D-684

Effects of Varying MIC parameters on the *in vitro* Activity of Finafloxacin

S. HAWSER[1], M. HACKEL[2], S. BOUCHILLON[2], A. VENTE[3]

[1]IHMA Europe Sàrl, Epalinges, Switzerland
[2]International Health Management Associates, Inc., Schaumburg, IL, USA
[3]MerLion Pharmaceuticals, Berlin, Germany

IHMA, Inc.
2122 Palmer Dr.
Schaumburg, IL
60173
Tel: 847.303.5003
www.ihmainc.com

Revised Abstract

Background: Finafloxacin is a novel member of the fluoroquinolone class of antibiotics. Specifically, finafloxacin belongs to a new 8-cyano subclass. The agent contains a novel base component which confers improved antibacterial activity under acidic conditions, where the activity of many existing fluoroquinolones is impaired. **Methods:** This CLSI M23 Tier 1 study investigated the effects of thirteen different MIC parameters: atmosphere, bovine serum albumin, calcium, fresh versus frozen plates, incubation time, inoculum size, medium, magnesium, plasma, pH, polysorbate 80, serum and temperature. The effect of varying each parameter on MICs was determined according to CLSI M07-A8 microdilution methodology. **Results:** Of the 13 different parameters studied, only pH consistently affected activity against all species, addition of plasma or serum was more species specific (see Table): All other parameters had no effect on the activity.

Species (n)	Standard Controls*	pH 5.8	pH 6.5	pH 8.5	50% plasma	25% serum	50% serum
H. influenzae (30)	0.008	0.03	0.04	0.36	NG**	0.009	0.006
K. pneumoniae (30)	2.2	0.644	0.401	6.8	10.037	3.81	1.54
P. aeruginosa (30)	12.1	4.6	6.1	24.8	5.104	10.752	13.803
S. aureus (30)	0.75	0.55	0.27	7.1	5.879	2.175	4.467
S. pneumoniae (30)	5.7	0.015	1.883	9.4	0.019	5.9	8.333
S. pyogenes (30)	0.558	0.015	0.224	2.4	0.015	0.625	1.4

Values shown are mean MICs expressed in mcg/ml. *Standard control conditions run at pH 7.2 with no added plasma or serum; **NG, no growth. Shading represents statistically significant differences (p<0.05) as compared to standard conditions.

Conclusions: Compared with the activity of finafloxacin under standard CLSI M07-08 conditions, pH affected the activity of the drug against all species studied. At lower pH, finafloxacin was more active while at higher pH the drug was less active. Plasma and serum also affected finafloxacin activity though the effects were more species specific.

Introduction

Finafloxacin is a novel member of the fluoroquinolone class of antibiotics. Specifically, finafloxacin belongs to a new 8-cyano subclass. The agent contains a novel base component which confers improved antibacterial activity under acidic conditions, where the activity of many existing fluoroquinolones is impaired. Finafloxacin is currently in clinical development with MerLion Pharmaceuticals. Under license with Alcon Pharmaceuticals, USA, finafloxacin is being developed for treating ear infections, including acute otitis externa (outer ear and ear canal) and acute otitis media (middle ear). In addition to otic and ophthalmic indications, finafloxacin, which has already shown potential for the treatment of a range of infections in Phase IIa clinical studies, is also being evaluated in critical care and hospital based infection settings

In order to thoroughly understand the activity of finafloxacin, an M23-A3 Tier 1 study in line with the requirements set out by the Clinical and Laboratory Standards Institute (CLSI) was performed. Quality control testing in the present study includes investigating the effects of thirteen different MIC parameters on the activity of finafloxacin against standard CLSI QC organisms and clinical isolates, namely, medium, fresh versus frozen panels, incubation time, calcium, magnesium, polysorbate 80, serum, plasma, pH, inoculum size, temperature, atmosphere and bovine serum albumin.

Materials & Methods

Parameter Modifications

Test media composition (finafloxacin and moxifloxacin): Modifications to the test media composition were made to result in the following systematic changes: 25 mcg/ml Ca^{++}, 50 mcg/ml Ca^{++}, 100 mcg/ml Ca^{++}, 12.5 mcg/ml Mg, 25 mcg/ml Mg, pH 5.8, pH 6.5, pH 7.2, pH 8.5, 25% human serum, 50% human serum, 50% human plasma, 0.002% polysorbate 80, and supplemented with bovine serum albumin at 0.001%, 0.05%, 0.02%, and 0.1%.

Incubation time and conditions (finafloxacin and moxifloxacin): The effects of incubation conditions were evaluated by incubating plates in ambient air, 5% CO_2 and under anaerobic conditions. Standard test panels were incubated at 30º C, 35º C and 40º C. Variations to inoculum preparation were evaluated by testing concentrations of 10^4, 10^5, 10^6, and 10^7 CFU/ml. All concentrations were confirmed by colony count. Standard test panels were read at 16, 18, 20, 24, and 48 hours to evaluate incubation time.

Panels (finafloxacin and moxifloxacin): Standard panels frozen for 48 hours at -80ºC were set up under standard testing conditions.

