Selection and Characterisation of Finafloxacin, Ciprofloxacin and Levofloxacin Resistant Mutants of Escherichia coli

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Introduction

Finafloxacin (FIN, Figure 1) is a novel, broad spectrum fluoroquinolone (FQ) belonging to a new class of anti-infective agents. It 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 exhibits superior activity to comparator FQs against antibiotic resistant bacteria in vitro [3] and in a wide range of rodent infection models [4,5]. Additionally, FIN displays an excellent safety profile in a wide range of primate, toxicity assays [6] and was well tolerated in healthy human volunteers [7]. These attributes suggest that FIN warrants clinical evaluation for bacterial infections that are associated with low pH such as urinary tract infection and Helicobacter pylori eradication.

The propensity for the development of resistance to FIN in E. coli was investigated in comparison to ciprofloxacin (CIP) and levofloxacin (LVX). Resultant FQ mutants were characterized at a molecular level.

Results and Discussion

The propensity for the development of resistance to FIN, CIP and LVX in E. coli was investigated through comparison of three-step, stepwise resistance frequencies (Figure 2) and mutants selection concentrations (Figure 3). Both FIN and LVX were determined according to the CLSI protocol for broth micro-dilution in sodium-adjusted Mueller Hinton broth (MHB) at pH 7.2 and pH 5.8.

Resistance frequencies

Single-step resistance frequencies of E. coli ATCC 25922 were determined on sodium phosphate buffered Muscle-Veal agar by plating 10⁷ CFU per plate. Three-step resistance frequencies were performed with plate dilution procedures at pH 7.2 and pH 5.8. Target mutation in gene segments of gyrA (of gyrA) for FIN and CIP mutants were identified from PCR produced DNA sequences of mutants were aligned with parent.

Results: Resistance frequencies (n = 3) of three-step mutants to FIN, CIP and LVX were 4.1 × 10⁻³; 2.2 × 10⁻² and 1.3 × 10⁻⁷ respectively. First-step mutants exhibited a 8 – 9-fold increase in susceptibility over the parent and mutants selected for FIN, CIP and LVX were identified in FIN, CIP and LVX, respectively. FIN, CIP and LVX were isolated with the gyrA resistance determination region (QRDR) of gyrA. The following substitutions were identified (G81D, S83L and D87N). No mutants were detected in the QRDR of gyrB. This suggests that FIN shares a common target with CIP and LVX.

Suscceptibility of characterised FQ resistant mutants of common parent were determined. All combinations of target mutations, to FIN, CIP and LVX were determined at pH 7.2 and pH 5.8. Single-step, agar selected, FIN, CIP and LVX mutants are listed in Table 1. Mutants to each drug exhibited a similar susceptibility profile, comprising a 8 – 32 fold increase in MIC in each of the three FQs tested. Mutants to each drug selected: single point mutations conferring one of the following series of substitutions within the QRDR of gyrA: G81D, S83L and D87N. No mutants were detected in the QRDR of gyrB. This suggests that FIN shares a common target with CIP and LVX.

MIC concentrations of mutants selected by FIN, CIP and LVX was each equivalent to 16 x MIC to FIN, CIP and LVX. MICs increased to 256 x MIC of FIN, CIP and LVX for mutants selected by FIN, CIP and LVX, respectively.

In summary, the propensity for the development of FIN resistance in E. coli was very similar to that of CIP or LVX when compared at concentrations relative to the MIC under the prevailing conditions of pH. In general, dual target mutants exhibited a further 4 – 16 fold increase in MIC.

By 2.5-fold increase in MIC.

Conclusions

• FIN exhibited improved antibacterial activity at low pH whereas CIP and LVX (along with other marketed FQs) lose activity. The mutant selection window for these FQs changed accordingly (and relative to potency) with pH.
• Resistance frequencies for FIN in E. coli were comparable to those for CIP and LVX at 8 x MIC; 16 x MIC and LVX was determined at pH 7.2 and pH 5.8.
• Single-step mutants to FIN, CIP and LVX exhibited relative decreases in susceptibility and common target amino acid sites, indicating gyrA as the primary target.

These findings imply that FIN would have an advantage, in terms of its mutant selection window, under conditions of low pH, reflecting its unusual pH activation profile.

Literature