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# Assessment of the *In Vivo* Efficacy of WCK 5222 (Cefepime-Zidebactam) against Carbapenem-Resistant *Acinetobacter baumannii* in the Neutropenic Murine Lung Infection Model

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**ABSTRACT** We evaluated the *in vivo* efficacy of human-simulated WCK 5222 (cefepime-zidebactam) against cefepime-resistant *Acinetobacter baumannii* strains ($n = 13$) in the neutropenic murine lung infection model. Twelve isolates were meropenem resistant. In control animals and those that received cefepime or zidebactam alone, the mean bacterial growth at 24 h was $>2$ $\log_{10}$ CFU/lung compared with 0-h controls ($6.32 \pm 0.33$ $\log_{10}$ CFU/lung). WCK 5222 produced a decline in the bacterial burden for all isolates (mean reduction, $-3.34 \pm 0.85$ $\log_{10}$ CFU/lung) and demonstrated remarkable potency.

**KEYWORDS** *Acinetobacter*, carbapenem resistant, lung infection

Pneumonia caused by carbapenem-resistant *Acinetobacter baumannii* is associated with increased mortality compared with carbapenem-susceptible cases (1, 2). Combination antimicrobial therapy is a preferred treatment modality, yet synergy observed *in vitro* has inconsistently translated to improved patient outcomes (1). Thus, novel agents are needed to combat these multidrug-resistant pulmonary pathogens. One such agent undergoing development, cefepime-zidebactam combination (WCK 5222; Wockhardt Bio AG, Switzerland), has displayed increased *in vitro* activity compared with cefepime alone against Gram-negative pathogens (3). Zidebactam inhibits Ambler class A and C $\beta$-lactamases and has intrinsic activity against *Enterobacteriaceae* and *Pseudomonas aeruginosa* via the inhibition of penicillin binding protein 2 (PBP-2) (3, 4). Although zidebactam has no direct antibacterial activity against *A. baumannii*, bacterial killing is observed when combined with cefepime. This activity is secondary to the $\beta$-lactam-enhancing effect of zidebactam, mediated through complementary PBP binding, as cefepime inhibits PBP-1a and PBP-3, while zidebactam inhibits PBP-2 (5).

The purpose of this study was to describe the *in vivo* efficacy of human-simulated WCK 5222 over 24 h against *A. baumannii* strains ($n = 13$, Table 1) in a neutropenic murine lung infection model (6). All strains were meropenem resistant, with the exception of ACBN 163. Female ICR mice weighing 20 to 22 g (Envigo RMS, Inc., Frederick, MD), cefepime hydrochloride with arginine (Qilu Antibiotics, Jinan, China), and zidebactam (Wockhardt Bio AG, Switzerland) were used throughout the study. For *in vivo* assessments, six mice comprised each pharmacokinetic time point and each treatment and control group in the efficacy analyses. Animals were administered all treatments by subcutaneous injection (0.1 to 0.2 ml), and the protocol was approved by the Hartford Hospital Institutional Animal Care and Use Committee. The MICs of cefepime, zidebactam, and WCK 5222 (cefepime and zidebactam, 1:1 concentration ratio) were determined in triplicate by broth microdilution using cation-adjusted Mueller-Hinton broth, in accordance with Clinical and Laboratory Standards Institute procedures (7).

Plasma and lung epithelial lining fluid (ELF) pharmacokinetic analyses were performed following intranasal inoculation with a meropenem-susceptible *A. baumannii*

