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Short communication

Mutant prevention concentrations of pradofloxacin for susceptible and mutant strains of *Escherichia coli* with reduced fluoroquinolone susceptibility

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ABSTRACT

Pharmacodynamic and mutant prevention properties of the fluoroquinolone pradofloxacin (PRA) were measured against a set of 17 *Escherichia coli* strains carrying no, one or two known mutations conferring reduced fluoroquinolone susceptibility. The strains included susceptible wild-types, isogenic constructed mutants, isogenic selected mutants and clinical isolates. The effectiveness of PRA was determined with regard to preventing the selection of resistant mutants, using static and changing concentrations of drug. Ciprofloxacin was used as a reference drug. Minimum inhibitory concentrations (MICs) and mutant prevention concentrations (MPCs) of PRA for the susceptible wild-type strains were in the range 0.012–0.016 mg/L and 0.2–0.3 mg/L, respectively, giving a mean ± standard deviation mutant prevention index (MPI = MPC/MIC) of 17.7 ± 1.1. The mean MPI PRA of the 14 mutant strains was 19.2 ± 12, and the mean MPI across all 17 strains was 18.9 ± 10.8. In an in vitro kinetic model in which PRA was diluted with a half-life of 7 h to mimic in vivo conditions, an initial concentration of PRA of 1.6–2.4 mg/L (8–10× MPC), giving a PRA AUC/MPC ratio of 73–92, and a $T_{>MPC}$ of 21–23 h was sufficient to prevent the selection of resistant mutants from the three susceptible wild-type strains. Dosing to reduce selection for antibiotic resistance in veterinary therapy has a role in reducing the reservoir of resistant mutants. We conclude that a level of dosing that prevents the selection of resistant mutants during therapy should be achievable in vivo.

1. Introduction

Antibiotic dosage regimens need to be optimised with regard to a drug's efficacy and safety but should also consider its propensity to select for resistance. Because bacterial populations are large, an infection with drug-susceptible bacteria may contain first-step mutant variants with reduced susceptibility. A dosing regimen that is designed for effectiveness only against the susceptible population, by dosing above the minimum inhibitory concentration (MIC), may selectively amplify less-susceptible mutant variants. The mutant selection window (MSW) concept is used to describe the range of antibiotic concentrations in which the growth of susceptible bacteria is suppressed while populations of first-step resistant mutants are selectively amplified [1]. The antibiotic concentration range included in the standard MSW stretches from the MIC up to the mutant prevention concentration (MPC) at the upper end [2]. The MPC is empirically defined as the drug concentration that prevents the growth of mutants in a 'susceptible population' of 10^{10} bacteria [1]. The potential problem of mutant selection during therapy could be reduced if dosing regimens used drug levels that inhibited not only the susceptible bacterial population but also the subpopulations of first-step resistant mutants.

Dosing regimens taking into account MPCs, without imposing unacceptable risks of increased toxicity, could be motivated from the principle of efficacy in clearing infections and of antibiotic stewardship by reducing the probability of selecting resistant mutants. The size of the MSW cannot be predicted a priori as a simple multiple of the MIC [3] but must be measured experimentally for relevant combinations of drug and bacteria.

Pradofloxacin (PRA) is a third-generation fluoroquinolone with a cyano substitution at C-8 that has been developed by Bayer

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HealthCare AG (Leverkusen, Germany) and was recently approved for the treatment of a range of bacterial infection indications in dogs and cats in the European Union (EU) and for skin infections in cats in the USA [4]. In the EU, approved indications include acute infections caused by susceptible strains of *Escherichia coli* [urinary tract infections (UTIs) in dogs and upper respiratory tract infections in cats]. Previous studies suggested that PRA might combine a high therapeutic activity with a low probability for selection of fluoroquinolone resistance [5,6]. These features make PRA interesting in terms of its potential as a very efficacious antimicrobial. Here we have determined the mutant prevention properties of PRA against a set of 17 *E. coli* strains carrying no, one or two mutations that decrease fluoroquinolone susceptibility. Ciprofloxacin (CIP) was used as a reference drug. The strains tested included clinical UTI isolates, selected mutants with decreased susceptibility, and strains constructed to carry mutations that decrease fluoroquinolone susceptibility. The aim of this study was to determine the pharmacodynamic properties of PRA in relation to preventing the selection of less-susceptible mutants in these genetically diverse strains. Measurements were made in relation to both static and changing concentrations of PRA, the latter reflecting its pharmacokinetic profile in dogs [7,8].

