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In Vitro Spectrum of Activity of Finafloxacin, a Novel, pH-Activated Fluoroquinolone, under Standard and Acidic Conditions▽

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Finafloxacin is a novel fluoroquinolone that exhibits enhanced antibacterial activity under acidic conditions. The aim of this study was to define the *in vitro* pH-activity relationship. Finafloxacin exhibited optimal antibacterial activity between pH 5.0 and 6.0 at which MICs were 4- to 8-fold lower than those determined at neutral pH. These observations were then confirmed against a larger collection of bacteria. These data suggest that finafloxacin could potentially offer a therapeutic advantage within acidic foci of infection.

Fluoroquinolones are a widely utilized class of antibacterial agent. However, a number of attempts to develop new, more potent, members of this class have failed due to concerns over safety and tolerability that have resulted in a halt to development, withdrawal from the market, or restriction of the market (12). Finafloxacin is a new fluoroquinolone belonging to a novel 8-cyano subclass that exhibited a low potential for toxicity or tolerability issues in preclinical tests (14) and in later clinical trials (13). Finafloxacin was also highly effective when tested in *in vivo* infection models, perhaps more so than would have been predicted from its *in vitro* MIC (7, 8). This effect was attributed, at least in part, to the enhancement of finafloxacin activity at slightly acidic pH (5, 6, 9), which is a distinctive property of finafloxacin in contrast to other marketed fluoro-

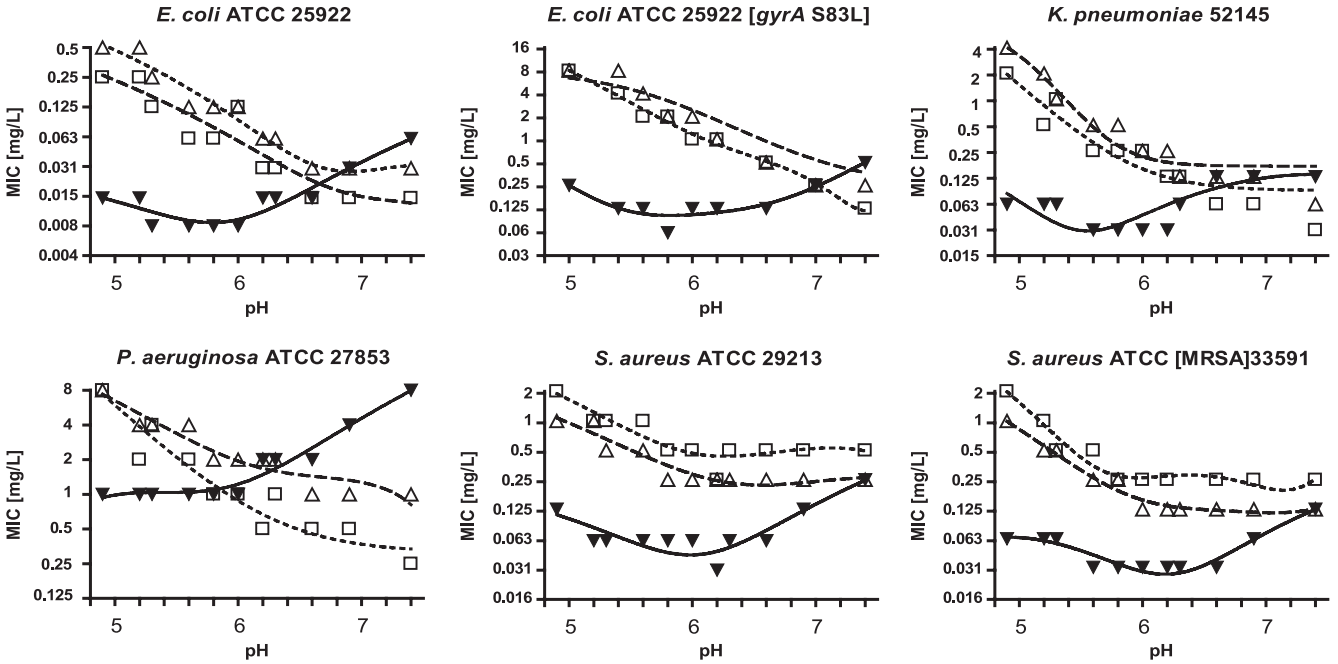

FIG. 1. MICs of finafloxacin, ciprofloxacin, and levofloxacin at pH values of 7.4 and below. Key: ▼, finafloxacin; □, ciprofloxacin; △, levofloxacin.