Antimicrobial Susceptibility Testing

Minimum inhibitory concentration (MIC) endpoints were determined by broth microdilution according to CLSI guidelines (CLSI, *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically; Approved Standard- Eighth Edition*, M07-A8, 2009). All panels were produced at IHMA.

Cation-adjusted Mueller-Hinton broth (CAMHB), cation-adjusted Mueller-Hinton broth supplemented with 3% laked horse blood (MHB/LHB) and *Haemophilus* Test Medium broth (HTM) were used as the test media. Endpoints were determined following CLSI guidelines (CLSI, *Performance Standards for Antimicrobial Susceptibility Testing; Twenty-First Informational Supplement* M100-S21, 2011).

Results

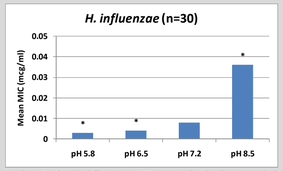
Of the thirteen different MIC parameters tested, only pH and to a lesser extent plasma and serum affected the activity of finafloxacin. Atmospheric conditions, calcium, magnesium, incubation time, inoculums size, medium, polysorbate 80, temperature and the use of fresh or frozen susceptibility plates had no effect on the activity of finafloxacin. Consequently, only data for pH, serum and plasma are shown below.

Table 1. Effect of pH on finafloxacin and moxifloxacin MIC activity (mcg/ml) against ATCC Quality Control Strains.

	Finafloxacin MICs (mcg/ml)			
Organism	**pH 5.8**	**pH 6.5**	**pH 7.2**	**pH 8.5**
E. coli ATCC 25922	≤.015	≤.015	.03	.25
E. faecalis ATCC 29212	.5	.25	.5	2
H. influenzae ATCC 49247	NG	≤.004	.008	NG
P. aeruginosa ATCC 27853	.25	2	4	32
S. aureus ATCC 29213	.12	.03	.12	1
S. pneumoniae ATCC 49619	≤.015	.25	.5	2
	Moxifloxacin MICs (mcg/ml)			
	pH 5.8	**pH 6.5**	**pH 7.2**	**pH 8.5**
E. coli ATCC 25922	1	.06	.03	≤.015
E. faecalis ATCC 29212	2	.25	.25	.12
H. influenzae ATCC 49247	NG	.03	.015	NG
P. aeruginosa ATCC 27853	32	16	4	2
S. aureus ATCC 29213	1	.12	.06	.06
S. pneumoniae ATCC 49619	≤.015	.12	.12	.06

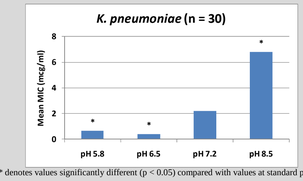
NG, no growth.

Figure 1. Effect of pH on activity of finafloxacin against clinical isolates of *H. influenzae*.



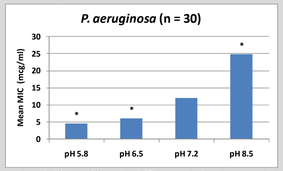
* denotes values significantly different (p < 0.05) compared with values at standard pH 7.2

Figure 2. Effect of pH on activity of finafloxacin against clinical isolates of *K. pneumoniae*.



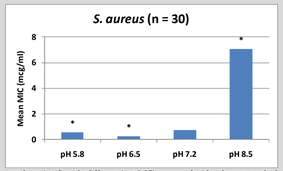
* denotes values significantly different (p < 0.05) compared with values at standard pH 7.2

Figure 3. Effect of pH on activity of finafloxacin against clinical isolates of *P. aeruginosa*.



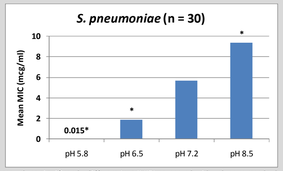
* denotes values significantly different (p < 0.05) compared with values at standard pH 7.2

Figure 4. Effect of pH on activity of finafloxacin against clinical isolates of *S. aureus*.



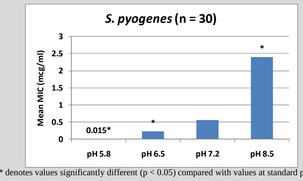
* denotes values significantly different (p < 0.05) compared with values at standard pH 7.2

Figure 5. Effect of pH on activity of finafloxacin against clinical isolates of *S. pneumoniae*.



* denotes values significantly different (p < 0.05) compared with values at standard pH 7.2

Figure 6. Effect of pH on activity of finafloxacin against clinical isolates of *S. pyogenes*.



* denotes values significantly different (p < 0.05) compared with values at standard pH 7.2

Table 2. Effect of serum / plasma on finafloxacin and moxifloxacin MIC values against ATCC Quality Control Strains.

