The ability of broad-spectrum β-lactamase inhibitor VNRX-5133 to restore bactericidal activity of cefepime in Enterobacteriaceae- and *P. aeruginosa*-expressing Ambler class A, B, C and D enzymes is demonstrated using time-kill kinetics

**Background**

VNRX-5133 is a novel cyclic boronate-based broad-spectrum β-lactamase inhibitor with potent and selective direct inhibitory activity against both serine- and metallo-β-lactamases (Ambler Classes A, B, C and D). VNRX-5133, combined with cefepime, has the potential to address the unmet medical need for a safe and effective therapy for infections caused by multi-drug resistant (MDR) extended spectrum β-lactamase producing and carbapenem-resistant Enterobacteriaceae (CRE) and *Pseudomonas aeruginosa*.

**Methods**

Time-kill experiments were conducted in MHII broth at 37°C. Overnight cultures were diluted to an initial inoculum of 10^6 CFU/mL. Cefepime was tested at 0.5, 1, 2, 4, and 8X the MIC when combined with VNRX-5133 fixed at 4 μg/mL. Activity was compared to cefepime alone. Viable counts were determined at 0, 2, 4, 6, 8, and 24 hours and kill curves generated by plotting Log_{10} CFU/mL versus time. Tests were conducted in 6 strains of Enterobacteriaceae (1 wild-type and 5 β-lactamase expressing) and 4 strains of *P. aeruginosa* (1 wild-type and 3 β-lactamase expressing). The presence of β-lactamase genes was verified using PCR with expression determined phenotypically.

**Results**

In five strains of β-lactamase expressing Enterobacteriaceae (1 each of CTX-M-15, KPC, VIM-1, NDM-1 and OXA-48) the addition of VNRX-5133 reduced the MIC of cefepime from 8 to ≥ 2048-fold. VNRX-5133 alone had no intrinsic antibacterial activity. VNRX-5133 restored bactericidal activity of cefepime through 24 hours at 0.5-2x the MIC against 5 Enterobacteriaceae isolates in time-kill assays. In three *P. aeruginosa* (1 each AmpC, VIM-2 and GES-1) the MIC of cefepime was reduced 8-fold in one strain and ≥64-fold in the remaining two. In the wild-type *P. aeruginosa*, no shift in MIC was noted. With the addition of VNRX-5133, bactericidal activity was achieved at 2x the MIC in all three β-lactamase expressing strains. Enzyme class did not affect the rescue of cefepime by VNRX-5133.

**Conclusions**

VNRX-5133 fully restores the bactericidal activity of cefepime against cephalosporin and carbapenem-resistant Enterobacteriaceae and *P. aeruginosa* expressing Ambler Class A, C and D serine-β-lactamases and Class B metallo-β-lactamases.

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