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WCK 5222 (Cefepime-Zidebactam) *In Vitro* Time-kill Studies against *Pseudomonas aeruginosa* and *Acinetobacter baumannii* Isolates with Characterized β-lactamases

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Abstract

Background: Zidebactam (ZID) is a new bicyclo-acyl hydrazide with a dual mechanism of action including selective binding to Gram-negative PBP2 and β-lactamase inhibition for Class A and C but not of Class B. We evaluated the activity of cefepime (FEP) and ZID alone and in combination against 3 ACB and 7 PSA isolates expressing Class A (KPC), B (IMP or VIM), C (AmpC) or D (OXA) β-lactamases by MIC and time-kill studies.

Methods: Broth microdilution MIC values for FEP, ZID and FEP-ZID (1:1 ratio and ZID at fixed 4, 8 and 16 μg/mL) were determined according to CLSI guidelines. Isolates were selected for time-kill based on β-lactamase content and MIC values. Time-kill testing employed sub-inhibitory concentrations of FEP and ZID as well as MIC multiples. Time-kill studies were sampled for colony counts at T_0, T_2, T_4, T_6, T_8 and T_{24}.

Results: Time-kill studies showed that ≤32 μg/mL of FEP or ≤16 μg/mL of ZID tested alone were not bactericidal against PSA (VIM-2). However, FEP-ZID combinations (32/8 and 8-32/16 μg/mL) were bactericidal against VIM-2 producing PSA by T_8. Other FEP-ZID combinations were bactericidal against PSA producing IMP-13 (32/8 and 8-32/16 μg/mL) and AmpC (8-32/8 μg/mL) by T_8 and T_{24}, respectively. Two $\log_{10}$ CFU/mL colony count reductions were observed for FEP-ZID combinations against OXA-23 (16-32/8 μg/mL) and OXA-24 (32/8 μg/mL) producing ACB by T_8.

Conclusion: FEP-ZID combinations showed potent activity in time-kill studies against PSA and ACB isolates expressing clinically relevant β-lactamases including AmpC, IMP, KPC, OXA, and VIM enzymes for which limited treatment options may be available. These results support further clinical development studies with FEP-ZID (WCK 5222).

Introduction

Widespread use of β-lactam antimicrobials commonly used to treat Gram-negative bacterial infections has resulted in the evolution and spread of clinical isolates expressing novel β-lactamases (including carbapenemases [KPC], metallo-β-lactamases [IMP, VIM, NDM] and ESBL enzymes) with resistance to current β-lactam therapies. This has created an unmet medical need for new and novel antibacterial compounds and combinations.

Zidebactam is a new bicyclo-acyl hydrazide ($C_{13}H_{21}N_5O_7S$ [**Figure 1**]) antimicrobial agent with a dual mechanism of action including selective and high affinity binding to Gram-negative PBP2, including *Pseudomonas aeruginosa* and *Acinetobacter* spp. along with β-lactamase inhibition (Class A and C, but not Class B enzymes). Cefepime is a parenteral fourth-generation oxyimino-cephalosporin with a broad-spectrum of activity against aerobic Gram-positive and Gram-negative bacteria, including *Pseudomonas aeruginosa*, approved by the United States Food and Drug Administration (US-FDA) in 1997. Current CLSI breakpoint interpretive criteria for cefepime against both *P. aeruginosa* and *Acinetobacter* spp. published in the M100-S26 document are ≤8, 16 and ≥32 μg/mL for susceptible, intermediate and resistant, respectively.

The purpose of this study was to evaluate the activity of cefepime and zidebactam alone and in combination (cefepime-zidebactam; WCK 5222) by MIC and *in vitro* time-kill testing against three *Acinetobacter baumannii* species complex (two producing Class D enzymes) and seven *P. aeruginosa* (six producing carbapenemases). Within the group of ten isolates selected, Class A (KPC), B (IMP or VIM), C (AmpC) and D (OXA) β-lactamases were represented.

