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Comparative Inhibitory and Bactericidal Activities of Finafloxacin and Ciprofloxacin against Gram-Negative and Gram-Positive UTI-pathogens under Physiological Conditions and at Varying pH-values

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Revised Abstract

Background: FIN is a novel fluoroquinolone (FQ) belonging to a new 8-cyano subclass which exhibits improved *in vitro* activity at slightly acidic pH and is therefore intended for treatment of UTI. The antibacterial and bactericidal activities of FIN and CIP were compared in artificial urine medium which reflects the physiological conditions of pH, ionic strength and chemical composition, encountered *in vivo*.

Methods: The MICs of FIN and CIP were determined against 34 strains (*S. aureus, S. saprophyticus,* Enterobacteriaceae, *P. aeruginosa,* incl. CIP-res and ESBL producers) using CLSI methodology in cation adjusted Mueller-Hinton Broth (CAMHB) at pH 7.2 and 5.8 and in artificial urine (pH 5.8). Bactericidal activity was determined against 10 strains exposed to 1 x, 4 x and 16 x MIC. During the initial log-linear phase of CFU-decline, single point kill rates (k= -ln(N/No))/t) were calculated.

Results: FIN MICs were 1 - 3 dilutions lower at pH 5.8 compared to those at pH 7.2, whereas CIP MICs increased by 1 - 3 dilutions at the lower pH. In artificial urine (pH 5.8), FIN exhibited MICs similar to those obtained in CAMHB pH 7.2, whereas CIP MICs increased by 10 - >100-fold. On average, FIN MICs were 4 - 5 dilutions lower than CIP in artificial urine, regardless of Gram type or susceptibility profile. Bactericidal activities of both FIN and CIP (kill-rates normalised to concentration) demonstrate that FIN is about 2- to >20-fold more active than CIP in both media.

Conclusions: The bacteriostatic (MICs) and bactericidal activities (time kill curves) of FIN differ favourably from those of CIP under conditions mimicking UTIs. The activity of FIN in artificial urine is quantitatively and qualitatively different from that of CIP. These findings indicate that FIN may be effective in the treatment of UTIs.

Introduction

Finafloxacin (FIN, Figure 1) is a novel, broad spectrum fluoroquinolone (FQ) belonging to a new 8-cyano subclass [1]. FIN contains a novel chiral base component which confers improved antibacterial activity at slightly acidic pH (pH 5.0 – 6.0). Other marketed FQs have significantly reduced activity over this pH range [2].

FIN exhibited superior activity compared with comparator FQs against adherent bacteria *in vitro* [3] and in a wide range of rodent infection models [4,5]. Additionally, FIN displayed an excellent safety profile in a wide range of predictive, *in vitro,* toxicity assays [6] and was well tolerated in healthy human volunteers [7]. These attributes suggest that FIN warrants clinical investigation for bacterial infections that are associated with low pH such as urinary tract infection and *Helicobacter pylori* eradication.

The antibacterial activity of FIN and ciprofloxacin (CIP) were compared in a medium that mimics, in part, the environment encountered during UTI.

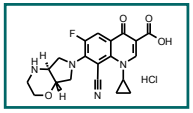


Figure 1. Finafloxacin hydrochloride.

Methods

MIC determinations

MIC testing was performed using a microdilution method according to CLSI (formerly NCCLS) guidelines [8]. MICs were determined in cation adjusted Mueller Hinton broth (CAMHB) at pH 7.2 and pH 5.8 and in artificial urine pH 5.8 [9]. The final inoculum was 5 x 10^4 CFU/mL.
35 strains of Gram-positive and Gram-negative bacteria were tested; these included a number with resistance determinants.

