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Serum Bactericidal Activity of Aztreonam, Cefoperazone, and Amikacin, Alone or in Combination, Against *Escherichia coli*, *Klebsiella pneumoniae*, *Serratia marcescens*, and *Pseudomonas aeruginosa*

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Serum samples from volunteers receiving (per kilogram) 20 mg of aztreonam, 20 mg of cefoperazone, 7.5 mg of amikacin, 20 mg of cefoperazone plus 20 mg of aztreonam, or 20 mg of aztreonam plus 7.5 mg of amikacin were evaluated for bactericidal activity against *Escherichia coli*, *Klebsiella pneumonia*, *Serratia marcescens*, and *Pseudomonas aeruginosa*. Serum bactericidal activities were similar for aztreonam alone or in combination but were lower for amikacin and cefoperazone alone, especially against *S. marcescens* and *P. aeruginosa*. Killing studies, performed with serum samples diluted 1:8, demonstrated a high killing rate for aztreonam plus amikacin, especially against *P. aeruginosa*.

Gram-negative infections are common in cancer patients, especially during neutropenia. Under these circumstances, a combination of a beta-lactam drug with an aminoglycoside is often used as an empiric treatment for febrile patients (3); even after the identification of the infecting organism, such combination therapy is often continued to take advantage of possible synergy (4). However, the potential nephro- and ototoxicity of the aminoglycosides may be a limiting factor for this approach.

Aztreonam (AZT) is a monobactam antibiotic with a broad spectrum of activity against most *Enterobacteriaceae* spp. and *Pseudomonas aeruginosa* but no significant activity against cocci or anaerobic organisms (7). In the present study, serum samples obtained from healthy volunteers 1 and 6 h after treatment with AZT at 20 mg/kg, cefoperazone (CEP) at 20 mg/kg, amikacin (AMK) at 7.5 mg/kg, or combinations of AZT plus CEP (20 mg/kg plus 20 mg/kg) and AZT plus AMK (20 mg/kg plus 7.5 mg/kg) were evaluated for serum bactericidal activity (SBA) against five selected strains each of *Escherichia coli*, *Klebsiella pneumoniae*, *Serratia marcescens*, and *P. aeruginosa* that were isolated from cancer patients. The rate of killing of these strains by the antibiotic-containing sera was also investigated.

MATERIALS AND METHODS

The test strains were isolated from clinical specimens from cancer patients at the Institut Jules Bordet. Ten healthy volunteers gave written informed consent and were selected for the study. As complement is not inactivated in the test sera, volunteers were screened beforehand for their intrinsic SBA against all the test strains. Only subjects showing a <1:2 titer, or at most a 1:2 titer, were selected. Each received, at 3-day intervals, one of the following treatments: AZT at 20 mg/kg; CEP at 20 mg/kg; AMK at 7.5 mg/kg; AZT plus AMK (20 mg/kg plus 7.5 mg/kg); or CEP plus AZT (20 mg/kg plus 20 mg/kg). The antibiotics were dissolved in 50 ml of 5% dextrose in water and were infused intravenously over 15 min. Blood was obtained before and 1 and 6 h after the end of the infusion. Serum samples were separated and stored at −70°C until used. All the measurements of serum levels and SBA were made in the sera of individual volunteers. Serum titration was done in duplicate in a microtiter system (4). Twofold dilutions of the test sera were made with 25-μl microdiluters in a 1:1 mixture of Mueller-Hinton (MH) broth supplemented with Ca^{2+} (50 mg/liter) and Mg^{2+} (20 mg/liter) and inactivated pooled normal human serum. Mid-logarithmic-phase cultures were adjusted by nephelometry to a 0.5 McFarland standard and further diluted in MH broth to yield a final concentration of 5×10^5 CFU/ml for each well. Microtiter plates were wrapped in tin foil and incubated overnight at 37°C. After the plates were mixed for 5 s on a microshaker, 2 μl from each well was subcultured on MH agar.

Bactericidal activity was defined as the highest dilution of serum showing no regrowth (99.9% killing of the original inoculum). MBCs of the drugs were determined in a similar fashion. Checkerboard tests were done in supplemented MH broth by using microtiter plates. Twofold dilutions of antibiotics were prepared beforehand in test tubes. With 25-μl pipette droppers, one antibiotic was dispensed horizontally and the other vertically, one dilution for each row; 50 μl of the inoculum was added to each well to achieve a final concentration of 5×10^5 CFU/ml. After overnight incubation and resuspension, 2 μl from each well was subcultured on MH agar.