Methods

Isolate collection: Molecular characterized clinical isolates were chosen for *in vitro* time-kill testing based on their β-lactamase content and susceptibility profile. Selected carbapenem-resistant *P. aeruginosa* isolates consisted of: Class A (one KPC-2), Class B (one IMP-13, one IMP-15 and one VIM-2) and Class C (one Amp-C hyper-expressing and one AmpC overexpressing plus loss of OprD). Two Class D expressing *A. baumannii* species complex (one OXA-23 and one OXA-24) isolates were also selected. An imipenem-susceptible/cefepime-resistant *A. baumannii* (ATCC 19606) and β-lactam susceptible *P. aeruginosa* (ATCC 27853) reference strain were also included.

Susceptibility testing: MIC values were determined using Clinical and Laboratory Standards Institute (CLSI) broth microdilution and macrodilution methodology as described in CLSI document M07-A10 (2016). Cefepime, the combination of cefepime + zidebactam (at a 1:1 ratio and fixed zidebactam concentrations of 4, 8, and 16 μg/mL) and zidebactam alone were tested against each isolate (**Table 1**) in 96-well, frozen-form panels produced by JMI Laboratories (North Liberty, Iowa, USA). Broth macrodilution MIC values were also generated for the seven *P. aeruginosa* isolates for zidebactam alone (**Table 1**). The carbapenems, imipenem and meropenem, were used as comparators for the *A. baumannii* and *P. aeruginosa* isolates, respectively. Quality control (QC) strains including *P. aeruginosa* ATCC 27853, *Escherichia coli* NCTC 13353 and *A. baumannii* ATCC 19606 were tested and inoculum densities were monitored by colony counts. QC ranges and interpretive criteria for the comparator compounds were as published in CLSI M100-S26 (2016), where available.

Time-kill testing: *In vitro* time-kill testing was performed according to Moody & Knapp (2010) using the 10 selected isolates for each of the cefepime and cefepime-zidebactam combinations. Additionally, zidebactam alone was tested for *in vitro* activity against the seven *P. aeruginosa* isolates. Starting inoculum density for *in vitro* time-kill testing was approximately 1.0×10^6 $\log_{10}$ CFU/mL. Time-kill curve samples were plated for colony counts at T_0, T_2, T_4, T_6, T_8, and T_{24}. The compound testing conditions and isolates selected are listed in **Table 2.**

Results

A. baumannii

- Stand-alone zidebactam was inactive in antibacterial susceptibility testing against the three *A. baumannii* isolates (MIC, >128 μg/mL; **Table 1**).
- Cefepime-zidebactam combinations demonstrated ≥four-fold and ≥two-fold MIC decreases against OXA-23 and OXA-24 producing *A. baumannii*, respectively, when compared to cefepime or zidebactam tested alone (**Table 1**). These *in vitro* time kill combination studies employing sub-MIC or MIC concentrations showed initial killing of up to 2.8-$\log_{10}$ by T_8 followed by regrowth at T_{24} (**Table 1** and **Figures 2-3**).
- Cefepime-zidebactam (fixed 16 μg/mL) demonstrated a two-fold lower MIC compared to cefepime alone against *A. baumannii* ATCC 19606 and four-fold lower MICs when combined with zidebactam at fixed concentrations of 4 and 8 μg/mL or in a 1:1 ratio (**Table 1**). In *in vitro* time kill testing, sub-inhibitory concentrations of cefepime-zidebactam showed initial bactericidal killing of 4.0- to 4.6-$\log_{10}$ by T_8 followed by regrowth at T_{24} (**Table 3**).
- Initial killing of 1.7 $\log_{10}$ (T_0) followed by rebound growth was observed for cefepime (32 μg/mL) against *A. baumannii* ATCC 19606 (**Table 3**). No reductions in CFU/mL were observed for cefepime alone at T_8 against OXA-23 or -24 producing *A. baumannii* (**Table 1** and **Figures 2-3**).
- These *A. baumannii in vitro* time-kill results are also corroborated by a recent study by Moya et.al. that demonstrated an enhancer effect due to complementary PBP binding by cefepime-zidebactam combinations.

