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Effect of pH on the *In Vitro* Activity of Finafloxacin against Gram-negative and Gram-positive Bacteria

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Revised Abstract

Background: Finafloxacin (FIN) is a novel 8-cyano-fluoroquinolone, that exhibits an *in vitro* spectrum of activity similar to that of ciprofloxacin (CIP). The present study was performed to study the effect of pH on the *in vitro* activity of FIN in comparison to CIP against selected strains of various aerobic Gram- and Gram+ bacterial species known to cause genito-urinary tract infections.

Methods: The susceptibilities of 100 clinical isolates to FIN and CIP were tested at pH 5, 6, 7.3, and 8. There were 22 *Escherichia coli* (ECO), 13 *Klebsiella pneumoniae* (KPN), 11 *Morganella morganii* (MOM), 10 *Proteus mirabilis* (PRM), 10 *Pseudomonas aeruginosa* (PSA), 12 *Staphylococcus aureus* (SAU), 11 *Staphylococcus saprophyticus* (SSA), and 11 *Streptococcus agalactiae* (SAG). Of these, 66 were sensitive and 34 exhibited reduced susceptibilities to CIP. MICs were determined using the CLSI broth microdilution method.

Results: Results are presented in Table 1.

Conclusions: Overall, FIN demonstrated superior activity to CIP under acidic conditions against isolates of all species including resistant strains. Furthermore, FIN showed comparable activity to CIP against Gram+ cocci at pH 7.3. Hence, FIN appears to be a promising new antimicrobial agent for the treatment of infections at acidic sites.

Methods

Bacterial strains: A total of 100 clinical isolates predominantly collected from 15 microbiology laboratories during a multi-centre study conducted between April and August 2005 in Germany were tested: *Escherichia coli* (n=22), *Klebsiella pneumoniae* (n=13), *Proteus mirabilis* (n=10), *Morganella morganii* (n=11), *Pseudomonas aeruginosa* (n=10), methicillin-susceptible *Staphylococcus aureus* (MSSA, n=6), methicillin-resistant *S. aureus* (MRSA, n=6), *Staphylococcus saprophyticus* (n=11), and *Streptococcus agalactiae* (n=11). Of these, 66 were susceptible to CIP (CIP-S) and 34 were either intermediate or resistant to CIP (non-susceptible, CIP-NS) according to the interpretive criteria defined by EUCAST [5].

Antibacterial agents: FIN (batch no. CBC000288; potency 84.5%) and CIP (batch no. CBC000290; potency 84.8%) were provided by MerLion Pharmaceuticals.

Susceptibility testing: The CLSI broth microdilution procedure with geometric twofold serial dilutions in cation-adjusted Mueller-Hinton broth (CAMHB) purchased from Becton Dickinson (Heidelberg, Germany; BBL™ Cation Adjusted Mueller Hinton II Broth, lot no. 6317238) was used to determine MICs [6]. Each strain was tested at the following pH values: 5.0, 6.0, 7.3 (standard), and 8.0. The pH was adjusted by adding drop by drop 1N HCl or 1N NaOH to the test medium. The pH was checked before autoclaving, after autoclaving and after addition of each antimicrobial agent.

Introduction

Finafloxacin (FIN, Figure 1) is a novel, broad spectrum fluoroquinolone (FQ) that belongs to a new 8-cyano subclass. FIN contains a novel base component which confers improved antibacterial activity at slightly acidic pH (pH 5.0 – 6.0) under which other FQs exhibit significantly reduced activity [1].

In addition, FIN exhibited superior activity to comparator FQs against adherent bacteria, *in vitro*, that was especially notable at low pH [2]. FIN also exhibited superior activity in rodent infection models [3,4] which involved inflammation, abscess formation or other acidic foci of infection.

The present study was performed to study the effect of the pH on the *in vitro* activity of FIN and CIP against 100 clinical isolates of various aerobic Gram-positive and Gram-negative bacterial species known to cause genito-urinary tract infections.

Results and Discussion

Results are presented in Table 1. Overall, FIN exhibited the highest *in vitro* activity at acidic conditions, while CIP was most active at pH 7.3 or 8.0. MICs of FIN and CIP against CIP-S *E. coli* are also illustrated in Figure 2 to demonstrate the opposing effect of pH on the inhibitory activity of these FQs.

***Enterobacteriaceae*:** Median MICs of FIN for CIP-S isolates of *E. coli*, *K. pneumoniae*, *P. mirabilis* and *M. morganii* were ≤0.25 mg/L each at pH 5.0 and pH 6.0, 0.125-1 mg/L at pH 7.3 and 0.25-2 mg/L at pH 8.0. FIN was more active than CIP against CIP-S isolates of all species at pH 5.0, while it was less active than CIP at pH 7.3 and 8.0. At pH 6.0, FIN showed superior activity to CIP against *E. coli* and *K. pneumoniae* and comparable activity to CIP against *P. mirabilis*, but was 4-fold less active than CIP against *M. morganii*. Similar differences were found for CIP-NS isolates.

***P. aeruginosa*:** Based on median MICs, FIN showed comparable activity to CIP at acidic pH, but was less active than CIP at pH 7.3 and 8.0, respectively.

***S. aureus*:** At pH 5.0 and 6.0, median MICs of FIN for CIP-S isolates (0.125 mg/L and 0.031 mg/L) were four dilution steps lower than those of CIP. Moreover, FIN was 2-fold more active than CIP at pH 7.3 and 8.0. This trend was also observed for CIP-NS isolates.

