AMERICAN SOCIETY FOR MICROBIOLOGY | Antimicrobial Agents and Chemotherapy®

EXPERIMENTAL THERAPEUTICS

Translational Efficacy of Humanized Exposures of Cefepime, Ertapenem, and Levofloxacin against Extended-Spectrum-β-Lactamase-Producing *Escherichia coli* in a Murine Model of Complicated Urinary Tract Infection

Marguerite L. Monogue,[a] David P. Nicolau[a,b]

Center for Anti-infective Research and Development, Hartford Hospital, Hartford, Connecticut, USA[a]; Division of Infectious Diseases, Hartford Hospital, Hartford, Connecticut, USA[b]

ABSTRACT Validated animal models are required as bridging tools to assess the utility of novel therapies and potential microbiologic outcomes. Herein, we utilized uropathogenic extended-spectrum-β-lactamase (ESBL)-producing and non-ESBL-producing *Escherichia coli* in the neutropenic murine complicated urinary tract infection (cUTI) model with humanized exposures of cefepime, ertapenem, and levofloxacin to assess its translational value to human outcomes. Our data support the translational utility of this murine model to cUTI in humans as humanized exposures produced microbiologic outcomes consistent with the phenotypic profiles of the organisms.

KEYWORDS ESBL, *Escherichia coli*, urinary tract infection, cefepime, ertapenem, levofloxacin

Urinary tract infections (UTIs) are one of the most common types of community-acquired and nosocomial infections, impacting 150 million people per year globally (1). *Escherichia coli* is the leading cause of UTIs, and often displays resistance to currently available antibiotics due to the production of various enzymes, such as extended-spectrum β-lactamases (ESBLs) (1). ESBLs are enzymes produced by Gram-negative bacteria, namely, *E. coli*, and are responsible for the resistance against penicillins, cephalosporins, and aztreonam (2). Carbapenems, such as ertapenem, are standard for the treatment of infections caused by ESBL-producing organisms, although the potential clinical utility of extended-spectrum cephalosporins, notably cefepime, has yet to be clearly defined. Herein, we used humanized exposures of cefepime, 2 g every 8 h (q8h) (0.5-h infusion), ertapenem, 1 g every 24 h (q24h), and levofloxacin, 500 mg q24h, against uropathogenic ESBL- and non-ESBL-producing *E. coli* and assessed antimicrobial efficacy after 24 and 48 h of therapy in a neutropenic murine complicated UTI (cUTI) model.

Commercially available cefepime 1g (lot no. 106014C), ertapenem 1g (lot no. m027105), and levofloxacin 500 mg (lot no. CLF150003) vials were acquired from Cardinal Health, Inc. Vials were reconstituted according to the package insert and further diluted with 0.9% normal saline solution (Hospira, Inc., Lake Forest, IL). Doses were administered as 0.2-ml subcutaneous injections to achieve humanized exposures of 2 g cefepime q8h (0.5-h infusion), 1 g ertapenem q24h, and 500 mg levofloxacin q24h, as previously described (3–5).

Two clinical uropathogenic *E. coli* isolates (EC 429, ESBL; EC 430, non-ESBL) were used in this study. The MICs of cefepime, ertapenem, and levofloxacin were determined

Received 27 June 2017 **Returned for modification** 27 July 2017 **Accepted** 22 August 2017

Accepted manuscript posted online 28 August 2017

Citation Monogue ML, Nicolau DP. 2017. Translational efficacy of humanized exposures of cefepime, ertapenem, and levofloxacin against extended-spectrum-β-lactamase-producing *Escherichia coli* in a murine model of complicated urinary tract infection. Antimicrob Agents Chemother 61:e01329-17. https://doi.org/10.1128/AAC.01329-17.

Address correspondence to David P. Nicolau, david.nicolau@hhchealth.org.

TABLE 1 MICs of EC 429 and EC 430

Isolate	MIC (μg/ml)		
	Cefepime	Ertapenem	Levofloxacin
EC 429, ESBL	≥64	≤0.016	16
EC 430, non-ESBL	≤0.063	≤0.016	≤0.063

in triplicates by broth microdilution as described by the Clinical and Laboratory Standards Institute guidelines (6).

