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In vivo pharmacokinetics and pharmacodynamics of ceftibuten/ledaborbactam, a novel oral β-lactam/β-lactamase inhibitor combination

Andrew J. Fratoni[1], Lindsay M. Avery[2], David P. Nicolau [1] and Tomefa E. Asempa [1]*

[1]*Center for Anti-Infective Research and Development, Hartford Hospital, 80 Seymour Street, Hartford 06102, CT, USA;*
[2]*Venatorx Pharmaceuticals, Malvern, PA, USA*

*Corresponding author. E-mail: tomefa.asempa@hhchealth.org

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Objectives: Oral β-lactam treatment options for MDR Enterobacterales are lacking. Ledaborbactam (formerly VNRX-5236) is a novel orally bioavailable β-lactamase inhibitor that restores ceftibuten activity against Ambler Class A-, C- and D-producing Enterobacterales. We assessed the ledaborbactam exposure needed to produce bacteriostasis against ceftibuten-resistant Enterobacterales in the presence of humanized ceftibuten exposures in the neutropenic murine thigh infection model.

Methods: Twelve ceftibuten-resistant clinical isolates (six *Klebsiella pneumoniae*, five *Escherichia coli* and one *Enterobacter cloacae*) were utilized. Ceftibuten/ledaborbactam MICs ranged from 0.12 to 2 mg/L (ledaborbactam fixed at 4 mg/L). A ceftibuten murine dosing regimen mimicking ceftibuten 600 mg q12h human exposure was developed and administered alone and in combination with escalating exposures of ledaborbactam. The $\log_{10}$ cfu/thigh change at 24 h relative to 0 h controls was plotted against ledaborbactam $fAUC_{0–24}$/MIC and the Hill equation was used to determine exposures associated with bacteriostasis.

Results: The mean ± SD 0 h bacterial burden was 5.96 ± 0.24 $\log_{10}$ cfu/thigh. Robust growth (3.12 ± 0.93 $\log_{10}$ cfu/thigh) was achieved in untreated control mice. Growth of 2.51 ± 1.09 $\log_{10}$ cfu/thigh was observed after administration of humanized ceftibuten monotherapy. Individual isolate exposure–response relationships were strong (mean ± SD R^2 = 0.82 ± 0.15). The median ledaborbactam $fAUC_{0–24}$/MIC associated with stasis was 3.59 among individual isolates and 6.92 in the composite model.

Conclusions: Ledaborbactam $fAUC_{0–24}$/MIC exposures for stasis were quantified with a ceftibuten human-simulated regimen against β-lactamase-producing Enterobacterales. This study supports the continued development of oral ceftibuten/ledaborbactam etzadroxil (formerly ceftibuten/VNRX-7145).

Introduction

Antimicrobial resistance among Gram-negative pathogens is compromising the efficacy of existing therapeutic options.[1] Among these concerning resistance mechanisms is the production of β-lactamases that hydrolyse certain β-lactam antibiotics, rendering them ineffective.[2] Multiple parenteral β-lactam/β-lactamase inhibitor (β-lactam/BLI) combinations have come to market in recent years that have helped to address this concern.[3–6] Badly missing from the current β-lactam armamentarium is an oral agent with activity against these challenging pathogens to serve as a step-down option or to treat infections, including complicated urinary tract infections (cUTIs), in an outpatient setting. Furthermore, the unsuccessful recent attempts to bring new oral carbapenems, sulopenem and tebipenem,[7,8] to market have heightened the need for advancements in this space.

To help meet this need, ceftibuten,[9] an oral third-generation cephalosporin, is being explored in combination with a new oral cyclic boronate BLI, ledaborbactam etzadroxil (formerly VNRX-7145). Ledaborbactam etzadroxil is the pro-drug of the active compound ledaborbactam (formerly VNRX-5236).[10] Ceftibuten is a viable β-lactam partner due to excellent bioavailability (75%–90%), high fractional urinary excretion, and enhanced stability against hydrolysis by ESBLs relative to other oral third-generation cephalosporins.[9] Ledaborbactam restores the *in vitro* activity of ceftibuten against Enterobacterales expressing serine β-lactamases including ESBLs, KPC, OXA and Ambler class C enzymes.[10] Herein, we determined the ceftibuten/ledaborbactam pharmacokinetic/pharmacodynamic (PK/PD) target

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expected to be predictive of clinical efficacy in cUTI (i.e. bacteriostasis)[11] against serine β-lactamase-producing Enterobacterales *in vivo*.

