**Background**

The β-lactam antibiotics are excreted via the bile duct into the intestine where they can disrupt the intestinal microflora. In clinical trials, P1A given orally with IV penicillins preserved the diversity of the intestinal microflora, reduced the selection for antibiotic-resistant coliforms, efficiently degraded piperacillin/tazobactam in the intestine, and did not alter plasma antibiotic levels. However, P1A has limited utility as it does not degrade cephalosporins, a major risk factor for C. difficile infection.

Therefore, P1A was selectively modified following comparison to other cephalosporin-hydrolyzing β-lactamases, including the CTX/M enzymes. In addition to single aa differences, two major areas of sequence divergence were observed. Block 1 [3 (aa) and Block 2 [4 (aa)]. A panel of single and multiple P1A aa changes were generated and characterized in kinetic and antibiotic hydrolysis assays.

**Results**

The first P1A mutant characterized contained a single aa change, D276N, and was designated P3A.

**Kinetics:** P1A and P3A

**Activity:** P1A and P3A

Michaelis-Menten kinetics were determined using non-linear regression analyses. P3A displayed a dramatic improvement in cefotaxime degradation while maintaining activity against the penicillins.

Relative antibiotic hydrolysis was evaluated using a microtiter plate assay that used E. coli growth as the read-out for antibiotic degradation that was designed to model the activity of β-lactamases in the gut in the presence of high antibiotic concentrations. P3A displayed improved activity against cefotaxime, ceftaxime, ceftriaxone, and cefotaxime while retaining activity against ampicillin.

Additional P1A mutants were screened by kinetic analysis. Some mutants containing the Block 2 changes (219 and 221) displayed improved degradation of cefotaxime. Some mutants containing the D276N change (230, 232, and 234), displayed improved degradation of both cefotaxime and cefotaxime.

The Block 2 and D276N (P3A) mutations were combined to generate P4A.

**Conclusions**

- Modification of five amino acids in P1A boosted its cephalosporin-degrading activity 1000-fold.
- P4A displayed improved degradation of all cephalosporins evaluated while retaining its activity against penicillins.
- Oral administration of P4A is anticipated to protect the gut flora from commonly used cephalosporins and to be used prophylactically to prevent C. difficile infection.

**References**