WCK 4282 (High-dose Cefepime-Tazobactam): Complementary Features Drive Efficacy against KPC-producing Pathogens

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INTRODUCTION AND PURPOSE

- Overuse of carbapenems has led to worldwide emergence/spread of carbapenem-resistant enterobacteriaceae (CRE) such as *Klebsiella pnuemoniae* carbapenamases (KPCs) which is of major health concern. KPC prevalence has been reported from North and South America, Europe, Middle East, Asia and Australia[1]. Though currently less frequent, KPC β-lactamases are also associated with *E. coli, Enterobacter, Citrobacter and Pseudomonas*[1].
- To date, limited therapies are available to treat KPC infections. Non-β-lactam agents such as tigecycline and colistin are associated with safety, efficacy and now even resistance concerns[2,3]. β-lactam-based therapy involving ceftazidime (CAZ)-avibactam (AVI) combination has been shown to possess comprehensive coverage of Class A and C β-lactamases and therapeutically relevant activity for KPCs. Therefore, its longer term utility in the treatment of CRE needs to be preserved through judicious use.
- Considering limited therapeutic options for KPCs and wide prevalence of Class A and C β-lactamases, development of an alternative cephalosporin based combination of clinically established agents is an attractive strategy for providing safe, effective treatment besides minimising the prospects of carbapenem resistance.
- WCK 4282 [cefepime (FEP)-tazobactam (TAZ)] is currently under development for treating MDR Gram-negative infections and has been recently evaluated in Phase-I studies in Europe.
- Recent large-scale surveillance study (JMI labs: P1262, P1263, ECCMID 2016, Amsterdam) established potent activity of WCK 4282 against *Enterobacteriaceae* (n=6219) with MICs of WCK 4282 being comparable to imipenem (IPM) and superior to piperacillin (PIP) -TAZ ($MIC_{50/90}$: WCK 4282- ≤0.03/0.25 µg/mL; IPM- 0.12/1 µg/mL; PIP-TAZ- 2/32 µg/mL). Here, we report the diverse complementary features of FEP and TAZ that form the basis of KPC coverage by WCK 4282 'extended infusion' product.

METHODS

In vitro studies

PBP binding

- The affinity of FEP for various PBPs in *K. pneumoniae* (Strain 52145) was evaluated in a competition assay with Bocillin FL using membrane preparations. Briefly, PBP containing solution was incubated with the serial concentration of FEP (0.0156–2 mg/L) and separated on SDS-PAGE. The Bocillin labeled PBPs were visualised using Molecular Imager FX-Pro (Bio-RAD) and IC_{50} for each PBP was determined using Quantity One software (Bio-RAD).

FEP and TAZ KPC stability

- Relative stability of FEP, TAZ and IPM against KPC enzyme was studied in cell free system. Briefly, KPC enzyme was isolated from KPC-producing cultures and clinically relevant concentrations of FEP (80 µg/mL), TAZ (32 µg/mL), FEP-TAZ (80+8 µg/mL) and IPM (40 µg/mL) were added to enzyme preparation and incubated at 35°C. After the specified time interval, the residual, unhydrolysed, drug concentrations were estimated by microbiological assays employing quantitative drug diffusion method using *E. coli* ATCC 25922 (for FEP, IPM) and *E. coli* NCTC 13352 harbouring TEM 10 (for TAZ, employing CAZ 10 µg/mL incorporated agar medium).

Enzyme inhibition

- Comparative KPC enzyme inhibition potential (IC_{50}) of β-lactamase inhibitors (BLIs)- TAZ, sulbactam and clavulanic acid was assessed by using enzyme preparation employing repeated freeze-thaw method. For nitrocefin assay, this preparation was pre-incubated with serially diluted BLI for 15 min at 37°C. The enzyme reaction mixture was further incubated with nitrocefin for 5 min to estimate the residual enzyme activity. Spectrophotometric readings were measured at 482 nm and IC_{50} values were determined using GraphPad Prism software.

