ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Feb. 1984, p. 212-215
0066-4804/84/020212-04$02.00/0

Vol. 25, No. 2

In Vitro Antimicrobial Activity of Aztreonam Alone and in Combination Against Bacterial Isolates from Pediatric Patients

HARRIS R. STUTMAN, DAVID F. WELCH, RONALD K. SCRIBNER, AND MELVIN I. MARKS*

Department of Pediatrics, Division of Infectious Disease, University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma 73190

Received 15 September 1983/Accepted 22 November 1983

We examined 134 pediatric clinical isolates of *Enterobacteriaceae, Pseudomonas aeruginosa*, and gram-positive cocci for susceptibility to aztreonam alone and in combination with seven other antibiotics. All 98 gram-negative isolates were susceptible to aztreonam with similar inhibitory and bactericidal activity. Combinations of aztreonam with cefoxitin, ampicillin, or clindamycin were generally indifferent or additive. Synergism was occasionally seen against enteric organisms with aztreonam plus cefoxitin or clindamycin. Combinations of tobramycin and aztreonam were synergistic (62%) against *P. aeruginosa*; aztreonam plus piperacillin or ticarcillin was additive. Aztreonam did not affect the activity of nafcillin against *Staphylococcus aureus*, or of ampicillin against species of *Streptococcus* group B or D. Antagonism was seen only with aztreonam plus cefoxitin against *Enterobacter* species, but not at clinically significant concentrations. Several combinations of antibiotics with aztreonam should be appropriate for initial therapy of infections in children without major risks of antibacterial antagonism.

Aztreonam, a monobactam (17), has excellent activity against aerobic gram-negative bacteria, especially *Enterobacteriaceae* (4, 16) and *Pseudomonas aeruginosa* (2, 9). Because of its lack of activity against gram-positive and anaerobic organisms, it will likely be used with other agents in the initial therapy of serious infections in appropriate pediatric patients, such as newborns and immunocompromised and post-surgical patients. Important pathogens in these patients include group B *Streptococcus, Staphylococcus, Listeria*, and various gram-negative bacteria, including *P. aeruginosa*. Therefore, initial antimicrobial combinations with aztreonam are likely to include ampicillin, cefoxitin, nafcillin, and clindamycin, as well as aminoglycosides and other antipseudomonal antibiotics. Hence, the susceptibilities of common pediatric pathogens to aztreonam alone and in appropriate antimicrobial combinations were examined in our laboratory.

MATERIALS AND METHODS

Antibiotics. Aztreonam was supplied as the anhydrous, crystalline, β-form by E. R. Squibb & Sons, Princeton, N.J. It was dissolved in a saturated solution of sodium bicarbonate, diluted to a stock concentration of 1,280 μg/ml with distilled water, and frozen in small samples at −70°C. All stock solutions were used within 4 weeks of preparation. Ampicillin, cefoxitin, clindamycin, nafcillin, tobramycin, piperacillin, and ticarcillin were provided by their respective manufacturers. Stock solutions were prepared in concentrations of 1,000 to 2,000 μg/ml, frozen, and used within 4 weeks of preparation.

Bacteria. All bacteria were initially isolated and identified in the Clinical Microbiology Laboratory at the Oklahoma Children's Memorial Hospital. Strains were stored in one-half strength nutrient agar at room temperature and subcultured onto appropriate solid media to confirm purity before testing. We tested 12 isolates each of *Escherichia coli, Klebsiella* spp., *Salmonella* spp., *Shigella* spp., *Enterobacter* spp., *Staphylococcus aureus, Streptococcus agalactiae* (group B), and *Streptococcus faecalis* (group D), 10 isolates each of *Proteus mirabilis* and *Serratia marcescens*, and 18 isolates of *P. aeruginosa* (mucoid and non-mucoid).

