and activity under infection relevant conditions.

The antibacterial activity of finafloxacin was investigated in a series of *in vitro* experiments that mimic the conditions of lower respiratory tract infection and compared to antibiotics already on the market. This included determination of antibacterial activity in a artificial sputum media designed to resemble CF sputum at varying pH and oxygen availability. Bactericidal activity against *P. aeruginosa* was also investigated in real CF sputum and against biofilms grown *in vitro*.

Methods

- MICs were determined in pH adjusted cation adjusted Mueller-Hinton broth using CLSI methodology for broth microdilution. Mucin (from porcine stomach), DNA (from salmon sperm) or sterilized sputum (from cystic fibrosis patients) was added to investigate the effects of these individual components on antibacterial activity.
- The following antibiotics (with abbreviations) were tested: ciprofloxacin (CIP), ceftazidime (CAZ), finafloxacin (FIN), levofloxacin (LVX), meropenem (MEM), moxifloxacin (MXF) and tobramycin (TOB).
- Artificial sputum (AS) composition that closely resembles CF sputum was used to determine MICs: 5g/L of mucin, 4g/L of DNA, 5.9 mg/L of DTPA (diethylentriamene pentaacetate), 5g/L of NaCl, 2.2g/L of KCl, 50g/L of amino acids, 5mL/L of egg emulsion as described by Sriramulu *et al.* 2000 [9]. AS was supplemented with 400 µM of KNO3 to facilitate bacterial growth under anaerobic conditions and 3% laked horse blood for *S. pneumoniae*. Because it was difficult to score growth after incubation by visual means, a drop plate method was employed where 10 µL of each well was transferred onto agar and growth scored after overnight incubation.
- Minimum biofilm eradication concentrations of the test drugs were determined using the modified Calgary device method published by Moskowitz *et al.*, 2004 [10].
- Anaerobic conditions were applied to the above susceptibility testing methods using GasPak™ EZ Anaerobe Container System Sachets (Becton Dickinson, UK).

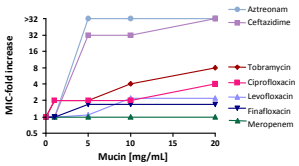
Results

Median MIC* [mg/L] for finafloxacin and comparator antibiotics in artificial sputum, determined at pH 7.2, pH 6.2 and pH 5.2 with and without oxygen

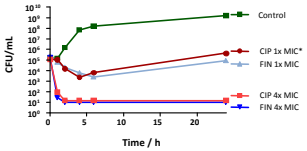
Species tested and number	Finafloxacin		Ciprofloxacin		Levofloxacin		Moxifloxacin		Tobramycin		Ceftazidime		Meropenem	
pH 5.2	O_2	AnO_2	O_2	AnO_2	O_2	AnO_2	O_2	AnO_2	O_2	AnO_2	O_2	AnO_2	O_2	AnO_2
Escherichia coli [n=5]	0.03	0.03	0.25	0.5	0.5	1	1	2	32	64	0.5	1	0.25	0.25
Stenotrophomonas maltophilia [n=5]	2	8	64	64	32	32	16	16	64	64	16	32	8	16
Pseudomonas aeruginosa [n=5]	32	8	16	32	32	32	64	64	16	64	64	64	32	32
Staphylococcus aureus [n=5]	0.25	1	4	8	2	4	1	1	32	32	64	64	32	64
Streptococcus pneumoniae [n=5]	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Klebsiella pneumoniae [n=5]	64	8	64	64	64	64	64	64	64	64	8	16	0.25	0.5
pH 6.2	O_2	AnO_2	O_2	AnO_2	O_2	AnO_2	O_2	AnO_2	O_2	AnO_2	O_2	AnO_2	O_2	AnO_2
Escherichia coli [n=5]	0.03	0.03	0.06	0.25	0.125	0.25	0.25	0.5	16	64	0.25	0.5	0.125	0.125
Stenotrophomonas maltophilia [n=5]	2	4	32	64	8	32	8	16	64	64	64	64	64	64
Pseudomonas aeruginosa [n=5]	16	1	4	2	2	4	32	16	32	32	32	64	32	16
Staphylococcus aureus [n=5]	0.125	0.25	1	2	0.5	1	0.25	0.5	32	48	64	64	64	64
Streptococcus pneumoniae [n=5]	0.5	n.d.	2	n.d.	1	n.d.	0.5	n.d.	64	n.d.	2	n.d.	0.25	n.d.
Klebsiella pneumoniae [n=5]	4	4	64	64	8	64	32	64	64	64	0.5	16	0.5	1
pH 7.2	O_2	AnO_2	O_2	AnO_2	O_2	AnO_2	O_2	AnO_2	O_2	AnO_2	O_2	AnO_2	O_2	AnO_2
Escherichia coli [n=5]	0.25	0.125	0.008	0.06	0.06	0.125	0.06	0.5	2	16	0.25	0.5	0.125	0.06
Stenotrophomonas maltophilia [n=5]	4	8	16	64	4	16	2	16	64	64	16	64	64	64
Pseudomonas aeruginosa [n=5]	32	0.5	2	1	1	2	8	8	16	32	32	64	32	4
Staphylococcus aureus [n=5]	0.25	0.25	0.5	2	0.25	1	0.125	0.5	2	32	64	64	16	32
Streptococcus pneumoniae [n=5]	1	0.5	1	4	1	2	0.25	1	64	64	4	4	0.25	0.25
Klebsiella pneumoniae [n=5]	16	4	8	64	4	16	8	32	16	64	0.5	64	1	2

