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BRIEF REPORT

In Vitro Hollow-Fiber Studies Assessing Antibacterial Activity of Ceftolozane/Tazobactam Against Multidrug-Resistant *Pseudomonas Aeruginosa*

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Our hollow-fiber infection model simulated the projected steady-state pharmacokinetics of ceftolozane and tazobactam in lung epithelial lining fluid of patients with pneumonia receiving 3 g of ceftolozane/tazobactam every 8 hours. Results confirmed the previously established in vitro activity of ceftolozane/tazobactam at and above approved breakpoints against multidrug-resistant *Pseudomonas aeruginosa*, regardless of *Pseudomonas*-derived cephalosporinase allele.

Keywords. antibacterial resistance; hollow-fiber infection model; pharmacokinetics/pharmacodynamics; *Pseudomonas aeruginosa*.

Nosocomial pneumonia is often associated with mechanical ventilation and carries a high mortality rate, with infections caused by multidrug-resistant bacteria having an increased mortality risk [1, 2]. In particular, infections caused by multidrug-resistant *Pseudomonas aeruginosa* add to the significant burden of hospital-acquired bacterial pneumonia [3, 4].

Ceftolozane/tazobactam (C/T), a fixed-dose combination of ceftolozane, an antipseudomonal cephalosporin, and tazobactam, a β-lactamase inhibitor, is active against gram-negative pathogens, including many multidrug-resistant *P. aeruginosa* strains [5–7]. Most recently, a 3-g dose of C/T (ceftolozane 2 g/tazobactam 1 g) every 8 hours (q8h) was approved for the treatment of ventilated patients with nosocomial pneumonia, based on results from a recent phase 3 study showing C/T to be noninferior to a meropenem (1 g) q8h regimen [5, 8].

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The purpose of this in vitro study was to describe the activity of C/T against *P. aeruginosa* strains using a pharmacokinetic/pharmacodynamic hollow-fiber (HF) cell culture system. Pharmacology is dictated by free drug concentration at the site of action; therefore, in contrast to other HF studies that focus on plasma concentrations [9], our study was designed to simulate the projected steady-state concentration–time profiles of ceftolozane and tazobactam in human lung epithelial lining fluid (ELF) after administration of 3 g of C/T.

All *P. aeruginosa* strains tested were clinical isolates sourced from International Health Management Associates, Inc. (IHMA; Schaumburg, IL, USA). Isolates were collected from respiratory tract specimens and were from North America, Europe, Asia Pacific, and the Middle East. Minimum inhibitory concentrations (MICs) were determined at IHMA and in house, and the higher experimentally determined MIC was used for pharmacokinetic/pharmacodynamic calculations. The C/T MICs of the 8 *P. aeruginosa* isolates were 8 μg/mL, which is 1 dilution above the susceptible breakpoints determined by the Clinical and Laboratory Standards Institute and European Committee on Antimicrobial Susceptibility Testing breakpoints of 4/4 μg/mL and 4 μg/mL, respectively [10, 11].

The *P. aeruginosa* isolates in this study were all multiply resistant; all 8 were resistant to aztreonam, cefepime, ceftazidime, and piperacillin/tazobactam, and 5 of 8 were resistant to levofloxacin and meropenem. None of the isolates were positive for extended-spectrum β-lactamases or metallo-β-lactamases. The isolates were chosen to provide a variety of *Pseudomonas*-derived cephalosporinase (*PDC*) alleles, also known as AmpC, a chromosomally encoded class C β-lactamase.

A custom-made, 2-compartment HF system [9, 12] was used to evaluate 8 distinct *P. aeruginosa* clinical isolates exposed to simulated clinical steady-state ELF exposures of ceftolozane and tazobactam from the C/T 3-g dose. This HF system has been described previously [12]. Pharmacokinetic profiles were designed to simulate the projected steady-state ELF pharmacokinetic profiles of C/T in the lungs of patients with pneumonia. This methodology was based on previous modeling of lung ELF concentrations that were achieved after 1-hour intravenous infusions of 3 g C/T q8h in ventilated patients with confirmed or suspected pneumonia [13]. The observed ELF concentration data informed the population pharmacokinetics model for ceftolozane, which simultaneously described the disposition of ceftolozane in both the plasma and ELF compartments [14]. The model projected a relatively flat ELF concentration–time profile for a typical pneumonia patient with normal renal function. The model-projected ceftolozane ELF concentration–time profile was simulated in the present HF experiment. By

