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PK/PD of the Novel Fluorocycline Eravacycline in a Murine *E. coli* Thigh Model

J. V. Newman[1], M. E. Pulse[2], W. J. Weiss[2]

[1]Tetraphase Pharmaceuticals, Watertown, MA; [2]UNT Health Science Center, Fort Worth, TX

Contact:
Tetraphase Pharmaceuticals
medinfo@tphase.com

Abstract

Background:

Eravacycline (ERV) is a novel, fully-synthetic fluorocycline antibiotic being developed for the treatment of serious infections, including those caused by multidrug-resistant (MDR) pathogens. This study evaluated the efficacy of eravacycline in a neutropenic mouse thigh infection model with *Escherichia coli*. The objective of the study was to (1) determine the pharmacokinetic/ pharmacodynamic (PK/PD) indices best-correlated with eravacycline efficacy and (2) determine the relative PK/PD magnitudes required for efficacy versus three isolates of *E. coli*.

Methods:

Female CD-1 mice were rendered neutropenic by IP injection of cyclophosphamide at 150 mg/kg (Day -4) and 100 mg/kg (Day -1) pre-infection. Mice were infected intramuscularly with approximately 6 $\log_{10}$ CFU for each of the *E. coli* isolates. Intravenous (IV) eravacycline administration was initiated at 2hr post-infection and was administered QD (q24h), BID (q12h) and QID (q6h) in the dose fractionation study and BID (q12h) for the dose response studies. Groups of infected mice were used for determining free plasma concentrations. The exposure for each dose group was determined using the mean values for each time point in a 2-compartment model. The delta in CFU/thigh was determined for each animal in the efficacy group and used in the PK/PD analysis.

Results:

Eravacycline free plasma exposures were non-linear across the IV dose range employed. Eravacycline demonstrated significant efficacy against all three *E. coli* isolates. Based upon free plasma levels, fAUC/MIC was the PK/PD index best-associated with efficacy. Eravacycline fAUC/MIC ratios associated with a static response were 16.1 ± 5.2 and for a 1-log reduction were 23.8 ± 9.9.

Conclusions:

Eravacycline showed dose-dependent efficacy *in vivo* against three *E. coli* isolates in neutropenic mice. The PK/PD parameter best-associated with efficacy was the free AUC/MIC and is considered to be the major parameter associated with *in vivo* activity.

Background

Eravacycline is a novel, fully-synthetic fluorocycline antibiotic in phase 3 clinical development for the IV treatment of complicated intra abdominal infections (cIAI) and complicated urinary tract infections (cUTI). The purpose of this study was to evaluate the *in vivo* efficacy of eravacycline in a neutropenic mouse thigh infection model with *Escherichia coli*. The objectives of the study were to (1) determine the pharmacokinetic/pharmacodynamic (PK/PD) indices best-correlated with eravacycline efficacy and (2) determine the relative PK/PD magnitudes required for efficacy versus three isolates of *E. coli*, including two isolates with known tetracycline resistance mechanisms.

Methods

Strain source

Three *E. coli* isolates were utilized in dose fractionation and dose response studies to assess the fAUC/MIC magnitudes associated with efficacy (Table1).

Table 1. *E. coli* isolates utilized in the murine neutropenic thigh model with IV administration.

Strains	Phenotype	ERV Modal MIC (µg/mL)
ATCC25922	CLSI QC strain	0.125
UNT244	*tet*(B), CTX-M-9/14	0.125
UNT245	*tet*(M), ESBL	0.25

Experimental Design

Female CD-1 mice (22 ± 2 g) were rendered neutropenic by IP injection of cyclophosphamide (Cytoxan) at 150 mg/kg (Day -4) and 100 mg/kg (Day -1) pre-infection. Mice were infected intramuscularly (IM) in a volume of 100 µL of a predetermined inoculum (~6 $\log_{10}$ CFU) into the right rear thigh of the test mice at time = 0.

Eravacycline was dosed IV starting at 2hr post-infection. Eravacycline was administered QD (q24h), BID (q12h) and QID (q6h) in the *E. coli* ATCC25922 dose fractionation study. Total daily doses equivalent to 10, 5, 2.5, 1 and 0.5 mg/kg/day were administered. Dose response studies with *E. coli* UNT244 and UNT245 utilized a BID(q12h) regimen and total daily doses equivalent to 40, 20, 10, 5, 2.5 and 1 mg/kg/day.

Groups of satellite mice infected with *E. coli* ATCC25922 as described above were used for determining plasma

Methods (cont'd)

concentrations. The plasma profile for each dose group was obtained by calculating the average plasma concentration of compound in each of the three animals per time point following a single IV administration of eravacycline. Free plasma concentrations were calculated using the free plasma relationship derived previously [Thabit]. The PK properties for each dose group was determined by non-compartmental analysis (WinNonLin 5.2, Pharsight).

The correlation between efficacy and the PK/PD indices total AUC/MIC, C_{max}/MIC and %T>MIC were determined by non linear regression (WinNonlin 5.2, Pharsight). The data were modelled using a sigmoidal E_{max} shown below, where E_{max} is the maximum growth observed in the absence of drug, E_0 is the maximum kill, EC_{50} is the concentration that gives 50% of response, and N is the Hill factor.

$$E = E_{max} - (E_{max} - E_0) \cdot \frac{C^N}{C^N + EC_{50}{}^N}$$

The PK/PD analysis was performed using the individual responses. The goodness of fit was determined by evaluating R^2 value for each PK/PD indices.

