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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 (revised)

Background: WCK 4282 (cefepime 2g/tazobactam 2g) 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 efficacy against various serine β-lactamase-producing Enterobacterales and *Pseudomonas aeruginosa* in a murine pneumonia model.

Methods: Clinical cefepime-resistant isolates (17 Enterobacterales and 2 *P. aeruginosa*) were utilized. Isolates expressed ESBLs, cephalosporinases, and/or serine carbapenemases (KPC, OXA-48-like). WCK 4282 MICs were 4-32 mg/L. For *in vivo* experiments, lungs of neutropenic mice were inoculated using standard inoculum (10^7 $\log_{10}$ cfu/mL). Serine-carbapenemase-producing isolates were also assessed using a low inoculum (1:5 dilution). Treatment mice received 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 $\log_{10}$ cfu/lung at 24h compared with 0h controls.

Results: At standard inoculum, mean 0h bacterial burden was 6.65 ± 0.23 $\log_{10}$ cfu/lung and increased at 24h by 2.48 ± 0.60 $\log_{10}$ cfu/lung among untreated controls. Lower inoculums initial bacterial burdens ranged from 5.81 ± 0.12 - 6.39 ± 0.13 $\log_{10}$ cfu/lung. At standard and/or low inoculums, cefepime and meropenem provided minimal activity. WCK 4282 produced >1-$\log_{10}$ reduction against 9/9 ESBL/cephalosporinase-producing strains. WCK 4282 provided variable activity among mice infected with standard or lower inoculums of OXA-48-like-producers. WCK 4282 exposures provided 0.53 ± 1.07 $\log_{10}$ cfu/lung growth against KPC-producers at standard versus bacteriostasis (-0.15 ± 0.54 change in $\log_{10}$ cfu/lung) at low inoculum.

Conclusion WCK 4282 produced potent *in vivo* activity against ESBL- and cephalosporinase-producing Enterobacterales and *P. aeruginosa*, and potential activity against OXA-48-like-producing Enterobacterales in a neutropenic pneumonia model.

BACKGROUND

- Pneumonia is the largest contributor to infectious mortality due to difficulties in lung penetration and incidence of multi-drug resistance[1-3].
- Bacterial pneumonia is commonly caused by Enterobacterales and *Pseudomonas aeruginosa* strains that may harbor one or more extended-spectrum β-lactamase (ESBL), cephalosporinase, or carbapenemase[1-3].
- WCK 4282 is an optimally designed high dose cefepime/tazobactam (2g/2g) that has shown potent activity against penicillinase-, cephalosporinase-, and ESBL-harboring Gram negative bacteria *in vitro*[4].
- Additional *in vitro* studies have shown potential utility of WCK 4282 against OXA-48-like-producing and KPC-producing isolates[5-6]

OBJECTIVE

The objective of this study was to evaluate WCK 4282 efficacy against serine-β-lactamase-producing Enterobacterales and *P. aeruginosa* in a neutropenic murine lung infection model.

METHODS

Antibiotic Compounds

- Commercially available cefepime and meropenem 1 g vials were reconstituted according to the package insert recommendations and further diluted in normal saline to respective concentrations.
- Analytical grade tazobactam was reconstituted and diluted in 50 mM sodium phosphate buffer.
- All antibiotics were administered as separate 0.1 mL subcutaneous injections.

Bacterial Isolates

- Nineteen clinical isolates consisting of Enterobacterales (n=17) and *P. aeruginosa* (n=2) with previously determined MIC were utilized in this study (**Table 1**).
- ESBL/cephalosporinase-producing *Escherichia coli* conferred cefepime-, ceftolozane/tazobactam-, and piperacillin/tazobactam-resistance.

Neutropenic Pneumonia Model

- Female CD-1 mice weighing 20-22 g were utilized for all studies.
- Neutropenia was induced with cyclophosphamide 250 mg/kg on day-4 and 100 mg/kg on day-1.
- Uranyl nitrate 5 mg/kg was given to reduce renal clearance of study compounds to permit human-simulated dosing.
- Mice were anesthetized with isoflurane and inoculated intranasally with 0.05 mL of 10^7-10^8 (or 10^6-10^7 for low inoculum studies) cfu/mL bacterial suspensions in 3% mucin 2 h before antibiotic dosing.
- Euthanasia was performed via CO_2 inhalation and ultimately cervical dislocation.

Plasma Pharmacokinetic Studies

- Cefepime monotherapy and cefepime-tazobactam murine simulated regimens were developed using previous healthy-volunteer data.
- Murine pharmacokinetic parameters were used from previous studies
- Blood was collected via cardiac puncture and centrifuged to separate plasma.
- Cefepime and tazobactam were measured with LC-MS/MS.
- Dosing regimens simulating human plasma exposures were determined and confirmed for cefepime as monotherapy and in the presence of tazobactam as well as tazobactam in the presence of cefepime.

In Vivo Efficacy Studies with Standard Inoculum

- For isolates at standard inoculum, 4 groups of 6 mice were utilized.
- Control groups were sacrificed at 0 h and 24 h.
- Treatment groups received human-simulated regimens of either cefepime or the combination of cefepime and tazobactam for 24 h.
- All lobes of both lungs were aseptically harvested, homogenized in normal saline (NS), and serially diluted before plating to measure bacterial burdens in lung tissue.
- Efficacy was defined as $\log_{10}$ change in cfu/lung at 24 vs. 0 h controls.

In Vivo Efficacy Studies with Low Inoculum

- Studies using low inoculums were conducted in similar fashion to standard inoculum with a few exceptions.
- An additional 1:5 inoculum dilution occurred prior to inoculation.
- 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 human simulated regimen (HSR) of meropenem simulating a 1g q8h 30 minutes infusion to serve as a control for serine carbapenemase expression [7].

