In Vivo Activity of WCK 4282 (High-Dose Cefepime/Tazobactam) against Serine- β -Lactamase-Producing *Enterobacterales* and *Pseudomonas aeruginosa* in the Neutropenic Murine Lung Infection Model

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ABSTRACT WCK 4282 (cefepime 2 g-tazobactam 2 g) maximizes systemic exposure of tazobactam and restores cefepime activity against various extended-spectrum β -lactamase (ESBL)- and cephalosporinase-producing strains *in vitro*. We describe clinical WCK 4282 exposure efficacies against various serine β -lactamase-producing *Enterobacterales* and *Pseudomonas aeruginosa* isolates in a murine pneumonia model. Clinical cefepime-resistant isolates (17 *Enterobacterales* and 2 *P. aeruginosa*) were utilized. Isolates expressed ESBLs, cephalosporinases, and/or serine carbapenemases (KPC and OXA-48-like). WCK 4282 MICs were 4 to 32 μ g/ml. For *in vivo* experiments, lungs of neutropenic mice were inoculated using standard inoculum (10^7 log₁₀ CFU/ml). Serine carbapenemase-producing isolates were also assessed using a low inoculum (1:5 dilution). Treatment mice received a human-simulated regimen (HSR) of cefepime, meropenem (control for serine carbapenemase expression with low inoculum experiments), or WCK 4282 human-simulated regimens. Efficacy was assessed as change in log₁₀ CFU/lungs at 24 h compared with 0-h controls. At standard inoculum, the mean 0-h bacterial burden was 6.65 ± 0.23 log₁₀ CFU/lungs, and it increased at 24 h by 2.48 ± 0.60 log₁₀ CFU/lungs among untreated controls. Initial bacterial burdens of lower inocula ranged from 5.81 ± 0.12 to 6.39 ± 0.13 log₁₀ CFU/lungs. At standard and/or low inocula, cefepime and meropenem provided minimal activity. WCK 4282 produced a >1 log₁₀ reduction against 9/9 ESBL-/cephalosporinase-producing strains. WCK 4282 provided variable activity among mice infected with standard or lower inocula of OXA-48-like-producers. WCK 4282 exposures provided 0.53 ± 1.07 log₁₀ CFU/lungs growth against KPC producers at a standard inoculum versus bacteriostasis (-0.15 ± 0.54 change in log₁₀ CFU/lungs) at a low inoculum. WCK 4282 produced potent *in vivo* activity against ESBL- and cephalosporinase-producing *Enterobacterales* and *P. aeruginosa* isolates and potential activity against OXA-48-like-producing *Enterobacterales* isolates in a neutropenic pneumonia model.

KEYWORDS ESBL, *Escherichia coli*, pharmacodynamics, pharmacokinetics

Pneumonia is the largest contributor to infectious mortality due to difficulties in antimicrobial lung penetration and the incidence of multidrug resistance (1–3). Bacterial pneumonia is commonly caused by *Enterobacterales* and *Pseudomonas aeruginosa* strains that may harbor one or more extended-spectrum β -lactamases (ESBLs), cephalosporinases, or carbapenemases (1–6). In fact, from 2012 to 2017, there has been a 53.3% increase in incidence in infections caused by ESBL-producing bacteria (7). Often, carbapenems will be initiated as therapy to treat pneumonia caused by ESBL-producing isolates; however, their use is associated with the emergence of carbapenemase-producing

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TABLE 1 Comparison of predicted pharmacodynamic index ($\%fT_{>MIC}$) in healthy volunteers and murine human-simulated regimens (HSR)

