POSTER # 1532

HUMAN TARGET ATTAINMENT PROBABILITIES FOR DELAFLOXACIN AGAINST *ESCHERICHIA COLI* AND *PSEUDOMONAS AERUGINOSA*

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ABSTRACT

Background: Delafloxacin (DLX) is a broad-spectrum fluoroquinolone antibiotic which has been approved by FDA for the treatment in adults with ABSSSI caused by designated susceptible bacteria. Previous studies determined DLX bacterial stasis and 1-$\log_{10}$ bacterial reduction free $AUC_{0\text{-}24}$ / MIC (ƒ$AUC_{0\text{-}24}$/MIC) targets for *Escherichia coli* (EC) and *Pseudomonas aeruginosa* (PA) in a mouse thigh infection model. The resulting PK/PD targets were used to predict DLX target attainment probabilities (TAP) in humans.

Material/methods: Monte Carlo simulations were used to estimate TAP with DLX 300 mg IV q12hr. Human DLX plasma pharmacokinetics were determined in patients with ABSSSI in a Phase 3 clinical trial. Individual AUC values were analyzed and determined to be log-normally distributed. The parameters of the AUC distribution were used to simulate random values for ƒAUC_{24}, which then were combined with random MIC values based on 2014-2015 US distributions of skin and soft tissue isolates of EC (n=108) and PA (n=40), to calculate PK/PD TAPs.

Results: DLX ƒ$AUC_{0\text{-}24}$/MIC targets for bacterial stasis and 1-$\log_{10}$ bacterial reduction for EC were 14.5 and 26.2, and for PA were 3.81 and 5.02, respectively. The Monte Carlo simulations for EC predicted TAPs of 97.8% for stasis at an MIC of 0.25 µg/mL, and 99.3% for 1-$\log_{10}$ bacterial reduction at an MIC of 0.12 µg/mL. The simulations for PA predicted TAPs of 97.3% for stasis and 86.5% for 1-$\log_{10}$ bacterial reduction at an MIC of 1 µg/mL.

E. coli	Target Attainment Prediction							
Target MIC (µg/mL)	0.008	0.015	0.03	0.06	0.12	0.25	0.5	1
Stasis	100	100	100	100	100	97.8	50.4	2.0
1-Log Kill	100	100	100	100	99.3	60.4	5.8	0.0

P. aeruginosa	Target Attainment Prediction								
Target MIC (µg/mL)	0.03	0.06	0.12	0.25	0.5	1	2	4	8
Stasis	100	100	100	100	100	97.3	45.9	1.7	0.5
1-Log Kill	100	100	100	100	100	86.5	17.8	0.3	0.1

Conclusions: DLX 300 mg IV q12hr, should achieve ƒAUC_{24}/MIC ratios that are adequate to treat ABSSSI caused by most contemporary isolates of EC and PA. For EC, isolates with DLX MICs ≤0.25 µg/mL comprised 73% of all isolates. For PA, isolates with DLX MICs ≤1 µg/mL comprised 88% of all isolates. Similar results would be expected for TAP with oral DLX 450 mg q12hr.

INTRODUCTION

- Delafloxacin is a new fluoroquinolone recently approved by the FDA for the treatment of acute bacterial skin and skin structure infections (ABSSSI) in adults caused by susceptible bacteria, including both Gram-positive and Gram-negative organisms.
- Delafloxacin has demonstrated activity against *Escherichia coli* (*E. coli*) and *Pseudomonas aeruginosa* (*P. aeruginosa*) both *in vitro* and in animal models.
- For delafloxacin, like other fluoroquinolones, the free-drug AUC_{24}/MIC ratio (ƒAUC_{24}/MIC) correlates with optimal clinical and microbiological outcomes.
- ƒAUC_{24}/MIC ratios determined in animal infection models for bacterial stasis and for a 1-$\log_{10}$ bacterial reduction can be combined with delafloxacin human pharmacokinetics in Monte Carlo simulations to yield estimates of target attainment probabilities for selected bacterial MIC values.