Pathogen-free female ICR mice weighing 20 to 22 g were obtained from Envigo RMS, Inc. (Indianapolis, IN). The protocol was reviewed and approved by the Institutional Animal Care and Use Committee at Hartford Hospital. Mice were rendered transiently neutropenic by injecting cyclophosphamide intraperitoneally at a dose of 150 mg/kg of body weight 4 days before and 100 mg/kg 1 day before inoculation. Uranyl nitrate 5 mg/kg was administered 3 days prior to inoculation to produce a controlled degree of renal impairment.

A previously published direct inoculation model was used to establish UTIs in mice (7, 8). Two transfers of the bacterial isolate were performed on Trypticase soy agar with 5% sheep blood (Becton, Dickinson & Co., Sparks, MD) and placed into an incubator at 37°C for approximately 24 h. A bacterial suspension of approximately 10^7 CFU/ml was prepared in sterile saline. Anesthesia was established with a 0.2-ml intraperitoneal dose of ketamine 100 mg/kg of body weight, xylazine 10 mg/kg, and acepromazine 3 mg/kg. The areas over each kidney (flanks) were shaved and prepped for surgical incisions. Surgical preps proceeded as follows: surgical scrub, blot with sterile gauze, surgical solution, and blot with sterile gauze. Incisions were made in the skin on each flank to visualize the kidneys. A volume of 0.05 ml of the bacterial isolate was injected directly into each kidney. The skin was closed with 4.0 Vicryl suture material with an RB-1 needle. Mice were kept in a warm oxygen-enriched environment and monitored during the postoperative period until they fully recovered from anesthesia (3 h). Control animals received sterile normal saline of the same volume with the same route and schedule as the most frequently dosed drug regimen. For each isolate tested, 3 untreated mice were used as 0-h controls and 3 additional mice (receiving normal saline) were used as 24- and 48-h controls each. All regimens were studied in groups of 3 mice over 24- and 48-h treatment periods for each bacterial isolate. After inoculation, one group for each bacterial isolate was euthanized by CO_2 exposure followed by cervical dislocation. At 24 and 48 h after the initiation of dosing, the treatment and control groups were euthanized. After sacrificing, the kidneys were removed and homogenized in normal saline. Serial dilutions were plated on an appropriate agar medium for CFU determination.

Table 1 presents the phenotypic profile of cefepime, ertapenem, and levofloxacin for EC 429 and EC 430 isolates. EC 429, an ESBL producer, was resistant to cefepime and levofloxacin but susceptible to ertapenem. EC 430 was pansusceptible to all three agents.

The initial bacterial density in the kidneys 3 h postinoculation (0 h) was 5.2 ± 0.03 $\log_{10}$ CFU/ml. Both test organisms displayed sustained growth over the 48-h study period in the kidneys of control animals, reaching 9.7 ± 0.1 $\log_{10}$ CFU/ml. Figure 1 illustrates the antibacterial efficacy of humanized exposures of cefepime, ertapenem, and levofloxacin against EC 429 and EC 430 after 24 and 48 h of treatment. Consistent with their phenotypic profiles, the cefepime and levofloxacin regimens demonstrated poor microbiologic responses against EC 429, whereas ertapenem exhibited >2 log kill over the 48-h treatment period. Based on the pansusceptibility of EC 430, humanized exposures of cefepime, ertapenem, and levofloxacin resulted in >2 log kill over the 48-h treatment period for all regimens.

The use of noncarbapenem antimicrobials for the treatment of ESBL UTIs remains a clinical controversy. Herein, using ESBL- and non-ESBL-producing organisms, we assessed the translational value of this murine UTI model and humanized exposures for