Drug	Regime	$\%fT_{>MIC}$ ($\mu\text{g/ml}$)											$fAUC$ ($\text{mg} \cdot \text{h/liter}$)	fC_{max} ($\mu\text{g/ml}$)
		0.125	0.25	0.5	1	2	4	8	16	32	64	128		
Cefepime	Human, 2 g	100	100	100	100	100	100	97	67	38	9	0	730	79
	Murine HSR	100	100	100	100	100	100	93	63	33	10	0	703	75
Tazobactam	Human, 2 g	100	100	89	73	58	45	34	23	9	0	0	223	39
	Murine HSR	95	85	75	65	55	45	34	24	10	0	0	222	39

strains and increased drug resistance (8–11). Other available broad-spectrum antibiotics (i.e., ceftazidime, cefepime, ceftolozane-tazobactam, and piperacillin-tazobactam) that can potentially treat pneumonia due to ESBL-producing isolates have mixed efficacy results (12–15). Moreover, the MERINO trial recently demonstrated the inferiority of piperacillin-tazobactam compared with meropenem treatment for ceftriaxone nonsusceptible *Escherichia coli* and *Klebsiella pneumoniae* (16). There is a clear need for a novel carbapenem-sparing antibiotic that can treat pneumonia caused by ESBL-producing isolates.

WCK 4282 (cefepime 2 g-tazobactam 2 g, administered every 8 h as a 1.5-h infusion) is an antibiotic that has shown potent activity against penicillinase-, cephalosporinase-, and ESBL-harboring Gram-negative bacteria *in vitro* (17). Additional *in vitro* studies have shown the potential utility of WCK 4282 against OXA-48-like-producing and KPC-producing isolates; however, there are sparse data *in vivo* (18, 19). Given the promising *in vitro* “carbapenem-like” spectrum of WCK 4282, we sought to evaluate WCK 4282 efficacy against serine β -lactamase-producing *Enterobacterales* and *P. aeruginosa* isolates in a neutropenic murine lung infection model.

(This data was presented virtually at IDWeek 2020.)

RESULTS

Human-simulated plasma pharmacokinetic studies. The free antibiotic time above the MIC ($fT_{>MIC}$), the free area under the concentration-time curve ($fAUC$), and the maximum free antibiotic concentration (fC_{max}) achieved with the murine cefepime and tazobactam human-simulated regimens (HSRs) were comparable to those achieved in healthy volunteers receiving cefepime-tazobactam (2 g/2 g) every 8 h as a 1.5 h infusion (Table 1). The murine cefepime HSR regimen consisted of 16, 18.5, 8, and 4 mg/kg doses at 0, 1, 3.5, and 5.75 h during each 8 h interval as monotherapy and in combination with tazobactam. The tazobactam component consisted of one 20 mg/kg dose given at 0 h during each 8-h interval. Confirmatory pharmacokinetic studies demonstrated that the observed concentrations were equivalent to the simulated regimens for cefepime as monotherapy, as well as to cefepime and tazobactam in combination (Fig. 1).

Standard and low *in vivo* efficacy studies. Starting average 0-h bacterial burden (mean $\pm$ standard deviation [SD]) for the standard inoculum experiments was $6.65 \pm 0.23 \log_{10}$ CFU/lungs across all isolates. Bacterial burdens increased to $9.13 \pm 0.59 \log_{10}$ CFU/lungs in the 24-h controls. For the ESBL/cephalosporin-producing strains, the change in the bacterial burdens after 24 h among control, cefepime HSR, and WCK 4282 HSR groups is shown in Fig. 2. The bacterial densities among the ESBL/cephalosporinase-producing *E. coli* increased by $2.12 \pm 0.82 \log_{10}$ CFU/lungs after cefepime HSR exposure. Comparatively, WCK 4282 HSR produced a potent -2.70 ± 0.63 (range, -1.80 ± 0.48 to -3.27 ± 0.42) $\log_{10}$ CFU/lungs average reduction in bacterial burden against the same isolates. WCK 4282 HSR achieved at least 1 $\log_{10}$ and 2 $\log_{10}$ reductions against 7 and 6 of the 7 examined ESBL-producing *E. coli* isolates, respectively. For ESBL-/AmpC-overproducing *P. aeruginosa* isolates, cefepime and WCK 4282 exposures resulted in average bacterial burden reductions of -0.23 ± 1.01 and $-2.04 \pm 0.18 \log_{10}$ CFU/lungs, respectively.

