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PHARMACOLOGY

In Vivo Efficacy of WCK 5222 (Cefepime-Zidebactam) against Multidrug-Resistant *Pseudomonas aeruginosa* in the Neutropenic Murine Thigh Infection Model

Marguerite L. Monogue,[a,b] Jennifer Tabor-Rennie,[a] Kamilia Abdelraouf,[a] David P. Nicolau[a,c]

[a]Center for Anti-Infective Research and Development, Hartford Hospital, Hartford, Connecticut, USA
[b]Department of Pharmacy, University of Texas Southwestern Medical Center, Dallas, Texas, USA
[c]Division of Infectious Diseases, Hartford Hospital, Hartford, Connecticut, USA

ABSTRACT We describe the *in vivo* efficacy of human-simulated WCK 5222 (cefepime-zidebactam) exposure against multidrug-resistant *Pseudomonas aeruginosa* (meropenem MICs 8 to >256 μg/ml) in a neutropenic murine thigh infection model. WCK 5222 MICs ranged from 4 to 32 μg/ml. Substantial *in vivo* WCK 5222 activity was observed against all isolates, further enhancing the efficacy of zidebactam alone in 11/16 isolates (WCK 5222 mean reduction, -1.62 ± 0.58 $\log_{10}$ CFU/thigh), and a lack of activity was observed with cefepime monotherapy.

KEYWORDS human simulated, pharmacodynamics, pharmacokinetics

Pseudomonas aeruginosa is a leading cause of health care-associated infections (HAIs), ranking in the top five causative organisms for ventilator-associated pneumonia (VAP) (no. 2), catheter-associated urinary tract infections (no. 3), and surgical-site infections (no. 5) between the years 2011 and 2014 (1). Among VAP *P. aeruginosa* isolates, approximately 28% were carbapenem resistant, and 20% were deemed multidrug resistant (MDR) (1). Multidrug resistance among *P. aeruginosa* is often multifactorial due to the organism's ability to acquire multiple resistance mechanisms, including β-lactamase production, overexpression of efflux pumps, downregulation of porin production, and target site modifications (2). These properties render *P. aeruginosa* infections a serious threat, as clinicians are often compelled to adopt more aggressive treatment strategies, such as the use polymyxins, despite their toxicity.

Zidebactam is a non-β-lactam, bicyclo-acyl hydrazide antibiotic derived from diazabicyclooctane scaffold, with dual mechanisms of action, demonstrating high affinity for penicillin-binding protein 2 (PBP2) binding in Gram-negative bacteria, including *P. aeruginosa* isolates, and exhibiting β-lactamase inhibition against classes A and C enzymes (3). When paired with an appropriate β-lactam (i.e., cefepime), zidebactam has been shown to function as a β-lactam enhancer (4). *In vivo*, a human-simulated regimen (HSR) of the combination of cefepime and zidebactam (WCK 5222) caused extensive (i.e., >1 to 2 log) eradication of carbapenem-resistant *Acinetobacter* spp. from neutropenic mouse lungs and thighs (5, 6). *In vitro*, WCK 5222 inhibited 99.5% of *P. aeruginosa* isolates at concentrations of $\leq$8 μg/ml (1:1 ratio), including carbapenem-resistant *P. aeruginosa* isolates (WCK 5222 MIC_{90}s against *P. aeruginosa* of 4 μg/ml and against AmpC-overproducing and metallo-β-lactamase-producing *P. aeruginosa* of 8 μg/ml); however, *in vivo* data against *P. aeruginosa* are currently lacking (7, 8).

We sought here to evaluate the *in vivo* efficacy of the WCK 5222 HSR compared to cefepime HSR or zidebactam HSR alone against MDR *P. aeruginosa* isolates in the neutropenic murine thigh infection model. In order to demonstrate the robustness of the WCK 5222 combination against the most challenging *P. aeruginosa* strains, we were

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

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TABLE 1 Phenotypic profiles and resistance mechanisms of the isolates selected for the *in vivo* efficacy studies