Fractional bactericidal indexes (FBCs) were calculated as follows (5): [(MBC *A* in combination/MBC *A* alone) + (MBC *B* in combination/MBC *B* alone)] = FBC index. Synergy was defined as an FBC index of ≤0.50.

The levels of AZT, CEP, and AMK in serum were measured by the agar diffusion method described by Bennet et al. (1). For AZT, the standard curve and samples were diluted 1:20 in phosphate buffer (pH 6.0); the assay was performed in nutrient agar (pH 7.8) with *E. coli* SC 12155 (E. R. Squibb & Sons) as the test organism. For CEP, nutrient agar (pH 6.8) was used with *Sarcina lutea* ATCC 9341 as the test organism. For AMK, the assay was done in nutrient agar (pH 8.0) with the *Bacillus subtilis* spore suspension (Difco Laboratories). For serum samples obtained after

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TABLE 1. MBCs of drugs alone or in combination

Test organism and isolate	MBCs[a] (μg/ml) of:					FBC index of:	
	AZT	CEP	AMK	AZT/CEP	AZT/AMK	AZT/CEP	AZT/AMK
E. coli							
1	0.05	0.20	6.25	0.002/0.10	0.01/3.10	0.540	0.696
2	0.05	0.10	0.80	0.005/0.05	0.02/0.40	0.700	0.900
3	0.10	0.20	3.10	0.01/0.10	0.005/0.80	0.600	0.308[b]
4	1.60	0.20	1.60	0.006/0.20	0.40/0.80	1.25	1.0
5	0.02	0.10	1.60	0.01/0.05	0.01/0.80	1.0	1.0
K. pneumoniae							
1	0.05	0.20	0.80	0.01/0.10	0.005/0.40	0.750	0.625
2	0.10	0.40	1.60	0.002/0.20	0.025/0.40	0.520	0.500[b]
3	0.10	12.5	1.60	0.02/0.80	0.025/0.050	0.264[b]	0.281[b]
4	0.10	12.5	0.80	0.01/3.10	0.025/0.40	0.350[b]	0.750
5	0.20	3.10	0.80	0.05/0.80	0.05/0.40	0.500[b]	0.750
S. marcescens							
1	0.40	25.0	50.0	0.10/6.25	0.05/6.25	0.500[b]	0.250[b]
2	0.20	3.10	6.25	0.02/0.80	0.02/1.60	0.375[b]	0.375[b]
3	0.20	50.0	6.25	0.10/0.80	0.05/0.80	0.515	0.375[b]
4	0.40	12.5	6.25	0.10/6.25	0.10/0.80	0.750	0.500[b]
5	0.40	25.0	25.0	0.20/0.40	0.05/6.25	0.515	0.375[b]
P. aeruginosa							
1	25.0	25.0	12.5	25.0/3.1	12.5/0.80	1.124	0.532
2	25.0	50.0	25.0	3.1/12.5	6.25/12.5	0.374[b]	0.750
3	12.5	100.0	12.5	1.6/12.5	3.10/0.80	0.253[b]	0.312[b]
4	6.25	6.25	6.25	1.6/25.0	1.60/0.40	4.25	0.320[b]
5	12.5	6.25	12.5	6.25/3.10	1.60/3.10	1.0	0.376[b]

[a] Underlined values were considered to represent resistance.
[b] The FBC index indicates synergy.

combined drug administration (AZT plus AMK), sodium polyanethol sulfonate (1%) was added to the agar medium when determining AZT levels (7). Amikacin levels were determined by a fluorescent immunoassay (Ames TDA kit). Levels of AZT and CEP in serum when the drugs were used in combination were not tested, as the two drugs are not distinguishable by bioassay.

