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In Vitro Activity of Aztreonam Combined with Tobramycin and Gentamicin against Clinical Isolates of *Pseudomonas aeruginosa* and *Pseudomonas cepacia* from Patients with Cystic Fibrosis

JOHN A. BOSSO,[1,2]* BARBARA A. SAXON,[3] AND JOHN M. MATSEN[2,3]

Department of Pharmacy Practice, College of Pharmacy,[1] and Departments of Pediatrics[2] and Pathology,[3] School of Medicine, University of Utah, Salt Lake City, Utah 84112

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The in vitro activity of aztreonam combined with tobramycin and with gentamicin was assessed in 78 clinical isolates of *Pseudomonas aeruginosa* and 11 clinical isolates of *Pseudomonas cepacia* from patients with cystic fibrosis. Synergy was detected in 56.4% of *P. aeruginosa* isolates and 60% of *P. cepacia* isolates with the aztreonam-tobramycin combination and in 49.3% of *P. aeruginosa* isolates and 81.8% of *P. cepacia* isolates with the aztreonam-gentamicin combination. No antagonism was observed. These combinations merit clinical evaluation in the treatment of patients with cystic fibrosis.

Aztreonam is a monobactam antibiotic with in vitro activity against a number of gram-negative aerobic bacteria (8). This activity extends to *Pseudomonas aeruginosa*, including isolates from patients with cystic fibrosis (19). Early experience with the use of aztreonam in patients with cystic fibrosis has yielded promising results (2, 21). However, the customary treatment for the pneumonia associated with pulmonary exacerbations of cystic fibrosis generally includes both a beta-lactam and an aminoglycoside antibiotic. Such combinations are used to take advantage of potential synergy and to help prevent or delay the emergence of resistance which is often observed with single-drug therapy. It is likely that aztreonam will find a place in such combination therapy with an aminoglycoside. We therefore studied the in vitro effects of combinations of aztreonam with tobramycin and with gentamicin against clinical isolates of *P. aeruginosa* and *Pseudomonas cepacia* from patients with cystic fibrosis.

MATERIALS AND METHODS

A total of 78 isolates of *P. aeruginosa* and 11 isolates of *P. cepacia* obtained from the sputum of patients with cystic fibrosis were identified by standard microbiological methods (5) and stored at room temperature on tryptic soy agar slants without dextrose before being studied. Isolates were transferred to duplicate blood agar plates and checked for purity. They were subsequently transferred to Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.) and incubated overnight at 35°C. The turbidity of each bacterial broth suspension was then standardized to that of a 0.5 McFarland standard. From each standardized suspension, 0.1 ml was transferred to 9.9 ml of Mueller-Hinton broth, supplemented with the divalent cations calcium and magnesium (final concentrations, 50 and 25 mg/ml, respectively), yielding a 1:100 dilution.

The antibiotics studied were supplied by the following sources: aztreonam, E. R. Squibb & Sons, Princeton, N.J.; gentamicin, Schering Corp., Kenilworth, N.J.; tobramycin, Eli Lilly & Co., Indianapolis, Ind. Stock solutions were prepared from dry powders and used immediately or frozen at −32°C. Frozen solutions of aztreonam were used within 1 month, and aminoglycoside solutions were used within 3 months. The antibiotic concentrations tested were twofold dilutions ranging from 1,024 to 0.0156 μg/ml for aztreonam and 1,024 to 0.062 μg/ml for tobramycin and gentamicin.

MICs were determined by microbroth dilution. Microtiter plates (Dynatech Laboratories, Inc., Alexandria, Va.) were prepared with the antibiotic preparation and either used immediately or stored in sealed plastic bags at −32°C for no longer than 1 month before use. Of the 1:100 bacterial broth dilution, 50 μl was added to each microtiter well with a multichannel pipette (final inoculum, $\geq 5 \times 10^5$). Plates were resealed in plastic bags and incubated overnight at 35°C. For the isolates in which synergistic effects were noted (on the basis of a fourfold or greater decrease in MIC of both drugs), MBCs were also determined. The MBC was defined as the lowest concentration of drug yielding no more than five colonies after a subculture of 0.01 to 0.02 ml from each well on the microtiter plate onto Columbia agar and overnight incubation at 35°C in sealed plastic bags.

Synergy was defined as a fourfold or greater reduction of the MIC or MBC (or both) of both antibiotics (22). Antagonism was defined as a fourfold or greater increase in the MIC of either drug.