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TABLE 1. *In vitro* susceptibility to finafloxacin and other fluoroquinolones under standard testing conditions and at slightly acidic pH

Organism	Susceptibility to ciprofloxacin[a]	No. of isolates tested	Antibiotic	MIC (mg/liter) at:[b]					
				pH 7.2–7.4			pH 5.8–6.2		
				Range	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}
Community-associated MRSA	Susceptible	33	Finafloxacin	0.125–0.25	0.125	0.25	0.06–0.125	0.06	0.125
			Ciprofloxacin	0.25–1	0.5	1	1–4	1	4
			Moxifloxacin	0.03–0.125	0.06	0.125	0.25–0.5	0.25	0.5
Staphylococcus aureus	Resistant	30	Finafloxacin	0.25–32	2	16	0.25–32	1	4
			Ciprofloxacin	4–>64	32	>64	16–>64	32	>64
			Levofloxacin	4–>64	2	32	4–>64	16	>64
			Moxifloxacin	2–32	2	32	0.5–64	8	32
Coagulase-negative staphylococcus	Susceptible	26	Finafloxacin	0.015–0.5	0.25	0.5	0.008–1	0.06	0.125
			Ciprofloxacin	0.06–1	0.25	0.5	0.125–4	0.5	1
	Resistant	16	Finafloxacin	0.06–>16	8	16	0.125–16	1	16
			Ciprofloxacin	2–>16	>16	>16	8–>16	>16	>16
Streptococcus pneumoniae	Mixed	21	Finafloxacin	0.5–4	1	2	ND	ND	ND
			Ciprofloxacin	1–4	2	4	ND	ND	ND
			Levofloxacin	0.5–2	1	2	ND	ND	ND
			Moxifloxacin	0.125–0.5	0.5	1	ND	ND	ND
Streptococcus pyogenes	Susceptible	22	Finafloxacin	0.25–1	0.5	0.5	0.125–0.5	0.25	0.25
			Ciprofloxacin	0.125–1	0.5	1	0.25–2	1	2
			Moxifloxacin	0.125–0.5	0.25	0.25	0.25–1	0.5	0.5
Streptococcus agalactiae	Mixed	11	Finafloxacin	0.5–4	1	2	0.125–4	0.25	0.5
			Ciprofloxacin	0.5–4	1	2	0.5–4	1	2
Enterococcus faecalis	Mixed	10	Finafloxacin	0.5–32	1	32	0.25–16	0.5	16
			Ciprofloxacin	0.5–128	1	64	2–>256	4	>256
Enterococcus faecium	Mixed	9	Finafloxacin	1–128	NC	NC	0.5–32	NC	NC
			Ciprofloxacin	1–256	NC	NC	2–>256	NC	NC
Escherichia coli	Resistant	75	Finafloxacin	16–>256	128	256	2–64	8	32
			Ciprofloxacin	8–>256	128	>256	>256	>256	>256
			Levofloxacin	8–128	32	64	32–>256	256	>256
	Susceptible	12	Finafloxacin	0.03–1	0.125	0.25	≤0.008–0.125	0.016	0.03
			Ciprofloxacin	≤0.008–0.125	0.016	0.03	0.06–2	0.125	0.25
Klebsiella spp.	Susceptible	16	Finafloxacin	0.06–4	0.25	2	0.008–1	0.06	0.5
			Ciprofloxacin	0.016–1	0.03	0.5	0.125–8	0.5	8
	Resistant	7	Finafloxacin	2–>32	NC	NC	0.5–>32	NC	NC
			Ciprofloxacin	2–>16	NC	NC	8–>16	NC	NC
Salmonella spp.	Mixed	8	Finafloxacin	0.5–16	NC	NC	0.06–4	NC	NC
			Ciprofloxacin	0.125–32	NC	NC	1–>32	NC	NC
			Levofloxacin	0.25–16	NC	NC	0.5–>32	NC	NC
			Moxifloxacin	0.25–16	NC	NC	1–>32	NC	NC
Proteus mirabilis	Mixed	10	Finafloxacin	0.5–>32	1	16	0.06–8	0.25	4
			Ciprofloxacin	≤0.008–1	0.016	1	0.06–>16	0.125	8
Providencia spp.	Mixed	11	Finafloxacin	0.06–16	8	16	≤0.03–8	1	8
			Ciprofloxacin	≤0.03–16	1	4	0.125–>16	16	>16
			Levofloxacin	≤0.03–16	1	2	0.06–>16	8	>16
			Moxifloxacin	≤0.03–16	0.5	2	0.125–>16	8	>16
Enterobacter spp.	Susceptible	10	Finafloxacin	0.06–0.5	0.125	0.125	≤0.03–0.125	≤0.03	≤0.03
			Ciprofloxacin	≤0.03	≤0.03	≤0.03	0.06–0.5	0.125	0.25
			Levofloxacin	≤0.03–0.06	≤0.03	0.06	0.125–0.5	0.25	0.5
			Moxifloxacin	≤0.03–0.25	≤0.03	0.06	0.125–2	0.25	0.5