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

| Isolate[a] | β-Lactamases encoded | Resistance genes detected | MIC by treatment (mode [range]) (μg/ml)[b] | |
|---|---|---|---|---|
| | | | Cefepime | WCK 5222 |
| ACBN 160 | OXA-24, OXA-65, TEM-1B | *aac(3)-IIa, strA, strB, sul2* | >512 (512, >512) | 32 (32, 32) |
| ACBN 163 | TEM-1D, ADC-25, OXA-66 | *aac(3)-Ia, aph(3′)-Ic, strA, strB, sul1* | 32 (32, 64) | 16 (16, 16) |
| ACBN 171 | ADC-25, OXA-23, OXA-66 | *armA, catB8, mph*(E), *msr*(E), *strA, strB, sul1* | 256 (256, 256) | 64 (64, 64) |
| ACBN 179 | ADC-25, OXA-23, OXA-223 | *aadA2, aadB, sul1* | 256 (256, 256) | 32 (32, 32) |
| ACBN 182 | PER-7, OXA-23, OXA-203 | *aph(3′)-VIa, armA, ARR-3, cmlA1, mph*(E), *msr*(E), *strA, strB, sul1, sul2, tet*(B) | 256 (256, 512) | 16 (16, 32) |
| ACBN 189 | OXA-24, OXA-65, TEM-1B | *aac(3)-IIa, strA, strB, sul2* | 128 (128, 256) | 32 (32, 32) |
| ACBN 194 | ADC-25, OXA-23, OXA-82 | *aph(3′)-Ic, armA, catB8, mph*(E), *msr*(E), *strA, strB, sul1* | 512 (512, >512) | 16 (16, 32) |
| ACBN JJ1-1 | ADC-81-like, OXA-24, OXA-65, TEM-1 | *aac(3)-IIa, aac(6′)-Ian, aph(3′)-VIa*-like, *aph(6)-Ia, aph(6)-Id, sul2* | >512 (>512, >512) | 64 (64, 64) |
| ACBN JJ3-20 | ADC-81-like, OXA-24, OXA-65, TEM-1 | *aac(3)-IIa, aac(6′)-Ian, aph(6)-Ia, aph(6)-Id, sul2* | 512 (512, 512) | 32 (32, 32) |
| ACBN JJ4-25 | ADC-30, OXA-66, OXA-72 | *aac(3)-I, aacA16, aadA1, aph(6)-Ia, aph(6)-Id, sul2, tet*(B) | 256 (256, 256) | 64 (64, >64) |
| ACBN JJ5-13 | ADC-33, OXA-23, OXA-82 | *aac(3)-I, ant(3″)-Ia, sul1* | 256 (256, 512) | 32 (32, 64) |
| ACBN JJ12-1 | ADC-81-like, OXA-23, OXA-69 | *armA, mph*(E), *msr*(E), *sul1* | 256 (128, 256) | 32 (32, 32) |
| ACBN JJ13-11 | ADC-96-like, CARB-16, OXA-10, OXA-23-like, OXA-58, OXA-68, OXA-72 | *aac(3)-IId, aadA1, ant(2″)-Ia, aph(6)-Ia, aph(6)-Id, arr-2, cmlA5, floR, mph*(E), *msr*(E), *sul1, sul2, tet*(X) | >512 (>512, >512) | 32 (32, 32) |

[a]Isolates with prefix "JJ" originated from a clinical respiratory culture (each from a different U.S. hospital system to minimize the inclusion of clonal isolates) and were selected from the Center for Anti-Infective Research and Development library; genotypic profiling was performed by JMI Laboratories (North Liberty, IA). All other isolates were acquired from and phenotypically and molecularly characterized by the FDA-CDC Antimicrobial Resistance Isolate Bank (Atlanta, GA).
[b]Modal zidebactam MIC was >512 μg/ml for all isolates studied.

strain, and the methods were consistent with those we have previously described (6, 8). Drug concentrations in plasma and bronchoalveolar lavage (BAL) fluid were determined using a validated liquid chromatography-tandem mass spectrometry method (9); drug concentrations in ELF were determined following normalization of drug concentrations in BAL fluid to the urea content in BAL fluid and plasma (8). Following administration of zidebactam single doses at 12.5 mg/kg and 37.5 mg/kg of body weight (based on mean body weight of the study population), the ELF-to-plasma penetration ratios were 0.81 and 0.91, respectively, calculated by dividing the ELF 24-h area under the concentration-time curve ($AUC_{0-24}$) by the calculated murine plasma unbound (free) drug $AUC_{0-24}$ ($fAUC_{0-24}$). Murine zidebactam plasma pharmacokinetic parameters were best described by a one-compartment model (WinNonlin v5.0.1; Pharsight Corp., Mountain View, CA), yielding a volume of distribution ($V$) of 0.23 liter/kg, absorption rate constant ($k_a$) of 6.95 $h^{-1}$, and elimination rate constant ($k_{e1}$) of 0.72 $h^{-1}$.

