In Vitro Activity of Aztreonam in Combination with Newer β-Lactams and Amikacin Against Multiply Resistant Gram-Negative Bacilli

MARY A. BUESING† AND JAMES H. JORGENSEN2*

Infectious Diseases Service, Department of Medicine, Brooke Army Medical Center, San Antonio, Texas 78234,† and Department of Pathology, The University of Texas Health Science Center at San Antonio, 2 San Antonio, Texas 78284

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The in vitro synergistic activity of aztreonam in combination with piperacillin, moxalactam, cefotaxime, cefoperazone, and amikacin was examined against multiply resistant isolates of the family Enterobacteriaceae and Pseudomonas aeruginosa. Aztreonam in combination with amikacin demonstrated synergy against 71% of the isolates, whereas combinations of aztreonam plus a second beta-lactam demonstrated synergy against 42% of the isolates.

Empirical therapy of suspected infections in neutropenic patients often consists of antibiotic combinations: usually an antipseudomonal penicillin with an aminoglycoside or possibly a cephalosporin with an aminoglycoside, especially if increased coverage of gram-positive organisms is desired. The rationale for this approach has been the need not only to provide broad-spectrum coverage of potential pathogens, but also to exploit the potentially synergistic action of two antibiotics as opposed to one in the therapy of gram-negative bacteremia (1, 3).

Aztreonam is a new beta-lactam antimicrobial agent which belongs to the family of monobactams (8). It has a high degree of beta-lactamase stability and excellent activity against aerobic gram-negative bacteria (5, 8). Aztreonam has previously demonstrated synergy when used in combination with gentamicin against certain strains of Pseudomonas aeruginosa and Klebsiella species (9). More recently, synergy was observed in 7 of 31 combinations of aztreonam and tobramycin and 2 of 31 combinations of aztreonam plus moxalactam against P. aeruginosa (C. Starkey, C. Corlett, G. S. Hall, T. L. Gavan, and S. Rehm, Abstr. Annu. Meet. Am. Soc. Microbiol. 1983, C348, p. 369).

In the present study, the activity of aztreonam with piperacillin, moxalactam, cefotaxime, cefoperazone, and amikacin was compared against selected multiply resistant clinical isolates of P. aeruginosa, Escherichia coli, Klebsiella pneumoniae, Enterobacter cloacae, and Serratia marcescens, using both growth inhibition and bactericidal determinations. Isolates were selected because of previously demonstrated resistance by disk diffusion testing to primary antibiotics such as carbenicillin, gentamicin, tobramycin, and first-generation cephalosporins. P. aeruginosa ATCC 27853 was included as a control strain to verify the potency of antibiotics and the adequacy of test methods.

Antibiotic powders of known potencies were obtained from their manufacturers for preparation of stock solutions for the tests each day. MICs for each isolate and antibiotic were determined by use of an Abbott MS-2 research system, which incorporates automated turbidimetric determinations. MS-2 research cuvettes which contained 11 separate wells accommodating a volume of 1 ml of Mueller-Hinton broth (Difco Laboratories) were utilized. Each test isolate was grown overnight on sheep blood agar; then an inoculum of 5 × 10^5 CFU/ml was prepared by suspending growth to a predetermined optical density. Viable cell counts were performed with each test to verify the actual inoculum density which was later utilized for MBC determinations. The cuvettes were immediately placed in an MS-2 analysis module and incubated at 35.5°C for 16 h with constant agitation. Optical density-versus-time plots were programmatically derived by the MS-2 system for each bacterial strain and antibiotic. The MIC was defined as the lowest concentration of drug which yielded consistent growth inhibition during the test period as evidenced by review of the kinetic plots. MBCs were determined at the conclusion of the incubation period by removing 0.1-ml samples from each cuvette well demonstrating growth inhibition and spreading the samples over the entire surface of duplicate Trypticase soy agar (BBL Microbiology Systems) plates. Resultant colonies were counted after 18 to 20 h of incubation at 35°C. The MBC was defined as the lowest concentration of antibiotic resulting in a ≥3-log decrease in the number of viable organisms (99.9% kill) based on the previously determined inoculum density for each test.

To determine possible synergistic effects, the separate MICs for each drug tested separately were combined in MS-2 research cuvettes as follows: 1/4 MIC, 1/4 MIC, 1/2 MIC, 2 × MIC, 4 × MIC, and 8 × MIC. Inocula and test conditions were the same as described above for testing of drugs separately. Kinetic inhibition curves were examined, and MBCs were also determined as described above. The effects of antibiotic combinations were determined as follows: synergy, at least a fourfold reduction in the MICs or MBCs of each antibiotic; antagonism, a fourfold or greater increase in the MICs or MBCs of each antibiotic; indifference, an effect intermediate between synergy and antagonism. However, MBCs beyond the concentration range tested of either antibiotic or antibiotic combination were considered indeterminate.

The individual MICs for each antibiotic and organism and the corresponding MBCs are listed in Table 1. The ranges of the MICs (in micrograms per milliliter) for each antibiotic were as follows: aztreonam, 0.03 to 16; moxalactam, 0.06 to 64; cefotaxime, 0.015 to 128; cefoperazone, 0.25 to 128; piperacillin, 1 to 2,048; and amikacin, 0.25 to 4. Table 2
summarizes the effects realized when aztreonam was combined with the other beta-lactams or amikacin. In 36 of 70 antimicrobial combinations, a synergistic effect was observed based upon the criteria defined previously. Aztreonam in combination with amikacin provided a synergistic effect against the majority of organisms tested (71%, 10 of 14). Synergy was more frequently between aztreonam and the other beta-lactam antibiotics (42%, 6 of 14). The effectiveness of a particular aztreonam–beta-lactam combination did not necessarily apply to other isolates, although two of the P. aeruginosa isolates were affected synergistically by combinations of aztreonam and the three cephalosporins, Cefotaxime, and aztreonam. Cefoperazone, unlike moxalactam or cefotaxime, demonstrated synergy with aztreonam against all three S. marcescens isolates. Piperacillin plus aztreonam provided synergy with four of six isolates which were resistant to piperacillin alone, i.e., MIC of ≥256.

As previously noted by Hallander et al. (2), synergistic or indifferent outcomes based on inhibitory criteria were not necessarily predictive of the killing effects of the drug combinations; e.g., a synergistic effect based on MIC criteria could result in an indifferent effect by combined MBCs, as with aztreonam and moxalactam against P. aeruginosa 54 or aztreonam and amikacin against S. marcescens 7. In two other instances, aztreonam and amikacin with E. cloacae 17 and aztreonam and piperacillin with S. marcescens 3, different MIC effects were associated with synergistic MBCs. Thus, at times an enhanced bactericidal effect was achieved with certain antibiotic combinations which provided an indifferent combined inhibitory effect.

Antagonism was not observed with any combination based on MIC determinations, but was seen with three combinations based on MBC results (Table 2). Two instances involved aztreonam with moxalactam against K. pneumoniae 9 and P. aeruginosa 21. The latter organism was also associated with antagonism involving aztreonam and piperacillin.

The results of this investigation are encouraging with respect to potential clinical applications of combined therapy with aztreonam plus either an aminoglycoside or another beta-lactam antibiotic. However, this effect could not be predicted entirely by knowledge of the species involved, nor was any particular aztreonam–beta-lactam combination more likely to provide a synergistic effect. Certain previous studies have likewise demonstrated variable in vitro synergy results with other new beta-lactams (4, 6, 7), even including strain- and test-method-dependent variation with P. aeruginosa (10). Thus, animal model or clinical studies will be required to clarify the importance of these observations.

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**LITERATURE CITED**