P. aeruginosa

- Cefepime, imipenem and meropenem MIC values were within published CLSI QC ranges for *P. aeruginosa* ATCC 27853 (**Table 1**).
- Zidebactam was intrinsically active against the six carbapenem-resistant *P. aeruginosa* isolates and ATCC 27853 reference strain (MIC, 8-16 μg/mL) as well as *E. coli* NCTC 13353 (MIC, 0.12 μg/mL) and *K. pneumoniae* ATCC BAA-1705 (MIC, 0.5 μg/mL; **Table 1**).
- Cefepime in combination with sub-inhibitory concentrations of zidebactam (fixed 4 μg/mL) showed ≥ 2-fold MIC improvements against *P. aeruginosa* expressing AmpC (64-fold), AmpC + OprD loss (2-fold), IMP-13 (4-fold), KPC-2 (≥4-fold), and VIM-2 (2-fold; **Table 1**).
- Cefepime-zidebactam combinations in which each single agent was present at an inhibitory or sub-inhibitory concentration were bactericidal (≥3-$\log_{10}$ reduction in viable bacterial counts) at T_6 or T_8 against *P. aeruginosa* isolates expressing AmpC, AmpC + OprD loss, IMP-13 and VIM-2 β-lactamases (**Table 3** and **Figures 4-5**). This bactericidal activity was maintained at T_{24} by cefepime-zidebactam against *P. aeruginosa* isolates expressing AmpC and AmpC + OprD loss (**Table 3** and **Figure 5**).
- A 1.9 to 2.3 $\log_{10}$ reduction in viable organism counts was observed for sub-inhibitory cefepime-zidebactam combinations (≤0.125x/0.5x MIC) at T_8 against the KPC-2 producing *P. aeruginosa* (**Table 3** and **Figure 6**), followed by regrowth at T_{24}.
- Time-kill testing of cefepime at 1x MIC reduced viable *P. aeruginosa* (ATCC 27853) bacterial counts by 2.3 $\log_{10}$ at T_8. Interestingly, cidality triggered by 0.5x MIC concentrations of cefepime-zidebactam (1/1 μg/mL), paralleled the cidality observed with 1x MIC of cefepime alone. Similarly, cefepime-zidebactam combinations (0.5/1, 1/1 and 2/1 μg/mL) reduced viable organism numbers by 2.3 to 2.9 $\log_{10}$ at T_8 followed by regrowth by T_{24} (**Table 3**).
- *P. aeruginosa* (IMP-15) displayed a 0.8 to 1.8 $\log_{10}$ CFU/mL reduction in viable counts with cefepime-zidebactam combinations of ≤0.125x/1x and ≤0.25x/1x MIC at T_8 (**Table 3**).
- Initial stasis in time-kill studies followed by regrowth was observed for zidebactam at 1x MIC against *P. aeruginosa* isolates producing AmpC, IMP-13, IMP-15, KPC-2 and VIM-2 (**Table 3**) and AmpC + OprD loss (**Table 3** and **Figure 5**).

Figure 1. Chemical structure of zidebactam.

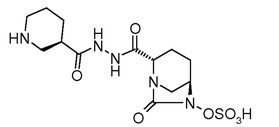


Table 1. Cefepime, cefepime-zidebactam, zidebactam and comparator compound MIC results for isolates included in this study.

