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Bactericidal Activity Of Finafloxacin Against Difficult To Kill Growth Forms of *Escherichia coli*

C. Y. GOH[1], F. GOH[1], **W. STUBBINGS[1]**, H. P. KROLL[2], H. LABISCHINSKI[1];

[1]MerLion Pharmaceuticals Pte Ltd, Singapore, Singapore, [2]Bayer HealthCare AG, Elberfeld, Germany.

Contact information:

WILL STUBBINGS
MerLion Pharmaceuticals Pte Ltd,
05-01 The Capricorn
Science Park 2,
Singapore 117528

Tel: +65 6829 5600

will.stubbings@merlionpharm.com

Revised Abstract

Background: FIN is a novel fluoroquinolone (FQ) belonging to a new 8-cyano subclass. FIN exhibits optimal efficacy at slightly acidic pH (5.0 - 6.0), under which other FQ lose activity. Therefore, FIN is intended for bacterial infections associated with an acidic environment. During infection, bacteria may exist as adherent populations and form persistent subpopulations. This study assessed the ability of FIN to kill these difficult to treat growth forms.

Methods: Adherent populations of *E. coli* C600 were grown on 0.45µm membrane filters perfused with BHI (pH 6.2) to a steady state of 10^7 - 10^8 CFU/mL of perfusate. FIN, ciprofloxacin (CIP), levofloxacin (LVX) or moxifloxacin (MXF) (all 5mg/L) were then perfused for 3d, followed by 1d of drug free media. Persistent subpopulations (persister frequencies) were defined as the fraction of viable cells that were recovered following exposure of high cell densities of *E. coli* ATCC 25922 (1 - 5 x10^8 CFU/mL) to FIN, CIP or LVX (10mg/L, 24h) in Mueller-Hinton, pH 7.2. Aderent populations of *E. coli* 25922 and *S. aureus* 29213 were also grown on segments of Foley catheters suspended in artificial urine before exposure to FQs.

Results: FIN resulted in a 5-log reduction of adherent *E. coli*, to below the limit of detection (<10^2 CFU/mL) within 5h, the nearest comparator to this was LVX (2-log reduction). All drugs had significantly reduced viability by day 3, however rapid regrowth was then observed following perfusion with drug-free media in the comparator-treated populations but no regrowth was observed after FIN treatment. The frequency of persisters that remained following high cell density killing in MH were FIN (5.2 x 10^{-7}) , CIP (1.6 x 10^{-3}) and LEV (1.6 x 10^{-4}). Overall, FIN eradicated catheter adherent populations by Δ 1 log greater than CIP.

Conclusions: Adherent populations of *E. coli* were killed more rapidly by FIN and did not regrow following cessation of treatment, which was observed with the comparators. Such superior degree of killing may be related to the lower numbers of persistent bacteria isolated following exposure to FIN.

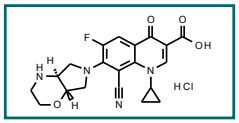
Introduction

Finafloxacin (FIN, Figure 1) is a novel, broad spectrum fluoroquinolone (FQ) that belongs 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) under which other marketed FQs exhibit significantly reduced activity [2]. FIN also exhibited superior activity to comparator FQs in a wide range of rodent infection models, including several models in which adherent bacterial populations are formed [3,4].

Additionally, FIN displayed an excellent safety profile in a wide range of predictive, *in vitro*, toxicity assays [5] and was well tolerated in healthy human volunteers [6]. 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.

Bacterial growth, *in vivo*, is thought to be often associated with slow generation time and formation of adherent populations or biofilms that exhibit phenotypic resistance to antibiotics and disinfectants. Despite this, most *in vitro* assessment of antibacterials involves testing only planktonic cultures. FIN and other FQs were evaluated in a series of *in vitro* models that involved formation of stationary or adherent populations to determine how effectively they eradicated these difficult to treat growth forms.

Figure 1.
Finafloxacin hydrochloride.



Methods

Membrane filter model

E. coli C600 were inoculated onto 0.45 µm filter cartridges under a continuous flow of brain heart infusion broth (BHI), pH 6.2. Once steady state had been established (10^7 – 10^8 CFU/mL of perfusate), FIN, ciprofloxacin (CIP), moxifloxacin (MXF) or levofloxacin (LVX) were perfused at 5mg/L for 3 days followed by drug-free media for a further 24h. Samples of perfusate were taken for CFU determination at T_0, T_{5h}, T_{3d} and T_{3d+1}.

Stationary-phase killing

E. coli 25922 were grown in cation-adjusted Mueller-Hinton broth (CAMHB) for 24h. FIN, CIP or LVX (all 10 mg/L) were then added to these stationary-phase broths for a further 24h. Viable counts were performed on washed culture samples before and after drug exposure. Counts plates were incubated for 72h before reading and the recovered (persistent) bacteria expressed as a fraction of the starting cell number.