Table 1. Isolates included in neutropenic murine lung infection model *in vivo* efficacy studies and their respective MICs.

CAIRD ID #	Known genotype	Cefepime (mg/L)	WCK 4282 [TZB 8mg/L] (mg/L)	Piperacillin/ tazobactam (mg/L)	Ceftolozane/ tazobactam (mg/L)	Imipenem (mg/L)
EC 741	Not determined	>128	4	>128	32	0.25
EC 739	MIR-1/ACT-1, DHA-1/DHA-2, CTX-M GR-1	>128	4	64	32	0.5
EC 737	CMY, TEM	>128	8	>128	>128	0.25
EC 731	TEM, PBP3 insert	>128	8	>128	64	0.5
EC 732	CTX-M Gr-1/2, PBP3 insert	>128	8	>128	64	0.12
EC 740	CMY, TEM	>128	8	>128	64	0.25
EC 728	CMY, TEM, PBP3 insert	64	16	>128	>128	1
PSA 1881	AmpC, VEB	>128	16	16	>128	1
PSA 1882	AmpC, VEB, CTX-M, OXA-1, OXA-2	>128	16	16	>128	16
EA 59	**KPC**, TEM	16	16	>128	16	32
KP 909	**KPC-3**, SHV, TEM	32	16	>128	128	16
KP 910	**KPC**, SHV, TEM	32	16	>128	>128	16
KP 906	**KPC**, SHV, TEM	32	32	>128	128	16
KP 813	**OXA-48**,CTXM-15, TEM-1, SHV-12	>512	8	N/A	N/A	N/A
KP 733	**OXA-48**,CTXM-15, TEM-1, SHV-12	>512	8	N/A	N/A	N/A
EC 734	**OXA-48/181**, TEM, PBP3 insert	>128	8	>128	128	1
KP 911	**OXA-181**, CMY, SHV, TEM, CTXM Gr-1	64	16	>128	128	8
KP 908	**OXA-181**, CTXM Gr-1, CMY, SHV	128	16	>128	>128	8
ECL 123	**OXA-48**, CTXM-15, ACT, TEM-OSBL	>512	64	N/A	N/A	N/A

TZB, tazobactam; EC, *E. coli*; PSA, *P. aeruginosa*; EA, *Enterobacter aerogenes* (now *Klebsiella aerogenes*); KP, *Klebsiella pneumoniae*; ECL, *Enterobacter cloacae*; N/A, not available

RESULTS

Figure 1. Observed murine free cefepime plasma concentration (mean ±SD) for WCK 4282 HSR and cefepime HSR compared with the expected human and murine exposures: (**A**) cefepime alone and in presence of tazobactam and (**B**) tazobactam in presence of cefepime.

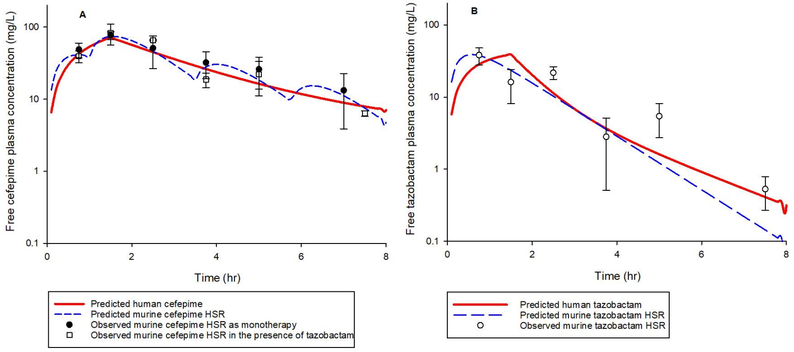
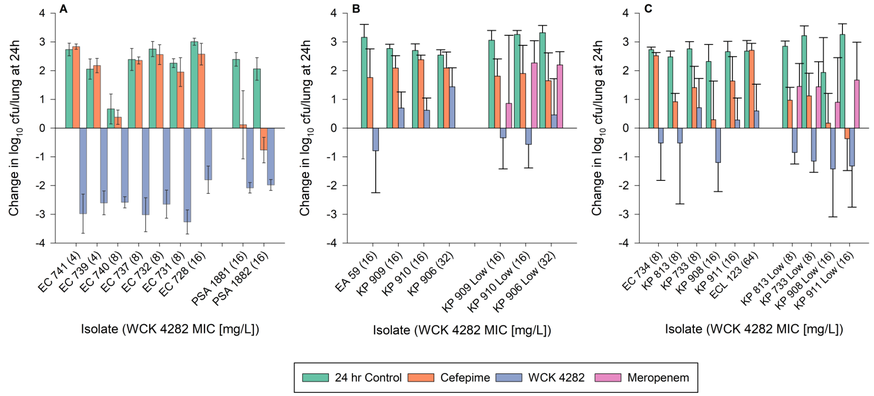


Figure 2. Change in bacterial density (mean±SD) at 24 h for mice receiving control, cefepime HSR, WCK 4282 HSR, and meropenem HSR (low inoculum for isolates producing (**A**) ESBL/cephalosporinase at standard inoculum, (**B**) KPC- and (**C**) OXA-48- producing Enterobacterales with or without ESBLs for standard and low inoculum.



DISCUSSION AND CONCLUSIONS

- **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 mg/L.**
- **At both the standard and the low inoculum, the addition of tazobactam to cefepime had a suppressive effect on KPC-producing isolates (~0.5 log10 cfu/lung growth and net stasis); however, this does not translate into efficacy against this infection entity.**
- **WCK 4282 could have a place in therapy for OXA-48-like-producing strains in lower inoculum infections such as complicated urinary tract infections.**

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