MATERIALS AND METHODS

ƒAUC24/MIC PK/PD Target Determination in Mice

Three strains of *E. coli* and three strains of *P. aeruginosa* were used for the determination of PK/PD targets. The *E. coli* strains were 1705874 (an extended spectrum β-lactamase-producing [ESBL+], multi-drug resistant [MDR] strain), 255010, and 1705884, with delafloxacin MICs of 2, 0.25 and 0.016 µg/mL, respectively. The three *P. aeruginosa* strains were ATCC 27853, PA01, and 1705889, with delafloxacin MICs of 0.25, 0.25 to 0.5, and 1 to 2 µg/mL, respectively.

The activity of delafloxacin against *E. coli* and *P. aeruginosa* was tested using a neutropenic CD-1 female mouse thigh infection model. Animals were made neutropenic by administration of cyclophosphamide on Day -4 (150 mg/kg, PO), and on Day -1 (100 mg/kg, PO). On Day 0, animals were inoculated into each thigh muscle with a preparation of the bacteria and then either left untreated (controls), or dosed subcutaneously with delafloxacin at various dosage levels at 2 hr and 9 hr after inoculation. Colony-forming units (CFUs) in thigh tissue were quantitated at 2 hr (untreated controls only) and 24 hr (controls and delafloxacin-dosed) after inoculation. The changes in $\log_{10}$ CFU relative to the 2 hr control group were determined.

The single-dose subcutaneous plasma pharmacokinetics of delafloxacin in mice were determined in a series of experiments with doses ranging from 0.5 to 320 mg/kg. The plasma concentration was dose-normalized and evaluated for statistical outliers. The resulting naïve-pooled data set was fitted to a 3-compartment extravascular PK model without lag time, with residual error modeled with a log-additive model. The PK model was used to simulate delafloxacin AUC_{24} exposures in the mouse efficacy experiments. Predicted free delafloxacin exposures were derived by applying the value for percent unbound in mouse plasma (2.77%) determined by *in vitro* plasma protein binding experiments with delafloxacin.

PK/PD targets were determined by fitting curves to the results for changes in $\log_{10}$ CFU in mice versus predicted delafloxacin $\log_{10}$ ƒAUC_{24}/MIC. A sigmoid curve, with variable slope and without fixed top or bottom was fitted to each data set. The resulting curves were used to estimate the ƒAUC_{24}/MIC ratios needed to achieve bacterial stasis and a 1-$\log_{10}$ bacterial reduction, for each organism and strain.

Delafloxacin Human Pharmacokinetics

Plasma delafloxacin concentrations from 1-hour intravenous infusions of 300 mg were determined in a Phase 3 study on the treatment of patients with ABSSSI (n=201). A noncompartmental pharmacokinetic analysis of these data was performed by Melinta. The individual steady-state AUC_{12} exposure data were analyzed for distributional characteristics and found to fit well to a unimodal log-normal distribution. The parameters of the log-normal distribution were used in the prediction of target attainment in humans. Predicted free delafloxacin exposures were derived by applying the value for percent unbound in human plasma (16.28%) determined by *in vitro* plasma protein binding experiments with delafloxacin.

Target Attainment Probability Determinations

The estimates of delafloxacin ƒAUC_{24}/MIC values needed to achieve target pharmacodynamic effects were combined with the free delafloxacin exposure estimates in humans to generate predicted target attainment probabilities in humans.

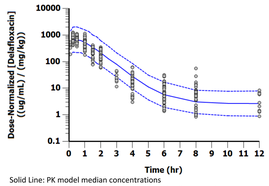
In addition, Monte Carlo simulations were performed to generate weighted target attainment probabilities against observed delafloxacin *E. coli* and *P. aeruginosa* MIC distributions from clinical skin and soft tissue isolates from North American patients (2014-2015 delafloxacin surveillance program). The observed MIC distributions were used to generate 100,000 random MIC values, which were combined with 100,000 random values for delafloxacin ƒAUC_{24} generated with the parameters of the Phase 3 log-normal distribution. Simulated ratios for ƒAUC_{24}/MIC were calculated and the fractions above the target ratios for stasis and 1-$\log_{10}$ bacterial reduction were determined.