* It was not possible to read the MIC by visual means, therefore growth was scored by drop plate method. [O_2] = aerobic, [AnO_2] = anaerobic

Effect of mucin (porcine stomach) on MIC of finafloxacin and comparator antibiotics against *P. aeruginosa* ATCC 27853 determined at at pH 7.2



Time kill profile of finafloxacin and ciprofloxacin against *P. aeruginosa* ATCC 27853 determined in CAMHB with 20% CF sputum at pH 7.2



Finafloxacin MIC; 4 mg/L, ciprofloxacin MIC; 0.5 mg/L

Minimum Biofilm Inhibitory Concentration of finafloxacin and comparator antibiotics against biofilms of *P. aeruginosa* ATCC 27853, determined at pH 7.2, 6.2 and 5.2

	pH 7.2	pH 6.2	pH 5.2
Ciprofloxacin	1.5	3	1.5
Levofloxacin	6	12	8
Finafloxacin	16	4	2
Tobramycin	2	4	4
Ceftazidime	≥128	≥128	≥128
Meropenem	2	0.75	1

Conclusions

- **Finafloxacin exhibited pH activation in artificial sputum, in contrast the other fluoroquinolones and tobramycin lost activity under acidic pH in artifical sputum**
- **Consequently, finafloxacin was more active (2- to 32-fold) than the comparator compounds (except ciprofloxacin with *P. aeruginosa*) in artificial sputum at pH 6.2 and pH 5.2 under aerobic conditions.**
- **Finafloxacin was the most active of all of the tested compounds under anaerobic conditions, even more notably at pH 6.2 and pH 5.2**
- **Finafloxacin exhibited bactericidal activity against planktonic *P. aeruginosa* in 20% CF sputum and against biofilms of *P. aeruginosa***
- **Finafloxacin exhibits antibacterial activity in the presence of sputum and sputum components which can inactivate other antibiotics. Furthermore, the enhanced antibacterial activity of finafloxacin under acidic and anaerobic conditions, against important respiratory pathogens, could be of advantage for the treatment of chronic and severe lower respiratory tract infection.**

References

(1) Stubbings *et al.* 2010. *Antimicrobial Agents and Chemotherapy* In submission.
(2) Kresken *et al.* 2008. 48th ICAAC / 46th IDSA Washington DC. Poster F1-2037.
(3) Goh *et al.* 2008. 48th ICAAC / 46th IDSA Washington DC. Poster F1-2042.
(4) Endermann *et al.* 2008. 48th ICAAC / 46th IDSA Washington DC. Poster F1-2044.
(5) Endermann *et al.* 2008. 48th ICAAC / 46th IDSA Washington DC. Poster F1-2045.
(6) Schmuck *et al.* 2008. 48th ICAAC / 46th IDSA Washington DC. Poster F1-2047.
(7) Patel *et al.* 2010. Antimicrobial Agents and Chemotherapy In In submission.
(8) Moony *et al.* 2010. 50th ICAAC. Poster A1-685
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Finafloxacin Exhibits Enhanced Activity Under Acidic And Anaerobic Conditions