Killing curves were performed on the 1-h specimens:

TABLE 2. Median SBAs of serum samples from 10 volunteers

Test organism	Drug	Median SBA titers against 5 isolates:									
		1 h after dosage[a]					6 h after dosage[b]				
		1	2	3	4	5	1	2	3	4	5
E. coli	AZT	≥1/2,048	≥1/2,048	1/1,024	1/64	1/1,024	1/256	1/128	1/128	1/8	1/64
	CEP	≥1/2,048	1/512	1/256	1/128	1/256	1/32	1/32	1/16	1/16	1/16
	AMK	1/4	1/8	1/8	1/8	1/8	1/2	1/2	1/2	1/4	1/2
	AZT/CEP	≥1/2,048	≥1/2,048	≥1/2,048	1/256	1/1,024	1/256	1/256	1/128	1/16	1/64
	AZT/AMK	≥1/2,048	≥1/2,048	≥1/2,048	1/64	≥1/2,048	1/256	1/128	1/128	1/8	1/128
K. pneumoniae	AZT	≥1/2,048	≥1/2,048	≥1/2,048	1/1,024	1/256	1/128	1/64	1/128	1/64	1/16
	CEP	1/64	1/64	1/8	1/2	1/8	1/4	1/8	1/2	<1/2	1/4
	AMK	1/16	1/16	1/16	1/16	1/8	1/4	1/4	1/2	1/4	1/2
	AZT/CEP	≥1/2,048	≥1/2,048	≥1/2,048	1/1,024	1/512	1/128	1/64	1/128	1/64	1/32
	AZT/AMK	≥1/2,048	≥1/2,048	≥1/2,048	1/1,024	1/1,024	1/128	1/128	1/128	1/64	1/32
S. marcescens	AZT	1/128	1/128	1/512	1/32	1/512	1/16	1/16	1/16	1/8	1/32
	CEP	1/4	1/8	1/4	1/4	1/2	<1/2	1/2	1/2	<1/2	1/2
	AMK	1/2	1/8	1/4	1/4	1/2	<1/2	1/2	1/2	1/2	1/2
	AZT/CEP	1/256	1/128	1/256	1/64	1/256	1/16	1/16	1/16	1/16	1/16
	AZT/AMK	1/16	1/512	1/1,024	1/512	1/512	1/16	1/32	1/32	1/32	1/16
P. aeruginosa	AZT	<1/2	1/2	1/16	1/16	1/8	<1/2	1/2	1/2	<1/2	<1/2
	CEP	<1/2	1/4	<1/2	1/2	1/4	<1/2	1/2	<1/2	<1/2	<1/2
	AMK	1/2	1/2	1/2	1/2	1/2	<1/2	<1/2	<1/2	<1/2	<1/2
	AZT/CEP	1/2	1/4	1/16	1/16	1/8	<1/2	1/2	1/2	<1/2	<1/2
	AZT/AMK	1/4	1/4	1/16	1/16	1/16	<1/2	<1/2	1/2	1/2	1/4

[a] AZT, 57 μg/ml; CEP, 59 μg/ml; AMK, 19 μg/ml; AZT plus CEP, not tested; AZT plus AMK, 59 μg/ml plus 24 μg/ml.
[b] AZT, 7 μg/ml; CEP, 6 μg/ml; AMK, 4 μg/ml; AZT plus CEP, not tested; AZT plus AMK, 6 μg/ml plus 3 μg/ml.

serum samples obtained at that time were pooled for each regimen. A 1:8 dilution of each pool was made with supplemented MH broth to a fixed volume of 2 ml. A control tube containing a mixture of normal human serum and MH broth in equal proportions was prepared for each strain. Bacteria were added to yield a final inoculum of 5×10^5 CFU/ml at time zero. All tubes were incubated at 37°C on a rotator. At 2-h intervals, 10 μl was removed with a calibrated loop, further diluted, and subcultured on MH agar. Colonies were counted after an overnight incubation at 37°C.

RESULTS

MBCs of each drug alone or in combination are listed in Table 1.

All *E. coli* and *K. pneumoniae* strains were susceptible to the investigated antibiotics. Two *S. marcescens* strains were resistant to amikacin, and three were resistant to cefoperazone, whereas all five strains were susceptible to aztreonam. Among *P. aeruginosa* strains, two were resistant to aztreonam, three to cefoperazone, and one to amikacin; the other test strains were only moderately susceptible to these drugs.

Synergism, as defined by FBCs of ≤0.5, was observed with the combination of aztreonam plus amikacin against all *S. marcescens* strains. This combination was also synergistic against three strains of *P. aerugonisa*, two strains of *K. pneumoniae*, and one strain of *E. coli*. Aztreonam associated with cefoperazone was mainly synergistic against *K. pneumoniae* (three of five strains). Two strains of *S. marcescens* and two of *P. aeruginosa* were also synergistically killed by this combination.

