β-LACTAMASE INDUCTION BY *N*-FORMIMIDOYLTHIENAMYCIN

Sir:

Development of resistance during therapy has been observed with the newer β-lactamase-resistant cephalosporins. The resistant bacteria, usually of the genera *Enterobacter*, *Serratia* and *Pseudomonas*, produce Type I cephalosporinases and are resistant to most β-lactams, including the third generation cephalosporins and aztreonam. Several investigators have shown that these strains remain susceptible to *N*-formimidoylthienamycin, NFT (imipenem), mecillinam and penems[1,2].

Clinical isolates were grown in the presence of subinhibitory concentrations of *N*-formimidoylthienamycin (NFT) in Mueller-Hinton broth in microtiter plates. β-Lactamase activity was detected using nitrocefin according to the method described by NEU[3]. β-Lactamase induction was demonstrated in 21/24 strains of Gram-negative bacteria tested, including strains of *Enterobacter*, *Serratia*, *Pseudomonas*, *Morganella*, *Providencia* and *Proteus*.

The minimum inhibitory concentrations for NFT and cefotaxime were determined by microtiter two-fold dilution tests in Mueller-Hinton broth. When combined with cefotaxime, NFT antagonized the activity of this third generation cephalosporin against isolates of the genera *Enterobacter*, *Serratia* and *Pseudomonas* in which NFT-inducible β-lactamase activity had been demonstrated. For example, the MIC for cefotaxime increased from 0.4 to 25 μg/ml for *Enterobacter cloacae* 2598, from 0.1 to 1.6 μg/ml for *Serratia marcescens* 1187, and from 12.5 to 100 μg/ml for *Pseudomonas aeruginosa* 3198 when tested in combination with a concentration of NFT equivalent to 1/4 its MIC.

Strains of *S. marcescens*, *P. aeruginosa*, and *E. cloacae* were passaged in the presence of subinhibitory concentrations of NFT. It was possible to select NFT-resistant mutants of *Serratia* and *Pseudomonas* strains with NFT-inducible β-lactamases after passage three times *in vitro*. The mutants were isolated on gradient plates containing 20 μg/ml of NFT in Mueller Hinton agar at the highest concentration. Increase in NFT-resistance was not observed with the *E. cloacae* strains passaged the same way. The increased resistance of mutants of *P. aeruginosa* and *S. marcescens* appeared to be associated with β-lactamase activity since the more resistant mutants became susceptible to NFT when it was combined with a β-lactamase inhibitor such as sulbactam. For example, the MIC for NFT against the mutant strain of *P. aeruginosa* 3198 was 32 μg/ml. When NFT was tested in combination with sulbactam, the resistant mutant became susceptible. The MIC for NFT was 4.0 μg/ml, which was similar to its MIC for the parental strain.

β-Lactamase was isolated from the NFT-resistant mutant of *P. aeruginosa* 3198 following induction by NFT (1.0 μg/ml). Cells grown in Trypticase soy broth were washed with phosphate-buffered saline, pH 7.0. A cell paste was frozen in liquid nitrogen and thawed. The broken cell suspension was clarified by centrifugation at 45,000×*g* at 4°C. The supernatant containing the β-lactamase activity was collected. The crude β-lactamase antagonized the activity of ceftazidime, ceftizoxime and moxalactam as well as NFT. The antibiotics were used at concentrations which gave approximately 20 mm zones of inhibition when tested by agar disc diffusion tests against an assay strain, *S. aureus* 209P. The discs containing the enzyme, 60 μg of protein, plus the antibiotics gave no zones of inhibition following incubation at 37°C, overnight.

Preliminary determinations of the approximate isoelectric point of the *Pseudomonas* β-lactamase were made using the LKB Multiphor with Ampholine PAG plates. The Type-I β-lactamase from *E. cloacae* P-99 was run as a control. The pI for the control was 7.4 as reported by others[4]. The enzyme from the NFT-induced resistant mutant of *P. aeruginosa* had a pI of 8.0.

These studies indicate that the problem of emergence of resistance observed in the clinic with the third generation cephalosporins may also occur with NFT. Our studies indicate that the β-lactamase induced by NFT can interfere with the activity of NFT and the third generation cephalosporins.

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