Evaluation of the Bactericidal Activity of Delafloxacin against *Neisseria gonorrhoeae* isolates by Time-Kill Methodology

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Abstract

Background

Gonorrhea has become increasingly difficult to treat due to the development of resistance to a number of antimicrobial agents, and new treatment options are desperately needed. Delafloxacin (DLX), a potent fluoroquinolone (FQ) demonstrates excellent *in vitro* antimicrobial activity against *Neisseria gonorrhoeae* (Ng), including those isolates that are ciprofloxacin-resistant (CIP-R), and has been designated as a QIDP for this indication. There are limited published data for the determination of bactericidal activity of FQs against Ng, mainly due to the difficulty in growing this fastidious organism in broth culture. With the availability of a broth that supports the growth of Ng, the evaluation of the bactericidal activity of DLX against this organism is possible.

Methods

CIP-S (ATCC49226, 255123, 255124) and CIP-R (255126) Ng isolates were tested. MICs for DLX, CIP, and ceftriaxone (CRO) were determined by the broth microdilution method using GC broth with 10 mL of GCHI supplement (Remel) added per liter. Time-kill kinetics were determined at 2X, 4X and 8X MIC for DLX, 4X and 8X MIC for CIP, and 4X MIC for CRO. An inoculum of ~5x10^5 cfu/mL was used in all experiments. Samples were removed from all flasks at 20 and 40 mins, 1, 2, 4, 6, 8, and 24 hrs, serially diluted in PBS and plated to determine the number of viable cells in each flask. Plates were incubated at 35˚ C in 5% CO_2 for 48 hours, colonies were counted and results graphed using GraphPad Prism.

Results

DLX and CIP demonstrated concentration-dependent bactericidal activity against all isolates of Ng. Against Ng ATCC 49226, DLX and CIP were bactericidal at 4X MIC by 4 hr, whereas CRO was bactericidal by 6 hr. Against the CIP-S clinical isolates 255123 and 255124, CIP and DLX killed quickly, demonstrating bactericidal activity by 2 to 4 hr. CRO was bactericidal by 8 and 6 hr for strains 255123 and 255124 respectively. Against 255126 (CIP-R) DLX was more rapidly bactericidal than CIP, killing within 6 hr compared to 8 hr for CIP. CRO showed a >3-log$_{10}$ decrease by 8 hr, but rebounded at 24 hr.

Conclusion

Against all four isolates of Ng, DLX demonstrated rapid bactericidal activity, similar to that seen for CIP against CIP-S strains. Ceftriaxone was slower to kill these organisms overall. DLX demonstrates in vitro antimicrobial potency and bactericidal activity against both CIP-S and CIP-R Ng.

Introduction

Fluoroquinolone agents, once widely used for the treatment of gonococcal infections, are no longer recommended by the CDC as of 2007[1]. In 2012 oral cephalosporins were also removed from the list of recommended therapies for these infections, leaving intramuscularly-administered ceftriaxone combined with either azithromycin or doxycycline as the only options[2]. Delafloxacin, a potent fluoroquinolone, is currently being evaluated in a Phase 3 clinical trial for uncomplicated *Neisseria gonorrhoeae* infections, and has received QIDP designation for this indication. Delafloxacin has been shown to have excellent *in vitro* activity against *N. gonorrhoeae*, including ciprofloxacin-resistant isolates[3]. Delafloxacin's enhanced potency has been attributed to its unique physical-chemical properties, including its structure and polarity[4]. Targeting both gyrase A and topoisomerase IV, delafloxacin has demonstrated a low likelihood for *in vitro* resistance development in MRSA[5]. With the availability of a broth medium that would sustain growth of this fastidious organism, we evaluated the bactericidal activity of delafloxacin against *N. gonorrhoeae*.

