Pharmacokinetics-Pharmacodynamics of Efficacy for Eravacycline Against *Escherichia coli* in an *In Vitro* Infection Model

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ABSTRACT

Background: Eravacycline is a novel synthetic fluorocycline with activity against pathogens commonly associated with complicated intra-abdominal and urinary tract infections. While tetracyclines have been utilized for >30 years, there are limited PK-PD data available in the literature. We carried out a series of dose-fractionation and dose-ranging studies using a one-compartment *in vitro* infection model to determine the PK-PD index that best describes eravacycline activity and the magnitude of the PK-PD associated with efficacy.

Methods: 48-hour one-compartment *in vitro* infection models were utilized for all studies. Dose-fractionation studies were completed in which 10^6 CFU/mL of *E. coli* 25922 (MIC, 0.125 mg/L) was exposed to eravacycline PK profiles mimicking the human free-plasma concentration-time profile for three doses (free-drug AUC_{0-48} values of 3.49, 8.17 and 17.5 mg*h/L). For each dose, eravacyline was administered as one total dose or fractionated doses q48h, q24h, q12h or q6h. In the dose-ranging studies, four additional *E. coli* clinical isolates (MIC values of 0.125 to 0.25 mg/L) were exposed to eravacycline dosing regimens with free-drug AUC_{0-48} values ranging from 0.10 to 21.1 mg*h/L q12h. Relationships between change in $\log_{10}$ CFU from baseline and free-drug C_{max}:MIC ratio, AUC_{0-24}:MIC ratio and %Time>MIC were fit using Hill-type models.

Results: As evidenced by an r^2 of 0.903 and the dispersion of data, data from the dose-fractionation studies showed that free-drug AUC_{0-48}:MIC ratio was the PK-PD index most associated with eravacycline efficacy. Figure 1 shows the relationship between free-drug AUC_{0-24}:MIC ratio and change in $\log_{10}$ CFU from baseline at 24 h based on data from the dose-ranging studies; free-drug AUC_{0-24}:MIC ratios associated with net bacterial stasis, and 1- and 2-$\log_{10}$ CFU reductions from baseline were 15.3, 20.5 and 28.8, respectively.

Conclusions: These data provide insight about the PK-PD index associated with eravacycline efficacy.

INTRODUCTION

- Eravacycline is a novel synthetic fluorocycline with activity against pathogens commonly associated with complicated intra-abdominal and urinary tract infections.
- Despite tetracyclines being utilized for over 30 years, there are limited pharmacokinetic-pharmacodynamic (PK-PD) data available in literature.
- A series of dose-fractionation and dose-ranging studies using a one-compartment *in vitro* infection model were used to determine the PK-PD index that best describes eravacycline activity and the magnitude of the PK-PD associated with efficacy.

OBJECTIVES

- The objectives of these analyses were the following:
  - To identify the PK-PD index associated with the efficacy of eravacycline against *Escherichia coli*; and
  - To determine the magnitude of the PK-PD index associated with the efficacy of eravacycline using a multiple *E. coli* isolate challenge panel.

METHODS

Antimicrobial Agents and Challenge Isolates

- Eravacycline was provided by Tetraphase Pharmaceuticals (Watertown, MA) and Tetracycline was purchased from Sigma-Aldrich (Natick, MA).
- Two *E. coli* isolates (ATCC 25922 and BAA-2452) were purchased from American Type Culture Collection (Manassas, VA), and two *E. coli* isolates (1135 and 355) were provided by Tetraphase Pharmaceuticals, while the remaining isolate (2692) was provided by JMI Laboratories (North Liberty, IA).

METHODS

Susceptibility Testing

- Eravacycline susceptibility studies were carried out for five *E. coli* isolates using freshly prepared cation-adjusted Mueller-Hinton microbroth- and agar-dilution methodologies (BD Laboratories, Franklin Lakes, New Jersey) in accordance with Clinical Laboratory Standards Institute (CLSI) guidelines [1].
- Tetracycline was utilized as an internal standard for all susceptibility assays.
- All minimum inhibitory concentrations (MIC) were determined in triplicate over a two-day period and presented as the modal value.

Dose-Fractionation Studies

- Dose-fractionation studies were performed in duplicate using a 48-hour one-compartment *in vitro* infection model.
  - An inoculum of 10^6 CFU/mL of *E. coli* 25922 (MIC, 0.125 mg/L) was exposed to eravacycline free-drug concentration-time profiles for three sets of dosing regimens providing free-drug AUC_{0-48} values of 3.49, 8.17 and 17.5 mg*h/L.
  - For of the above-described AUC_{0-48} values, eravacycline was administered as one total dose or fractionated doses, once every 48, 24, 12, or 6 hours (q48h, q24h, q12h or q6h, respectively).
  - Relationships between change in $\log_{10}$ CFU from baseline and free-drug C_{max}:MIC ratio, AUC_{0-24}:MIC ratio and %Time>MIC were evaluated using non-linear least regression and Hill-type models.

Dose-Ranging Studies

- A series of dose-ranging studied were performed in duplicate using the one-compartment *in vitro* infection model and each of the five *E.coli* isolates evaluated. Free-drug AUC_{0-24} values ranging from 0.10 to 21.1 mg•h/L were administered q12h.
  - All eravacycline dosing regimens were compared to a no-treatment control over a 48-hour period.

Drug Assay

- All eravacycline samples in Mueller-Hinton broth collected for chemical analysis were assayed by Frontage Laboratories (Exton, PA) using liquid chromatography-tandem mass spectrometry. The lower limit of quantitation was 1 ng/mL and the standard curve was linear over a range from 1 to 2,000 ng/mL [2].

