P1991

Efficacy of Cefpodoxime proxetil and ETX0282 in a murine UTI model with *E. coli* and *K. pneumoniae*

W. J. WEISS[1], K. CARTER[1], M. E. PULSE[1], P. NGUYEN[1], D. VALTIERRA[1], K. PETERSON[1], R. TOMMASI[2], J. P. MUELLER,[2] J. O'DONNELL[2]

[1]UNTHSC PreClinical Services, Ft. Worth, TX, [2]Entasis Therapeutics, Waltham, MA

* Contact Information:
UNT Health Science Center
3500 Camp Bowie Blvd.
Fort Worth, TX 76107
william.weiss@unthsc.edu
www.unthsc.edu/research/preclinical

ECCMID
Amsterdam, Netherlands
13-16 April 2019

UNT HEALTH SCIENCE CENTER

Abstract

Background: The increase in ESBL-producing uropathogenic *E coli* (Eco) and *K. pneumoniae* (Kpn) in urinary tract infections (UTI) is an important health concern. This study was conducted to evaluate the efficacy of cefpodoxime proxetil w/wo co-administration of ETX0282, an orally bioavailable prodrug of ETX1317, a broad spectrum inhibitor of Class A and C β-lactamases, in a murine ascending UTI model against CTX-M-14 Eco and KPC-2 Kpn clinical isolates.

Methods: The *in vitro* MIC values were determined by broth microdilution methods in accordance with CLSI guidelines. Female C3H/HeJ mice were placed on 5% glucose water for 6 days and transurethrally infected with 9.00 log_{10} of the Eco or 8.95 log_{10} CFU of the Kpn. Treatments of cefpodoxime proxetil (CPDP) alone and CPDP:ETX0282 (varying ratios) were initiated 4 days post-infection and administered orally (PO) every 6 hours for 3 days. Kidneys, bladders, and urine were collected and processed for CFU counts ~12 hours after the final dose.

Results: Bacterial titers for the Eco were 6.53, 6.02 and 6.64 log_{10} CFU in kidneys, bladders and urine for the untreated controls on Day 7. Administration of CPDP or ETX0282 alone resulted in an approx. 1 log CFU decrease in Eco titers in kidneys and urine. The combination of CPDP (50 mg/kg) + ETX0282 (5 – 50 mg/kg) reduced titers to 2.62-2.93 log_{10} CFU in the kidneys and 2.05–2.73 log_{10} CFU in urine.
Bacterial titers for the Kpn were 6.38, 3.45 and 5.19 log_{10} CFU in kidneys, bladders and urine for the untreated controls on Day 7. Administration of CPDP or ETX0282 alone resulted in an approx. 1-2 log CFU decrease in Kpn titers in kidneys and urine. The combination of CPDP (50 mg/kg) + ETX0282 (5 – 50 mg/kg) reduced titers to 2.67-2.79 log_{10} CFU in the kidneys and 2.05–2.39 log_{10} CFU in urine.

Conclusion: In this study with Eco and Kpn, co-administration of CPDP and ETX0282 significantly reduced bacterial titers in all three tissues as compared with CPDP or ETX0282 alone. The results demonstrate that ETX0282 rescues cefpodoxime efficacy in a urinary tract infection model with both *E. coli* CTX-M-14 and *K. pneumoniae* KPC-2 clinical isolates.

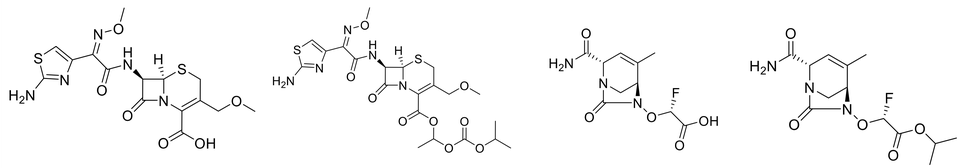
Introduction

Extended-spectrum β-lactamase (ESBL) producing Gram-negative bacteria have been increasingly reported as causes of urinary tract infections (UTI). CTX-M and KPC type β-lactamases have proved by far the most successful in disseminating in the clinical setting and have overall become the most prevalent ESBLs worldwide. Limited treatment options are available for UTIs caused by these organisms.
ETX0282 is an orally bioavailable prodrug of ETX1317, a broad spectrum inhibitor of Class A and C β-lactamases. ETX0282, the oral prodrug of ETX1317, is used in combination with cefpodoxime proxetil (CPDP), which is hydrolyzed *in vivo* to release cefpodoxime (CPD). The current study was conducted to determine the efficacy of CPDP w/wo co-administration of ETX0282 in a murine urinary tract infection model using an ESBL producing *E.coli* and *K. pneumoniae* clinical isolate.