RESULTS

Delafloxacin Pharmacokinetics in Mice

Delafloxacin exposures in mice were fairly dose-proportional across the range of subcutaneous doses of 0.5 to 320 mg/kg. A visual predictive check of the mouse PK model with dose-normalized delafloxacin concentrations from mice dosed with 1.25 to 160 mg/kg demonstrated a good fit by the model (Figure 1). The model was used to estimate free delafloxacin AUC_{24} values at the doses used in the mouse efficacy studies (Table 1).

FIGURE 1. DOSE-NORMALIZED DELAFLOXACIN PLASMA CONCENTRATIONS IN MICE



Solid Line: PK model median concentrations
Dashed Lines: PK model concentration 5th and 95th percentiles

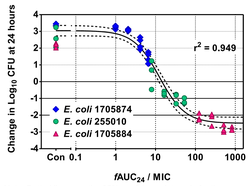
TABLE 1. ESTIMATED FREE $AUC_{0\text{-}24}$ IN MOUSE EFFICACY EXPERIMENTS

BID Dose (mg/kg)	Free $AUC_{0\text{-}24}$ (hr*µg/mL)
1	0.0807
5	0.407
10	0.810
20	1.62
25	1.98
40	3.26
50	4.10
80	6.57
100	8.19
160	13.4

Mouse PK/PD Experiments

Delafloxacin demonstrated dose-dependent killing of *E. coli* in the mouse infection model. Delafloxacin's *in vivo* activity correlated with its *in vitro* activity, with the degrees of *in vivo* efficacy after doses of 25 to 160 mg/kg correlating with the MIC values of the three *E. coli* strains. The range of doses tested against each *E. coli* strain did not define a complete PK/PD curve for each strain, so a curve was fit to combined data from all three strains (Figure 2). A variable-slope sigmoid curve was fit that indicated ƒAUC_{24}/MIC ratios of 14.5 for bacterial stasis, and 26.2 for a 1-$\log_{10}$ bacterial reduction (Table 2).

FIGURE 2 DELAFLOXACIN PK/PD VS THREE *E. COLI* STRAINS IN MOUSE



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

TABLE 2 PARAMETERS (STD ERROR) OF SIGMOID CURVE FIT TO *E. COLI* MOUSE PK/PD DATA, AND PREDICTED VALUES OF ƒAUC_{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 ƒAUC_{24}/MIC**
Stasis	14.5
1-Log_{10} CFU Reduction	26.2

Delafloxacin demonstrated dose-dependent killing of *P. aeruginosa* in the mouse infection model. Delafloxacin *in vivo* activity correlated with its *in vitro* activity, with efficacy demonstrated at doses ≥ 20 mg/kg against the ATCC 27853 and PAO1 strains (delafloxacin MICs = 0.25 – 0.5 µg/mL) and at 160 mg/kg for the 1705889 strain (delafloxacin MIC = 1 – 2 µg/mL). PK/PD analyses were performed on data from each strain individually and with all data combined (Figure 3). Sigmoid, variable-slope curves indicated ƒAUC_{24}/MIC targets for bacterial stasis (3.23 to 5.22) and a 1-$\log_{10}$ reduction in bacterial burden (4.18 to 6.01) (Table 3).

FIGURE 3. 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.