Time-kill experiments

These were performed with the following panel of 10 strains:

- *Enterobacter cloacae* ATCC 13047
- *Enterococcus faecalis* ATCC 29212
- *Escherichia coli* ATCC 25922
- *Escherichia coli* WT-2 (CIP^{BS})
- *Escherichia coli* M1-4 (CIP^{R})
- *Escherichia coli* WT-4-M2-1 (CIP^{R})
- *Proteus mirabilis* ATCC 9240
- *Pseudomonas aeruginosa* ATCC 10145
- *Staphylococcus aureus* ATCC 29213
- *Staphylococcus saprophyticus* ATCC 15305

CIP^{BS}; ciprofloxacin - borderline susceptible, CIP^{R}; ciprofloxacin resistant, as determined under standard MIC test conditions.
The strains were stored frozen at – 80°C in a volume of 100 µL.

Time-Kill curve kinetics

Kill curve kinetics were carried out using a modified CLSI method [10]. FIN and CIP were tested at multiples (x 1, x 4, and x 16) of the MIC value in mg/L against each strain.
Samples were taken at 0h, 1h, 2h, 4h, 6h, 8h and 24 h after incubation. Ten-fold serial dilutions were inoculated onto Mueller-Hinton agar and colonies enumerated following 24 h incubation at 37°C.

Results and Discussion

Effect of pH and medium on activity of FIN and CIP

The MIC values in mg/L of FIN and CIP against the 35 strains tested in CAMHB at pH 7.2 and 5.8 and in artificial urine at pH 5.8 are shown in Figure 2.

FIN MICs were lower at an acidic pH value in CAMHB (pH 5.8) and were also low in artificial urine (pH 5.8), despite the high levels of divalent cations which inactivate most of the commercially available FQs like CIP. In contrast CIP MICs increased strain dependently from 2- to greater than 10-fold in acidic CAMHB and increased >10- to >100-fold in artificial urine (pH 5.8).

Bactericidal effects of FIN and CIP

The bactericidal activity of FIN against two of the strains, at multiples of the MIC, in CAMBH pH 7.2 and artificial urine pH 5.8 are shown in Figure 3 (*E. coli*) and Figure 4 (*P. mirabilis*).

When compared on the basis of MIC (under the prevailing conditions) the bactericidal activities of both FIN and CIP were comparable. However, the concentration normalised kill-rates (basis 1mg/L) clearly demonstrate that FIN is approximately 2-fold to >20-fold more active than CIP in CAMHB or synthetic urine. Normalised kill rates for selected organisms are illustrated in Fig. 5 where it can be seen that FIN is more active than CIP.

Results and Discussion

Bacterial Strain	CAMHB pH 7.2 FIN	CAMHB pH 7.2 CIP	CAMHB pH 5.8 FIN	CAMHB pH 5.8 CIP	Art. urine pH 5.8 FIN	Art. urine pH 5.8 CIP
S. aureus ATCC 29213	0,25	0,5	0,125	2	0,25	8
E. coli ATCC 25922	0,03	0,03	0,015	0,06	0,06	0,5
E. faecalis ATCC 29212	1	1	0,25	2	1	8
K. pneumoniae ATCC 13883	0,06	0,03	0,015	1	0,25	2
E. coli ATCC 11775	0,25	1	0,015	0,06	0,125	2
P. mirabilis ATCC 9240	0,5	0,015	0,25	0,06	0,5	2
E. cloacae ATCC 13047	0,25	0,015	0,03	0,015	0,25	2
S. marcescens ATCC 13880	2	0,03	0,5	0,5	0,125	2
P. aeruginosa ATCC 10145	4	0,06	1	0,5	1	4
S. aureus ATCC 13709	0,25	0,5	0,015	1	0,5	8
S. saprophyticus ATCC 15305	0,25	0,5	0,015	0,5	0,5	8
S. agalactiae ATCC 13813	0,25	0,5	0,125	0,5	1	8
E faecalis ATCC 19433	1	1	0,5	4	2	16
C. freundii ATCC 8090	0,0075	≤0,0075	≤0,0075	≤0,0075	0,06	≤0,0075
K. pneumoniae ESBL ATCC 700603	4	0,25	1	4	4	32
K. pneumoniae ESBL 20 SHV 27 TEM116	32	4	4	64	16	>128
K. oxytoca ESBL 23 SHV 12 TEM 1	128	>128	16	>128	128	>128
E. coli ESBL CIA TEM 7	0,5	0,06	0,03	4	64	>128
E. coli ESBL 85 (U-473108 from Kiel)	64	64	8	64	64	>128
S. aureus 133	0,25	0,06	0,015	0,5	0,25	8
S. aureus clone 16 (CIP^{R})	0,25	1	0,03	2	2	64
S. aureus 105-11 (CIP^{R})	1	4	0,5	16	4	128
S. aureus 104-13 (CIP^{R})	2	16	1	32	8	>128
S. aureus 103-17 (CIP^{R})	8	64	4	128	32	>128
E. coli WT	0,015	0,015	0,015	0,015	0,06	2
E. coli WT-2 (CIP^{BS})	1	0,125	0,125	2	1	16
E. coli WT-3-M4 (CIP^{R})	128	32	16	>128	128	>128
E. coli WT-3-1-M4 (CIP^{R})	32	2	4	32	32	>128
E. coli WT-3-M21 (CIP^{R})	64	8	8	64	64	>128
E. coli WT-4-M2-1 (CIP^{R})	0,25	0,03	0,015	0,03	0,25	2
E. coli M6 (CIP^{BS})	4	0,5	0,5	4	4	64
E. coli M6-4 (CIP^{R})	4	0,5	1	8	4	128
E. coli M9 (CIP^{R})	16	2	2	32	16	>128
E. coli M9 (CIP^{R})	>128	128	64	>128	>128	>128