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TABLE 1—*Continued*

Organism	Susceptibility to ciprofloxacin[a]	No. of isolates tested	Antibiotic	MIC (mg/liter) at:[b]					
				pH 7.2–7.4			pH 5.8–6.2		
				Range	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}
Morganella morganii	Mixed	11	Finafloxacin	0.125–16	1	16	0.03–16	0.25	4
			Ciprofloxacin	≤0.008–>16	0.008	4	0.016–>16	0.06	>16
Serratia marcescens	Susceptible	12	Finafloxacin	0.125–8	1	8	0.06–4	0.25	2
			Ciprofloxacin	≤0.03–1	≤0.03	1	0.125–>16	1	16
			Levofloxacin	≤0.03–4	0.125	1	0.25–>16	1	16
			Moxifloxacin	0.06–4	0.125	2	1–>16	4	>16
Stenotrophomonas maltophilia	Mixed	19	Finafloxacin	0.5–32	2	4	0.125–16	0.5	1
			Ciprofloxacin	0.5–64	4	8	2–256	8	64
Pseudomonas aeruginosa	Susceptible	22	Finafloxacin	1–32	4	16	0.25–8	0.5	2
			Ciprofloxacin	0.03–1	0.25	0.5	0.125–2	0.5	1
	Resistant	9	Finafloxacin	>32	NC	NC	16–>32	NC	NC
			Ciprofloxacin	8–32	NC	NC	8–>32	NC	NC
Haemophilus influenzae	Susceptible	35	Finafloxacin	≤0.004–0.06	0.008	0.03	ND	ND	ND
			Ciprofloxacin	0.008–0.03	0.008	0.016	ND	ND	ND
			Levofloxacin	0.008–0.03	0.016	0.03	ND	ND	ND
			Moxifloxacin	≤0.004–0.125	0.016	0.06	ND	ND	ND
Neisseria gonorrhoeae	Mixed	10	Finafloxacin	≤0.03–0.25	0.06	0.125	≤0.03–0.125	0.06	0.06
			Ciprofloxacin	≤0.03–0.125	0.06	0.06	ND	ND	ND
			Levofloxacin	0.06–0.25	0.06	0.25	0.06–0.125	0.06	0.125
			Moxifloxacin	≤0.03–0.125	0.06	0.06	ND	ND	ND

[a] According to the CLSI susceptibility breakpoint for ciprofloxacin (3). Mixed, susceptible and resistant.
[b] ND; not determined (usually strains grew poorly at low pH); NC, MIC_{50} and MIC_{90} were not calculated for groups of less than 10 strains.

quinolones, which generally lose activity at pH below neutral (4, 15). The present study had two aims. First, the pH-antibacterial activity relationship for finafloxacin was investigated over a range of pH 4.8 to 7.4 in order to better define the optimal pH range for activity. Second, the activity of finafloxacin was investigated against strains from several bacterial collections, comprising 445 isolates belonging to 19 species, to determine the reproducibility of the pH effect across different species of pathogenic bacteria and to provide an initial description of the spectrum of finafloxacin activity.

(Part of the research reported in this paper was presented in a poster session at the 48th Annual Interscience Conference on Antimicrobial Agents and Chemotherapy-Infectious Diseases Society of America 46th Annual Meeting, Washington, DC, 25 to 28 October 2008 [11]).

