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Serum Bactericidal Activity of Ceftazidime and Cefoperazone Alone or in Combination with Amikacin Against *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*

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Sera of volunteers receiving ceftazidime (2 g) or amikacin (500 mg), alone or in combination, or cefoperazone (2, 4, or 6 g) or cefoperazone (2 g) with amikacin (500 mg) were evaluated for bactericidal activity against *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. Serum bactericidal activities were similar for ceftazidime and ceftazidime plus amikacin, but were definitely lower for amikacin alone. Against *P. aeruginosa*, a 6-g dose of cefoperazone resulted in a higher frequency of peak serum bactericidal activities greater than or equal to 1:8 than a 2-g dose of cefoperazone plus amikacin. Killing studies, performed in 1:8 diluted serum, demonstrated a higher killing rate for cefoperazone plus amikacin than for a 6-g dose of cefoperazone, the more resistant *P. aeruginosa* excepted. Emergence of resistance was found with a 2-g dose of cefoperazone for *K. pneumoniae* and with a 6-g dose of cefoperazone for *P. aeruginosa*, but not with cefoperazone plus amikacin.

Combinations of an aminoglycoside with beta-lactam antibiotics are frequently synergistic in vitro (7) and because of this have been often used for empirical treatment of febrile patients. There is some evidence that this in vitro synergism (6) and serum bactericidal activity (SBA) greater than 1:8 (5) correlate with a favorable clinical outcome. Recently developed cephalosporins with expanded spectra of activity have been investigated as a single-drug therapy for gram-negative bacillary infection (9, 11, 15). The overall cure rate in non-neutropenic patients has been satisfactory. However, there has been a significant rate of superinfection with resistant strains in patients treated with these broad-spectrum cephalosporins (4, 10, 13, 15). Cefoperazone and ceftazidime, two of the most active of these cephalosporins, have high activities against *Enterobacteriaceae* and are especially active against *Pseudomonas aeruginosa*; 90% of the *Pseudomonas* strains are inhibited by 16 μg of cefoperazone per ml and by 1.8 μg of ceftazidime per ml (2, 3, 14).

In the present study, serum obtained from healthy volunteers 1 and 6 h after various doses of cefoperazone and ceftazidime alone and in combination with amikacin was evaluated for SBA against selected strains of *P. aeruginosa* and *Klebsiella pneumoniae* isolated from cancer patients. The rate of killing of *K. pneumoniae* and *P. aeruginosa* by sera containing cefoperazone alone or in combination with amikacin was also investigated, with special attention to the possible emergence of resistant strains.

MATERIALS AND METHODS

Test strains were isolated from clinical specimens in cancer patients at the Institut Jules Bordet. Ceftazidime (2 g) and amikacin (0.5 g), alone or in combination, were tested for activity against seven strains of *K. pneumoniae* and *P. aeruginosa*. Cefoperazone in doses of 2, 4, or 6 g alone and in 2-g doses in combination with amikacin (0.5 g) were tested against nine strains of *K. pneumoniae* and *P. aeruginosa*. Minimal inhibitory concentrations (MICs) were determined in Mueller-Hinton broth supplemented with calcium chloride (50 mg/liter) and magnesium sulfate (20 mg/liter), using a twofold dilution technique in microtiter plates. A final concentration of 5×10^4 viable organisms per ml was included in each well. The MIC was defined as the lowest concentration in which there was no visible growth after 18 h of incubation at 37°C. All wells which remained clear were subcultured on Mueller-Hinton agar plates (1 μl). The lowest concentration of drug that produced a 98% reduction in the original inoculum after 18 h of incubation at 37°C was defined as the minimal bactericidal concentration (MBC). Sera for measurements of drug concentration and bactericidal activity were obtained from 10 volunteers. These were healthy males and females (five of each) 22 to 35 years of age and weighing 45 to 65 kg. Each volunteer received randomly, on separate days, one of the following regimens: 2 g of cefoperazone; 4 g of cefoperazone; 6 g of cefoperazone; 2 g of cefoperazone plus 500 mg of amikacin; 2 g of ceftazidime; 500 mg of amikacin; or 2 g of ceftazidime plus 500 mg of amikacin. Each antibiotic was dissolved in 50 ml of 5%

TABLE 1. MBCs of cefoperazone, ceftazidime, and amikacin for test strains of *K. pneumoniae* and *P. aeruginosa*

Drug	MBCs (μg of drug/ml of culture medium) against:			
	K. pneumoniae		*P. aeruginosa*	
	Range	Median	Range	Median
Cefoperazone	0.2–12.5	0.4	0.8–25	3.1
Ceftazidime	0.1–3.1	0.2	1.5–12.5	3.1
Amikacin	0.4–6.25	3.1	3.1–25	25

glucose in water and infused intravenously over 15 min. Blood was obtained 1 and 6 h after the beginning of the infusion. Serum was separated promptly and stored at −70°C until assayed. All the measurements of serum level and serum inhibitory and bactericidal activities were made on the sera of individual volunteers.

