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Serum Bactericidal Activity and Postantibiotic Effect in Serum of Patients with Urinary Tract Infection Receiving High-Dose Amikacin

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Ten patients received a 30-min infusion of amikacin (30 mg/kg) on day 1 and 15 mg/kg on day 2. Mean serum creatinine was 1.1 ± 0.3 (standard deviation) mg/dl before and 1.0 ± 0.3 mg/dl 3 days after the second infusion. Mean serum amikacin concentrations before, at the end of infusion, and 1, 6, 12, and 24 h after 30 and 15 mg/kg were 0, 157, 79, 31, 16, 5, 5, 85, 51, 19, 12, and 5 mg/liter, respectively. Five strains each of *Staphylococcus aureus*, *Staphylococcus epidermidis* susceptible and resistant to oxacillin, *Streptococcus (Enterococcus) faecalis*, *Corynebacterium* sp. strain JK, *Listeria monocytogenes*, *Mycobacterium fortuitum* (three strains), *Klebsiella pneumoniae*, *Serratia marcescens*, *Acinetobacter calcoaceticus*, and *Pseudomonas aeruginosa* were tested. Serum bactericidal activities (SBAs) were ≥1:8 in ≥80% of the sera 1 and 6 h after 30 mg/kg and in ≥60% of the sera 1 and 6 h after 15 mg/kg against *Staphylococcus aureus* and *Staphylococcus epidermidis* susceptible to oxacillin, *A. calcoaceticus*, and *K. pneumoniae*. *L. monocytogenes*, *Serratia marcescens*, and *P. aeruginosa* had lower SBAs. Very low or no activity was observed against oxacillin-resistant staphylococci and *Streptococcus faecalis*. The study of the killing rate in serum confirmed these results. Postantibiotic effect was studied by incubating a strain from each species in serum samples obtained 1 and 6 h after both regimens for 0.5, 1, or 2 h. The duration of postantibiotic effect depended on the duration of contact and the concentration of amikacin for the following organisms: oxacillin-susceptible staphylococci, *L. monocytogenes*, *P. aeruginosa*, *A. calcoaceticus*, *K. pneumoniae*, and *Serratia marcescens*. *M. fortuitum* was killed after 30 min of contact. No postantibiotic effect was observed with *Streptococcus faecalis*, *Corynebacterium* sp. strain JK, or oxacillin-resistant staphylococci. Amikacin at 30 mg/kg provided high levels and SBAs against susceptible pathogens. Prolonged postantibiotic effects were observed. No signs of nephrotoxicity occurred.

Bacterial infections are still a major cause of morbidity and mortality in cancer patients, especially in neutropenic patients (25). Treatment of these infections often consists of administration of a broad-spectrum beta-lactam in combination with an aminoglycoside (7), usually amikacin. It was shown that the response rate diminishes and mortality increases when microorganisms resistant to the beta-lactam antibiotics are responsible for the infection. The increasing prevalence of multiply beta-lactam-resistant gram-negative bacilli and the occurrence of clinical failures during treatment with third-generation cephalosporins due to constitutive mutation of the cephalosporinase gene are of major concern (23). Resistance to amikacin is rare, but at conventional doses, this antibiotic is not an optimal therapy for gram-negative bacillary septicemia. It has been reported that the outcome of patients with gram-negative bacillary bacteremia (17) or pneumonia (18) who were treated with aminoglycosides was related to the drug concentration in their serum. Therefore, higher doses of amikacin than usually recommended (15 mg/kg per day) might be more successful (2); the mean dose used by Finley et al. (8) to treat febrile granulocytopenic cancer patients was 29 mg/kg per day.

Studies in animals (1, 10, 11, 15, 21, 27) have suggested that nephrotoxicity was lower when the daily dose was given as a single administration than as two or three injections. Furthermore, prolonged postantibiotic effects (PAE) have been observed with aminoglycosides in vitro and in experimental infections (4, 5, 9, 12; S. Gudmundson, J. D. Turnidge, and W. A. Craig, Clin. Res. 30:777A, 1982).

The purpose of the present investigation was to evaluate the efficacy, as measured by serum bactericidal activity (SBA) (16, 24, 30, 31), the rate of killing in serum (6), and the PAE in the serum of patients receiving amikacin parenterally in higher than usual doses. The tests were performed against common pathogens isolated during episodes of severe infections in neutropenic or immunocompromised patients.

MATERIALS AND METHODS

Patients. The protocol was reviewed and approved by the Ethical Committee of the Institut Jules Bordet. Ten cancer patients were included in the study after having given their informed consent. They were eligible for the study if they had received an aminoglycoside for urinary tract infection due to multiresistant bacteria. Exclusion criteria were abnormal renal (serum creatinine ≥1.5 mg/dl) or hepatic (serum bilirubin ≥2 mg/dl) function.

