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BACTERICIDAL ACTIVITY OF DELAFLOXACIN AGAINST RECENT ISOLATES OF *STAPHYLOCOCCUS AUREUS*

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ABSTRACT

Background

Delafloxacin, an anionic fluoroquinolone antimicrobial agent, demonstrates excellent *in vitro* activity against Gram-positive and Gram-negative pathogens and is currently undergoing evaluation as a potential treatment for acute bacterial skin and skin structure infections. Despite increased infection control efforts in the clinic, MRSA remains an important pathogen in skin infections. MRSA isolates from a recently-completed delafloxacin Phase 3 clinical trial for the treatment of skin infections were selected for experiments to determine the bactericidal activity of delafloxacin by time-kill methodology.

Methods

Delafloxacin, levofloxacin, vancomycin, daptomycin, and linezolid MICs were determined by the broth microdilution method against MRSA isolates that were identified as harboring zero (MRSA 110), three (MRSA 124), or four (MRSA 165) mutations in the QRDR. MBCs were determined after sampling from sub-MIC and subsequent concentrations. Time-kill experiments were performed at MIC multiples for each antibiotic to include human free C_{max} concentrations, with an inoculum of ~5x10^5 CFU/mL of each organism. At 0, 1, 2, 4, 6, 8, and 24 hours, a sample was removed from each flask, centrifuged, washed and diluted in PBS, and plated to determine the number of viable cells. Plates were incubated overnight at 35˚ C in ambient air. Colonies were counted and kill-curves were plotted using GraphPad Prism. Bactericidal activity was interpreted as a 3-$\log_{10}$ or greater decrease in CFU/mL.

Results

At 16X MIC, delafloxacin killed MRSA 124 more rapidly than the same concentration of levofloxacin, and was bactericidal at 16X and 32X MIC against MRSA 165 at 24 hours. Against all three strains, daptomycin at 8X MIC and above demonstrated the most rapid killing of all agents tested. Vancomycin was bactericidal at 24 hours against these 3 isolates at concentrations above 8X MIC. Linezolid was bactericidal against MRSA 165 at 24 hours but was bacteriostatic against MRSA 110 and 124 at all concentrations tested. Bactericidal activity was observed for delafloxacin and levofloxacin against MRSA 110 at six hours for most concentrations.

Conclusions

Delafloxacin, with MICs and MBCs more potent than those of levofloxacin, demonstrates excellent cidal activity in vitro against MRSA, including those isolates that are levofloxacin-resistant.

INTRODUCTION

Methicillin-resistant *S. aureus* (MRSA), less prevalent today than in the past due to vital infection control measures, continues to be an important pathogen contributing to skin and other infections. Delafloxacin, an investigational anionic fluoroquinolone, has demonstrated excellent *in vitro* activity against *S. aureus*, including MRSA isolates that are resistant to other fluoroquinolones. In a recent delafloxacin Phase 3 acute bacterial skin and skin structure infection (ABSSSI) trial where 50.9% of *S. aureus* isolates were identified as MRSA, delafloxacin was found to be more active than levofloxacin with an MIC_{90} of 0.25 µg/mL compared with 8 µg/mL for levofloxacin.[1] Mutations in the quinolone-resistance determining region (QRDR) were identified in these MRSA isolates. The aim of the present study was to determine the bactericidal activity of delafloxacin against selected MRSA isolates harboring up to four mutations in the QRDR from this clinical trial.

METHODS

Broth microdilution susceptibility testing using cation-adjusted Mueller Hinton Broth (CAMHB,BD) was performed on the MRSA isolates and *S. aureus* ATCC 29213 using CLSI recommended methods[2,3]. Antimicrobial agents were dissolved and diluted to 100X the highest desired final concentration and serially diluted in water or DMSO (Table 1) in a 96 well microtiter tray. The 100X tray was diluted 1:10 in CAMHB; daughter plates containing 10 µL of compound in each well were dispensed. Additional Ca++ was added to CAMHB as required for daptomycin.