Materials and methods

Antimicrobial agents

Ceftibuten (batch number CHAN200002, Covalent Laboratories Pvt. Ltd., Telangana, India) and ledaborbactam (batch number YD00113-010) were supplied by Venatorx Pharmaceuticals, Inc. For *in vivo* studies, ceftibuten and ledaborbactam were reconstituted with a sodium phosphate buffer (50 mM, pH 7). Subsequent dilutions in buffer were made to attain final concentrations that would deliver mean weight-based doses to study mice. Both agents were administered via subcutaneous injections of 0.1 mL.

Bacterial isolates and in vitro susceptibility testing

In total, 12 ceftibuten-resistant (per EUCAST, >1 mg/L based on 400 mg once-daily oral dosing for infections originating in the urinary tract) Enterobacterales isolates were included in these studies—*Klebsiella pneumoniae* ($n=6$), *Escherichia coli* ($n=5$) and *Enterobacter cloacae* ($n=1$). Broth microdilution was performed for each strain in triplicate or until a modal value was achieved in accordance with CLSI guidance to determine ceftibuten/ledaborbactam MICs, with ledaborbactam concentrations fixed at 4 mg/L.[12] Fixing the ledaborbactam concentration at 4 mg/L is consistent with the methodology used in early characterization of the compound.[10,13,14] The rationale for choosing 4 mg/L for *in vitro* testing is based on *in vitro* broth microdilution assessments across a range of fixed concentrations of ledaborbactam and correlation of *in vitro* results with *in vivo* antibacterial activity seen in earlier investigations with the neutropenic mouse thigh infection model.[10,13,14] *K. pneumoniae* BAA-1705 (ceftibuten/ledaborbactam MIC range 0.12–0.5 mg/L) and *E. coli* ATCC 25922 (ceftibuten/ledaborbactam MIC range 0.03–0.12 mg/L) were used as quality control strains on each day of study.

WGS and genome analysis for β-lactamases and other genes

A genomic analysis was conducted on all 12 strains utilized in PK/PD analyses. DNA extraction, Illumina library preparation and raw WGS data from Illumina HiSeq 2×150 bp were provided by GENEWIZ (South Plainfield, NJ, USA). WGS analysis was performed using Geneious Prime version 2022.1.1 (Biomatters Inc., San Diego, CA, USA). The reads were trimmed with BBDUK Adapter/Quality Trimming Version 38.84 (Brian Bushnell). *De novo* assembly was performed with the Geneious Assembler using 0.8 to 3.6 million reads per genome to give 5.3 to 6.7 million nucleotides per assembly. β-Lactamases in each genome were annotated using a search set of 84 representative β-lactamases and cut-off of 40% identity. This search set successfully identifies all ∼2000 β-lactamases included in ResFinder. If an annotated β-lactamase gene was incomplete or split over two contigs or had a premature stop codon, 100% of trimmed reads were mapped to the standard sequence and then analysed further to verify the presence of intact gene, truncated gene or multiple alleles. The *ftsI* gene encoding PBP3 and the genes encoding major porins (OmpE36/OmpC/OmpK36 and OmpE35/OmpF/OmpK35) were annotated using the reference genes listed in Table 1.

Laboratory animals

Animals were maintained and utilized in accordance with National Research Council recommendations. The study protocol was reviewed and approved by the Institutional Animal Care and Use Committee at Hartford Hospital. Pathogen-free, female ICR (CD-1) mice weighing approximately 20–22 g were obtained from Charles River Laboratories, Inc. (Wilmington, MA, USA). The mice underwent a 48 h acclimatization period upon delivery. Animals were housed in groups of six at controlled room temperature in HEPA-filtered cages (Innovive, San Diego, CA, USA). Cages were supplemented with paper nesting material for enrichment purposes. Study rooms were maintained with diurnal cycles (12 h light/12 h dark) and food and water were provided *ad libitum*.

Neutropenic thigh infection model

Mice were rendered transiently neutropenic by injecting cyclophosphamide intraperitoneally at a dose of 150 mg/kg 4 days before inoculation and 100 mg/kg 1 day before inoculation. In addition, uranyl nitrate 5 mg/kg was administered intraperitoneally 3 days prior to inoculation to produce a controlled degree of renal impairment to assist with humanizing the target exposure of ceftibuten.

Isolates were frozen in skimmed milk and stored at −80°C. Prior to experimentation, isolates were subcultured twice onto Trypticase soy agar with 5% sheep blood (Becton, Dickinson & Co., Sparks, MD, USA) and incubated for 18 to 24 h. After 18 to 24 h incubation of the second subculture, a bacterial suspension of approximately 10^7 cfu/mL was made for thigh inoculations. The thigh infection was produced by intramuscular injection of 0.1 mL of the inoculum into the left thigh only of each mouse 2 h prior to the initiation of antimicrobial therapy, resulting in an initial bacterial burden of approximately 10^5 to 10^6 cfu/thigh.

