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In vivo Pharmacodynamic Evaluation of Delafloxacin against *K. pneumoniae* (KPN) and *P. aeruginosa* (PSA) in the Neutropenic Murine Pneumonia Model

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ABSTRACT

Background: Delafloxacin is a broad-spectrum anionic fluoroquinolone with promising results from a Phase 3 study in patients with community acquired bacterial pneumonia. The purpose of this study was to examine the PK/PD activity of delafloxacin in a neutropenic murine pneumonia model against a diverse group of GNR organisms that commonly cause pneumonia.

Methods: 12 KPN and 5 PSA clinical strains were used. MICs were determined by BMD at JMI Laboratories. The neutropenic murine pneumonia model was used for all treatment studies. Delafloxacin dosing was by subcutaneous (SC) route. Dose-ranging efficacy studies were performed against all strains (dose range 0.0039 - 320 mg/kg/6h). Treatment outcome was determined by organism burden in the lungs (CFU) at the end of each experiment (24 h). The dose-response data was analyzed using the Emax Hill equation. Data was fit to the PK/PD index AUC/MIC. Static and cidal target exposures were calculated for each strain.

Results: MICs were lower for KPN organisms and ranged from 0.03-4 mg/L; whereas, for PSA the range was 0.125-4 mg/L. Delafloxacin exhibited strong dose-dependent activity with maximal cidal activity of up to 3-log kill. The PK/PD index AUC/MIC fit the treatment efficacy data well (R2 0.66-0.84). Median PK/PD targets are shown in the table and were not significantly different by Mann-Whitney Rank Sum Test (p=.142).

Group	Static Dose (mg/kg/24h)	Stasis 24h tAUC/MIC	Stasis 24h fAUC/MIC	1-log kill dose (mg/kg/24h)	1-log kill tAUC/MIC	1-log kill 24h fAUC/MIC
KPN	109	1192	28.6	235	2672	64.1
PSA	294	236	5.66	538	598	14.3

Conclusions: Delafloxacin demonstrated efficacy in the neutropenic murine pneumonia model against a diverse group of clinical KPN and PSA organisms. The PK/PD index AUC/MIC was robustly linked with efficacy, similar to previous fluoroquinolone PK/PD studies. However, delafloxacin exhibited therapeutic efficacy at lower AUC/MIC target exposures compared to other fluoroquinolones. For example, net stasis was noted at free AUC/MIC exposures of 28.6 for KPN and 5.66 for PSA. These results suggest delafloxacin may be a useful addition to the armamentarium for GNR organisms involved in pneumonia and will be useful for designing dosing regimens for clinical use.

BACKGROUND

- Delafloxacin is an anionic fluoroquinolone antibiotic approved for skin soft tissue infection and under review for approval for community acquired bacterial pneumonia
- Delafloxacin exerts broad gram-positive and gram negative coverage including *Enterobacteriaceae* and *Pseudomonas aeruginosa*
- The purpose of this study was to examine the PK/PD activity of delafloxacin in a neutropenic murine pneumonia model against a diverse group of GNR organisms that commonly cause pneumonia.

METHODS

Strains and susceptibility testing: 12 KPN strains, 4 strains commonly utilized in animal model studies (43816, 4105, 4110, 81-1260A) and 8 strains obtained from patients with pneumonia from the SENTRY surveillance program, and 5 PSA strains, 4 from the delafloxacin CAP trial (71, 62, 65, 724), were utilized (see Table 1). All isolates were tested in accordance with CLSI methodology using BMD. MICs were performed on at least three separate occasions in duplicate.

Murine pneumonia infection model: Six week-old, specific pathogen-free, female ICR/Swiss mice weighing 23 to 27 g were used for all studies. Mice were rendered neutropenic by injecting cyclophosphamide. Broth cultures of freshly plated bacteria were grown to logarithmic phase overnight to an absorbance of 0.3 at 580 nm. After a 1:10 dilution into fresh media, bacterial counts of the inoculum 6.7 ±0.3 $\log_{10}$ CFU/mL. Lung infections with each of the strains were produced by pipetting 50 µL of inoculum into the anterior nares of isoflurane-anesthetized mice held upright to allow for inhalation into the lungs. Antimicrobial therapy was initiated 2 h after the infection procedure. After 24 h, the animals were euthanized and lungs removed, homogenized, and plated for CFU determination. No treatment and zero-hour controls were included.

Pharmacokinetic studies and analysis: Plasma pharmacokinetic study in mice was previously performed in our lab (Lepak and Andes, AAC 2016 Jul 22;60).