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Revised Abstract

Background Finafloxacin (FIN) is a novel fluoroquinolone that exhibits improved antibacterial and pharmacodynamic properties at pH values below neutral which often characterize infection sites. Deep seated and chronic infection sites e.g. intraabdominal abscesses and in cystic fibrosis airway can also be comprised of areas with low oxygen. The aim of this study was to determine the effect of pH and oxygen on the activity of FIN and comparator antibiotics.

Methods MICs were performed aerobically and anaerobically at pH 7.2, 6.2 and 5.2 against 176 clinical isolates.

Results Comparative aerobic and anaerobic median MICs (MIC_{50})are shown in the Table. The activity of tobramycin (TOB) decreased under anaerobic conditions whereas FIN activity was increased; pH readings confirmed this effect was not due to changes in pH during incubation.

Under aerobic conditions, FIN activity increased by a factor of 2-8 at pH 6.2 / 5.2 compared to at pH 7.2. Conversely, the activities of ciprofloxacin (CIP), levofloxacin (LVX), moxifloxacin (MXF) and TOB were decreased by a factor of 2-32 in the acidic media. Meropenem (MEM) and ceftazidime (CAZ) activity was not affected by pH.

Under anaerobic and low pH conditions, the activity of FIN was similar to at pH 7.2 (anaerobic). CIP, LVX, MXF and TOB, all exhibited decreased activity at lower pH, compared to at pH 7.2, under anaerobic conditions.

Conclusions These data highlight the impact of environmental conditions on antibiotic activity, and that pH and oxygen are important parameters. FIN demonstrated enhanced activity under both acidic and anaerobic conditions, and warrants clinical investigation for indications where these conditions prevail.

Background

- Finafloxacin is a novel pH activated, broad spectrum fluoroquinolone in development for infection indications in the hospital and critical care setting
- Finafloxacin exhibits enhanced activity at low pH and under other environmental conditions associated with infection [1, 2]
- Finafloxacin exhibits bactericidal activity against forms of quiescent growth, thought to be relevant *in vivo* e.g. non-growing cells, biofilms and persisters [3]
- Other fluoroquinolones lose activity under such conditions. Consequently, Finafloxacin exhibited superior activity in a series of infection models [4, 5]
- The activity of finafloxacin under infection relevant conditions and against infection relevant growth forms in combination with the high dosing potential predicted from its safety profile [6, 7, 8], suggest finafloxacin will offer improved properties over currently marketed fluoroquinolones

Background and aim

The potency of antibiotics against organisms that grow aerobically is routinely performed at pH 7.2-7.4 and in atmospheric conditions (or 5% CO_2 for more fastidious organisms). However, the pH and oxygen availability at the site of infection could be quite different and thus standard susceptibility testing may under- or overestimate the capacity of an antibiotic to work in certain locations.

The enhancing effect of acidic pH on the activity of finafloxacin (and the negative effect on activity of other fluoroquinolones) has been described before. The aim of this study was to investigate the activity of finafloxacin and other antibiotics against a selection of clinically relevant facultative anaerobes, using variables of pH and oxygen availability.

Methods

- MICs were determined in pH adjusted cation adjusted Mueller-Hinton broth (MHB) using CLSI methodology for broth microdilution. Anaerobic conditions were applied methods using GasPak™ EZ Anaerobe Container System Sachets (Becton Dickinson, UK).
- The following antibiotics (with abbreviations) were tested: ciprofloxacin (CIP), ceftazidime (CAZ), finafloxacin (FIN), levofloxacin (LVX), meropenem (MEM), moxifloxacin (MXF) and tobramycin (TOB).
- Clinical isolates were obtained from the National University Hospital (NUH); Singapore, *Pseudomonas aeruginosa* ATCC 27853 from the ATCC and *Staphylococcus aureus* NRS384 (USA-300) from NARSA.
- Time kill curves were performed with drug at 0.5x, 1x, and 4x MIC according to CLSI defined protocols. A hypoxic chamber was used for time kill cultures under anaerobic conditions.
- Anaerobic incubator strips were used in all experiments and pH indicators strips were used to monitor pH in MIC wells before and after incubation.