comparison, tazobactam exhibited a clear elimination phase after reaching maximum concentration (C_{max}). To simulate the flat projected pharmacokinetic profile of ceftolozane in the lung ELF of a patient with pneumonia, a constant infusion of ceftolozane at 32 μg/mL (half-life >24 hours) was used. Tazobactam was delivered via syringe pump under computer control to create the appropriate steady-state pharmacokinetic profile in the lung ELF (C_{max}, 4.2 μg/mL at 2.75 hours; half-life, 3.5 hours). Free drug concentrations were measured at time points corresponding to every tazobactam C_{max} in samples taken from the extracapillary space of the HF cartridges to assess whether target drug concentrations had been reached. Liquid chromatography–tandem mass spectometry was used to assess drug levels. The lower limit of quantitation for ceftolozane and tazobactam was 100 ng/mL, and the calibration range of the assay was 0.05 to 100 μg/mL.

Although ceftolozane was delivered as a constant infusion, the system was brought to the steady-state concentration at the time of the first dose by delivery of a 5-mL bolus of drug solution containing 960 μg/mL of ceftolozane to the central compartment (150 mL). The variability of the individual measured concentration values was within the error of the detection assay. In the case of the strain that grew back at 24 hours, strain 1389713, the decrease in ceftolozane concentration observed was likely due to β-lactamase activity. For tazobactam, the syringe pump infusion method allowed the tazobactam concentrations to remain within a target range [15], with drug clearance to the projected C_{min} minimum concentration value occurring between each dose; however, this study was not a pharmacokinetics/pharmacodynamics study designed to propose a dose.

Cultures were incubated within the HF system and achieved a density of 1×10^4 colony-forming units (CFU)/mL to 1×10^6 CFU/mL at the time of the first dose, which is similar to the methodology used in previous HF studies [9, 16, 17]. The duration and sampling schedule was also similar to that used by MacGowan et al.; viable bacterial counts were determined from samples taken from the extracapillary space of the HF cartridge at prespecified time intervals (0, 2.75, 8, 16, 24, 32, 40, 48, 56, 64, and 69 hours) [16]. Activity was defined as a 3-log decrease in CFU/mL from baseline with no regrowth up to 69 hours (the time at which the studies were terminated). Although some HF studies continue past 3 days, this study followed the protocol established by Wu et al. [9]. Ceftolozane/tazobactam broth dilution assays were used to evaluate MICs of regrown colonies.

Activity was measured by change in $\log_{10}$ CFU/mL from the starting inoculum to a 24-hour time point. Activity (ie, a 3-log decrease in CFU/mL) was demonstrated in 7 of 8 *P. aeruginosa* strains (4 of 4 at a C/T MIC of 4 μg/mL and 3 of 4 at a C/T MIC of 8 μg/mL) tested at the steady-state ELF exposures in the HF model, and all *P. aeruginosa* isolates carried a *PDC* allele (Table 1). Only 1 isolate (1389713) showed an initial decrease in bacterial counts, followed by regrowth at 16 hours. This isolate encoded a *PDC-3* allele; however, the other *PDC-3* allele-containing isolate did not show regrowth (1390214). All ceftolozane and tazobactam concentrations were similar to the targets (ceftolozane, 32 μg/mL constant infusion; tazobactam, C_{max} of 4.2 μg/mL q8h). For 7 of the 8 *P. aeruginosa* isolates tested, the first 8 hours showed >3-log decreases in CFU/mL. These organisms reached the limit of detection within 8–40 hours and showed no regrowth at up to 69 hours. Figure 1, A and D shows representative time-kill study results for a strain with a C/T MIC of 4 μg/mL (strain 1404343) and a strain with a C/T MIC of 8 μg/mL (strain 1485137), respectively. The growth control had reached approximately 1×10^9 and 1×10^8 CFU/mL for strains 1404343 and 1485137, respectively, by the 8-hour time point; however, bacterial growth was abrogated in both strains under conditions that simulated ceftolozane and tazobactam concentrations at steady-state ELF with 3 g of C/T dosed q8h. In the extracapillary space of the HF cartridges, ceftolozane and tazobactam free drug concentrations were near or above the target (strain 1404343, Figure 1B and C; strain 1485137,

Table 1. *Pseudomonas aeruginosa* Strains Evaluated in the Hollow-Fiber System With C/T