Organism	MIC (μg/ml)									β-Lactamase(s) encoded
	Cefepime	Zidebactam	WCK 5222	Meropenem	Piperacillin-tazobactam	Ceftolozane-tazobactam	Aztreonam	Amikacin	Ciprofloxacin	
PSA 1716	>64	4	4	≥64	≥128	≥64	32	>32	≥8	VIM-28
PSA 1719	>64	4	4	≥8	≥128	≥64	≥16	>32	≥8	VIM-1
PSA 1727	>64	8	4	≥8	≥64	≥64	8	>32	≥8	PDC-3, VIM-2
PSA M23-21	64	512	4	8	128	8	128	256	1	NA[a]
PSA 1730	>64	8	8	≥64	≥128	≥64	≥16	>32	≥8	VEB-14, VIM-49
PSA 1559	>64	8	8	16	>32	64	>128	32	>16	NA
PSA 1728	>64	16	8	≥64	≥128	≥64	4	>32	≥8	VIM-2
PSA 1729	>64	16	8	≥64	≥64	≥64	≥16	>32	≥8	PDC-11, VEB-14, VIM-49
PSA M1-13	>64	16	8	32	128	128	128	128	32	NA
PSA M1-18	64	16	8	16	512	8	128	32	0.5	NA
PSA 1780	>256	16	16	>256	256	>256	>64	128	>16	NDM, VEB, CTX-M
PSA 1781	>256	16	16	>256	>256	>256	>64	>256	>16	VIM, OXA-4, CTX-M
PSA 1782	>256	16	16	>256	>256	>256	>64	>256	>16	NDM, CTX-M
PSA 1775	>256	32	16	>256	>256	>256	32	>256	>16	NDM, CTX-M
PSA M29-24	>64	32	16	32	256	2	32	256	4	NA
PSA 1735	>64	32	32	≥64	≥64	64	≥16	>32	4	PDC-3

[a]NA, not available.

specifically interested in examining the efficacy against isolates with a WCK 5222 MIC of $\geq$MIC$_{90}$ against *P. aeruginosa* (4 μg/ml). To guide the isolate selection, a large collection of clinical *P. aeruginosa* isolates ($n = 70$) were initially screened for the WCK 5222 MICs (cefepime-zidebactam at a 1:1 ratio), and 16 isolates were then selected for the *in vivo* efficacy assessment. All 16 isolates were MDR (defined as nonsusceptibility to at least one agent in three or more antimicrobial classes) and meropenem resistant (meropenem MICs of 8 to >256 μg/ml). Several isolates produced β-lactamases, including *Pseudomonas*-derived cephalosporinase (PDC), metallo-β-lactamases (NDM and VIM), and extended-spectrum β-lactamases (VEB and CTX-M).

The MICs of cefepime, zidebactam WCK 5222, meropenem, piperacillin-tazobactam, ceftolozane-tazobactam, aztreonam, amikacin, and ciprofloxacin were determined in triplicate using the broth microdilution as outlined by the Clinical and Laboratory Standards Institute (9).

Female ICR mice weighing 20 to 22 g (Charles River Laboratories, Inc., Wilmington, MA) were utilized in the study, and the protocol was approved by the Hartford Hospital Institutional Animal Care and Use Committee. The neutropenic murine thigh model was used as previously published (5). Treatment and control groups were composed of three mice each. Cefepime (Qilu Antibiotics, Jinan, China), zidebactam (Wockhardt Bio AG, Switzerland), and WCK 5222 treatment groups received previously established HSRs administered by subcutaneous injections equivalent to clinical doses of 2 g every 8 h (q8h; 1-h infusion), 1 g q8h (1-h infusion), or cefepime HSR coadministered with zidebactam HSR, respectively (6, 10). A confirmatory pharmacokinetic study was completed to validate human WCK 5222 exposures according to a published regimen (6). Control mice were vehicle dosed. The initial CFU burden was assessed prior to dose administration (0 h) for each isolate as mice were euthanized and their thighs harvested, as previously described (11). Efficacy was quantified by the change in bacterial density ($\Delta\log_{10}$ CFU/thigh) obtained in the treatment mice after 24 h relative to the 0-h untreated controls. The values corresponding to the $\log_{10}$ change in CFU at 24 h for zidebactam and WCK 5222 were compared using a Student *t* test (equal variances were assumed) using SigmaPlot 14.0 (Systat Software, Inc., San Jose, CA). A *P* value of $\leq$0.05 was considered significant.