Methods

Three ciprofloxacin-susceptible (CIP-S) isolates and one ciprofloxacin-resistant (CIP-R) isolate were included in this study. *N. gonorrhoeae* ATCC 49226 was purchased from ATCC, Manassas, VA, and three clinical isolates were acquired from David Farrell (GR Micro, UK, currently at JMI Laboratories, North Liberty, IA). A sample of each isolate was removed from frozen stock and passaged twice on Chocolate II Agar (GC II Agar, with Hemoglobin and BD IsoVitaleXTM) (CA; Becton Dickinson) at 35˚ C in 5% CO_2 prior to testing to ensure viability and sterility. Minimum inhibitory concentrations (MICs) for delafloxacin, ciprofloxacin, and ceftriaxone were determined in GC broth by the broth microdilution method[6] and are shown in Table 1 below.

Table 1 MICs for test compounds against *N. gonorrhoeae* isolates

Isolate	Delafloxacin MIC (µg/mL)	Ciprofloxacin MIC (µg/mL)	Ceftriaxone MIC (µg/mL)
ATCC 49226	0.002	0.004	0.016
255123	0.004	0.125	0.015
255124	0.015	0.25	0.004
255126	0.125	8	0.015

The GC broth medium was prepared as follows: 15 g Special Peptone (Oxoid), 1 g corn starch (Argo), 2 g NaCl (JT Baker), 4 g K_2HPO_4 (Alfa Aesar), and 1g KH_2PO_4 (BDH) were dissolved in 990 mL deionized water and autoclaved. Once cooled, 10 mL of GCHI supplement (Remel) was added to the broth and mixed.

Methods

Bactericidal activity was determined by time-kill methodology as described by CLSI[7]. Once dissolved, compounds were diluted in GC broth to a volume of 1 mL at 25X the desired final concentration; a flask containing 1 mL of GC broth without compound was prepared as a growth control. Compounds were added to test flasks at concentrations of 2X, 4X and 8X MIC for delafloxacin, 4X and 8X MIC for ciprofloxacin, and 4X MIC for ceftriaxone. For the test organism, a 0.5 McFarland equivalent was prepared, diluted 1:200 in pre-warmed GC broth, and incubated in 5% CO_2 at 35˚ C for 30 minutes prior to exposure to compound. After the 30 minute pre-incubation, 24 mL was removed and added to each test flask for a final volume of 25 mL. A sample was removed from the growth control flask, diluted in Phosphate Buffered Saline (PBS) and plated on CA to confirm an inoculum of approximately 5x10^5 CFU/mL. Samples were then removed from all flasks at 20 and 40 minutes, 1, 2, 4, 6, 8, and 24 hours, diluted in PBS and plated on CA to determine the number of viable cells in each flask. Plates were incubated at 35˚ C in 5% CO_2 for 48 hours and colonies were counted. Plate counts were graphed using GraphPad Prism.

Results

Delafloxacin demonstrated concentration-dependent bactericidal activity against all isolates of *N. gonorrhoeae* (Figures 1a, 2a, 3a, and 4a).

Against *N. gonorrhoeae* ATCC 49226, delafloxacin and ciprofloxacin were bactericidal at 4X MIC by 4 hours, whereas ceftriaxone was bactericidal by 6 hours, as shown in Figures 1a and 1b.

Figure 1a: Delafloxacin kill curves at 2X, 4X, and 8X MIC for CIP-S *N. gonorrhoeae* ATCC49226

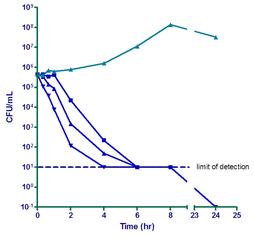
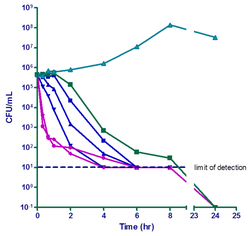
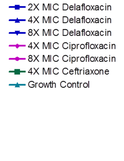


Figure 1b: Delafloxacin, Ciprofloxacin, and Ceftriaxone kill curves for CIP-S *N. gonorrhoeae* ATCC49226





Against *N. gonorrhoeae* 255123 (Figures 2a and 2b), delafloxacin and ciprofloxacin had nearly identical kill curves. Ceftriaxone was slower to kill.