Cefepime, zidebactam, and WCK 5222 human-simulated regimens used in the efficacy analyses were constructed to describe free drug concentrations observed in humans following 1-h intravenous infusions of 2 g cefepime and 1 g zidebactam administered every 8 h in phase 1 studies (Wockhardt, Ltd., unpublished data). Specifically, these murine regimens were based on the percentage of the dosing interval during which the unbound drug concentrations exceed the MIC (%$fT_{>MIC}$) in human plasma (Table 2) following administration of the aforementioned target doses. Murine cefepime pharmacokinetic parameters that we derived previously were used to determine the cefepime regimen, accounting for differences in protein binding in humans and mice (10); for zidebactam, the parameters reported above were utilized, accounting for murine (12.6%) and human (4.7%) protein binding (Wockhardt, Ltd., unpublished data). Confirmatory plasma pharmacokinetic studies demonstrated that murine free plasma exposures predicted by the mathematical model were achieved following administration of the human-simulated regimens. The cefepime human-simulated regimen was confirmed first (Fig. 1), and the zidebactam regimen was confirmed in a separate study dosed in combination with the confirmed cefepime regimen (Fig. 2). There was no evidence of pharmacokinetic interaction between cefepime and zidebactam in plasma or ELF (Fig. 1).

In the 24-h efficacy studies, groups of animals received either human-simulated

**TABLE 2** Comparison of $\%fT_{>MIC}$ values achieved in human plasma and murine plasma after treatment[a]

| | Plasma $\%fT_{>MIC}$ for: | | | |
|---|---|---|---|---|
| | Cefepime | | Zidebactam | |
| MIC ($\mu$g/ml) | Humans | Mice | Humans | Mice |
| 4 | 100.00 | 100.00 | 92.50 | 92.50 |
| 8 | 92.92 | 92.50 | 67.50 | 70.00 |
| 16 | 66.25 | 66.25 | 42.08 | 41.25 |
| 32 | 41.25 | 41.25 | 19.17 | 20.42 |
| 64 | 18.33 | 19.58 | 0.00 | 3.75 |
| 128 | 0.00 | 0.00 | 0.00 | 0.00 |

[a]Cefepime (2 g) and zidebactam (1 g) were administered to humans intravenously over 1 h every 8 h in a phase 1 study. Human-simulated regimens were administered subcutaneously to mice.

cefepime alone, human-simulated zidebactam alone, saline injections at each treatment time point (i.e., 24-h controls), or human-simulated WCK 5222 (i.e., the combination of cefepime and zidebactam human-simulated regimens, administered as a cefepime dose followed immediately by a separate injection of the corresponding zidebactam dosage). Efficacy was defined as the change in $\log_{10}$ CFU/lung at 24 h from the 0-h control groups, composed of animals sacrificed 2 h postinoculation with each isolate (mean among all isolates, 6.32 $\pm$ 0.33 $\log_{10}$ CFU/lung). While the control and monotherapy groups experienced bacterial growth over 24 h for each isolate, WCK 5222 produced a decline in bacterial burden (mean bacterial reduction, $-3.34 \pm 0.75$ $\log_{10}$ CFU/lung; Fig. 3). WCK 5222 produced substantial bacterial killing against 13 genotypically diverse *A. baumannii* isolates, inclusive of the isolates that expressed OXA-23/24, oxacillinases often responsible for carbapenem resistance in *A. baumannii* (11). Moreover, four isolates harbored *armA*, which encodes a methyltransferase that confers high-level resistance to all aminoglycosides (12).

