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MECHANISMS OF ACTION: PHYSIOLOGICAL EFFECTS

The Novel β-Lactam Enhancer Zidebactam Augments the *In Vivo* Pharmacodynamic Activity of Cefepime in a Neutropenic Mouse Lung *Acinetobacter baumannii* Infection Model

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ABSTRACT WCK 5222 is a combination of cefepime and the high-affinity PBP2-binding β-lactam enhancer zidebactam. The cefepime-zidebactam combination is active against multidrug-resistant Gram-negative bacteria, including carbapenemase-expressing *Acinetobacter baumannii*. The mechanism of action of the combination involves concurrent multiple penicillin binding protein inhibition, leading to the enhanced bactericidal action of cefepime. The aim of the present study was to assess the impact of the zidebactam-mediated enhanced *in vitro* bactericidal action in modulating the percentage of the time that the free drug concentration remains above the MIC (percent *fT*>MIC) for cefepime required for the *in vivo* killing of *A. baumannii*. Cefepime and cefepime-zidebactam MICs were comparable and ranged from 2 to 16 mg/liter for the *A. baumannii* strains ($n = 5$) employed in the study. Time-kill studies revealed the improved killing of these strains by the cefepime-zidebactam combination compared to that by the constituents alone. Employing a neutropenic mouse lung infection model, exposure-response analyses for all the *A. baumannii* strains showed that the cefepime *fT*>MIC required for 1-$\log_{10}$ kill was 38.9%. In the presence of a noneffective dose of zidebactam, the cefepime *fT*>MIC requirement dropped significantly to 15.5%, but it still rendered a 1-$\log_{10}$ kill effect. Thus, zidebactam mediated the improvement in cefepime's bactericidal effect observed in time-kill studies, manifested *in vivo* through the lowering of cefepime's pharmacodynamic requirement. This is a first-ever study demonstrating a β-lactam enhancer role of zidebactam that helps augment the *in vivo* activity of cefepime by reducing the magnitude of its pharmacodynamically relevant exposures against *A. baumannii*.

KEYWORDS *Acinetobacter baumannii*, *in vivo* mouse model, WCK 5222, zidebactam

The emergence of diverse carbapenemases in *Enterobacteriaceae*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii* poses a heightened therapeutic challenge (1). In the United States, the scenario is even more difficult when considering *A. baumannii* infections, since >50% of *A. baumannii* isolates are reported to be carbapenem resistant due to acquisition of carbapenem-hydrolyzing class D β-lactamases, such as OXA-23, OXA-24/40, and OXA-58. The percentage of multidrug-resistant (MDR) *Acinetobacter* spp. causing hospital-acquired infections was found to range from 5.0% to 88.1% across U.S. states, with the national average being 54.8% (2).

The recently approved drugs ceftazidime-avibactam, meropenem-vaborbactam, and plazomicin, which promise clinical coverage of certain carbapenem-resistant Gram-negative bacteria, however, are not able to offer therapeutically relevant activity against MDR *A. baumannii* strains. The pharmacophores of β-lactamase inhibitors employed in ceftazidime-avibactam and meropenem-vaborbactam are structurally quite different (diazabicyclooctane and cyclic boronic acid, respectively), yet both lack

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broad-spectrum β-lactamase-inhibitory activity; specifically, they lack activity against the class B carbapenemases expressed in *Enterobacteriaceae* and *P. aeruginosa* and the class D carbapenemases encountered in *A. baumannii* (3, 4). Thus, discovery of a β-lactamase inhibitor that inhibits all four classes of β-lactamases remains elusive, pointing toward the limitation of a β-lactamase inhibitor-based approach. Therefore, alternative approaches independent of β-lactamase inhibition are required to resurrect well-accepted β-lactam agents.

WCK 5222 is a combination of cefepime (FEP) and the β-lactam enhancer antibiotic zidebactam (ZID), which is based on a novel bicycloacyl hydrazide pharmacophore derived from diazabicyclooctane. The combination operates through a novel mechanism of action involving the concomitant inactivation of multiple penicillin binding proteins (PBPs) (5, 6). As a result, the FEP-ZID combination circumvents the need for β-lactamase inhibition and has been shown to be active against all four classes of β-lactamase producers (7). Zidebactam, being a non-β-lactam, is stable to β-lactamases, including those of class B and D, and therefore provides unhindered PBP2 binding even in β-lactamase-producing strains. Though ZID inhibits certain class A and C β-lactamases, however, it is not an inhibitor of class B β-lactamases and the *A. baumannii*-associated class D carbapenemases. Zidebactam's PBP2 binding in *A. baumannii* was evidenced through spheroplast formation upon exposure to low concentrations of ZID (5, 8, 9), as well as through cell-free PBP-binding studies (5, 6, 8, 9). Studies have shown that FEP causes PBP3 inactivation at sublethal concentrations, even in the presence of FEP-hydrolyzing β-lactamases, which is manifested as cell elongation. Upon concurrent PBP2 and PBP3 inactivation by FEP-ZID, a rapid bactericidal action is triggered even against isolates producing ZID-noninhibitable β-lactamases (5, 6, 8, 9). As a result, the FEP-ZID combination is associated with bactericidal action faster than that of FEP alone. Enhanced cell killing by combinations of β-lactam agents that provide multiple PBP occupancy was previously described (10). Even carbapenems that are known to possess a high affinity to multiple PBPs display rapid bactericidal action and have been reported to require a lower percentage of the time that the free drug concentration remains above the MIC (percent *fT*>MIC) of 20% to 30% for efficacy. In contrast, penicillins and cephalosporins are associated with a higher percent *fT*>MIC requirement owing to their sole PBP3-mediated slow bactericidal action.

