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IN VIVO PK/PD OF DELAFLOXACIN AGAINST *ESCHERICHIA COLI* AND *PSEUDOMONAS AERUGINOSA* IN THE MOUSE THIGH INFECTION MODEL

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ABSTRACT

Background: Delafloxacin (DLX) is a broad-spectrum fluoroquinolone (FQ) antibiotic under FDA review for the treatment of ABSSSI. These studies determined DLX PK/PD targets in the neutropenic mouse thigh infection model for *Escherichia coli* (EC) and *Pseudomonas aeruginosa* (PA).
Methods: MIC values were determined according to CLSI guidelines. Three EC isolates (1705874, 255010, 1705884), and three PA isolates (ATCC® 27853™, PA01, 1705889) were used in a neutropenic mouse thigh infection model, with subcutaneous (SC) DLX dosing. DLX mouse SC PK was measured over doses of 0.5 to 320 mg/kg. A PK model was developed to estimate 24-hr free AUC ($fAUC_{24}$) in the PK/PD tests. For the PK/PD studies, DLX was dosed at 1 to 160 mg/kg/dose at 2 hr and 9 hr after inoculation. Outcomes were determined by the change in colony-forming units (CFUs) in the thigh at 24 hr compared to controls at 2 hr. A variable-slope sigmoid equation was fit to the exposure-response data. The PK/PD index, $fAUC_{24}$/MIC, for net stasis and 1-$\log_{10}$ reduction in CFUs was determined for individual isolates and for combined data.
Results: For EC, MIC values ranged from 0.016 to 2 µg/mL. At 2 hr post-inoculation, mice had mean values of 5.76- to 6.88-$\log_{10}$ CFU/thigh. In control mice at 24 hr, mice had mean values of 8.41- to 9.64-$\log_{10}$ CFU/thigh. The data from the individual EC strains were insufficient to determine individual curves, so the data were combined. Predicted $fAUC_{24}$/MIC values for net stasis and 1-$\log_{10}$ reduction in CFUs were 14.5 and 26.2, respectively. The curve estimate for maximum reduction in CFUs was 5.53-$\log_{10}$. The Hill slope was -1.35.
For PA, MIC values ranged from 0.25 to 2 µg/mL. At 2 hr post-inoculation, mice had mean values of 4.79- to 5.08-$\log_{10}$ CFU/thigh. In control mice at 24 hr, mice had mean values of 7.15- to 9.29-$\log_{10}$ CFU/thigh. Curve estimates for maximum reductions in CFUs ranged from 5.48- to 6.70-$\log_{10}$. Steep Hill slopes of -1.99 to -5.45 were observed for the individual strains, and -2.60 for combined data.
Conclusion: DLX had potent *in vitro* and *in vivo* activity against selected strains of EC and PA. $fAUC_{24}$/MIC targets for net stasis and 1-$\log_{10}$ reduction in CFUs for EC were somewhat lower than reported for other FQs. $fAUC_{24}$/MIC targets for PA were markedly lower than other FQs. These results may inform clinical dose selection and susceptibility breakpoints for DLX for the treatment of EC and PA infections.

P. aeruginosa strain	DLX MIC (mg/mL)	Stasis $fAUC_{24}$/MIC	1-Log_{10} Reduction $fAUC_{24}$/MIC
ATCC 27853	0.25	3.23	4.75
PAO1	0.25 – 0.5	3.49	4.18
1705889	1 - 2	5.22	6.01
Combined	-	3.81	5.02

MATERIALS AND METHODS

Mouse Pharmacokinetic Studies The plasma pharmacokinetics of subcutaneous delafloxacin in mice was studied in several experiments using three mouse strains (CD-1, ICR, and BALB/c) over a range of delafloxacin doses. Dose-normalized delafloxacin plasma concentrations and mean profiles did not appear to be markedly different among the mouse strains, so the data were pooled across all the strains and studies. The pooled, dose-normalized plasma concentration data were log-transformed and the values were analyzed at each time point for statistical outliers (*FindOutliersESDtest*). Eight values were excluded as statistical outliers, with an additional four points excluded as pharmacokinetically implausible. A 3-compartment, extravascular PK model, without lag time, was fit to the naïve-pooled data using Phoenix NLME, Version 1.3 (Certara, Princeton NJ). Residual error was modeled with a log-additive model. Goodness-of-fit assessments indicated the model fit the data adequately. The final PK model was used to simulate exposures at the doses used in the mouse efficacy studies (1, 5, 10, 20, 40, 80, and 160 mg/kg, BID).