References

(1) Stubbings *et al.* 2010. *Antimicrobial Agents and Chemotherapy* In submission.
(2) Kresken *et al.* 2008. 48th ICAAC / 46th IDSA Washington DC. Poster F1-2037.
(3) Goh *et al.* 2008. 48th ICAAC / 46th IDSA Washington DC. Poster F1-2042.
(4) Endermann *et al.* 2008. 48th ICAAC / 46th IDSA Washington DC. Poster F1-2044.
(5) Endermann *et al.* 2008. 48th ICAAC / 46th IDSA Washington DC. Poster F1-2045.
(6) Schmuck *et al.* 2008. 48th ICAAC / 46th IDSA Washington DC. Poster F1-2047.
(7) Patel *et al.* 2010. *Antimicrobial Agents and Chemotherapy* In submission.
(8) Moony *et al.* 2010. 50th ICAAC. Poster A1-685.

Results

MIC_{50} [mg/L] for finafloxacin and comparator antibiotics, determined at pH 7.2, pH 6.2 and pH 5.2 with and without oxygen

	Finafloxacin						Ciprofloxacin					
	pH 7.2		pH 6.2		pH 5.2		pH 7.2		pH 6.2		pH 5.2	
	O_2	AnO_2	O_2	AnO_2	O_2	AnO_2	O_2	AnO_2	O_2	AnO_2	O_2	AnO_2
Staphylococcus aureus [n=16]	0.25	0.125	0.125	0.125	0.125	0.25	0.5	0.5	1	1	4	4
Coagulase negative staphylococci [n=8]	0.125	0.125	0.06	0.06	0.06	0.125	0.25	0.25	0.25	0.5	2	2
Streptococcus pneumoniae [n=13]	1	0.25	n.d.	n.d.	n.d.	n.d.	1	1	n.d.	n.d.	n.d.	n.d.
Non-pneumococcal *Streptococcus spp.* [n=18]	0.5	0.25	0.25	0.125	n.d.	n.d.	0.5	0.5	0.5	0.5	n.d.	n.d.
Enterococcus spp. [n=15]	1	1	1	1	2	2	2	4	4	32	32	32
Escherichia coli [n=15]	0.125	0.008	0.008	0.008	0.03	0.008	0.008	0.06	0.125	0.125	0.5	0.5
Pseudomonas aeruginosa [n=15]	4	0.5	1	0.25	0.5	0.5	0.125	0.125	0.25	0.25	2	2
Stenotrophomonas maltophilia [n=10]	2	0.5	0.5	0.25	0.5	n.d.	4	8	16	8	64	n.d.
Klebsiella spp. [n=15]	4	1	1	2	2	2	2	16	32	32	32	32
Proteus spp. [n=13]	1	0.25	0.25	0.125	0.25	0.25	0.03	0.06	0.125	0.125	2	1
Acinetobacter baumannii [n=11]	8	1	2	1	4	4	32	32	32	32	32	32
Enterobacter spp. [n=6]	0.25	0.06	0.06	0.06	0.06	0.125	0.03	0.06	0.125	0.25	2	2
Serratia marcescens [n=6]	0.5	0.25	0.25	0.25	0.5	0.5	0.06	0.125	0.5	0.5	4	2