In a large surveillance study, the MIC_{90} of FEP-ZID against *Enterobacteriaceae*, *P. aeruginosa*, and *A. baumannii* was 0.12, 4, and 32 mg/liter, respectively (11, 12). The *in vivo* efficacy of the FEP-ZID combination against MDR *Acinetobacter* strains has been established through several studies (13). Recently, Avery et al. (14) and Abuhussain et al. (15) demonstrated the 2- to 4-$\log_{10}$ kill of several carbapenem-resistant *A. baumannii* strains (meropenem MICs, 8 to >64 mg/liter) by a simulated human regimen of FEP-ZID in neutropenic murine lung and thigh infection models. Interestingly, most of these MDR *A. baumannii* isolates expressed a carbapenem-hydrolyzing oxacillinase (OXA-23 or OXA-24), and FEP-ZID MICs ranged from 16 to 64 mg/liter.

The objective of the present study was to explore the significance of ZID-mediated enhancement of FEP's *in vitro* bactericidal activity in modulating FEP's *in vivo* pharmacodynamic (PD) activity. A standard neutropenic mouse lung infection model was employed, wherein the efficacy of FEP alone and of FEP-ZID against five *A. baumannii* strains with FEP MICs ranging from 2 to 16 mg/liter was determined. Employing strains with lower FEP MICs enabled us to determine the magnitude of the percent *fT*>MIC for FEP monotherapy, which could then be compared with the FEP requirement in the presence of ZID. Demonstration of an impact of ZID on the FEP percent *fT*>MIC would not have been possible by employing MDR *A. baumannii* (FEP-resistant) strains, as FEP would fail to provide an antibacterial response at clinically relevant doses. In order to investigate the impact of ZID on the FEP percent *fT*>MIC required for 1-$\log_{10}$ kill, ZID was combined with various fractionated dose regimens of FEP. The percent *fT*>MIC for cefepime as monotherapy and in combination with ZID required for 1-$\log_{10}$ kill was derived from exposure-response analyses.

TABLE 1 Modal MICs of cefepime, zidebactam, cefepime-zidebactam, and meropenem against *A. baumannii* strains evaluated in time-kill and *in vivo* studies

A. baumannii strain	β-Lactamases	MIC (mg/liter)[a]			
		FEP	ZID	FEP-ZID (1:1)	MEM
ATCC 19606	ADC, TEM, PER, OXA-51	16	>512	16	2
SL1	ADC, TEM, PER, OXA-51	16	>512	16	1
NCTC 10303	ADC, TEM, PER, OXA-51	2	>512	2	1
ATCC BAA 747	ADC, TEM, OXA-51	2	>512	2	0.5
S 630	ADC, TEM, OXA-51	4	>512	4	1

[a]FEP, cefepime; ZID, zidebactam; MEM, meropenem.

(This study was presented in part at ASM Microbe, New Orleans, LA, 1 to 5 June 2017 [16].)

RESULTS

The MICs of the antibacterial agents against five *A. baumannii* strains were determined by broth microdilution. Zidebactam MICs were >512 mg/liter, consistent with the reported lack of intrinsic growth-inhibitory activity of zidebactam against *A. baumannii* (13). Cefepime and FEP-ZID (1:1 ratio) modal MICs were identical for all the strains and ranged from 2 to 16 mg/liter. All these strains showed meropenem MICs in the range of 0.5 to 2 mg/liter (Table 1).

Time-kill studies were undertaken for FEP, ZID, and FEP-ZID against all *A. baumannii* strains. ZID alone did not show killing activity, even at the highest concentration of 64 mg/liter. FEP alone at 0.5× MIC was largely static, while 1× MIC induced 0.5- to 1.5-$\log_{10}$ kill across the strains studied. However, in the presence of ZID, 0.5× MIC of FEP turned bactericidal and caused ≥2-$\log_{10}$ kill for all strains except *A. baumannii* NCTC 10303. For *A. baumannii* NCTC 10303, enhanced killing to the extent of 2 $\log_{10}$ was observed with FEP-ZID at 1× MIC. Thus, although no MIC-based synergistic effect was observed, the FEP-ZID combination showed enhanced kill compared to that of FEP and ZID alone (Fig. 1a to e).

Table 2 describes the plasma pharmacokinetic (PK) parameters of FEP and ZID in healthy mice at doses ranging from 25 to 200 mg/kg of body weight for FEP and 12.5 to 100 mg/kg for ZID administered through the subcutaneous route. For both FEP and ZID, a dose-dependent increase in the maximum concentration in plasma and the area under the concentration-time curve (AUC) was observed. The half-life for both FEP and ZID was in the range of 0.2 to 0.4 h. The mouse plasma protein binding of both FEP and ZID was <10%. Combination PK studies showed a lack of an interaction between FEP and ZID, as reported previously (data not shown) (17). The study comparing the PK profiles of FEP and ZID in healthy and infected mice showed no significant difference between the two groups (Fig. 1; see also Table S1 in the supplemental material).

In order to determine the total plasma/epithelial lining fluid (ELF) penetration ratio, mouse ELF PK were determined by employing 50- and 100-mg/kg doses of FEP and 25- and 50-mg/kg doses of ZID. The FEP and ZID ELF exposures in terms of the AUC for these doses were in the range of 13.66 ± 1.47 to 31.68 ± 3.54 μg·h/ml and 6.69 ± 0.42 to 15.49 ± 1.64 μg·h/ml, respectively (Table S2). Thus, the total plasma/ELF penetration ratio for FEP and ZID was in the range of 0.3 to 0.5. The PK/PD analyses were undertaken based on the plasma concentrations, as PK/PD requirements for β-lactams are invariably expressed by employing readily and robustly measurable plasma concentrations.