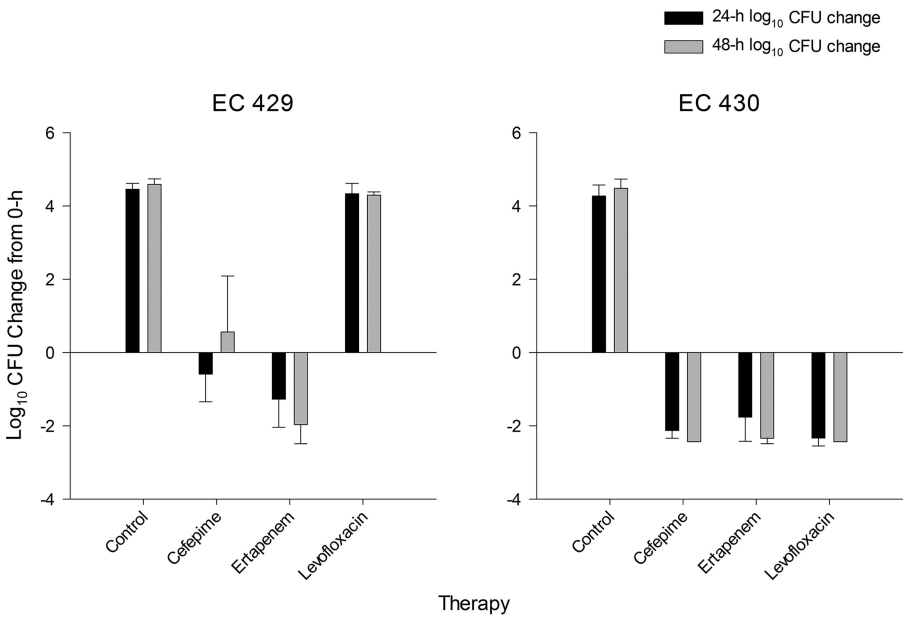

FIG 1 Efficacy (mean change in $\log_{10}$ CFU from 0-h control) of human simulated treatment with cefepime 2 g q8h (0.5-h infusion), ertapenem 1 g q24h, and levofloxacin 500 mg q24h against EC 429 (left) and EC 430 (right) after 24 and 48 h.

several frequently employed antimicrobial regimens for cUTI in humans. Levofloxacin displays poor clinical and microbiologic outcomes in the face of phenotypic resistance (9, 10). Similar to these clinical outcomes, data derived in the current study show a lack of efficacy in an organism defined as nonsusceptible when exposed to a humanized regimen. In an attempt to provide maximum *in vivo* exposures for cefepime, we utilized the 2-g q8h exposure as opposed to the lower 1-g dose that is often clinically utilized for cUTIs. Interestingly, despite this higher cefepime exposure, which initially demonstrated slight bacterial kill at 24 h, regrowth was noted during the following 24 h of therapy. These findings are reflective of clinical data in patients with ESBL-derived cUTIs (11). As expected, ertapenem was effective against both isolates, producing a large and sustained bacterial kill over 48 h. Our observations are consistent with the clinical and microbiologic outcomes reported for this compound in the setting of cUTIs (11–13).

The use of murine infection models during the anti-infective research and development process has been a standard practice since the 1950s (14). The selection of a model is dependent on the model's ability to mimic the disease pathogenesis observed in humans in relation to the antimicrobial's target therapeutic area (i.e., pneumonia, UTI, skin and soft tissue, etc.). To further enhance the translatability of these murine models to that of humans, the antimicrobial exposures in each species should be similar (humanized exposures). Since this murine model is intended to simulate a cUTI, a disease of the kidney/tissue, humanizing the plasma exposure is a more relevant target profile than that of urine. While it is expected that this plasma profile will result in sufficiently high urinary concentrations, additional studies may be undertaken to define the urinary profile of the compound of interest to ensure clinical applicability of the antimicrobial in the setting of both upper and lower urinary tract diseases.

To our knowledge, this is the first murine model of cUTI using completely humanized regimens to assess bacterial efficacy. In conclusion, the translatability of humanized antimicrobial exposures in murine cUTI models to human outcomes will be beneficial in determining optimal therapeutic options against these drug-resistant bacteria.

ACKNOWLEDGMENTS

We thank Jennifer Tabor-Rennie, Sara Robinson, Debora Santini, Elizabeth Cyr, Christina Sutherland, Kimelyn Greenwood, Islam Ghazi, Abrar Thabit, Kamilia Ab-

delraouf, and Mordechai Grupper from the Center for Anti-Infective Research and Development, Hartford, CT, for their assistance with conducting the study.

This study was internally funded by the Center for Anti-infective Research and Development.