Administration of antibiotics. The first dose of the treatment was a high dose of amikacin (30 mg/kg) given as a slow infusion over 30 min in 50 ml of 5% glucose in water. A second dose of 15 mg/kg was given after 24 h. From the third day to the end of therapy, the patients received conventional doses (7.5 mg/kg twice a day). Blood samples were obtained before administration, at the completion of administration, and 1, 6, 12, and 24 h after the end of the infusion of both the 30- and 15-mg/kg doses. The patients were asked to empty their bladder prior to the beginning of infusion. Urine was collected from the beginning of the infusion to 1 h after, 1 to

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6 h after, 6 to 12 h after, and 12 to 24 h after the end of infusion.

Test strains. Five strains each of *Staphylococcus aureus* (oxacillin susceptible and resistant), *Staphylococcus epidermidis* (oxacillin susceptible and resistant), *Streptococcus (Enterococcus) faecalis*, *Listeria monocytogenes*, *Corynebacterium* sp. strain JK, *Klebsiella pneumoniae*, *Serratia marcescens*, *Pseudomonas aeruginosa*, and *Acinetobacter calcoaceticus* and three strains of *Mycobacterium fortuitum* were selected for the study. These 57 strains were recent isolates from the Institut Jules Bordet.

Susceptibility testing. MICs and MBCs were measured in microtiter plates with Mueller-Hinton broth supplemented with calcium (50 mg/liter) and magnesium (20 mg/liter) (22); for *Corynebacterium* sp. strain JK and *M. fortuitum*, the tests were performed in brain-heart infusion broth (BHIB) (Difco Laboratories) supplemented with 10% fetal calf serum (supplemented BHIB). The initial inoculum in each well was 10^6 CFU/ml. MBC determinations were done by subculturing 4 μl from each well on drug-free agar, each test being done in duplicate wells. The MBC was defined as the concentration causing a 99.9% reduction of the initial inoculum (19, 29).

SBS and SBA. The serum bacteriostatic activity (SBS) and SBA against all strains were measured for each serum sample taken after 1 and 6 h. The strains of *M. fortuitum* were not tested. Serum titration was done in a microtiter system with a 1:1 mixture of Mueller-Hinton broth and normal human serum as the diluent (22) (or supplemented BHIB). Inoculum concentration and sampling for bactericidal determinations were done as described above. Results were expressed as the median reciprocal SBS and SBA for each species at a given time and as the percentage of sera with a reciprocal SBS or SBA ≥8.

Expected reciprocal SBS (or SBA) at 1 h was calculated by dividing the serum concentration of amikacin for each patient 1 h after administration by the MIC (or MBC) for each strain studied. The expected SBS or SBA was compared with the actual SBS or SBA. A Spearman coefficient of correlation was calculated for the whole set of data as well as for each species.

Serum and urine assays. Levels of amikacin in serum and urine were measured by fluorescence polarization immunoassay (Abbott) (13). The sensitivity of the assay was 0.1 mg/liter. Control serum samples were spiked with 160, 80, and 20 mg/liter and assayed 10 times for the intra-assay reproducibility study and 5 times for the interassay reproducibility study. The serum containing the highest concentration was diluted eightfold and the one containing 80 mg/liter was diluted fourfold in saline; therefore, the study of reproducibility included the variation due to the dilution procedure. Control urine samples were prepared identically to study the reproducibility of the amikacin assay for urine specimens. Serum samples had a coefficient of variation of 0.5 to 2.4% (intraassay) and 4 to 7.5% (interassay). Urine samples had a coefficient of variation of 2 to 3.1% (intraassay) and 3.9 to 8.1% (interassay). Control serum and urine specimens obtained from the patients prior to their inclusion into the study were assayed, and none of them had amikacin or interfering substances. The TDX assay of amikacin has a very high specificity (13).

Pharmacological and statistical calculations. Serum levels were fit with time by a single-exponential decay with the Pharmacologic Calculation System PHARM/PCS version 3 program (Life Science Associates, Bayport, N.Y., 1984) (26). Area under the curve (AUC) between 30 min and 24 h was calculated by the trapezoidal Simpson rules with the same program. Results obtained after 30 and 15 mg/kg were compared by variance analysis and the Wilcoxon matched-pairs rank test. The following null hypothesis was tested to compare the 30- and 15-mg/kg doses: log intercept (30 mg/kg) = log 2× intercept (15 mg/kg); and log AUC (30 mg/kg) = log 2× AUC (15 mg/kg).

TABLE 1. Serum concentration of amikacin

Amikacin dose (mg/kg)	Time postinfusion (h)	Mean drug concn (mg/liter) ± SD	Exponential constant[a] ± SD	AUC (30 min–24 h) (mg · h/liter) ± SD
30	0.5	157.2 ± 32.3	0.184 ± 0.059	685.4 ± 284
	1	79.6 ± 14.9		
	6	30.6 ± 16.9		
	12	15.8 ± 13.2		
	24	5.0 ± 7.5		
15	0.5	84.6 ± 16.7	0.174 ± 0.059	419.2 ± 254
	1	50.6 ± 14.8		
	6	19.3 ± 14.8		
	12	11.6 ± 11.6		
	24	4.6 ± 7.0		

[a] The relationship between concentration and time was fit to a single-exponential decay.

