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Mutations in the β3-β4 loop of penicillin-binding protein 2 from cephalosporin-resistant Neisseria gonorrhoeae hinder cephalosporin acylation via restriction of protein dynamics

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Background

Neisseria gonorrhoeae strains with decreased susceptibility to the extended-spectrum cephalosporins (ESCs) cefixime and ceftriaxone pose an increasing threat to human health. A major contributor to the ESC resistance of N. gonorrhoeae is acquisition of mosaic variants of penA, encoding penicillin-binding protein 2 (PBP2). penA from the ESC-resistant strain H041 contains over 60 mutations compared to the gene from the FA19 susceptible strain. Of these, 8 are known to confer the majority of ESC resistance. Two of the 8 mutations, F504L and N512Y, are present on the β3-β4 loop of the protein, which moves toward the active site when PBP2-FA19 is acylated by ceftriaxone but not when PBP2-H041 is similarly acylated. Knowing the mechanism of action of these mutations will enable design of new anti-gonococcal agents capable of bypassing resistance mechanisms.

Aim/Methods

Based on the hypothesis that F504L and N512Y hinder loop movement of β3-β4, we mutated these residues in the forward and reverse directions (i.e. F504L and N512Y in the background of FA19 and L504F and Y512N in the background of H041) and then measured acylation kinetics for ceftriaxone and determined crystal structures.

Results

We found that introducing the two H041 resistance mutations into PBP2-FA19 lowers the rate of acylation by ceftriaxone by nearly 60-fold, and a crystal structure of the mutated protein bound to ceftriaxone shows that the β3-β4 loop now occupies an extended position in contrast to its inward position in PBP2-FA19. In the reverse direction, introducing L504F and Y512N into PBP2-H041 increases the rate of acylation by 22-fold for both ceftriaxone and cefixime, and a crystal structure shows the loop now moves toward the active site upon acylation by cefixime.

Conclusions

These data provide strong evidence that mutations present in ESC-resistant strains of N. gonorrhoeae hinder protein movement of the β3-β4 loop and that such movement is necessary for efficient binding and acylation by ESCs. In turn, this suggests that agents capable of overcoming the conformational barrier created by these mutations will be more effective inhibitors of PBP2 and potentially exhibit better antimicrobial activity.

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