	Finafloxacin MICs (mcg/ml)			
Organism	**Control**	**25% Serum**	**50% Serum**	**50% Plasma**
E. coli ATCC 25922	.03	.12	.06	≤.015
E. faecalis ATCC 29212	.5	1	2	≤.015
H. influenzae ATCC 49247	.008	.008	NG	NG
P. aeruginosa ATCC 27853	8	8	4	2
S. aureus ATCC 29213	.12	.25	.5	≤.015
S. pneumoniae ATCC 49619	.5	1	2	≤.015
	Moxifloxacin MICs (mcg/ml)			
	Control	**25% Serum**	**50% Serum**	**50% Plasma**
E. coli ATCC 25922	.03	≤.015	≤.015	≤.015
E. faecalis ATCC 29212	.12	.12	.06	≤.015
H. influenzae ATCC 49247	.008	.008	NG	NG
P. aeruginosa ATCC 27853	4	1	.25	.03
S. aureus ATCC 29213	.03	.03	.03	≤.015
S. pneumoniae ATCC 49619	.12	.12	.12	≤.015

NG, no growth.

Table 3. Effect of serum / plasma on finafloxacin and moxifloxacin activity against clinical isolates.

			Finafloxacin MICs (mcg/ml)	
	Control	50% Plasma	25% Serum	50% Serum
H. influenzae	0.009	NG	0.009	0.006
K. pneumoniae	2.286	10.037	3.818	1.542
P. aeruginosa	13.067	5.104	10.752	13.803
S. aureus	1.355	5.879	2.175	4.467
S. pneumoniae	3.95	0.019	5.9	8.333
S. pyogenes	0.317	≤0.015	0.625	1.4

			Moxifloxacin MICs (mcg/ml)	
	Control	50% Plasma	25% Serum	50% Serum
H. influenzae	0.01	NG	0.008	0.005
K. pneumoniae	1.192	0.214	0.29	0.066
P. aeruginosa	10.967	0.069	2.33	1.899
S. aureus	2.43	3.346	2.021	2.421
S. pneumoniae	0.944	0.019	0.731	0.787
S. pyogenes	0.142	≤0.015	0.185	0.172

*Values represent arithmetic means. NG, no growth. Grey shading represents values that were statistically significantly different (p < 0.05) as compared with control values.

Conclusions

- Of the thirteen parameters studied, differences in pH most commonly affected the activity of finafloxacin.
- The activity of finafloxacin at low pH (pH 5.8 and 6.5) was generally superior (p < 0.05) against all isolates tested when compared with control pH (7.2).
- The addition of plasma caused an increase in activity against three species and a decrease in activity for two species.
- The addition of 25% and 50% serum caused a decrease in finafloxacin activity against *S. aureus*, *S. pneumoniae* and *S. pyogenes*. However, there was no effect on finafloxacin activity against *K. pneumoniae* and *P. aeruginosa*.
- All other parameters including atmospheric conditions, calcium, magnesium, incubation time, inoculums size, medium, polysorbate 80, temperature and the use of fresh or frozen susceptibility panels had no effect on the activity of finafloxacin.

Comparative Activity of Finafloxacin and Moxifloxacin against Anaerobes

E-113

S. HAWSER[1], M. HACKEL[2], S. BOUCHILLON[2], A. VENTE[3]

[1]IHMA Europe Sàrl, Epalinges, Switzerland
[2]International Health Management Associates, Inc., Schaumburg, IL, USA
[3]MerLion Pharmaceuticals, Berlin, Germany

IHMA, Inc.
2122 Palmer Dr.
Schaumburg, IL
60173
Tel: 847.303.5003
www.ihmainc.com

Revised Abstract

Background: Finafloxacin is a novel member of the fluoroquinolone class of antibiotics. Specifically, finafloxacin belongs to a new 8-cyano subclass. The agent contains a novel base component which confers improved antibacterial activity under acidic conditions, where the activity of many existing fluoroquinolones is impaired. The current study compares the activity of finafloxacin (FIN) and moxifloxacin (MOX) against recent anaerobic clinical isolates. **Methods:** The activity of FIN and MOX against 216 anaerobic isolates from 2009-2010 were investigated under standard CLSI conditions by agar dilution. **Results:**

Species (n)	Drug	Min	Max	Mean	Median	MIC_{90}
Anaerococcus prevotii (6)	FIN	≤.015	0.5	0.145	0.03	0.5
	MOX	0.03	1	0.41	0.12	1
Bacteroides fragilis group (108)	FIN	0.06	8	1.492	0.5	4
	MOX	0.12	16	2.29	0.25	8
Clostridium difficile (27)	FIN	0.06	8	2.812	2	8
	MOX	0.5	16	3.407	1	8
Clostridium perfringens (31)	FIN	≤.015	1	0.129	0.06	0.12
	MOX	0.12	1	0.338	0.25	0.5
Finegoldia magna (11)	FIN	≤.015	8	1.704	0.12	4
	MOX	0.03	8	1.757	0.12	4
Peptostreptococcus spp.(24)	FIN	≤.015	4	0.239	0.06	0.25
	MOX	≤.015	4	0.308	0.06	0.5
Peptoniphilus asaccharolyticus (9)	FIN	≤.015	0.25	0.058	0.03	0.25
	MOX	0.06	1	0.198	0.12	1

Values shown are MICs expressed in mcg/ml.

Conclusions: FIN was generally more active than MOX against the seven different anaerobic species studied. Against five of the seven species, FIN MIC_{90} values were 1 to 2 dilutions lower than MIC_{90} values for MOX. Analysis of the mean MICs further showed that FIN was more active than MOX against most of the species tested.