Serum was assayed in triplicate for contents of cefoperazone and ceftazidime by the bioassay method of Bennett et al. (1), using *Sarcina lutea* ATCC D341 as the test organism for cefoperazone and *Bacillus subtilis* 1DO4E for ceftazidime. Serum levels of amikacin were assayed by fluorescent immunoassay with an Ames TDA fluorescent immunoassay kit.

Serum inhibitory and bactericidal activities were measured in microtiter plates. Serum to be tested was diluted with a 1:1 mixture of heat-inactivated pooled human serum in Mueller-Hinton broth supplemented with calcium and magnesium as recommended by Reller and Stratton (12); the final inoculum was 5×10^4 CFU/ml. After overnight incubation at 37°C, plates were mixed for 5 s on a microshaker before reading. Serum inhibitory activity was defined as the highest dilution showing no turbidity. Using a hand inoculator, we subcultured 1 μl of each well on drug-free Mueller-Hinton agar which was incubated further. SBA was the highest dilution which resulted in no growth (98% killing of initial inoculum).

Killing curves were made in a 1:8 dilution of sera obtained 1 h after the administration of the antibiotic(s). The antibiotic concentrations were equal to one-eighth of the mean values reported in Table 2. The sera of 10 volunteers were pooled for each regimen, and the mixture was tested against nine strains of *P. aeruginosa* and nine strains of *K. pneumoniae*. The 1:8 dilution was made with Mueller-Hinton broth supplemented with Ca^{2+} and Mg^{2+} to a volume of 2 ml. Bacteria were added to yield an inoculum of 10^5 CFU/ml. At 1-h intervals, 10 μl were removed with a calibrated loop and further diluted and subcultured on Mueller-Hinton agar. Colonies were counted after overnight incubation at 37°C.

RESULTS

The MBC values for each species are listed in Table 1. All of these *K. pneumoniae* strains were fully susceptible to ceftazidime, cefoperazone, and amikacin; the *P. aeruginosa* strains were, on the contrary, often resistant or of intermediate susceptibility to amikacin and sometimes resistant to cefoperazone. Table 2 summarizes the serum levels of the three drugs. A direct relationship was found between the dose of cefoperazone administered and serum levels. Serum levels were similar after administration of 2-g doses of ceftazidime and cefoperazone. The results of SBA determinations are presented in Table 3. The values of the median SBA were identical for ceftazidime alone and ceftazidime plus amikacin. These two regimens exhibited a similar percentage of sera with SBA ≥ 1:8 at 1 and 6 h for *K. pneumoniae* (≥97%). Against *P. aeruginosa*, both regimens had a similar rate (97%) of SBAs ≥ 1:8 at 1 h; at least 75% of the sera had SBAs ≥ 1:8 at 6 h.

All the cefoperazone regimens gave similarly good results against *K. pneumoniae* at 1 h; after 6 h, 6 g of cefoperazone significantly more often resulted in SBAs ≥ 1:8 than 2 g of cefoperazone plus amikacin ($P = 0.02$). With *P. aeruginosa*, there was a significant trend toward a higher frequency of SBAs ≥ 1:8 with increasing dosage of cefoperazone ($P < 0.01$); 6 g of cefoperazone produced more frequently SBAs ≥ 1:8 than 2 g of cefoperazone plus amikacin ($P = 0.02$). At 6 h, there were no significant differences between the different regimens. Figure 1 summarizes the data on the killing rate of *K. pneumoniae* by pooled sera obtained from individuals receiving 2- and 6-g doses of cefoperazone and the combination of 2 g of cefoperazone plus 500 mg of amikacin. Cefoperazone alone (2 and 6 g) result-

TABLE 2. Serum levels of cefoperazone, ceftazidime, and amikacin, alone or in combination

Drug dose (g)			Serum level (μg/ml) ± SD after:	
Cefoperazone	Ceftazidime	Amikacin	1 h	6 h
2			93.0 ± 10	7.0 ± 3
4			191.0 ± 36	16.0 ± 7
6			293.0 ± 37	28.0 ± 14
	2		72.0 ± 20	9.0 ± 3
		0.5	19.0 ± 4	3.0 ± 1
2		0.5	96.0 ± 18 (cefoperazone)	8.5 ± 4 (cefoperazone)
			17.0 ± 6 (amikacin)	2.5 ± 1 (amikacin)
	2	0.5	71.0 ± 17 (ceftazidime)	8.0 ± 2 (ceftazidime)
			21.0 ± 7 (amikacin)	3.0 ± 1 (amikacin)

