AAR-726

Efficacy of Ceftibuten + VNRX-7145, a Novel β-lactamase Inhibitor, Against KPC-2 and ESBL *E. coli* Strains in a Murine UTI Model

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ASM Microbe
San Francisco, CA
20-24 June 2019

UNT HEALTH SCIENCE CENTER

Abstract

Background: The increase in ESBL- and/or carbapenemase-producing uropathogenic *E. coli* among community-onset urinary tract infections (UTI) is an important public health concern. VNRX-7145 is an orally bioavailable compound that undergoes biotransformation *in vivo* to the active β-lactamase inhibitor (BLI), VNRX-5236. This study was conducted to evaluate the efficacy of ceftibuten w/wo co-administration of VNRX-5236 in the murine ascending UTI model against *E. coli* strains expressing ESBLs and/or carbapenemases.

Methods: MIC values against three clinical strains of *E. coli* were determined by broth microdilution following CLSI guidelines. Female C3H/HeJ mice were placed on 5% glucose water for 6 days and then transurethrally infected with ~9.0 $\log_{10}$ CFU of each *E. coli* isolate. Treatments of ceftibuten alone, ceftibuten:VNRX-5236 (1:1), or amoxicillin:clavulanate (2:1) were initiated 4 days post-infection and administered subcutaneously BID (q12hr) for 3 days. Kidneys, bladders, and urine were collected and processed for bacterial titer determination 18 hours after the final dose. Efficacy was determined by comparing the mean CFU of treated groups to untreated controls.

Results: Ceftibuten MICs were reduced from 8 – 64 μg/mL to ≤ 0.06 – 0.12 μg/mL with fixed VNRX-5236 concentrations of 1 – 4 μg/mL for each of the strains. At Day 7 post-infection in the UTI model, mean bacterial titers for the 3 bacterial strains were 6.5 – 7.1 $\log_{10}$ CFU in kidney, 5.7 – 6.8 $\log_{10}$ CFU in bladders and 5.9 – 7.2 $\log_{10}$ CFU/mL in urine for the untreated controls. Administration of ceftibuten alone from 1 – 300 mg/kg resulted in a dose response. There were minimal CFU reductions in the kidneys observed and up to 2 $\log_{10}$ CFU lower titers in the bladder and urine at ceftibuten doses of 100 and 300 mg/kg. The addition of VNRX-5236 to ceftibuten treatment resulted in increased efficacy with bacterial titers that were up to 2 $\log_{10}$ CFU lower in the kidneys, 3.2 $\log_{10}$ CFU lower in bladders and up to 4 $\log_{10}$ CFU lower in urine than ceftibuten alone at doses of 3, 10, 30, 100 and 300 mg/kg.

Conclusion: In this UTI study with *E. coli* isolates expressing ESBLs or a combination of ESBLs and KPC carbapenemase, ceftibuten alone exhibited a dose dependent reduction in bacterial titers. co-administration of ceftibuten and VNRX-5236 (1:1) further reduced the bacterial titers in kidneys, bladders, and urine compared with ceftibuten alone. The results demonstrate that VNRX-5236 rescues ceftibuten activity in a UTI model against uropathogenic strains of *E. coli* expressing TEM-1 + CTX-M-15, CTX-M-15 or KPC-2 + SHV-12.

Introduction

Extended-spectrum β-lactamase (ESBL) producing Gram-negative bacteria have been increasingly reported as causes of urinary tract infections (UTI), which was recently highlighted in a cross-sectional study where 23% of ~700 bacterial UTI isolates were ESBL positive (1). Uropathogenic *Escherichia coli* or UPEC accounts for the majority of all UTI isolates and up to 81% of ICU-associated UPEC have been reported as ESBL producers (2). CTX-M-type ESBLs have significantly emerged among UPEC isolates due to CTX-M's ability to rapidly spread on mobile elements in clinically relevant strains, including multidrug-resistant (MDR) strains (3). CTX-M producing MDR strains often have multiple ESBL genes, as well as aminoglycoside and quinolone resistance genes, thereby limiting available treatment options for UTIs. Therefore, additional treatment options are urgently needed in order to provide sufficient coverage of UTIs caused by MDR strains.

VNRX-7145 is an orally bioavailable compound that undergoes biotransformation *in vivo* to the active β-lactamase inhibitor (BLI), VNRX-5236. VNRX-5236 is a novel broad-spectrum BLI with potent activity against both ESBLs and serine carbapenemases. Here are described the results of an investigation conducted to determine the ability of VNRX-5236 to restore the efficacy of ceftibuten in an ascending murine UTI model infected with ESBL and KPC expressing *E.coli* strains.

