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EXPERIMENTAL THERAPEUTICS

In Vivo Efficacy of Humanized WCK 5222 (Cefepime-Zidebactam) Exposures against Carbapenem-Resistant *Acinetobacter baumannii* in the Neutropenic Thigh Model

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ABSTRACT Herein, we describe the *in vivo* efficacy of human-simulated WCK 5222 (cefepime-zidebactam) exposure against carbapenem-resistant *Acinetobacter baumannii* strains in a neutropenic murine thigh infection model. Five of the six isolates examined expressed OXA-23 or OXA-24. WCK 5222, despite showing MICs of 16 to 64 mg/liter, produced remarkable *in vivo* activity; human-simulated exposure showed a decline in the bacterial burden for all isolates (mean reduction, -2.09 ± 1.01 $\log_{10}$ CFU/thigh), while a lack of activity was observed with cefepime and zidebactam monotherapies.

KEYWORDS human simulated, pharmacodynamics, pharmacokinetics

Nosocomial infections due to *Acinetobacter baumannii* are prevalent worldwide and include serious infections, such as ventilator-associated pneumonia and bloodstream infections (1, 2). *A. baumannii* has the capacity to acquire almost all bacterial resistance mechanisms, including those resulting in carbapenem resistance (1) and multidrug resistance. The main mechanisms contributing to carbapenem resistance in *A. baumannii* are the production of carbapenem-hydrolyzing β-lactamases (carbapenemases, predominantly oxacillinases belonging to Ambler class D enzymes, such as OXA-23-like and OXA-24-like), the overexpression of efflux pumps, as well as a reduced expression of outer membrane porins that modulate cellular permeability (3). Thus, *A. baumannii* is considered one of the ESKAPE (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species) organisms, representing one of the greatest challenges in clinical practice (4).

A novel diazabicyclooctane non-β-lactam antibiotic, zidebactam (Wockhardt Bio AG, Switzerland), has been found to exhibit a dual mode of action that includes a β-lactam-enhancing effect mediated via selective and high-affinity binding to penicillin binding protein 2 (PBP-2) and inhibitory activity against Ambler class A and C β-lactamases (5, 6). Cefepime is a fourth-generation cephalosporin with activity against *A. baumannii* through binding to PBP-1a and PBP-3 (7, 8). Compared with cefepime alone, the combination of cefepime with zidebactam (WCK 5222; Wockhardt Bio AG, Switzerland) exhibited improved *in vitro* and *in vivo* activity against *A. baumannii* (5, 9).

We evaluated the *in vivo* efficacy of the human-simulated regimen (HSR) of WCK 5222 (9) for 24 h against clinical carbapenem-resistant *A. baumannii* isolates ($N = 6$) in a neutropenic murine thigh infection model. The protocol was approved by the Hartford Hospital Institutional Animal Care and Use Committee. All examined isolates were meropenem resistant with MICs of 8 to >64 mg/liter, including 5 isolates expressing the carbapenem-hydrolyzing oxacillinases OXA-23 or OXA-24. Cefepime, zidebactam, and WCK 5222 (cefepime-zidebactam at 1:1 ratio) MICs were assessed in

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TABLE 1 Phenotypic profiles and resistance mechanisms of the *Acinetobacter baumannii* isolates selected for the *in vivo* efficacy studies[a]

Organism	β-Lactamases	Additional positive molecular test results	Modal MIC (mg/liter)[b]		WCK 5222 $\%fT_{>MIC}$[c]	
			Cefepime	WCK 5222	Cefepime	Zidebactam
ACBN 194	ADC-25, OXA-23, OXA-82	*aph*(3′)-Ic, *armA*, *catB8*, *mph(E)*, *msr(E)*, *strA*, *strB*, *sul1*	512	16	66.25	41.25
ACBN 179	ADC-25, OXA-23, OXA-223	*aadA2*, *aadB*, *sul1*	256	32	41.25	20.42
ACBN 160	OXA-24, OXA-65, TEM-1B	*aac(3)-IIa*, *strA*, *strB*, *sul2*	>512	32	41.25	20.42
ACBN 189	OXA-24, OXA-65, TEM-1B	*aac(3)-IIa*, *strA*, *strB*, *sul2*	128	32	41.25	20.42
ACBN JJ4-25	ADC-30, OXA-66, OXA-72	*aac(3)-I*, *aacA16*, *aadA1*, *aph(6)-Ia*, *aph(6)-Id*, *sul2*, *tet(B)*	256	64	19.58	3.75
ACBN 171	ADC-25, OXA-23, OXA-66	*armA*, *catB8*, *mph(E)*, *msr(E)*, *strA*, *strB*, *sul1*	256	64	19.58	3.75