Table 3 illustrates the plasma percent *fT*>MIC for FEP and ZID, taking into account the FEP-ZID MIC range of 2 to 16 mg/liter and employing FEP at a dose of 25 mg/kg administered every 2 h (q2h) and ZID doses of 12.5 and 37.5 mg/kg q2h. The ZID dose of 12.5 mg/kg q2h in the mouse yields about 1/3 of the clinical exposure, while the higher dose of 37.5 mg/kg q2h is equivalent to the clinical exposures attained with a selected dose regimen of ZID at 1 g every 8 h (q8h) in FEP-ZID. Our previous studies have shown that varying the *in vivo* exposure of one constituent of FEP-ZID modulates

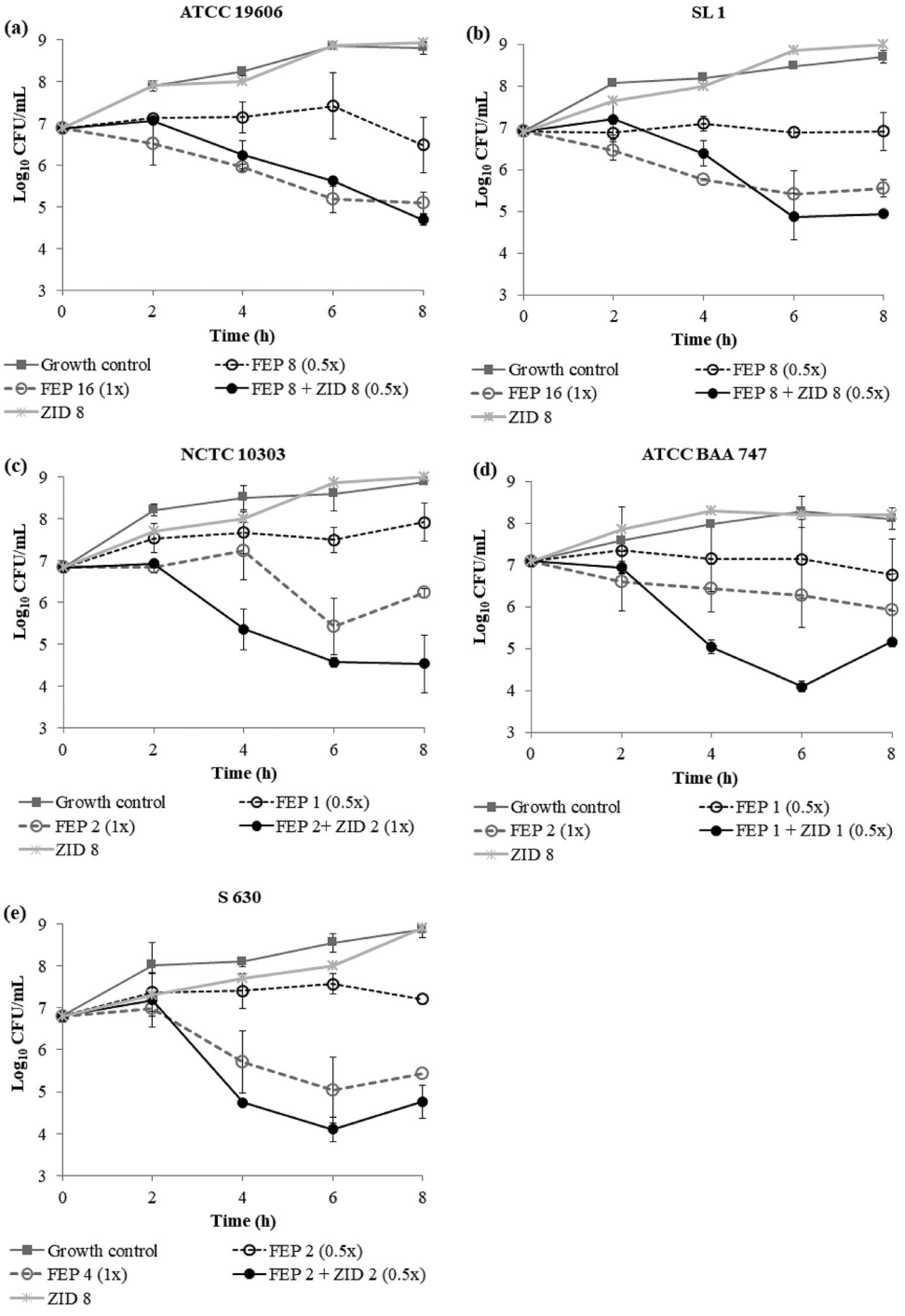

FIG 1 Time-kill study results for FEP-ZID against various *A. baumannii* strains (a to e). ZID alone at 64 mg/liter did not elicit even a bacteriostatic response. The numbers after the abbreviations FEP and ZID are concentrations of drug (in milligrams per kilogram) and the multiple of the MIC (which is in parentheses).

the efficacy-linked exposure of the second constituent. In this context, it was envisaged that use of ZID at 12.5 mg/kg q2h would impose a considerable PK/PD stretch on to FEP and, in turn, on the enhancer mechanism.

In neutropenic mouse lung infection studies, across the strains, the mean ± standard deviation bacterial lung burden at the initiation of treatment (0 h) was 7.12 ± 0.43 $\log_{10}$ CFU/lung. At the 25-h endpoint, net growth of >2 $\log_{10}$ CFU/lung was observed in untreated control mice and mice treated with ZID alone (12.5 and 37.5 mg/kg q2h), indicating a lack of *in vivo* efficacy of ZID.

TABLE 2 Mouse plasma pharmacokinetic parameters of single doses of cefepime or zidebactam[a]

Drug[a]	Dose (mg/kg)	C_{max} (mg/liter)	T_{max} (h)	AUC (μg·h/ml)	CL (liters/h/kg)	V (liters/kg)	$t_{1/2}$ (h)
FEP	25	31.9 ± 3.6	0.1 ± 0.0	15.7 ± 0.9	1.6 ± 0.1	0.6 ± 0.1	0.2 ± 0.0
	50	58.2 ± 0.3	0.25 ± 0.0	38.8 ± 0.0	2.6 ± 0.0	1.2 ± 0.6	0.3 ± 0.2
	100	151.8 ± 11.6	0.25 ± 0.0	68.42 ± 4.38	1.47 ± 0.1	0.56 ± 0.15	0.27 ± 0.1
	200	229.00 ± 0.85	0.25 ± 0.0	142.9 ± 18.0	1.41 ± 0.18	0.67 ± 0.2	0.34 ± 0.1
ZID	12.5	19.8 ± 3.4	0.3 ± 0.0	12.8 ± 0.3	1.0 ± 0.0	0.4 ± 0.0	0.3 ± 0.0
	25	38.2 ± 0.6	0.3 ± 0.0	24.4 ± 2.1	1.0 ± 0.1	1.9 ± 0.4	0.3 ± 0.0
	50	93.8 ± 19.9	0.1 ± 0.1	43.2 ± 4.3	1.2 ± 0.1	0.6 ± 0.1	0.4 ± 0.0
	100	201.6 ± 55.9	0.1 ± 0.1	90.0 ± 11.0	1.1 ± 0.2	0.47 ± 0.2	0.28 ± 0.1