	Levofloxacin						Moxifloxacin					
	pH 7.2		pH 6.2		pH 5.2		pH 7.2		pH 6.2		pH 5.2	
	O_2	AnO_2	O_2	AnO_2	O_2	AnO_2	O_2	AnO_2	O_2	AnO_2	O_2	AnO_2
Staphylococcus aureus [n=16]	0.25	0.25	0.5	0.5	2	2	0.125	0.125	0.25	0.5	1	1
Coagulase negative staphylococci [n=8]	0.25	0.25	0.25	0.5	2	2	0.125	0.125	0.25	0.5	1	1
Streptococcus pneumoniae [n=13]	1	1	n.d.	n.d.	n.d.	n.d.	0.25	0.125	n.d.	n.d.	n.d.	n.d.
Non-pneumococcal *Streptococcus spp.* [n=18]	0.5	0.5	0.5	1	n.d.	n.d.	0.125	0.25	0.25	0.25	n.d.	n.d.
Enterococcus spp. [n=15]	2	4	4	4	16	16	0.5	1	1	2	8	8
Escherichia coli [n=15]	0.03	0.125	0.125	0.25	1	1	0.06	0.25	0.25	0.5	2	2
Pseudomonas aeruginosa [n=15]	0.5	0.5	1	1	4	4	2	2	4	4	16	16
Stenotrophomonas maltophilia [n=10]	2	2	4	4	32	n.d.	1	1	4	2	16	n.d.
Klebsiella spp. [n=15]	1	2	8	8	32	32	2	8	8	16	32	32
Proteus spp. [n=13]	0.06	0.125	0.25	0.25	1	1	0.5	2	2	4	16	16
Acinetobacter baumannii [n=11]	8	8	16	32	32	32	16	8	32	32	32	32
Enterobacter spp. [n=6]	0.06	0.25	0.25	0.5	4	4	0.125	0.5	1	1	8	8
Serratia marcescens [n=6]	0.125	0.25	0.5	0.5	4	4	0.5	1	2	4	16	8

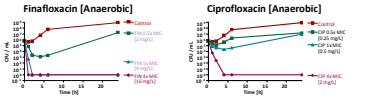
	Tobramycin						Ceftazidime					
	pH 7.2		pH 6.2		pH 5.2		pH 7.2		pH 6.2		pH 5.2	
	O_2	AnO_2	O_2	AnO_2	O_2	AnO_2	O_2	AnO_2	O_2	AnO_2	O_2	AnO_2
Staphylococcus aureus [n=16]	1	8	4	16	16	32	32	32	32	16	8	8
Coagulase negative staphylococci [n=8]	4	32	32	32	64	64	32	32	32	16	2	2
Streptococcus pneumoniae [n=13]	16	2	n.d.	n.d.	n.d.	n.d.	4	2	n.d.	n.d.	n.d.	n.d.
Non-pneumococcal *Streptococcus spp.* [n=18]	16	16	16	8	n.d.	n.d.	0.25	0.25	0.25	0.25	n.d.	n.d.
Enterococcus spp. [n=15]	32	32	32	32	32	32	64	32	64	32	32	32
Escherichia coli [n=15]	1	4	4	8	16	32	0.25	0.5	0.5	0.5	2	1
Pseudomonas aeruginosa [n=15]	0.5	2	2	4	16	16	4	16	4	16	8	16
Stenotrophomonas maltophilia [n=10]	64	64	64	64	64	n.d.	32	32	32	32	8	n.d.
Klebsiella spp. [n=15]	8	4	16	8	32	32	2	8	2	16	4	4
Proteus spp. [n=13]	1	8	4	16	32	32	0.125	4	0.125	8	32	16
Acinetobacter baumannii [n=11]	16	8	16	4	32	32	32	32	32	32	32	32
Enterobacter spp. [n=6]	0.5	2	2	4	16	32	1	1	2	32	32	32
Serratia marcescens [n=6]	4	16	16	16	32	32	0.5	0.5	1	32	32	32

	Meropenem					
	pH 7.2		pH 6.2		pH 5.2	
	O_2	AnO_2	O_2	AnO_2	O_2	AnO_2
Staphylococcus aureus [n=16]	8	8	16	8	0.5	0.25
Coagulase negative staphylococci [n=8]	0.5	1	4	1	0.125	0.06
Streptococcus pneumoniae [n=13]	0.125	0.125	n.d.	n.d.	n.d.	n.d.
Non-pneumococcal *Streptococcus spp.* [n=18]	0.008	0.008	0.008	0.008	n.d.	n.d.
Enterococcus spp. [n=15]	8	8	4	4	4	4
Escherichia coli [n=15]	0.03	0.03	0.06	0.06	0.125	0.125
Pseudomonas aeruginosa [n=15]	1	0.5	0.5	0.5	1	1
Stenotrophomonas maltophilia [n=10]	64	64	64	64	64	n.d.
Klebsiella spp. [n=15]	0.06	0.06	0.125	0.125	0.25	0.125
Proteus spp. [n=13]	0.25	0.5	1	1	4	1
Acinetobacter baumannii [n=11]	32	32	32	32	4	8
Enterobacter spp. [n=6]	0.06	0.06	0.06	0.125	0.25	0.125
Serratia marcescens [n=6]	0.06	0.125	0.125	0.25	0.25	0.5

