Delafloxacin exerts potent anti-gonococcal activity despite mutations that decrease antibiotic susceptibility due to target modification or drug efflux

S.G. Kurz1,2, E. Duffy3, J Balthazar4, R.A. Bonomo2, W. M. Shafer1,5
1Tufts Medical Center, Boston, MA, 2VA-Medical Center, Cleveland, OH, 3Melinta Therapeutics, New Haven, CT, 4Emory University, Atlanta, GA, 5VA-Medical Center, Decatur, GA

Abstract

Background: The emergence of Neisseria gonorrhoeae (NG) strains resistant to extended-spectrum cephalosporins represents a major public health problem that requires the development of new drugs for effective treatment. Accordingly, we examined the susceptibility of gonococcal clinical isolates to delafloxacin (DFX) and compared its properties to those of other quinolones. Methods: We examined the structure-activity relationship of DFX against NG strains using a range of target-directed compounds. Results: We found DFX is highly active against NG (MIC = 0.06 µg/ml) even if it expresses a fourfold increase in quinolone resistance compared to other agents (e.g., CIP, Mox 2-4 µg/ml). Furthermore, DFX is highly effective against mutant strains that overproduce efflux pumps. Conclusions: These results show that delafloxacin (DFX) is an effective drug for the treatment of NG. Delafloxacin (DFX) is an effective drug for the treatment of NG.

Methods

GC cultures: material form frozen aliquots was streaked out on chocolate agar plates and grown at 37° C in an atmosphere supplemented with 5% CO2. Pseudomonas cultures were used starting on solid medium. Mueller-Hinton plates supplemented with esculin (0.2%) and for the GC cultures supplemented with different concentrations of DFX. Strains: CDC and CDC13 isolates from China, CDC 19 from Lansing, MI, USA. Genetic derivatives bearing mutations that enhance mtrCDE gene expression or inactivate the MtrCDE efflux pumps were from previous studies initialicated by the Shafer laboratory (2002). Mtr CDE toxicity was used for ager (agar) selection. DNA isolation and sequencing: Single colonies were heat killed and used as DNA templates. PCR of target genes was performed, including upstream promoter regions.

Results

1) Chemical properties of delafloxacin

2) Quinolone target gene mutations

3) The efflux pump mtrCDE operon is negatively regulated by MtrR and expression can be enhanced by promoter mutations (strains KH15 or FA19mtr120) or loss of MtrR

4) Strains with different levels of mtrC expression

5) Correlation of genetic background and MIC data of tested strains

Differential expression, as assessed by RNA slot-blot (Data from Hagman et al. 1995) of mtr in 4 different strains: FA19 with wild type mtrCDE sequence and mtr R, promotor, KH15 with an enhancing deletion to the promoter region, KH15 with a mtrR deletion, and KH9 with mtrR insertion mutant. Similarly, mtrR deficient strains were constructed (expression not shown but not impaired by mtrR mutations)(4). Expression levels of mtr in FA19mtr120 is similar to that of strain KH15 (2).

Conclusion

• Drug resistance is a widespread phenomenon and poses continuous challenges on the treatment of gonorrhea.
• Currently, oral therapeutic options are not available, and resistance to current systemic cephalosporin therapy is emerging.
• Delafloxacin is a novel FQ with superior bactericidal effect compared to currently clinically available FQs and is currently being phase II clinical trials for treatment of uncomplicated gonorrhea.
• While it is a potential substrate for Mtr-mediated efflux, it still retains its activity. It does not appear to be a target for Mtr.
• In conclusion, we show that delafloxacin, which is now in phase III clinical trials for treatment of uncomplicated gonorrhea, is likely value in an era when multidrug resistant strains of NG continue to emerge.

Acknowledgement

Work in the Shafer laboratory was supported by NIH grant R01 AI021150-29 and a VHA Merit Award. JS was a recipient of a Senior Research Career Scientist Award from the Department of Veterans Affairs Medical Research Service. CDC strains were kindly provided by Johan Melendez, Johns Hopkins University School of Medicine, Baltimore.

References