[a]FEP, cefepime; ZID, zidebactam; C_{max}, maximum concentration of drug in plasma; T_{max}, time to C_{max}; AUC, area under the concentration-time curve; CL, clearance; V, volume of distribution; $t_{1/2}$, half-life.

Figure 2 illustrates the pharmacodynamic response observed with FEP monotherapy and FEP-ZID combination therapy at a total daily dose of 600 mg/kg for *A. baumannii* SL1. Based on the mouse plasma PK, FEP monotherapy at a total dose of 600 mg/kg fractionated into regimens administered every 2 h (q2h), every 4 h (q4h), every 6 h (q6h), and every 12 h (q12h) provides an FEP *fT*>MIC of 48.3%, 29.7%, 22.9%, and 12.3%, respectively. Cefepime at the most frequent regimen of 50 mg/kg q2h produced 1.03 ± 0.4-$\log_{10}$ kill, which was associated with an *fT*>MIC of 48.3%. Regimen-dependent erosion in the FEP bactericidal action was observed with less frequent fractionation of q4h (6 doses), q6h (4 doses), and q12h (2 doses). For instance, q4h, q6h, and q12h fractionations of FEP provided bacteriostatic, 0.5 ± 0.5-$\log_{10}$, and 1.48 ± 0.42-$\log_{10}$ net growth effects, respectively, due to lowering of the percent *fT*>MIC with these infrequent regimens. Interestingly, addition of ZID (12.5 mg/kg q2h) to such infrequent FEP regimens of q4h and q6h enabled FEP to achieve 1.74 ± 0.5-$\log_{10}$ kill and 1.02 ± 0.52-$\log_{10}$ kill, respectively, which were associated with FEP *fT*>MIC of 29.7% and 22.9%, respectively. These requirements are significantly lower than those for FEP monotherapy, which is associated with an *fT*>MIC of 48.3%, which is linked with 1.03-$\log_{10}$ kill, thereby demonstrating ZID's enhancer action. Notably, ZID rendered a bacteriostatic effect even at the most infrequent q12h FEP regimen, which otherwise was inefficacious. A similar trend was noted for the other strains as well.

For all five *A. baumannii* strains (strain-wise total daily FEP dose range, 50 to 600 mg/kg), bacterial eradication ranging from 0.67 to 1.41 $\log_{10}$ was observed with the regimen of FEP alone q2h. However, addition of an inefficacious dose of ZID (12.5 mg/kg q2h) to the less frequent FEP regimens of q6h or q12h yielded 1.02- to 2.96-$\log_{10}$ kill, respectively (Fig. 3). Thus, ZID was able to substantially reduce the number of FEP doses (without altering the total daily dose) and still provide a favorable pharmacodynamic effect.

Table 4 provides the percent *fT*>MIC of FEP as monotherapy and in combination with ZID, identified from a nonlinear sigmoidal maximum-effect (E_{max}) model for each of the five strains. The exposure-response curves obtained for these strains are provided in Fig. 4a to k. For 1-$\log_{10}$ kill, the *fT*>MIC for FEP monotherapy ranged from 32 to 47.8%, but in the presence of ZID, this value was significantly reduced to 10.4 to 24.9% (Table 4), signifying the role of the enhancer action of ZID. Further, the pharmacodynamic responses obtained for all the strains were combined using the nonlinear

TABLE 3 Percent *fT*>MIC for cefepime and zidebactam in mouse plasma for an MIC range of 2 to 16 mg/liter

	% *fT*>MIC for:		
MIC (mg/liter)	Cefepime at 25 mg/kg q2h	Zidebactam at 12.5 mg/kg q2h	Zidebactam at 37.5 mg/kg q2h
2	73.8	63.5	79.8
4	63.6	48.7	66.5
8	48.4	33.1	51.2
16	31.5	10.1	37.3

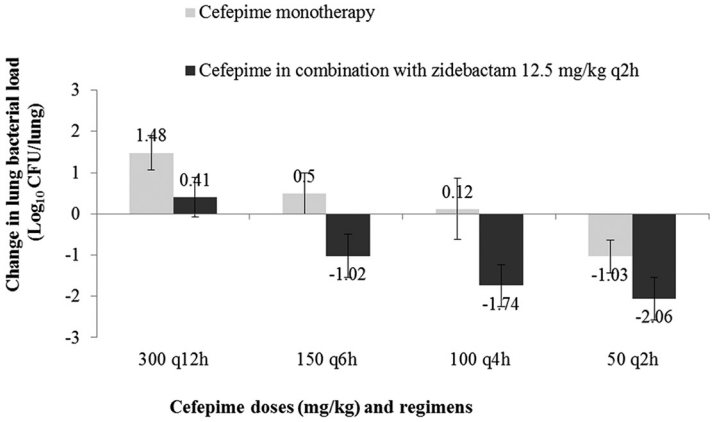

FIG 2 Pharmacodynamic efficacy of cefepime monotherapy and the cefepime-zidebactam combination against *A. baumannii* SL1.

sigmoidal E_{max} model (Fig. 5a and b). For 1-$\log_{10}$ kill, this composite fit-based exposure-response analyses showed an *fT*>MIC requirement of 38.9% for FEP alone, which dropped to 15.5% in combination with ZID (Table 4).