Treatment Studies: Delafloxacin was administered by subcutaneous route. Four-fold increasing doses (range 0.039 – 320 mg/kg/6h) were administered to groups (n=3 for each dose group) of infected mice. The burden of organisms were quantified at the beginning of therapy (0 h), end of therapy in each treatment group, and at 24h after infection in untreated control animals. The difference between 24h burden in treatment groups and the starting burden (0 h) was utilized in the analysis. Growth in 24h no treatment controls was utilized to ensure each organism was equally fit in the model.

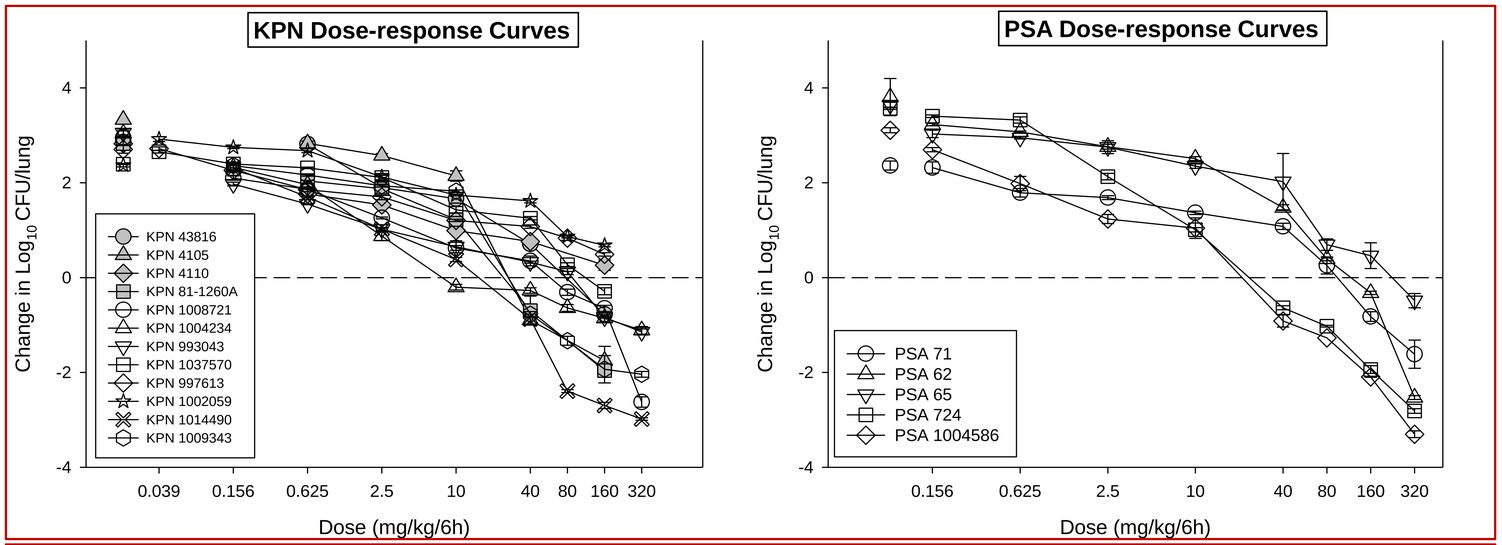
PK/PD Analysis: The exposure-response data was analyzed utilizing the sigmoid Emax Hill equation. AUC/MIC was the PK/PD parameter of interest given previous studies have demonstrated this is the PK/PD driver efficacy for fluoroquinolones. The AUC/MIC exposure associated with net stasis (no change in bacterial burden between start and end of therapy) and 1-log kill (a 1-log reduction in CFU burden from start to end of therapy) were determined for each organism.

RESULTS

Table 1. KPN and PSA strains used in the study, *in vitro* susceptibility results, and resistance genotype where known

Organism		Delafloxacin MIC (mg/L)	Levofloxacin MIC (mg/L)	Ciprofloxacin MIC (mg/L)	Comments
KPN	43816	0.06	0.06		WT
	4105	1	1		TEM26, SHV4
	4110	0.5	1		TEM1, SHV1
	81-1260A	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	
KPN	1008721	0.12	0.12	0.12	
	1009343	0.25	0.12	0.25	
	1014490	0.25	0.12	0.06	
PSA	71	1		2	WT
	62	2		4	WT
	65	4		>8	*parC* S87L, *gyrA* T83I
	724	0.12		≤0.06	WT
	1004586	0.5	0.5	0.12	

RESULTS (cont.)