Rate of killing in serum. Portions of each serum sample obtained at 1 and 6 h after the administration were pooled and frozen at −80°C until used. After a 1:2 dilution in supplemented Mueller-Hinton broth (final volume, 2 ml) (or supplemented BHIB), time-kill curves were started for all test strains by the method of Dracke et al. (6). Only one strain of *M. fortuitum* was tested. Bacterial inoculum was 5×10^5 CFU/ml at time zero. All tubes were put on a rotator at 37°C and agitated throughout the experiment; 10-μl samples were obtained at 0, 2, 4, 6, and 24 h. Suitable dilutions were made and plated on Mueller-Hinton agar (or blood tryptic soy agar), and colonies were counted after overnight incubation.

PAE in serum. The PAE was tested by the dilution technique of Craig et al. (5). Portions of each serum sample obtained at 1 and 6 h after administration were pooled. The killing rate was measured as above with one strain of each species, and the drug susceptibility pattern was tested in tubes containing 1 ml plus 10 μl of broth containing the bacterial inoculum. The starting inoculum was 10^6 CFU/ml. After 0.5, 1, and 2 h of contact with amikacin-containing sera collected at 1 and 6 h or fresh human pooled serum for the control tube, the tubes were diluted 1,000-fold with antibiotic-free fresh medium. The growth was followed afterwards by sampling at 0, 0.5, 2, 4, and 24 h after dilution. A control tube without antibiotic was included and treated similarly. The concentration of amikacin was measured in the sample collected at time zero after the 1,000-fold dilution. The PAE was expressed as the difference between the time required for the test tubes submitted to the antibiotic to reach 1 log CFU/ml higher than that obtained after 1,000-fold dilution and the time required for the control tube to reach a similar inoculum level.

RESULTS

Pharmacokinetics. The concentrations of amikacin in serum after doses of 15 and 30 mg/kg are shown in Table 1. The relationship between serum concentrations versus time was fit to a single-exponential decay; coefficients of correlation

TABLE 2. In vitro susceptibility to amikacin

Species (no. of strains)	MIC (mg/liter)		MBC (mg/liter)	
	Median	Range	Median	Range
Staphylococcus aureus				
Oxacillin susceptible (5)	0.8	0.4–1.6	1.6	0.8–3.1
Oxacillin resistant (5)	3.1	0.8–6.2	6.2	6.2–12.5
Staphylococcus epidermidis				
Oxacillin susceptible (5)	6.2	0.2–6.2	6.2	0.2–6.2
Oxacillin resistant (5)	1.6	0.8–6.2	12.5	1.6–12.5
Streptococcus faecalis (5)	>50	>50	>50	>50
Corynebacterium sp. strain JK (5)	>50	>50	>50	>50
Mycobacterium fortuitum (3)	0.4		0.8	
Listeria monocytogenes (5)	6.2	1.6–12.5	12.5	6.2–12.5
Acinetobacter calcoaceticus (5)	3.1	0.8–6.2	3.1	0.8–12.5
Pseudomonas aeruginosa (5)	1.6	0.8–3.1	3.1	3.1–6.2
Serratia marcescens (5)	1.6	0.4–3.1	3.1	0.4–6.2
Klebsiella pneumoniae (5)	0.8	0.4–1.6	1.6	0.8–3.1

were always >0.97 ($P < 0.01$). Serum concentrations at 0.5, 1, and 6 h after 30 mg/kg were not significantly different from twice the corresponding concentrations after 15 mg/kg (Wilcoxon test, $P > 0.2$). The exponential constants of the single-exponential decay fit calculated for 30- and 15-mg/kg doses were not significantly different (Wilcoxon). The AUC after 30 mg/kg was not significantly different from twice the AUC after 15 mg/kg. These results suggested that the small period of time between the two doses did not cause excessive accumulation that could have influenced the results observed after the dose of 15 mg/kg given on day 2. The small increase in urinary excretion during the first hour after 15 mg/kg (28.9 ± 13.8% [standard deviation] versus 16.6 ± 17.6% after 30 mg/kg) could reflect a small residual amount of amikacin from the dose of 30 mg/kg given on day 1. Almost 100% of the amikacin was eliminated in the urine within 24 h after 30 mg/kg (99.2 ± 12.1%) or 15 mg/kg (98.1 ± 9.3%). No renal toxicity could be detected before or after the administration of three doses of amikacin (30, 15, and 7.5 mg/kg).

In vitro susceptibility. Table 2 shows the in vitro susceptibility of the microorganisms tested. All strains were susceptible to amikacin in vitro except *Streptococcus faecalis* and *Corynebacterium* sp. strain JK, for which the MICs were >50 mg/liter.

SBS and SBA. Table 3 shows the reciprocal of the SBS and SBA titers in serum 1 and 6 h after the end of amikacin infusion. For both times and each organism, the median titers after 30 mg/kg were twice those obtained after 15 mg/kg. High activity was observed against *Staphylococcus aureus* and *Staphylococcus epidermidis* susceptible to oxacillin and against *Serratia marcescens* and the *K. pneumoniae* after 15 and 30 mg/kg 1 and 6 h after the administration of amikacin. The activity was very low against oxacillin-resistant staphylococci, even 1 h after 30 mg/kg. Moderate activity was observed against *L. monocytogenes* and *A. calcoaceticus*. Against *P. aeruginosa*, amikacin at 30 mg/kg provided moderate SBA after 1 h, with 78% of the sera having reciprocal titers of ≥8; however, 1 and 6 h after 15 mg/kg, <12% of the sera had titers of ≥8. As expected, no activity was observed against *Streptococcus faecalis* even 1 h after 30 mg/kg. SBA titers were usually identical to or half the corresponding SBS titers, except for oxacillin-resistant staphylococci.