Introduction

Finafloxacin is a novel member of the fluoroquinolone class of antibiotics. Specifically, finafloxacin belongs to a new 8-cyano subclass. The agent contains a novel base component which confers improved antibacterial activity under acidic conditions, where the activity of many existing fluoroquinolones is impaired. Finafloxacin is currently in clinical development with MerLion Pharmaceuticals. Under license with Alcon Pharmaceuticals, USA, finafloxacin is being developed for treating ear infections, including acute otitis externa (outer ear and ear canal) and acute otitis media (middle ear). In addition to otic and ophthalmic indications, finafloxacin, which has already shown potential for the treatment of a range of infections in Phase IIa clinical studies, is also being evaluated in critical care and hospital based infection settings.

One of the most extensively used therapies for the treatment of anaerobic infections is moxifloxacin. In order to better understand the utility of finafloxacin against anaerobes, the current study has evaluated a direct head to head comparison of the activity of finafloxacin as compared with moxifloxacin against a range of anaerobic species from a European population.

Materials & Methods

Clinical Isolates

A total of 216 clinical anaerobic isolates were tested comprising *Anaerococcus prevotii* (n = 6), *Bacteroides fragilis* group (n = 108), *Clostridium difficile* (n = 27), *C. perfringens* (n = 31), *Finegoldia magna* (n = 11), *Peptostreptococcus* spp. (n = 24) and *Peptinophilus asaccharolyticus* (n = 9). In addition the following CLSI ATCC isolates were tested: *B. fragilis* ATCC 25285, *B. thetaiotaomicron* ATCC 29741, *C. difficile* ATCC 700057 and *Eubacterium lentum* ATCC 43055

Anaerobic Antimicrobial Susceptibility Testing

Susceptibility testing of anaerobes with finafloxacin and moxifloxacin was performed in line with the Clinical and Laboratory Standards Institute (CLSI) guideline using agar dilution (Clinical and Laboratory Standards Institute. 2007. Methods for Antimicrobial Susceptibility Tests of Anaerobic Bacteria; Approved Standard—Seventh Edition. CLSI Document M11-A7. Wayne, PA.).

Results

Table 1. Activity of finafloxacin and moxifloxacin against *B. fragilis* ATCC 25285, *B. thetaiotaomicron* ATCC 29741, *C. difficile* ATCC 700057 and *E. lentum* ATCC 43055.

QC Organism	Drug	N	Min	Max	Mean	Median
B. fragilis ATCC 25285	Finafloxacin	10	0.12	0.12	0.12	0.12
	Moxifloxacin	10	0.12	0.12	0.12	0.12
B. thetaiotaomicron ATCC 29741	Finafloxacin	10	1	1	1	1
	Moxifloxacin	10	1	1	1	1
C. difficile ATCC 700057	Finafloxacin	10	1	1	1	1
	Moxifloxacin	10	1	1	1	1
E. lentum ATCC 43055	Finafloxacin	10	0.12	0.12	0.12	0.12
	Moxifloxacin	10	0.12	0.25	0.146	0.12

Values shown are MICs expressed in mcg/ml.

Table 2. Comparison of the activity of finafloxacin and moxifloxacin against seven anaerobic species by MIC range.

Species (n)	Drug	Min	Max
Anaerococcus prevotii (6)	FIN	≤.015	0.5
	MOX	0.03	1
Bacteroides fragilis group (108)	FIN	0.06	8
	MOX	0.12	16
Clostridium difficile (27)	FIN	0.06	8
	MOX	0.5	16
Clostridium perfringens (31)	FIN	≤.015	1
	MOX	0.12	1
Finegoldia magna (11)	FIN	≤.015	8
	MOX	0.03	8
Peptostreptococcus spp.(24)	FIN	≤.015	4
	MOX	≤.015	4
Peptoniphilus asaccharolyticus (9)	FIN	≤.015	0.25
	MOX	0.06	1

Values shown are MICs expressed in mcg/ml.

Figure 1. Comparison of the activity of finafloxacin and moxifloxacin against seven anaerobic species by mean MIC.

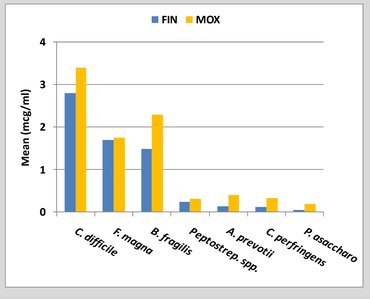


Figure 2. Comparison of the activity of finafloxacin and moxifloxacin against seven anaerobic species by median MIC.