For *A. baumannii* SL1, the impact of a higher ZID dose providing clinically equivalent exposures was also assessed. Figure 4c shows an exposure (percent *fT*>MIC)-response curve based on the pharmacodynamic responses obtained for FEP in combination with ZID at 37.5 mg/kg q2h. The lower-dose regimen of 12.5 mg/kg q2h reduced the FEP requirement from 35.5% (Fig. 4a and Table 4) to 17.7% (Fig. 4b and Table 4) for 1-$\log_{10}$ kill. Likewise, the higher ZID dose regimen of 37.5 mg/kg q2h resulted in a reduction to 15.8%. This shows that even the lower ZID dose was able to provide a significant reduction in the FEP percent *fT*>MIC and that the higher dose maintained the enhancer effect.

DISCUSSION

The objective of the present study was to investigate the effect of PBP2-binding ZID on the pharmacodynamic activity of PBP3-binding FEP against five *A. baumannii* strains. The cefepime MIC against these strains ranged from 2 to 16 mg/liter, suggesting a lack of significant expression of FEP-impacting β-lactamases. The addition of ZID did not lower the MIC of FEP against any of these *A. baumannii* strains. However, time-kill studies revealed that ZID mediated the enhancement of bactericidal activity at sub-MICs of FEP. Even though high concentrations of ZID (64 mg/liter) were not bactericidal on a stand-alone basis, the ZID concentrations of 1 to 8 mg/liter in combination with

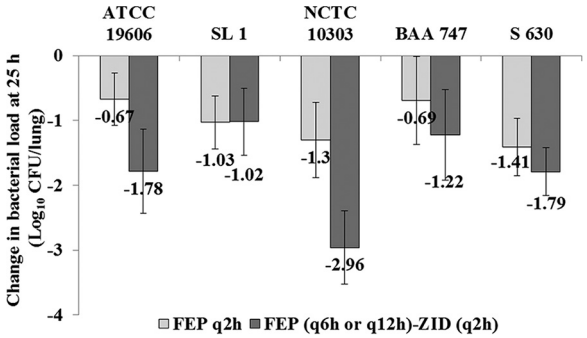

FIG 3 Pharmacodynamic response of *A. baumannii* strains to FEP and FEP-ZID in various dose regimens in an immunocompromised murine lung infection model. The lung bacterial load is represented as the mean number of $\log_{10}$ CFU per lung ± standard deviation. The strain-wise total daily doses of FEP were 600 mg/kg (ATCC 19606), 600 mg/kg (SL1), 50 mg/kg (NCTC 10303), 50 mg/kg (BAA 747), and 200 mg/kg (S 630).

TABLE 4 Percent *fT*>MIC of cefepime alone and in the presence of zidebactam for bacteriostatic and 1-$\log_{10}$-kill effects against five *A. baumannii* strains

	% *fT*>MIC requirement for the indicated effect[a]			
	Cefepime		Cefepime in presence of zidebactam	
A. baumannii strain	Bacteriostatic	1-$\log_{10}$ kill	Bacteriostatic	1-$\log_{10}$ kill
NCTC 10303	26.7	32	9.9	10.4
ATCC 19606	31.9	47.8	7.7	16.6
SL1	25.4	35.5	7.6	17.7
BAA 747	34	39.9	4.9	13.5
S630	37.4	45.4	22.7	24.9
Composite fit for all strains	30.9	38.9	7.3	15.5

[a]Identified from a sigmoidal nonlinear E_{max} model (GraphPad Prism [version 7] software)-based exposure-response analysis.

FEP induced a higher degree of killing. This corroborates the previous observation demonstrating the induction of pleomorphic spheroplasts by ZID at concentrations as low as 0.5 to 1 mg/liter, an indicator of efficient PBP2 binding in *A. baumannii* (5). Previous studies have also shown that ZID alone does not bring about consistent bactericidal action, an observation also reported for the PBP2-binding penicillin amdinocillin alone (18). In view of the reported high affinity of ZID to PBP2 and FEP to PBP3, the augmented killing observed with FEP in the presence of ZID is attributable to the simultaneous inactivation of PBP3 and PBP2 by FEP and ZID, respectively. Satta et al. previously showed that the simultaneous saturation of PBP2 and PBP3 by a combination of amdinocillin and aztreonam, respectively, produced an enhanced killing of *Escherichia coli* compared to that of single PBP2 or PBP3 saturation by amdinocillin and aztreonam alone, respectively (10).

The zidebactam-mediated enhancement of FEP killing activity observed in time-kill studies was also manifested in neutropenic mouse lung infection studies. For each strain, FEP monotherapy administered q2h was associated with 1-$\log_{10}$ kill, and less frequent q4h, q6h, and q12h regimens did not consistently result in a bactericidal effect. However, combining less frequent FEP dose regimens (q4h, q6h, or q12h) with ZID consistently resulted in a $\geq$1-$\log_{10}$ kill, despite the significantly reduced percent *fT*>MIC of FEP with these regimens. The composite fit of the FEP pharmacodynamic responses for all five *A. baumannii* strains revealed that the *fT*>MIC linked with a 1-$\log_{10}$ kill was 38.9%, which in the presence of ZID dropped to 15.5%, thus demonstrating ZID's enhancer effect *in vivo*. A similar reduction in the FEP percent *fT*>MIC was also associated with bacteriostasis as the efficacy endpoint. Thus, ZID mediated enhancement of FEP's *in vitro* bactericidal activity duly translated *in vivo* in terms of a significant reduction of the FEP pharmacodynamic requirement for efficacy. It is notable that despite the limited availability of ZID, a significant enhancement of FEP pharmacodynamic activity was observed. For instance, ZID at a dose of 12.5 mg/kg administered q2h provides a ZID *fT*>MIC of merely 10.1% for a FEP-ZID MIC of 16 mg/liter (Table 3). Such a short-duration requirement for ZID indicates that, possibly, for enhancer action, even sub-MICs drive pharmacodynamic action.