There was an excellent correlation between the measured SBS or SBA and the calculated SBS or SBA both for all strains pooled together and for each species ($P \leq 0.001$).

TABLE 3. Reciprocal SBS and SBA titers

Species (no. of strains)	Amikacin dose (mg/kg)	Reciprocal titer (% of samples with titer ≥ 8) at time postadministration:			
		1 h		6 h	
		Median SBS	Median SBA	Median SBS	Median SBA
Staphylococcus aureus					
Oxacillin susceptible (5)	30	128 (100)	64 (98)	32 (92)	16 (88)
	15	64 (100)	32 (100)	8 (66)	8 (54)
Oxacillin resistant (5)	30	32 (98)	<2 (32)	8 (74)	<2 (8)
	15	16 (90)	<2 (18)	4 (42)	<2 (2)
Staphylococcus epidermidis					
Oxacillin susceptible (5)	30	512 (84)	512 (76)	128 (78)	64 (72)
	15	256 (80)	128 (76)	32 (76)	32 (66)
Oxacillin resistant (5)	30	32 (78)	<2 (14)	4 (50)	<2 (12)
	15	8 (70)	<2 (10)	4 (24)	<2 (8)
Streptococcus faecalis (5)	30	<2 (0)	<2 (0)	<2 (2)	<2 (0)
	15	<2 (0)	<2 (0)	<2 (0)	<2 (0)
Corynebacterium sp. strain JK (3)	30	<2 (0)	<2 (0)	<2 (0)	<2 (0)
	15	<2 (0)	<2 (0)	<2 (0)	<2 (0)
Listeria monocytogenes (5)	30	32 (100)	16 (98)	8 (62)	4 (44)
	15	16 (89)	8 (80)	4 (36)	2 (28)
Acinetobacter calcoaceticus (5)	30	32 (96)	32 (86)	8 (76)	8 (64)
	15	16 (88)	16 (80)	4 (52)	4 (64)
Pseudomonas aeruginosa (5)	30	16 (88)	8 (78)	4 (32)	2 (12)
	15	8 (78)	4 (38)	4 (12)	2 (6)
Serratia marcescens (5)	30	32 (100)	32 (96)	16 (94)	8 (74)
	15	16 (98)	16 (86)	8 (70)	4 (46)
Klebsiella pneumoniae (5)	30	128 (100)	128 (100)	32 (96)	32 (92)
	15	64 (100)	64 (98)	16 (78)	16 (74)

TABLE 4. Rate of killing after 2 h of incubation with serum samples (diluted 1:2) collected 1 and 6 h after amikacin administration[a]

Species (no. of strains)	Mean maximal rate of killing (log_{10} CFU/ml) ± SD			
	30-mg/kg		15-mg/kg dose	
	1-h sample	6-h sample	1-h sample	6-h sample
Oxacillin-susceptible *Staphylococcus aureus* (5)	2.8 ± 0.5[a]	1.8 ± 0.2[b]	2.3 ± 0.2[a,b]	1.6 ± 0.2
Oxacillin-susceptible *Staphylococcus epidermidis* (5)	1.3 ± 0.7[c,d]	0.8 ± 0.5[c]	1.0 ± 0.8	0.5 ± 0.6[d]
Listeria monocytogenes (5)	4.4 ± 0.4[e,f]	3.5 ± 0.7	3.8 ± 0.5[e]	3.1 ± 1.1[f]
Acinetobacter calcoaceticus (5)	2.3 ± 1.1	2.4 ± 1.5	2.4 ± 1.5	2.1 ± 1.6
Pseudomonas aeruginosa (5)	3.4 ± 0.5[g]	2.5 ± 0.5[h]	3.1 ± 0.4	2.1 ± 0.7[g,h]
Serratia marcescens (5)	2.5 ± 1.1	2.2 ± 0.5	2.3 ± 0.8	2.2 ± 0.4
Klebsiella pneumoniae (5)	3.9 ± 0.7[i,j]	3.0 ± 1.0	3.1 ± 0.7[i]	2.4 ± 0.7[j]

[a] Values with the same superscript were significantly different ($P < 0.05$, Wilcoxon test). Species for which little or no killing was observed are not included.