Ex vivo protein binding studies in mice

Three different ledaborbactam doses (0.2, 1 and 5 mg/kg) were administered to result in plasma concentrations spanning the range associated

Table 1. Reference genes used in genotypic analysis

Organism	Strain	Gene name	Nucleotide accession	Locus tag/CDS	Protein accession
E. cloacae	ATCC 13047	*ompE36*	NC_014121	ECL_03519	WP_013098006.1
E. cloacae	ATCC 13047	*ompE35*	NC_014121	ECL_02724	WP_164928068.1
E. cloacae	ATCC 13047	*ftsI*	NC_014121	ECL_00881	WP_013095569.1”
E. coli	K-12 MG1655	*ompC*	NC_000913.3	b2215	NP_416719.1
E. coli	K-12 MG1655	*ompF*	NC_000913.3	b0929	NP_415449.1
E. coli	K-12 MG1655	*ftsI*	NC_000913.3	b0084	NP_414626.1
K. pneumoniae	ATCC 13883	*ompK36*	NZ_KN046818	DR88_RS05545	WP_004149145.1
K. pneumoniae	ATCC 13883	*ompK35*	NZ_KN046818	DR88_RS17700	WP_004195943.1”
K. pneumoniae	ATCC 13883	*ftsI*	NZ_KN046818	DR88_RS22130	WP_002888559.1

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with anticipated humanized PK exposures. Protein binding studies were conducted *ex vivo* in triplicate as follows: mice were prepared as described in the neutropenic murine thigh infection model as above. Two hours after bacterial inoculation, 36 mice (nine groups of 4 mice each) were administered ledaborbactam (three groups/12 mice total for each respective ledaborbactam dose). One hour after dosing, mice were euthanized by CO_2 asphyxiation followed by blood collection via intracardiac puncture and ultimately cervical dislocation. Blood samples from each group of mice (four mice) were pooled in vials containing K_2EDTA. The whole blood was processed within 15 min of collection via centrifugation at 10 000 rpm at 4°C for 10 min to separate the plasma. An aliquot of each of the pooled plasma samples was reserved for total drug concentration determination. Approximately 0.9 mL of each of the pooled plasma samples was then transferred into Centrifree®ultrafiltration devices (Millipore Corporation, Billerica, MA, USA) with a 30 kDa molecular weight cut-off according to the manufacturer's recommendation and centrifuged for 45 min at 4°C at 2000×g to generate an ultrafiltrate volume of approximately 250 μL. The resulting ultrafiltrate solution and corresponding aliquots of plasma were transferred into polypropylene tubes and stored at −80°C until shipped on dry ice to Absorption Systems (Exton, PA, USA) for analysis. In addition, *in vitro* non-specific binding to the Centrifree devices was assessed in triplicate utilizing vehicle solution spiked with ledaborbactam to achieve a concentration of 5 mg/L, and devices were processed under the same conditions as the plasma samples. Triplicate concentrations of ledaborbactam in both the initial plasma solutions and ultrafiltrates were determined by Absorption Systems via LC-MS/MS.

The protein binding at each administered dose was calculated using the following formula: % Protein Binding = 100−($Concentration_{ultrafiltrate}$/$Concentration_{plasma}$ × 100). The non-specific binding was calculated similarly using the following formula: % Non-specific Binding = 100−($Concentration_{processed}$/$Concentration_{stock}$ × 100). Any ultrafiltrate concentration that was reported as greater than the corresponding plasma concentration, likely due to accepted assay variability, was considered 0% protein binding.

PK studies

Previously generated ceftibuten and ledaborbactam PK model parameters in mice were used to simulate (Phoenix WinNonlin, version 8.3, Certara, Princeton, NJ, USA) murine dosing regimens that approximate the human free plasma exposures for both agents.[13] The targeted murine exposure of ceftibuten simulated the human concentration–time profile of an oral dose of 600 mg q12h at steady-state. Human exposure profiles of ceftibuten and ledaborbactam were targeted based on simulations using the typical values from preliminary population PK models that were developed from Phase 1 data (VNRX Clinical Study Protocol VNRX-5024-101 version 4.0; NCT04314206) for ceftibuten, and for ledaborbactam (VNRX Clinical Study Protocol VNRX-7145-101, version 7.0; NCT04243863). These Phase I studies provide supportive clinical safety data for a range of ceftibuten doses including ceftibuten 600 mg by mouth q12h, which is expected to provide plasma concentrations capable of attaining previously derived ceftibuten preclinical PK/PD targets.[9]

The developed murine dosing regimens were then administered in confirmatory PK studies to ensure the predicted exposures were achieved *in vivo* prior to dose-ranging studies. Briefly, mice were prepared as previously described and infected with a WT *K. pneumoniae* isolate. At timepoints ranging from 0.5 to 22 h after dosing of ceftibuten and ledaborbactam, groups of six mice were euthanized by CO_2exposure followed by blood collection in K_2EDTA vials, via intracardiac puncture and cervical dislocation. The blood was centrifuged for 10 min at 4°C at 10 000 rpm and the plasma was stored at −80°C until analysed; plasma drug concentrations were determined by Absorption Systems using qualified LC-MS/MS methods.