Methods and Materials

Bacterial strains: *E. coli* UNT057-1 (CTX-M-15), UNT167-1 (KPC-2, SHV-12), and UNT204-1 (TEM-1, CTX-M-15).

***In vitro* MICs**: Susceptibility of the *E. coli* strains to ceftibuten, VNRX-5236, and ceftibuten plus 1, 2 or 4 μg/mL of VNRX-5236 was determined using the broth microdilution method as defined by CLSI guidelines.

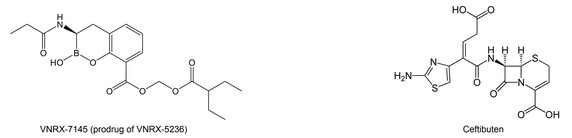
Animals & Housing: Starting 6 days before infection, female C3H/HeJ mice (5-6 weeks old) were maintained on 5% (w/v) glucose water for the duration of each study.

Inoculum preparation and infection: Each *E. coli* strain was cultured in ambient conditions overnight on tryptic soy agar (TSA) at 37ºC. Plate growth was suspended in tryptic soy broth to an optical density that resulted in ~9 $\log_{10}$ CFU being trans-urethrally inoculated in anesthetized mice.

Treatment: Infected animals were randomly separated into groups of 5 and each study had 18 groups. Starting 4 days after infection, treatment groups were SC administered doses of ceftibuten, ceftibuten:VNRX-5236 (1:1), or amoxicillin:clavulanate (2:1) BID for 3 consecutive days.

Study Endpoint. Urine was collected from each group prior to being euthanized by approved methods at 4 and 7 days post-infection. The kidneys and bladder of each euthanized animal were aseptically removed and homogenized in PBS. Urine and tissue homogenates were serially diluted and plated onto BHI agar plates containing 0.5% activated charcoal. Plates were incubated at 37ºC and colony counts were recorded ~20 hours later. Results represent the combination of 2 separate efficacy studies conducted with each *E. coli* strain.

Figure 1. Structures of VNRX-7145 and Ceftibuten



VNRX-7145 (prodrug of VNRX-5236) Ceftibuten

Table 1. *In vitro* Activity of Ceftibuten, VNRX-5236, & Ceftibuten + VNRX-5236 against ESBL Producing *E. coli*

Strain	MIC (μg/mL) Ceftibuten	VNRX-5236	Ceftibuten + 4 μg/mL VNRX-5236	Ceftibuten + 2 μg/mL VNRX-5236	Ceftibuten + 1 μg/mL VNRX-5236
UNT 057-1 (CTX-M-15)	16	>64	0.12	0.02	0.12
UNT 167-1 (KPC-2, SHV-12)	64	>64	0.12	0.12	0.12
UNT 204-1 (TEM-1, CTX-M-15)	8	>64	0.06	0.06	0.06

Panel 1. Efficacy of Ceftibuten, Ceftibuten:VNRX-5236, and Amoxicillin:Clavulanate in a Murine UTI Model Challenged with *E. coli*

