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In vivo PK/PD of Delafloxacin Against *Staphylococcus aureus* (SA), *Streptococcus pneumoniae* (SPN), and *Klebsiella pneumoniae* (KPN) in the Murine Lung Infection Model

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ABSTRACT

Background: Delafloxacin is a broad-spectrum anionic fluoroquinolone under development for the treatment of bacterial pneumonia. The goal of the study was to determine the PK/PD targets in the murine lung infection model for SA, SPN, and KPN.

Methods: 4 isolates of each species were utilized for in vivo studies: SA (1 MSSA, 3 MRSA), SPN (2 PCN-S, 2 PCN-R), KPN (3 ESBL, 1 WT). MICs were determined using CLSI methods. A neutropenic murine lung infection model was utilized for all treatment studies and drug dosing was by subcutaneous route. Single dose plasma PK was determined in the mouse model after administration of 2.5, 10, 40 and 160 mg/kg. For in vivo studies, four fold increasing doses of delafloxacin (range 0.03 to 160 mg/kg) were administered q6h to infected mice. Treatment outcome was measured by determining organism burden in the lung (CFU) at the end of each experiment (24 h). The Emax Hill equation was used to model the dose-response data. The magnitude of the PK/PD index AUC/MIC associated with net stasis and 1-log kill were determined in the lung model for all isolates.

Results: MICs ranged from 0.06-1 mg/L. Single dose PK parameter ranges include: Cmax 2-70.7 mg/L, $AUC_{0-\infty}$ 2.8-152 mg*h/L, $T_{1/2}$ 0.7-1 h. At the start of therapy mice had 6.3 ± 0.09 log_{10} CFU/lung. In control mice the organism burden increased 2.1 ± 0.44 log_{10} CFU/lung over the study period. There was a relatively steep dose-response relationship observed with escalating doses of delafloxacin. Maximal organism reductions ranged from 2- to more than 4-log_{10}. The median AUC/MIC magnitude associated with each endpoint for each species group is shown in the table.

Organism	Stasis			1-log kill		
	Dose (mg/kg/24h)	24h AUC/MIC	24h free drug AUC/MIC	Dose (mg/kg/24h)	24h AUC/MIC	24h free drug AUC/MIC
SA Median	0.34	59.2	1.42	1.74	330	7.92
SPN Median	1.36	23.5	0.56	4.97	140	3.36
KPN Median	106	1681	40.3	217	2298	55.2

Conclusions: Delafloxacin demonstrated in vitro and in vivo potency against a diverse group of pathogens including those with phenotypic drug-resistance to other classes. Median free drug AUC/MIC targets associated with net stasis were very low for all pathogen groups: SA 0.04, SPN 0.4, and KPN 9.68. 1-log kill targets were 2- to 5-fold higher. These results have potential relevance for clinical dose selection and evaluation of susceptibility breakpoints for delafloxacin for the treatment of lower respiratory tract infections involving these pathogens.

BACKGROUND

- Delafloxacin (RX-3341, ABT-492, WQ-3034) is a novel fluoroquinolone antibiotic in development for treatment of respiratory tract infections
- Delafloxacin exhibits broad spectrum activity that includes *S. aureus* (MSSA and MRSA), *S. pneumoniae*, and *K. pneumoniae*
- Clinical trials have shown potency and efficacy in patients with acute bacterial skin and skin structure infection
- The objectives of our experiments were to characterize the *in vivo* efficacy of delafloxacin using a neutropenic murine lung infection model for three common respiratory tract pathogen groups including *S. aureus*, *S. pneumoniae*, and *K. pneumoniae*.
  - Specifically, the pharmacokinetic/pharmacodynamic targets of delafloxacin were examined to provide a framework for further development of drug-dosing regimens to optimize delafloxacin therapy for respiratory infections.

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METHODS

Strains and susceptibility testing: 4 *S. aureus* (1 MSSA and 3 MRSA), 4 *S. pneumoniae* (1 pen-S and 3 pen-R), and 4 *K. pneumoniae* (1 wild-type and 3 ESBL) strains were utilized. All isolates were tested in accordance with CLSI methodology. MICs were performed on three separate occasions in duplicate.

Pharmacokinetic studies and analysis: Single dose plasma PK of delafloxacin was performed by the sponsor following SC administration of delafloxacin at 2.5, 10, 40, and 160mg/kg. Plasma from groups of three mice per time point were collected. Drug concentration measurements were performed by LC-MS/MS. A non-compartmental model was used for PK analysis.