The demonstration of FEP-ZID efficacy in the lung, despite lower ELF exposure requirements, is in agreement with the findings of a previous study, wherein *A. baumannii* strains with even higher FEP-ZID MICs were employed (14). In this study, by Avery et al., the authors refer to spheroplast- and elongation-inducing concentrations of ZID and FEP, respectively, as the pharmacodynamic drivers of efficacy (14). The fact that ZID brought down the percent *fT*>MIC of FEP, despite not being able to lower the FEP MICs, signifies the relevance of time-kill studies in revealing the enhancer mechanism of action. Though the sub-MIC killing eventually diminished by 24 h *in vitro*, bacterial eradication *in vivo* was sustained due to the replenishment of FEP-ZID through repeat dose administration in mice, a scenario similar to clinical dosing.

Previously, for multiple-PBP-binding carbapenems, MacVane et al. (19) reported an

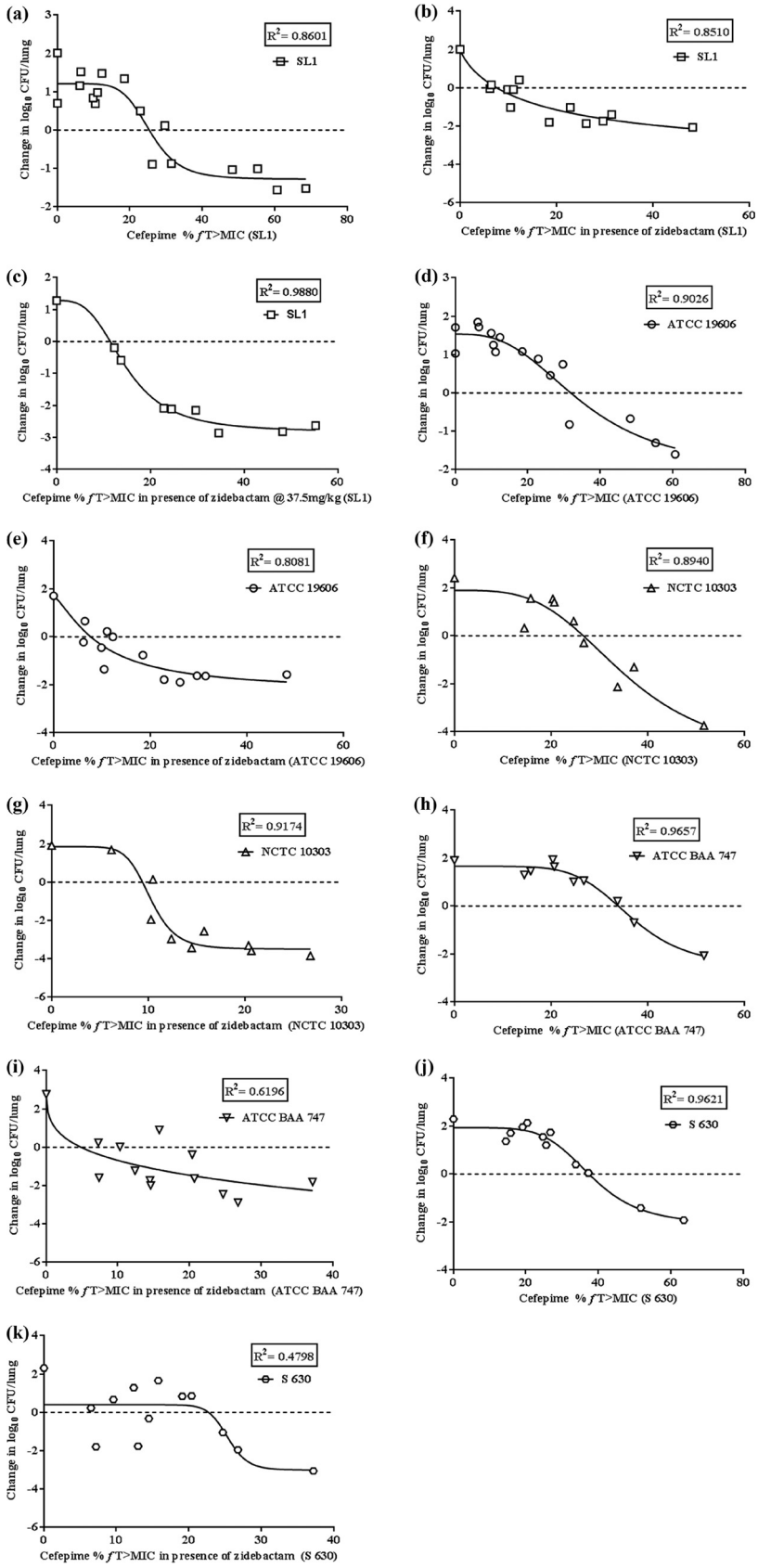

FIG 4 Exposure-response analysis for identifying the percent *fT*>MIC of cefepime alone and in the presence of zidebactam for static and 1-$\log_{10}$ kill effects. (a) Cefepime alone for *A. baumannii*

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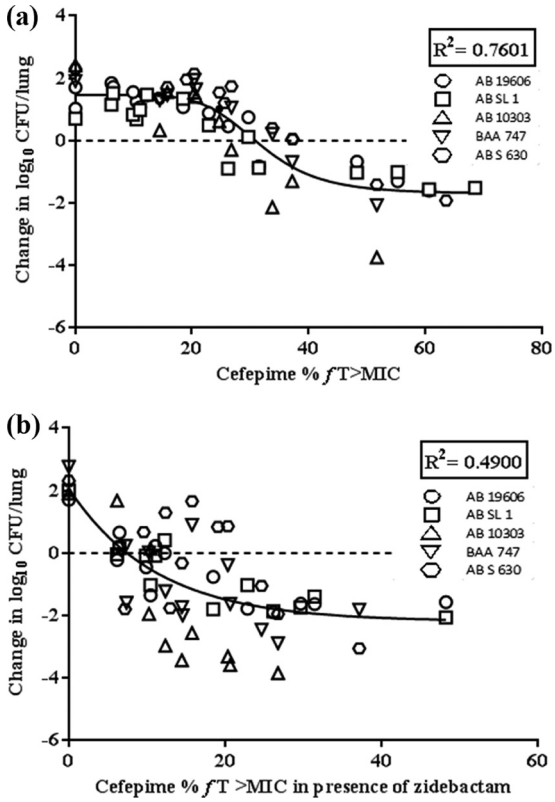