Kidney Mean $\log_{10}$ CFU ± Standard Deviations

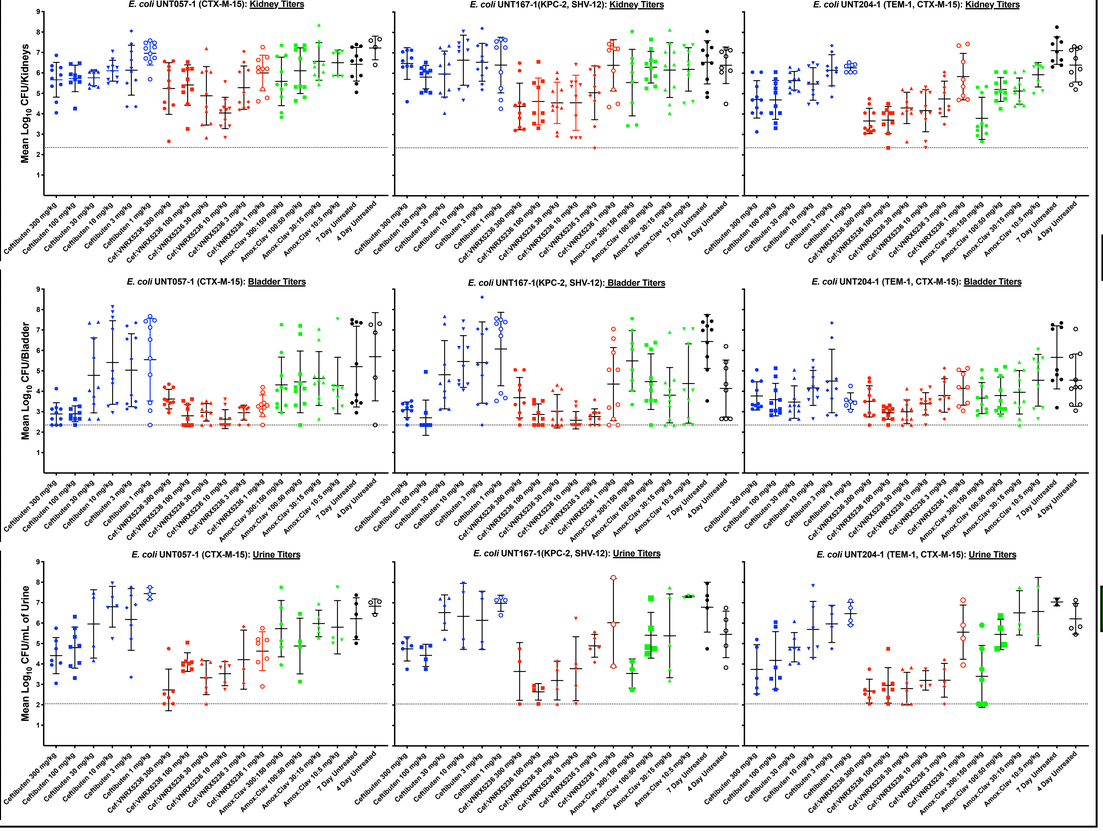
Test Articles	Dose (mg/kg)	UNT057-1 n=	UNT057-1 Mean Values	UNT057-1 SD	UNT167-1 n=	UNT167-1 Mean Values	UNT167-1 SD	UNT204-1 n=	UNT204-1 Mean Values	UNT204-1 SD
Ceftibuten	300	10	5.66	0.85	10	6.09	1.41	10	4.72	0.92
	100	9	5.73	0.65	10	5.50	1.14	10	4.69	0.95
	30	10	5.55	0.77	10	5.96	1.13	10	5.63	0.45
	10	10	6.10	0.51	9	6.64	1.22	9	5.47	0.80
	3	10	6.13	1.22	10	6.54	0.93	9	6.15	0.76
	1	10	6.96	0.57	9	6.40	1.36	7	6.27	0.21
Ceftibuten:VNRX5236	300:300	10	5.23	1.26	10	4.38	1.14	10	3.66	0.62
	100:100	10	5.41	0.99	9	4.62	1.15	10	3.99	1.11
	30:30	10	4.88	1.43	10	4.55	1.01	10	4.48	0.91
	10:10	10	4.04	0.76	10	4.56	1.35	10	4.17	1.04
	3:3	10	5.28	1.06	10	5.05	1.32	9	4.74	0.87
	1:1	10	5.99	0.87	10	6.39	1.26	8	5.84	1.14
Amoxicillin:Clavulanate	300:150	10	5.58	1.19	8	5.55	1.63	10	3.79	1.03
	100:50	10	6.11	1.12	10	6.30	0.77	10	5.20	0.59
	30:15	10	6.56	0.92	10	6.16	1.34	10	5.12	0.64
	10:5	8	6.32	0.78	9	6.19	1.06	9	6.17	0.92
7 Day Untreated Controls	na	10	6.42	0.82	10	6.54	1.06	9	7.11	0.67
4 Day Untreated Cotnrols	na	5	7.10	2.23	8	6.19	0.90	10	6.40	0.83

Bladder Mean $\log_{10}$ CFU ± Standard Deviations

Test Articles	Dose (mg/kg)	UNT057-1 n=	UNT057-1 Mean Values	UNT057-1 SD	UNT167-1 n=	UNT167-1 Mean Values	UNT167-1 SD	UNT204-1 n=	UNT204-1 Mean Values	UNT204-1 SD
Ceftibuten	300	10	2.89	0.55	10	3.22	0.52	10	3.77	0.69
	100	9	2.90	0.38	10	2.70	0.82	10	3.59	0.80
	30	10	4.78	1.84	10	5.14	1.88	10	3.48	0.80
	10	10	5.41	2.05	9	5.46	1.27	9	4.17	0.85
	3	10	5.03	1.79	10	5.41	1.99	9	4.51	1.55
	1	10	5.55	2.02	9	6.07	1.80	7	3.83	0.90
Ceftibuten:VNRX5236	300:300	10	3.62	0.47	10	2.96	0.67	10	3.51	0.77
	100:100	10	2.80	0.54	9	2.87	0.51	10	2.95	0.31
	30:30	10	2.97	0.43	10	3.03	0.82	10	3.00	0.58
	10:10	10	2.64	0.46	10	2.58	0.42	10	3.39	0.55
	3:3	10	2.95	0.37	10	2.76	0.38	9	3.80	0.82
	1:1	10	3.29	0.52	10	4.35	1.79	8	4.14	0.83
Amoxicillin:Clavulanate	300:150	10	4.31	1.35	8	5.36	1.47	10	3.67	0.76
	100:50	10	4.46	1.51	10	4.47	1.36	10	3.79	0.90
	30:15	10	4.63	1.32	10	3.81	1.35	10	3.95	1.07
	10:5	8	4.28	1.38	9	4.38	1.94	9	4.54	1.27
7 Day Untreated Controls	na	10	5.21	1.98	10	6.44	1.32	9	5.66	1.55
4 Day Untreated Cotnrols	na	4	6.53	1.25	8	4.14	1.39	10	4.54	1.28