Murine lung model: Six week-old, specific pathogen free, female ICR/Swiss mice weighing 23-27 g were used. Mice were rendered neutropenic by cyclophosphamide injection. Broth cultures of freshly plated bacteria were grown overnight to logarithmic phase. The inoculum ranged from $10^{8.1\text{-}8.2}$ CFU/ml, $10^{8.1\text{-}8.4}$ CFU/ml, and $10^{8.0\text{-}8.3}$ CFU/ml for *S. aureus*, *S. pneumoniae*, and *K. pneumoniae*, respectively. Lung infections with each of the strains were produced by administration of 50 µl of inoculum into the nares of isofluorane-anesthetized mice. Mice were then held upright to allow for aspiration into the lungs. Therapy with delafloxacin was initiated 2 h after induction of infection. Organism burden was determined by CFU quantitation from lung homogenates after 24 h.

Treatment efficacy - pharmacodynamic target determination: *In vivo* treatment studies were performed with all isolates. Seven (*S. aureus* and *S. pneumoniae*) and five (*K. pneumoniae*) four-fold increasing dosing regimens (range 0.156 to 640 mg/kg/24 h) of delafloxacin were administered by SC route to groups of three neutropenic infected mice per dose level. Drug was fractionated into an every 6 hour administration schedule. The correlation between efficacy and the PK/PD parameter AUC/MIC was determined by nonlinear least-squares multivariate regression. AUC/MIC was chosen as the predictive pharmacodynamic as previous studies have demonstrated this index to be predictive for fluoroquinolones. The mathematical model used was derived from the Hill equation. The coefficient of determination (R^2) was used to estimate the variance that might be due to regression with the PK/PD parameter AUC/MIC. The dose required to produce net static effect (Static Dose) and 1 log_{10} kill (1 log kill dose) compared to the start of therapy was calculated for each drug-organism combination. The associated 24 h total and free drug AUC/MIC targets were calculated.

RESULTS

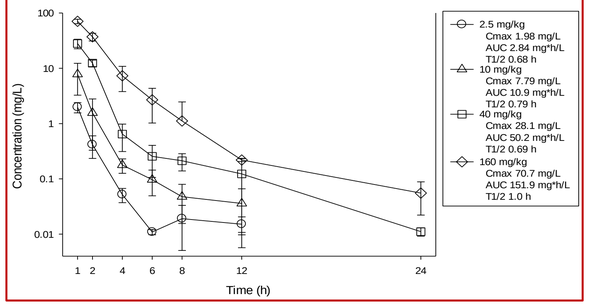
Table 1: Select Strains used in study and *In vitro* Susceptibility Results:

Organism	Comment	Delafloxacin MIC (mg/L)	Levofloxacin MIC (mg/L)
S. aureus ATCC 29213	MSSA	0.008	0.25
S. aureus ATCC 33591	MRSA (USA200)	0.008	0.25
S. aureus MW2	MRSA (USA400)	0.004	0.25
S. aureus R2527	MRSA (USA300)	0.004	0.125
S. pneumoniae ATCC 10813	Pen-S	0.03	1
S. pneumoniae ATCC 49619	Pen-R	0.125	1
S. pneumoniae 145	Pen-R	0.016	0.5
S. pneumoniae 1329	Pen-R	0.016	0.5
K. pneumonia ATCC 43816	Wild-type	0.06	0.06
K. pneumonia 4105	TEM26, SHV4	1	1
K. pneumonia 4110	TEM1, SHV1	0.5	1
K. pneumonia 81-1260A	CTX-M, AmpC	0.06	0.06

RESULTS (cont.)

Pharmacokinetics: Plasma PK of delafloxacin and select PK parameters including $AUC_{0-\infty}$, Cmax, and elimination half-life (T1/2) are shown in **Figure 1**. $AUC_{0-\infty}$ was linear across the dose range (R^2 = 0.99)

Figure 1.



Pharmacodynamic target determination: Bacterial growth in untreated control animals is shown in **Table 2** and increased from 1.5-3.3 log_{10} CFU/lung over 24 h. The dose response curves for delafloxacin against each organism group based on the burden at the start of therapy (zero hour) is shown in **Figures 2a-c**. Each symbol represents the mean ± standard deviation of organism burden in three mice.