RESULTS

The aztreonam-tobramycin combination was tested against 78 *P. aeruginosa* isolates and 10 *P. cepacia* isolates. The MICs of aztreonam for the *P. aeruginosa* isolates ranged from 0.0312 to 128 μg/ml (mean, 9.22 μg/ml). The MBCs for the isolates affected synergistically on the basis of MIC reduction ranged from 0.25 to 32 μg/ml. The MICs of tobramycin for all *P. aeruginosa* isolates tested ranged from 0.25 to 64 μg/ml (mean, 5.73 μg/ml). The MBCs of tobramycin for the isolates that were synergistically affected ranged from 0.5 to 64 μg/ml. The MICs of aztreonam for the *P. cepacia* isolates ranged from 1 to 1,024 μg/ml (mean, 180.1 μg/ml). The MBCs of the *P. cepacia* isolates showing a synergistic effect on the basis of MIC reduction ranged from 8 to 1,024 μg/ml. The MICs of tobramycin for all *P. cepacia* isolates tested ranged from 32 to 128 μg/ml (mean, 67.2 μg/ml), and the MBCs for the isolates affected synergistically ranged from 64 to 256 μg/ml.

Of the *P. aeruginosa* isolates, 44 (56.4%) were affected

* Corresponding author.

TABLE 1. In vitro activity of aztreonam combined with tobramycin and gentamicin

Drug combined with aztreonam and organism tested	No. of isolates	Synergy[a] (%)
Tobramycin		
P. aeruginosa	78	56
Aztreonam-resistant *P. aeruginosa*	9	33
Tobramycin-resistant *P. aeruginosa*	26	42
P. cepacia	10	60
Aztreonam-resistant *P. cepacia*	6	50
Tobramycin-resistant *P. cepacia*	10	60
Gentamicin		
P. aeruginosa	75	49
Aztreonam-resistant *P. aeruginosa*	9	56
Gentamicin-resistant *P. aeruginosa*	47	47
P. cepacia	11	82
Aztreonam-resistant *P. cepacia*	5	100
Gentamicin-resistant *P. cepacia*	10	90

[a] Synergy is defined as a fourfold or greater reduction in the MICs of both drugs.

synergistically by this combination on the basis of a fourfold reduction in the MIC of both antibiotics (Table 1). Of these isolates, 42 (53.8%) were affected synergistically on the basis of fourfold reductions in the MIC and MBC of both antibiotics. Six *P. cepacia* isolates (60%) were affected synergistically on the basis of a fourfold reduction in the MIC of both drugs. Four (40%) isolates were synergistically affected on the basis of a fourfold reduction in the MIC and MBC of both antibiotics. No antagonism was observed. A synergistic effect was observed in three of nine aztreonam-resistant (MIC, ≥32 μg/ml) isolates of *P. aeruginosa* and three of six *P. cepacia* isolates. Likewise, synergy was detected in 11 of 26 tobramycin-resistant (MIC, ≥8 μg/ml) *P. aeruginosa* isolates and in 6 of 10 tobramycin-resistant isolates of *P. cepacia*. For 28 *P. aeruginosa* isolates, MICs of aztreonam were greater than the theoretically achievable concentration in sputum of ≤5 μg/ml. Of the isolates affected synergistically, the synergistic MIC of aztreonam was <5 μg/ml for 14. For 33 isolates, MICs of tobramycin were greater than the theoretically achievable concentration in sputum of ≤2 μg/ml. Of the isolates that were affected synergistically, the synergistic MIC of tobramycin was ≤2 μg/ml for 15. For nine *P. cepacia* isolates, MICs of aztreonam were greater than 5 μg/ml. Of the isolates that were affected synergistically, synergistic MICs of aztreonam for two were <5 μg/ml. For all 10 *P. cepacia* isolates, tobramycin MICs were greater than 2 μg/ml, and there were no synergistic MICs below this concentration.

The aztreonam-gentamicin combination was tested against 75 *P. aeruginosa* isolates and 11 *P. cepacia* isolates. The MICs of aztreonam for the *P. aeruginosa* isolates ranged from 0.0312 to 128 μg/ml (mean, 12.23 μg/ml). The MBCs of aztreonam for those isolates affected synergistically ranged from 0.5 to 128 μg/ml. The MICs of gentamicin for all *P. aeruginosa* isolates tested ranged from 2 to 512 μg/ml (mean, 19.04 μg/ml). The MBCs of gentamicin for the organisms that were affected synergistically ranged from 2 to 1,024 μg/ml. The MICs of aztreonam for *P. cepacia* isolates ranged from 1 to 1,024 μg/ml (mean, 137.18 μg/ml). For the isolates that were affected synergistically, the MBCs of aztreonam ranged from 8 to 1,024 μg/ml. The MICs of gentamicin for these isolates ranged from 64 to 256 μg/ml (mean, 104.73 μg/ml), and the MBCs for the isolates that were affected synergistically ranged from 64 to 256 μg/ml.

