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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.
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(4) Endermann *et al.* 2008. 48th ICAAC / 46th IDSA Washington DC. Poster F1-2044.
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(6) Schmuck *et al.* 2008. 48th ICAAC / 46th IDSA Washington DC. Poster F1-2047.
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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

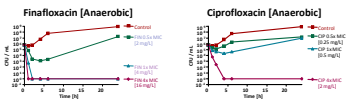
	Finafloxacin						Ciprofloxacin						Levofloxacin						Moxifloxacin					
	pH 7.2		pH 6.2		pH 5.2		pH 7.2		pH 6.2		pH 5.2		pH 7.2		pH 6.2		pH 5.2		pH 7.2		pH 6.2		pH 5.2	
	O_2	AnO_2	O_2	AnO_2	O_2	AnO_2	O_2	AnO_2	O_2	AnO_2	O_2	AnO_2	O_2	AnO_2	O_2	AnO_2	O_2	AnO_2	O_2	AnO_2	O_2	AnO_2	O_2	AnO_2
Staphylococcus aureus [n=16]	0.25	0.125	0.125	0.125	0.125	0.25	0.5	0.5	1	1	4	4	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]	0.125	0.125	0.06	0.06	0.06	0.125	0.25	0.25	0.25	0.5	2	2	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	0.25	n.d.	n.d.	n.d.	n.d.	1	1	n.d.	n.d.	n.d.	n.d.	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	0.25	0.25	0.125	n.d.	n.d.	0.5	0.5	0.5	0.5	n.d.	n.d.	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]	1	1	1	1	2	2	2	4	4	32	32	32	2	4	4	4	16	16	0.5	1	1	2	8	8
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	0.03	0.125	0.125	0.25	1	1	0.06	0.25	0.25	0.5	2	2
Pseudomonas aeruginosa [n=15]	4	0.5	1	0.25	0.5	0.5	0.125	0.125	0.25	0.25	2	2	0.5	0.5	1	1	4	4	2	2	4	4	16	16
Stenotrophomonas maltophilia [n=10]	2	0.5	0.5	0.25	0.5	n.d.	4	8	16	8	64	n.d.	2	2	4	4	32	n.d.	1	1	4	2	16	n.d.
Klebsiella spp. [n=15]	4	1	1	2	2	2	2	16	32	32	32	32	1	2	8	8	32	32	2	8	8	16	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	0.06	0.125	0.25	0.25	1	1	0.5	2	2	4	16	16
Acinetobacter baumannii [n=11]	8	1	2	1	4	4	32	32	32	32	32	32	8	8	16	32	32	32	16	8	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	0.06	0.25	0.25	0.5	4	4	0.125	0.5	1	1	8	8
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	0.125	0.25	0.5	0.5	4	4	0.5	1	2	4	16	8

	Tobramycin						Ceftazidime						Meropenem					
	pH 7.2		pH 6.2		pH 5.2		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	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	8	8	16	8	0.5	0.25
Coagulase negative staphylococci [n=8]	4	32	32	32	64	64	32	32	32	16	2	2	0.5	1	4	1	0.125	0.06
Streptococcus pneumoniae [n=13]	16	2	n.d.	n.d.	n.d.	n.d.	4	2	n.d.	n.d.	n.d.	n.d.	0.125	0.125	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.	0.008	0.008	0.008	0.008	n.d.	n.d.
Enterococcus spp. [n=15]	32	32	32	32	32	32	64	32	64	32	32	32	8	8	4	4	4	4
Escherichia coli [n=15]	1	4	4	8	16	32	0.25	0.5	0.5	0.5	2	1	0.03	0.03	0.06	0.06	0.125	0.125
Pseudomonas aeruginosa [n=15]	0.5	2	2	4	16	16	4	16	4	16	8	16	1	0.5	0.5	0.5	1	1
Stenotrophomonas maltophilia [n=10]	64	64	64	64	64	n.d.	32	32	32	32	8	n.d.	64	64	64	64	64	n.d.
Klebsiella spp. [n=15]	8	4	16	8	32	32	2	8	2	16	4	4	0.06	0.06	0.125	0.125	0.25	0.125
Proteus spp. [n=13]	1	8	4	16	32	32	0.125	4	0.125	8	32	16	0.25	0.5	1	1	4	1
Acinetobacter baumannii [n=11]	16	8	16	4	32	32	32	32	32	32	32	32	32	32	32	32	4	8
Enterobacter spp. [n=6]	0.5	2	2	4	16	32	1	1	2	32	32	32	0.06	0.06	0.06	0.125	0.25	0.125
Serratia marcescens [n=6]	4	16	16	16	32	32	0.5	0.5	1	32	32	32	0.06	0.125	0.125	0.25	0.25	0.5

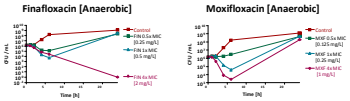
pH of plate wells (at MIC) of QC strains *S. aureus* 29213 (*Sa*), *E. coli* 25922 (*Ec*) and *P. aeruginosa* 27853 (*Pa*) following 24 h anaerobic incubation.

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	6.3	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.