Urine Mean $\log_{10}$ CFU / mL ± Standard Deviations

Test Articles	Dose (mg/kg)	UNT057-1 n=	UNT057-1 Mean Values	UNT057-1 SD	UNT167-1 n=	UNT167-1 Mean Values	UNT167-1 SD	UNT204-1 n=	UNT204-1 Mean Values	UNT204-1 SD
Ceftibuten	300	7	4.41	0.88	5	4.75	0.59	5	3.74	1.20
	100	8	4.80	1.01	5	3.95	1.16	6	4.18	1.41
	30	4	5.96	1.68	5	6.52	0.86	7	4.82	0.72
	10	5	6.80	1.00	4	6.34	1.61	6	5.69	1.37
	3	7	6.17	1.51	4	6.14	1.42	4	5.96	0.91
	1	3	7.44	0.29	4	5.71	2.47	4	6.47	0.55
Ceftibuten:VNRX5236	300:300	6	2.73	1.02	3	3.64	1.41	7	3.00	0.99
	100:100	7	4.09	0.46	4	2.65	0.41	7	2.96	0.87
	30:30	7	3.33	0.83	4	3.20	0.95	7	2.80	0.79
	10:10	5	3.52	0.59	5	3.78	1.56	3	3.20	0.48
	3:3	3	4.21	1.45	5	4.90	0.56	5	3.21	0.82
	1:1	7	4.62	0.95	3	6.03	2.17	4	5.56	1.32
Amoxicillin:Clavulanate	300:150	6	5.73	1.38	3	3.54	0.71	8	3.27	1.45
	100:50	4	4.88	1.36	5	5.41	1.12	4	5.45	0.74
	30:15	5	5.98	0.65	5	5.39	2.05	3	6.50	1.09
	10:5	4	5.79	1.31	3	7.30	0.05	2	6.57	1.67
7 Day Untreated Controls	na	5	6.21	1.03	5	6.79	1.22	2	7.03	0.19
4 Day Untreated Cotnrols	na	3	6.82	0.36	5	5.46	1.14	5	6.21	0.75



Summary and Conclusions

- The MIC values of ceftibuten (8 to 64 μg/mL) were decreased 128- to 512-fold when combined with 3 fixed concentrations of the β-lactamase inhibitor, VNRX-5236, thereby rendering all 3 *E. coli* strains susceptible to ceftibuten.
- When compared to groups treated with ceftibuten alone, the mean bacterial titers of groups treated with equivalent doses of ceftibuten plus VNRX-5236 at a 1:1 ratio were reduced by up to 1.41 – 2.08 $\log_{10}$ in the kidneys, 0.78 – 2.88 $\log_{10}$ in the bladders and 2.75 – 3.32 $\log_{10}$ in the urine.
- Approximately 78% of the mean CFU titers determined for all 3 sample types (kidneys, bladder, urine) collected from groups co-administered doses of ceftibuten and VNRX-5236 were significantly lower than mean counts determined for untreated controls 7 days after infection (p<0.05; one-way ANOVA with Bonferroni's multiple comparison test).
- By comparison, only 33% of the mean CFU titers determined for samples collected from groups administered doses of ceftibuten alone or 2:1 combined doses of amoxicillin and clavulanate were significantly lower than the mean counts of untreated controls 7 days post-infection (p<0.05; one-way ANOVA with Bonferroni's multiple comparison test).
- Overall, the results demonstrate that VNRX-5236 rescues ceftibuten activity in a mouse UTI model against 3 β-lactamase expressing UPEC strains, and therefore, further development of this BLI for the treatment of these severe infections is warranted.

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Acknowledgments

Animal studies conducted in support of the work presented here were done in accordance with protocol 2017-0047 approved by the Institutional Animal Care and Use Committee (IACUC) at the University of North Texas Health Science Center (UNTHSC). The authors gratefully acknowledge the support of the UNT System College of Pharmacy.

This project has been sponsored by VenatoRx Pharmaceuticals, Inc. and funded in part with Federal funds from the National Institute of Allergy and Infectious Diseases, National Institutes of Health, Department of Health and Human Services (Contract No. HHSN272201300019C).