The mean serum levels and the results of the SBA determinations are indicated in Table 2. Aztreonam levels were similar whether the drug was given alone or in combination with amikacin. The same applies for amikacin. Aztreonam and cefoperazone levels, when the drugs were given in combination, could not be compared with the levels determined after administration of the drugs singly. SBAs (median value and range) reflect the activity of the sera of the 10 volunteers against each isolate of the various test species.

Figure 1 summarizes the data on the killing rates of *E. coli*, *K. pneumoniae*, and *S. marcescens* by pooled sera diluted to 1:8, obtained 1 h after the administration of the different drugs alone or in combination. Drug concentrations in the diluted pools were as follows: AZT, 6.25 μg/ml; CEP, 7.0 μg/ml; AMK, 2.5 μg/ml; AZT plus AMK, 7.0 μg/ml plus 2.9 μg/ml. Levels of AZT plus CEP were not measured. Similar rates of killing were achieved with all regimens, with the exceptions of AMK and AZK plus AMK, which showed a better killing rate on *K. pneumoniae* at 2 h after administration.

Figure 2 summarizes the same experiment described above, except with *P. aeruginosa*. The combination of AZT plus AMK had increased effectiveness when compared with AZT alone; AMK alone was ineffective. As far as CEP and AZT alone or in combination were concerned, the killing rate was directly related to the MBC; no increased activity was observed with this combination.

DISCUSSION

Treatment with AZT resulted more often in a higher median SBA against each *E. coli* test strain than did treatment with CEP. However, all the serum samples gave SBAs of ≥1:8 at 1 and 6 h with both drugs, and the killing rates were comparable. No additional effect was observed with the

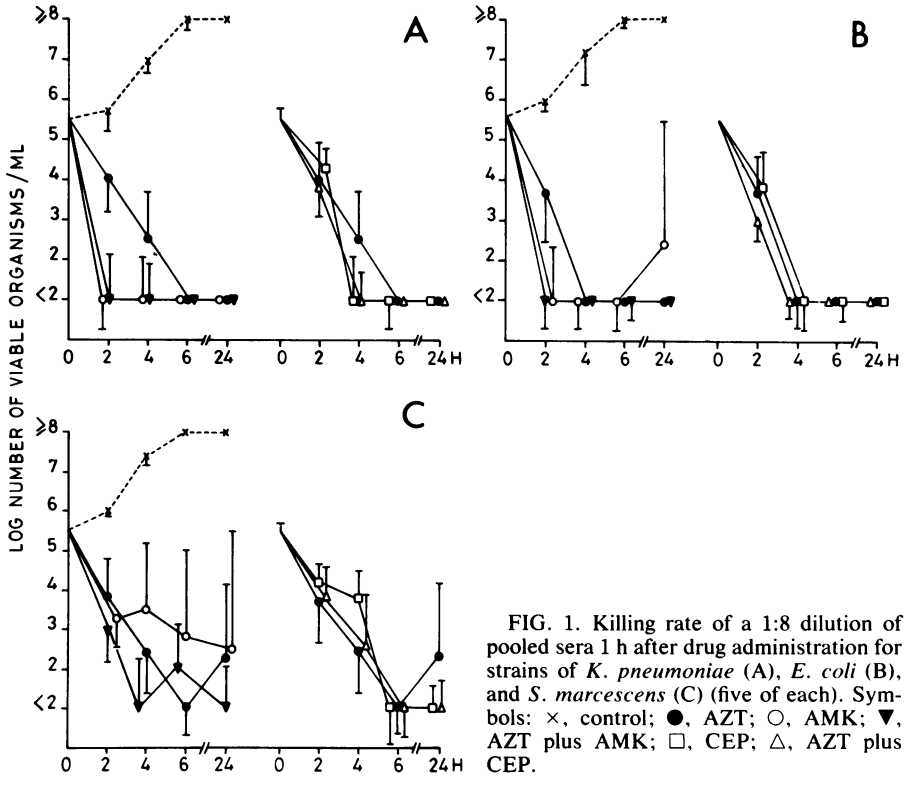

FIG. 1. Killing rate of a 1:8 dilution of pooled sera 1 h after drug administration for strains of *K. pneumoniae* (A), *E. coli* (B), and *S. marcescens* (C) (five of each). Symbols: ×, control; ●, AZT; ○, AMK; ▼, AZT plus AMK; □, CEP; △, AZT plus CEP.

