(1) Submission ID#1539738

β-lactams with an R1-ureido moiety are potent inhibitors of penicillin-binding protein 2 (PBP2) of Neisseria gonorrhoeae

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Background

A major contributor to the extended-spectrum cephalosporin (ESC) resistance of Neisseria gonorrhoeae is acquisition of mosaic penA alleles encoding the essential transpeptidase penicillin-binding protein 2 (PBP2). Commensurate with this, PBP2 from the ESC-resistant strain H041 exhibits lower binding and acylation activity for ceftriaxone compared to PBP2 from the susceptible strain, FA19. Crystal structures show that lowered activity correlates with displacement of the β3-β4 loop away from the active site in PBP2-H041, compared to its inward position in PBP2-FA19. These data suggest that β-lactams capable of eliciting inward movement of the β3-β4 loop will be superior inhibitors of PBP2-H041. Previously, we found that cefoperazone exhibits a significantly higher rate of acylation against PBP2-H041 than ceftriaxone. One feature of cefoperazone is the presence of a ureido group and thus the goal of this study was to see whether other β-lactams containing ureido moieties were also highly reactive toward PBP2 and to determine their effects on

the conformation of the β3-β4 loop.

Aim/Methods

Piperacillin, azlocillin and mezlocillin are penicillins that contain a ureido moiety. We measured their rates of acylation against PBP2-H041 and also determined binding affinities using an acylation-incompetent S310A mutant. In addition, crystal structures of PBP2-H041 in complex with piperacillin, azlocillin and cefoperazone were solved, and MICs for all four antibiotics were determined against H041.

Results

Piperacillin and azlocillin show significantly higher activity against PBP2-H041, with piperacillin having an 86-fold increase in acylation rate compared with ceftriaxone. In addition, MICs for piperacillin against H041 are lower (0.75 μg/mL vs. 2 μg/mL for ceftriaxone). Surprisingly, the elevated acylation rate appears to be driven entirely through intrinsic reactivity of the β-lactam, as these antibiotics show negligible non-covalent binding to the S310A mutant of PBP2-H041. Structures of PBP2-H041 in complex with cefoperazone, piperacillin, and azlocillin show that the β3-β4 loop occupies the inward conformation.

Conclusions

These data suggests that ureido β-lactams are able to overcome resistance mutations in PBP2-H041 by promoting movement of the β3-β4 loop toward the active site, thereby enhancing the rate of acylation. This study also highlights the potential of piperacillin to replace ceftriaxone, as ESC-resistant strains of Neisseria gonorrhoeae spread globally.

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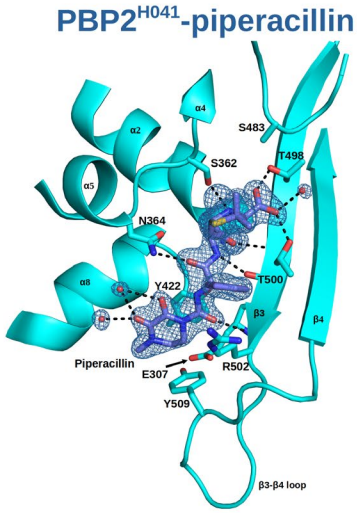
Supplemental Document Upload

PBP2-H041 in complex with piperacillin


PBP2H041-piperacillin
α4
α2
S483
T498
S362
α5
N364
α8
Y422
T500
β3
β4
Piperacillin
E307
R502
Y509
β3-β4 loop