Finafloxacin (manufactured by MerLion Pharmaceuticals GmbH, Berlin, Germany, or by Bayer HealthCare AG [now Bayer-Schering AG], Elberfeld, Germany) and ciprofloxacin, levofloxacin, and moxifloxacin (Sigma-Aldrich, Republic of Singapore, or Bayer HealthCare AG, Leverkusen, Germany) were tested against strains from the culture collections of MerLion Pharmaceuticals and their research partners. Susceptibility testing was performed according to the CLSI protocol for broth microdilution (2). The pH of broth was adjusted by the addition of hydrochloric acid prior to autoclaving and was remeasured afterwards.

The MICs of finafloxacin, ciprofloxacin, and levofloxacin were determined against six reference strains (including an *in vitro* selected mutant of *Escherichia coli* ATCC 25922 with reduced susceptibility to finafloxacin) at pH values ranging from pH 4.8 to 7.4 (Fig. 1). Data were plotted in GraphPad Prism, version 4, software (La Jolla, CA), and trend lines were drawn with the nonlinear regression (polynomial) tool. Finafloxacin exhibited a 4- to 8-fold increase in activity (denoted by a 4- to 8-fold lowering of the MIC) at pH 6.0 compared to activity at pH 7.4. The pH range in which finafloxacin exhibited optimal activity was pH 5.0 to 6.0. Conversely, the activities of both ciprofloxacin and levofloxacin decreased at increasingly acidic pH. Both exhibited a 2- to 8-fold decrease in activity at pH 6.0, compared to activity at pH 7.4, and a further 8-fold decrease at pH 5.0 compared to activity at pH 6.0. These contrasting pH-dependent effects on the antibacterial activities of the different fluoroquinolones had the net result that finafloxacin exhibited MICs at pH 5.0 to 6.0 that were 8- to 16-fold lower than those of ciprofloxacin or levofloxacin against *E. coli* ATCC 25922, *E. coli* 25922 (*gyrA* S83L) (an *in vitro* selected mutant exhibiting reduced susceptibility to fluoroquinolones) and *Klebsiella pneumoniae* 52145 and 4- to 8-fold lower against *Staphylococcus aureus* ATCC 29213 and *S. aureus* ATCC 33591 (methicillin-resistant *S. aureus* [MRSA]). Finafloxacin MICs against *Pseudomonas aeruginosa* ATCC 27853 were 2- to 4-fold lower than those of ciprofloxacin or levofloxacin at pH 5.0 to 6.0.

The activity of finafloxacin was also determined against a panel of 19 bacterial species under both the standard susceptibility testing conditions (pH 7.2 to 7.4) (2) and slightly acidic pH (pH 5.8 to 6.2) (Table 1). This acidic pH range was chosen for this study to represent a slightly acidic pH that would be found in a range of indications including respiratory, intra-abdominal, urinary tract, and skin and soft tissue infection (1).

Finafloxacin activity was increased at the slightly acidic pH, compared to activity at pH 7.2 to 7.4, with the magnitude of this increase differing between species but consistent among strains of the same species. Conversely, at pH 5.8 to 6.2, ciprofloxacin, levofloxacin, and moxifloxacin MICs were generally 2- to 8-fold higher than those determined at pH 7.2 to 7.4.

These preliminary *in vitro* findings suggest that finafloxacin may have an advantage over other fluoroquinolones in terms of potency within acidic foci of infection. Bacteria infect body sites, including those which are typically at pH values below neutral, e.g., the urinary tract, skin, or respiratory epithelia (1). The environmental conditions and pH experienced by the invading bacteria could be further diversified if the bacteria were localized within, e.g., host phagocytotic cells or inflammatory compartments such as abscesses. The host immune response may also play a role in lowering the pH, e.g., during infections that result in chronic inflammation as experienced during airway infections of cystic fibrosis (CF) or chronic obstructive pulmonary disease (COPD) patients (10, 16). The relevance of this pH-dependent activity needs to be further explored using *in vivo* infection models to determine the pharmacokinetic/pharmacodynamic drivers of activity and the contribution of infection site pH to these. Furthermore, clinical trials to demonstrate the pharmacokinetics and efficacy of finafloxacin during treatment of bacterial infections, especially those within acidic foci, will be required in order to understand the clinical relevance of this effect.

This paper is dedicated to Harald Labischinski, who died on 24 August 2010. Harald was a valued colleague and advisor on the finafloxacin project and is missed by all who worked with him.

W.S., P.L., F.G., and G.C.Y. are all current or previous employees of MerLion Pharmaceuticals GmbH or MerLion Pharmaceuticals Pte Ltd.

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