Isolate	C/T MIC Used in PK/PD Analyses,[a,b] μg/mL	β-Lactamase–Encoding Genes	Δ $\log_{10}$ CFU/mL From Starting Inoculum at 24 Hours[c]
1390214	4	*PDC-3*	–3.48
1404343	4	*PDC-31*	–3.99
1411850	4	*PDC-8*	–3.83
1469708	4	*PDC-8*	–4.65
1389468	8	*PDC-44*	–3.29
1399172	8	*PDC-1*	–4.36
1485137	8	*PDC-1*	–3.88
1389713	8	*PDC-3*	2.94

Abbreviations: Δ, change; CFU, colony-forming units; C/T, ceftolozane/tazobactam; MIC, minimum inhibitory concentration; PD, pharmacodynamics; PDC, *Pseudomonas*-derived cephalosporinase; PK, pharmacokinetics.

[a]C/T MICs were determined experimentally by microbroth dilution assays with tazobactam 4 μg/mL added to varying concentrations of ceftolozane.

[b]Isolates were tested at International Health Management Associates, Inc. (Schaumburg, IL, USA) and in house, and the higher experimentally determined MIC was used for PK/PD calculations; C/T MICs were determined to be near the Clinical and Laboratory Standards Institute and European Committee on Antimicrobial Susceptibility Testing susceptibility breakpoints.

[c]Isolates 1390214 and 1389468 were tested twice due to a low starting inoculum for the first experiment. Results presented are from the second experiment.