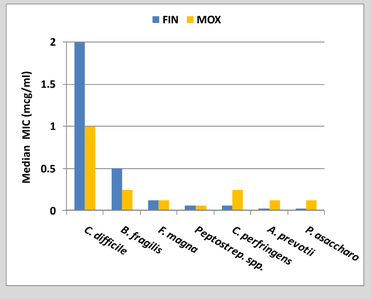
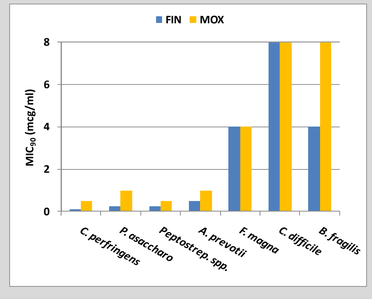


Figure 3. Comparison of the activity of finafloxacin and moxifloxacin against seven anaerobic species by MIC_{90}.



Conclusions

- Against the seven different anaerobic species, finafloxacin was generally 1 to 2 doubling dilutions more active than moxifloxacin by MIC_{90}.
- Analyses of means showed that finafloxacin was generally more active than moxifloxacin, while median MIC values were similar for both drugs.
- MIC ranges for both drugs were similar in terms of numbers of dilutions though minimum and maximum MIC values for finafloxacin were generally lower than those for moxifloxacin.
- Further studies with larger numbers of clinical isolates and other species are warranted to further characterize the anti-anaerobic activity of finafloxacin.

E-1834

Spectrum of *in Vitro* Activity of Finafloxacin, a Novel 8-cyano Fluoroquinolone, against 985 Aerobic Gram-Positive and Gram-Negative Bacteria

S. HAWSER[1], M. HACKEL[2], S. BOUCHILLON[2], A. VENTE[3]

[1]IHMA Europe Sàrl, Epalinges, Switzerland
[2]International Health Management Associates, Inc., Schaumburg, IL, USA
[3]MerLion Pharmaceuticals, Berlin, Germany

IHMA, Inc.
2122 Palmer Dr.
Schaumburg, IL
60173
Tel: 847.303.5003
www.ihmainc.com

Revised Abstract

Background: Finafloxacin is a novel member of the fluoroquinolone class of antibiotics. Specifically, finafloxacin belongs to a new 8-cyano subclass. The agent contains a novel base component which confers improved antibacterial activity under acidic conditions, where the activity of many existing fluoroquinolones is impaired. The current report describes the broad-spectrum of activity of this agent. **Methods:** A total of 985 isolates were tested and comprised of 410 gram-positive and 575 gram-negative clinical isolates from 2009-2010. MICs were determined according to CLSI M07-A8 broth microdilution methodology. **Results:** Activity of finafloxacin against the study isolates is shown below in the Table.

Species (n)	Median MIC	MIC_{90}	Species (n)	Median MIC	MIC_{90}
E. faecalis (25)	2	>32	*A. baumannii* (25)	8	>32
E. faecium (25)	>32	>32	*C. freundii* (24)	0.25	>32
S. aureus (101)	0.12	8	*E. coli* (99)	0.12	>32
MSSA (76)	0.12	0.12	*H. influenzae* (100)	0.008	0.03
MRSA (25)	4	>32	*K. pneumoniae* (102)	0.25	>32
S. epidermidis (100)	0.12	>32	*M. catarrhalis* (26)	0.03	0.03
MSSE (50)	0.12	0.25	*M. morganii* (25)	1	>32
MRSE (50)	4	>32	*P. mirabilis* (25)	1	>32
S. agalactiae (24)	0.5	1	*Providencia* spp. (24)	4	>32
S. pneumoniae (100)	1	1	*P. aeruginosa* (100)	4	>32
S. pyogenes (100)	0.5	1	*S. marcescens* (25)	2	8
Viridans streptococci (25)	1	4			

Values shown are MICs expressed in mcg/ml.

Conclusions: By analysis of all median MIC values, the spectrum of *in vitro* activity of finafloxacin included all species tested, with the exception of *E. faecium* for which the median MIC was >32 mcg/ml. By MIC_{90}, finafloxacin was most active against *H. influenzae* and *M. catarrhalis* (MIC_{90} 0.03 mcg/ml) and streptococci (MIC_{90} range 1 – 4 mcg/ml). As expected with all fluoroquinolones, activity against methicillin-resistant staphylococci was limited.

Introduction

Finafloxacin is a novel member of the fluoroquinolone class of antibiotics. Specifically, finafloxacin belongs to a new 8-cyano subclass. The agent contains a novel base component which confers improved antibacterial activity under acidic conditions, where the activity of many existing fluoroquinolones is impaired. Finafloxacin is currently in clinical development with MerLion Pharmaceuticals. Under license with Alcon Pharmaceuticals, USA, finafloxacin is being developed for treating ear infections, including acute otitis externa and acute otitis media. In addition to otic and ophthalmic indications, finafloxacin, which has already shown potential for the treatment of a range of infections in Phase IIa clinical studies, is also being evaluated in critical care and hospital based infection settings.

In order to more thoroughly investigate the spectrum of activity of finafloxacin, a total of 985 aerobic gram-positive and gram-negative clinical isolates were tested for susceptibility to finafloxacin and comparator agents.

Materials & Methods

Antimicrobial Susceptibility Testing
Minimum inhibitory concentration (MIC) endpoints were determined by broth microdilution according to CLSI guidelines (Clinical and Laboratory Standards Institute (CLSI), *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically; Approved Standard- Eighth Edition*, M07-A8, Wayne, PA. USA, 2009). All panels were produced at IHMA.