For the 16 MDR-*P aeruginosa* studied, the cefepime, zidebactam, and WCK 5222 MICs were $\geq$64, 4 to 512, and 4 to 32 μg/ml, respectively (Table 1). For one isolate (M23-21), WCK 5222 showed considerably improved *in vitro* potency compared to the use of zidebactam alone, with zidebactam and WCK 5222 MICs of 512 and 4 μg/ml,

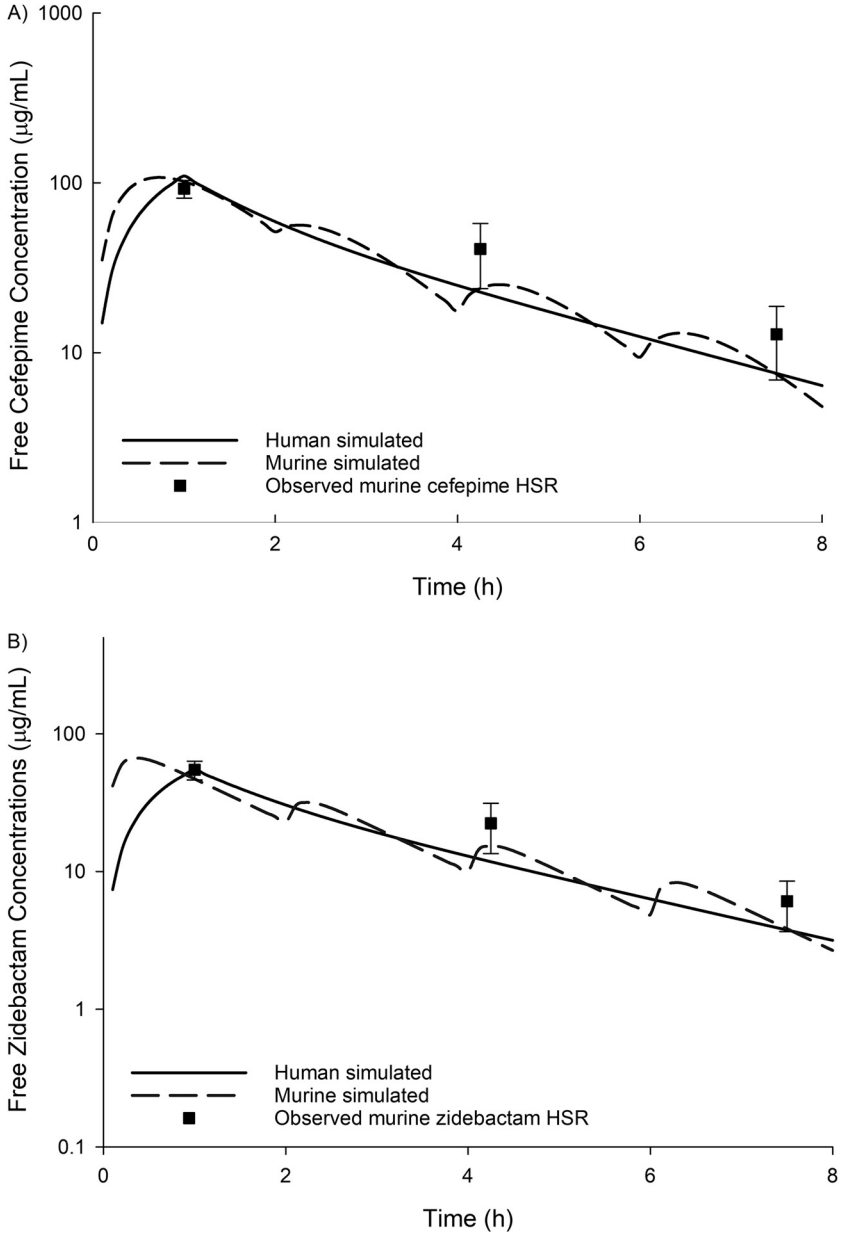

FIG 1 Observed cefepime (A) and zidebactam (B) free plasma concentrations in the neutropenic thigh infection model following human-simulated regimens.

respectively. The results from the confirmatory pharmacokinetic study are presented in Fig. 1. The mean $\log_{10}$ CFU/thigh at 0 h was 4.75±0.43. Compared to the 0-h control, the changes in mean bacterial density at 24 h in untreated control mice and in mice treated with cefepime, zidebactam, and WCK 5222 arms were +3.36±0.71, +2.12±1.17, −0.85±0.90, and −1.62±0.58 $\log_{10}$ CFU/thigh, respectively (Fig. 2). A reduction in bacterial burden was observed with the WCK 5222 HSR for all isolates. Compared to treatment with zidebactam alone, WCK 5222 demonstrated enhanced antibacterial activity in 11/16 isolates ($P < 0.05$), most notably for M23-21 (+1.34±0.54 $\log_{10}$ CFU bacterial growth with zidebactam HSR, −0.97±0.12 $\log_{10}$ CFU bacterial kill with WCK 5222 HSR) and 1775 (+0.57±0.48 $\log_{10}$ CFU bacterial growth with zidebactam HSR, −1.58±0.00 $\log_{10}$ CFU bacterial kill with WCK 5222 HSR). For isolates with a WCK 5222 MIC of <32 μg/ml, WCK 5222 achieved >1 $\log_{10}$ CFU kill against 13/15 isolates inclusive of 4/5 with MICs of 16 μg/ml.

