A-015
53rd Annual ICAAC
10-13 September, 2013
Denver, CO

Plasma Protein Binding of Eravacycline in Mouse, Rat, Rabbit, Cynomolgus Monkey, African Green Monkey and Human Using Microdialysis

R.S.P. SINGH[1], N.M.S. FALCAO[1], J. SUTCLIFFE[2], H. DERENDORF[1]
[1]University of Florida, Gainesville, FL; [2]Tetraphase Pharmaceuticals, Inc., Watertown, MA

Contact:
Ravi S. P. Singh
University of Florida
ravi.singh@ufl.edu

Abstract

Background: Eravacycline (TP-434), a novel fluorocycline antibiotic under development, demonstrated anti-infective activity against a wide range of microorganisms including ESKAPE pathogens. To test the efficacy of eravacycline against some highly infectious pathogens, animal models are used. Since only the unbound fraction of drug is responsible for pharmacodynamic activity, the determination of plasma protein binding is necessary to calculate the dose that will give the desired *f*AUC/MIC ratio in these species. Microdialysis is a sampling technique that determines the unbound fraction of a drug and it has been used for *in vivo*, *ex vivo* and *in vitro* sampling. In the present study, we determined the plasma protein binding of eravacycline in mouse, rat, rabbit, cynomolgus monkey, African green monkey ,and human using the microdialysis technique. **Methods:** The microdialysis probe recovery was determined using the extraction efficiency technique at a perfusate flow rate of 1.5 µL/min. The eravacycline plasma protein binding was determined at 0.1, 1, 10 and 100 µg/mL in triplicate in pooled heparinized plasma of different species at 37ºC. The collected dialysate was analyzed using a LC-MS/MS method developed with deuterated internal standards. **Results:** The average probe recovery was 86.4%. The LC-MS/MS method had accuracy, precision and linearity from a range of 10 – 1000 ng/mL eravacycline with a %CV ≤15%. The range of unbound fraction of eravacycline varied among the species and decreased with the increase in the total drug concentration. The free fractions of eravacycline were 20.7±3.7, 11.7±1.3, 5.3±0.8, 31.3±3.6, 39.7±7.4, 49.2±8.7% at 0.1 µg/mL in human, African green monkey, cynomolgus monkey, New Zealand white rabbit, Sprague-Dawley rat and BALB/c mouse, respectively, while the free fractions were 10.5±2.0, 3.1±1.4, 1.0±0.5, 17.4±2.7, 2.1±1.3 and 13.6±2.7 %, respectively, at 10 µg/mL. **Conclusion:** Eravacycline displays atypical nonlinear plasma protein binding in all tested species. The cause of the class-specific inverse characteristic is currently unknown.

Introduction

Eravacycline (TP-434), a novel fluorocycline antibiotic currently under development at Tetraphase Pharmaceuticals, Inc., is effective against tetracycline-susceptible and tetracycline-resistant bacteria. The pharmacokinetics of eravacycline suggests its potential for intravenous, once a day therapy. Additionally, eravacycline is orally bioavailable and has demonstrated anti-infective activity against a wide range of microorganisms including ESKAPE pathogens (Tables 1 and 2). This makes eravacycline suitable as a first-line IV antibiotic for severe infections with a potential option of a step-down oral therapy [1-3].

To test the efficacy of eravacycline against some highly infectious pathogens (*e.g., Bacillus anthracis, Francisella tularensis, Yersinia pestis*), where efficacy testing in humans is not possible, animal models are used in place of human clinical trials. Since only the unbound fraction of drug is responsible for pharmacodynamic activity, the determination of plasma protein binding is necessary to calculate the dose that will give desired *f*AUC/MIC ratio in these species.

Microdialysis is a sampling technique that determines the unbound fraction of a drug and it has been used for *in vivo*, *ex vivo* and *in vitro* sampling [4-6]. In the present study, we determined the plasma protein binding of eravacycline in mouse, rat, rabbit, cynomolgus monkey, African green monkey and human using the microdialysis technique.

Table 1: Activity of Eravacycline against "ESKAPE" Pathogens

Organism	N	MIC_{50}/MIC_{90} in µg/ml MIC range					
		Eravacycline	Tigecycline	Daptomycin	Linezolid	Vancomycin	Fluoro-quinolone[a]
Enterococcus faecalis	194	**0.06/0.13** ≤0.016-0.13	**0.13/0.25** ≤0.016 - 0.5	**2/4** 0.13 - 8	**2/2** ≤0.5 - 32	**2/>64** 0.5->64	**>8/>32** 0.25->32
Enterococcus faecium	153	**0.06/0.06** ≤0.016 - 0.5	**0.06/0.13** ≤0.016 - 0.5	**4/8** 1 - 16	**2/4** ≤0.5 - 32	**2 />64** ≤0.5 - >64	**>32/>32** 0.25 - >32
Staphylococcus aureus	408	**0.06/0.25** ≤0.016 - 4	**0.13/0.25** ≤0.016 - 16	**1/1** 0.063 - 4	**2/2** 1 - 64	**1/1** 0.5 - 8	**1/32** 0.06 - >64
Staphylococcus aureus (MRSA)	284	**0.06/0.13** 0.016-4	**0.13/0.25** ≤0.016 - 1	**1/1** 0.063 - 4	**2/4** 1 - 64	**1/1** 0.5-8	**8/>32** 0.06 - >64