Drug Formulations Delafloxacin meglumine was prepared in 5% dextrose in water for subcutaneous (SC) administration. Cyclophosphamide monohydrate (Sigma) was dissolved in sterile water for oral (PO) administration.

Mouse Efficacy Studies

Microorganisms The minimal inhibitory concentrations (MIC) for the strains used in this study were determined using CLSI guidelines for the broth microdilution method using cation-adjusted Müeller-Hinton (MH) broth. The three *E. coli* strains were *E. coli* 1705874 (an extended spectrum β-lactamase producing [ESBL+], multi-drug resistant [MDR] strain); *E. coli* 255010; and *E. coli* 1705884. The delafloxacin MIC values were 2, 0.25 and 0.016 µg/mL, respectively. The three *P. aeruginosa* strains used in this study were 27853 (ATCC), PAO1 (ATCC), and 1705889 (Eurofins Medinet). The delafloxacin MIC values were 0.25, 0.25 to 0.5, and 1 to 2 µg/mL, respectively. Inocula were prepared by streaking from frozen stocks onto Tryptic Soy Agar + 5% Sheep Blood plates (TSA + 5% SB plates, Becton Dickinson) and incubating overnight at 35˚ C. From these plates, bacterial aliquots were transferred into pre-warmed MH broth and grown for approximately 2 hours at 35˚ C with shaking. Following the 2 hour incubation, the optical density was measured and the culture diluted to an OD_{625} value of approximately 0.025, and then again diluted either at 1:20 or at 1:10 for inoculation.

Neutropenic Thigh Infection Model Female CD-1 mice (Charles River Labs) weighing 25 – 35g were rendered neutropenic by PO administration of 150 and 100 mg/kg cyclophosphamide on Days -4 and -1, respectively. At time zero, mice were injected with 0.1 mL of the final cultures into both caudal thigh muscles under isoflurane inhalation anesthesia. Mice then were dosed subcutaneously at 2 and 9 hours post-infection with delafloxacin.

Determination of Bacterial Burdens A group of untreated control mice was sacrificed at the start of therapy (2 hours post-infection), and the treatment groups were sacrificed at 24 hours after the initiation of treatment (26 hours post-infection). Mice were euthanized by CO_2 inhalation and both caudal thigh muscles harvested and individually homogenized in sterile bags. Serial dilutions of the homogenates were plated on TSA + 5% BA plates and incubated overnight for enumeration of bacterial burdens. The change in colony forming units was defined as the change in bacterial density 24 hours after treatment initiation compared to bacterial density at the onset of treatment.

INTRODUCTION

Delafloxacin, a new fluoroquinolone under development by Melinta Therapeutics, has good activity against *Escherichia coli* and *Pseudomonas aeruginosa* both *in vitro* and in animal infection models. Delafloxacin was demonstrated to be effective against *E. coli* and *P. aeruginosa* in a mouse peritoneal infection model with similar ED_{50} values to trovafloxacin and ciprofloxacin.

Like other fluoroquinolones, the delafloxacin free-drug AUC_{24}/MIC ratio ($fAUC_{24}$/MIC) correlates with optimal clinical and microbiological outcomes (Wispelwey, 2005). In studies on delafloxacin activity against methicillin-resistant *Staphylococcus aureus* (MRSA) in a neutropenic mouse thigh infection model, the median $fAUC_{24}$/MIC for a 1-$\log_{10}$ bacterial reduction was shown to be 14.3. In a neutropenic mouse lung infection model, median 1-$\log_{10}$ bacterial reduction $fAUC_{24}$/MIC ratios of 7.9, 3.4, and 55 were estimated for *Staphylococcus aureus, Streptococcus pneumoniae, and Klebsiella pneumoniae*, respectively (Lepak & Andes, 2016).

This poster presents a PK/PD analysis of data obtained using a neutropenic CD-1 female mouse thigh infection model for delafloxacin versus three strains of *E. coli*, and three strains of *P. aeruginosa*.