Organism[a]	Isolate (Resistance)	MIC (μg/mL)							
		FEP[b]	FEP-ZID			ZID			
			Fixed 4[c]	Fixed 8	Fixed 16	1:1 ratio	Micro[d]	Macro[e]	IMI	MEM
PSA	635502 (IMP-13)	64	16	≤0.06	≤0.06	8	8	8	--	16
PSA	708365 (IMP-15)	>128	>128	>128	≤0.06	16	16	16	--	>64
PSA	665999 (VIM-2)	32	16	16	≤0.06	16	16	16	--	>64
PSA	552227 (KPC-2)	>128	64	0.12	≤0.06	16	16	8	--	>64
PSA	645702 (AmpC[g] + OprD loss)	32	16	2	≤0.06	8	16	16	--	16
PSA	651180 (AmpC[h])	16	0.25	≤0.06	≤0.06	8	8	8	--	4
ACB	374978 (OXA-23)	>64	32	32	32	32	>128	--	32	--
ACB	393295 (OXA-24)	>64	64	64	64	32	>128	--	128	--
ACB	ATCC 19606	32	8	8	16	8	>128	--	1	--
PSA	ATCC 27853	2 (0.5-4)[f]	≤0.06	≤0.06	≤0.06	1	2	2	1 (1-4)[f]	0.5 (0.25-1)[f]
EC	NCTC 13353 (CTX-M-15)	64	≤0.06	≤0.06	≤0.06	0.12	0.12	--	0.12	≤0.03
KPN	ATCC BAA-1705 (KPC-2)	32	≤0.06	≤0.06	≤0.06	0.25	0.5	--	--	16

a. ACB = *A. baumannii* species complex, EC = *Escherichia coli*, KPN = *Klebsiella pneumoniae* and PSA = *P. aeruginosa*
b. FEP = cefepime, ZID = zidebactam, FEP-ZID = cefepime-zidebactam, IMI = imipenem and MEM = meropenem
c. ZID at fixed 4, 8, or 16 μg/mL
d. Micro = broth microdilution MIC
e. Macro = broth macrodilution MIC
f. CLSI QC range (M100-S26)
g. AmpC = AmpC derepressed

Table 2. Summary of *in vitro* time-kill curve test conditions for cefepime, cefepime-zidebactam and zidebactam.

Organism[a]	Isolate (Resistance)	Test Concentrations μg/mL		
		FEP[b]	FEP-ZID	ZID
PSA	635502 (IMP-13)	8, 16, 32	8/8, 16/8, 32/8, 8/16, 16/16, 32/16	8[c] 16, 32
PSA	708365 (IMP-15)	8, 16, 32	8/8, 16/8, 32/8, 8/16, 16/16, 32/16	8, 16[c], 32
PSA	665999 (VIM-2)	8, 16, 32[c]	8/8, 16/8, 32/8, 8/16, 16/16, 32/16	8, 16[c], 32
PSA	552227 (KPC-2)	8, 16, 32	8/4, 16/4, 32/4, 8/8, 16/8, 32/8	8, 16[c], 32
PSA	645702 (AmpC[d] + OprD loss)	8, 16, 32[c]	8/8, 16/8, 32/8, 8/16, 16/16, 32/16	8, 16[c], 32
PSA	651180 (AmpC[d])	8, 16[c], 32	8/4, 16/4, 32/4, 8/8, 16/8, 32/8	4, 8[c], 16
PSA	ATCC 27853	0.5, 1, 2[c]	0.5/0.5, 1/0.5, 2/0.5, 0.5/1, 1/1, 2/1	0.5, 1[c], 2
ACB	374978 (OXA-23)	8, 16, 32	8/8, 16/8, 32/8	--
ACB	393295 (OXA-24)	8, 16, 32	8/8, 16/8, 32/8	--
ACB	ATCC 19606	8, 16, 32[c]	8/8, 16/8, 32/8	--

a. ACB = *A. baumannii* species complex and PSA = *P. aeruginosa*
b. FEP = cefepime, ZID = zidebactam and FEP-ZID = cefepime-zidebactam
c. Bold represents the isolates MIC value
d. AmpC = AmpC derepressed

Figure 2. *In vitro* time-kill results for FEP and FEP + ZID combinations against *A. baumannii* (OXA-23)