Methods and Materials

Mice: 5 - 6 week old, Female C3H/Hej mice (20-22 gm).
Strain: *Escherichia coli* UNT209-1 (CTX-M-14) and *Klebsiella pneumoniae* UNT170-1 (KPC-2).
Minimum Inhibitory Concentration Assays (MIC): MICs of CPD w/wo ETX1317 at a 1:2 ratio were determined by broth microdilution in accordance with CLSI guidelines.
Preparation: Mice were kept on water with 5% glucose starting 6 days prior to infection and for the duration of the study.
Infection: Anesthetized mice were trans-urethrally inoculated with 50 μL of a prepared inoculum (~9 Log_{10} CFU) via a PE_{10} catheter. The bacteria ascend the urinary tract and localize in the kidneys by 4 days after infection.
Treatment: CPDP, ETX0282 and CPDP + ETX0282 were administered orally (PO), q6hrs x 3 days starting 4 days post-infection. Meropenem was administered by subcutaneous (SC) injection, q6hrs x 3 days, starting 4 days after infection.
Sampling / Endpoint: Urine was collected before animals were euthanized on days 4 and 7 after infection. Kidneys and bladder were collected after euthanasia in sterile PBS, homogenized, diluted and plated for the determination of bacterial CFU titers ~ 18 hrs. after the last administered dose. Plates were incubated at 37° C overnight and colony counts were generated for each sample the next day.

Figure 1. Chemical Structures



Cefpodoxime (CPD) | Cefpodoxime Proxetil (CPDP) | ETX1317 | ETX0282

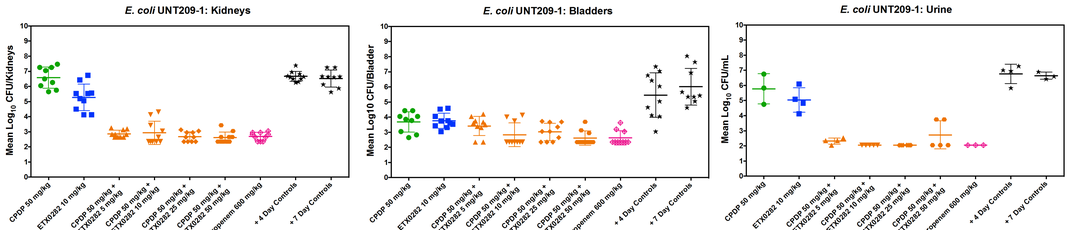
Table 1. Minimum Inhibitory Concentrations of Cefpodoxime w/wo ETX1317

	MIC (ug/mL)	
Test Article	*E. coli* UNT209-1	*K. pneumoniae* UNT170-1
Cefpodoxime	128	> 128
ETX1317	0.125	4
Cefpodoxime + ETX1317*	≤ 0.0625	0.25
Meropenem	≤ 0.0625	32

* Combination tested at 1:2 ratio

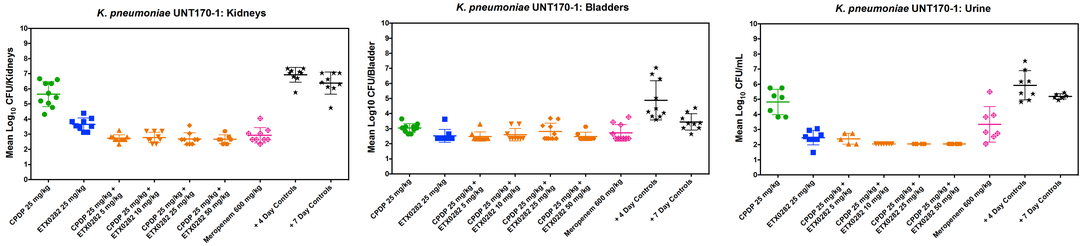
Panel 1. Mean log_{10} CFU of *E. coli* UNT209-1 in Kidneys, Bladder & Urine

