Vaborbactam (VAB) is not affected by KPC-2 and KPC-3 Variants Containing Asp179Tyr Amino Acid Substitution that are Resistant to Ceftazidime (CAZ) Potentiation with Avibactam

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Abstract

Background: Many recent developments in the field of combating antibiotic resistance are based on the introduction of novel molecules that can be combined with existing antibiotics. The strategy of combining two antibiotics (BLI) in vitro is based on the premise that their individual MIC values would be additive, which is not true due to the presence of resistant genotypes. VAB and Avibactam (AVI) are β-lactamase inhibitors (BLI) that have been developed to be potentiated by CAZ. KPC-2 and KPC-3 are resistant to potentiation when paired with CAZ and ceftazidime (CAZ). Although Avibactam and VAB have been shown to be potentiated against KPC-2 and KPC-3, their activity is affected by the D179Y mutation. The objectives of this study were to: 1. Characterize the activity of VAB and Avibactam against KPC-2 and KPC-3 strains with or without the D179Y mutation in vivo and in vitro. 2. Characterize the activity of these inhibitors against KPC-3 strains with a high MIC of cephalosporins and aztreonam, the latter of which is not affected by KPC-3. 3. Evaluate the kinetics of inhibition of D179Y mutants by either AVI or VAB.

Methods:

1. Antibiotic susceptibility testing was performed using the broth microdilution method and the agar dilution method. 
2. Inhibition kinetics were studied using the steady state method.
3. A modified nitrocefin plate assay was used to determine initial inhibition rates.
4. Inactivation kinetic parameters were determined using continuous monitoring of turbidity.

Results:

1. MIC values for VAB and Avibactam against KPC-2 and KPC-3 mutants were determined.
2. The D179Y mutation in both KPC-2 and KPC-3 resulted in significant changes in MIC values: a 16-64-fold decrease in MIC values. 
3. KPC-3 differs from KPC-2 by a single H274Y substitution.
4. KPC-3, respectively (64-128-fold effect); against the concomitant 32-64-fold decrease in aztreonam to various antibiotics and BLI combinations were determined spectrophotometrically using nitrocefin (NCF) and CAZ as substrates.
5. Evaluation of the wild type KPC and D179Y mutants as substrates for CAZ and Avibactam showed that KPC-3 was more susceptible to CAZ than KPC-2, while Avibactam showed a 16-fold decrease in MIC values.
6. VAB showed a similar potency in reducing CAZ MIC against KPC-2 and KPC-3; however, VAB showed a 256-fold decrease in MIC values for KPC-2 and KPC-3 mutants.
7. Avibactam resistance in Enterobacteriaceae with KPC-3 carbapenemase. The presence of resistance was associated with resistance to avibactam, consistent with a new mode of binding to the active site for vaborbactam compared to avibactam. 

Conclusions:

Both microbiological and biochemical data confirm that the wild type KPC-2 and KPC-3 that carry D179Y mutation have increased catalytic activity for ceftazidime hydrolysis and cause resistance to avibactam but not vaborbactam inhibition.

Vaborbactam resistance activity of KPC enzymes with the D179Y mutation associated with resistance to avibactam is consistent with a different mode of binding to the active site for vaborbactam.

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References


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