FIG 5 (a) Comodeling-based cefepime exposure-response analysis for all *A. baumannii* strains. (b) Comodeling-based cefepime-zidebactam exposure-response analysis for all *A. baumannii* strains.

fT>MIC of 23.67% and 32.82% for bacteriostatic and bactericidal effects, respectively, against *A. baumannii* in a neutropenic mouse thigh infection model. These values are lower than those reported for cephalosporins and penicillins. The characteristic features of carbapenems, such as multiple PBP binding, rapid bactericidal action, and low percent *fT*>MIC requirements, are also evident with FEP-ZID, which shows a mechanism of action similar to carbapenems. For instance, in recently reported studies involving MDR *A. baumannii* strains with an FEP-ZID MIC in the range of 16 to 64 mg/liter, FEP-ZID in a simulated human regimen showed 2- to 3-$\log_{10}$ kill against class D carbapenemase-producing *A. baumannii* strains in neutropenic mouse lung and thigh infection models (14, 15). Interestingly, in these studies, FEP availability of as low as an ~20% *fT*>MIC in combination with ZID was adequate to exert 2- to 4-$\log_{10}$ kill effects. Even for ZID, availability of as low as an ~4% *fT*>MIC was able to evoke enhancer action (14, 15).

This study demonstrated that ZID exerted a reduction in the percent *fT*>MIC of FEP, and in conjunction with the high FEP-ZID clinical doses selected, this feature could impart a substantial PK/PD leeway to the combination. This PK/PD attribute could be helpful in providing consistent clinical efficacy even for therapeutically challenging patients, such as those with reduced drug exposures.

The β-lactam enhancer action of ZID in augmenting FEP efficacy is distinct from the

FIG 4 Legend (Continued)
SL1; (b) cefepime in the presence of zidebactam for *A. baumannii* SL1; (c) cefepime in the presence of zidebactam (at a higher dose of 37.5 mg/kg q2h) for *A. baumannii* SL1; (d) cefepime alone for *A. baumannii* ATCC 19606; (e) cefepime in the presence of zidebactam for *A. baumannii* ATCC 19606; (f) cefepime alone for *A. baumannii* NCTC 10303; (g) cefepime in the presence of zidebactam for *A. baumannii* NCTC 10303; (h) cefepime alone for *A. baumannii* ATCC BAA 747; (i) cefepime in the presence of zidebactam for *A. baumannii* ATCC BAA 747; (j) cefepime alone for *A. baumannii* S 630.

action of β-lactamase inhibitors, as the latter are not known to alter the PK/PD attributes of the partner β-lactam drug. This is the first study to reveal the augmentation of FEP's pharmacodynamic action by the β-lactam enhancer ZID against *A. baumannii*.

MATERIALS AND METHODS

Antimicrobial test agents. Cefepime hydrochloride with arginine analytical powder (batch no. 7040DK86DA; Qilu Antibiotics Pharmaceutical, China) and ZID (batch no. ESS10928, synthesized by Wockhardt, India) were used throughout the studies. A commercially available formulation of meropenem (batch no. ZID17018; Zuventus, India) was used. For *in vivo* studies, drug stock solutions (20 mg/ml) were prepared with sterile water for injection, and subsequent working dilutions were prepared in sterile 0.9% saline solution. All doses were administered as milligrams per kilogram of body weight. Control animals were administered the vehicle at a volume equivalent to the highest volume used in the study group. FEP and ZID were given as two separate injections.

Bacterial isolates and *in vitro* susceptibility testing. Five carbapenemase-negative *A. baumannii* isolates (ATCC 19606, SL1, NCTC 10303, ATCC BAA 747, and S 630) that showed meropenem MICs of ≤2 mg/liter were employed. *A. baumannii* ATCC 19606 and ATCC BAA 747 were procured from ATCC. *A. baumannii* NCTC 10303 was procured from Public Health England, UK. The other two *A. baumannii* strains, SL1 and S 630, were clinical isolates obtained from Indian tertiary care hospitals. For all isolates, the modal MICs of FEP, ZID, and FEP-ZID were determined from multiple replicates, each from a separate subculture, by broth microdilution, in accordance with Clinical Laboratories and Standards Institute procedure M07-A10 (20). Quality control isolate *A. baumannii* NCTC 13304 was used for validation of FEP-ZID MICs (quality control range, 4 to 16 mg/liter) (21). Since both the constituents of FEP-ZID are antibacterially active, the MIC determination method involves the use of a 1:1 FEP-ZID ratio, which has been accepted by CLSI and published in the 28th edition of the CLSI M100 document (22). The M23 study, which involves independent MIC determinations in multiple replicates undertaken in eight U.S. laboratories, showed that the FEP-ZID MICs determined at a 1:1 ratio are highly reproducible, and the quality control ranges were successfully established (21). Moreover, the use of a 1:1 ratio for MIC determination eliminates activity bias due to either of the components.

Time-kill studies. Time kill studies were initiated by adding drugs to cation-adjusted Mueller-Hinton broth (CA-MHB) medium containing exponentially growing cultures at the targeted inoculum, and the cultures were incubated at 37°C under shaking conditions (120 rpm). Viability counts were determined at various time points by plating serial dilutions (1:10) of the cultures. Time-kill studies were initiated with an inoculum (~7 $\log_{10}$ CFU/ml) 1 $\log_{10}$ higher than the generally employed cell density. The change in the number of $\log_{10}$ CFU over that in the starting inoculum was monitored for up to 8 h upon exposure to FEP, ZID, or FEP-ZID. All time-kill studies were performed in duplicate, and mean values from two experiments with standard deviations are shown.