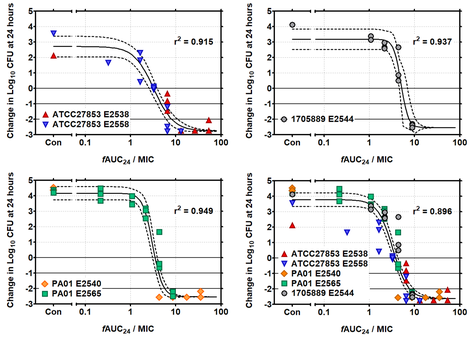


TABLE 3. PARAMETERS OF SIGMOID CURVES FIT TO *P. AERUGINOSA* MOUSE PK/PD DATA, AND PREDICTED VALUES OF ƒAUC_{24}/MIC FOR EFFICACY TARGETS

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

Delafloxacin Human Pharmacokinetics

Plasma concentrations of IV delafloxacin in patients from a Phase 3 study on the treatment of ABSSSI were subjected to noncompartmental analysis (Table 4). The parameters of a log-normal distribution of the clinical AUC data were used to predict steady-state free plasma delafloxacin AUC_{24}, after BID dosing of 300 mg.

TABLE 4. NONCOMPARTMENTAL PLASMA PHARMACOKINETIC PARAMETERS FOR PHASE 3 STUDY 302 (300 MG BID, STEADY STATE).

	AUC_{tau} (hr*µg/mL)	C_{max} (µg/mL)
n	201	201
Mean	24.0	7.32
Std Dev	8.50	2.04
CV%	35.5	27.9
Geo Mean	22.6	7.02
Geo CV%	35.2	30.8
Min	7.57	2.56
Median	22.2	7.14
Max	60.3	12.9

Human PK/PD Target Attainment

The probability of *E. coli* target attainment for stasis in patients receiving delafloxacin 300 mg BID was high (≥ 97.8%) for MICs up to and including 0.25 µg/mL (Table 5). Likewise, the probability of achieving a 1-$\log_{10}$ bacterial reduction was high (≥ 99.3%) for MIC values up to and including 0.125 µg/mL.

TABLE 5. PROBABILITIES OF TARGET ATTAINMENT (%) AGAINST *E. COLI* FOR STASIS (ƒAUC_{24}/MIC=14.5), OR 1-LOG_{10} BACTERIAL REDUCTION (ƒAUC_{24}/MIC=26.2)

Target	MIC (µg/mL)						
	0.015	0.03	0.06	0.125	0.25	0.5	1
Stasis	100.0	100.0	100.0	100.0	97.8	49.5	2.0
1-Log_{10} CFU reduction	100.0	100.0	100.0	99.3	61.2	4.0	0.0

The probability of *P. aeruginosa* target attainment for stasis in patients receiving delafloxacin 300 mg BID was high (≥ 97.3%) for MICs up to and including 1 µg/mL (Table 6). Likewise, the probability of achieving a 1-$\log_{10}$ bacterial reduction was high (≥ 99.9%) for MIC values up to and including 0.5 µg/mL.

TABLE 6. PROBABILITIES OF TARGET ATTAINMENT (%) AGAINST *P. AERUGINOSA* FOR STASIS (ƒAUC_{24}/MIC=3.81), OR 1-LOG_{10} BACTERIAL REDUCTION (ƒAUC_{24}/MIC=5.02)

Target	MIC (µg/mL)									
	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8
Stasis	100	100	100	100	100	100	97.3	45.8	1.6	0.0
1-Log_{10} CFU Reduction	100	100	100	100	100	99.9	86.8	18.2	0.2	0.0

Delafloxacin MIC Distributions and Weighted PK/PD Target Attainment

Delafloxacin MIC values were determined for skin and soft tissue isolates of *E. coli* (Table 7) and *P. aeruginosa* (Table 8) collected from North American patients in 2014-2015. The delafloxacin MIC values against *E. coli* exhibited a bimodal distribution, with most isolates exhibiting MIC values ≤ 0.25 µg/mL (79 out of 108). The majority of *P. aeruginosa* isolates (35 out of 40) exhibited MIC values ≤ 1 µg/mL. The observed distributions were used to calculate weighted estimates of PK/PD target attainment. For *E. coli*, the predicted target attainment probabilities were 98% for stasis at an MIC of 0.25 µg/mL and 99% for 1-$\log_{10}$ bacterial reduction at an MIC of 0.12 µg/mL (Table 9). For *P. aeruginosa*, the predicted target attainment probabilities were 97% and 86% for stasis and 1-$\log_{10}$ bacterial reduction, respectively, at an MIC of 1 µg/mL (Table 10). The target attainment probability curves are shown overlaid on the MIC distributions in Figure 4 and Figure 5.