Catheter adherent population

E. coli 25922 or *S. aureus* 29213 were grown for 24h – 6d on segments of silicone coated Foley urinary catheters suspended in artificial urine medium [7]. Catheter adherent cultures of different ages were washed and exposed to concentration ranges of FIN or CIP for 24h. The surviving catheter-adherent cells were recovered in PBS by sonication and vortexing and plated out for CFUs.

Results and Discussion

Membrane filter model

The comparative bactericidal activity of FIN, CIP, LVX or MXF (5 mg/L) against membrane adherent populations of *E. coli* are summarised in Figure 2.

FIN had a rapid effect on the viability of adherent *E. coli* populations, causing a 5-log reduction to below the limit of detection (<10^2 CFU/mL) within 5 h, the nearest comparator to this was LVX which caused a 2-log reduction. All drugs had significantly reduced viability by day 3, however rapid regrowth of the adherent population was then observed following perfusion with drug-free media in the CIP, LVX or MXF-treated populations but no regrowth was observed after FIN treatment.

Stationary-phase killing

Saturated, stationary-phase cultures of *E. coli* 25922 were exposed to FIN, CIP or LVX for 24h. The extent of killing (Δ $\log_{10}$ CFU), determined from the survival rate are shown in Figure 3. The greater number of surviving CFUs were recovered following exposure to CIP (persister frequency, 1.6 x 10^{-3}), then LVX (1.6 x 10^{-4}) then FIN (5.2 x 10^{-7}). These data indicate, that FIN induces a more thorough eradication of non-dividing, high density *E. coli* populations than CIP or LVX.

Results and Discussion

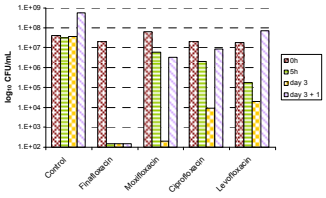


Figure 2. Viability (CFU/mL perfusate) of adherent *E. coli* following 5h ( ) and 3d ( ) exposure to FIN, CIP, MXF or LVX (5 mg/L). Following exposure, the adherent populations were perfused with drug free media for a further 24h and viability measured to determine the extent of recovery ( ).

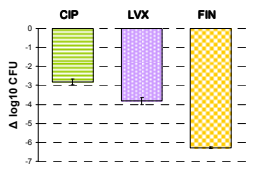


Figure 3. Killing (Δ $\log_{10}$ CFU) of stationary phase *E. coli*. Saturated 24h broth cultures were exposed to CIP ( ), LVX ( ) or FIN ( ) (10 mg/L) for 24h, at which point surviving (persistent) cells were recovered.

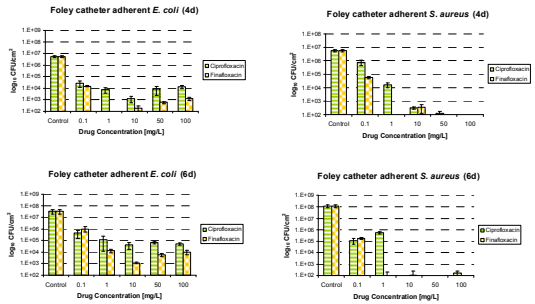


Figure 4. Catheter-adherent populations of *E. coli* and *S. aureus* following exposure to CIP ( ) and FIN ( ). Populations were grown for 4 or 6 days on silicon-coated Foley catheters in artificial urine medium, before drug exposure to concentration ranges of FIN or CIP for 24h. Following treatment, viable cells were recovered and enumerated. FIN exhibited a greater degree of killing than CIP, in terms of eradication, on both populations.

Catheter-adherent populations

Catheter-adherent populations of *E. coli* and *S. aureus* exhibited age-dependent susceptibilities to antibiotics. For example at 3 days, adherent populations of both species were completely eradicated from the catheter following exposure to 0.1 mg/L of FIN or CIP. Older populations began to exhibit phenotypic resistance to these drugs and hence were more difficult to treat. FIN exhibited superior bactericidal activity to CIP at concentrations of 1mg/L and above against 4- and 6- day old catheter adherent populations of *E. coli* and *S. aureus* (Figure 4). On average, FIN reduced such populations to 1 – 2 $\log_{10}$ CFU lower than equivalent CIP treated populations.

Conclusions

- FIN exhibited superior killing to CIP, LVX and MXF against filter membrane-adherent *E. coli*. Killing was faster and FIN was the only drug to prevent the treated population from re-growing in drug-free media.
- FIN also exhibited superior bactericidal activity to CIP against catheter adherent *E. coli* and *S. aureus*. Killing was superior in terms of the lower numbers of surviving cells.
- Exposure of stationary-phase *E. coli* to FIN also resulted in a more extensive killing than CIP and LVX.
- These findings show that FIN is superior to other FQs in terms of the speed and extent of its bactericidal activity against non-growing and adherent *E. coli*.

Literature

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