Susceptibility testing. Antimicrobial susceptibilities were determined by microbroth dilution in Mueller-Hinton broth supplemented with calcium and magnesium (3). Appropriate dilutions of antibiotic were added with a calibrated 50-μl pipette to the appropriate wells of a sterile, 96-well microtiter plate and serially diluted with an automatic microdilutor (Dynatech Laboratories, Inc., Alexandria, Va.). The inoculum was prepared from an overnight culture in supplemented Mueller-Hinton broth and diluted to contain 4×10^5 to 5×10^5 CFU/ml by the use of a McFarland nephelometer standard, and the colonies were counted after culture onto solid antibiotic-free media. The MIC was the lowest concentration showing no visible growth after overnight incubation (18 to 24 h) at 37°C. Then 10 μl was transferred from each well onto appropriate antibiotic-free agar and re-incubated for another 18 to 24 h. The MBC was the lowest antibiotic concentration permitting growth of fewer than 5 colonies (>99.9% kill).

For comparative purposes, 1.5-μl subcultures from these wells were plated onto antibiotic-free agar by means of an automatic inoculator (Dynatech). The MBC by this alternate method was defined as the lowest antibiotic concentration permitting no bacterial growth (>99% kill). Appropriate wells without antibiotics served as growth controls, and a sterility control was included on each microtiter plate.

Synergy and time-kill studies. Synergy between antibiotics was evaluated for selected strains and antibiotics. The checkerboard pattern microtiter broth dilution method in divalent cation-supplemented Mueller-Hinton broth was used (10). An inoculum of 1.0×10^5 to 2.0×10^5 CFU/ml, prepared as described above, was added to serial twofold dilutions of one antibiotic in combination with similar dilutions of the other tested antibiotic. Appropriate initial antibiotic concentrations were chosen on the basis of previous MIC studies. The lowest concentration of each single antibiotic inhibiting visible growth after overnight incubation was defined as the MIC. Synergism was present when the MIC of each antibiotic in combination was one-fourth or less of its MIC alone. Antagonism was present if the MIC of either

* Corresponding author.

antibiotic was increased fourfold or more over its MIC alone, or if the MICs of both antibiotics were increased twofold or more over their respective MICs alone.

This is comparable to the fractional inhibitory concentration index as described by Elion et al., (1), in which synergism occurs if the fractional inhibitory concentration is ≤0.5 and antagonism occurs when the fractional inhibitory concentration is >2. For gram-positive organisms, all of which were resistant to aztreonam, synergy or antagonism was defined as a fourfold change in the MIC of the gram-positive antibiotic at a pharmacokinetically attainable concentration of aztreonam (128 to 256 μg/ml) (15).

Timed bacterial killing of selected isolates was measured on organisms grown overnight and diluted to a concentration of 10^5 CFU/ml in Mueller-Hinton broth (18). Antibiotics, alone and in combination, were added at concentrations equal to the MIC of each drug alone. The mixture was incubated at 37°C on a rotary shaker at 100 rpm for 24 h. Samples were removed, serially diluted, and plated on agar for determination of viable organism counts before the addition of antibiotics and 2, 6, and 24 h after incubation with antibiotics. Synergism was defined as a 100-fold decrease in the number of bacteria killed by the combination as compared with the most effective single antibiotic. Antagonism was present if the number of bacteria increased 10-fold with the combination as compared with either single antibiotic.

RESULTS

Gram-negative isolates were susceptible to aztreonam at therapeutically achievable concentrations (Table 1). At the inoculum size of approximately 5×10^5 CFU/ml, 27 of 98 isolates showed a difference between MIC and MBC values with the criterion of >99.9% kill. Only five of these (one *E. coli*, two *P. mirabilis*, two *P. aeruginosa*) had as much as a fourfold difference. In comparison, only 12 isolates had MIC-MBC differences under the >99% kill criterion, of which two were fourfold increases. The 36 gram-positive isolates were resistant to aztreonam, although 4 of 12 group B *Streptococcus* isolates had MICs of 64 μg/ml.

Synergism of aztreonam and cefoxitin against *E. coli*, *Klebsiella* spp., *Shigella* spp., and *Salmonella* spp. was common (Table 2). Combinations of aztreonam with ampicillin were noninteractive. Clindamycin plus aztreonam was frequently synergistic against *E. coli* and *Klebsiella* spp. Antagonism was seen only with cefoxitin plus aztreonam against *Enterobacter* species (three *E. cloacae* and two *E. aerogenes*) and against one isolate of *S. marcescens*. In these instances, cefoxitin increased the aztreonam MIC values four- to eightfold, which made them, therefore, no greater than 2 μg/ml.