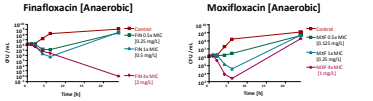
pH of plate wells (at MIC) of QC strains *S. aureus* 29213 (*Sa*), *E. coli* 25922 (*Ec*) and *P. aeruginosa* 27853 (*Pa*) following 24 h anaerobic incubation.

Drug & Starting pH		Final pH		
		Sa	*Ec*	*Pa*
CIP	5.2	5.3	5.3	6.2
	6.2	6.3	6.3	6.5
	7.2	7.3	7.3	7.3
FIN	5.2	5.3	5.3	5.5
	6.2	6.7	6.7	6.7
	7.2	7.3	7.3	7.3
TOB	5.2	5.3	5.3	5.3
	6.2	6.7	6.7	6.7
	7.2	7.3	7.3	7.3

Time kill of *P. aeruginosa* determined under anaerobic conditions at pH 7.2



Time kill of *S. aureus* NRS384 determined under anaerobic conditions at pH 7.2



Conclusions

- In addition to pH activation, finafloxacin activity was enhanced under anaerobic, compared to aerobic conditions. This effect was most pronounced at pH 7.2, suggesting that there may be an overlapping mechanism for pH and anaerobic activation of finafloxacin.
- Acidic pH had a negative effect on the activity of other fluoroquinolones and tobramycin.
- Tobramycin also exhibited reduced activity under anaerobic conditions (compared to aerobic) against most species tested. In general, the activities of ciprofloxacin, levofloxacin and moxifloxacin were unaffected by oxygen with several exceptions e.g. *Klebsiella spp.* and *Enterobacter spp.*
- These data suggest that finafloxacin could exhibit greater antibacterial and bactericidal activity at infection sites with low pH or oxygen availability, than would be predicted from its MIC (at pH 7.2); whereas other fluoroquinolones and tobramycin could exhibit worse than expected activities.

Antibacterial Activity of Finafloxacin Against Isogenic *Pseudomonas aeruginosa* (*Pa*) Isolates Expressing Combinations of Defined Mechanisms of Fluoroquinolone (FQ) Resistance and Propensity to Select for Resistance.

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Abstract

Introduction
Finafloxacin (FIN) is a novel fluoroquinolone (FQ) that exhibits improved antibacterial and pharmacodynamic properties at pH values below neutral which often characterize infection sites. The aim of this study was to determine the propensity of FIN and other FQs to select for resistance in the nosocomial pathogen *Pa* and to determine the effects of defined target and efflux resistance mechanisms on activity.

Methods
Mutation prevention concentrations (MPC) for FIN, ciprofloxacin (CIP), levofloxacin (LVX) were determined for *Pa* 27853 from an inoculum of 10^{10} CFU/mL at pH 7.2, 6.2 and 5.2. Susceptibility testing was also performed at these pHs (CLSI method).

Results
MPCs for FIN were 64, 8 and 8 mg/L at pH 7.2, 6.2 and 5.2 respectively, for CIP (2, 8 and 64) and for LVX (8, 32 and 64). MPC/MIC ratios were in the range 4-16, resistance frequencies were in the range 3.4 x10^{-8} - 1.9 x10^{-9}, and mutant MICs were 4-16-fold higher than that of the parent. The susceptibility of isogenic sets of *Pa* harboring defined mutations in efflux regulators, target mutations and combinations thereof are shown in the Table.

Conclusions
Unlike marketed FQs, FIN activity increases at low pH. This was reflected in the greater potency of FIN towards FQ resistant *Pa* and having a lower propensity, than CIP or LVX, to select for resistant *Pa* at the more acidic pH. These data suggest that FIN could have an advantage over other FQs at sites acidified by infection and inflammation processes.

