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EXPERIMENTAL THERAPEUTICS

In Vivo Pharmacodynamic Target Determination for Delafloxacin against *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* in the Neutropenic Murine Pneumonia Model

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ABSTRACT Delafloxacin is a broad-spectrum anionic fluoroquinolone that has completed a phase 3 study for community-acquired bacterial pneumonia. We investigated the pharmacodynamic target for delafloxacin against 12 *Klebsiella pneumoniae* and 5 *Pseudomonas aeruginosa* strains in the neutropenic murine lung infection model. The median 24-h free-drug area under the curve (*f*AUC)/MIC values associated with net stasis and 1-log kill were 28.6 and 64.1 for *K. pneumoniae*, respectively. The 24-h *f*AUC/MIC values associated with net stasis and 1-log kill for *P. aeruginosa* were 5.66 and 14.3, respectively.

KEYWORDS *Klebsiella*, *Pseudomonas aeruginosa*, delafloxacin, pharmacodynamics

Delafloxacin is a novel fluoroquinolone antibiotic indicated in adults for the treatment of acute bacterial skin and skin structure infections, and a phase 3 study for community-acquired bacterial pneumonia has been completed (1–3). Delafloxacin has a broad spectrum of activity that includes Gram-positive and Gram-negative bacteria (4–7). We previously characterized the pharmacokinetic and pharmacodynamic (PK/PD) activity of delafloxacin against *Staphylococcus aureus* and *Streptococcus pneumoniae* using a neutropenic murine pneumonia infection model (8). In the current studies, we explored the *in vivo* activity of delafloxacin against multiple strains of *K. pneumoniae* and *P. aeruginosa* to delineate target PK/PD exposures for stasis and 1-log reduction in the neutropenic murine pneumonia infection model.

Eight *K. pneumoniae* strains and five *P. aeruginosa* strains were utilized in the current *in vivo* study. Analysis of previous data in this model (our laboratory) with four *K. pneumoniae* strains was integrated into the present data set. The strains, MIC phenotypes, and genotypes (when available) are presented in Table 1. MICs were determined in triplicate according to CLSI guidelines (9). The MICs ranged widely from 0.03 to 4 mg/liter for *K. pneumoniae* and from 0.12 to 4 mg/liter for *P. aeruginosa*, which are similar to the MIC ranges for delafloxacin identified in an *in vitro* surveillance study (4). The neutropenic murine lung infection model was used for *in vivo* study of delafloxacin. Animals were maintained in accordance with American Association for Accreditation of Laboratory Animal Care criteria. All animal studies were approved by the Animal Research Committee of the William S. Middleton Memorial VA Hospital and the University of Wisconsin. Mice were infected with 6.7 ± 0.3 $\log_{10}$ CFU of each strain via nasal inhalation. The *in vivo* fitness of each strain was assessed, and study demonstrated robust growth over 24 h. Two hours after lung infection, delafloxacin was administered to mice subcutaneously every 6 h for the duration of the 24-h experiment. Treatment included a dose range of 0.0156 to 1,280 mg/kg in 24 h. The highest doses of delafloxacin reduced the lung organism burden by up to 3 $\log_{10}$ compared with the burden

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TABLE 1 Susceptibility results of delafloxacin and comparators against *K. pneumoniae* and *P. aeruginosa* strains

Organism and strain	MIC (mg/liter)			Strain description
	Delafloxacin	Levofloxacin	Ciprofloxacin	
K. pneumoniae				
43816[a]	0.06	0.06		Wild type
4105[a]	1	1		TEM26, SHV4
4110[a]	0.5	1		TEM1, SHV1
81-1260A[a]	0.06	0.06		CTX-M, AmpC
1037570	0.5	1	2	
997613	1	0.5	0.25	
1002059	4	1	0.5	
993043	0.03	0.03	0.03	
1004234	0.06	0.06	0.03	
1008721	0.12	0.12	0.12	
1009343	0.25	0.12	0.25	
1014490	0.25	0.12	0.06	
P. aeruginosa				
71	1		2	Wild type
62	2		4	Wild type
65	4		>8	*parC* S87L, *gyrA* T83I
724	0.12		≤0.06	Wild type
1004586	0.5	0.5	0.12	

[a]Results for these strains were retrieved from our previous study (8).

at the start of therapy (Fig. 1). A 1-$\log_{10}$ kill was achieved against 7 of 12 *K. pneumoniae* strains, and the dose-response curves correlated well with our previous studies on *K. pneumoniae* (8). A 1-$\log_{10}$ kill was achieved for all but the highest MIC strain of *P. aeruginosa*.