Cation-adjusted Mueller-Hinton broth (CAMHB), cation-adjusted Mueller-Hinton broth supplemented with 3% laked horse blood (MHB/LHB) and *Haemophilus* Test Medium broth (HTM) were used as the test media. Endpoints were determined following CLSI guidelines (CLSI, *Performance Standards for Antimicrobial Susceptibility Testing; Twenty-First Informational Supplement* M100-S21, Wayne, PA. USA, 2011).

As finafloxacin is known to possess improved activity at acidic pH, unlike other fluoroquinolones, susceptibility tests were performed at standard pH (pH 7.2) and acidic pH (pH 5.8).

Quality control (QC)
QC testing was performed each day of testing as specified by CLSI using *P. aeruginosa* ATCC 27853, *S. aureus* ATCC 29213, *E. coli* ATCC 25922, *E. faecalis* ATCC 29212, *S. pneumoniae* ATCC 49619, and *H. influenzae* ATCC 49247.

Data Handling and Analysis
The mean MIC (arithmetic), median (mcg/ml), and MIC_{90} (mcg/ml) were determined for all antimicrobial agents tested.

Results

Table 1. Comparison of finafloxacin MIC_{90}s and median MICs against gram-positive bacteria.

	MIC_{90} (mcg/ml)			
	E.faecalis (25)	*E.faecium* (25)	*S.aureus* (101)	*S. epidermidis* (100)
pH 7.2	>32	>32	8	>32
pH 5.8	32	>32	4	16

	S. agalactiae (24)	*S. pneumoniae* (100)	*S.pyogenes* (100)	Viridans group streptococci (25)
pH 7.2	1	1	1	4
pH 5.8	0.25	0.5	0.25	1

	Median MIC(mcg/ml)			
	E.faecalis (25)	*E.faecium* (25)	*S.aureus* (101)	*S. epidermidis* (100)
pH 7.2	2	>32	0.12	0.12
pH 5.8	1	32	0.06	0.06

	S. agalactiae (24)	*S. pneumoniae* (100)	*S.pyogenes* (100)	Viridans group streptococci (25)
pH 7.2	0.5	1	0.5	1
pH 5.8	0.12	0.25	0.12	0.5

Table 2. Comparison of finafloxacin MIC_{90}s and median MICs against gram-negative bacteria.

	MIC_{90} (mcg/ml)					
	A. baumannii (25)	*C. freundii* (24)	*E. coli* (99)	*H. influenzae* (100)	*K. pneumoniae* (102)	*M. catarrhalis* (26)
pH 7.2	>32	>32	>32	0.03	>32	0.03
pH 5.8	32	8	16	0.008	16	≤0.015

	M. morganii (25)	*P. mirabilis* (25)	*Providencia spp.* (24)	*P. aeruginosa* (100)	*S. marcescens* (25)
pH 7.2	>32	>32	>32	>32	8
pH 5.8	16	32	32	32	1

	Median (mcg/ml)					
	A. baumannii (25)	*C. freundii* (24)	*E. coli* (99)	*H. influenzae* (100)	*K. pneumoniae* (102)	*M. catarrhalis* (26)
pH 7.2	8	0.25	0.12	0.008	0.25	0.03
pH 5.8	1	0.06	0.03	≤0.004	0.06	≤0.015

	M. morganii (25)	*P. mirabilis* (25)	*Providencia spp.* (24)	*P. aeruginosa* (100)	*S. marcescens* (25)
pH 7.2	1	1	4	4	2
pH 5.8	0.25	0.25	0.5	0.5	0.5

Figure 1. Activity of finafloxacin and moxifloxacin against streptococci: MIC_{90} at standard pH (7.2) and acidic pH (5.8).

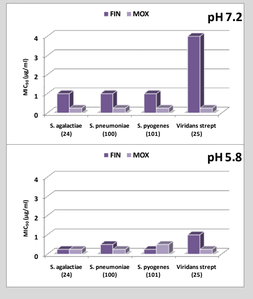


Figure 2. Activity of finafloxacin and moxifloxacin against streptococci: Mean MIC at standard pH (7.2) and acidic pH (5.8).

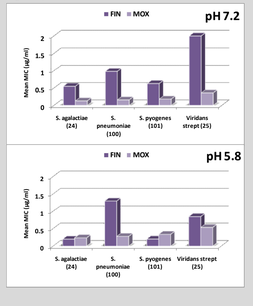


Figure 3. Activity of finafloxacin and moxifloxacin against *H. influenzae* and *M. catarrhalis*: MIC_{90} at standard pH (7.2) and acidic pH (5.8).

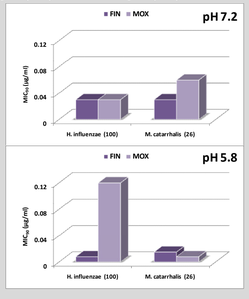
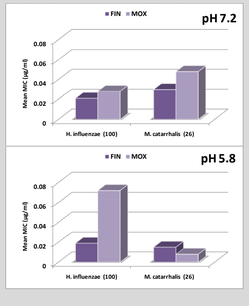


Figure 4. Activity of finafloxacin and moxifloxacin against *H. influenzae* and *M. catarrhalis*: Mean MIC at standard pH (7.2) and acidic pH (5.8).