Ceftibuten/ledaborbactam dose-ranging studies

The purpose of these studies was to assess the ability of various exposures of ledaborbactam (ranging from $fAUC_{0-24}$ 1–90 mg·h/L; informed from preliminary *in vivo* studies focused on dose selection)[14] to potentiate the activity of ceftibuten 600 mg q12h humanized exposures against 12 β-lactamase-producing Enterobacterales isolates *in vivo*.

Control mice (0 h; $n = 6$), representing initial bacterial burden, were harvested 2 h post-inoculation and prior to antibiotic initiation. Treatment mice ($n = 6$) received a ceftibuten 600 mg q12h human-simulated regimen (HSR) alone or in combination with four different ledaborbactam regimens (achieving ledaborbactam $fAUC_{0-24}$of 1, 5, 50 and 90 mg·h/L) and were subsequently sacrificed after 24 h. The 24 h control mice ($n = 6$) received vehicle dosing. Antimicrobial activity was assessed by the 24 h change in $\log_{10}$ cfu/thigh relative to the 0 h control bacterial density and reported as mean ± SD.

PD analyses

Previous studies determined that the PD index best correlated with the efficacy of ledaborbactam when co-administered with ceftibuten is the $fAUC_{0-24}$/MIC (i.e. MIC of ceftibuten potentiated by ledaborbactam at a fixed concentration of 4 mg/L).[13] In the present study, plots of 24 h $\log_{10}$ change in cfu/thigh versus ledaborbactam $fAUC_{0-24}$/MIC in the presence of humanized ceftibuten exposures were constructed. An E_{max} model was fitted to the cfu response data from each of the 12 Enterobacterales isolates individually and together as a composite to determine the $fAUC_{0-24}$/MIC required for bacteriostasis (the translational endpoint for efficacy in cUTI).[11]

Results

In vitro susceptibility

The 12 isolates utilized in this study exhibited a wide range of ceftibuten/ledaborbactam MICs (0.12 to 2 mg/L), with ledaborbactam concentrations fixed at 4 mg/L (Table 2). Ceftibuten MICs ranges were >32 to 128, >32, and >64–128 for *E. coli*, *E. cloacae* and *K. pneumoniae*, respectively. In combination with 4 mg/L ledaborbactam, the MICs decreased for all isolates with a range of reduction of >32- to 1024-fold.

WGS and genome analysis for β-lactamases and other genes

Complete results of genome sequencing and analyses are presented in Table 3. Isolates expressed various β-lactamases (such as SHV, CTX-M, KPC and OXA), PBP3 variants and OmpC and OmpK porin status.

Ex vivo protein binding studies in mice

The non-specific binding of ledaborbactam to the Centrifree devices was 0%. Ledaborbactam protein binding was not concentration dependent across the studied exposure range, with a mean of 6.7%.

PK studies

Based on $\%fT_{>MIC}$ and $fAUC$, estimated free (unbound) ceftibuten exposures in humans following dosages of 600 mg q12h were successfully recapitulated in the neutropenic murine thigh infection model (Table 4). The ceftibuten 600 mg q12h HSR

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Table 2. Bacterial burden (cfu/thigh) at 0 h and after 24 h of ceftibuten 600 mg q12h HSR in combination with various ledaborbactam exposures against 12 Enterobacterales in the neutropenic murine thigh infection model