N = number of isolates; [a]Fluoroquinoline = Levofloxacin/Ciprofloxacin

Table 2: Activity of Eravacycline against "ESKAPE" Pathogens

Organism	N	MIC_{50}/MIC_{90} in µg/mL MIC range						
		Eravacycline	Tigecycline	Carbapenem[a]	Fluoro-quinolone[b]	3rd Gen Ceph[c]	Amino-glycoside[d]	Piperacillin/ Tazobactam
Klebsiella pneumoniae	394	**0.5/2** 0.03 - 16	**0.5/2** 0.13 - 16	**0.25/>8** ≤0.002 - >32	**0.5/>32** ≤0.25 - >64	**8/>32** ≤0.016 - >64	**0.5/>32** ≤0.25 - >64	**8/>128** ≤0.5 - >128
Klebsiella pneumoniae 3rd Gen I/R	210	**0.5/2** 0.03 -16	**1/4** 0.13 - 16	**1/16** ≤1 - >32	**>4/>32** ≤0.25 - >64	**>32/>64** 4 - >64	**4/16** ≤0.25- >32	**>64/>128** 0.5- >128
Carbapenem-resistant *K. pneumoniae*	90	**0.5/2** 0.13 - 16	**1/2** 0.25 - 16	**2/>8** 0.13 - >32	**>4/>32** 0.06 - >64	**>32/>32** 1 - 64	**4/>8** 0.25 - >32	**>64/>128** 4 - >128
Acinetobacter baumannii	188	**0.25/1** ≤0.016 - 8	**0.5/4** ≤0.016 - 8	**2/32** 0.13 - >32	**>2/>2** 0.016 - >32	**>16/>32** 0.13 - >32	**8/>32** ≤0.25 - >32	**>64/>128** ≤0.5 - >128
Pseudomonas aeruginosa	145	**8/32** 1 - >32	**16/32** 1 - >32	**2/>8** 0.13 - >32	**1/>4** 0.06 - >32	**>16/>32** 1 - >64	**2/>8** 0.13 - >32	**8/>128** >64 - >128
Enterobacter cloacae	270	**0.5/2** 0.03 - 4	**0.5/2** 0.06 - 8	**0.5/2** 0.03 - >32	**≤0.25/>4** 0.008 - >32	**2/>64** 0.03 - >64	**0.5/8** ≤0.25 - >32	**4/>64** 0.5 - >128

N = number of isolates; [a]Carbapenem = Ertapenem/Imipenem/Meropenem; [b]Fluoroquinoline = Levofloxacin/Ciprofloxacin; [c]Third generation cephalosporin (Ceftazidime/Cefotaxime/Ceftriaxone; [d]Aminoglycoside = Gentamicin/Tobramycin

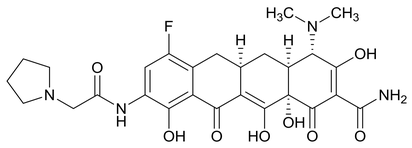

Figure 1. The chemical structure of eravacycline

Methods

BIOANALYTICAL METHOD: A sensitive HPLC-MS/MS method for the quantification of eravacycline in saline and plasma was developed using API 4000 (AB Sciex, Framingham, MA, USA) coupled with PerkinElmer Series 200 pump and auto sampler. The chromatographic conditions were optimized to chromatographically separate eravacycline from its C4-epimer TP-498. The linearity range of eravacycline in saline was established. Plasma samples were precipitated by the addition of 0.5% formic acid in acetonitrile. The linearity ranges of eravacycline in plasma from different species were established.

MD PROBE AND SETUP: The CMA 63 probe (P/N: 8010514, M Dialysis, Solna, Sweden) MD probe was used in the protein binding studies. The probe consists of a polyarylethersulfone (PAES) membrane (20,000 Da cut-off) and the polyurethane shaft, inlet and outlet tubes. The length of the probe membrane was 30 mm and outer diameter (OD) was 0.6 mm. Before the start of the experiment on each day, the integrity of the probe was ascertained by pumping perfusate (blank saline) and collecting dialysate while the probe was placed in the blank saline. The perfusate was pumped using a Harvard syringe pump 22 (Harvard Apparatus, Holliston, Massachusetts, USA) at the flow rate 1.5 µL/min and dialysate was collected on ice in an amber-colored 0.5 mL polypropylene microcentrifuge tube. The temperature of the sample (drug solution in saline in the case of probe recovery or plasma) was maintained at 37ºC for the duration of the experiment and the entire set up was covered to protect from any light sensitivity of the compound (Figure 2).