Background

- Finafloxacin is a novel pH activated, broad spectrum fluoroquinolone in development for infection indications in the hospital and critical care setting [1]
- Finafloxacin exhibits enhanced activity at low pH and under other environmental conditions associated with infection [1, 2]
- Finafloxacin exhibits bactericidal activity against forms of quiescent growth, thought to be relevant *in vivo* e.g. non-growing cells, biofilms and persisters [3]
- Other fluoroquinolones lose activity under such conditions. Consequently, finafloxacin exhibited superior activity in a series of infection models [4,5]
- The activity of finafloxacin under infection relevant conditions and against infection relevant growth forms in combination with the high dosing potential predicted from its safety profile [6, 7, 8], suggest finafloxacin will offer improved properties over currently marketed fluoroquinolones.

References

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Background and aim

Pseudomonas aeruginosa is an important hospital pathogen which is becoming increasingly difficult to treat due to the emergence and selection of resistant strains. Resistance to fluoroquinolones is usually mediated through combinations of target mutations in *gyrA* and mutations giving rise to increased activity of efflux pumps.

The aims of this study were to investigate the propensities of finafloxacin and other marketed fluoroquinolones to select for resistance in *P. aeruginosa* and to measure their activities against strains harboring combinations of mutations within target and regulatory genes affecting drug efflux.

Methods

- MICs were determined in pH adjusted cation adjusted Mueller-Hinton broth (MHB) using CLSI methodology for broth microdilution.
- The following antibiotics (with abbreviations) were tested: ciprofloxacin (CIP), finafloxacin (FIN) and levofloxacin (LVX). The putative efflux pump inhibitor (EPI) phenylalanyl-arginyl-ß-naphthylamide (PAßN) was added to 8 µg/mL to examine the role of efflux in wild type and MDR backgrounds.
- *P. aeruginosa* strain ATCC 27853 was used for determination of mutation prevention concentration (MPC) and mutation frequency. Inocula of 10^{10} colony forming units were spread onto a series of Mueller-Hinton agar (MHA) plates containing 2-fold dilutions of the test drug. The lowest concentration at which no mutants grew following 48 h incubation was the MPC.
- To examine the effects of target mutations, regulatory mutations affecting multiple drug efflux (MDE) pumps and combinations thereof, *P. aeruginosa* strain ML5087 was used. Mutants containing MDE resistance markers in: *nfxB* (overexpression of MexCD-OprJ), *nalB* / *mexR* (overexpression of MexAB-OprM) and *nfxC* (overexpression of MexEF-OprN) were constructed with and without further quinolone resistance mutations within the target gene *gryA*. Mutations were confirmed by sequencing, details of which are listed in Table 2.

Results

	pH 5.2			pH 6.2			pH 7.2		
	FIN	CIP	LVX	FIN	CIP	LVX	FIN	CIP	LVX
MIC [mg/L]	1	4	4	1	1	2	4	0.25	1
MPC [mg/L]	8	64	64	8	8	32	64	2	8
MPC/MIC	8	16	16	8	8	16	16	8	8
Mutation frequency (at 1/2 MPC)	8.2 x 10^{-9}	2.7 x 10^{-8}	4.3 x 10^{-8}	2.0 x 10^{-8}	3.4 x 10^{-8}	7.4 x 10^{-9}	1.6 x 10^{-8}	1.8 x 10^{-9}	5.1 x 10^{-9}

Table 1: Mutation prevention concentrations and mutation frequencies of finafloxacin, ciprofloxacin and levofloxacin, determined at pH 5.2 pH 6.2 and pH 7.2 determined with *Pa* ATCC 27853

- The MPC of FIN decreased at acidic pH compared to pH 7.2, conversely the MPCs of CIP and LVX increased. Mutants to FIN, CIP and LVX exhibited proportional 4-16-fold increases in MIC to the other FQs.