Starting 0-h bacterial burden for OXA-48-like-producing isolates using the standard inoculum ranged from 6.54 ± 0.18 to $6.83 \pm 0.17 \log_{10}$ CFU/lungs. Averaged observed changes in bacterial density after cefepime HSR and WCK 4282 HSR exposures were

FIG 1 Observed murine free cefepime plasma concentration (mean $\pm$ standard deviation [SD]) for WCK 4282 human-simulated regimen (HSR) and cefepime HSR compared with the expected human and murine exposures. (A) cefepime alone and in the presence of tazobactam, and (B) tazobactam in the presence of cefepime.

1.58 ± 0.93 and $-0.11 \pm 0.76 \log_{10}$ CFU/lungs, respectively (Fig. 3) with only 1 isolate achieving a $>1 \log_{10}$ reduction. In the KPC-producing isolates, the standard starting 0-h inoculum ranged from 6.49 ± 0.30 to $7.02 \pm 0.16 \log_{10}$ CFU/lungs. Modest WCK 4282 activity ($0.53 \pm 1.07 \log_{10}$ CFU/lungs average growth) was observed compared with that in the cefepime HSR counterpart ($2.08 \pm 0.59 \log_{10}$ CFU/lungs average growth), as shown in Fig. 4.

Three isolates (EC 734, ECL 123, and EA 59) were excluded from the low-inoculum studies due to inadequate isolate fitness resulting in the inability of these isolates to induce infection at the low starting bacterial burden, as evidenced by the lack of bacterial growth among the control groups. The ranges of the 0-h bacterial burden for the lower inoculum among the OXA-48-like- and KPC-producing isolates were 5.82 ± 0.37 to 6.39 ± 0.13 and 5.81 ± 0.12 to $6.18 \pm 0.30 \log_{10}$ CFU/lungs, respectively. For the OXA-48-like-producing isolates, the observed increases in bacterial burden were

FIG 2 Change in bacterial density (mean $\pm$ SD) at 24 h for mice receiving control, cefepime HSR, and WCK 4282 HSR for isolates harboring various ESBLs and/or cephalosporinases for *E. coli* and *P. aeruginosa*.

0.94 ± 1.20 and 0.83 ± 1.13 log₁₀ CFU/lungs after cefepime and meropenem HSR exposures, respectively. On the other hand, the WCK 4282 HSR exhibited greater activity with the lower inoculum relative to that with the standard inoculum; the average reduction in bacterial burden was -1.16 ± 0.22 log₁₀ CFU/lungs (Fig. 3). For KPC-producing isolates at the lower inoculum, similar growth was seen between cefepime (1.79 ± 0.12 log₁₀ CFU/lungs) and meropenem (1.78 ± 0.79 log₁₀ CFU/lungs) HSR groups (Fig. 4). Consistent with the findings from the experiments with the standard inoculums, modest and variable degrees of *in vivo* activity was observed with WCK

FIG 3 Change in bacterial density (mean $\pm$ SD) at 24 h for mice receiving control, cefepime HSR, WCK 4282 HSR, and meropenem HSR for isolates harboring an OXA-48-like enzyme with or without ESBLs/cephalosporinases for standard and low inocula.

FIG 4 Change in bacterial density (mean $\pm$ SD) at 24 h for mice receiving control, cefepime HSR, WCK 4282 HSR, and meropenem HSR for isolates harboring KPC with or without ESBLs for standard and low inocula.

4282 HSR (-0.15 ± 0.54 log₁₀ CFU/lungs average reduction).

DISCUSSION

For years, the empirical antibiotic workhorse for bacterial pneumonia infections has been piperacillin-tazobactam; however, with the rising incidence of Gram-negative bacteria expressing different variants of ESBLs, there is a clear need for empirical therapy replacement. Other potential options, such as carbapenems, may be effective treatment options, but they are associated with long-term multidrug-resistant repercussions. Choosing an appropriate empirical option is key to improving patient outcomes. A retrospective study by Cheng and colleagues evaluated the risk factors associated with increased mortality for ESBL-producing *E. coli* and *K. pneumoniae* bacteremic pneumonia among 111 patients (20). Using univariate and multivariate analyses, severe sepsis, critical illness, and the presence of rapidly fatal underlying disease were found to be associated with increased 30-day mortality. On the other hand, selecting appropriate empirical therapy was associated with a decrease in mortality for the univariate (odds ratio [OR], 0.21; 95% confidence interval [CI], 0.09 to 0.49) and the multivariate (OR, 0.19; 95% CI, 0.07 to 0.55) analyses. Other studies reported similar findings (21, 22), which further highlights the need for novel empirical therapies for the successful management of the infection due to ESBL phenotypes.

