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Paper Poster Session
Cefepime-tazobactam

WCK 4282 (high-dose Cefepime-Tazobactam): Complimentary features drive efficacy against KPC-producing pathogens

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Background: 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. Emerging KPC prevalence rates in certain geographies compel clinicians to resort to either compromised or *ad hoc* combination therapies. Development of novel combination therapy based on clinically established agents is an attractive strategy of providing safe and effective treatment. Here, we report the diverse complementary features through which FEP-TAZ provides activity against KPC pathogens.

Material/methods: To gain a comprehensive insight into complementary features of FEP and TAZ, we performed an all-rounded analysis including: first ever FEP *Klebsiella pneumonia* (KP) PBP-binding profile, FEP stability towards KPC; TAZ (8 µg/mL) inhibition of KPC enzyme and its ability to rescue FEP. Time-kill studies and agar well-diffusion assays were also performed. PBP binding was determined by Bocillin FL competition assay. KPC stability was estimated by incubating enzyme-drug mixture and subsequent estimation of the un-hydrolysed FEP and TAZ. Enzyme inhibition (IC_{50}) was determined by using nitrocefin as substrate. FEP-TAZ MICs were determined by CLSI broth micro-dilution method, against 54 KPC+ESBL-producing isolates collected during 2013-14 from SENTRY surveillance. Time-kill studies employed log-phase culture (starting inoculum of 6.25-6.78 log_{10} CFU/mL). The viable counts were enumerated by plating aliquots of serially diluted culture. Quantitative drug diffusion assays were undertaken with and without TAZ (8 µg/mL) incorporation in agar medium. Proof of concept i*n vivo* efficacy studies (2h post infection, TID for two days) involving lung infection were conducted in neutropenic mouse (bacterial count at therapy initiation 6.0-6.54 log_{10} CFU/lung).

Results: In KP, FEP bound to multiple PBPs-1a, 2 and 3 (IC_{50}s: 1.2±0.47, 0.74±0.27 and 0.19±0.08 µg/mL, respectively). KPC IC_{50} for TAZ, sulbactam and clavulanic acid were 1.3, 7.3 and 3.2µg/mL respectively. FEP stability studies showed its relative stability towards KPC enzyme (50% un-hydrolysed at 4h), while imipenem was 100% hydrolysed by just 1h. Addition of TAZ (8 µg/mL) provided significant protection to FEP (>50% spared up to extended period of 6h). Furthermore, FEP-TAZ provided synergistic killing (1.5-2 log_{10} CFU/mL) by 4-6h. Similar synergistic effect of FEP-TAZ was also observed in drug diffusion assays, wherein, FEP provided clear zones of inhibition in the presence of TAZ, while ceftazidime (CAZ) failed to do so. MIC data showed that ~77.7% of the KPC strains (n=42/54) were inhibited at or below the proposed PK-PD breakpoint (≤16 µg/mL) of

extended-infusion high-dose WCK 4282. *In vivo*, FEP-TAZ showed bacteriostatic effect at 100 mg/kg and bactericidal effect at 200 mg/kg (1.77 $\log_{10}$ CFU/mL). CAZ-TAZ did not provide lung KPC eradication.

Conclusions: Cumulatively, our results demonstrate that TAZ effectively spares FEP enabling *in vivo* efficacy of WCK 4282 against KPC.