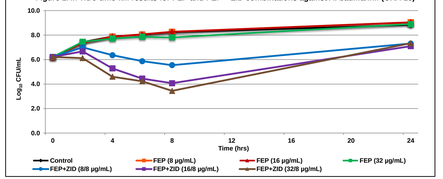


Figure 3. *In vitro* time-kill results for FEP and FEP + ZID combinations against *A. baumannii* (OXA-24)

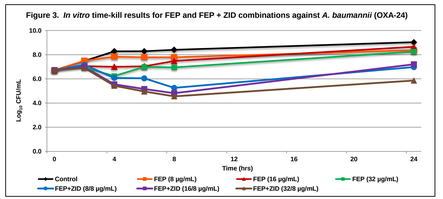


Table 3. Reductions in viable bacterial counts for cefepime, cefepime-zidebactam and zidebactam in *in vitro* time kill studies.

Isolate[a] (Resistance)	Drug[b]	Concentration Tested μg/mL	$\log_{10}$ CFU/mL reductions in viable counts from T_0 (hours)				
			T_2	T_4	T_6	T_8	T_{24}
ACB ATCC 19606	FEP	32	0.4	1.2	1.4	1.7	+1.3[c]
	FEP-ZID	8/8	0.0	2.8	**3.8***	**4.4***	+0.1
		16/8	0.7	**3.4***	**4.1***	**4.0***	2.1
		32/8	1.4	**3.4***	**4.1***	**4.6***	0.2
ACB (OXA-23)	FEP	32	+1.2	+1.7	+1.9	+2.1	+2.8
	FEP-ZID	8/8	+0.8	+0.2	0.3	0.7	+0.9
		16/8	+0.5	0.9	1.8	2.1	+0.9
		32/8	0.1	1.6	2.0	2.8	+0.9
ACB (OXA-24)	FEP	32	+0.2	0.5	+0.3	+0.2	+1.6
	FEP-ZID	8/8	+0.5	0.6	0.7	1.4	+0.3
		16/8	+0.3	1.2	1.5	1.9	+0.5
		32/8	+0.2	1.3	1.7	2.1	0.8
PSA (IMP-13)	FEP	32	0.0	+0.3	+0.9	+1.2	+4.1
	ZID	8	+0.2	1.2	2.0	2.0	1.2
	FEP-ZID	8/8	0.1	0.9	2.3	2.8	1.0
		16/8	0.0	1.0	2.7	2.7	1.2
		32/8	0.9	1.8	**3.2***	2.7	1.6
PSA (IMP-15)	FEP	32	+0.5	+1.3	+1.8	+2.0	+3.4
	ZID	16	+0.2	+0.3	+0.7	+1.0	+1.2
	FEP-ZID	8/16	+0.6	+0.1	+0.3	+0.5	+0.8
		16/16	+0.5	+0.1	0.2	0.8	+0.5
		32/16	+0.1	0.2	1.0	1.8	+1.8
PSA (VIM-2)	FEP	32	0.3	0.6	1.5	2.0	+0.2
	ZID	16	+0.4	+0.4	+0.7	+0.6	+1.6
	FEP-ZID	8/16	0.2	1.7	2.4	**3.1***	1.8
		16/16	0.3	2.2	2.5	**3.3***	2.6
		32/16	0.5	2.7	**3.2***	**3.4***	**3.1***
PSA (KPC-2)	FEP	32	+1.0	+1.7	+2.1	+2.0	+3.1
	ZID	16	+0.6	+0.6	+0.5	+0.5	+1.5
	FEP-ZID	8/8	+0.2	0.4	1.6	2.0	+2.2
		16/8	0.0	1.4	1.8	1.9	2.4
		32/8	0.5	1.5	1.8	2.3	+0.9
PSA (AmpC + OprD loss)	FEP	32	0.1	+0.4	+0.7	+0.1	+2.8
	ZID	16	+0.4	0.0	+0.3	+0.3	+1.6
	FEP-ZID	8/16	1.1	2.1	2.4	**3.0***	**4.0***
		16/16	1.5	1.9	2.6	2.8	**3.0***
		32/16	2.0	1.8	2.5	2.7	**3.6***
PSA (AmpC)	FEP	16	0.2	0.3	0.2	+0.1	+2.3
	ZID	8	+0.1	0.3	0.5	0.3	+0.4
	FEP-ZID	8/8	0.9	2.1	**3.0***	**4.0***	**4.7***
		16/8	0.7	1.5	2.4	2.6	**4.0**
		32/8	2.0	2.2	2.7	1.9	**3.9**
PSA ATCC 27853	FEP	2	0.9	1.9	2.4	2.3	+0.7
	ZID	1	+0.8	+1.5	+1.6	+2.0	+2.8
	FEP-ZID	0.5/1	0.2	0.1	0.8	2.3	+1.7
		1/1	0.8	1.5	1.9	2.7	+0.8
		2/1	1.2	1.9	2.2	2.9	+0.4