Three methicillin-resistant *S. aureus* (MRSA) isolates evaluated in this study were obtained from JMI Labs (North Liberty, IA) and were collected in 2013 from US patients participating in a delafloxacin ABSSSI Phase 3 clinical trial. PCR and sequencing were performed on fluoroquinolone-resistant isolates by the JMI Labs to identify mutations in the quinolone resistance-determining region (QRDR). Isolates were removed from frozen stocks and subcultured twice to ensure viability and sterility. A 0.5 McFarland equivalent of each isolate was prepared and diluted approximately 1:200 in CAMHB; 90 µL was added to each well of the test tray for a final volume of 100 µL. Negative and growth controls were included, and plate counts confirmed an inoculum ~5E+05 CFU/mL. Microtiter trays and plate counts were incubated for 20 hours in ambient air at 35ºC. Following incubation the MICs were determined as the lowest concentration of compound with no visible growth, and plate counts were read and recorded.

Minimal bactericidal concentrations (MBCs) were determined for all agents but linezolid by removing 10 µL from sub-MIC wells and all subsequent wells showing no growth, plated, incubated, and interpreted as described by CLSI[4].

TABLE 1. C_{MAX} VALUES FOR ANTIMICROBIAL AGENTS

Antimicrobial Agent	Manufacturer	Solvent/Diluent	Mean Human C_{max} (µg/mL)	Human Protein Binding	Human Free C_{max} (µg/mL)
Delafloxacin	Melinta Therapeutics, Inc.	NaOH + water/water	9.82	84%	1.57
Levofloxacin	Fluka	NaOH + water/water	9.7	24-38%	6.0-7.4 (average 6.7)
Daptomycin	Cubist	water/water	96	90 – 93%	6.7-9.6 (average 8.2)
Vancomycin	Sigma	DMSO/DMSO	63	55%	28
Linezolid	Melinta Therapeutics, Inc.	DMSO/DMSO	12	31%	8.28

Time-kill curves were performed as described by CLSI to determine bactericidal activity[4]. The drug concentrations tested ranged from 4X MIC to as much as 256X MIC, to include the human free C_{max} concentrations, shown in Table 1[5-10]. Compounds were dissolved as described above and diluted in CAMHB at 25X the desired final concentration to 1 mL total volume; 1 mL CAMHB alone was used for each growth control. For each isolate, a 0.5 McFarland equivalent was prepared then diluted 1:400 into two flasks of pre-warmed CAMHB; one flask contained additional CA++ as required for daptomycin. The flasks were incubated with shaking at 35ºC in ambient air for 30 minutes. Following the 30 minute incubation, 24 mL was removed from the appropriate flask and added to each test flask for a final volume of 25 mL. Flasks were incubated with shaking in ambient air at 35ºC. At 0, 1, 2, 4, 6, 8, and 24 hours, an aliquot was removed from each flask and centrifuged to pellet the cells. In order to eliminate drug carry-over, the supernatant was removed from each tube; the cells were reconstituted in the same volume of PBS, serially diluted and plated to determine the number of viable cells in each flask. BA plates were incubated at 35ºC in ambient air for 24-48 hours after which the colonies were counted and recorded. A 3-$\log_{10}$ decrease in CFU/mL within 24 hours was considered to be bactericidal. GraphPad Prism was used to plot the kill-curves. Up to three replicates were performed for each agent/organism combination.

RESULTS

TABLE 2. MICS AND MBCS FOR DELAFLOXACIN AND COMPARATORS AGAINST S. AUREUS ISOLATES.

Isolate Number	QRDR genotype	MIC/MBC (µg/mL)				
		Delafloxacin	Levofloxacin	Vancomycin	Daptomycin	Linezolid
ATCC 29213	n.t.	0.004/0.008	0.25/0.25	1/2	0.5/1	4/n.t.
110	n.t.	0.008/0.008	0.5/0.5	1/1	0.5/1	4/n.t.
124	*gyrA* E88K, S84L; *parC* S80F; *parE* WT	0.5/0.5	8/8	1/1	0.5/1	4/n.t.
165	*gyrA* E88K, S84L; *parC* E84G, S80Y; *parE* WT	4/8	>32/>32	1/1	0.5/0.5	4/n.t.

Abbreviations: MIC, minimum inhibitory concentration; MBC, minimal bactericidal concentration; QRDR, quinolone resistance determining region; n/a, not available; WT, wild type; n.t., not tested.