On the basis of a fourfold reduction in MIC, 37 (49.3%) *P. aeruginosa* isolates and 9 (81.8%) *P. cepacia* isolates were affected synergistically (Table 1). Thirty-three *P. aeruginosa* isolates (44%) and nine *P. cepacia* isolates (72.7%) were affected synergistically on the basis of a fourfold reduction in MIC and MBC for both antibiotics. No antagonism was observed. Synergy was detected in five of nine aztreonam-resistant isolates of *P. aeruginosa* and all five aztreonam-resistant isolates of *P. cepacia*. Synergy was also detected in 22 of 47 gentamicin-resistant (MIC, ≥8 μg/ml) *P. aeruginosa* isolates and 9 of 10 gentamicin-resistant isolates of *P. cepacia*. For 27 *P. aeruginosa* isolates, aztreonam MICs were greater than 5 μg/ml. Of the isolates that were synergistically affected, synergistic aztreonam MICs for 18 were less than 5 μg/ml. For 67 *P. aeruginosa* isolates, gentamicin MICs were greater than 2 μg/ml. Of the isolates that were affected synergistically, synergistic gentamicin MICs were less than 2 μg/ml for 28. For nine *P. cepacia* isolates, aztreonam MICs were greater than 5 μg/ml. Of the isolates that were affected synergistically, synergistic aztreonam MICs were less than 5 μg/ml for four. For all 11 *P. cepacia* isolates, gentamicin MICs were greater than 2 μg/ml, and none were reduced below that concentration by combination with aztreonam.

DISCUSSION

In vitro synergism of various aminoglycoside-beta-lactam combinations against isolates of *P. aeruginosa* and *P. cepacia* from patients with cystic fibrosis has been evaluated by other investigators. Scribner et al. studied a number of aminoglycoside (amikacin, tobramycin, and gentamicin) and beta-lactam (ticarcillin, moxalactam, cefoperazone, azlocillin, piperacillin, and ceftazidime) combinations against 60 *P. aeruginosa* isolates (20). The highest rate of synergy was observed with the amikacin-azlocillin combination (65%); the lowest rate was the tobramycin-ceftazidime combination (23.3%). Aronoff and Klinger studied synergy with amikacin combined with aztreonam, piperacillin, and ticarcillin in 22 isolates each of amikacin-resistant *P. aeruginosa* and *P. cepacia* (1). The amikacin-aztreonam combination was associated with synergy in 81.8% of isolates of both *P. cepacia* and *P. aeruginosa*. Our findings are similar to these results. Synergy in some isolates that were resistant to aztreonam, tobramycin, or gentamicin is also noteworthy.

The use of potentially synergistic antibiotic combinations in the treatment of pulmonary infections associated with *Pseudomonas* species in patients with cystic fibrosis is theoretically attractive and a common practice for a number of reasons. The use of combinations of two or more antibiotics that have different mechanisms of bactericidal action and in vitro synergy might result in clinical results that are more favorable than those produced by a single agent. The emergence of antibiotic resistance during therapy may be delayed or prevented with the use of a synergistic combination. Moreover, multiply drug-resistant bacteria may be susceptible to synergistic combinations. Last, the use of a synergistic combination may allow for the use of lower doses of each individual antibiotic and, thereby, a lower rate of side effects. Unfortunately, convincing data to support any of these theories, at least in the context of cystic fibrosis, are lacking. The data that have been published are often contradictory. The rapid development of resistance on the part of *P. aeruginosa* during therapy of patients with cystic fibrosis with only a beta-lactam antibiotic has been documented by numerous investigators (3, 11, 12–15, 17, 18). However, whereas some have reported less emergence of resistance

with combination therapy (13, 15), others have observed similar rates of resistance developing with single-drug and combination therapies (9, 12, 17, 18).

Several investigators have reported superior clinical outcomes for patients with cystic fibrosis who were treated with combination antibiotic therapy, compared with results of single-antibiotic treatment (4, 10, 15, 16). Once again, however, others have reported equivalent clinical results when comparing single-agent regimens with combination regimens (6, 7, 9, 13, 17, 18). It is unfortunate that many of these studies were flawed by small study populations, and thus a large chance for beta-error, and failure to ensure adequate aminoglycoside serum concentrations. Thus, we are left with unsupported theories to justify or refute the value of potentially synergistic antibiotic combinations in this setting. Nonetheless, the use of such combinations in the treatment of patients with cystic fibrosis is commonplace.

One recent study of aztreonam therapy in patients with cystic fibrosis in our own institution detected no change in the susceptibility patterns of *P. aeruginosa* isolates with therapy even though the drug was used alone (2). However, the appearance of resistant isolates did occur in individual patients. This was also a small, uncontrolled trial, and it is open to the same criticisms listed above. The results of the present study indicate that in vitro synergy between aztreonam and aminoglycosides against isolates of *P. aeruginosa* and *P. cepacia* is frequent, even in aztreonam- or aminoglycoside-resistant isolates. Such combinations merit evaluation in this setting.

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