Killing rate in serum. Table 4 shows the rate of killing of all tested strains during the first 2 h of incubation in serum samples obtained 1 and 6 h after both doses. Figures 1 and 2 show the killing curves in serum for a representative strain of each group of five strains belonging to the same species. Against oxacillin-susceptible staphylococci (Fig. 1A and C), the sera collected 1 h after either 30 or 15 mg/kg had a slightly higher killing rate than the corresponding sera collected at 6 h; however, almost no viable organisms were recovered after 24 h of incubation, except for the strain of oxacillin-susceptible *Staphylococcus aureus* incubated in

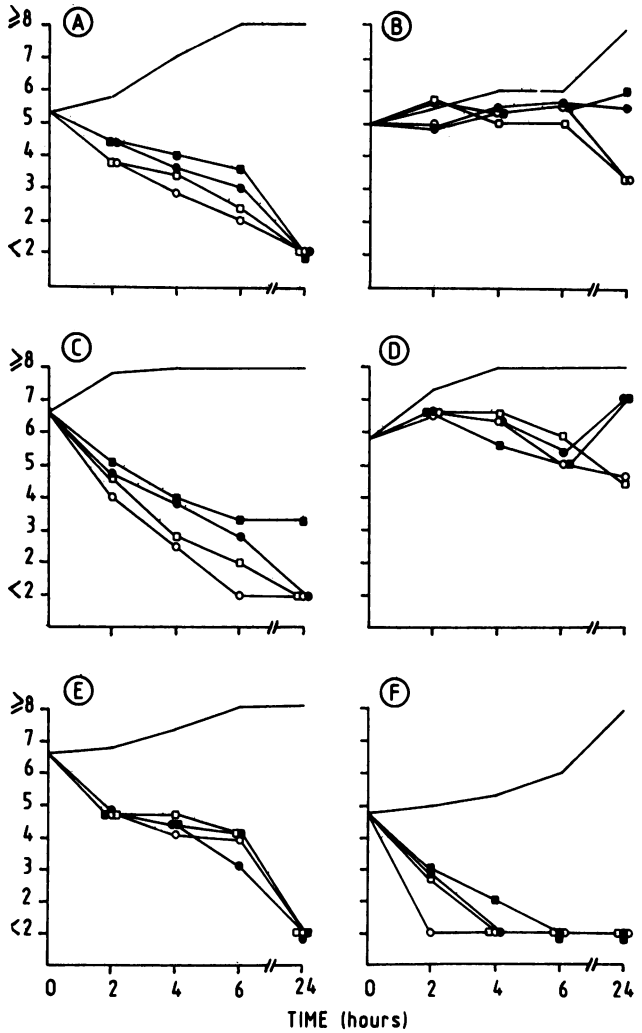

FIG. 1. Rate of killing of *Staphylococcus epidermidis* susceptible to oxacillin (A) and resistant to oxacillin (B), *Staphylococcus aureus* susceptible to oxacillin (C) and resistant to oxacillin (D), *Serratia marcescens* (E), and *K. pneumoniae* (F) in serum of patients receiving amikacin. Symbols: ——, control; samples taken 1 h (○, □) and 6 h (●, ■) after 30-mg/kg (○, ●) and 15-mg/kg (□, ■) doses of amikacin.

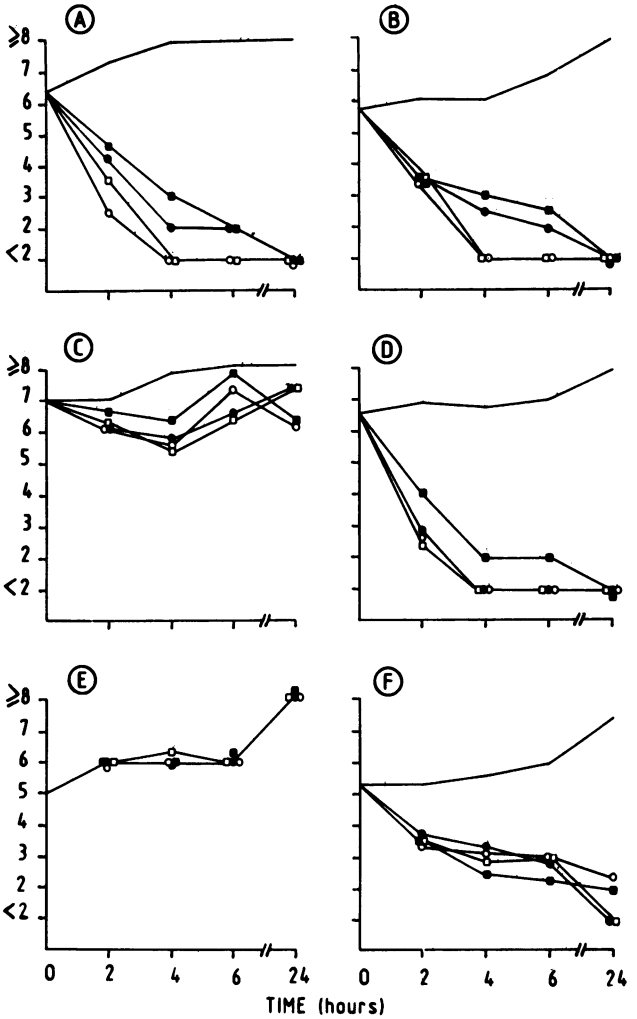

FIG. 2. Rate of killing of *P. aeruginosa* (A), *A. calcoaceticus* (B), *Streptococcus faecalis* (C), *L. monocytogenes* (D), *Corynebacterium* sp. strain JK (E), and *M. fortuitum* (F) in serum of patients receiving amikacin. The symbols are the same as in Fig. 1.