MIC determination

- FEP-TAZ MICs were determined as per CLSI M07-A9 broth micro-dilution method and additionally in CA-MHB medium supplemented with 0.85% NaCl by employing TAZ at fixed concentration (8 µg/mL) against 54 KPC+ESBL-producing isolates [*K. pneumoniae* (KP)-43, *K. oxytoca*-1, *E. coli*-3, *E. cloacae species complex*-2, *E. aerogenes*-1, *S. marcescens*-2, *C. freundii species* complex-1, *P. mirabilis*-1] collected during 2013-14 from SENTRY surveillance.

Diffusion assays

- FEP-TAZ disk and agar well diffusion assays were performed according to the reference CLSI M02-A11 method. Briefly, bacterial suspensions set to 0.5 McFarland turbidity were spread on MHA plates. Desired concentrations of drugs were added either to agar wells or disks. The plates were incubated for 18h at 35°C and inhibition zones measured.

Time-kill kinetics

- Time-kill studies were initiated by adding drugs to CA-MHB medium (with or without NaCl) containing exponentially growing cultures at a starting count of 6.3-6.6 log_{10} CFU/mL and incubated at 37°C under shaking conditions (120 rpm). Viability counts at various time points were determined by plating serial dilutions (1:10) of the culture on the TSA (Tryptone Soya Agar - HiMedia, India) medium.

In vivo efficacy studies

Murine systemic infection

- Male/female Swiss albino mice were intraperitoneally infected (3-5×10^6 CFU/mouse) with a bacterial inoculum in 5% hog gastric mucin that resulted in the death of untreated mice within 24h. Subcutaneous treatment was initiated 2h post-infection for 1 day (KP H521) or 2 days (KP H528). FEP, FEP-TAZ was given t.i.d. (3h apart) and IPM was given b.i.d. (3h apart). Survival pattern was monitored for 7 days. $ED_{50/90}$ was determined by probit analysis.

Neutropenic mouse lung eradication study

- Lung eradication studies were conducted in mice which were rendered neutropenic (cyclophophamide, 200 mg/kg at -4 days, pre-infection) that ensured sustained neutropenia throughout the study period. Lung infection was initiated by intranasal instillation of 10^6 CFU of KP H521 under light anesthesia. FEP, FEP-TAZ (1:1), CAZ-TAZ (1:1) treatment was initiated 2h post-infection (2h count: 6.39 log_{10} CFU/lung) t.i.d. (3h apart) for 2 days. However PIP-TAZ was given six times (2h apart). Bactericidal effect in lung was assessed 48h post-infection for all the treatment groups.

RESULTS

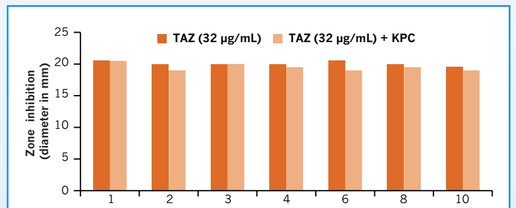
PBP binding

- First time ever FEP PBP binding profile in *Klebsiella* showed binding to PBPs-1a, 2 and 3 (IC_{50}s: 1.2±0.47, 0.74±0.27 and 0.19±0.08 µg/mL, respectively). The multiple PBP binding profile observed for *Klebsiella* is expected to translate in strong cidal effect as reported for *E. coli.*

TAZ stability to KPC

- TAZ was relatively stable to KPC, as evidenced by detection of significant proportion of un-hydrolysed TAZ until 10h (**Figure 1**). Under similar conditions IPM was completely hydrolysed within 1h suggesting potent KPC activity. Similar relative stability of TAZ against KPC compared to clavulanic acid and sulbactam has been previously reported[4].

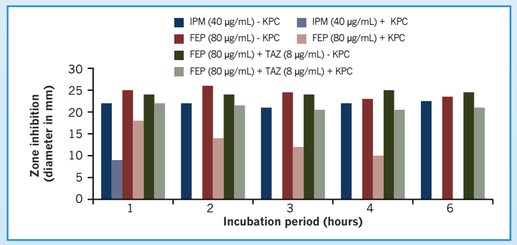
Figure 1. Stability of TAZ towards KPC-mediated hydrolysis



FEP stability towards KPC-mediated hydrolysis and FEP sparing effect of TAZ:

- FEP showed relatively better stability towards KPC enzyme, while IPM was hydrolysed completely within 1h. Further, addition of TAZ at modest level (8 µg/mL) provided significant protection to FEP which was spared (>50%) up to an extended period of 6h (**Figure 2**) as observed in quantitative microbiological assay.