				Log_{10}cfu/thigh						
Isolate	CTB MIC (mg/L)	CLB MIC[a] (mg/L)	β-Lactamase(s) encoded	0 h control	24 h control	CTB HSR (600 mg q12h)	CTB HSR + ledaborbactam *f*AUC 1	CTB HSR + ledaborbactam *f*AUC 5	CTB HSR + ledaborbactam *f*AUC 50	CTB HSR + ledaborbactam *f*AUC 90
EC 614	>64	1	EC-1149 (AmpC), CTX-M-55, OXA-1, OXA-48	6.17 (0.07)	7.03 (0.57)	6.41 (1.35)	5.19 (0.31)	4.40 (0.25)	4.38 (0.24)	4.72 (0.08)
EC 617	>64	0.5	EC-204 (AmpC), CTX-M-14, TEM-1	5.87 (0.19)	9.33 (0.16)	9.13 (0.22)	6.37 (1.16)	5.00 (0.12)	4.61 (0.33)	4.84 (0.40)
EC 636	>32	0.12	EC-690 (AmpC), CTX-M-15	6.04 (0.26)	10.05 (0.29)	9.21 (0.22)	5.41 (0.39)	5.22 (0.16)	5.08 (0.11)	5.07 (0.30)
EC 639	128	0.12	EC-690 (AmpC), CTX-M-15, TEM-1, OXA-1	6.30 (0.15)	10.40 (0.15)	9.61 (0.27)	5.88 (0.39)	5.78 (0.26)	5.68 (0.33)	5.91 (0.15)
EC C11-23	>64	0.5	EC-725 (AmpC), CTX-M-15, TEM-1	5.52 (0.10)	9.04 (0.35)	9.00 (0.11)	5.06 (0.25)	5.28 (0.74)	4.78 (0.23)	4.73 (0.13)
ECL 150	>32	0.25	ACT-24 (AmpC), CTX-M-15, OXA-1	6.01 (0.04)	9.23 (0.35)	6.83 (0.62)	6.14 (0.82)	5.32 (0.07)	5.54 (0.49)	5.36 (0.00)
KP 774	>64	1	KPC- 3, TEM-1, SHV-11, OXA-9 truncation	6.01 (0.08)	9.51 (0.16)	8.67 (0.36)	7.20 (1.11)	6.13 (0.46)	5.49 (0.08)	5.52 (0.08)
KP 780	>64	2	CTX-M-15, CTX-M-2, TEM-1, SHV-11, OXA-9	6.02 (0.11)	9.42 (0.04)	9.11 (0.32)	8.61 (0.29)	7.79 (0.38)	6.00 (0.18)	6.68 (1.05)
KP 785	>64	0.25	CTX-M-15, CTX-M-14, TEM-1, SHV-1, CMY-4, OXA-1, OXA-204 (OXA-48 type)	6.12 (0.10)	7.56 (0.34)	8.26 (0.08)	6.82 (0.63)	5.94 (0.29)	5.64 (0.16)	5.59 (0.12)
KP 786	>64	0.5	CTX-M-15, TEM-1, SHV-11, CMY-4, OXA-48	6.00 (0.06)	8.97 (0.23)	8.69 (0.46)	7.48 (0.12)	6.29 (0.35)	6.22 (0.05)	6.16 (0.29)
KP 816	128	0.12	CTX-M-15, SHV-11, SHV-12, TEM-1, OXA-1	5.88 (0.07)	9.05 (0.04)	8.68 (0.46)	6.97 (1.27)	5.97 (0.14)	6.08 (0.26)	5.72 (0.20)
KP 1171	>64	1	CTX-M-14, KPC-2, SHV-11, TEM-1	5.64 (0.14)	9.41 (0.13)	8.06 (0.39)	7.08 (0.44)	6.82 (1.43)	5.57 (0.11)	5.87 (0.37)

EC, *E. coli*; ECL, *E. cloacae*; KP, *K. pneumoniae*; CTB, ceftibuten; CLB, ceftibuten/ledaborbactam. Data presented as mean (SD). *f*AUC represents *f*$AUC_{0–24}$ and is expressed in mg·h/L.
[a]Based on the MICs of ceftibuten/ledaborbactam combination at a fixed ledaborbactam concentration of 4 mg/L.

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Table 3. Results of genomic analysis, including assessments for variants in the ceftibuten target site (PBP3) and porin channels

Isolate	CTB MIC (mg/L)	CLB MIC[a] (mg/L)	β-Lactamase(s) encoded	PBP3 variant	OmpC (porin) status	OmpF (porin) status
EC 614	>64	1	EC-1149 (AmpC), CTX-M-55, OXA-1, OXA-48	None	Intact	Intact
EC 617	>64	0.5	EC-204 (AmpC), CTX-M-14, TEM-1	I332V	Intact	Truncated at residue 20
EC 636	>32	0.12	EC-690 (AmpC), CTX-M-15	A233T, I332V	Intact	Lesion
EC 639	128	0.12	EC-690 (AmpC), CTX-M-15, TEM-1, OXA-1	A233T, I332V	Intact	Intact
EC C11-23	>64	0.5	EC-725 (AmpC), CTX-M-15, TEM-1	D149E, I332V	Intact	Intact
ECL 150	>32	0.25	ACT-24 (AmpC), CTX-M-15, OXA-1	None	Intact	Intact
KP 774	>64	1	KPC- 3, TEM-1, SHV-11, OXA-9 truncation	None	GD (Gly115-Asp116) insertion	Truncated at residue 41
KP 780	>64	2	CTX-M-15, CTX-M-2, TEM-1, SHV-11, OXA-9	None	Intact	Truncated at residue 114
KP 785	>64	0.25	CTX-M-15, CTX-M-14, TEM-1, SHV-1, CMY-4, OXA-1, OXA-204 (OXA-48 type)	None	Intact	Intact
KP 786	>64	0.5	CTX-M-15, TEM-1, SHV-11, CMY-4, OXA-48	None	Intact	Intact
KP 816	128	0.12	CTX-M-15, SHV-11, SHV-12, TEM-1, OXA-1	None	Intact	Truncated at residue 34
KP 1171	>64	1	CTX-M-14, KPC-2, SHV-11, TEM-1	None	GD (Gly115-Asp116) insertion	Truncated at residue 41