TABLE 1. Antimicrobial activity of aztreonam against gram-negative isolates (microbroth dilution method)

Organism (*n*)	Geometric mean MIC (μg/ml)	MIC range	MBC range[a]
E. coli (12)	0.07	0.03–0.50	0.03–0.50 (1)
Klebsiella spp. (12)	0.04	0.015–0.12	0.03–0.12 (0)
Enterobacter spp. (12)	0.05	0.015–1.0	0.015–1.0 (0)
S. marcescens (10)	0.19	0.06–1.0	0.06–2.0 (0)
P. mirabilis (10)	0.009	≤0.008–0.03	≤0.008–0.12 (2)
Shigella spp. (12)	0.04	0.008–0.12	0.008–0.25 (0)
Salmonella spp. (12)	0.07	0.03–0.25	0.03–0.50 (0)
P. aeruginosa (18)	1.70	0.25–32	0.25–32 (2)

[a] Values in parentheses indicate the number of strains for which MBC > 2× MIC (see the text).

TABLE 2. Activity of aztreonam in combination with other antibiotics against *Enterobacteriaceae* (checkerboard microbroth method)

Organism (*n*)	No. of isolates with combination effects; aztreonam plus[a]:					
	Ampicillin		Cefoxitin		Clindamycin	
	Synergy	Antag	Synergy	Antag	Synergy	Antag
E. coli (12)	0	0	6	0	6	0
Klebsiella spp. (12)	0	0	5	0	6	0
Enterobacter spp. (12)	0	0	3	5	3	0
P. mirabilis (10)	0	0	0	0	ND	ND
S. marcescens (10)	0	0	1	1	ND	ND
Shigella spp. (12)	0	0	7	0	ND	ND
Salmonella spp. (12)	1	0	3	0	0	0

[a] Antag, Antagonism. ND, Not done.

The synergistic combinations of bacterial strains and antibiotics were randomly distributed within strains. Susceptibility to one antibiotic combination was not predictive of susceptibility to another.

The combinations of aztreonam and tobramycin, aztreonam and ticarcillin, and aztreonam and piperacillin were synergistic against 10 of 16 (62%), 1 of 16 (6%), and 3 of 16 (18%) *P. aeruginosa* isolates, respectively. No antagonism was seen with any combination. Two of the three strains susceptible to the synergistic activity of piperacillin and aztreonam were also synergistically susceptible to tobramycin and aztreonam. No differences in synergistic activity were seen between mucoid and nonmucoid strains.

Aztreonam did not antagonize the activity of any tested antimicrobial agent against gram-positive cocci. Although aztreonam alone had no appreciable activity against any gram-positive strain, aztreonam plus ampicillin and aztreonam plus cefoxitin were synergistic against one and two isolates of group B *Streptococcus*, respectively. Aztreonam plus clindamycin was synergistic against 2 of 12 isolates of group D *Streptococcus*. The activity of nafcillin against 12 isolates of *S. aureus* was unaffected by the presence of aztreonam.

The bactericidal effects of selected antibiotics were also determined in time-kill experiments. These studies confirmed the results of the checkerboard assays with *Pseudomonas*. Figure 1 shows the synergistic activity of tobramycin and aztreonam against *P. aeruginosa* strain 2; this pattern was confirmed with two other *P. aeruginosa* isolates. With a strain of *Enterobacter* which had shown antagonism to the combination of cefoxitin and aztreonam in checkerboard experiments, time-kill analysis revealed that the combination was equivalent to the activity of cefoxitin alone (Fig. 2). *Enterobacter* strain 17 showed similar killing curves (data not shown). Both of these isolates were also tested at inoculum sizes of 2×10^6 to 3×10^6 CFU/ml, and the killing curves were similar.

Time-kill curves prepared for isolates of *E. coli*, *Klebsiella* spp., and *Salmonella* spp. did not reveal interactions not shown by the checkerboard microdilution studies.

