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Unraveling the cefepime-zidebactam synergy basis against metallo-beta-lactamase (MBL)-producing Pseudomonas aeruginosa through penicillin-binding protein (PBP) binding dynamics

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Background: Zidebactam (WCK5107) is a new Bicyclo-acyl Hydrazide (BCH) discovered at the Wockhardt Research Centre (India). The objectives of this work were to determine the *P. aeruginosa* PAO1 penicillin-binding protein (PBP) binding dynamics of cefepime and imipenem and the binding affinities of zidebactam in combination with cefepime, including PBP binding affinities in the presence of the MBL VIM-1.

Material/methods: VIM-1 kinetics study to determine the relative stability of cefepime and imipenem was undertaken. PBPs of PAO1 were obtained and binding kinetics for cefepime and imipenem were estimated in a series of competition experiments over time (0, 5, 10, 15 and 30 min) with Bocillin FL. 50% inhibitory concentrations (IC_{50}) of cefepime, imipenem, and zidebactam were determined in the presence of different concentrations of purified VIM-1. IC_{50} of the cefepime combination with fixed concentrations of zidebactam (4 and 8 mg/L) were also determined.

Results: Standard IC_{50} experiments (in which Bocillin FL is added at t = 30 min after the test β-lactam), evidenced the potent inhibition of all PBPs by imipenem (highest for PBP2) and the potent inhibition of PBP1a and PBP3 by cefepime. PBP binding studies in the presence of VIM-1 at concentrations that led to 100% cefepime hydrolysis over 30 min, yielded just a 2-6 times increase in PBP3 IC_{50} of cefepime. In contrast, under similar conditions, imipenem PBP2 IC50 rose by 50-100 folds, thus suggesting that binding of cefepime to PBP3 is a significantly faster process. Zidebactam PBP2 binding was unaffected in the presence of VIM-1. As shown in Figure 1, experiments measuring the PBP binding kinetics over time, confirmed that cefepime binding to PBP3 is a rapid phenomenon as only 3.6 times lowering of PBP3 IC_{50} was observed from initial t = 0 min (PBP3 IC_{50} = 0.26 mg/L) to final t = 30 min (PBP3 IC_{50} = 0.07 mg/L). Similarly, even for PBP1a, just 2 times lowering of cefepime IC_{50} was observed at 30 min. However, for imipenem, 56 times lowering of PBP2 IC_{50} suggests a much slower rate of binding (0 min PBP2 IC_{50} = 1.69 mg/L and 30 min PBP2 IC_{50} = 0.03 mg/L). Likewise, even for PBP1a and 1b, a 16 and 28 times lowering of IC_{50} was observed at 30 min for imipenem. Finally, cefepime-zidebactam combination showed potent complementary PBP2 and PBP3 inhibition.

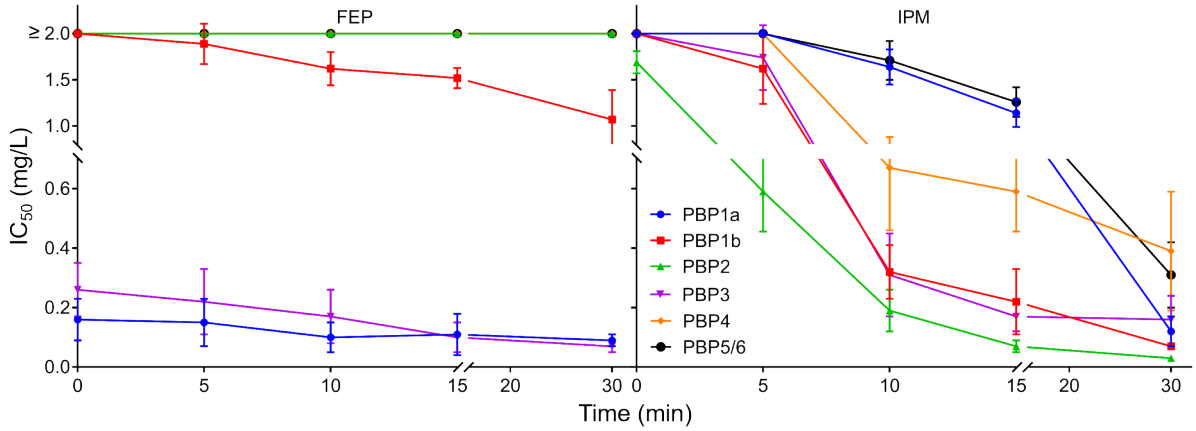

Figure 1. PBP binding dynamics for cefepime (FEP) and imipenem (IPM).

Conclusions: Our results demonstrate that cefepime has a fast binding kinetics allowing it to effectively engage PBP1a and PBP3 even in the presence of MBL. On the other hand, zidebactam shows a potent, MBL stable, PBP2 inhibitory activity. Thus, the obtained results provide a mechanistic basis for the observed synergy between cefepime and zidebactam against MBL-producing Gram-negatives.