Strain	Fluoroquinolone resistance marker	Upregulated efflux pump	MIC [mg/L]pH 5.2			MIC [mg/L]pH 6.2			MIC [mg/L]pH 7.2		
			FIN	CIP	LVX	FIN	CIP	LVX	FIN	CIP	LVX
ML5087	Wild type		0.5	1	4	0.5	0.25	0.5	8	0.125	0.5
ML5087-M1a	*gyr*AT83I		16	16	64	16	16	32	>64	8	32
ML5087	nfxBD11bp	MexCD-OprJ	0.5	4	8	0.5	0.125	0.5	4	0.125	0.5
ML5087nfxB-M13a	nfxBD11bp, *gyr*AT83I	MexCD-OprJ	8	64	>64	16	4	16	>64	8	16
ML5087	nalB (=*mex*RT130P)	MexAB-OprM	2	16	32	4	0.5	2	64	0.5	4
ML5087nalB-M6a	nalB (=*mex*RT130P), *gyr*AT83I	MexAB-OprM	16	>64	>64	32	32	64	>64	16	32
ML5087-M4	nfxC	MexEF-OprN	2	16	32	4	2	8	32	0.5	2
ML5087nfxC-M4-M8	nfxC, *gyr*A T83I	MexEF-OprN	16	64	>64	32	32	64	>64	16	32

Table 2: MICs of finafloxacin, ciprofloxacin and levofloxacin, at pH 5.2 pH 6.2 and pH 7.2, against isogenic *P. aeruginosa* harboring combinations of target and MDE fluoroquinolone resistance mutations.

- Mutations in nalB and nfxC, resulting in increased expression of MexAB-OprM and MexEF-oprN respectively, resulted in MIC increases for all FQs by 4 to 32-fold.
- Combination of a *gyrA* and MDE mutation resulted in MIC increases of 16 to >128-fold, relative to wild type.
- Lowering pH from 7.2 to 6.2 and 5.2 increased the activity of finafloxacin by a factor of 4 to 32-fold. Conversely, the activities of ciprofloxacin and levofloxacin decreased at the lower pHs by a factor of up to 32-fold.

Strain	FQ resistance marker	pH 5.2						pH 6.2						pH 7.2					
		FIN		CIP		LVX		FIN		CIP		LVX		FIN		CIP		LVX	
			+EPI		+EPI		+EPI		+EPI		+EPI		+EPI		+EPI		+EPI		+EPI
Wild type		0.5	0.125	1	0.5	4	1	0.5	0.06	0.25	≤0.06	0.5	≤0.06	8	1	0.125	≤0.06	0.5	≤0.06
nfxBD11bp	mexCD-oprJ	0.5	0.125	4	1	8	2	0.5	0.125	0.125	0.125	0.5	0.125	8	2	0.125	≤0.06	0.5	0.125
nalB (=*mex*RT130P)	mexAB-oprM	2	0.25	16	4	32	8	4	0.5	0.5	0.25	2	0.5	64	16	0.5	0.25	4	1
nfxC	mexEF-oprN	2	1	32	16	32	16	4	1	2	2	4	1	32	8	0.5	0.5	2	1

Table 3: MICs of isogenic strains harboring MDR mutations, in the presence and absence of efflux pump inhibitor (EPI)

- Addition of the putative efflux pump inhibitor PAßN partially restored sensitivity in both wild type and MDE backgrounds, suggesting that efflux plays a role both in the intrinsic resistance of the strain and in the reduced susceptibility of the MDE mutants.

CIP; ciprofloxacin, EPI; efflux pump inhibitor (PAßN at 8 mg/L) FIN; finafloxacin, LVX; levofloxacin, MPC; mutation prevention concentration

Conclusions

- **Finafloxacin activity against *P. aeruginosa* was reduced by the presence of mutations in the target gene (*gyrA*) and MDE backgrounds *nfxB*, *nfxC* and *nalB*, to a similar degree to other fluoroquinolones. Despite this, the pH activation exhibited by finafloxacin resulted in an overall greater potency than ciprofloxacin and levofloxacin against fluoroquinolone resistant *P. aeruginosa* under acidic conditions (Table 2).**
- **The pH activation also translated into finafloxacin exhibiting a lower potential than ciprofloxacin or levofloxacin to select for resistance under acidic conditions (Table 1).**
- **Based on these findings, finafloxacin could exhibit improved antibacterial properties over other fluoroquinolones, against *P. aeruginosa* in infection sites acidified by inflammation and other physiological processes relating to infection.**