PBP3 sequence was identical to the reference sequence in instances where no variation (i.e. "None") is listed. OmpE36 (OmpC) and OmpE35 (OmpF), and OmpK36 (OmpC) and OmpK35 (OmpF) proteins were analysed in *E. cloacae* and *K. pneumoniae* strains, respectively.
[a]Based on the MICs of ceftibuten/ledaborbactam combination at a fixed concentration of 4 mg/L.

Table 4. Comparison of the PK profiles and %$fT_{>MIC}$ values achieved with ceftibuten at each doubling MIC dilution in humans versus mice receiving HSRs

Regimen	Species	%$fT_{>MIC}$ for an MIC (mg/L) of:								$fAUC_{0-24}$ (mg·h/L)	fC_{max} (mg/L)
		0.06	0.12	0.25	0.5	1	2	4	8		
Ceftibuten 600 mg q12h	Human	100	100	100	100	94	71	47	13	101.7	8.7
	Mouse	100	100	100	95	85	69	45	15	101.2	11.4

consisted of 2.85, 3.8, 2.85 and 0.46 mg/kg at 0, 1.75, 4 and 8 h, respectively, for 0–12 h, and 1.43, 1.9, 1.43 and 0.23 mg/kg at 12, 13.75, 16 and 20 h for the second 12 h interval. The ceftibuten concentration–time profiles observed with ceftibuten monotherapy and co-administered with ledaborbactam doses to achieve exposures equivalent to $fAUC_{0-24}$of 50 and 90 mg·h/L were comparable and no appreciable drug interaction was observed (Figure 1). Ledaborbactam dosing regimens that provided free plasma exposures of $fAUC_{0-24}$of 50 and 90 mg·h/L were developed and confirmed when co-administered with the ceftibuten HSR (Figure 2) and additional regimens providing predicted $fAUC_{0-24}$of 5 and 1 mg·h/L were extrapolated.

Ceftibuten/ledaborbactam dose-ranging and PD analyses

The mean ± SD 0 h bacterial burden in the thighs for the 12 isolates was 5.96 ± 0.24 log_{10} cfu/thigh. Robust growth was achieved in the neutropenic murine thigh infection model, as the bacterial burdens increased over 24 h by an average magnitude of 3.12 ± 0.93 log_{10} cfu/thigh in the untreated vehicle-dosed control mice. *In vivo* growth was concordant with ceftibuten *in vitro* resistance as all isolates displayed net 24 h growth (2.51 ± 1.09 log_{10} cfu/thigh) after administration of ceftibuten HSR monotherapy. The results of the ledaborbactam dose-ranging studies for each of the isolates are presented in Table 2. Mean 24 h log_{10} cfu/thigh changes of 0.55, −0.14, −0.54 and −0.45

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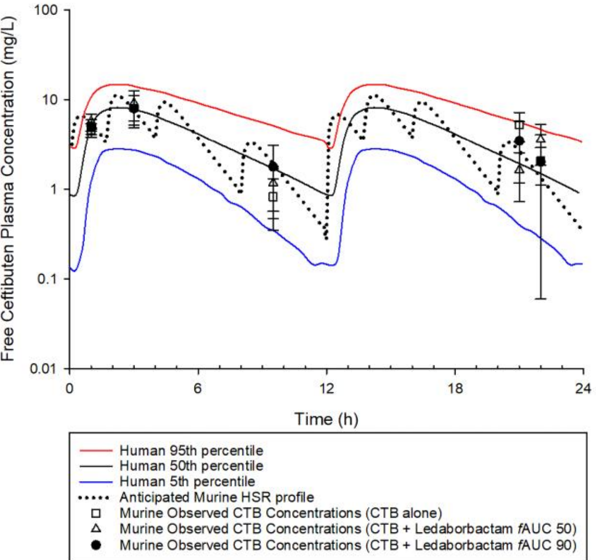

Figure 1. Ceftibuten (CTB; 600 mg q12h) human-simulated free plasma concentration–time profile and achieved concentrations when administered alone and in combination with various ledaborbactam $fAUC_{0-24}$ exposures in a neutropenic murine thigh infection model compared with human monotherapy exposure. Observed data are means ± SDs. This figure appears in colour in the online version of *JAC* and in black and white in the print version of *JAC*.

