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# Vaborbactam (RPX7009) plus meropenem is active against the newly discovered BKC-1 and FR-1 carbapenemases

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**Background:** Vaborbactam (formerly known as RPX7009) is a new beta-lactamase inhibitor with potent activity against class A serine carbapenemases such as KPC, SME and NMC-A. It is in Phase 3 clinical development in combination with meropenem (Carbavance®). BKC-1 and FRI-1 are new serine carbapenemases recently discovered in clinical isolates of *K. pneumoniae* and in *E. cloacae*, respectively. These enzymes show low sequence homology with majority of clinically relevant serine carbapenemases. The objective of this study was to elucidate biochemical mechanism of KPC-2, BKC-1 and FRI-1 inhibition by vaborbactam.

**Material/methods:** Genes encoding BKC-1 and FR-1 were synthesized and cloned into the vector pUCP-24. Recombinant plasmids expressing KPC-2, BKC-1 and FR-1 were transformed into the strain of *P. aeruginosa* PAM1154 lacking major efflux pumps. MICs of various antibiotics with or without vaborbactam were determined. KPC-2, BKC-1 and FRI-1 enzymes were purified from overexpressing recombinant *E. coli* strains. $K_i$s were determined spectrophotometrically using nitrocefin and various carbapenems as substrates. Inactivation efficiency parameters ($k_2/K$) were calculated after fitting enzyme progressive inactivation curves using nitrocefin as a substrate. $K_{off}$ values were determined by jump-dilution method. Stoichiometry of carbapenemase inhibition was studied by measuring enzyme inhibition with various enzyme/inhibitor ratios.

**Results:** Vaborbactam (4 µg/ml) reduced meropenem MICs to that observed for the vector only strain (0.06-0.125 µg/ml) in isogenic strains containing BKC-1 (8 µg/ml), FR-1 (4 µg/ml), and KPC-2 (32 µg/ml). Vaborbactam but not tazobactam or clavulanic acid reduced the MICs for carbapenems, aztreonam and cephalosporins against BKC-1 or FR-1 expressing strains. Analysis of initial rates of nitrcocefin hydrolysis by the purified enzymes demonstrated that vaborbactam inhibited KPC-2, BKC-1 and FRI-1 with $K_i$ of 0.075, 0.019 and 0.18 µM, respectively. Detailed biochemical experiments demonstrated important differences in inhibition kinetics between KPC-2 and two other carbapenemases. Kinetic curves of nitrocefin hydrolysis in the presence of vaborbactam were consistent with progressive inactivation of all three enzymes. Inactivation efficiency ($k_2/K$) by vaborbactam was $7.3 \times 10^3$, $1.2 \times 10^4$ and $3.4 \times 10^3$ $M^{-1}s^{-1}$ for KPC-2, BKC-1 and FRI-1, respectively. $K_{off}$ values were extremely low for KPC-2 giving residence time of ~17 hours, while for BKC-1 and FRI-1 residence time of RPX7009 was 45 and 9 min, respectively. Stoichiometry of inhibition was 1:1 for KPC-2 and BKC-1 and 1:8 for FRI-1.

**Conclusions:** Vaborbactam potentiated activity of meropenem and other antibiotics against engineered strains overexpressing newly discovered BKC-1 and FRI-1carbapenemases, and inhibited nitrocefin hydrolysis driven by each of these enzymes.