We utilized the PK of delafloxacin from this model recently reported from our lab to estimate the plasma area under the curve (AUC) over the current dose range (8). Murine protein binding of 97.6% (Melinta Therapeutics, Inc; data on file) was used to determine free-drug concentrations. The resultant AUC/MIC exposures are shown in Fig. 2. The PK/PD index AUC/MIC correlated well with the therapeutic effect (R^2 = 0.66 for *K. pneumoniae* and 0.84 for *P. aeruginosa*) when modeled using the sigmoid maximum effect (E_{max}) model.

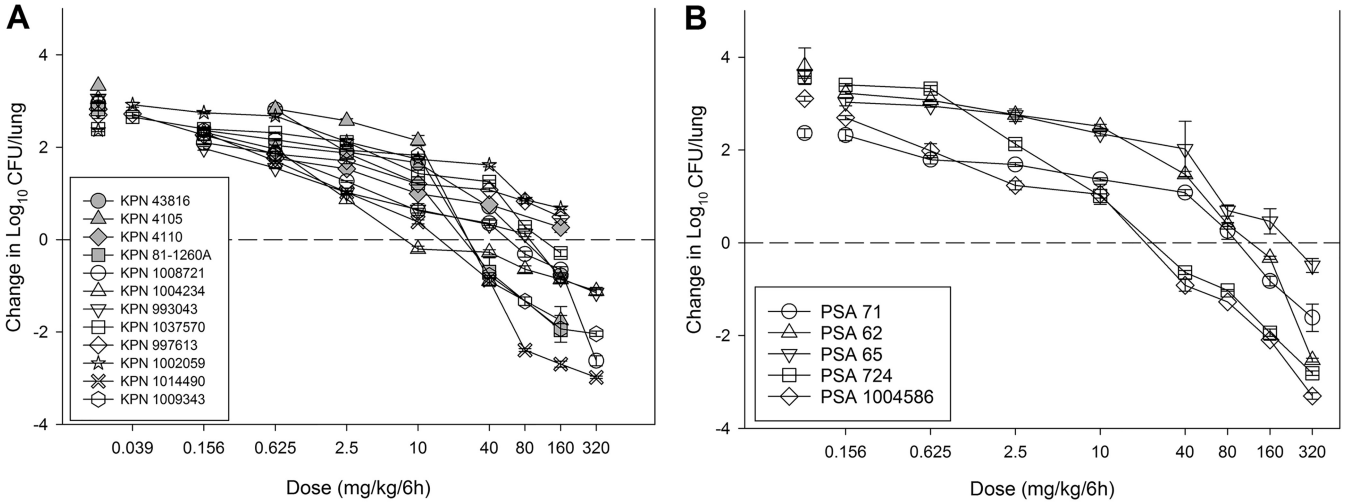

FIG 1 Dose-response curves for delafloxacin against 12 *K. pneumoniae* strains (A) and 5 *P. aeruginosa* strains (B) in the neutropenic murine lung infection model. Symbols represents means and standard deviations from three lung infection replicates. Gray symbols, strains retrieved from our previous study (8). Eight different dose levels were administered by subcutaneous route every 6 h. The burden of organisms was enumerated at the start and end of therapy over a 24-h study period. Horizontal dashed line at 0, burden of organisms at the start of therapy. Data points above the line, net growth (i.e., increase) in burden; data points below the line, reduction in bacterial burden.