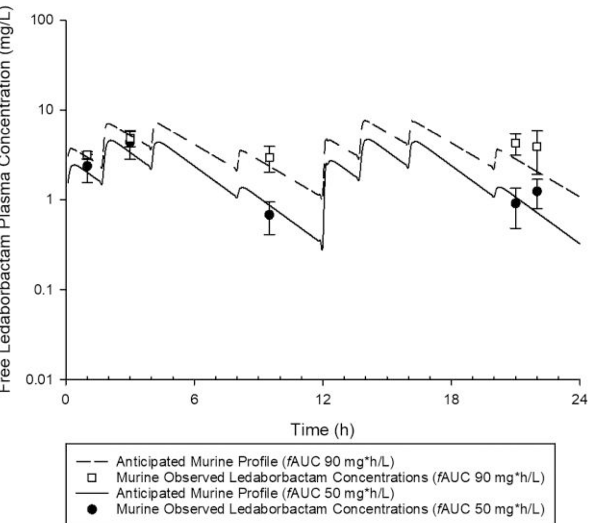

Figure 2. Ledaborbactam free plasma concentration–time profile and achieved concentrations when co-administered with a ceftibuten (600 mg q12h) HSR in a neutropenic murine thigh infection model.

were observed after administration of the ceftibuten HSR and ledaborbactam plasma exposures of $fAUC_{0-24}$1, 5, 50 and 90 mg·h/L, respectively. Among the 12 individual model fits (mean $R^2=0.82$; range: 0.57 to 0.98), the median ledaborbactam $fAUC_{0-24}$/MIC associated with stasis was 3.59 (IQR, 0.92–38.00). The coefficient of determination of the composite model (Figure 3) describing the relationship between ledaborbactam $fAUC_{0-24}$/MIC and change in bacterial density at 24 h via an inhibitory sigmoid E_{max} dose–response curve was 0.62. The $fAUC_{0-24}$/MIC associated with stasis was 6.92.

Discussion

Oral options for serine β-lactamase-producing Enterobacterales including ESBLs and KPCs are limited, and the rising level of cross-resistance to multiple classes of antibiotics, including trimethoprim/sulfamethoxazole and fluoroquinolones is concerning.[15–18] The urinary tract represents the most common site of infection caused by ESBL-producing organisms, resulting in hospitalizations to receive IV antibiotics and a corresponding appreciable economic and public health burden.[19,20] While tetracycline derivatives with activity against serine β-lactamase-producing Enterobacterales have come to market in recent years, their utility in treating these uropathogens may be limited by poor urinary exposure and tolerability, and none carry an FDA-approved indication for urinary tract infections.[21] With the above taken into consideration, the repurposing of a highly bioavailable oral β-lactam with the ability to achieve high urinary concentrations, combined with a novel BLI, is an attractive approach for the safe and efficacious treatment of serine β-lactamase-producing Enterobacterales infections.

Ledaborbactam, the active component of orally bioavailable ledaborbactam etzadroxil, is a boronic acid-based BLI that restores the activity of ceftibuten *in vitro* against these organisms.[22] While early efforts with boron-based inhibitors have focused on IV administration, such as vaborbactam and taniborbactam, ledaborbactam etzadroxil is poised to potentially become the first boron-based oral agent.[22] This study in the neutropenic murine thigh infection model determined the PD exposure of ledaborbactam expected to be predictive of a positive clinical outcome in cUTI patients when co-administered with ceftibuten 600 mg orally q12h.

A previously reported MIC distribution of 100 clinical Enterobacterales expressing ESBLs, KPCs, class C cephalosporinases and OXA-48, yielded MIC_{90} values ranging from 0.25 to 1 mg/L.[10] A separate dataset focusing on isolates relevant to a cUTI indication included 1066 clinical urinary tract-derived Enterobacterales isolates that were resistant to both amoxicillin/clavulanic acid and levofloxacin.[23] In that study, the $MIC_{90}$of ceftibuten (>32 mg/L) was reduced to 2 mg/L with the fixed addition of 4 mg/L ledaborbactam.[23] Coupled with these MIC distribution datasets, the PK/PD stasis targets identified in this current study should prove valuable in informing clinical dose selection, especially for a cUTI indication. The totality of data suggests that attainment of these identified PK/PD targets with clinically achievable exposures of ceftibuten/ledaborbactam may provide therapeutic benefit against Enterobacterales isolates, even among those with multiple mechanisms of resistance including β-lactamases, substitutions in PBP3 and alterations in major porins.

