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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).

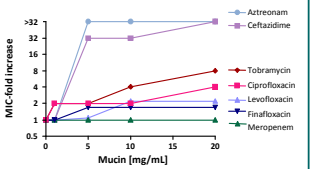
Results

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

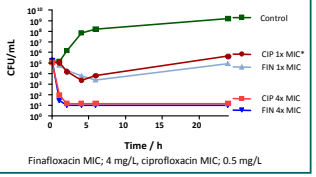
Species tested and number	Finafloxacin		Ciprofloxacin		Levofloxacin		Moxifloxacin		Tobramycin		Ceftazidime		Meropenem	
pH 5.2	O_2	AnO_2	O_2	AnO_2	O_2	AnO_2	O_2	AnO_2	O_2	AnO_2	O_2	AnO_2	O_2	AnO_2
Escherichia coli [n=5]	0.03	0.03	0.25	0.5	0.5	1	1	2	32	64	0.5	1	0.25	0.25
Stenotrophomonas maltophilia [n=5]	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.**

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