[a]Adapted from reference 9.
[b]For all isolates studied, the modal zidebactam MIC was ≥512 mg/liter.
[c]Estimated for the murine human-simulated regimens.

triplicate using the broth microdilution methodology as outlined by the Clinical and Laboratory Standards Institute (9, 10). Quality control isolate *Pseudomonas aeruginosa* ATCC 27853 was used for the validation of WCK 5222 (range, 0.5 to 2 mg/liter) and zidebactam (range, 1 to 8 mg/liter) MICs; additionally, *Staphylococcus aureus* ATCC 29213 was used for the validation of cefepime (range, 1 to 4 mg/liter). The modal MIC was utilized to characterize the isolates for the final analyses. Cefepime and WCK 5222 MICs were 128 to >512 and 16 to 64 mg/liter, respectively; zidebactam MICs were >512 mg/liter for all isolates (Table 1). Female ICR mice weighing 20 to 22 g (Envigo RMS, Inc., Frederick, MD) were rendered transiently neutropenic via intraperitoneal (i.p.) injections of 150 and 100 mg/kg cyclophosphamide (Sigma-Aldrich Co., St. Louis, MO) 4 days and 1 day prior to bacterial inoculation, respectively. In addition, a single i.p. injection of 5 mg/kg uranyl nitrate was administered 3 days prior to inoculation to induce a predictable degree of renal impairment. Mice were inoculated intramuscularly in each thigh with 0.1 ml of a bacterial suspension of 10^7 CFU/ml 2 h prior to antibiotic dosing to achieve an initial inoculum of $\sim 10^6$ CFU/thigh. Treatment and control groups were composed of 3 mice each. The 0-h control groups were sacrificed 2 h postinoculation. Treatment groups received a previously established HSR of either cefepime (Qilu Antibiotics, Jinan, China) equivalent to an intravenous (i.v.) clinical dose of 2 g every 8 h (q8h) as a 1-h infusion (9), zidebactam (Wockhardt Bio AG, Switzerland) equivalent to an i.v. clinical dose of 1 g q8h as a 1-h infusion (9), or WCK 5222 (doses of cefepime HSR coadministered with those of zidebactam HSR). All treatments were administered by subcutaneous injections (0.1 ml/agent) for 24 h. All HSRs mimicked the exposures in human plasma on the basis of the percentage of the dosing interval during which the free drug concentrations remained above the MIC ($\%fT_{>MIC}$) (9, 11). The WCK 5222 $\%fT_{>MIC}$ of cefepime and zidebactam for each isolate are reported in Table 1 (9). Control mice were vehicle dosed for 24 h. Treatment and 24-h control mice were sacrificed at the end of the study period, and thighs were harvested and processed as previously described (12). To assess efficacy, changes in the $\log_{10}$ CFU/ml at 24 h relative to the initial bacterial burdens of the 0-h groups were calculated.

The average $\log_{10}$ CFU/thigh at 0 h across all isolates was 5.85 ± 0.22. Mean increases in bacterial burden at 24 h in the untreated control, cefepime HSR-treated, and zidebactam HSR-treated groups were 2.34 ± 0.93, 1.36 ± 1.40, and 2.04 ± 0.80 $\log_{10}$ CFU/thigh, respectively. A decline in bacterial burden was observed with the WCK 5222 HSR for all isolates, with a mean reduction of −2.09 ± 1.01 $\log_{10}$ CFU/thigh across all isolates. Four out of six isolates achieved a >2-$\log_{10}$ reduction with WCK 5222 HSR, while a >1-$\log_{10}$ reduction was attained in the remaining two isolates (Fig. 1).

Infections caused by carbapenem-resistant *A. baumannii* remain a challenge to treat effectively. In the present murine thigh study, WCK 5222 displayed potent *in vivo* activity against carbapenem-resistant *A. baumannii* expressing OXA carbapenemases. These *in vivo* potency results are in general agreement with those reported by Avery et al. using the murine lung model (9). In the neutropenic lung model, the authors