a. ACB = *A. baumannii* species complex and PSA = *P. aeruginosa*
b. FEP = cefepime, ZID = zidebactam and FEP-ZID = cefepime-zidebactam
c. $\log_{10}$ CFU/mL increase in viable colony counts designated by "+"
d. Bactericidal $\log_{10}$ CFU/mL reductions in are listed in bold

Figure 4. *In vitro* time-kill results for FEP, ZID and FEP + ZID combinations against *P. aeruginosa* (VIM-2)

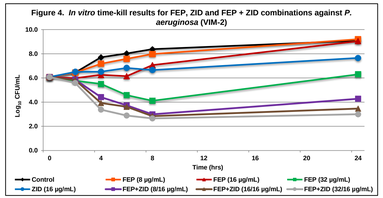


Figure 5. *In vitro* time-kill results for FEP, ZID and FEP + ZID combinations against *P. aeruginosa* (AmpC derepressed+OprD loss)

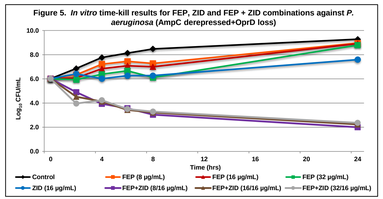
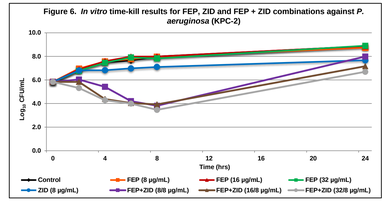


Figure 6. *In vitro* time-kill results for FEP, ZID and FEP + ZID combinations against *P. aeruginosa* (KPC-2)



Conclusions

- **Zidebactam demonstrated intrinsic *in vitro* antibacterial activity against all seven *P. aeruginosa* isolates tested.**
- **Cefepime-zidebactam combinations (generally at sub-inhibitory concentrations) demonstrated potent activity in *in vitro* time-kill studies against *A. baumannii* species complex and *P. aeruginosa* isolates expressing clinically relevant β-lactamases including AmpC, IMP, KPC, OXA and VIM for which only limited treatment options may be available.**
- **Cefepime-zidebactam combinations in which each single agent was present at an inhibitory or sub-inhibitory concentration were bactericidal (≥3-$\log_{10}$ reduction in viable bacterial counts) against *P. aeruginosa* isolates expressing AmpC, AmpC + OprD loss, IMP-13 and VIM-2 β-lactamases as well as *A. baumannii* ATCC 19606. This bactericidal activity was maintained at T_{24} by cefepime-zidebactam against *P. aeruginosa* isolates expressing AmpC and AmpC + OprD loss.**
- **These *in vitro* results highlighting cefepime-zidebactam (WCK 5222) sub-MIC killing may, possibly, contribute significantly to the *in vivo* PD effects against serious Gram-negative infections caused by *Acinetobacter* spp. and *P. aeruginosa*, including MDR organisms. Additional *in vivo* PK-PD studies directed towards further understanding of the WCK 5222 PK-PD aspects are warranted.**

Acknowledgements

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