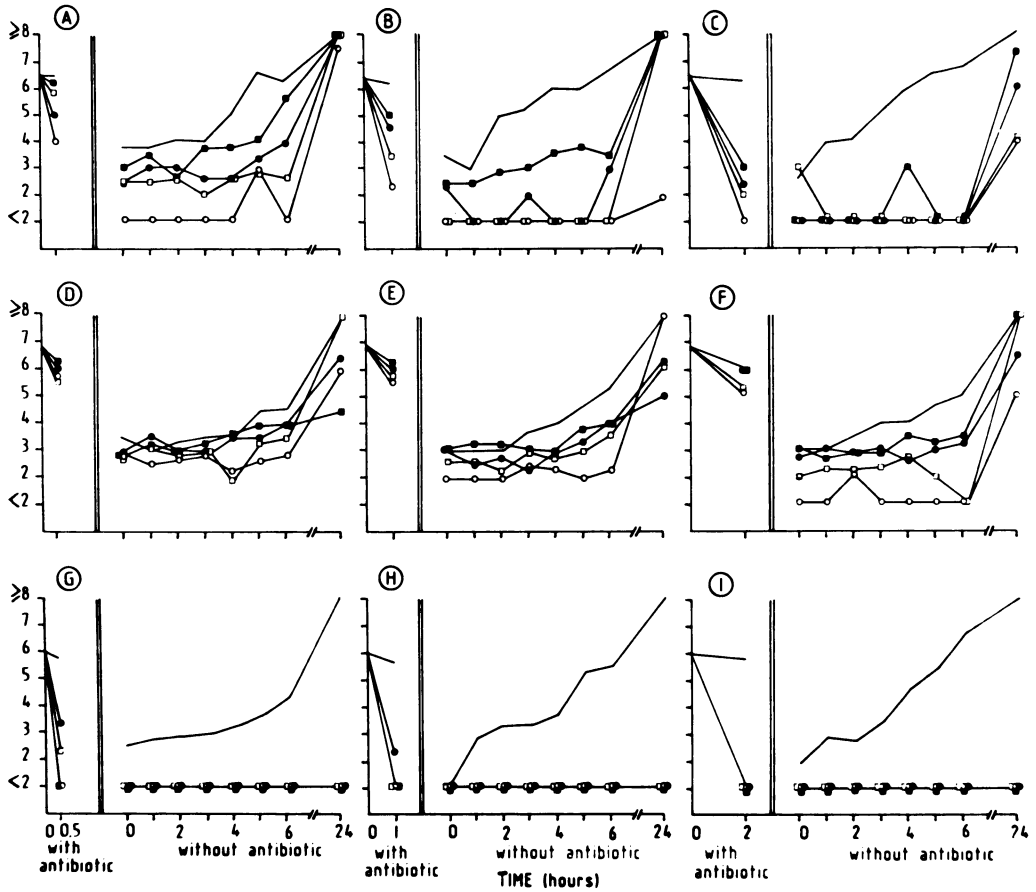

FIG. 3. PAE observed in sera collected from patients receiving 15 and 30 mg of amikacin per kg after various durations of incubation. *Staphylococcus aureus* susceptible to oxacillin (A, B, C), *L. monocytogenes* (D, E, F), and *M. fortuitum* (G, H, I) were incubated for 30 min (A, D, G), 1 h (B, E, H), or 2 h (C, F, I) with the serum samples. The symbols are the same as in Fig. 1.

the serum obtained 6 h after 15 mg/kg. Very little or no killing was observed against oxacillin-resistant staphylococci (Fig. 1B and D). Oxacillin-resistant staphylococci regrew in sera collected 6 h after 15 and 30 mg/kg without significant change in their in vitro susceptibility to amikacin as measured in broth. *Serratia marcescens* was rapidly killed (Fig. 1E) independently of the dose of amikacin, whereas *K. pneumoniae* (Fig. 1F) was more rapidly killed in sera collected 1 h after 30 mg/kg (Wilcoxon, $P \leq 0.05$). *P. aeruginosa* and *A. calcoaceticus* (Fig. 2A and B) were more rapidly killed in sera collected 1 h after 15 or 30 mg/kg than in those collected after 6 h (Wilcoxon, $P \leq 0.05$). *Streptococcus faecalis* (Fig. 2C) and *Corynebacterium* sp. strain JK (Fig. 2E) were not affected by the very high concentration of amikacin present in the peak sera. *L. monocytogenes* was very rapidly killed in the four sera (Fig. 2D). *M. fortuitum* was less rapidly killed (Fig. 2F), independently of the dose.