TABLE 7. MIC FREQUENCIES (CUMULATIVE PERCENTAGE) OF SKIN AND SOFT TISSUE ISOLATES FROM 2014-2015 NORTH AMERICAN DELAFLOXACIN SURVEILLANCE

E. coli

MIC (µg/mL)						
0.008	0.015	0.03	0.06	0.12	0.25	0.5
2 (1.9%)	23 (23.1%)	40 (60.2%)	5 (64.8%)	7 (71.3%)	2 (73.1%)	1 (74.1%)

MIC (µg/mL)						
1	2	4	> 4	Total	MIC_{50}	MIC_{90}
8 (81.5%)	10 (90.7%)	7 (97.2%)	3 (100.0%)	108	0.03	2

P. aeruginosa

MIC (µg/mL)						
0.008	0.015	0.03	0.06	0.12	0.25	0.5
--	0 (0.0%)	1 (2.5%)	1 (5.0%)	15 (42.5%)	10 (67.5%)	3 (75.0%)

MIC (µg/mL)						
1	2	4	>4	Total	MIC_{50}	MIC_{90}
5 (87.5%)	3 (95.0%)	1 (97.5%)	1 (100.0%)	40	0.25	2

TABLE 9. PERCENT PROBABILITIES OF STASIS OR 1-LOG_{10} BACTERIAL REDUCTION FOR *E. COLI* AND *P. AERUGINOSA* PK/PD TARGET ATTAINMENT BASED ON CLINICAL ISOLATE DISTRIBUTIONS

E. coli

	MIC (µg/mL)							
Target	**0.008**	**0.015**	**0.03**	**0.06**	**0.12**	**0.25**	**0.5**	**1**
Stasis	100 (100)	100 (100)	100 (100)	100 (100)	100 (100)	98 (100)	50 (99)	2 (90)
1-Log_{10} CFU Reduction	100 (100)	100 (100)	100 (100)	100 (100)	99 (100)	60 (99)	6 (98)	0 (89)

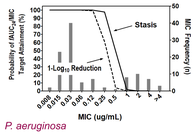
P. aeruginosa

	MIC (µg/mL)								
Target	**0.03**	**0.06**	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**>4**
Stasis	100 (100)	100 (100)	100 (100)	100 (100)	100 (100)	97 (100)	46 (96)	2 (93)	0.5 (91)
1-Log_{10} CFU Reduction	100 (100)	100 (100)	100 (100)	100 (100)	100 (100)	86 (98)	18 (92)	0.3 (90)	0.1 (87)

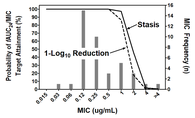
Note: Number in parenthesis is the cumaltive probability

FIGURE 4. PK/PD TARGET ATTAINMENT IN HUMANS BASED ON CLINICAL ISOLATE DISTRIBUTION

E. coli



P. aeruginosa



CONCLUSION

- 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 ƒAUC_{24}/MIC ratio of 14.5, and a 1-$\log_{10}$ bacterial reduction would be achieved at an ƒAUC_{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 ƒAUC_{24}/MIC ratio of 3.81, and a 1-$\log_{10}$ bacterial reduction would be achieved at an ƒAUC_{24}/MIC ratio of 5.02.
- Analyses using simulations based on the PK/PD targets and on human pharmacokinetics suggest delafloxacin 300 mg IV given twice daily should achieve ƒAUC_{24}/MIC ratios that are adequate to treat the majority of isolates of *E. coli* and *P. aeruginosa*.
- It has been demonstrated clinically that equivalent plasma exposures of delafloxacin are achieved with a 450-mg oral dose (tablet) and a 300-mg intravenous dose (1h infusion). It is plausible both dosing regimens will be adequate for the treatment of *E. coli* and *P. aeruginosa*.

Refer to full prescribing information at www.baxdela.com