Anti-MRSA Properties of Delafloxacin: Mutant Analysis and Characterization

C. Tow-Keogh, T. McConnell, J. Remy, J. Dalton, and *J.A. DeVito

Rib-X Pharmaceuticals, Inc., New Haven, CT, USA

ABSTRACT

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Background: Delafloxacin (DFX) is a broad-spectrum quinolone (3) antibiotics that has successfully completed Phase 3 clinical trials for the treatment of community-acquired pneumonia and skin and skin structure infections. DFX is highly potent, with MICs for TB and MRSA clinical isolates 16X lower than comparator drugs (4-8). As a result, DFX is considered a next-generation fluoroquinolone (9). This study was carried out to analyze DFX-R mutations in the QRDR of S. aureus with the aim to provide a comprehensive understanding of DFX-R resistance mechanisms to inform future clinical trials.

The study began with a naturalistic selection of DFX-R mutants for S. aureus by screening 25 ml CAMHB using Promega’s BacTiter Glo Assay kit in 125 ml Erlenmeyer flasks. For competitive growth analysis, mutant isolates were inoculated testing to ensure sterility and viability. For oxacillin susceptibility testing, CAMHB was supplemented with 2% NaCl according to CLSI. MICs were determined after incubation compared in water and 0.01M NaOH. Ten µl of the 10-fold compound dilution plate were added to 90 µl of a ~1:200 dilution of the 0.5 McFarland standard of each organism for a

Table 1. Delafloxacin is highly potent against MRSA.

Table 2. Characteristics of delafloxacin-resistant MRSA mutants selected in the laboratory.

Table 3. In vitro fitness of delafloxacin-resistant mutants is compromised.

Delafloxacin is a broad-spectrum quinolone antibiotic with excellent activity against quinolone-resistant and -sensitive MRSA and MSSA (1). High activity in vitro can translate to high clinical success, as experienced by large clinical trials as well as preclinical and animal models. A comprehensive understanding and identification of the mechanisms leading to DFX-R resistance is critical to confirm the drug’s efficacy in the clinic and prevent the spread of DFX-R strains. Mutations in the QRDR are reported to be the primary resistance mechanism to quinolones (2). This study was designed to comprehensively characterize the resistance mechanisms to DFX-R mutants harboring mutations in the QRDR.

METHODS

Delafloxacin-R mutants were isolated in 25 ml CAMHB using Promega’s BacTiter Glo Assay kit in 125 ml Erlenmeyer flasks. For competitive growth analysis, mutant isolates were inoculated testing to ensure sterility and viability. For oxacillin susceptibility testing, CAMHB was supplemented with 2% NaCl according to CLSI. MICs were determined after incubation compared in water and 0.01M NaOH. Ten µl of the 10-fold compound dilution plate were added to 90 µl of a ~1:200 dilution of the 0.5 McFarland standard of each organism for a

The results described in this work demonstrate that delafloxacin is highly active against both quinolone-resistant and quinolone-susceptible MRSA. The data show spontaneous mutations to delafloxacin-R resistance were selected in S. aureus in vitro. The presence of DFX-R mutants was confirmed using the BacTiter Glo Assay with BacTrack software to ensure at least 5 colonies were plated per dilution. The MICs for the DFX-R mutants were analyzed using the Clinical Laboratory Standards Institute (CLSI) broth microdilution method.

For each mutation, the MIC and resistance frequency were determined for a panel of 28 S. aureus strains (Table 2). The MICs for DFX-R mutants were 4-128X lower than comparator quinolones and delafloxacin is not subject to efflux by quinolone-specific pumps (e.g. NorA, NorB, NorC data not shown). The resistance frequency for delafloxacin-R mutants was observed to be approximately equal to 100% in all S. aureus strains, and the MICs were 4-128X lower than comparator quinolones. The MPC for delafloxacin varies among MRSA strains and is lower than other quinolones. The MICs for delafloxacin-R mutants were determined by the CLSI broth microdilution method using a 10-fold dilution series for 6 replicates.

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