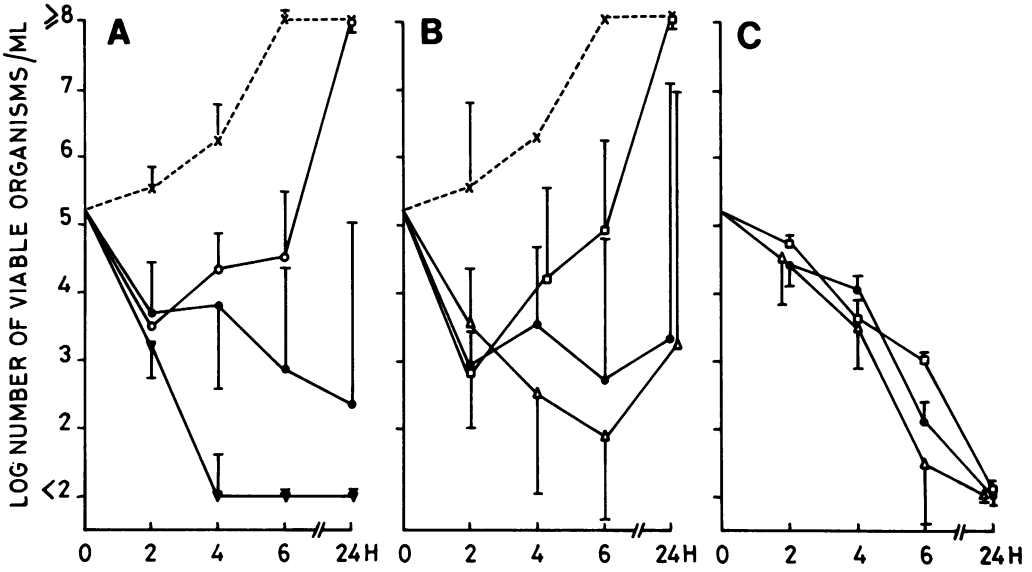

FIG. 2. Killing rate of a 1:8 dilution of pooled sera 1 h after drug administration for five strains of *P. aeruginosa* (A). Symbols in panel A: ×, control; ●, AZT; ○, AMK; ▼, AZT plus AMK. Data are presented separately for three cefoperazone-resistant (B) and two cefoperazone-susceptible (C) strains. Symbols in panels B and C: ×, control; ●, AZT; □, CEP; △, AZT plus CEP.

combination of AZT plus CEP. This latter observation correlates with the lack of synergy between AZT and CEP in the checkerboard studies.

With *K. pneumoniae*, AZT was associated with significantly better median SBAs against each test strain than was CEP; all serum samples except one gave SBAs of ≥1:8 at 1 or 6 h with AZT, in contrast to the results with CEP. These data are in accordance with the lower MBC values for AZT. The addition of CEP to AZT, contrary to the expectations derived from the low FBC indices, did not produce more effectiveness with respect to either the SBA or the killing rates. The addition of AMK to AZT, although it did not enhance the median SBA titers, increased the early killing rate of *K. pneumoniae* by AZT.

S. marcescens is a pathogen frequently involved in nosocomial bacteremia; its resistance to common antimicrobial agents often requires the use of aminoglycosides. AZT, with its very low MBCs, resulted in very good SBAs after 1 and 6 h, in contrast to CEP or AMK alone. The combination of AZT plus AMK was synergistic for all the *S. marcescens* strains by the in vitro checkerboard tests. That combination, although improving the median SBA at 1 h and 6 h against three test strains, did not change the killing rate compared with that of AZT alone.

P. aeruginosa strains were not as susceptible to AZT as the other gram-negative organisms tested here. Median SBAs of ≥1:8 were obtained with AZT against three test strains only at 1 h, whereas neither CEP nor AMK treatment resulted in such values.

The addition of AMK to AZT, which resulted in synergy in vitro for three out of five strains, did not significantly change the median SBA results but improved the killing rate for all strains.

In conclusion, AZT seems promising for single-drug therapy for various aerobic gram-negative infections. However, the increased killing rate observed when AMK was added may suggest a recommendation for the combination, especially for neutropenic patients (2). Dosages higher than 20 mg/kg might be necessary in clinical studies, especially in cases of *P. aeruginosa* infections, to obtain a higher frequency of SBAs that are ≥1:8.

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