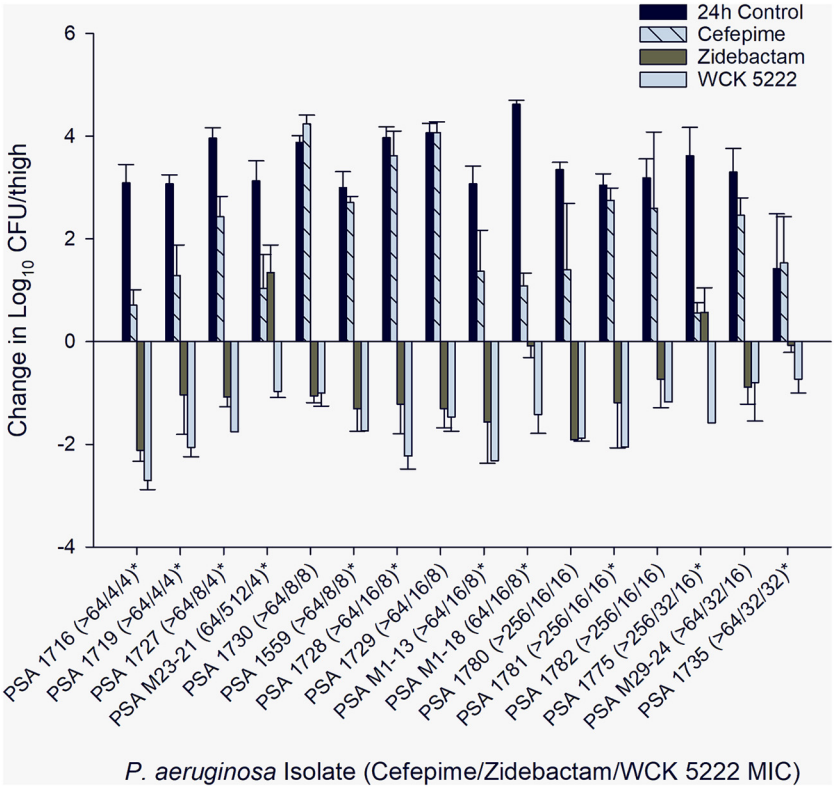

FIG 2 Mean bacterial changes in $\log_{10}$ CFU/thigh ± the standard deviations at 24 h relative to the starting inoculum in a neutropenic murine thigh infection model. MICs are displayed in μg/ml. *, statistically significant difference ($P < 0.05$) between zidebactam and WCK 5222.

Effective antimicrobials against MDR *P. aeruginosa* remain scarce, highlighting the need for agents with novel mechanisms of action. In our study, HSR of zidebactam alone demonstrated *in vivo* activity against MDR *P. aeruginosa*, inclusive of metallo-β-lactamase-producing high-risk clones, which reflected the compound's PBP binding capability in *P. aeruginosa*. WCK 5222 demonstrated enhanced efficacy over zidebactam alone in the majority of isolates up to an MIC of 32 μg/ml, a finding attributed to the β-lactam enhancing effect of zidebactam, driven by the complementary PBP binding of cefepime (PBP1 and PBP3) and zidebactam (PBP2) (3). Our findings support the clinical translatability of previous *in vitro* data demonstrating zidebactam's enhancement of the activity of β-lactams against *P. aeruginosa* exhibiting various resistance mechanisms, including isolates resistant to ceftolozane-tazobactam, an agent often reserved for infections caused by MDR *P. aeruginosa* (3). Furthermore, our data demonstrated potent WCK 5222 *in vivo* activity against *P. aeruginosa* isolates at the top 10% of the WCK 5222 MIC distribution (7, 8). In summary, data derived in the present study display the *in vitro* and *in vivo* activities of WCK 5222 against MDR and metallo-β-lactamase-producing *P. aeruginosa*, as well as assist with the delineation of the MIC susceptibility breakpoints. These results support the clinical evaluation of WCK 5222 for the management of infections due to MDR *P. aeruginosa*.

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