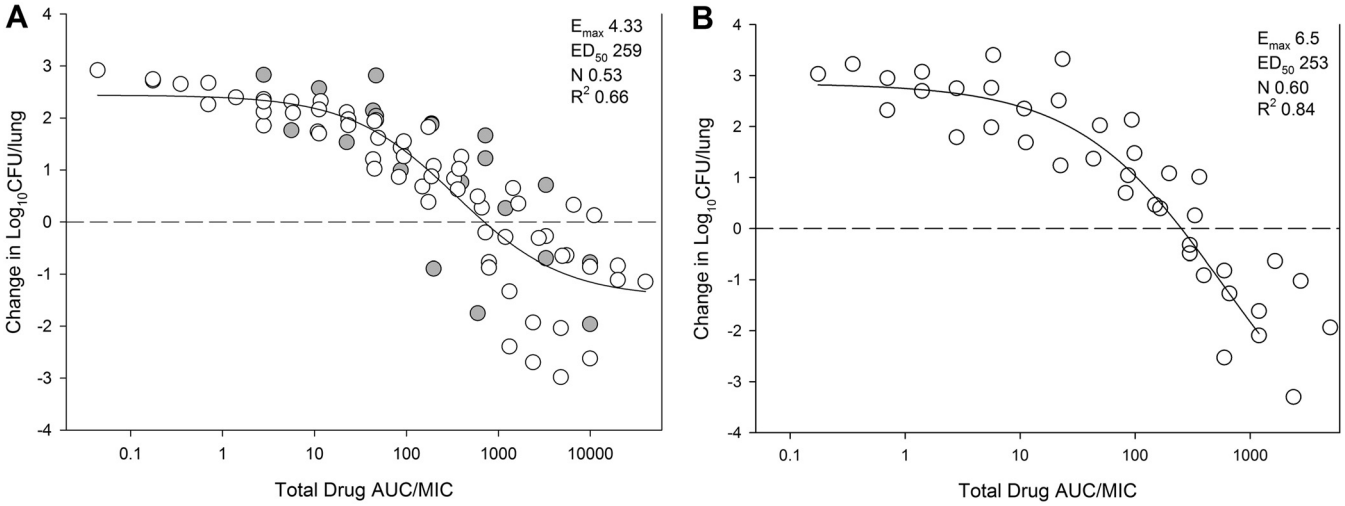

FIG 2 *In vivo* exposure-response relationship between the PK parameter 24-h AUC/MIC and treatment effect for 12 *K. pneumoniae* strains (A) and 5 *P. aeruginosa* strains (B) in the neutropenic murine lung infection model. Each symbol is the mean of three lung infection replicates. Eight total drug dosing regimens were fractionated into an every-6-h regimen. The delafloxacin exposure is represented on the *x* axis as plasma 24-h AUC/MIC. The burden of organisms was measured at the start and end of therapy over a 24-h period. Horizontal dashed line at 0, burden of organisms at the start of therapy. Data points above the line, net growth (i.e., increase) in burden; data points below the line, net reduction in bacterial burden. Line drawn through the data, best-fit line based on the sigmoid E_{max} model (Hill equation). Additional PD paremeters shown are E_{max}, 50% maximal effect point (ED_{50}), slope of the line (N), and R^2 (coefficient of determination).

The AUC/MIC exposures associated with net stasis and 1-log_{10} kill for each strain are shown in Table 2. The median PD target 24-h free-drug plasma AUC/MIC associated with net stasis and 1-log_{10} kill for *K. pneumoniae* were 28.6 and 64.1, respectively. The median PD target 24-h free-drug plasma AUC/MIC associated with net stasis and 1-log_{10} kill for *P. aeruginosa* were 5.66 and 14.3, respectively.