Figure 2. Addition of TAZ spares FEP against KPC-mediated hydrolysis



KPC inhibition by TAZ

- KPC IC_{50} for TAZ, sulbactam and clavulanic acid were 1.3, 7.3 and 3.2 µg/mL, respectively. A varying degree of KPC inhibition by TAZ has also been previously reported by various investigators (reported IC_{50}s of TAZ for KPC ranged from 0.37-43 µM)[5,6,7].

MIC and disk diffusion

- MIC data showed that 77.7% of the KPC strains (n=42/54) were inhibited at or below the PK/PD breakpoint (≤16 µg/mL) for high-dose extended-infusion FEP-TAZ (**Table 1**). The basis of breakpoint of ≤16 µg/mL has been arrived through *in vivo* PK/PD studies; PoP PK based on WCK 4282 Phase-I data and MCS analysis undertaken by Prof. J. W. Mouton (Mouton J.W. *et al.* "Pharmacodynamics of cefepime and tazobactam against carbapenemase positive strains in a neutropenic mouse model" ECCMID 2016, Amsterdam).

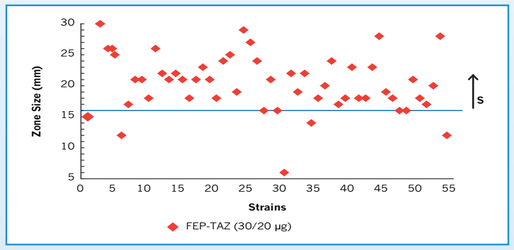
Table 1. FEP-TAZ inhibition profile against worldwide KPC strains collected during 2013-14

Drug	Percentage strains inhibited at MIC (µg/mL)								
	≤1	2	4	8	12	16	32	64	>64
FEP	7.4	13	18.5	37	51.8	70.37	87	96.3	100
FEP-TAZ (8 µg/mL)	13	20.3	33.3	51.8	61	77.7	94.4	98.14	100
FEP-TAZ (8 µg/mL) + NaCl	31.4	38.8	57.4	79.6	83.3	88.8	98.1	100	100
IPM	11.11	22.22	42.59	77.78	NT	94.44	98.15	100	100

NT=not tested; NaCl did not have an effect on IPM MICs, showing an optimal expression of KPC.

- For WCK 4282, a combination disk mass of FEP-30 µg and TAZ-20 µg has been accepted by CLSI for the determination of susceptibility. For majority of KPC strains, the range of zone sizes obtained were 16-30 mm (**Figure 3**).

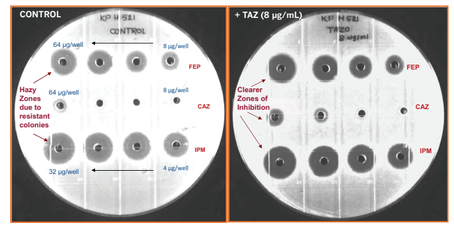
Figure 3. Zone dimensions for 54 KPC strains with FEP-TAZ disks.



S: Susceptible.

- Similar synergistic effect of FEP and TAZ was also observed in drug diffusion assays, wherein, FEP provided clear zones of inhibition in the presence of TAZ, while CAZ failed to do so (**Figure 4**).

Figure 4. Synergy of FEP and TAZ observed by well diffusion assays against KPC-expressing strain (KP H521)

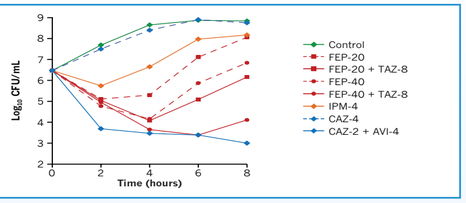


Time-kill studies

- FEP-TAZ provided synergistic killing (1.5-2 log_{10} CFU/mL) till 4-6h, at approximately 2-4x MIC (clinically relevant concentrations); CAZ-AVI was also cidal at its 2-4x MIC (**Figure 5a, 5b**).