Figure 2. Activity of FIN and CIP in CAMBH at pH 7.2 and 5.8 and in artificial urine pH 5.8. CIP^{BS}; ciprofloxacin - borderline susceptible, CIP^{R}; ciprofloxacin resistant, as determined under standard MIC test conditions.

Figure 5. Comparative bactericidal effects of FIN and CIP in synthetic urine, pH 5.8 at 1 x MIC (■), 4 x MIC (■) and 16 x MIC (■). Relative kill rates normalised to 1 mg/L.

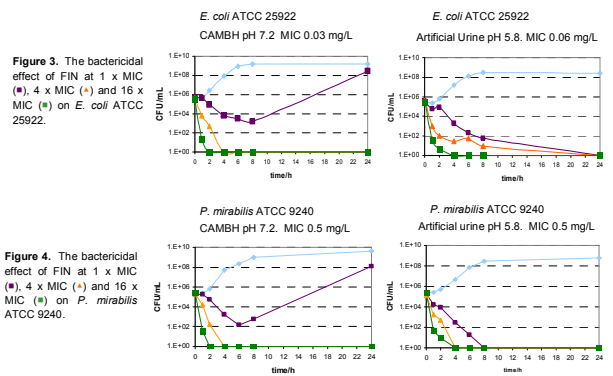
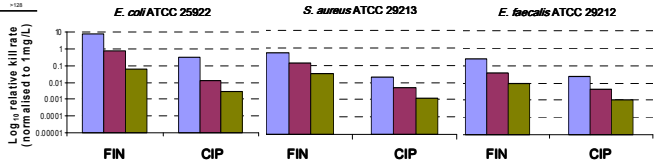


Figure 3. The bactericidal effect of FIN at 1 x MIC (■), 4 x MIC (▲) and 16 x MIC (■) on *E. coli* ATCC 25922.

Figure 4. The bactericidal effect of FIN at 1 x MIC (■), 4 x MIC (▲) and 16 x MIC (■) on *P. mirabilis* ATCC 9240.



Conclusions

- FIN was more active in CAMHB at an acidic pH (5.8) than at pH 7.2, unlike CIP, which had reduced activity at an acidic pH.
- These bacteriostatic (MICs) and bactericidal activities (time kill curves) of FIN also differ favourably from those of CIP under conditions mimicking UTIs. The activity of FIN in artificial urine was both quantitatively and qualitatively different from that of CIP.
- These properties, plus the excellent tolerance seen by the oral route in Phase I studies in man [7] and the lack of toxicity seen in predictive *ex vivo* toxicity tests [6], indicate that finafloxacin is an excellent candidate for progression to the clinic.

Literature

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