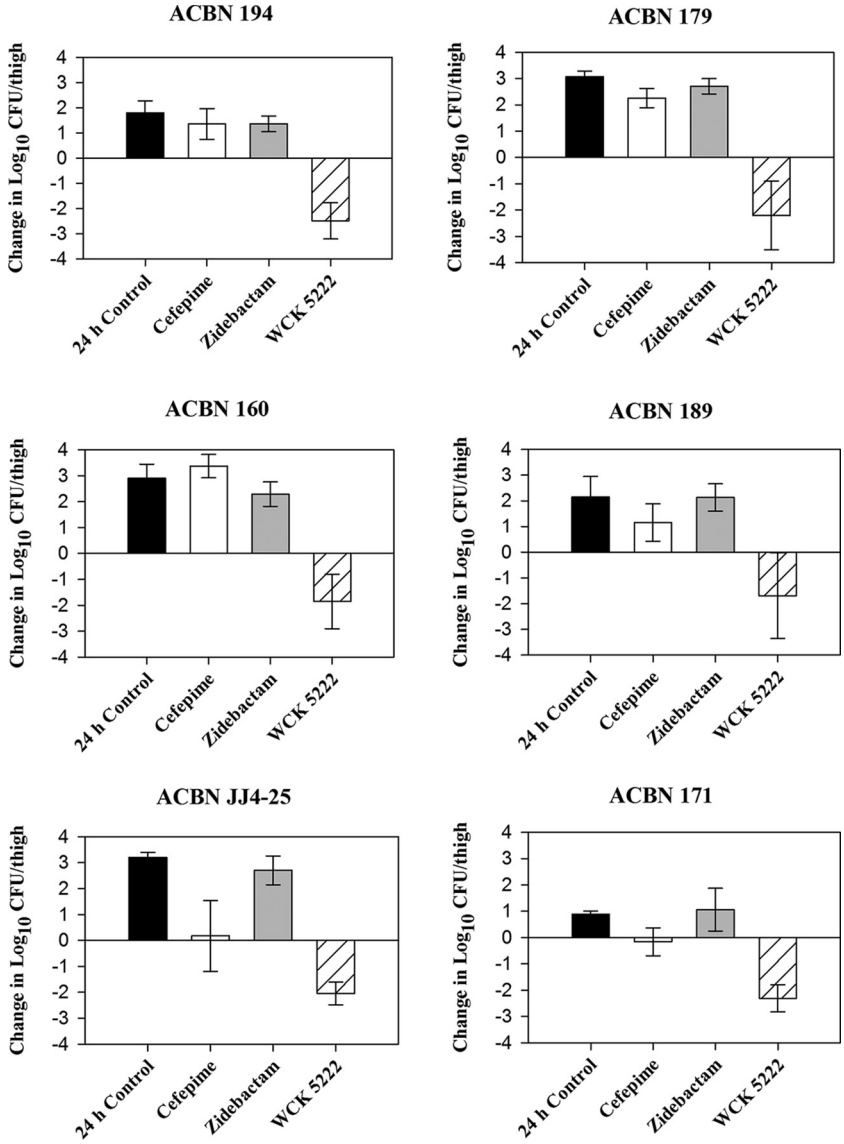

FIG 1 Mean bacterial growth or reduction in $\log_{10}$ CFU/thigh plus or minus the standard deviation (SD) at 24 h relative to the starting inoculum in a neutropenic murine thigh infection model.

demonstrated a $>$2-$\log_{10}$ reduction in bacterial burden upon the administration of WCK 5222 HSR against 12 meropenem-resistant *A. baumannii* isolates with WCK 5222 MICs of 16 to 64 mg/liter, despite achieving $\%fT_{>\mathrm{MICs}}$ of cefepime and zidebactam in plasma as low as ~20% and ~4%, respectively, as shown in Table 1 (9).

Previously published *in vitro* data demonstrated that zidebactam has the capability to potentiate the activity of cefepime against carbapenem-resistant *Enterobacteriaceae* and *Pseudomonas aeruginosa* isolates, as evidenced by the marked decline in WCK 5222 MICs relative to those of cefepime alone (13, 14). However, zidebactam has been shown to cause a modest potentiation of cefepime against *A. baumannii in vitro*, as the WCK 5222 MICs remained high ($\geq$16 mg/liter). Nevertheless, our results suggested that zidebactam could effectively enhance cefepime activity *in vivo*, inclusive of isolates with a WCK 5222 MIC of 64 mg/liter. Similar observations were reported by Bhagwat et al. and Avery et al., where cefepime exposures below the typical threshold required for efficacy were shown to produce significant bactericidal effects *in vivo* when combined with zidebactam, suggestive of significant potentiation *in vivo* (9, 15). Notably, this enhancer effect has been previously reported for other β-lactam/β-lactamase inhibitor combinations, such as nacubactam-based combinations (16).

In summary, WCK 5222 HSR showed potent *in vivo* activity against carbapenem-resistant *A. baumannii* expressing OXA carbapenemases in the murine thigh infection model, which is attributed to the β-lactam-enhancing effect of zidebactam driven by the complementary PBP binding of cefepime and zidebactam. These results support the clinical evaluation of WCK 5222 for the management of infections due to carbapenem-resistant *A. baumannii*.

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