PROBE RECOVERY: The probe recovery of eravacycline was established at 0.1 and 100 µg/mL using the extraction efficiency technique. The probe was placed in a reservoir tube containing an eravacycline solution in saline and blank saline was perfused at 1.5 µL/min. An aliquot of 20 µL was collected from the reservoir tube (RT) before its incubation at 37ºC. The probe was equilibrated for 20 minutes and the dialysates were collected after 40 and 60 min. An aliquot of 20 µL was collected from the RT at 20, 40 and 60 min. The probe recovery was calculated using equation 1, where $C_{Dialysate}$ (T1, T2) is the dialysate collected between time interval T1 and T2, C_{RT}(T1) and C_{RT}(T2) are the concentrations of eravacycline in the reservoir tube at times T1 and T2, respectively. The probe recoveries were averaged to calculate final percent probe recovery.

PROTEIN BINDING STUDIES: The plasma protein binding was studied at four concentrations of eravacycline (0.1, 1, 10 and 100 µg/mL). Eravacycline was spiked into mixed gender pooled plasma (N≥6) (previously frozen) and an aliquot of 100 µL was collected before incubation at 37ºC. The probe was placed in a reservoir tube containing test compounds in plasma and blank saline was perfused at 1.5 µL/min. The probe was equilibrated for 20 minutes and the dialysates were collected at 40 and 60 min. An aliquot of 100 µL was collected from the RT at 20, 40 and 60 min. The percent free fraction was calculated using equation 2, where $C_{Dialysate}$ (T1, T2) is the dialysate collected between time interval T1 and T2, CP_{RT}(T1) and CP_{RT}(T2) are the concentrations of eravacycline in plasma at time T1 and T2, respectively. The calculated percent free fractions were averaged to calculate final percent free fraction.

$$\%\,Probe\,Recovery = \frac{2 \times C_{Dialysate}(T1, T2)}{C_{RT}(T1) + C_{RT}(T2)} \times 100 \qquad \text{Equation 1}$$

$$\%\,Free\,Fraction = \frac{2 \times C_{Dialysate}(T1, T2)}{CP_{RT}(T1) + CP_{RT}(T2)} \times \frac{100}{\%\,Probe\,Recovery} \times 100 \qquad \text{Equation 2}$$

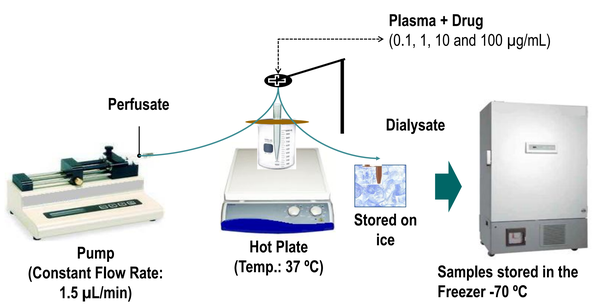

Figure 2. Schematic representation of microdialysis setup for the determination of plasma protein binding

Results

BIOANALYTICAL METHOD: Eravacycline was separated from its C4-epimer TP-498 using reverse phase liquid chromatography. A gradient method with varying proportions of 0.5% formic acid in water (A) and 0.5% formic acid in acetonitrile:methanol (50:50) (B) and a flow rate of 0.8 mL/min was used. The HPLC column used was a Varian Polaris C18-A, 50×3 mm, 3 µm column with Polaris 3 C18-A Metaguard 2.0 mm guard column. The column chromatography conditions separated eravacycline from TP-498. The eluate was split 1:1 before detection by the mass spectrometer. Q1/Q3 mass ion pairs used for eravacycline and internal standards were 280.2/84.0 and 284.0/92.2, respectively. The linearity range was 10-1000 ng/mL. The intra- and inter-day accuracy were within ±15 % of nominal value and the precision (CV) was below 15%.

PROBE RECOVERY: The probe recovery of eravacycline was 88.5±8.3 and 84.2±7.2 at 0.1 and 100 µg/mL. The average recovery (86.4%) was used for calculations.

PROTEIN BINDING : In all six species, the free fraction of eravacycline in plasma decreased as the total concentration of eravacycline increased in the plasma (Figure 3).