DISCUSSION

All 98 randomly selected, gram-negative isolates from ill children were sensitive to the bactericidal effects of aztreonam. These data confirm the activity of aztreonam

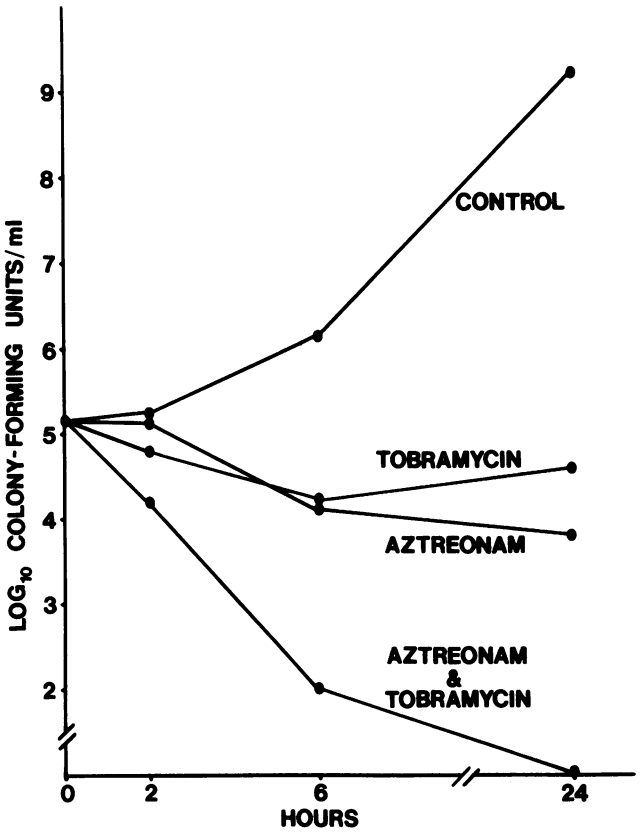

FIG. 1. Time-kill curves showing synergy of aztreonam (4 μg/ml) and tobramycin (2 μg/ml) against *P. aeruginosa* strain 2.

against clinically important gram-negative bacteria.

Since aztreonam is a narrow-spectrum drug, it is likely to be used in combination with antibiotics effective against gram-positive and anaerobic organisms in the initial therapy of suspected sepsis. Our experiments have shown that combinations of aztreonam with cefoxitin or clindamycin are commonly synergistic, particularly against *E. coli, Klebsiella-Enterobacter* spp., and *Shigella* spp. Tobramycin plus aztreonam is also frequently synergistic against *P. aeruginosa*. Furthermore, there was no adverse effect of aztreonam on the activity of several antibiotics active against gram-positive, aztreonam-resistant bacteria. This was previously noted for aztreonam and nafcillin against five isolates of *S. aureus* (S. H. Zinner, N. M. Ampel, L. Moon-McDermott, and M. H. Keating, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 22nd, Miami, Fla., abstr. no. 133, 1982).

Other preliminary reports indicate that aztreonam and other antimicrobials, such as moxalactam, imipenem, and aminoglycosides, are indifferent against susceptible organisms (J. A. Hindler, W. L. Hewitt, I. Ioka, R. L. Muench, and L. S. Young, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 22nd, Miami, Fla., abstr. no. 132, 1982). However, such combinations are unlikely to be used in pediatric practice, except against *Pseudomonas* spp., for which combination therapy is often advantageous.

The synergy noted between clindamycin and aztreonam against isolates of *E. coli* and *Klebsiella-Enterobacter* spp. is intriguing. Sanders et al. (14) have recently presented evidence that such interactions may be related to the role of clindamycin as an effective inhibitor of the derepression of β-lactamases. This would be especially important in strains such as those of *Enterobacter* spp. which frequently possess inducible β-lactamases. A similar mode of action has also been postulated for the synergy found between chloramphenicol, another protein inhibitor, and β-lactams against strains of *Klebsiella-Enterobacter-Serratia* spp. otherwise resistant to β-lactam antibiotics (8). The possibility that antibiotics without inherent anti-gram-negative activity (clindamycin), or with generally bacteriostatic activity (chloramphenicol), may commonly enhance the bactericidal activity of concurrently administered β-lactams merits further study.