In our study, we evaluated a variety of ESBL-/cephalosporinase-producing *E. coli* and ESBL-producing/AmpC-overproducing *P. aeruginosa* isolates. The results show, using plasma exposures, that WCK 4282 exhibited potent *in vivo* activity against all the

examined isolates, including the ceftolozane–tazobactam-, and piperacillin–tazobactam-resistant phenotypes. The addition of high-dose tazobactam restored ceftolozane activity by effectively inhibiting the ESBL variant enzymes, often contributing to increased ceftolozane MICs, as evidenced by the lower WCK 4282 MICs (17, 19). The ceftolozane component of WCK 4282 was pharmacodynamically optimized to achieve its pharmacodynamic target of $fT > MIC$ of 50 to 70% for isolates with MICs of up to $16 \mu\text{g/ml}$ relative to the combination (WCK 4282) MIC (23). Thus, our *in vivo* observations corroborate these findings by demonstrating potent WCK 4282 efficacy against ESBL- and cephalosporinase-producing *E. coli* and *P. aeruginosa* isolates, with WCK 4282 MICs of up to $16 \mu\text{g/ml}$. WCK 4282 could potentially be considered as a carbapenem-sparing empirical antibiotic for bacterial pneumonia infections.

Unlike ESBL-producing organisms, variable WCK 4282 efficacy was observed with OXA-48-like-producing isolates at a standard inoculum. Although only 1 isolate was able to achieve a $>1 \log_{10}$ CFU/lungs reduction after WCK4282 HSR exposure, all 6 isolates had suppressive growth. Infections caused by OXA-48-like-producing organisms continue to burden North African and European countries (24). While there are several novel antibiotics targeting KPC-producing bacteria, a few possess activity against OXA-48-like enzymes. Theoretically, both ceftazidime and ceftolozane are stable in the presence of OXA-48-like enzymes; however, when paired with ESBL enzymes, both antibiotics are rendered ineffective (24–26). The addition of tazobactam can potentially enhance ceftolozane activity when in the presence of OXA-48-like and ESBL enzymes. While some bacterial reductions were observed with the WCK HSR compared with the ceftolozane HSR against the OXA-48-like-producing strains, the efficacy observed against the majority of the strains examined in our *in vivo* studies at the standard inoculum did not achieve a degree that would be predictive of clinical cure. On the other hand, enhanced bacterial killing was observed when a lower inoculum of the OXA-48-like-producing organisms was utilized to induce infection. Interestingly, WCK 4282 had a $>1 \log_{10}$ CFU/lungs reduction with 3/4 of the OXA-48-like-producing isolates, despite isolate growth in the presence of meropenem and ceftolozane HSR exposures. In particular, the growth under a meropenem HSR points toward efficient expression of serine carbapenemases, even at a relatively lower infecting load. The observed activity with WCK 4282 with lower-bacterial-burden infections is likely attributed to the high-dose ceftolozane and tazobactam in the combination, which could overcome the lower magnitudes of ESBL and OXA-48-like production. Thus, the results from our studies provide evidence that WCK 4282 could have a place in therapy for OXA-48-like-producing strains in clinical scenarios where lower inoculum and high drug concentrations would predominate, such as complicated urinary tract infections or complicated intra-abdominal infection with good source control.

For KPC-producing isolates, there is some literature suggesting that pairing tazobactam to ceftolozane is synergistic and enhances ceftolozane activity, which allows for the treatment of KPC-producing isolates. At both the standard and the low inocula, the addition of tazobactam to ceftolozane had a suppressive effect on KPC-producing isolates ($\sim 0.5 \log_{10}$ CFU/lungs growth and net stasis); however, this did not translate into efficacy against this infection entity. The results from our *in vivo* observations, together with the lack of reduction in the MIC when ceftolozane is combined with tazobactam (Table 2), are consistent with the findings from previously published studies that demonstrated the instability of tazobactam in the presence of KPC-type β -lactamases (27).