Figure 2a: Delafloxacin kill curves at 2X, 4X, and 8X MIC for CIP-S *N. gonorrhoeae* 255123

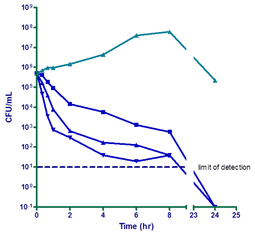
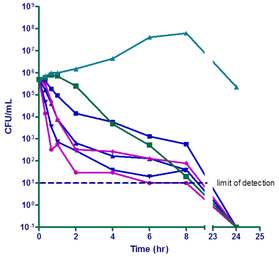
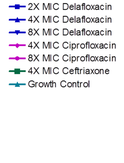


Figure 2b: Delafloxacin, Ciprofloxacin, and Ceftriaxone kill curves for CIP-S *N. gonorrhoeae* 255123





Results

Against *N. gonorrhoeae* 255124 (Figures 3a and 3b), delafloxacin and ciprofloxacin killed quickly at 4X MIC; ceftriaxone was bactericidal by 6 hours.

Figure 3a: Delafloxacin kill curves at 2X, 4X, and 8X MIC for CIP-S *N. gonorrhoeae* 255124

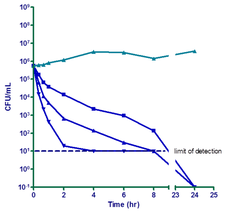
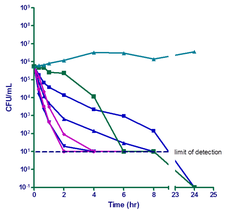
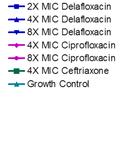


Figure 3b: Delafloxacin, Ciprofloxacin, and Ceftriaxone kill curves for CIP-S *N. gonorrhoeae* 255124





Against the CIP-R strain *N. gonorrhoeae* 255126, delafloxacin was bactericidal at 4X MIC by 6 hours and ciprofloxacin was by 8 hours (Figures 4a and 4b). Regrowth was noted for ceftriaxone at 24 hours, as shown in Figure 4b.

Figure 4a: Delafloxacin kill curves at 2X, 4X, and 8X MIC for CIP-R *N. gonorrhoeae* 255126

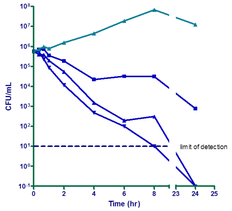
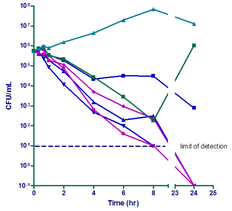
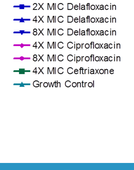


Figure 4b: Delafloxacin, Ciprofloxacin, and Ceftriaxone kill curves for CIP-R *N. gonorrhoeae* 255126





Conclusions

- **Against the CIP-S isolates of *N. gonorrhoeae*, delafloxacin demonstrated rapid bactericidal activity, similar to that seen for ciprofloxacin.**
- **Whereas the kill kinetics against CIP-R *N. gonorrhoeae* 255126 are similar for both agents, the MIC for delafloxacin against this isolate is significantly lower (64-fold) than that of ciprofloxacin.**
- **Ceftriaxone was slower to kill these organisms overall.**
- **Delafloxacin's *in vitro* antimicrobial potency and bactericidal activity against both CIP-S and CIP-R *N. gonorrhoeae*, combined with its expected low potential for resistance development as shown previously in MRSA, offer potential as a treatment for gonococcal infections.**

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