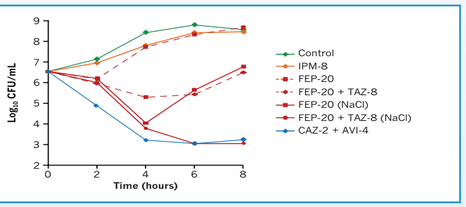
Figure 5. Cidal synergy of FEP-TAZ against KP strains

5a) KP H518 (SHV, TEM, KPC)



MICs (µg/mL) - FEP: 32; FEP-TAZ: 16; CAZ-AVI: 0.5; IPM: 8.

5b): KP J243 (KPC, TEM)



MICs (µg/mL) - FEP: 32; FEP-TAZ: 16; CAZ-AVI: 0.5; IPM-32 (NaCl did not have an effect on IPM cidality, showing an optimal expression of KPC).

Systemic infection studies

- For KP strains harbouring KPC+ESBL β-lactamases, ED_{90} for FEP-TAZ was found to be in the range of 152 to 199 mg/kg while ED_{90} for FEP was >200-400 mg/kg (**Table 2**).

Table 2. FEP-TAZ efficacy in murine systemic infection model

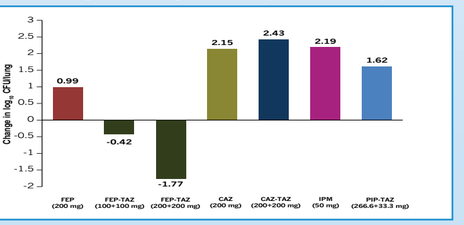
Strains (β-lactamases)	Drug	MIC (µg/mL)	ED_{50} (mg/kg)	ED_{90} (mg/kg)
KP H521 (KPC, SHV and TEM)	FEP	>32	>400	>400
	FEP+TAZ	16	199.09+102.58	199.09+199.09
	IPM*	32	>50	>50
KP H528 (KPC, SHV and TEM)	FEP	>32	>200	>200
	FEP+TAZ	16	151.71+73.31	151.71+151.71
	IPM*	32	>50	>50

* Overnight mortality in IPM-treated animals.

Lung eradication studies

- FEP-TAZ showed bacteriostatic effect at 100 mg/kg and bactericidal effect at 200 mg/kg (1.77 log_{10} CFU kill/lung) administered t.i.d. Unlike FEP and TAZ combination, combination of CAZ and TAZ failed to even inhibit the growth of KPC in lung (**Figure 6**). Likewise, PIP-TAZ and IPM also did not provide lung eradication effect.

Figure 6. FEP-TAZ lung eradication against KP H521 (KPC, SHV and TEM)



MICs (µg/mL) : FEP: >32; FEP-TAZ: 16; IPM: 32.

CONCLUSIONS

- **The complementary features of FEP and TAZ highlighted in the study led to *in vitro* activity and *in vivo* efficacy against KPC strains.**
- **KPC sparing effect of TAZ facilitates FEP mediated killing due to multiple PBP binding.**
- **The transient killing effects observed with FEP-TAZ combination appear to drive *in vivo* efficacy demonstrated through systemic efficacy and lung eradication studies.**
- **FEP-TAZ combination, in addition to the potent coverage of Class A and C β-lactamases, also provides *in vitro* and *in vivo* activity against KPC strains with MIC of ≤16 µg/mL and thus offers an attractive empiric carbapenem-sparing treatment option superior to PIP-TAZ.**

REFERENCES

1. Chen LF. *et al.* Infect Drug Resist. 2012; 5:133–141.
2. Van Duin D. *et al.* Diagn Microbiol Infect Dis. 2013; 75:115–120.
3. Fox JL. *et al.* Microbe. 2016; 2:52-53.
4. Papp-Wallace KM. *et al.* Antimicrob Agents Chemother. 2010; 54:890–897.
5. Stachyra T. *et al.* J Antimicrob Chemother. 2009; 64:326–329.
6. Yigit H. *et al.* Antimicrob Agents Chemother. 2001; 45:1151–1161.
7. Hecker SJ. *et al.* J Med Chem. 2015; 58:3682–3692.

DISCLOSURES

All authors are employees of Wockhardt Research Centre, Aurangabad, India.
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