WCK 4282 efficacy was previously evaluated in a neutropenic thigh infection model using the same isolates examined in the current investigation (C. G. Gill, K. Abdelraouf, and D. P. Nicolau, unpublished data). In the thigh infection model, 6 of the 7 ESBL-/cephalosporinase-producing isolates achieved a $>1 \log_{10}$ CFU/lungs reduction upon the administration of WCK 4282 human-simulated exposure. Similarly to the lung model, 3 of the OXA-48-like-producing isolates examined had variable suppressive growth with WCK 4282, whereas minimal suppressive activity was observed among the KPC-producing isolates. Thus, the parallel observations across two different

TABLE 2 Isolates included in neutropenic murine lung infection model *in vivo* efficacy studies and their respective MICs

β -lactamase class	Isolate ^a	Genotype	MIC (μ g/ml) ^b					
			Cefepime	WCK 4282 (TZB ^c 8 μ g/ml)	Piperacillin-tazobactam	Ceftolozane-tazobactam	Meropenem	Imipenem
ESBL and/or cephalosporinase	EC 741	Not determined	>128	4	>128	32	0.12	0.25
	EC 739	MIR-1/ACT-1, DHA-1/DHA-2, CTX-M GR-1	>128	4	64	32	0.06	0.5
	EC 737	CMY, TEM	>128	8	>128	>128	0.25	0.25
	EC 731	TEM, PBP3 insert	>128	8	>128	64	0.12	0.5
	EC 732	CTX-M Gr-1/2, PBP3 insert	>128	8	>128	64	0.06	0.12
	EC 740	CMY, TEM	>128	8	>128	64	0.06	0.25
	EC 728	CMY, TEM, PBP3 insert	64	16	>128	>128	0.06	1
	PSA 1881	AmpC, VEB	>128	16	16	>128	2	1
	PSA 1882	AmpC, VEB, CTX-M, OXA-1, OXA-2	>128	16	16	>128	16	16
KPC	EA 59	KPC, TEM	16	16	>128	16	32	32
	KP 909	KPC-3, SHV, TEM	32	16	>128	128	8	16
	KP 910	KPC, SHV, TEM	32	16	>128	>128	8	16
	KP 906	KPC, SHV, TEM	32	32	>128	128	16	16
OXA-48-like	KP 813	OXA-48, CTXM-15, TEM-1, SHV-12	>512	8	NA	NA	4	NA
	KP 733	OXA-48, CTXM-15, TEM-1, SHV-12	>512	8	NA	NA	4	NA
	EC 734	OXA-48/181, TEM, PBP3 insert	>128	8	>128	128	1	1
	KP 911	OXA-181, CMY, SHV, TEM, CTXM Gr-1	64	16	>128	128	32	8
	KP 908	OXA-181, CTXM Gr-1, CMY, SHV	128	16	>128	>128	32	8
	ECL 123	OXA-48, CTXM-15, ACT, TEM-OSBL	>512	64	NA	NA	4	NA

^aEC, *E. coli*; PSA, *P. aeruginosa*; EA, *Enterobacter aerogenes* (now *Klebsiella aerogenes*); KP, *Klebsiella pneumoniae*; ECL, *Enterobacter cloacae*.^bNA, not available.^cTZB, tazobactam.

infection models substantiate the utility of WCK 4282 for the treatment for ESBL and cephalosporinase producers, as well as its potential role for the management of low acute clinical infection due to OXA-48-like-producing *Enterobacteriales*.

In summary, infection caused by serine β -lactamase-producing *Enterobacteriales* spp. and *P. aeruginosa* continues to be problematic, especially bacterial pneumonia. WCK 4282 is a pharmacodynamically optimized treatment that has shown efficacy against ESBL-producing *E. coli* and *P. aeruginosa* up to an MIC of 16 μ g/ml, with potential to treat OXA-48-like-producing *Enterobacteriales* in lower-bacterial-burden infections. The *in vivo* potency of WCK 4282 using achievable human exposures suggests that this compound may play an important carbapenem-sparing role in the management of select β -lactamase-producing Gram-negative pathogens.