2. Materials and methods

2.1. Bacterial strains

The *E. coli* strains tested included 3 fluoroquinolone-susceptible reference strains and 14 mutants with reduced fluoroquinolone susceptibility (Table 1). The fully susceptible strains included: MG1655, a standard *E. coli* K12 laboratory strain used for genetic manipulations whose genome has been completely sequenced; Nu14, an *E. coli* UTI isolate used in previous studies of drug susceptibility [9]; and ATCC 8739, a completely sequenced (http://www.jgi.doe.gov/) reference wild-type *E. coli* strain. The 14 mutant strains with reduced susceptibility to fluoroquinolones included six UTI isolates, each carrying a single identified fluoroquinolone resistance mutation [10], two mutants with reduced fluoroquinolone susceptibility selected from ATCC 8739 (provided by H-G Wetzstein, Bayer HealthCare AG) and six mutants isogenic to MG1655 and constructed using λ-Red recombineering [11] and bacteriophage P1 transduction to carry one or two fluoroquinolone resistance mutations affecting DNA gyrase and/or drug efflux. All mutations introduced by recombineering and P1 transduction were verified by DNA sequencing.

2.2. Antimicrobial susceptibility testing

PRA and CIP powders were obtained from Bayer HealthCare AG. Stock solutions were prepared at 500 μg/mL in sterile distilled water (for PRA) or 0.1 M NaOH (for CIP) and were kept at room temperature shielded from the light (PRA) or at 4 °C (CIP). Stock solution concentrations were determined by high-performance liquid chromatography (HPLC) and were found to be within ±10% of the expected values. The MIC (mg/L) was measured by broth microdilution according to the recommendations of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (http://www.eucast.org/). The MPC (mg/L) was measured by agar dilution on Mueller–Hinton agar (Difco Becton Dickinson, Sparks, MD). To measure the MPC, each concentration point and experiment used a freshly grown overnight cell population of $\geq 10^{10}$ CFU distributed between 10 separate agar plates (ca. 10^9 CFU/agar plate). The MPC was measured after 96 h of incubation at 37 °C in sealed plastic bags, with drug concentration steps of 0.1 mg/L up to a concentration of 5 mg/L, and thereafter steps of 1 mg/L. Each reported data point is based on at least three independent experiments including the concentration steps above and below the MPC.

2.3. Statistical analysis

The Wilcoxon signed-rank test was used to compare the differences in MIC and MPC measured for CIP versus PRA across the set of 17 *E. coli* strains (two-tailed comparisons). The test was performed using a package available on the VasserStats: Website for Statistical Computation (http://vassarstats.net/).

2.4. In vitro kinetic model

An in vitro kinetic model [12] was used to measure the selection of resistant mutants as a function of initial PRA concentration and rate of PRA dilution simulating the serum concentration–time curve in dogs. PRA added to the culture flask was diluted according to first-order kinetics $C = C_{max}e^{-kt}$, where C_{max} is the initial concentration, C is the concentration at time t, k is the rate of elimination and t is the time that has elapsed since the addition of PRA. The apparatus was operated in a thermostatic room at 35 °C. The initial PRA concentrations tested were multiples of the MPC (2×, 4×, 6×, 8×, 10× and 12× MPC). PRA was diluted with a half-life of 7 h to mimic the in vivo serum half-life in dogs [8]. Each of the three different wild-type strains, one constructed first-step *gyrA* mutant, and one constructed first-step *marR* mutant were tested.

3. Results

3.1. Minimum inhibitory concentration and mutant prevention concentration measurements

The MIC and MPC for both PRA and CIP were measured for each of the 17 wild-type and mutant strains (Table 1). Comparing the MICs for PRA and CIP across all 17 strains showed that the PRA MIC was slightly lower than the CIP MIC for 10 strains (lower by only 1 step on the MIC scale in 7/10 cases), was equal for 6 strains, and was slightly higher for 1 strain. Pairwise comparison of all 17 strains showed that the differences in MIC, although small, are statistically significant ($P = 0.0121$). Thus, on average, the PRA MIC was slightly lower than the CIP MIC for these *E. coli* strains. Most strains (10/17) also had lower MPCs for PRA than for CIP (Table 1) but this difference was not statistically significant ($P = 0.0588$). Although the PRA and CIP MPCs were similar for most strains, there were a few strains for which the PRA MPC was much lower than the CIP MPC. These include two UTI isolates, C120 and C77, as well as the constructed *marR* mutant LM202 (Table 1).

3.2. Mutant selection window and mutant prevention index calculations

The MIC and MPC data were used to calculate the size of the mutant selection window (MSW = MPC − MIC) and of the mutant prevention index (MPI = MPC/MIC) for each strain and drug combination (Table 1). The mean ± standard deviation (S.D.) PRA and CIP MPI values for the 17 isolates were 18.9 ± 10.8 and 28.7 ± 38.3, respectively. The mean ± S.D. pairwise ratio of PRA/CIP MSW is close to unity (1.03 ± 0.81) showing that there is no significant difference between the PRA and CIP MSW for the 17 *E. coli* strains tested ($P = 0.0588$). The mean ± S.D. pairwise ratio of PRA/CIP MPI across the 17 strains is also close to unity (1.25 ± 0.84) ($P = 0.7642$). We conclude that, with the exception of a few strains noted above, PRA and CIP are approximately equal with respect to the static pharmacodynamic parameters MIC and MPC, and that both drugs are predicted to be similarly restrictive against the selection of additional *E. coli* mutants.