The PK/PD magnitudes associated with efficacy from the *E. coli* UNT244 and *E. coli* UNT245 dose response studies were calculated separately. To make a direct comparison, the *E. coli* ATCC25922 BID, q12h regimen data was analyzed independent from the QD (q24h) and QID (q6h) results.

Results

E. coli ATCC25922 dose fractionation study:

- From the *E. coli* ATCC25922 dose fractionation study, the relationship between the difference in the CFU/thigh for each animal and the pharmacokinetic parameter of fAUC/MIC, fC_{max}/MIC and %fT>MIC is shown in Figure 1.
- The correlation coefficients were 0.88, 0.78 and 0.85 for fAUC/MIC, fC_{max}/MIC and %fT>MIC, respectively. The weighted sum of the squared residuals was 40, 68 and 48 for fAUC/MIC, fC_{max}/MIC and %fT>MIC, respectively.
- The fAUC/MIC magnitudes associated with efficacy obtained in the full dose fractionation are depicted in Table 2. The fAUC/MIC required for the EC_{50} and a static effect were calculated to be 10.3 and 15.9, respectively.

Results (cont'd)

Figure 1. Relationship between the delta CFU/thigh and the PK/PD parameters of fAUC/MIC (A), fC_{max}/MIC (B), and the time the free plasma concentrations remain above MIC, %fT>MIC (C) associated with eravacycline efficacy following IV administration

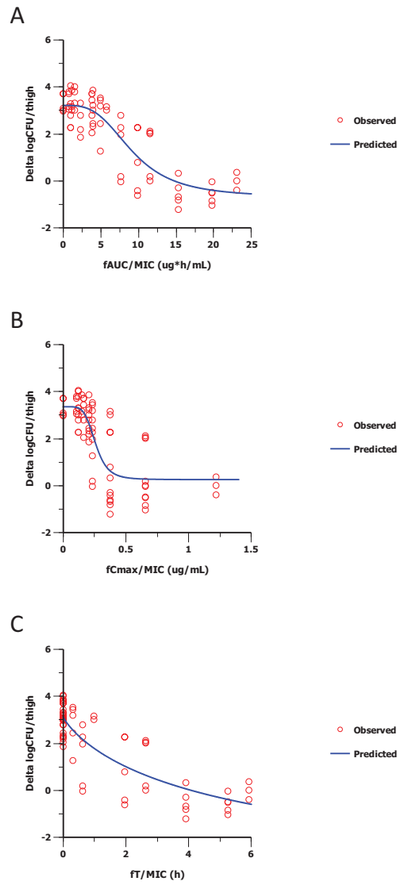

Table 2. Calculated free plasma PD/PK magnitudes associated with eravacycline efficacy in the *E. coli* ATCC25922 dose fractionation study following IV administration.

Indices	EC_{50}±SE (%CV)	Stasis	R^2	WSSR
fAUC/MIC	10.3 ± 1.2 (9.8)	15.9	0.88	40
fC_{max}/MIC	0.3 ± 0.02 (9.8)	NR	0.78	68
fT>MIC	5.9 ± 15.8 (269)	4.1	0.85	48

EC_{50}±SE, 50% effect concentration ± standard error; %CV, percent coefficient of variance; Stasis, free exposure required for a net static effect; R^2, correlation coefficient; WSSR, weighted sum of the squared residuals; NR, not reached.

E. coli dose response studies:

Table 3. Free plasma AUC/MIC magnitudes associated with eravacycline efficacy in the neutropenic murine thigh infection model following IV administration.

				fAUC/MIC magnitude	
Isolate	Burden	Vehicle (Mean ± SD)	E_{max}	Stasis	1-log reduction
ATCC25922	5.04	3.30 ± 0.38	-0.58	15.4	NR
UNT244	5.77	3.29 ± 0.28	-1.63	21.6	30.8
UNT245	5.85	3.31 ± 0.25	-2.08	11.3	16.8
			Mean ± SD	16.1 ± 5.2	23.8 ± 9.9

Burden, mean $\log_{10}$ CFU at the start of treatment; EC, *E. coli*; Emax, maximum CFU $\log_{10}$ reduction in the model; NR, not reached; SD, standard deviation; Vehicle, growth in $\log_{10}$ CFU above burden at start of treatment. NC not calculated

Conclusions

- Eravacycline showed dose–dependent efficacy *in vivo* against three *E. coli* isolates in neutropenic mice.
- The PK/PD parameter best-associated with efficacy was the free AUC/MIC and is considered to be the major parameter associated with the *in vivo* activity.
- The free AUC/MIC required for a bacteriostatic effect and 1 log kill in the neutropenic model ranged from 11 to 22 and from 17 to 31, respectively.

References

1. Thabit AL, Monogue ML, Nicolau DP. 2016. Eravacycline pharmacokinetics and challenges in defining humanized exposure *in vivo*. *Antimicrob Agents Chemother*. 60: 5072-5075.