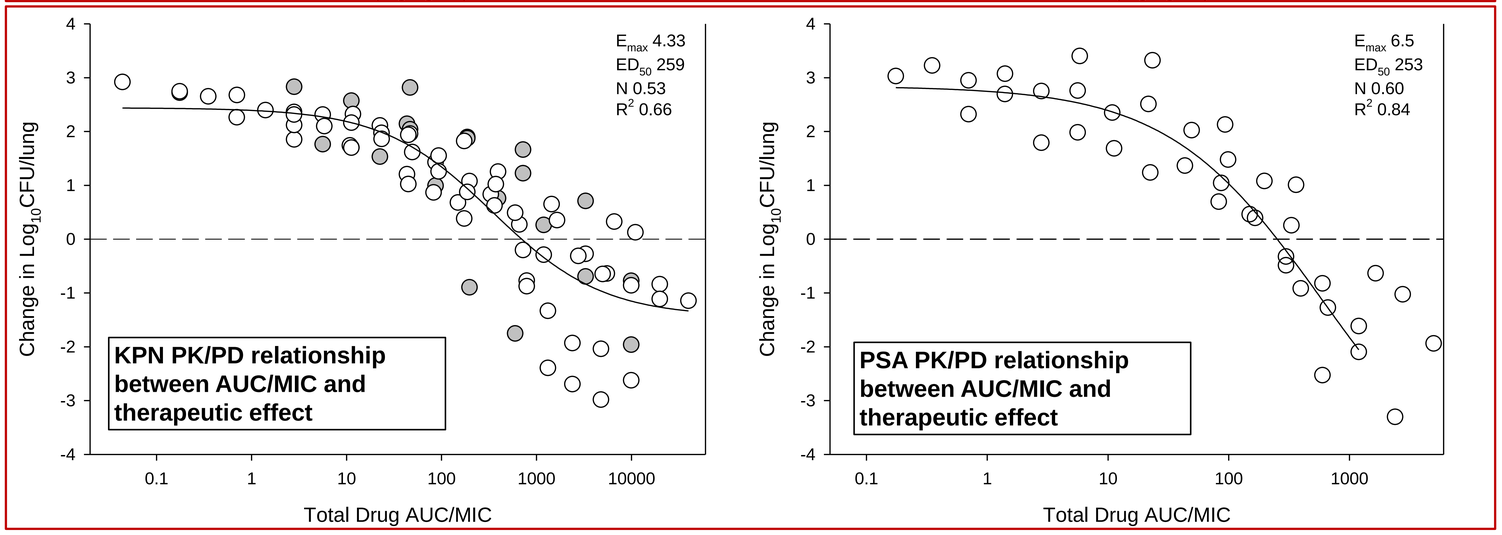


Table 2. Delafloxacin PK/PD target analysis for stasis and 1-log kill for KPN and PSA in the neutropenic mouse pneumonia model

	Organism	Growth in untreated controls ($\Delta\log_{10}$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
KPN	43816	2.86	304	5287	127			
	4105	3.33	106	128	3.08	196	228	5.47
	4110	2.82	NA					
	81-1260A	2.83	84.8	1681	40.3	238	4369	105
	1002059	2.95	NA					
	997613	3.04	NA					
	1037570	3.05	639	1192	28.6			
	1008721	2.39	134	1365	32.8	545	4312	103
	1004234	2.70	50.7	952	22.8	862	13394	321
	993043	2.37	157	6467	155			
	1009343	2.76	109	528	12.7	233	1032	24.8
	1014490	2.72	37.2	161	3.87	98.5	474	11.4
	Median		**109**	**1192**	**28.6**	**235**	**2672**	**64.1**
	SE		**63**	**764**	**18.3**	**117**	**2033**	**48.8**
PSA	71	2.36	294	309	7.41	830	774	18.6
	62	3.81	354	179	4.30	903	421	10.1
	65	3.64	1057	236	5.66	NA		
	724	3.57	107	1035	24.8	246	2152	51.6
	1004586	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**

CONCLUSIONS

1] Similar to previous studies, AUC/MIC was a strong predictor of therapeutic effect for delafloxacin in the mouse pneumonia model

2] Delafloxacin demonstrated potent *in vivo* activity against a diverse group of KPN and PSA strains.

3] Median PK/PD target 24h free drug AUC/MIC was 28.6 for KPN and 5.66 for PSA. These targets are numerically lower than comparator fluoroquinolones where stasis targets are, in general, noted at AUC/MIC values in excess of 100.

4] Integrating these results with known human PK of delafloxacin and MIC distribution within KPN and PSA suggests that PK/PD target exposures would be met for a majority of isolates.

5] These results, as well as our previous work with *S. pneumoniae*, will be useful to optimize dosing regimens and set preliminary susceptibility breakpoints

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