Conclusions

- Finafloxacin was active against all gram-positive and gram-negative species in the current study and was generally more active under acidic conditions as compared with standard pH (pH 7.2).
- Against gram-positive organisms, finafloxacin was most active against the streptococci with MIC_{90}s ranging from 1 – 4 mcg/ml (standard pH) to 0.25 – 1 mcg/ml (pH 5.8). Activity against staphylococci was generally limited. Certain *E.faecium* strains were susceptible to FIN (Min MIC = 0.5 at pH 5.8), however the majority of strains in this study demonstrated poor susceptibility (Median MIC = 32 at pH 5.8)
  Against gram-negative organisms, finafloxacin was most active against *H. influenzae* and *M. catarrhalis* with MIC_{90}s for both of 0.03 mcg/ml at standard pH and MIC_{90}s one to two dilutions lower at pH 5.8. Activity against *Enterobacteriaceae* and non-fermenters was limited.
- Finafloxacin is currently under clinical development for the treatment of systemic infections and local infections for which pharmacokinetic / pharmacodynamic data will help to establish the optimal target pathogens per each clinical indication.

51th ICAAC
Chicago 2011

E-1835

Investigation of *In Vitro* Antagonistic and Synergistic Effects of Finafloxacin in Combination with Other Antibiotics

C. Y. GOH[1], S. B. NG[1], M. EVERETT[1] and A. VENTE[2]

[1]MerLion Pharmaceuticals, Singapore [2]MerLion Pharmaceuticals, Berlin, Germany

Contact information:

Andreas Vente
Merlion Pharmaceuticals GmbH
Robert-Rossle-Strasse 10
13125 Berlin, Germany
Phone : +49 30 9489 4050
Fax: +49 30 9489 4051
E-mail: **vente@merlionpharma.de**

Revised Abstract

Background:
Finafloxacin (FIN) is a novel member of the fluoroquinolone class of antibiotics. Specifically, FIN belongs to a new 8-cyano subclass that contains a novel base component which confers improved antibacterial activity under acidic conditions. Combination antibiotic therapy has been one of the options considered when dealing with multi-drug resistant bacteria infection. The aim of this study was to identify potential antagonistic or synergistic interactions between FIN and other commonly used antibiotics.

Methods:
Chequerboard experiments were performed at pH 5.8 and 7.2 under otherwise standard CLSI conditions using FIN in combination with meropenem (MER), ceftazidime (CTZ), amikacin (AMK) or colistin (COL) against *P. aeruginosa* ATCC 27853 and tigecycline (TIG), vancomycin (VAN), daptomycin (DAP) or linezolid (LZD), against MRSA ATCC 33591. Combinations were scored on the basis of the fractional inhibitory concentration index (FICI). FIN in combination with AMK was also tested against three fluoroquinolone-resistant *E.coli* UTI isolates and one susceptible strain (ATCC 25922). Time-kill studies were performed at synergistic drug combinations observed in the chequerboard experiment, and also at combinations of sub-MIC concentrations.

Results:
None of the drug combination exhibited antagonism. Most antibiotic combinations showed partial synergistic or additive effects (FICI: > 0.5 ≤1) under both acidic and neutral conditions, except FIN-MER and FIN-AMK which showed synergy (FICI: ≤ 0.5) at pH 7.2. Synergy was also observed for FIN-AMK against one UTI isolate at pH 5.8. Time-kill studies confirmed that the synergistic effects were bactericidal rather than bacteriostatic. Higher drug concentrations were required to attain synergistic bactericidal effect.

Introduction

Finafloxacin (FIN) is a novel member of the fluoroquinolone class of antibiotics. Specifically, FIN belongs to a new 8-cyano subclass. The agent contains a novel base component which confers improved antibacterial activity under acidic conditions[1], where the activity of many existing fluoroquinolones is impaired.

Combination antibiotic therapy has been one of the options considered when dealing with multi-drug resistant bacteria infection. However, there is a possibility of antagonism arising from the combination of certain antibiotics. Therefore, it is important to ensure that the use of FIN in combination therapy does not cause antagonistic effects.

The aim of this study was to identify potential antagonistic or synergistic interactions between FIN and other commonly used antibiotics.

[1]W. Stubbings *et. al*., 2011. In vitro spectrum of activity of finafloxacin, a novel, pH-activated fluoroquinolone, under standard and acidic conditions. Antimicrob. Agents Chemother. 55: 4394-4397.

[2]RL. White *et. al*., 1996. Comparison of three different in vitro methods of detecting synergy: time-kill, chequerboard, and E test. Antimicrob. Agents Chemother. 40: 1914-1918.

Results

In the chequerboard experiments most antibiotic combinations showed additive effect (FICI: > 0.5 ≤1) under both acidic and neutral conditions. When tested against *P. aeruginosa* ATCC 27853 and *E. coli* UTI clinical isolate FIN-AMK showed synergy (FICI: ≤ 0.5) at pH 7.2 and pH 5.8, respectively. Borderline synergy (FICI = 0.5) was observed for FIN-MER against *P. aeruginosa* at pH 7.2 for two out of three experiments.