PAE in serum. The concentration of amikacin after 1,000-fold dilution in antibiotic-free fresh broth was always ≤0.1 mg/liter. This corresponded to one-fifth of the lowest MIC and one-tenth of the lowest median MIC for the tested strains. Figure 3 shows the regrowth curves of an oxacillin-susceptible strain of *Staphylococcus aureus*, *L. monocytogenes*, and *M. fortuitum* after various durations of contact with sera collected 1 and 6 h after 30 and 15 mg of amikacin per kg. The PAE was proportional to the duration of contact with the antibiotic-containing serum and its concentration in amikacin for oxacillin-susceptible *Staphylococcus aureus* and *L. monocytogenes*. Against the former organism, the peak serum level obtained after 30 mg/kg had a PAE of 2 h after 30 min, 4 h after 1 h, and more than 5 h after 2 h of exposure; on the other hand, the serum obtained 6 h after 15 mg/kg had a PAE of 90 min after 30 min, 135 min after 1 h, and more than 5 h after 2 h of exposure. Against *L. monocytogenes*, the peak-level serum after 30 mg/kg provided a longer PAE, ranging from 1 h after 30 min of exposure to more than 3 h after 2 h of exposure, than the serum collected 6 h after 15 mg/kg which ranged from no PAE after 30 min of exposure to more than 3 h after 2 h of exposure. However, even after 2 h of contact (Fig. 3C and F), the two strains regrew after 24 h of incubation in antibiotic-free broth. The MICs for the regrowing colonies showed no significant change in their in vitro susceptibility to amikacin. *M. fortuitum* was apparently completely killed even after 30 min of contact with amikacin-containing sera (Fig. 3G).

Figure 4 shows the results obtained with *P. aeruginosa*, *A. calcoaceticus*, and *K. pneumoniae*. The PAE also appeared to depend on the duration of contact and the concentration of amikacin. The PAE obtained in serum collected 1 h after 30 mg/kg ranged from 1 to more than 2 h depending on the time of exposure, whereas the PAE obtained in serum collected 6 h after 15 mg/kg ranged from no PAE to more than 75 min. Similar results were observed with *K. pneumoniae* and *A. calcoaceticus*. For the strain of *K. pneumoniae* studied, the PAE after 30 min of exposure to serum samples

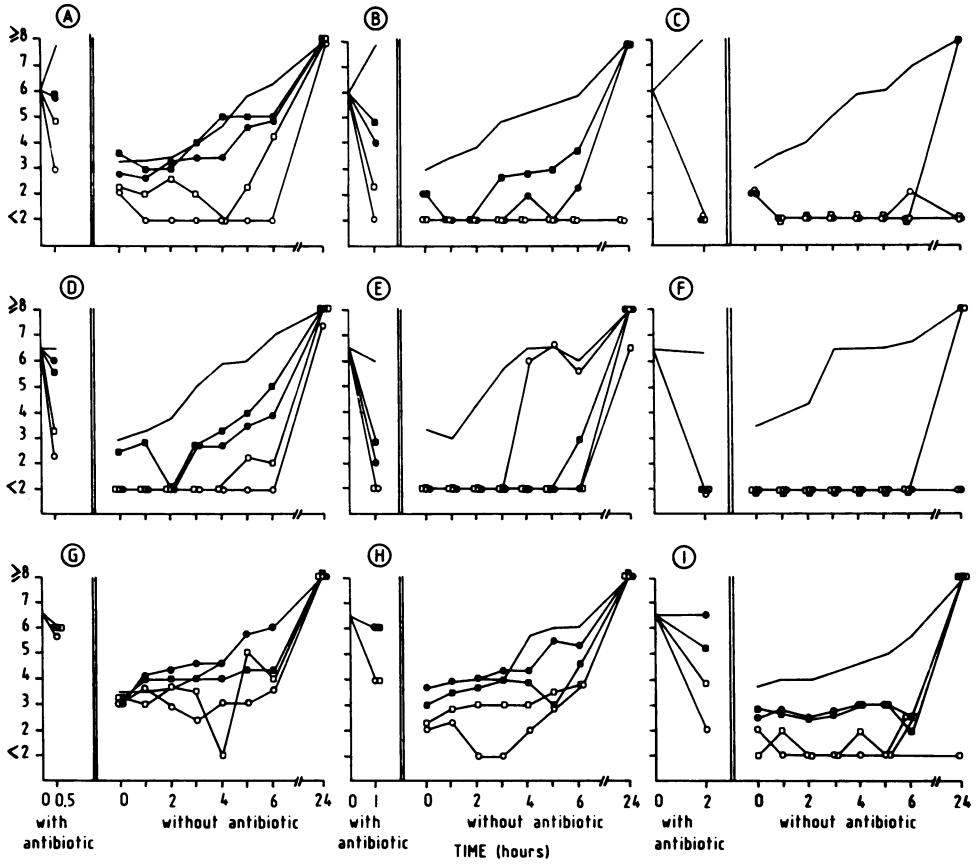

FIG. 4. PAE observed in sera collected from patients receiving 15 and 30 mg of amikacin per kg after various durations of incubation. *K. pneumoniae* (A, B, C), *A. calcoaceticus* (D, E, F), and *P. aeruginosa* (G, H, I) were incubated for 30 min (A, D, G), 1 h (B, E, H), or 2 h (C, F, I) with the antibiotic-containing serum samples. The symbols are the same as in Fig. 1.

containing amikacin were as follows: more than 150 min for serum collected 1 h after 30 mg/kg, 120 min for serum collected 1 h after 15 mg/kg, 60 min for serum collected 6 h after 30 mg/kg, and 0 for serum collected 6 h after 15 mg/kg.