TABLE 3. Median SBAs and percentage of sera with SBA ≥ 1:8

Organism	Antibiotic (dose)	SBA			
		1 h		6 h	
		Median titer	% Sera SBA ≥ 1:8	Median titer	% Sera ≥ 1:8
K. pneumoniae	Amikacin (0.5 g)	1:32	99	1:4	47
	Ceftazidime (2.0 g)	1:512	99	1:64	97
	Amikacin (0.5 g) + ceftazidime (2.0 g)	1:512	99	1:64	97
	Cefoperazone (2.0 g)	1:32	99	1:4	32[a]
	Cefoperazone (4.0 g)	1:64	100	1:8	51[a]
	Cefoperazone (6.0 g)	1:64	100	1:16	66[a,b]
	Amikacin (0.5 g) + cefoperazone (2.0 g)	1:32	100	1:8	51[b]
P. aeruginosa	Amikacin (0.5 g)	1:2	11	<1:2	1
	Ceftazidime (2.0 g)	1:64	97	1:8	74
	Amikacin (0.5 g) + ceftazidime (2.0 g)	1:64	100	1:8	81
	Cefoperazone (2.0 g)	1:8	65[a]	<1:2	22
	Cefoperazone (4.0 g)	1:16	75[a]	1:2	27
	Cefoperazone (6.0 g)	1:32	85[a,b]	1:4	33
	Amikacin (0.5 g) + cefoperazone (2.0 g)	1:16	72[b]	<1:2	23

[a] $P = 0.01$, chi-square test for trend.
[b] $P = 0.02$, Fisher's exact test.

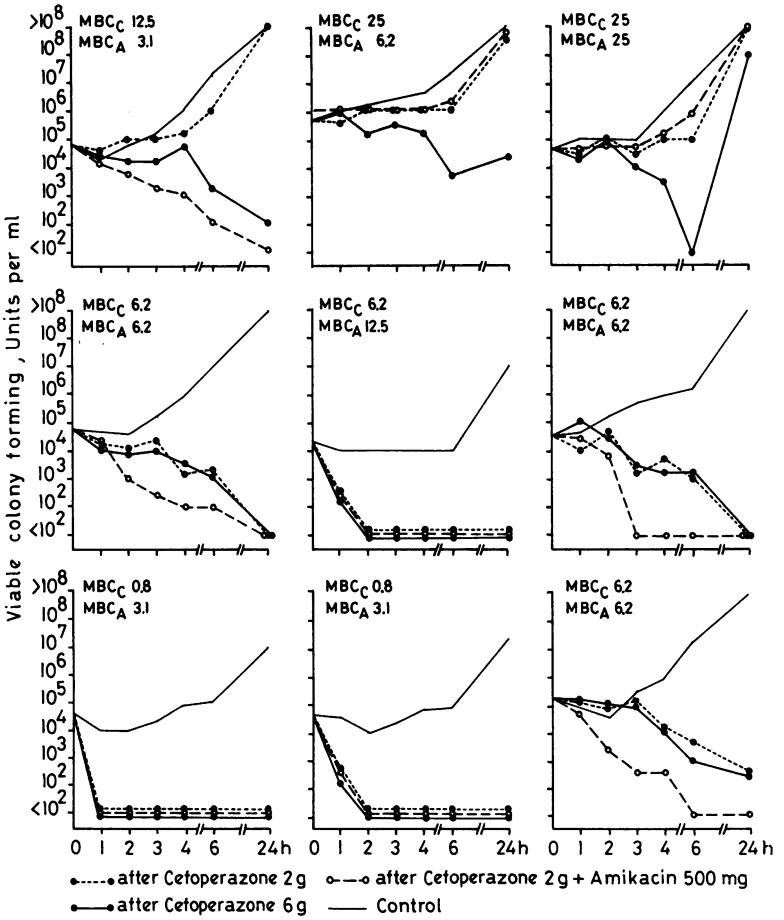

FIG. 1. Killing rates of *K. pneumoniae* by cefoperazone alone or in combination with amikacin. MBCs of cefoperazone (MBC_C) and amikacin (MBC_A) are shown for each of the nine strains tested.

ed in similar activity, significantly lower than the combination. For one strain exposed to serum obtained after administration of 2 g of cefoperazone, we observed the growth of a resistant strain (MBC ≥ 100 μg/ml) after 24 h. Killing rates of *P. aeruginosa* are shown in Fig. 2. Against susceptible strains, the combination of cefoperazone plus amikacin was equal or superior to the 2- and 6-g doses of cefoperazone, which were equal. The 6-g dose of cefoperazone had the highest killing rate against the resistant strains.