Animal infection model. (i) Laboratory animals. Male and female Swiss albino mice weighing approximately 22 to 24 g were obtained from the Wockhardt animal breeding facility. Mice were allowed to acclimate to the laboratory environment for at least 48 h prior to all experimental procedures and were provided food and water *ad libitum*. The study protocol was reviewed and approved by Wockhardt's Institutional Animal Ethics Committee.

(ii) Neutropenic mouse lung infection model. Male and female Swiss albino mice weighing 25 to 27 g were rendered neutropenic by intraperitoneal (i.p.) injections of cyclophosphamide at 150 and 100 mg/kg 4 days and 1 day, respectively, prior to infection. At 2 h prior to the initiation of antibacterial therapy, each animal was infected, while under anesthesia (xylazine at 8 mg/kg and thiopental sodium at 40 mg/kg i.p.), by the intranasal route with 0.08 ml of a bacterial suspension containing approximately 10^8 $\log_{10}$ CFU/ml of the infecting pathogen.

Pharmacokinetic studies. The PK of FEP and ZID alone and FEP-ZID in combination (2:1 ratio) were determined in healthy Swiss albino mice following subcutaneous administration of FEP single doses ranging from 25 mg/kg to 200 mg/kg and ZID single doses ranging from 12.5 to 100 mg/kg. For determination of the PK of the combination, FEP and ZID doses were combined at a 2:1 ratio. Following administration of the doses, blood samples were collected from groups of four mice each at 6 to 10 time points ranging from 0.25 to 24 h. Plasma FEP and ZID concentrations were determined by liquid chromatography-tandem mass spectrometry (LC-MS/MS), using a validated method. PK parameters for FEP and ZID were calculated using first-order input and elimination, by using a nonlinear least-squares technique with the help of Phoenix WinNonlin (version 6.4) software. The PK parameters obtained from these studies were used to derive the percent $fT>$MIC for FEP administered in different regimens. In a separate experiment, PK in infected and healthy mice were comparatively determined at a dose of 100 mg/kg for FEP and 50 mg/kg for zidebactam. The infection was induced as described above in "(ii) Neutropenic mouse lung infection model." Pulmonary PK studies were conducted in Swiss albino mice following subcutaneous administration of single doses of FEP alone at 25 mg/kg and ZID alone at 12.5 mg/kg. Following administration of the compounds ($n = 3$ mice/time point), samples of blood and bronchoalveolar lavage (BAL) fluid were concomitantly collected at various predetermined time points ranging from 0.0 to 8 h. Blood samples were collected through the retro-orbital sinus and placed in heparin-containing tubes, and the mice were later humanely sacrificed by cervical dislocation while they were under CO_2 anesthesia. The mice were then placed on a comfortable platform, and the trachea was exposed by making a 1-cm-long incision on the ventral neck skin for insertion of the cannula, which was secured in place. The lungs were instilled 3 times with 0.5 ml of

sterile normal saline solution (0.9% NaCl), and the fluid was immediately aspirated. Aspirates thus recovered were pooled in a single microcentrifuge tube and directly placed on ice. Collected blood and BAL fluid samples were centrifuged at 10,000 rpm for 10 min. Plasma and BAL fluid samples from the respective mice were split (according to matrix), arranged with respect to the scheduled time points, and stored at −70°C until bioanalysis. Plasma FEP and ZID concentrations were determined by LC-MS/MS using a validated method.

Bacterial density studies. In order to determine the exposure requirements of FEP alone, infected animals were treated with various FEP fractionated dose regimens that allowed creation of a range of percent *fT*>MIC values and identify the magnitude of the percent *fT*>MIC required for bacteriostasis and a 1-$\log_{10}$ drop for each strain. To assess the pharmacodynamic enhancement, FEP dose regimens were combined with a fixed ZID dose of 12.5 mg/kg, which produced about 1/3 of the clinical exposure of ZID in the FEP-ZID combination (23).

Bacterial eradication studies were performed to identify the exposures of FEP alone and FEP-ZID that provided bacteriostasis and a 1-$\log_{10}$ drop in the number of CFU of each of the five *A. baumannii* strains. At 2 h following infection, over a 24-h period, groups of 6 mice received subcutaneous doses (0.25 ml) of FEP alone in various fractionated regimens or FEP in combination with a nonefficacious 12.5-mg/kg q2h regimen of ZID. An additional ZID dose of 37.5 mg/kg q2h, which provides a clinically equivalent ZID exposure, was also tested against the SL1 strain. The control group included 6 animals that were untreated at 0 h and another 6 animals that received equivalent (0.25 ml) saline dosing at each time point for the period of 24 h. To assess the lung bacterial load at the initiation (0 h) and end (25 h) of the treatment, animals were humanely euthanized, and all lung lobes were harvested in sterile phosphate-buffered saline and homogenized. The normal practice is to determine the viable count at 24 h after the initiation of dosing; however, we delayed it by 1 h (25 h). The lung viable bacterial load (number of $\log_{10}$ CFU per lung) was determined by plating the serial dilutions of lung homogenates on Trypticase soya agar (TSA) plates, followed by incubation at 37°C for approximately 16 to 20 h. The efficacy was estimated as the mean change in the lung bacterial load at 25 h in the treatment groups compared to that at 0 h (at the initiation of therapy) in the control group.

The percent *fT*>MIC of FEP for each dose was determined from PK studies and employed in a nonlinear sigmoidal E_{max} model (GraphPad Prism [version 7] software) to derive the magnitude of the percent *fT*>MIC of FEP linked to bacteriostatic and 1-$\log_{10}$ kill effects against each strain. A comodeling approach was also undertaken, wherein the pharmacodynamic responses obtained from all strains were combined for the nonlinear sigmoidal E_{max} model to identify a unified exposure requirement for FEP alone and in the presence of ZID.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/AAC.02146-18.

SUPPLEMENTAL FILE 1, PDF file, 0.2 MB.

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