MATERIALS AND METHODS

Ethics. All animal experiments were conducted in concordance with the National Research Council of the National Academy of Sciences standards. The study protocol (no. HHC-2019-0112) was approved by the Institutional Animal Care and Use Committee of Hartford Hospital (assurance no. A3185-01).

Antimicrobial test agents. Commercially available cefepime (lots 108175c and 108011c; WG Critical Care, Paramus, NJ) and meropenem (lot 008D91; Fresenius Kabi, Lake Zurich, IL) 1-g vials and analytical grade tazobactam (lot 05/08, MicroConstants, Inc.; San Diego, CA; lot 89786-04-09; Ambeed, Inc., Arlington Heights, IL) were used for *in vivo* experiments. Cefepime and meropenem were diluted in concordance with the package insert and further diluted to the desired final concentrations in normal saline (NS). Tazobactam doses were reconstituted and diluted in 50 mM sodium phosphate buffer. All doses were administered subcutaneously as a final volume of 0.1 ml.

Isolates. Prior to study, all isolates were stored frozen at -80°C in skim milk. Isolates were subcultured twice on Trypticase soy agar with 5% sheep blood (Becton, Dickinson and Co., Sparks, MD) and incubated at 37°C for 18 to 24 h. A total of 19 clinical *Enterobacteriales* ($n = 17$) and *Pseudomonas aeruginosa* ($n = 2$) isolates were included for *in vivo* studies. The expressed β -lactamases and broth microdilution MICs of each strain are reported in Table 2. In total, 7 *E. coli* isolates used in experiments harbored one or more ESBL and/or cephalosporinase among other non-ESBL-type β -lactamases (TEM, $n = 4$; CTX-M, $n = 2$; CMY, $n = 2$; MIR-1/ACT-1 and/or DHA-1/DHA-2, $n = 1$; genotype not determined, $n = 1$). These *E. coli* isolates were cefepime, ceftolozane-tazobactam, and piperacillin-tazobactam resistant according to

CLSI and EUCAST breakpoints (28, 29). The 2 *P. aeruginosa* isolates harbored at least one ESBL, plus AmpC-overproduction (CTX-M, $n = 1$; VEB, $n = 2$; OXA-1/OXA-2, $n = 1$). Both *P. aeruginosa* were cefepime and ceftolozane-tazobactam resistant according to CLSI breakpoints (28) and were cefepime, ceftolozane-tazobactam, and piperacillin-tazobactam resistant according to EUCAST breakpoints (29). Four *Klebsiella pneumoniae*, one *E. coli*, and one *Enterobacter cloacae* isolate harbored an OXA-48-like enzyme with expression of additional β -lactamases (TEM, $n = 5$; CTX-M, $n = 5$; CMY, $n = 2$; ACT, $n = 1$; SHV, $n = 4$). The remaining *Enterobacterales* isolates (*K. pneumoniae*, $n = 3$ and *Enterobacter aerogenes* [*Klebsiella aerogenes*], $n = 1$) harbored a KPC with additional TEM- and/or SHV-derived enzymes.

Animals. Specific pathogen-free, female, CD-1 mice (20 to 22 g) were obtained from Charles River Laboratories, Inc. (Raleigh, NC). All animals were allowed to acclimatize for 48 h prior to study procedures and were housed in groups of six animals at controlled room temperature in HEPA-filtered cages (Innovive, San Diego, CA). Cages were supplemented with paper nesting material for enrichment purposes. Study rooms were maintained with diurnal cycles (12 h light/12 h dark), and food and water were provided *ad libitum*. Monitoring was conducted three times daily for signs of morbidity; mice were euthanized if found moribund and tissues were harvested.