Test Article	Dose (mg/kg)	Kidneys		Bladders		Urine	
		Mean Log $_{10}$ CFU	SD	Mean Log $_{10}$ CFU	SD	Mean Log $_{10}$ CFU	SD
CPDP	50	6.58	0.69	3.68	0.67	5.78	1.00
ETX0282	10	5.28	0.88	3.76	0.50	5.04	0.81
CPDP + ETX0282	50 + 5	2.87	0.25	3.42	0.62	2.32	0.20
	50 + 10	2.93	0.77	2.84	0.78	2.05	0.00
	50 + 25	2.68	0.32	3.03	0.57	2.05	0.00
	50 + 50	2.62	0.37	2.62	0.47	2.73	0.93
Meropenem	600	2.70	0.27	2.63	0.48	2.05	0.00
+4 Day Controls	NA	6.67	0.33	5.46	1.47	6.77	0.64
+7 Day Controls	NA	6.53	0.56	6.02	1.22	6.64	0.24



Panel 2. Mean log_{10} CFU of *K. pneumoniae* UNT170-1 in Kidneys, Bladder & Urine

Test Article	Dose (mg/kg)	Kidneys		Bladder		Urine	
		Mean Log $_{10}$ CFU	SD	Mean Log $_{10}$ CFU	SD	Mean Log $_{10}$ CFU	SD
CPDP	25	5.65	0.83	3.04	0.30	4.82	0.84
ETX0282	25	3.65	0.42	2.53	0.43	2.47	0.48
CPDP + ETX0282	25 + 5	2.74	0.24	2.48	0.31	2.39	0.35
	25 + 10	2.79	0.34	2.60	0.41	2.05	0.00
	25 + 25	2.70	0.40	2.81	0.56	2.05	0.00
	25 + 50	2.67	0.29	2.49	0.28	2.05	0.00
Meropenem	600	2.93	0.50	2.72	0.55	3.35	1.17
+4 Day Controls	NA	6.93	0.49	4.88	1.30	5.93	0.96
+7 Day Controls	NA	6.38	0.73	3.45	0.55	5.19	0.17



Summary and Conclusions

- In the mouse urinary tract infection model, Cefpodoxime proxetil alone was ineffective at reducing the bacterial titers of either the *E. coli* or *K. pneumoniae* isolate in the kidneys or urine.
- Administration of ETX0282 alone reduced bacterial titers in kidneys, bladder and urine by 1.5 – 3 log_{10} CFU when compared to the infection control groups for both strains.
- Co-administration of CPDP (50mg/kg) + ETX0282 (5-50 mg/kg) further reduced bacterial titers by up to 3 log_{10} CFU in the kidneys, bladders and urine when compared to CPDP or ETX0282 administered alone.
- In general CPDP + ETX0282 reduced the bacterial load in the kidneys, bladder and urine to near the limit of detection for each tissue.
- The results indicate that cefpodoxime proxetil + ETX0282 efficiently inhibits the ESBL producing organisms *in vivo* and ETX0282 restores cefpodoxime antimicrobial activity in a urinary tract infection model against the uropathogenic strains of *E. coli* expressing CTX-M-14 and *K. pneumoniae* expressing KPC-2.

References

1) Biosafety in Microbiological and Biomedical Laboratories, CDC web site (04/01/02) http://www.absa.org/riskgroups/index.htm
2) Cleeland, R. and E. Squires. 1991. Evaluation of New Antimicrobials in Vitro and in Experimental Animal Infections. In Antibiotics in Laboratory Medicine", 3rd. ed., edited by Victor Lorian. Willams and Wilkins Baltimore, Maryland. pp. 752 - 783.
3) Guidelines for the Welfare of Animals in Rodent Protection Tests (Lab Animals, 1994,28)
4) Lahlaoui, H., A. Khalifa, M. Moussa. 2014. Epidemiology of Enterobacteriaceae producing CTX-M type extended spectrum b-lactamase (ESBL). Medecine et maladies. 44:400-404.
5) Canton, R., T. Coque. 2006.. Current Opin. Microbiol. 9:466-475.
6) Porreca, A., K. Sullivan, J. Gallagher. 2018. The Epidemiology, Evolution, and Treatment of KPC-Producing Organisms. Curr Infect Dis Rep. 20: 13.

Acknowledgments

The mouse UTI model described herein was conducted in accordance with a protocol (IACUC#1017-0047) approved by the UNTHSC Institutional Animal Care and Use Committee.

The authors gratefully acknowledge the support of the UNT System College of Pharmacy.

This study was funded by Entasis Therapeutics, Waltham, MA

Powered by CARB-X