When exposed to 6 g of cefoperazone, some *P. aeruginosa* that were initially moderately resistant to cefoperazone (MIC, 25 μg/ml) became resistant (MIC, 100 μg/ml); resistance did not appear in sera obtained from recipients of cefoperazone plus amikacin.

DISCUSSION

SBAs ≥ 1:8 were obtained in 99 to 100% of the sera with all the regimens tested against *K. pneumoniae* 1 h after administration; at 6 h, all the ceftazidime-containing regimens resulted in a high frequency of SBAs ≥ 1:8. The 6-g dose of cefoperazone resulted in significantly more frequent SBAs ≥ 1:8 than a 4-g dose of cefoperazone or cefoperazone plus amikacin. A possible explanation may be the high levels of cefoperazone from the 6-g dose as compared with the low levels of cefoperazone (2 g) and amikacin 6 h after the administration. The same explanation may explain the better activity of increasing doses of cefoperazone in comparison with cefoperazone plus amikacin against *P. aeruginosa*.

Ceftazidime resulted in high median SBAs against *P. aeruginosa* 1 and 6 h after the injection. Addition of amikacin did not increase these median SBA values; an explanation may be found in the low susceptibility of our *P. aeruginosa* strains to amikacin. However, only clinical trials can establish the value of single-drug therapy; recent reviews of the literature have indicated that combinations of antibiotics, especially when synergistic, may be more effective than single-drug therapy in neutropenic patients with gram-negative bacillary infections (8).

Killing studies gave further information on the problem of single-drug versus combination therapy. They were performed only with cefoperazone but showed a significantly higher killing activity for the combination of cefoperazone plus amikacin compared with any of the cefoperazone regimens. Amikacin appeared to prevent the emergence of resistant strains.

In conclusion, ceftazidime seems to be promising as a single-drug therapy for *K. pneumoniae* and *P. aeruginosa* infections. Cefoperazone at high doses (6 g) might also be a safe regimen under the same circumstances. However, the increased killing observed when amikacin was added to cefoperazone may provide evidence for preferring the combination, especially in neutro-

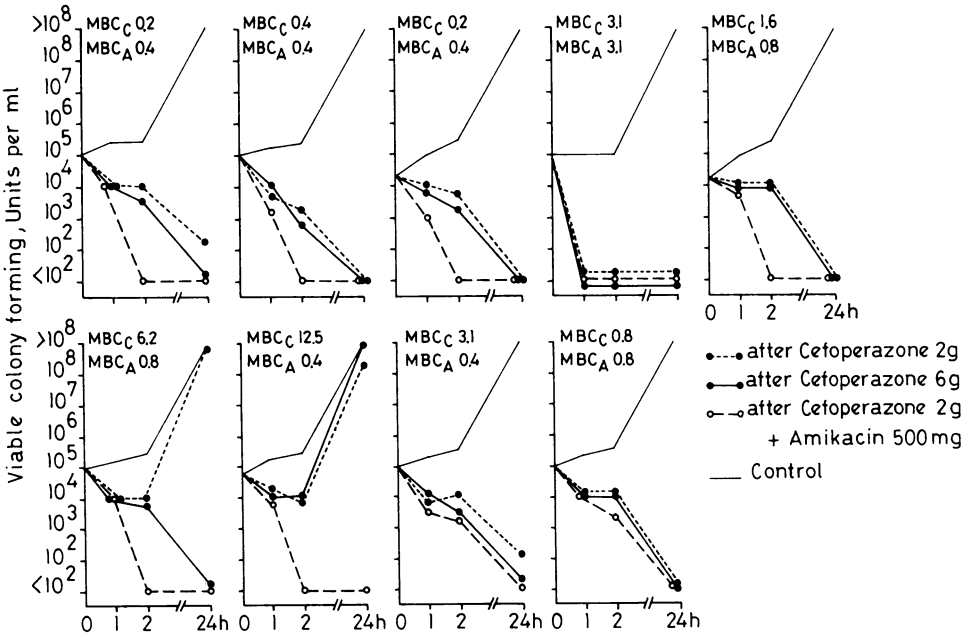

FIG. 2. Killing rates of *P. aeruginosa* by cefoperazone alone or in combination with amikacin. MBCs of cefoperazone (MBC_C) and amikacin (MBC_A) are shown for each of the nine strains tested.

penic patients. In addition, combination therapy may lower the emergence of strains resistant to the beta-lactam antibiotic.

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