Neutropenic lung infection model. Mice in all experiments were prepared as follows. To induce neutropenia, cyclophosphamide 250 mg/kg and 100 mg/kg was administered intraperitoneally (i.p.) on days -4 and -1 , respectively. To aid in developing the human-simulated regimens, a predictable degree of renal impairment was produced using 5 mg/kg of uranyl nitrate administered i.p. on day -3 (30). Mice were anaesthetized with isoflurane and inoculated intranasally with 0.05 ml of 10^7 to 10^8 CFU/ml (or 10^5 to 10^7 for lower-inoculum studies) bacterial suspensions in NS with mucin 3% (wt/vol). After anesthesia, mice recovered in an O_2 -enriched chamber, then randomized to treatment or control groups.

Human simulated plasma pharmacokinetic studies. A human-simulated regimen (HSR) was developed to simulate cefepime-tazobactam plasma pharmacokinetics of intravenous (i.v.) WCK 4282 (2 g/2 g every 8 h [q8h] administered over 1.5 h) based on a previous healthy volunteer study (31). Murine dosing regimens were developed using previously estimated pharmacokinetic parameters of cefepime (volume of the central compartment, 0.1460728 liters/kg; absorption constant, 1.4015008 h^{-1} ; elimination constant, 1.3485326 h^{-1} and tazobactam (volume of the central compartment, 0.33343189 liters/kg; absorption constant, 3.0973355 h^{-1} ; elimination constant, 0.85528953 h^{-1} from prior murine infection model studies in Phoenix WinNonlin (Centra, Princeton, NJ) (32, 33).

Following simulation, confirmatory pharmacokinetic experiments were conducted for cefepime monotherapy, as well as for cefepime and tazobactam combination, to demonstrate that the observed concentrations were comparable to the derived HSR profiles. Mice were prepared for pharmacokinetic experiments as described above. Groups of six mice per time point were euthanized by terminal cardiac puncture following CO_2 asphyxiation. Blood samples were collected at six time points (0.75 h, 1.5 h, 2.5 h, 3.75 h, 5 h, and 7 [or 7.5] h). Blood was collected in sodium-fluoride EDTA tubes (Becton, Dickinson and Co.) and centrifuged at 10,000 rpm for 10 min at $8^\circ C$. Separated plasma was stored at $-80^\circ C$ until drug concentrations were determined using a validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) method previously described (C. G. Gill et al., unpublished data). Free drug human and murine studies were corrected for cefepime (humans, 20%; mice, 0%) and tazobactam (humans, 30%; mice, 25%) protein binding (34, 35).

Standard and low inoculum *in vivo* efficacy studies. Standard inoculum efficacy studies were conducted as follows. Two hours after inoculation, one group of six mice per isolate was sacrificed using CO_2 asphyxiation followed by cervical dislocation. The remaining groups received one of the following regimens for 24 h: WCK 4282 HSR, cefepime HSR, or a normal saline control administered at the same frequency as the WCK 4282 regimen. Following 24 h of treatment, the mice were sacrificed using the same methods stated above. The lungs were aseptically harvested and homogenized in NS. Lung homogenates were serially diluted onto Trypticase soy agar with 5% sheep blood plates, and the colony counts (CFU/lungs) were determined after overnight incubation. Efficacy was assessed by the change in $\log_{10}$ CFU/lungs from the 0-h control and reported as mean $\pm$ standard deviation (SD). Similarly to previous studies, a $>1\log_{10}$ CFU/lungs reduction in bacterial burden was used as a surrogate to predict efficacy in humans (36–38).

Low-inoculum lung infection studies were conducted for 3 of the 4 KPC- and 4 of the 6 OXA-48-like-producing *Enterobacterales* isolates in a similar fashion as stated above, with a few exceptions. For one, the bacterial inoculums were subjected to a 1:5 dilution relative to the standard inoculum. Furthermore, in order to demonstrate adequate carbapenemase *in vivo* activity for mice infected with the lower inoculum, an additional group per isolate was administered a previously determined HSR of meropenem simulating a 1 g q8h 30-min infusion to serve as a negative control (39). All other dosing groups, harvesting techniques, and assessments of efficacy were conducted in the same fashion as described for the standard inoculum experiments.

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