TABLE 2 Delafloxacin pharmacodynamic target exposures for each *K. pneumoniae* and *P. aeruginosa* strain in the murine lung infection model

Organism and strain	Bacterial burden at start of therapy (log_{10} CFU/lung)	Growth in control at 24 h (Δlog_{10}CFU/lung)	Stasis			1-Log kill		
			Dose (mg/kg/24 h)	24-h AUC/MIC		Dose (mg/kg/24 h)	24-h AUC/MIC	
				Total drug	Free drug		Total drug	Free drug
K. pneumoniae								
43816[a]	6.30	2.86	304	5,287	127			
4105[a]	6.30	3.33	106	128	3.08	196	228	5.47
4110[a]	6.32	2.82	NA[b]					
81-1260A[a]	6.28	2.83	84.8	1,681	40.3	238	4,369	105
1037570	6.53	2.95	NA					
997613	6.56	3.04	NA					
1002059	6.64	3.05	639	1,192	28.6			
993043	6.99	2.39	134	1,365	32.8	545	4,312	103
1004234	6.64	2.70	50.7	952	22.8	862	13,394	321
1008721	6.71	2.37	157	6,467	155			
1009343	6.92	2.76	109	528	12.7	233	1,032	24.8
1014490	7.26	2.72	37.2	161	3.87	98.5	474	11.4
Median			109	1,192	28.6	235	2,672	64.1
SE			63.0	764	18.3	117	2,033	48.8
P. aeruginosa								
71	6.23	2.36	294	309	7.41	830	774	18.6
62	6.92	3.81	354	179	4.30	903	421	10.1
65	6.93	3.64	1,057	236	5.66	NA		
724	6.31	3.57	107	1,035	24.8	246	2,152	51.6
1004586	6.52	3.11	61.5	142	3.41	169	409	9.82
Median			294	236	5.66	538	598	14.3
SE			179	166	3.99	192	413	9.91

[a]The results for these strains were retrieved from our previous study (8).
[b]NA, not achieved.

Previous studies have demonstrated that PD targets of fluoroquinolones associated with clinical and microbiological responses vary for different bacterial species (10–14). These studies with older fluoroquinolones demonstrated that the AUC/MIC associated with clinical success was ≥100 for *S. aureus* isolates and Gram-negative bacteria, whereas an AUC/MIC of <40 appeared to be linked to treatment efficacy for *S. pneumoniae* isolates. This divergence in species targets holds true for delafloxacin as demonstrated by integrating the PK/PD targets identified in the current study with those of previous PK/PD studies with delafloxacin. The previous study with a murine pneumonia model demonstrated that PK/PD targets for *S. aureus* and *S. pneumoniae* were ~50- to 100-fold lower than those of comparative fluoroquinolones (8, 15). The delafloxacin PK/PD targets were somewhat lower than those described for other fluoroquinolones in this model (10). The mechanistic basis for this numeric difference is not clear.

As expected, free-drug AUC/MIC was strongly correlated with *in vivo* efficacy of delafloxacin, which is consistent with other fluoroquinolones (8, 10, 12–14). Previous preclinical and clinical evaluations have demonstrated the predictive value of stasis endpoints in the murine model with clinical outcome for patients with community-acquired respiratory tract infections (14). The 24-h free-drug AUC/MICs required for stasis for *K. pneumoniae* and *P. aeruginosa* infections were 28.6 and 5.66, respectively. The human steady-state PK of delafloxacin in healthy subjects using 450-mg oral (16) and 300-mg intravenous (17) clinical dosing regimens demonstrated free AUC from 0 to 24 h (AUC_{0-24}) of 9.9 and 7.5 mg · h/liter. The current PK/PD stasis targets indicate that human PK would predict efficacy against *K. pneumoniae* infection with MICs up to 0.25 mg/liter and against *P. aeruginosa* infection with MICs of ≤1 mg/liter. These MIC values included 78.2% of *K. pneumoniae* strains and 75% of *P. aeruginosa* strains from large surveillance studies with delafloxacin (4).

In conclusion, these results suggest that delafloxacin is a promising agent against *K. pneumoniae* and *P. aeruginosa* infections. The PK targets identified in the murine pneumonia model for net stasis are achievable in most cases when examining the targets in the context of human PK of approved dosing regimens and epidemiological MIC distribution. These animal model PK/PD targets should be useful for future designs of delafloxacin dosing regimens and the development of susceptibility breakpoints.

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