The high concentration of amikacin in the serum obtained 1 h after 30 mg/kg could apparently completely kill *P. aeruginosa* after 2 h of contact only (Fig. 4I). Sera obtained 1 and 6 h after 30 mg/kg completely killed the strain of *A. calcoaceticus* (Fig. 4F). Sera obtained 1 and 6 h after 30 mg/kg and 1 h after 15 mg/kg killed the strain of *K. pneumoniae* (Fig. 4C).

An oxacillin-susceptible strain of *Staphylococcus epidermidis* (data not shown) showed results identical to those observed with the oxacillin-susceptible strain of *Staphylococcus aureus*. The strain of *Serratia marcescens* provided results identical to those observed with *K. pneumoniae*. No PAE was observed with *Streptococcus faecalis*, *Corynebacterium* sp. strain JK, or oxacillin-resistant staphylococci.

DISCUSSION

Most authors agree that a multicompartment model best describes the distribution and elimination characteristics of aminoglycosides, but for practical purposes the use of an open-compartment model may be acceptable (20). In this study, serum levels and SBS and SBA titers were proportional to the dose of amikacin given. The results observed with the dose of 15 mg/kg were usually twice those obtained at a corresponding time after 7.5 mg/kg, such as tested in a previous study (29). High SBA titers (≥8) were observed up to 6 h after administration against susceptible pathogens such as oxacillin-susceptible staphylococci, *A. calcoaceticus*, and *K. pneumoniae*. Lower activity was observed against *L. monocytogenes*, *Serratia marcescens*, and *P. aeruginosa*. No activity was obtained against *Streptococcus faecalis* and oxacillin-resistant staphylococci. The rank correlations between actual SBS or SBA and calculated SBS or SBA were excellent for the whole set of data as well as for each species, suggesting that in vitro susceptibility may be easily extrapolated to the in vivo situation with aminoglycosides.

These results were confirmed by the study of the killing rate in serum. However, the rate of killing was correlated with amikacin concentration only for oxacillin-susceptible *Staphylococcus aureus*, *K. pneumoniae*, *P. aeruginosa*, *A. calcoaceticus*, and *L. monocytogenes*.

Although some of the rates of killing were significantly different, the difference was relatively small and probably not clinically significant.

Prolonged PAE was observed for most of the strains tested and was usually dependent on the species, the duration of contact with amikacin-containing sera, and the dose given. The dependence of PAE on the concentration has been reported for aminoglycosides and extensively discussed by Craig et al. (5). Prolonged PAE is probably clinically relevant since in the neutropenic mouse model, the

efficacy of gentamicin given continuously or discontinuously was identical, although the efficacy of a beta-lactam with no PAE was much higher when the drug was given continuously than when given discontinuously (9, 12; Gudmundson et al., 1982). PAE observed for *Escherichia coli*, *K. pneumoniae*, and *P. aeruginosa* were similar to those observed in the present study. The role of serum has been studied by Bundtzen et al. (4); they reported that at similar multiples of the MIC, the duration of the PAE was nearly identical in serum and in broth.

Although no nephrotoxicity was observed in the present study, it might occur with more prolonged treatments with high doses. In the study of Finley et al. (8), who administered a mean of 29.4 mg/kg per day, nephrotoxicity was not higher than in a previous study using conventional doses. Our patients, although presenting with an acute urinary tract infection, were not severely ill, and none of them was bacteremic. In the presence of gram-negative bacteremia the renal toxicity of aminoglycosides may be potentiated by endotoxinemia (3). Furthermore, ototoxicity, which was not assessed here, might also be a problem with longer treatment (8).

Studies performed in rats (1, 10, 11, 15, 27) and dogs (21) have suggested that gentamicin, tobramycin, netilmicin, and dibekacin were less nephrotic when the same daily dose was given once instead of two or three times per day by a variety of criteria such as serum creatinine, renal DNA synthesis, accumulation of myelin bodies, and other morphological modifications observed by electron microscopy, and which were correlated to the rate of cortical accumulation of the aminoglycosides. It is not possible to firmly extrapolate these observations to amikacin.

Aminoglycosides are usually administered in combination with various beta-lactam antibiotics. These combinations, especially when synergistic, have given better results than single-drug treatment in the therapy of infections in neutropenic patients (16, 24). It is most probable that the beneficial effect of the aminoglycoside is restricted to the first days of treatment, provided that the first peak levels are high enough. Therefore, the use of high doses of aminoglycosides during the first 2 or 3 days of treatment could be a valuable approach and might minimize the toxicity. Moreover, synergistic interactions with a beta-lactam are more likely to occur when the ratio between the concentration of the aminoglycoside and the MIC is high (14, 28).

We have investigated the efficacy of single infusion of high doses of amikacin (15 and 30 mg/kg) against a variety of pathogens isolated from immunocompromised patients, as measured by the SBA and SBS titers, the rate of killing in serum, and the duration of PAE. The results of each test were usually proportional to the concentration of amikacin in the serum. The results obtained with the dose of 30 mg/kg against susceptible pathogens suggested that a single injection a day might be a valuable treatment.

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