The pharmacodynamic driver of cephalosporin efficacy is $\%fT_{>MIC}$, and 50 to 70% is the target exposure for bactericidal activity (13). We demonstrated that WCK 5222 freely penetrated the murine ELF compartment without preferential accumulation. Thus, it is remarkable that potent bacterial killing with human-simulated WCK 5222 was

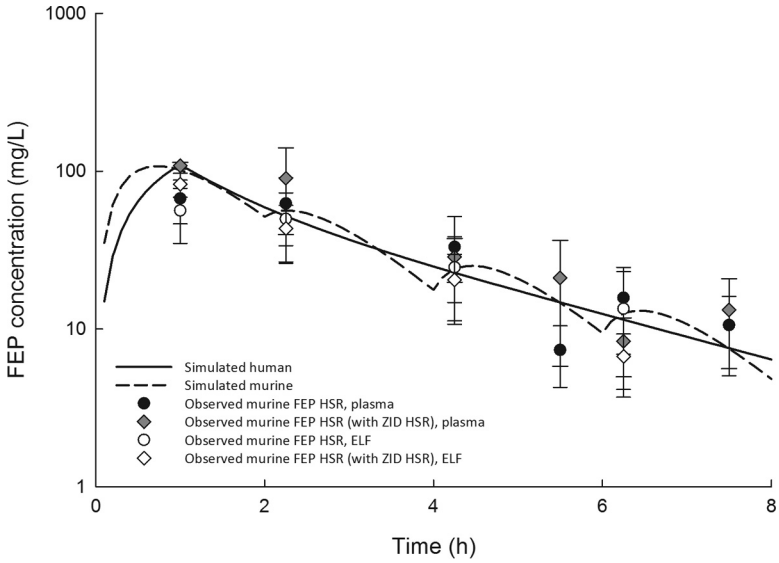


**FIG 1** Observed cefepime (FEP) free plasma and epithelial lining fluid (ELF) concentrations in the neutropenic lung infection model following dosing of the cefepime human-simulated regimen (42 mg/kg at 0 h, 9 mg/kg at 2 h, 6 mg/kg at 4 h, and 3 mg/kg at 6 h, every 8 h) alone or in combination with that of zidebactam compared with the simulated human cefepime exposure following a 2-g 1-hour intravenous infusion.

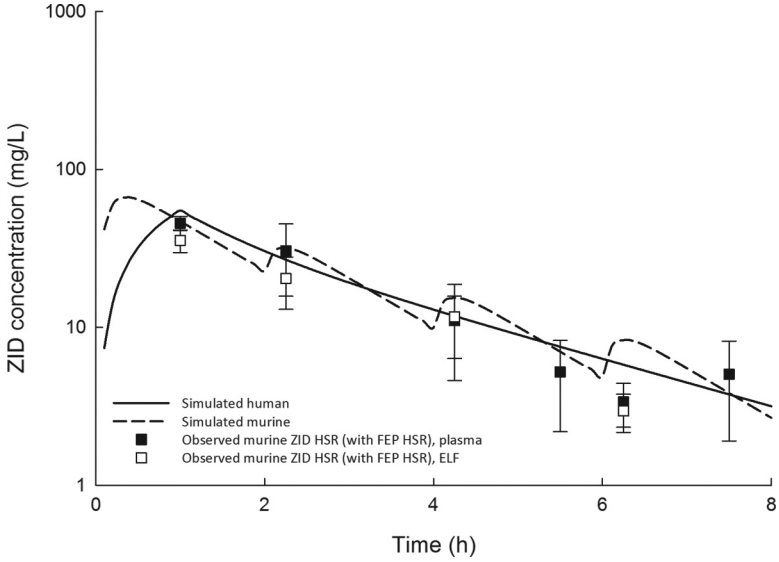


**FIG 2** Observed zidebactam (ZID) free plasma and epithelial lining fluid (ELF) concentrations in the neutropenic lung infection model following dosing of the zidebactam human-simulated regimen (22.5 mg/kg at 0 h, 4.5 mg/kg at 2 h, 2.5 mg/kg at 4 h, and 1.5 mg/kg at 6 h, every 8 h) and the cefepime human-simulated regimen compared with the simulated human zidebactam exposure following a 1-g 1-hour intravenous infusion.