Although rare with other combinations, antagonism was seen with the combination of cefoxitin and aztreonam. This has previously been reported with cefoxitin and other antimicrobial agents, both β-lactams and aminoglycosides (7). These effects may be due to cefoxitin induction of chromosomally mediated beta-lactamase activity (12). In this way, enzyme-resistant drugs, such as cefoxitin, may be capable of antagonizing concurrently administered beta-lactams, such as aztreonam, that are better substrates for the inducible lactamases. This phenomenon may not be due to hydrolysis of aztreonam but rather to binding of beta-lactamase to the antimicrobial agent with subsequent prevention of diffusion through bacterial membranes (11). *Enterobacter, Serratia,* and *Pseudomonas* are the genera most likely to possess inducible beta-lactamases (13). In fact, we noted antagonism only against *Enterobacter* and *Serratia* species. It is reassuring that inhibitory concentrations were still quite low, even

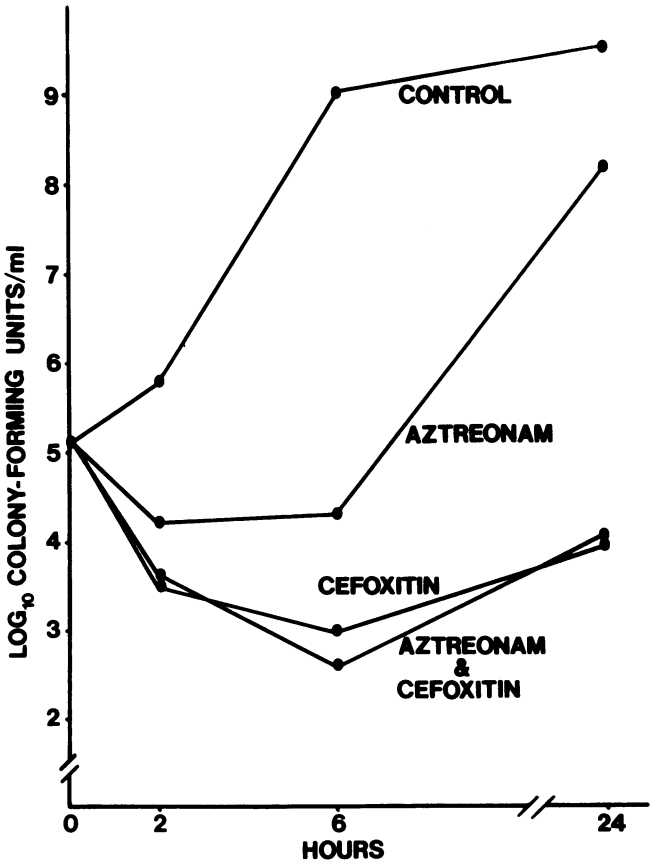

FIG. 2. Time-kill curves showing indifference of aztreonam (0.06 μg/ml) and cefoxitin (48 μg/ml) against *E. cloacae* strain 2.

with such antagonism. Even so, if the mechanisms postulated are correct, the adverse interaction may be expected to increase with prolonged exposure of the organism to cefoxitin, the inducing agent. Caution is probably indicated, therefore, in using aztreonam plus cefoxitin in *Enterobacter* or *Serratia* infections.

The dynamics of bacterial killing studied in time-kill experiments confirmed the synergism noted against many isolates of *P. aeruginosa* and some members of *Enterobacteriaceae* by the checkerboard method. The interaction seemed most effective during exponential growth. The timed killing studies against *Enterobacter* did not show the antagonism noted by the checkerboard method. This discrepancy may be related to the inherent differences between measurements of inhibitory and bactericidal activity. The prolonged exposure of bacteria to drugs in the kinetic experiments may have led to better penetration of antimicrobial agent into the bacterial cell, despite the postulated binding of aztreonam to beta-lactamases. Alternatively, the limited number of antibiotic concentrations tested in the time-kill experiments may have missed those most likely to reveal the antagonism (5). The time-kill curve is, however, generally still considered more reflective of in vivo activity (6).

Our results lend additional support for the potential role of aztreonam in treating pediatric infections. In association with ongoing pharmacokinetic and safety studies, they should provide valuable guidelines for selection of aztreonam regimens in future clinical trials.