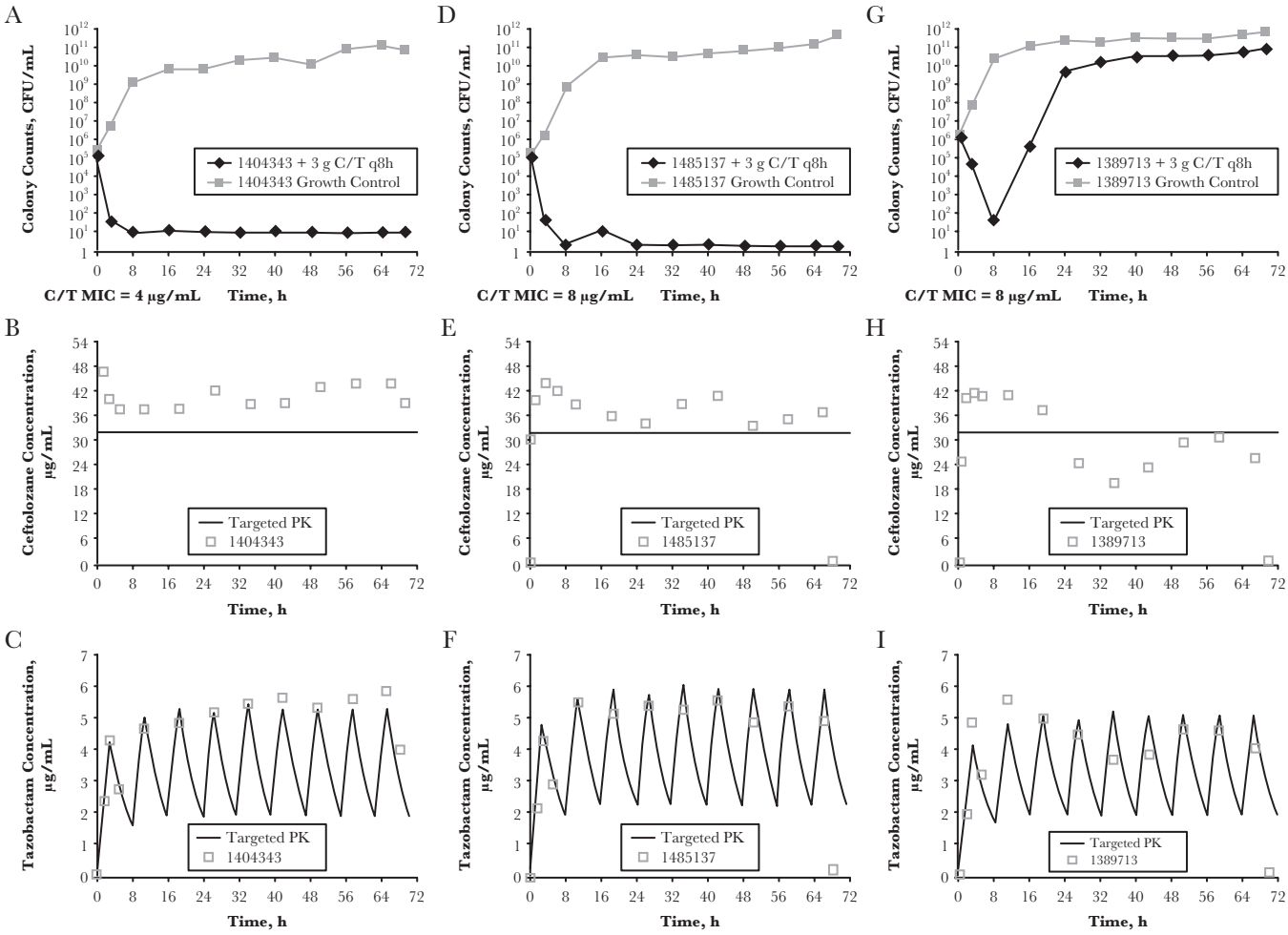

Figure 1. HF efficacy study of ceftolozane/tazobactam at simulated steady-state ceftolozane and tazobactam ELF PK profiles against *Pseudomonas aeruginosa* isolates. Depicted are the time-kill study results against *P. aeruginosa* 1404343 (A), 1485137 (D), and 1389713 (G) of 3 g C/T (ceftolozane 2 g/tazobactam 1 g) dosed q8h as an intravenous infusion over 1 hour to simulate the PK at steady-state ELF.[a] Results for 1404343 (A), 1485137 (D), and 1389713 (G) of C/T treatment q8h are shown as solid diamonds (◆), while untreated "Growth Control" results are shown as solid squares (■). The limit of detection in this assay was 10 CFU/mL. For 1404343 (B), 1485137 (E), and 1389713 (H): Ceftolozane concentrations at selected time points during the HF study (open squares [□], labeled with the organism name) vs target ceftolozane concentrations (solid lines) at steady-state ELF with 2 g of ceftolozane dosed q8h. For 1404343 (C), 1485137 (F), and 1389713 (I): Tazobactam concentrations at selected time points during the HF study (open squares [□], labeled with the organism name) vs target tazobactam concentrations (solid lines) at steady-state ELF with 1 g of tazobactam dosed q8h. [a]Ceftolozane PK at steady-state ELF was projected to most closely resemble a constant infusion; therefore, ceftolozane was delivered as a constant infusion. Tazobactam was dosed q8h by infusion over 165 minutes to simulate tazobactam PK at steady-state ELF. Abbreviations: CFU, colony-forming units; C/T, ceftolozane/tazobactam; ELF, epithelial lining fluid; HF, hollow fiber; MIC, minimum inhibitory concentration; PK, pharmacokinetic(s); q8h, every 8 hours.

Figure 1E and F). Only *P. aeruginosa* strain 1389713 had an initial 4-log CFU/mL decrease observed, followed by 24-hour regrowth of bacteria with an initial MIC of 8 µg/mL (Figure 1G–I). In strain 1389713, the C/T MIC was >64 µg/mL for resistant colonies at the end of the study (69 hours), compared with 8 µg/mL for the parent strain. Supplementary Figure 1 shows time-kill study results for additional strains evaluated and corresponding ceftolozane and tazobactam concentrations at selected time points during the HF study.

HF infection models are an established method of studying pharmacokinetic/pharmacodynamic parameters and have been used successfully for several different drugs [9, 18–21]. This report presents a novel approach that leverages an HF model and recent pharmacokinetic/pharmacodynamic data from critically ill patients with pneumonia to assess free drug concentration in ELF, not in plasma, to simulate drug exposure at the site of action. This method enables us to extend the established pharmacokinetic/pharmacodynamic profile of C/T beyond the clinical data collected and apply it to in vitro studies of resistant pathogens. A limitation to this study is the relatively low starting inoculum, which may influence bacterial kill and resistance development. Results from this in vitro HF study, which support the findings of the successful phase 3 clinical trial, show that the simulated human steady-state ELF exposures for ceftolozane and tazobactam in patients with pneumonia after administration of C/T 3 g q8h

represent adequate concentrations in the lung to provide antibacterial activity against multidrug-resistant *P. aeruginosa*.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

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Author contributions. All authors are responsible for the work described in this paper. All authors were involved in at least 1 of the following: conception, design of work or acquisition, analysis, interpretation of data, drafting the manuscript and/or revising/reviewing the manuscript for important intellectual content. All authors provided final approval of the version to be published. All authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Patient consent. This in vitro study does not include factors necessitating patient consent.

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