REFERENCES

1. Flores-Mireles AL, Walker JN, Caparon M, Hultgren SJ. 2015. Urinary tract infections: epidemiology, mechanisms of infection and treatment options. Nat Rev Microbiol 13:269–284. https://doi.org/10.1038/nrmicro3432.
2. Cantón R, Valverde A, Novais A, Baquero F, Coque T. 2007. Evolution and current situation of ESBL. Enferm Infecc Microbiol Clin 25(Suppl 2):2–10. (In Spanish.)
3. Crandon JL, Nicolau DP. 2015. *In vivo* activities of simulated human doses of cefepime and cefepime-AAI101 against multidrug-resistant Gram-negative *Enterobacteriaceae*. Antimicrob Agents Chemother 59:2688–2694. https://doi.org/10.1128/AAC.00033-15.
4. DeRyke CA, Banevicius MA, Fan HW, Nicolau DP. 2007. Bactericidal activities of meropenem and ertapenem against extended-spectrum-beta-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* in a neutropenic mouse thigh model. Antimicrob Agents Chemother 51:1481–1486. https://doi.org/10.1128/AAC.00752-06.
5. Ghazi IM, Crandon JL, Lesho EP, McGann P, Nicolau DP. 2015. Efficacy of humanized high-dose meropenem, cefepime, and levofloxacin against *Enterobacteriaceae* isolates producing Verona integron-encoded metallo-β-lactamase (VIM) in a murine thigh infection model. Antimicrob Agents Chemother 59:7145–7147. https://doi.org/10.1128/AAC.00794-15.
6. Clinical and Laboratory Standards Institute. 2017. Performance standards for antimicrobial susceptibility testing; 17th informational supplement. CLSI document M100-S17. Clinical and Laboratory Standards Institute, Wayne, PA.
7. U.S. Food and Drug Administration. Microbiology review. Drug approval package: Avycaz. http://www.accessdata.fda.gov/drugsatfda_docs/nda/2015/206494Orig1s000MicroR.pdf. Updated March 12, 2015. Accessed 26 January 2016.
8. Borgonovi M, Miossec C, Lowther J. 2007. The Efficacy of ceftazidime combined with NXL104, a novel β-lactamase inhibitor, in a mouse model of kidney infections induced by β-lactamase producing *Enterobacteriaceae*. Abstr Proceedings of the 17th European Congress of Clinical Microbiology and Infectious Diseases, Munich, Germany.
9. McGregor JC, Allen GP, Bearden DT. 2008. Levofloxacin in the treatment of complicated urinary tract infections and acute pyelonephritis. Ther Clin Risk Manag 4:843–853. https://doi.org/10.2147/TCRM.S3426.
10. Huntington JA, Sakoulas G, Umeh O, Cloutier DJ, Steenbergen JN, Bliss C, Goldstein EJ. 2016. Efficacy of ceftolozane/tazobactam versus levofloxacin in the treatment of complicated urinary tract infections (cUTIs) caused by levofloxacin-resistant pathogens: results from the ASPECT-cUTI trial. J Antimicrob Chemother 71:2014–2021. https://doi.org/10.1093/jac/dkw053.
11. Seo YB, Lee J, Kim YK, Lee SS, Lee JA, Kim HY, Uh Y, Kim HS, Song W. 2017. Randomized controlled trial of piperacillin-tazobactam, cefepime and ertapenem for the treatment of urinary tract infection caused by extended-spectrum beta-lactamase-producing *Escherichia coli*. BMC Infect Dis 17:404. https://doi.org/10.1186/s12879-017-2502-x.
12. Sevgi DY, Gunduz A, Sahin AM, Derin O, Konuklar AS, Oncul A, Uzun N, Sonmez E. 2014. Ertapenem for the treatment of complicated urinary tract infections caused by extended-spectrum β-lactamase-producing bacteria: a case series report. Dis Mol Med 2:7–11. https://doi.org/10.5455/dmm.20140416054553.
13. Fong JJ, Rosé L, Radigan EA. 2012. Clinical Outcomes with ertapenem as a first-line treatment option of infections caused by extended-spectrum β-lactamase producing Gram-negative bacteria. Ann Pharmacother 46:347–352. https://doi.org/10.1345/aph.1Q473.
14. Eagle H, Fleischman R, Musselman AD. 1950. The bactericidal action of penicillin *in vivo*: the participation of the host, and the slow recovery of the surviving organisms. Ann Intern Med 33:544–571. https://doi.org/10.7326/0003-4819-33-3-544.