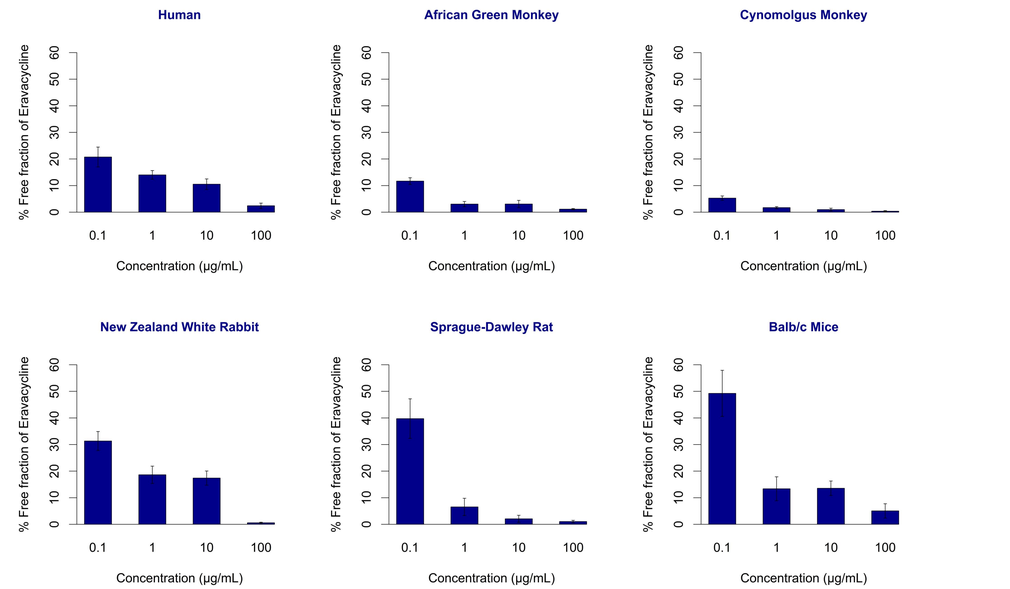

Figure 3. The percent free eravacycline in the pooled plasma from six different species at four different concentrations (0.1, 1, 10 and 100 µg/mL) of total eravacycline (n=3). The percent free fraction of eravacycline at 1, 10 and 100 µg/mL was significantly lower (p<0.001) than 0.1 µg/mL in all six species.

Conclusion

Eravacycline displays atypical nonlinear plasma protein binding in all tested species. In all species, the percent free fraction decreased with the increase in total concentration of eravacycline. Among the tested species, mice had the highest unbound fraction of eravacycline while cynomolgus monkey had the lowest unbound fraction. Tigecycline, another drug from the same class of compounds, also shows atypical nonlinear plasma protein binding [7]. The cause of the class-specific inverse characteristic is currently unknown and requires further investigation.

Acknowledgements

Authors thank Sebastian Pieper, Angelika Volk, Julia Richartz and Melissa Softa for their help with microdialysis experiments. Authors also thank Dr. David Christ for his insights during method development. This project has been funded in whole or in part with Federal funds from the Biomedical Advanced Research and Development Authority, Office of the Assistant Secretary for Preparedness and Response, Office of the Secretary, Department of Health and Human Services, under Contract No HHSO100201200002C.

References

1. Tetraphase Presents TP-434 Data accessed on 24th May 2012 at <http://www.dddmag.com/news/2010/09/tetraphase-presents-tp-434-data> updated on 15th September 2010.
2. Sutcliffe, J., Grossman, T., Ronn, M., Xiao, X, Leighton, A., TP-434 Has Potential to Treat Complicated Urinary Tract Infections (cUTI), accessed on 24th May 2012 at <http://www.tphase.com/files/P4%20TP-434%20for%20cUTIs.pdf>
3. Christ, D. and Sutcliffe, J., TP-434 is Metabolically Stable and Has Low Potential for Drug-Drug Interactions, accessed on 24th May 2012 at <http://www.tphase.com/files/F1-2162.pdf>
4. Schmidt, S., Banks, R., Kumar, V., Rand, K.H., and Derendorf ,H., Clinical microdialysis in skin and soft tissues: an update, **2008**, J Clin Pharmacol., 48 (3): 352–64
5. Quellec, A.L., Dupin, S., Tufenkji, A.E., Genissel, P., Houlin, G., Microdialysis: An alternative for in vitro and in vivo protein binding studies, Pharmaceutical Research, **1994**, 11(6): 835-838.
6. Sarre, S., Belle K.V., Smolders, I., Krieken, G., Michotte, Y., The use of microdialysis for the determination of plasma protein binding of drugs, J. Pharmaceut. Biomed Anal., **1992**, 10(10-12): 735-739.
7. Muralidharan, G., Micalizzi, M., Speth, J., Raible,D., Troy, S., Pharmacokinetics of Tigecycline after Single and Multiple Doses in Healthy Subjects. Antimicrob. Agents Chemother. **2005,** 49(1):220-229.