RESULTS

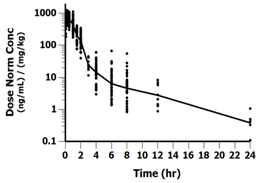
Mouse PK

Delafloxacin plasma concentration data from five pharmacokinetic studies using various mouse strains (CD-1, ICR and BALB/c) was dose-normalized (dose range: 0.5 to 320 mg/kg) and pooled for analysis (Figure 1). A 3-compartment, extravascular PK model, without lag time, was fit to the naïve-pooled data. Residual error was modeled with a log-additive model. Parameter estimates are given in Table 1. The model was used to simulate C_{max} and AUC_{24} exposures for BID dosing as performed in the animal efficacy experiments (Table 2).

Table 1: Parameter estimates for delafloxacin mouse 3-compartment PK model

Parameter	Estimate	Units
Ka	2.41	1/hr
V1	0.000149	L/kg
V2	0.702	L/kg
V3	29.1	L/kg
CL1	0.623	L/(kg*hr)
CL2	5.83	L/(kg*hr)
CL3	0.252	L/(kg*hr)
stdev0	0.651	--
Ka: Absorption constant		
V1, V2 and V3: Central and peripheral compartment volumes		
CL1, CL2 and CL3: Central and intercompartmental clearances		

Figure 1: Delafloxacin dose-normalized plasma concentrations in mice



Note: The line follows the geometric mean concentrations at each time point

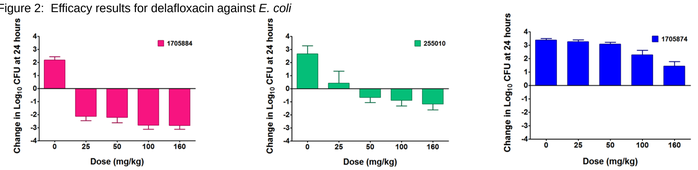
Table 2: Delafloxacin mean total exposures in simulations for efficacy experiments

	Simulated Exposures	
BID Dose (mg/kg)	C_{max} (µg/mL)	$AUC_{0\text{-}24}$ (hr*µg/mL)
1	0.872	2.91
5	4.56	14.7
10	8.85	29.3
20	16.9	58.6
40	33.3	118
80	69.1	237
160	131	483

Thigh Infection Model Results

Delafloxacin activity vs. *E. coli* in the neutropenic mouse thigh infection model (Figure 2) correlated with its *in vitro* activity, with efficacy demonstrated at doses ≥ 25 mg/kg/dose against the 1705884 and 255010 strains (delafloxacin MICs = 0.016 – 0.25 µg/mL) and a lack of efficacy up to 160 mg/kg/dose for the 1705874 strain (delafloxacin MIC = 2 µg/mL).

Figure 2: Efficacy results for delafloxacin against *E. coli*



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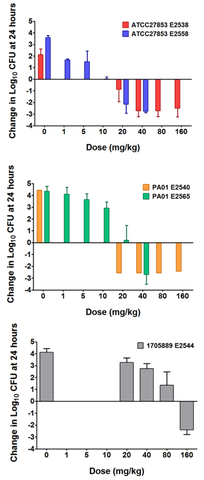
Lepak, A. & Andes, D., 2016. In vivo Pharmacodynamic Target Assessment of Delafloxacin against Staphylococcus aureus, Steptococcus pneumoniae, and Klebsiella pneumoniae in the Murine Lung Infection Model. *Antimicrob Agents Chemother,* 22 July, 60(8), pp. 4764-4769.

Wispelwey, B., 2005. Clinical Implications of Pharmacokinetics and Pharmacodynamics of Fluoroquinolones. *Clin Infect Dis,* Volume 41, pp. S127-135.

RESULTS

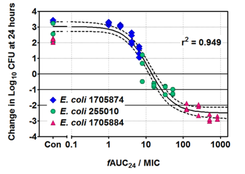
Delafloxacin activity vs. *P. aeruginosa* in the neutropenic mouse thigh infection model (Figure 3) correlated with its *in vitro* activity, with efficacy demonstrated at doses ≥ 20 mg/kg/dose against the ATCC 27853 and PAO1 strains (delafloxacin MICs = 0.25 – 0.5 µg/mL) and at 160 mg/kg/dose for the 1705889 strain (delafloxacin MIC = 1 – 2 µg/mL). Dose-dependent efficacy was observed against all three *P. aeruginosa* strains.

Figure 3: Efficacy results for delafloxacin against *P. aeruginosa*



Note: EXXXX indicates individual experiment numbers.