Pharmacokinetic-Pharmacodynamic Analysis

- Data were evaluated using non-linear least squares regression and Hill-type models.
- Relationships between the PK-PD index associated with efficacy based on the results of dose-fractionation studies and change in $\log_{10}$ CFU/mL from baseline at 24 and 48h were determined.
- The magnitude of the above-described PK-PD index associated with net bacterial stasis, and 1- and 2-$\log_{10}$ CFU/mL reductions from baseline were calculated.

RESULTS

Susceptibility Testing

- The eravacycline and tetracycline microbroth- and agar-dilution MIC values for the challenge *E.coli* isolate panel are presented in **Table 1.**
  - Eravacycline MIC values ranged from 0.125 to 0.25 mg/L.
  - Tetracycline MIC values ranged from 0.5 to 8 mg/L.

RESULTS

Table 1. Results of the eravacycline and tetracycline microbroth- and agar-dilution susceptibility studies for five *E. coli* isolates

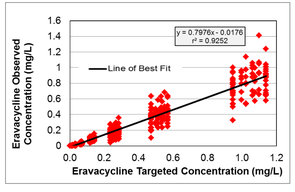
E. coli Isolate	Known Resistance Mechanisms	MIC (mg/L)[a]			
		Eravacycline		Tetracycline[b]	
		Microbroth	Agar	Microbroth	Agar
25922	Wild Type	0.125	0.125	0.5	1
BAA-2452	NDM-1	0.125	0.125	2	2
1135	ESBL[c]	0.25	0.25	8	4
355	CTX-M-9/14	0.125	0.25	4	8
2692	NDM-1, TEM-1, CTX-M-15	0.25	0.5	2	8

a. Represents modal MIC values.
b. Tetracycline non-susceptible isolates represented those with MIC values ≥ 4 mg/L [3].
c. Unknown ESBL enzyme.

Pharmacokinetics Assessment

- As evidenced by the good agreement between the observed and targeted eravacycline concentrations shown in **Figure 1**, the eravacycline PK profiles were simulated reasonably well in the one-compartment *in vitro* infection model.

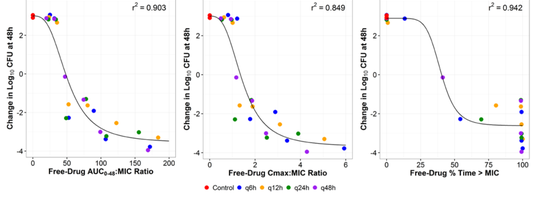
Figure 1. Relationship between observed and targeted eravacycline free-drug concentrations simulated within the one-compartment *in vitro* infection model



Dose-Fractionation Studies

- As evidenced by the dispersion of data across the fitted lines in **Figure 2**, data from the dose-fractionation studies showed that free-drug AUC_{0-48}:MIC ratio was the PK-PD index most associated with eravacycline efficacy.
  - While % Time > MIC provided a high r^2 value of 0.942, the range of data at 100% T>MIC was wide and thus, this PK-PD index poorly described the efficacy of eravacycline.

Figure 2. Relationships between change in $\log_{10}$ CFU from baseline at 48 hours and eravacycline free-drug AUC_{0-48}:MIC ratio, C_{max}:MIC ratio and the %Time > MIC based on data for *E. coli* ATCC 25922

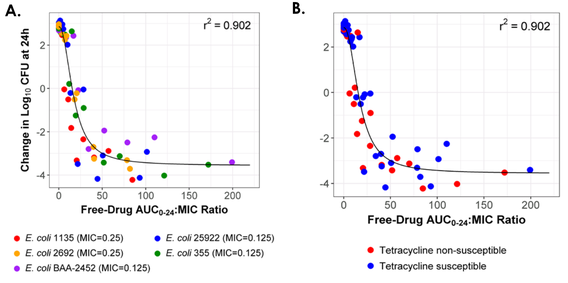


RESULTS

Dose-Ranging Studies

- The relationship between change in $\log_{10}$ CFU from baseline at 24 hours and the eravacycline free-drug AUC_{0-24}:MIC ratio based on data for five *E. coli* isolates is shown in **Figure 3a.**
  - The magnitude of eravacycline free-drug AUC_{0-24}:MIC ratio associated with net bacterial stasis and 1- and 2- $\log_{10}$ CFU reductions from baseline was 15.3, 20.5, and 28.8, respectively.
- **Figure 3B** shows the same relationship between change in $\log_{10}$ CFU from baseline at 24 hours and the eravacycline free-drug AUC_{0-24}:MIC ratio as shown in **Figure 3A** but with the five *E. coli* isolates color-coded by tetracycline susceptibility.

Figure 3. Relationships between change in $\log_{10}$ CFU from baseline at 24 hours and eravacycline free-drug AUC_{0-24}:MIC ratio based on data for five *E. coli* isolates (A) and tetracycline susceptibility (B)



CONCLUSIONS

- Eravacycline free-drug AUC_{0-48}:MIC ratio was the PK-PD index that was most associated with eravacycline efficacy.
- The magnitude of eravacycline free-drug AUC_{0-24}:MIC ratio associated with net bacterial stasis and 1- and 2- $\log_{10}$ CFU reductions from baseline was 15.3, 20.5, and 28.8, respectively, based on data from five *E. coli* isolates.
- These data will be useful to support eravacycline dose selection and recommendations for interpretive criteria for *in vitro* susceptibility testing of eravacycline against Enterobacteriaceae.

REFERENCES

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ACKNOWLEDGMENTS

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