LITERATURE CITED

1. **Elion, G. B., S. Singer, and G. H. Hitchings.** 1954. Antagonists of nucleic acid derivatives. VIII. Synergism in combination of biochemically related antimetabolites. J. Biol. Chem. **208**:477–488.
2. **Fainstein, V., S. Weaver, and G. P. Bodey.** 1982. Comparative in vitro study of SQ26,776. Antimicrob. Agents Chemother. **21**:294–298.
3. **Gavan, T. L., and A. L. Barry.** 1980. Microdilution test procedures, p. 459–462. *In* E. H. Lennette, A. Balows, W. J. Hausler, Jr., and J. P. Truant (ed.), Manual of clinical microbiology, 3rd ed. American Society for Microbiology, Washington, D.C.
4. **Jacobus, N. V., M. C. Ferreira, and M. Barza.** 1982. In vitro activity of azthreonam, a monobactam antibiotic. Antimicrob. Agents Chemother. **22**:832–838.
5. **King, T. C., and D. J. Krogstad.** 1983. Spectrophotometric assessment of dose-response curves for single antimicrobial agents and antimicrobial combinations. J. Infect. Dis. **147**:758–764.
6. **King, T. C., D. Schlessinger, and D. J. Krogstad.** 1981. The assessment of antimicrobial combinations. Rev. Infect. Dis. **3**:627–633.
7. **Markowitz, S. M., and D. J. Sibilla.** 1983. *In vitro* activity of cefoxitin, alone and in combination with aminoglycoside or other β-lactam antibiotics against common gram-negative pathogens. Chemotherapy **29**:89–98.
8. **Michel, J., H. Bornstein, R. Luboshitzky, and T. Sacks.** 1975. Mechanism of chloramphenicol-cephaloridine synergism on *Enterobacteriaceae*. Antimicrob. Agents Chemother. **7**:845–849.
9. **Reeves, D. S., M. J. Bywater, and H. A. Holt.** 1981. Antibacterial activity of the monobactam SQ 26,776 against antibiotic resistant enterobacteria, including *Serratia* spp. J. Antimicrob. Chemother. **8**(Suppl. E):57–68.
10. **Sabath, L. D., C. E. McCall, N. H. Steigbigel, and M. Finland.** 1967. Synergistic penicillin combinations for treatment of human urinary-tract infections, p. 149–155. Antimicrob. Agents Chemother. 1966.
11. **Sanders, C. C.** 1983. Novel resistance selected by the new expanded-spectrum cephalosporins: a concern. J. Infect. Dis. **147**:585–589.
12. **Sanders, C. C., and W. E. Sanders, Jr.** 1979. Emergence of resistance to cefamandole: possible role of cefoxitin-inducible beta-lactamases. Antimicrob. Agents Chemother. **15**:792–797.
13. **Sanders, C. C., and W. E. Sanders, Jr.** 1983. Emergence of resistance during therapy with the newer β-lactam antibiotics: role of inducible β-lactamases and implications for the future. Rev. Infect. Dis. **5**:639–648.
14. **Sanders, C. C., W. E. Sanders, Jr., and R. V. Goering.** 1983. Influence of clindamycin on derepression of β-lactamases in *Enterobacter* spp. and *Pseudomonas aeruginosa*. Antimicrob. Agents Chemother. **24**:48–53.
15. **Swabb, E. A., M. A. Leitz, F. G. Pilkiewicz, and A. A. Sugerman.** 1981. Pharmacokinetics of the monobactam SQ 26,776 after single intravenous doses in healthy subjects. J. Antimicrob. Chemother. **8**(Suppl. E):131–140.
16. **Sykes, R. B., D. P. Bonner, K. Bush, and N. H. Georgopapadakou.** 1982. Azthreonam (SQ 26,776), a synthetic monobactam specifically active against aerobic gram-negative bacteria. Antimicrob. Agents Chemother. **21**:85–92.
17. **Sykes, R. B., C. M. Cimarusti, D. P. Bonner, K. Bush, D. M. Floyd, N. H. Georgopapadakou, W. H. Koster, W. C. Liu, W. L. Parker, P. A. Principe, M. L. Rathnum, W. A. Slusarchyk, W. H. Trejo, and J. S. Wells.** 1981. Monocyclic β-lactam antibiotics produced by bacteria. Nature (London) **291**:489–491.
18. **Weinstein, R. J., L. S. Young, and W. L. Hewitt.** 1975. Comparison of methods for assessing *in vitro* antibiotic synergism against *Pseudomonas* and *Serratia*. J. Lab. Clin. Med. **86**:853–862.