Figure 4: Delafloxacin PK/PD vs. three *E. coli* strains in mouse

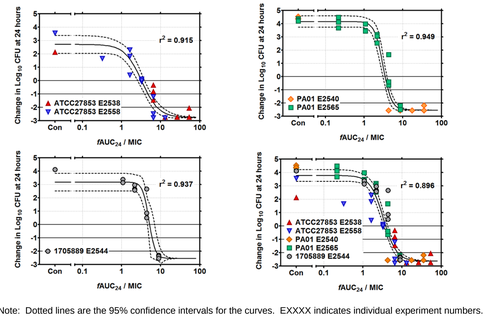


Note: Dotted lines are the 95% confidence intervals for the curve.

Table 3: Parameters (Std Error) of sigmoid curve fit to mouse *E. coli* PK/PD data, and predicted values of $fAUC_{24}$/MIC for efficacy targets

Curve Parameter	Value
Bottom	-2.48 (0.18)
Top	3.05 (0.15)
Hill Slope	-1.35 (0.18)
R^2	0.949
Log IC_{50}	1.10 (0.05)
IC_{50}	12.4
Target	**Predicted $fAUC_{24}$/MIC**
Stasis	14.5
1-Log_{10} CFU Reduction	26.2

Figure 5: Delafloxacin PK/PD vs. three *P. aeruginosa* strains in mouse



Note: Dotted lines are the 95% confidence intervals for the curves. EXXXX indicates individual experiment numbers.

Table 4: Parameters (standard error) of sigmoid curve fit to mouse *P. aeruginosa* PK/PD data, and predicted values of $fAUC_{24}$/MIC for efficacy targets

	***P. aeruginosa* Strain**			
Curve Parameter	1705889	PA01	ATCC27853	All Data Combined
Bottom	-2.54 (fixed)	-2.53 (fixed)	-2.76 (fixed)	-2.62 (fixed)
Top	3.19 (0.29)	4.17 (0.21)	2.72 (0.32)	3.78 (0.22)
Hill Slope	-5.45 (2.49)	-3.99 (0.70)	-1.99 (0.35)	-2.60 (0.36)
R^2	0.937	0.949	0.915	0.896
Log IC_{50}	0.699 (0.033)	0.489 (0.029)	0.513 (0.053)	0.520 (0.029)
IC_{50}	5.00	3.08	3.26	3.31
Target	**Predicted $fAUC_{24}$/MIC**			
Stasis	5.22	3.49	3.23	**3.81**
1-Log_{10} CFU Reduction	6.01	4.18	4.75	**5.02**

PK/PD

The results of the animal efficacy studies were combined with simulated delafloxacin exposures to generate PK/PD curves. The efficacy measure was correlated to the estimated free delafloxacin AUC_{24}/MIC ($fAUC_{24}$/MIC), which was obtained after multiplying the simulated total exposure values by the mean free fraction of delafloxacin in mouse plasma, 0.0277, determined *in vitro*.

For *E. coli*, none of the experiments with the three strains spanned a range of doses wide enough to define a PK/PD curve. Therefore, the data from all three strains was combined for a pooled PK/PD analysis. This approach yielded a smooth, composite PK/PD relationship that was used for curve fitting (Figure 4). A sigmoid, variable-slope curve was fit to the data and the parameters are given in Table 3. The curve was used to predict $fAUC_{24}$/MIC targets for bacterial stasis and a 1-$\log_{10}$ reduction in bacterial burden of 14.5 and 26.2, respectively.

CONCLUSIONS

- The activity of delafloxacin against *Escherichia coli* and *Pseudomonas aeruginosa* was tested *in vivo* in a neutropenic mouse thigh infection model.
- For *E. coli*, using strains with high, intermediate and low susceptibility to delafloxacin, the results from analysis of the pooled data suggested that bacterial stasis would be achieved at an $fAUC_{24}$/MIC ratio of 14.5, and a 1-$\log_{10}$ bacterial reduction would be achieved at an $fAUC_{24}$/MIC ratio of 26.2.
- For *P. aeruginosa*, using strains with varying susceptibility to delafloxacin, the results from analysis of the pooled data suggested that bacterial stasis would be achieved at an $fAUC_{24}$/MIC ratio of 3.81, and a 1-$\log_{10}$ bacterial reduction would be achieved at an $fAUC_{24}$/MIC ratio of 5.02.