Variability in PK/PD exposure targets from pre-clinical models is not unusual and driven by factors such as the underlying resistance mechanism and limitations of MIC testing.[24–26] AUC/MIC thresholds are especially vulnerable to the 2-fold inherent

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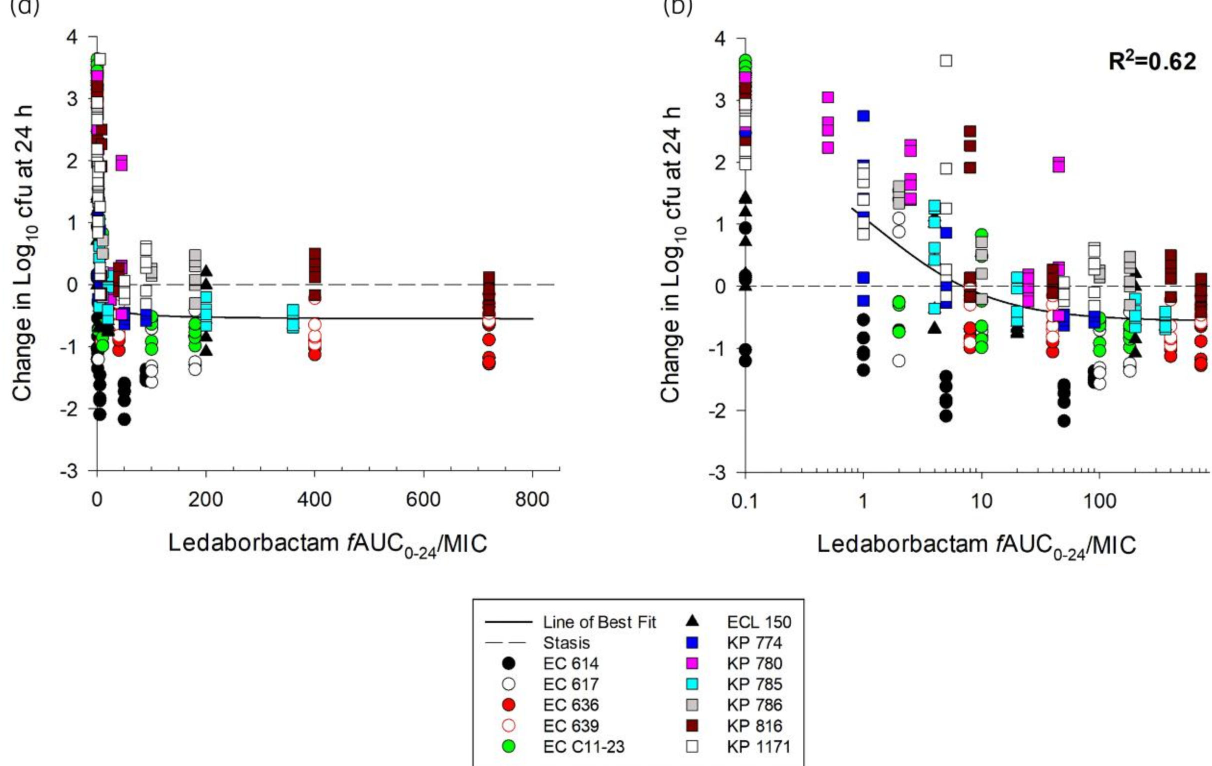

Figure 3. The composite curve of best fit to the ledaborbactam $fAUC_{0–24}$/MIC and change in log_{10} cfu/thigh at 24 h for all Enterobacterales isolates ($n = 12$) following co-administration of ledaborbactam and the ceftibuten 600 mg q12h HSR in the neutropenic murine thigh infection model. The solid line represents the curve of best fit ($R^2 = 0.62$), while the data points represent the observed changes in bacterial burdens in each infected thigh. Points on the *y*-axis represent the ceftibuten monotherapy groups with no ledaborbactam exposure. Presented in (a) linear and (b) log format. EC, *E. coli*; ECL, *E. cloacae*; KP, *K. pneumoniae*. This figure appears in colour in the online version of *JAC* and in black and white in the print version of *JAC*.

variability of MIC broth microdilution testing, which ultimately can alter the magnitude of the exposure target by multiples. Our study was no exception; while the median (3.92) and composite (6.92) $fAUC_{0–24}$/MIC exposure targets for stasis were similar, there was notable variability on an individual isolate basis (IQR, 0.92–38.00), which can be visually appreciated in the composite figure. When selecting clinical dosing regimens, it is imperative to consider this and not rely solely on average exposure targets.

In conclusion, in the presence of humanized unbound ceftibuten plasma exposures, a median ledaborbactam $fAUC_{0–24}$/MIC of 3.59 (IQR, 0.92–38.00) achieved bacteriostasis against 12 serine β-lactamase-expressing Enterobacterales isolates in the neutropenic murine thigh infection model. These data warrant further non-clinical studies in cUTI models as well as supporting the ongoing dose-selection and clinical development of ledaborbactam etzadroxil.

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Transparency declarations

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