In the time-kill studies no synergy was observed for either FIN-AMK or FIN-MER at the chequerboard synergistic drug concentrations. However, FIN-AMK showed enhanced bactericidal effects at early time points when it was used at a higher drug concentration of 0.5x MIC, as compared to the single most active agent. The enhanced effect was observed against both *P. aeruginosa* ATCC 27853 and *E. coli* UTI clinical isolate. Increasing the drug concentrations for FIN-MER to 0.5x MIC showed slight improvement in the bactericidal effect.

Table 1. Mean FICI of FIN-AB combination under both acidic and neutral conditions.

Antibiotic	Organism	AB MIC*	FIN MIC*	Mean FICI**	
		ph 5.8/7.2	pH 5.8/7.2	pH 5.8	pH 7.2
Meropenem	*P. aeruginosa* ATCC 27853	0.5/0.5	0.5/4	0.63	0.54
Amikacin	*P. aeruginosa* ATCC 27853	32/2	0.5/4	0.57	**0.50**
Colisitin (Pol E)	*P. aeruginosa* ATCC 27853	0.5/0.5	0.5/4	0.58	0.74
Ceftazidime	*P. aeruginosa* ATCC 27853	4/2	0.5/4	0.67	0.60
Daptomycin	*MRSA ATCC 33591*	1/0.5	0.06/0.13	0.59	0.63
Linezolid	*MRSA ATCC 33591*	1/1	0.06/0.13	1.00	0.63
Amikacin	UTI *E. coli 003/3*	32/4	16/64	0.53	0.83
Amikacin	UTI *E. coli 005/5*	32/8	4/16	0.54	0.71
Amikacin	UTI *E. coli 008/4*	16/2	16/128	**0.34**	0.55
Amikacin	*E. coli* ATCC 25922	16/4	0.0078/0.031	**0.40**	0.71

*MIC (mg/L) **Mean FICI (n = 3) of Best FIN-AB combination Synergy = 0.5 (bold)

Figure 1. Synergy time-kill of FIN-AMK against *E. coli* urinary tract infection (UTI) clinical isolate.

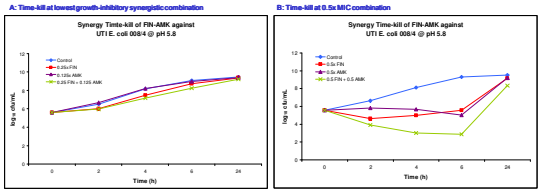
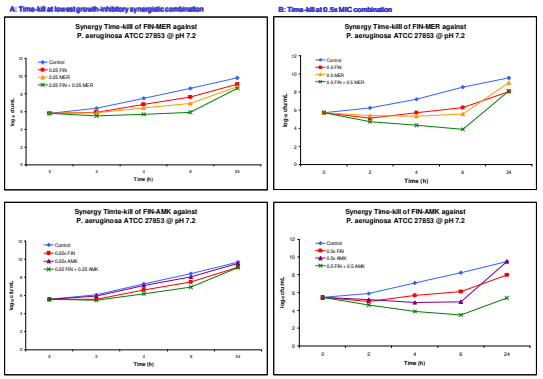


Figure 2. Synergy time-kill of FIN-MER & FIN-AMK against *P. aeruginosa* ATCC 27853.



Methods

Chequerboard synergy test
Chequerboard experiments were performed at pH 5.8 and 7.2 under otherwise standard CLSI conditions using FIN in combination with meropenem (MER), ceftazidime (CTZ), amikacin (AMK) or colistin (COL) against *P. aeruginosa* ATCC 27853 and tigecycline (TIG), vancomycin (VAN), daptomycin (DAP) or linezolid (LZD), against MRSA ATCC 33591. Combinations were scored on the basis of the fractional inhibitory concentration index (FICI). FIN in combination with AMK was also tested against three fluoroquinolone-resistant *E. coli* UTI isolates and one susceptible strain (ATCC 25922).

Time-kill studies
Kill kinetics of AMK, MER and FIN were determined by incubating an inoculum of ~5 x 10^5 cfu/mL of either *P. aeruginosa* or *E. coli* with the drug concentrations that showed synergy in the checkerboard experiments, and at 1 dilution below the MICs (0.5x MIC).

Synergy was defined as a ≥ 2 $\log_{10}$ decrease in colony count by the drug combination compared to the most active single agent. Indifference was defined as < 2 $\log_{10}$ increase or decrease in colony count; antagonism was defined as ≥ 2 $\log_{10}$ increase in colony count by the drug combination compared to the most active single agent[2].

Conclusions

- **No antagonism was observed when FIN was used in combination with the antibiotics tested**
- **FIN-AMK & FIN-MER showed synergistic bacteriostatic effects**
- **Both combinations at 0.5x MIC showed enhanced bactericidal effects at early time points, compared to the individual drugs at the same concentration**
- **Further studies at higher concentrations are warranted**