Figure 2a. *S. aureus*

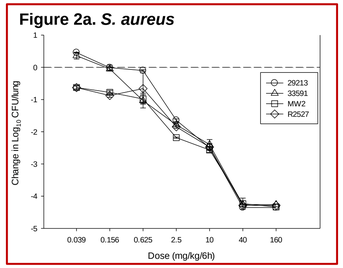


Figure 2b. *S. pneumoniae*

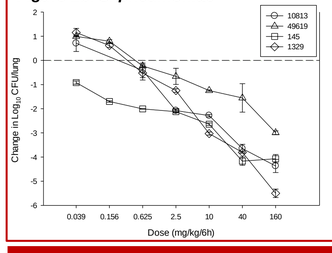
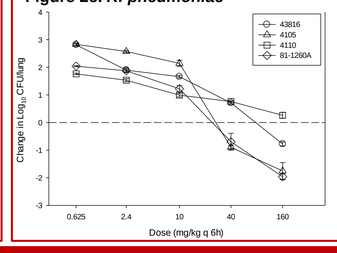


Figure 2c. *K. pneumoniae*



The relationship between PD index AUC/MIC and treatment efficacy is shown in **Figures 3a-c**. Each data point represents the mean of organism burden from three mice. A best-fit line based on the Hill equation is included. The PD parameters E_{max}, ED_{50}, slope (N), and coefficient of determination (R^2) are shown in the figure legend. Total daily doses necessary to achieve net stasis and 1 log kill for are shown in **Table 2** below.

Figure 3a. *S. aureus*

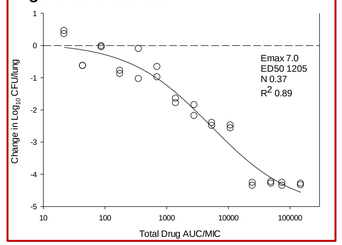


Figure 3b. *S. pneumoniae*

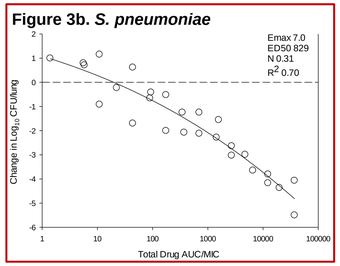


Figure 3c. *K. pneumoniae*

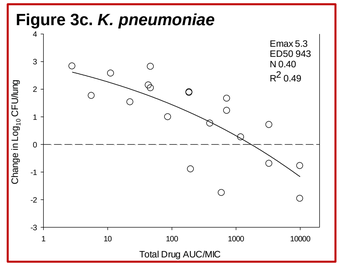


Table 2.

Organism	24h Control Growth (Log10 CFU/Lung)	Stasis			1 log kill		
		Dose (mg/kg/24h)	24h Total Drug AUC/MIC	24h Free Drug AUC/MIC	Dose (mg/kg/24h)	24h Total Drug AUC/MIC	24h Free Drug AUC/MIC
SA 29213	1.99	0.93	130	3.12	3.22	451	10.8
SA 33591	2.06	0.53	74.6	1.79	2.25	315	7.57
SA MW2	1.53	<0.16*	<43.8*	<1.05*	0.98	276	6.61
SA R2527	1.47	<0.16*	<43.8*	<1.05*	1.23	345	8.28
Median		**0.34**	**59.2**	**1.42**	**1.74**	**330**	**7.92**
SP 10813	2.23	0.93	34.8	0.84	3.83	143	3.43
SP 49619	1.8	1.36	12.2	0.29	15.5	137	3.29
SP 145	2.07	<0.16*	<10.9*	<0.26*	0.23	16.5	0.39
SP 1329	1.5	1.36	95.1	2.28	6.11	428	10.3
Median		**1.14**	**23.5**	**0.56**	**4.97**	**140**	**3.36**
KP 43816	2.86	304	5287	127			
KP 4105	3.33	106	128	3.08	196	228	5.47
KP 4110	2.82						
KP 81-1260A	2.83	84.8	1681	40.3	238	4369	105
Median		**106**	**1681**	**40.3**	**217**	**2298**	**55.2**

*Dose and drug exposure (AUC/MIC) were set to the lowest value studied for organisms in which only cidal activity was observed.

CONCLUSIONS

1] Delafloxacin demonstrated *in vitro* and *in vivo* potency against a diverse group of pathogens including those with phenotypic drug-resistance to other classes and in particular to MSSA and MRSA.

2] As with other fluoroquinolones, AUC/MIC was a robust predictor of treatment outcome with exception of KPN group

3] Free drug AUC/MIC targets associated with net stasis were very low for all pathogen groups: SA 1.42, SPN 2.28, and KPN 40.3. The 1-log kill targets were 2- to 5-fold higher. These are numerically lower than previous studies with other fluoroquinolones

4] These results have potential relevance for clinical dose selection and evaluation of susceptibility breakpoints for delafloxacin for the treatment of lower respiratory tract infections involving these pathogens.