***S. saprophyticus*:** Based on median MICs, FIN showed three and two dilution steps higher activity than CIP at pH values of 5.0 and 6.0, respectively, while it was one dilution step less active than CIP at pH 7.3 and 8.0.

***S. agalactiae*:** FIN demonstrated superior activity to CIP, at pH 5.0 and 6.0 and showed equal activity at pH 7.3, while it was one dilution step less active at pH 8.0.

Results and Discussion

Table 1: MICs of 66 Gram-negative and 34 Gram-positive organisms

Species	Pheno-type	No. of strains	Para-meter	pH 5.0		pH 6.0		pH 7.3		pH 8.0	
				FIN	CIP	FIN	CIP	FIN	CIP	FIN	CIP
Escherichia coli	CIP-S	12	Median	0.031	0.5	0.016	0.125	0.125	0.016	0.25	≤0.008
			Range	0.016-0.25	0.25-8	≤0.008-0.125	0.063-2	0.031-1	≤0.008-0.125	0.031-2	≤0.008-0.125
	CIP-NS	10	Median	8	≥16	8	≥16	≥32	≥16	≥32	≥16
			Range	4->16	≥16	4->16	≥16	≥32	≥16	≥32	8->16
Klebsiella pneumoniae	CIP-S	5	Median	0.063	1	0.031	0.25	0.125	0.031	0.5	≤0.016
			Range	0.062-0.125	0.5-2	0.016-0.063	0.125-0.5	0.063-0.25	0.031	0.25-0.5	≤0.016
	CIP-NS	8	Median	2	>16	1.5	>16	6	3	12	1
			Range	0.5->32	>16	0.25->32	8->16	0.5->32	1->16	1->32	0.25->16
Proteus mirabilis	CIP-S	7	Median	0.188	1	0.25	0.25	1	0.031	1.5	≤0.031
			Range	0.063-0.25	0.25-1	0.063-0.25	0.063-0.25	0.25-1	0.016-0.031	0.5-2	≤0.008-0.031
	CIP-NS	3	Median	4	>16	4	8	16	2	16	1
			Range	4	8->16	4-8	8->16	16->32	1-2	16->32	0.5-1
Morganella morganii	CIP-S	9	Median	0.25	1	0.25	0.063	0.5	≤0.008	2	≤0.008
			Range	0.125-0.5	0.25-1	0.031-0.25	0.016-0.125	0.125-1	≤0.008-0.063	0.5-2	≤0.008
	CIP-NS	2	Median	-	-	-	-	-	-	-	-
			Range	4->32	≥16	4-16	≥16	16	4-16	16->32	2-16
Pseudomonas aeruginosa	CIP-S	5	Median	1	1	0.5	0.5	4	0.125	8	0.063
			Range	0.25-2	0.125-2	0.25-2	0.125-1	2-16	0.063-0.5	2-16	0.031-0.5
	CIP-NS	5	Median	8	>16	8	>16	>32	>16	>32	8
			Range	4->32	4->16	4->32	2->16	>32	1->16	>32	0.5->16
Staphylococcus aureus	CIP-S	7	Median	0.125	2	0.031	0.5	0.25	0.5	0.25	0.5
			Range	0.031-0.125	0.5-8	0.031-0.125	0.25-2	0.125-0.25	0.25-1	0.25-16	0.25-4
	CIP-NS	5	Median	8	>16	2	>16	8	>16	>32	16
			Range	0.5-8	>16	0.125-4	>16	1-16	>16	2->32	8->16
Staphylococcus saprophyticus	CIP-S	11	Median	0.063	0.5	0.125	0.5	0.5	0.25	1	0.5
			Range	≤0.008-0.125	≤0.008-1	0.125	0.25-1	0.25-0.5	0.25-0.5	1	0.25-0.5
	CIP-S*	10	Median	0.25	2	0.25	1	1	1	2	1
			Range	0.125-0.5	2-4	0.125-0.5	0.5-2	0.5-2	0.5-2	1-4	0.5-2
Streptococcus agalactiae	CIP-NS*	1	Median	-	-	-	-	-	-	-	-
			Range	2	16	4	4	4	4	8	4

Abbreviations: CIP-S, ciprofloxacin-susceptible; CIP-NS, non-susceptible to ciprofloxacin; *Epidemiological cut off value defined by EUCAST

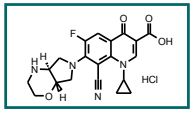

Figure 1. Finafloxacin hydrochloride.

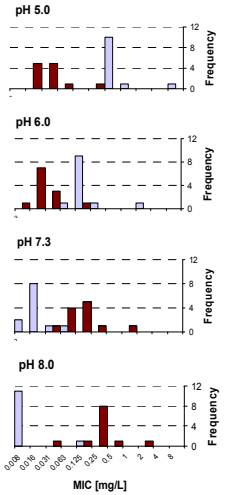

Figure 2. MIC distribution of FIN ( )and CIP ( ) at pH 5.0, 6.0, 7.3 and 8.0 against CIP-S *E. coli* (n = 12).

Conclusions

- FIN demonstrated superior activity to CIP under acidic conditions against isolates of all species, except *P. aeruginosa* for which both drugs showed similar potency under these conditions.
- FIN appears to be a promising new antimicrobial agent for the treatment of infections in acidic environments.

Literature

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