observed for isolates with MICs of 64 $\mu$g/ml when the $\%fT_{>MIC}$ in plasma was only ~20% and ~4% for cefepime and zidebactam, respectively. This *in vivo* potentiation of cefepime activity is discordant with a previously reported observation of minimal *in vitro* potentiation in *A. baumannii* (3). This may be secondary to the limitations of an MIC-based assessment of synergy for $\beta$-lactam–$\beta$-lactam enhancer combinations or the use of artificial media that may underestimate the *in vivo* potency of antimicrobials (14–16). Moreover, Bhagwat and colleagues proposed that WCK 5222 activity correlated

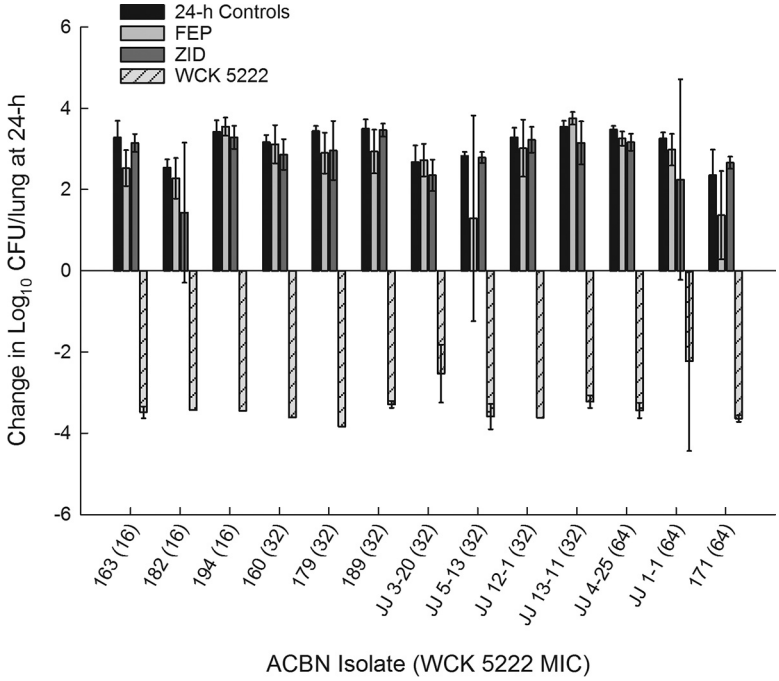


**FIG 3** Average change in $\log_{10}$ CFU/lung ($\pm$ standard deviation) at 24 h from 0 h burden in a neutropenic murine lung infection model.

with novel markers of antibacterial efficacy (17). Cefepime concentrations that were substantially lower than the MIC induced bacterial cell elongation, and this minimum elongation concentration (MEC) predicted efficacy in combination with zidebactam. Likewise, zidebactam induced spheroplast formation, and the minimum spheroplastation concentration (MSC) was reportedly associated with efficacy against *A. baumannii*. Evidence of pharmacodynamic relevance of these novel markers was based on the observation that rather than the MIC, these sub-MICs (MSC and MEC) provided an optimal exposure-response correlation (17). The MEC and MSC values were not assessed in this study, as our experiments were not designed to further validate this theory.

To summarize, human-simulated WCK 5222 demonstrated potent antibacterial activity against carbapenem-susceptible and carbapenem-resistant *A. baumannii* in the neutropenic murine lung infection model. Importantly, the extent of activity observed with the addition of zidebactam to cefepime was greater than expected, as each drug alone displayed a lack of antibacterial activity against all isolates tested. These observations support the role of zidebactam as a $\beta$-lactam enhancer. Overall, the results of this *in vivo* pharmacodynamic assessment support the continued clinical development of WCK 5222 for the treatment of lung infections caused by *A. baumannii*.

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