TABLE 3. TIME AT WHICH A 3-LOG_{10} DECREASE IN CFU/ML WAS OBSERVED FOR MRSA 110

MRSA 110 (FQ-S)	MIC/Human Free C_{max} (µg/mL)				
	Delafloxacin	Levofloxacin	Daptomycin	Vancomycin	Linezolid
	0.008/1.57	0.5/6.7	0.5/8.2	1/28	4/8.28
#-fold of MIC	Time of 3-$\log_{10}$ decrease in cfu/mL (hr)				
4x	not cidal	2	4	not cidal	*not cidal*
8x	6	4	2	24	not cidal
16x	6	6	*1*	24	not cidal
32x	6	6	1	*24*	not cidal
64x	6		1	24	
128x	6		1	24	
256x	*4*				

C_{max} concentrations are indicated by italics; if C_{max} was below the test range then the lowest concentration tested is indicated.

TABLE 4. TIME AT WHICH A 3-LOG_{10} DECREASE IN CFU/ML WAS OBSERVED FOR MRSA 124

MRSA 124 (Triple mutant)	MIC/Human Free C_{max} (µg/mL)				
	Delafloxacin	Levofloxacin	Daptomycin	Vancomycin	Linezolid
	0.5/1.57	8/6.7	1/8.2	0.5/28	4/8.28
#-fold of MIC	Time of 3-$\log_{10}$ decrease in cfu/mL (hr)				
4x	*not cidal*	*not cidal*	4	24	*not cidal*
8x	4	24	*2*	24	not cidal
16x	4	6	1	24	not cidal
32x	4		1	24	not cidal
64x			1	*24*	
128x			1	24	

C_{max} concentrations are indicated by italics; if C_{max} was below the test range then the lowest concentration tested is indicated.

TABLE 5. TIME AT WHICH A 3-LOG_{10} DECREASE IN CFU/ML WAS OBSERVED FOR MRSA 165

MRSA 165 (Quadruple mutant)	MIC/Human Free C_{max} (µg/mL)				
	Delafloxacin	Levofloxacin	Daptomycin	Vancomycin	Linezolid
	4/1.57	>32/6.7	0.5/8.2	1/28	4/8.28
#-fold of MIC	Time of 3-$\log_{10}$ decrease in cfu/mL (hr)				
4x	*not cidal*	not tested	4	not cidal	*not cidal*
8x	not cidal		2	24	24
16x	8		*1*	24	24
32x	24		1	*24*	24
64x			1	24	
128x			1	24	

C_{max} concentrations are indicated by italics; if C_{max} was below the test range then the lowest concentration tested is indicated.

FIGURE 1. BACTERICIDAL ACTIVITY AT 16X MIC AGAINST MRSA 110

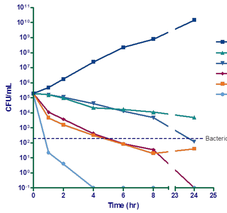


FIGURE 2. BACTERICIDAL ACTIVITY AT 16X MIC AGAINST MRSA 124

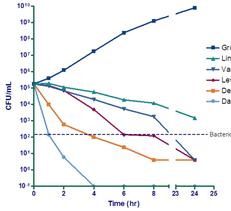
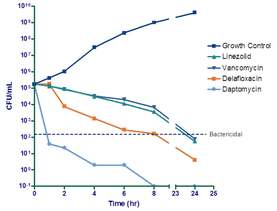


FIGURE 3. BACTERICIDAL ACTIVITY AT 16X MIC AGAINST MRSA 165



CONCLUSION

- Concentration-dependent killing of delafloxacin was found to be strain-dependent
- Bactericidal activity was observed for delafloxacin and levofloxacin against the levofloxacin-susceptible MRSA isolate 110 at six hours for most concentrations. Delafloxacin at 16X MIC killed MRSA 124 (triple mutant) more rapidly than the same concentration of levofloxacin, and was bactericidal at 16X and 32X MIC against the quadruple mutant MRSA 165 at 24 hours.
- Overall, daptomycin demonstrated the most potent killing of all agents tested. Vancomycin at 8X-128X MIC was bactericidal at 24 hours against these three isolates. Linezolid was bactericidal against MRSA 165 at 24 hours but did not kill MRSA 110 and 124 at any concentration tested.
- Delafloxacin, with MICs and MBCs lower than those of levofloxacin, demonstrates favorable bactericidal activity against MRSA, including those isolates that are levofloxacin-resistant.

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